ADDITION OF ENDOGENOUS TANNINS FOR IMPROVING THE QUALITY OF FERMENTED CIDER AND THE DEVELOPMENT OF A POMACE-DERIVED TANNIN CONCENTRATE

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

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August 2017

ABSTRACT

A consumer acceptability study was conducted to determine acceptability of ciders fortified with endogenous and exogenous tannins as availability of high-tannin cider apples is limiting cider market growth. The results indicate positive consumer preferences for cider fortified with tannin from apple and non-apple sources and consumers' willingness to pay an additional premium for these products.

There are no commercially available apple-derived tannins. Apple pomace, rich in tannins, is an unexploited waste product. Optimized extraction and concentration trials were conducted to determine conditions for enhanced phenolic recovery from pomace, yielding 41 °Brix concentrates with 1.3-2.4% tannins. Ciders prepared with a Red Delicious and Dabinett high-tannin concentrate received positive overall liking scores and showed insignificant changes other than phenolic augmentation.

Pomace for tannin production should be low in acidity, and moderate-to-high in fermentable sugars. At typical dosage applications, the increased cost to each 750 mL unit of cider would be $0.12-0.24.

BIOGRAPHICAL SKETCH

Micah Martin received his Bachelor of Science degree in Mathematics from Pepperdine University in 2009. He worked under the mentorship of Kendra Killpatrick researching Gaussian binomial identities.

Upon graduating, Micah went on to obtain his teaching certificate and work as a middle school teacher with Teach for America in Baltimore, MD. After the completion of the program, he decided to explore his passion for food. Collaborating with James Beard Award-winning Chef Spike Gjerde, Micah co-founded and managed an award-winning canning company that preserved local agricultural produce.

Later, in New Orleans, Micah was a chef at Shaya, 2016 James Beard 'Best New Restaurant' award winner. He then decided to a pursue his graduate degree in Food Science with a minor in Applied Economics and Management at Cornell University. His research has focused on exploring options for valorizing waste products from the cider making process, a crucial sector of NYS agriculture. As a member of the program, he received the prestigious departmental Kosi Award in Food Science. He was also a member of the IFTSA MARS product development team that placed first with their product Jack'd Jerky. He served as a teaching assistant for the two classes Product Development and Cider Production. For his role as teaching assistant for the Product Development class, Micah was supported by the Henry and Ruth Herzog Graduate Research Fund.

At graduation, he was given the honor of representing the Cornell graduate school as Degree Marshal for the Masters' candidates. He plans to return to New Orleans, LA where he hopes to work in an extension or a product development role.

In loving memory of James Alex Martin,

Roscoe Baker, and Rhon Davis.

ACKNOWLEDGMENTS

I would like to begin by thanking my advisor, Dr. Olga Padilla-Zakour, for providing me this incredible opportunity to pursue my master's degree at Cornell University. She has been an inspiring mentor, teacher, and unwavering friend throughout this entire process. I count myself immensely lucky to have worked under her tutelage. In addition, to my fellow lab members, you have been a fantastic support system, personally, and academically. I can't imagine having completed this research without you.

I would also like to express gratitude to Kyle Kriner, Alina Stelick, Cortni McGregor, and Chris Gerling, who were immeasurably valuable in providing technical assistance with experiments, sensory tests, and cider making. Thank you to my committee member, Dr. Josh Woodard, for his support and for teaching one of the most informative, cross-disciplinary, and challenging courses of my academic career.

To my mother, Lori Martin, you have been my staunchest supporter. You have taught me everything I know about writing and have answered my phone calls at any point, day or night. My gratitude will never recompense a lifetime of unconditional love. Hannah and Aaron, thank you for the kind and generous-hearted people you have become. No greater siblings could ever be wanted. And finally, to my partner, Sean Waters, with grace and hope you have helped me come out the other side. You have held in your eyes and your words the enchantment of life, and have shared it with me audaciously and absolutely.

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CHAPTER 1

INTRODUCTION

A distinction is to be made on the outset that cider, as Americans have come to know it, is an entirely different product than that known by the rest of the world. In America, cider refers to the fresh, unfermented juice from apples that is further distinct from 'apple juice' because it is unfiltered and not treated with clarifying enzymes. Cider, *sidra*, *cidre*, *apfelwein*, and others, are all used synonymously throughout the rest of the world to refer to the fermented apple juice beverage, as will be the convention in this paper.

Brief History of Cider

The current market for cider is a product of several significant events in its history. In the 17th century, it was popular among the lower orders of society as an economical, cold-hardy alternative to wine and was often used in its diluted form as a payment of wages (French, 1982). It didn't take long before sailors also noticed that, unlike beer, a flagon of cider could stave off scurvy on long voyages at sea. Cider, as with most fermented beverages, functioned as a method of preservation, extending the shelf life of apple juice, and providing a source of clean and safe drinking water, albeit with some measure of alcohol (Vallee, 1998).

English settlers brought this tradition with them when they arrived in colonial America, a region well-suited to growing apples. Stories of Johnny Appleseed's adventures (Price, 1954), John Adam's penchant for morning imbibing, or its featured role in William Henry Harrison's Presidential campaign (Boller, 2004), filled

newspapers and books with stories of cider's influence throughout the 18th and 19th centuries.

Its popularity ended with the beginning of Prohibition in 1918 (though the amendment was not ratified and in full effect until 1920) (Okrent, 2010). The perception of apples as primarily a foundation for cider changed suddenly, and Americans started consuming apples for their nutritional value and taste *en masse* (Means, 2011). At the brunt of the Federal Bureau of Investigation's axe, growers were faced with the eviscerated market for cider and the concomitant economic opportunity of apples for fresh market. What ensued was the permanent removal of trees bearing varieties that were suited for making cider and in their place, the substitution of fresh eating apples.

Following the end of Prohibition in 1933, beer, wine, and liquor sales all quickly recovered, while cider did not and this has persisted until recent history. Fast forward to the 21st century and cider sales have been booming. Over the period of 2009 to 2014, total gallons of cider bottled in the United States went from 6.9 million gallons to 53.6 million gallons (ATTTB, 2009). This growth was primarily driven by a few large producers with ties to already successful much larger beer companies (Boston Beer Company, MillerCoors, C&C Group). Since 2014, the exponential growth of the large producers has slowed somewhat, but local craft producers continue to see accelerating profits with a 39% growth in off-premise annual dollar sales from 2015 to 2016 (Brager & Crompton, 2017).

There is a similar picture in the state of New York, the second largest apple producing state in the US. New York is home to the largest domestic producer of cider, Angry Orchard (Boston Beer Co.) and an ever growing number of licensed small and medium-scale cider producers. With this influx of new producers, and the relatively recent boom of cider in general, consumers' expectations are still as of yet

unclear. Over time, brand familiarities will develop, the recommendations of friends and experts will become more common, and more nuanced palates will emerge from exposure to a diverse set of cider styles. Both small and large producers are diversifying their product portfolios to maintain differentiation and keep adventurous and at times fickle consumers engaged.

Though there has been much growth in the cider sector, still nearly 80% of the apples produced in New York State (NYS) are destined for fresh eating or for processing (not as juice) (National Agricultural Statistics Service, 2016). NYS in particular, due to its agricultural resources, is well-suited to take advantage of the growing cider market. There is also opportunity in mimicking the successful strategies of state wineries, as in the Finger Lakes region of NYS, in developing an agro-tourism industry around on-site winery/cidery tours. Angry Orchard, Beak & Skiff, and others have expanded their on-premise presence to take advantage of this growing trend.

Recently the Alcohol and Tobacco Tax and Trade Bureau (TTB) increased the minimum percent of alcohol by volume (ABV) of 'hard cider' from 7% to 8.5% (Imposition and Rate of Tax, 2016). The allowable carbonation level was also increased from 0.392 g/100 mL to 0.64 g/100 mL. The implications of this are that a greater number of ciders legally qualify as a 'hard cider' and are taxed at the substantially lower rate of 22.6 cents (before small producer tax credits). Those ciders with ABV's above 8.5% and/or carbonation levels above 0.64 g/100mL are considered either 'still wines' or 'effervescent wines' and are taxed at much higher rates. Changes in regulations such as these have and will continue to encourage the growth of small producers and the growth of the cider sector.

The TTB is not the first to struggle with how to define cider among its two largest competitors: should it be considered a wine or a beer and how should it thusly

be regulated? A case can be made for either, both, or neither, and these opinions are made evident by modern choices in packaging. Ciders are equally available in 750 mL 'wine' and champagne bottles and 12-oz cans and bottles. In the United Kingdom, ciders are primarily packaged in 12-oz cans or bottles and marketed to compete with beer, whereas in France, they are traditionally packaged in 750 mL 'champagne' or 'wine' glass bottles and are marketed to compete with these products. The starting material and the cider production process is more certainly on the side of wine production.

Due to the prolonged period of cider unpopularity in the United States, research interest at academic institutions was limited. Most of the research into cider up until the mid 1980's was due to the Long Ashton Research Station (LARS) in the United Kingdom (Lea & Drilleau, 2003). Some key contributions made by LARS that have dramatically improved the quality of cider and are in large part responsible for its modern success are: the importance of sulphur dioxide in controlling wild fermentations, insistence on rigorous standards of hygiene, and the development of pure yeast cultures (Beech, 1972).

Making of Cider

In the production of cider there are classically four different types of apples as originally classified by the LARS system **(**Table 1). This system of apple classification differentiates apples based on total polyphenolics and titratable acidity. Apples which are available for fresh eating are of the 'low tannin' variety, but vary depending on their acidity (tartness). On one end of this spectrum would be the ubiquitous Red Delicious apple with ample sweetness but sparse acidity. On the other end of the spectrum might be a Granny Smith, regarded for its exceptional acidity.

Table 1. Long Ashton Research Station system of apple categorization for use in cider making.

	Low Acid (<0.45% malic acid w/v)	**High Acid** (>0.45% malic acid w/v)
Low Tannin (<0.2% polyphenolics w/v)	Sweet	Sharp
High Tannin (>0.2% polyphenolics w/v)	Bittersweet	Bittersharp

As for the high tannin apples, all of these varieties bear names which are uncommon to most outside the cider making profession. However, 150 years ago in Civil War America, these would have been household names. 'Household names' is a literal description because many of these varieties are regionally relevant and the names arise from the area or name of the family orchard where that variety began such as Roxbury Russet, Albemarle Pippin, or Ben Davis (Calhoun, 1995).

These varieties have not been commercialized for fresh eating due to their high tannin content which makes them from moderately-to-extremely astringent. Unlike wine, where the varietal often dominates the label (especially in the new world wine growing regions), rarely does one apple cultivar produce an exceptional cider of its own right. Typically, different varieties of apples are blended to obtain a desired flavor profile with regard to acidity, astringency, and sweetness and are then marketed either as a mélange or without reference to the apples used.

It is in this way that the classical apple classification system (Table 1) is useful in creating these blends. 'Sweet' apples contribute the necessary fermentable sugars for the fermentation to reach the desired alcohol concentration. The 'sharps' provide acidity which is responsible for cider's piquancy while accentuating supporting flavors; it also balances any potential residual sweetness and it plays a dominant role in pairing cider with food. Finally, the acidity helps to prevent microbial spoilage by

decreasing the pH of the cider below 3.8. The direct relationship between pH and the effectiveness of sulfites which are used to help control microbial spoilage and prevent oxidation is also significant. With a lower the pH, sulfite additions are more effective, and thus less sulfites need to be added.

One category of cider apple that is distinctly omitted from the traditional apple classification system is 'aromatics.' Many cider producers include specific varieties in their ciders solely for their aromatic profile; however, all aromatic varieties can still be classified as in Table 1 and thus will contribute some other important qualities to the cider in addition to aroma. Sweetness is not measurably classified in the LARS system, but is acknowledged only as the implied opposite of acidity. There are many apples however, such as Jonathan or Newtown Pippin, which have both high acidity and high sweetness for which the additional category 'sharp-sweets' is often applied.

The 'bittersharps' and 'bittersweets' will contribute, respectively, a moderate degree of acidity and sweetness, but it is their astringency which sets them apart. The quality of astringency helps to balance other components in cider, as in wine, providing additional structure, and ensuring clarity in the final product. Apples such as Kingston Black and Northern Spy are notable exceptions to the 'single-variety rule,' each containing sufficient levels of sugar, acid, and polyphenolics to make acceptable single-varietal ciders.

The basic process of cider making (Figure 1) involves relatively few steps and resembles the wine making process. It begins with fresh fruit which is milled and pressed to produce fresh apple juice which is then fermented in a storage container and can then be deemed 'cider'. Many variations of this process exist (as shown) and each has important implications for the resulting product.

Prior to pressing, harvested apples can be milled immediately or they can be stored in cold storage for many months. Many cider producers prefer to 'sweat' their

fruit, allowing the apples to ripen and respire with concomitant loss in moisture content, before pressing which has the effect of naturally concentrating the juice and thus the flavor (Merwin, Valois, & Padilla-Zakour, 2008). Many types of mills exist for grinding the apples, but most in contemporary use are a type of hammer mill or rotating knives which reduce the apples to a fine pulp to increase juice yield (Beech, 1972).

After milling, apple pulp can be immediately conveyed, often with pumping, to the press for pressing into juice. Many cider makers will first treat the pulp with pectolytic enzymes to enhance juice extraction. The French style of keeved cider involves macerating the juice with the apple pomace at this point yielding a more oxidized juice with greater extraction of pectin and pectin methyl esterase (PME) into the juice (Lea & Piggott, 2012). The PME is responsible for de-methylating pectin but it does not hydrolyze the polysaccharide as do other pectolytic enzymes -- for example, those that are used for clarification or enhanced juice extraction. The de-methylated pectin is then able to cross-link with the assistance of Ca^{2+} to form a gel (Damodaran, Parkin, & Fennema, 2007). As this gel grows, it rises from the bottom of the fermenting vessel due to the release of carbon dioxide in the initial fermentation. As it rises, it will ensnare proteins and amino acids necessary for yeast fermentation. The intended result is a very slow fermentation process, conducted at very low temperatures that will not ferment to completion, resulting in a naturally sweet, effervescent, and low-alcohol cider.

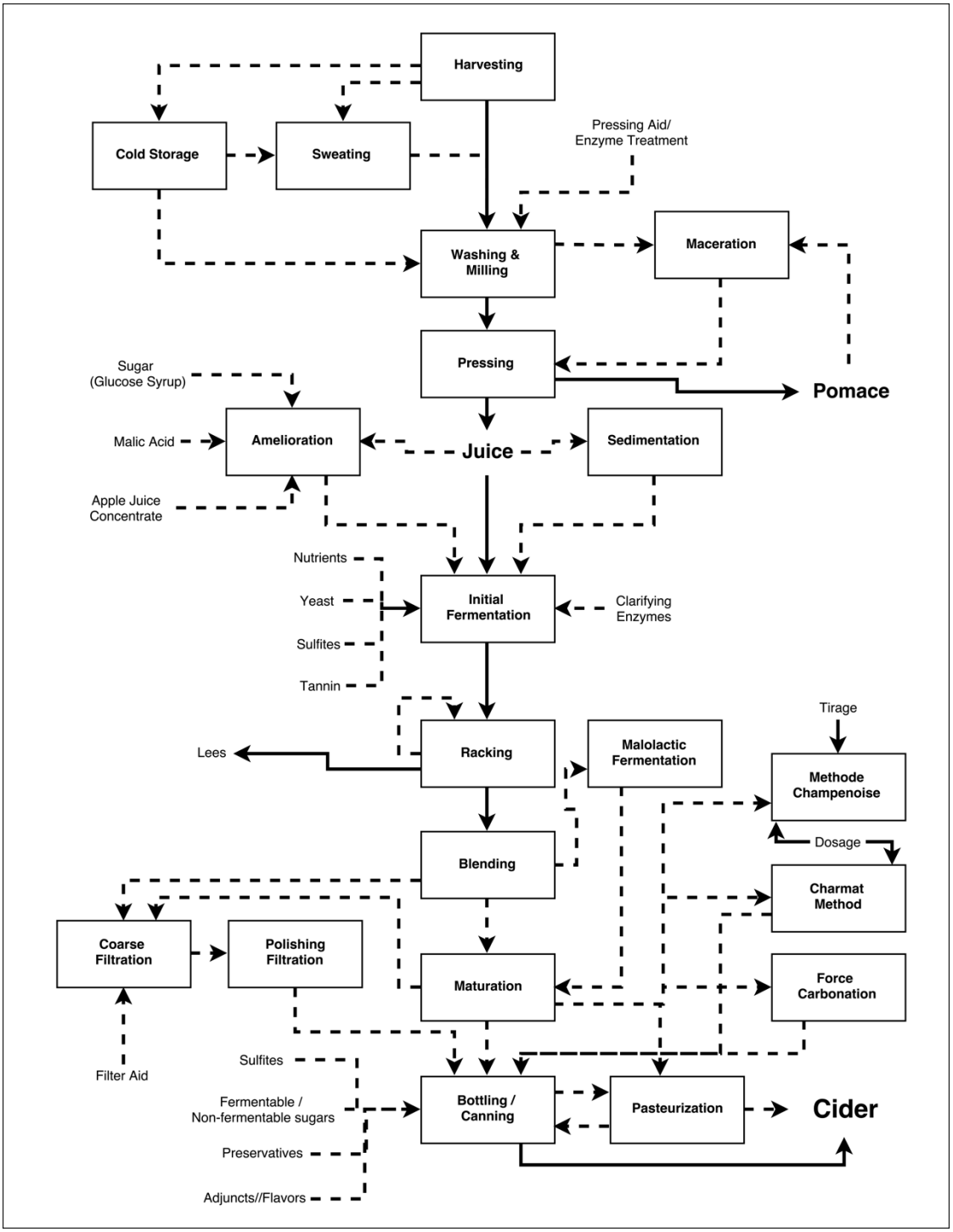

Figure 1. Flowchart of primary and secondary processes in production of cider.

Following pressing, the spent pomace can be extracted with water and then concentrated to capture the juice (and sugar) that remains in the spent pomace, though this is a practice often reserved for larger producers or years of lean harvest (Bump, 1989). The cider can then receive acid adjustments with the addition of malic acid to bring the pH below 3.8 and to the cider maker's preference. Sugar can be added to increase the eventual alcohol content of the cider which can then be diluted later in the process with water, finished cider, or apple juice concentrate to typical levels of alcohol. Sulfites are often added at this point with the addition of potassium metabisulfite (57.6% available SO_2) to reduce wild microflora and to allow for a cleaner fermentation with pitched (added) yeasts. Sulfites are strictly regulated and if the cider contains more than 10 ppm of total sulfur dioxide -- and nearly all do -- the label must contain a declaration stating "contains sulfites" (US Food and Drug Administration, 2016). Failure to do so can result in a recall of the product.

However, it is not uncommon to limit the use of sulfites at this point in the process to allow for the native 'wild' yeasts arising from the environment (processing area, orchard, and fruit) to begin the fermentation. Some cider makers will intend for the wild yeast population to finish the fermentation to dryness (0.0% residual sugar) while others will pitch a commercialized, standardized strain of *Saccharomyces cerevisiae* into the must after a brief period of 'wild' fermentation. This 'wild' character is seen by some as part of the *terroir* and as such, a necessary part of their creative process (Berry & Slaughter, 2003).

The most common practice, especially among large cider producers, is to use a commercialized, standardized strain of *Saccharomyces cerevisiae* because this will give consistent, predictable, and defect-free fermentations. Wild yeasts, on the other hand, are unpredictable, can lead to inconsistent fermentations, and are often responsible for cider defects. Most wild yeasts are also not tolerant of increasing

ethanol concentrations, will not finish a fermentation to completion, or will become sluggish (Boulton, Singleton, Bisson, & Kunkee, 2013). As yeast fermentation slows, with remaining fermentable sugars and nutrients in the must, it presents a ripe environment for competing defect organisms (*Lactobacillus*, *Acetobacter, Zymomonas, Oenococcus,* and other) to dominate (Merwin et al., 2008).

Yeast nutrients are often added prior to fermenting due to the low naturally occurring nitrogen levels in apple fruit (Boudreau, McGuire, Peck, & Stewart, 2016). Yeast assimilable nitrogen (YAN) and other nutrients are necessary for yeast reproduction and can vary widely in different apple varieties and due to different growing conditions. Without sufficient YAN, it has been shown that *Saccharomyces* yeasts will degrade amino acids containing sulfur to obtain nitrogen, which leads to the release of hydrogen sulfide (H_2S) (Jiranek, Langridge, & Henschke, 1995). This compound is typically described as smelling of 'rotten eggs' and has an odor threshold below 1 μg/L (ppb) (Rapp & Mandery, 1986).

After any yeast, nutrients, sulfites, acid/sugar adjustments are made, the juice is transferred to a fermenting stainless steel tank or wooden barrel and fermented typically until no residual sugar remains. The length of the primary fermentation depends on a large number of factors including temperature, yeast strain used, nutrient additions, sulfite additions, and other factors, but usually lasts from 2-4 weeks.

The options following primary fermentation are many. The cider maker will typically rack the cider, which is to separate the cider from the yeast which have expired and settled to the bottom of the tank (referred to as lees). The cider may then be filtered with a coarse filter, polishing filter (for clarification), and/or sterile filter (removal of all microorganisms with an absolute pore size < 0.2 μm) in succession. A filter aid such as bentonite may be added at this point to aid in clarification. In addition, or alternatively, the cider can be then stored for maturation (in oak or steel)

or undergo malolactic fermentation (MLF). MLF is a form of secondary fermentation in which lactic acid bacteria (LAB) convert the malic acid in the cider to lactic acid and carbon dioxide, which has the effect of producing a 'softer' flavor. It can also impart a buttery flavor from the concurrent production of diacetyl by LAB (Zhang & Lovitt, 2006). This practice is more typical among craft or traditional producers than at large scale (Lea & Drilleau, 2003).

The cider may be blended at any point in this process, but is typically done before final racking and storage. Sulfites can be added to stabilize the cider against further possible fermentation and oxidation. Additional acid or sugar adjustments may be made, but care must be taken in adding a fermentable sugar (glucose, fructose, sucrose) to a non-sterile product as remaining organisms will likely ferment it. If a sweetened cider is desired, a non-fermentable sugar (xylitol, sucralose, stevia, and others) must be used, or the product should be pasteurized. Preservatives may also be added to the cider, especially to unpasteurized ciders, to control the risk of spoilage. Adjuncts and/or flavors are incorporated typically at the end immediately prior to bottling. All of these options will result in a still cider. If a carbonated cider is desired, many options can be explored, which are described at length elsewhere (Jolicoeur, 2013).

Apple Composition and Phenolics

The common apple is a fruit from the family *Rosaceae* (the rose family), genus *Malus* and species *domestica*. Apples are on average 92.1% - 96.95% pulp, 3% - 7% skin, and 0.05% - 0.9% seeds with an average weight range of 25 - 180 g (Charley, Mumford, & Warcollier, 1949). The USDA in 2008 tabulated the macronutrient profile of apples at 13.8% carbohydrates, 0.17% fat, and 0.26% protein with the remaining 85.6% being water (Simmonds & Preedy, 2015).

The popular adage, “an apple a day keeps the doctor away,” can be ascribed to the abundant vitamins, micronutrients, and antioxidants in apples. A group of these compounds, the polyphenolics, have been lauded for their free radical scavenging abilities and have been shown to reduce the occurrence and risk of heart disease and cancer (Lee & Smith, 2000; Liu, Eberhardt, & Lee, 2001).

Mean total fermentable sugars for an apple are 10.4 g on a 100 g fresh-weight (FW) basis (Simmonds & Preedy, 2015). Of those sugars, around 20% are sucrose, 23% glucose, and 57% fructose, though this ratio varies significantly between varieties. Apples of the bittersweet and sweet varieties contain higher sugar concentrations which amounts to higher alcohol fermentations. Apples also contain upwards of 4% sorbitol which is a non-fermentable sugar alcohol (Eisele & Drake, 2005). To assess the ripeness of an apple or its suitability for fermenting, growers will often measure the total soluble solids (TSS) expressed in °Brix which has a high correlation with total sugars, but varies due to other soluble components such as acids or phenols. In a survey of the juice from over 175 apple varieties, TSS ranged from 10.26 to 21.62 °Brix with a mean value of 14.24; TA ranged from 0.23% to 1.82% as malic acid with a mean value of 0.87%; pH ranged from 3.37 to 4.24 with a mean value of 3.71 (Eisele & Drake, 2005). Malic acid constituted over 90% of the acids measured, however quinic, tartaric, citric, shikimic, fumaric, isocitric, and succinic acids have also been reported in appreciable amounts in apples (Eisele & Drake, 2005; Lee & Wrolstad, 1987; Wu et al., 2007). All of these findings are subject to immense variability and require large sample sizes for meaningful data which is standard practice for surveys of agricultural commodities.

Another component that plays a significant role in fermentations is concentration of pectin in pressed juice. The amount of pectin in apples has important implications for juice yield from pressing, viscosity of pre-enzyme treated juice,

premature fouling of filtration membranes, undesirable pectin hazes, and reduced astringency in juice (Downing, 2012). Pectin content in apples ranges from 0.14% to 1.15% (w/w) with typical values around 0.78% (Baker, 1997). This is substantially higher than for European wine grapes which tend to range from 0.2% to 0.6% (Silacci & Morrison, 1990).

The yeast assimilable nitrogen (YAN) content of the juice is also important for successful fermentations. Research conducted found that of 15 different apple cultivars surveyed, 14 of them had levels of YAN below the recommended concentration of 140 mg/L (Boudreau et al., 2016). Another survey found that only 13% of surveyed grape musts had levels below 140 mg/L YAN (Butzke, 1998). It is thus more imperative that cider makers supplement juice YAN than it is for vintners if a completed fermentation devoid of off-aromas is to be achieved (Boudreau et al., 2016; Boulton et al., 2013).

As mentioned prior, apples are abundant in phenolic compounds. Typical levels of phenolics range from 0.1% to 0.6% (w/w), but have been reported as high as in excess of 1% on a fresh weight basis (Shahidi & Naczk, 2003). This represents five times the concentration of phenolics that would be necessary to classify an apple as a cider apple (a 'bitter') versus a culinary apple (Table 1). On a concentration basis, most of the phenolic compounds are located in the epidermis, seeds, and stem of the fruit. However, since these parts of the apple cumulatively constitute less than 8% of the apple, the parenchyma (flesh) is also a moderate source for these compounds.

The phenolic compounds in apples are typically divided into six primary classes (Figure 2): the flavan-3-ols ((+)-catechin and (-)-epicatechin), the hydroxycinnamic/phenolic acids (chlorogenic acid), the dihydrochalcones (phloridzin and phloretin glycosides), the proanthocyanidins (polymerized flavan-3-ols), the

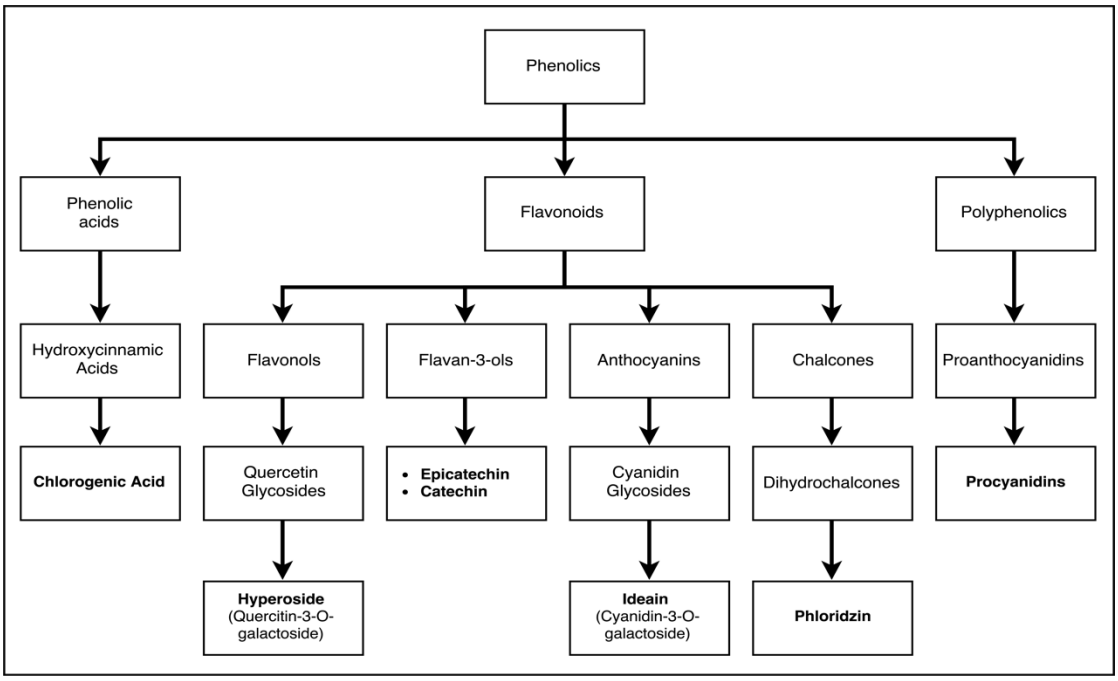

Figure 2. Categorical relationships of major phenolic compounds in apples.

anthocyanins (cyanidin-3-O-galactoside and other cyanidin glycosides), and the flavonols (quercetin-3-O-galactoside and other quercetin glycosides).

Proanthocyanidins are characterized by "hydroxylation pattern, stereochemistry, proportions of flavan-3-ol constitutive units, location and nature of interflavanyl linkages, and by degree of polymerization" (Guyot, Marnet, & Drilleau, 2001, p. 14). In apples, procyanidins are the dominant proanthocyanidins. They are characterized by a C4→C8 linkage between the flavan-3-ol constitutive units (-)-epicatechin and (+)-catechin, the former dominating in apples (Figure 3). The C4→C8 imposes a linear arrangement which dominates in apples, but C4→C6 branched-linkages can occur but are more common in grapes (Hemingway, 1989).

Hydroxylation on the B ring can occur in wine grapes at the 5' position, referred to as prodelphinidins. These galloylated polymers have been studied for their role in astringency in grapes, but are not typically synthesized in apples (Souquet, Cheynier, Brossaud, & Moutounet, 1996). Both prodelphinidins and procyanidins are

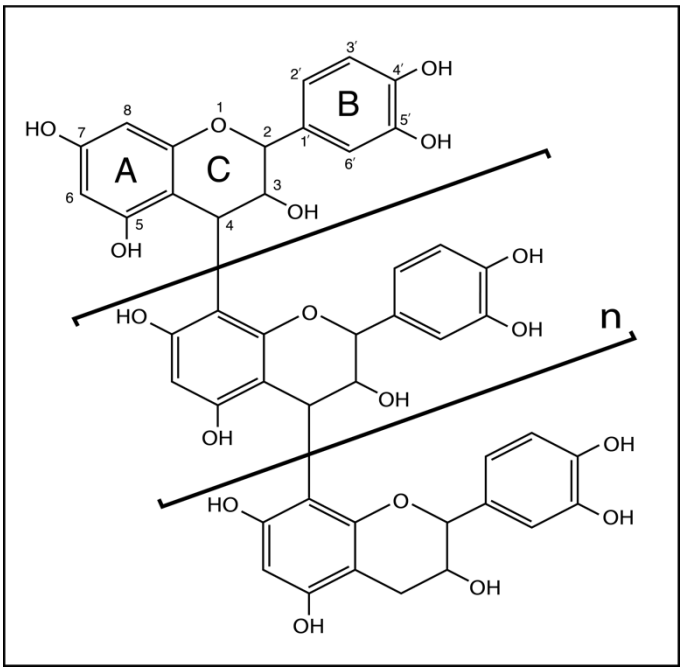

Figure 3. Generalized chemical structure and standardized referential numbering system of procyanidins with variable polymerization of constitutive flavan-3-ol units. Additional hydroxylation at 3' yields prodelphinidins.

part of the phenolic class proanthocyanidins because upon acid hydrolysis, they will yield red-pigmented anthocyanidins (Cheynier & Fulcrand, 2003).

Procyanidins also belong to a class of compounds called tannins. Tannins were functionally defined by Bate-Smith and Swain as "water soluble phenolic compounds having molecular weights between 500 and 3,000 (Da) and, besides giving the usual phenolic reactions, they have special properties such as the ability to precipitate alkaloids, gelatin, and other proteins" (1962). Since this definition was first proposed, multiple studies have since shown that it is likely too limited in scope. It has been shown that procyanidins also have affinities for polysaccharides such as dextrin, cyclodextrin, cellulose, and pectin that can form hydrophobic pockets to ensnare proanthocyanidins and firmly bind them through non-covalent interactions (Cai, Gaffney, Lilley, & Haslam, 1989). In addition, many phenolic compounds exist of much higher molecular weight that are able to bind and precipitate proteins (Haslam, 1996).

It is hypothesized that tannins are synthesized by plants as defensive mechanisms against herbivory and pathogenic microorganisms (Shahidi & Naczk, 2003). They have long been used to treat animal hides in the production of leather. These tannins are typically sourced from trees such as oak, but can be found throughout the plant kingdom. They are synthesized throughout the plant but are often concentrated in the stems and leaves, and for fruits, in the skin and seeds. Some notable food stuffs that are high in tannin are tea leaves, walnuts, spices, such as cinnamon or clove, grapes, quince, and legumes.

The ability of tannins to convert animal hide into leather is due to their susceptibility to oxidation and their ability to precipitate proteins. When eaten, a similar effect is observed with a 'drying' of the mucosal tissue in the mouth. The tannins bind to salivary proteins through non-covalent interactions producing a tannin-protein complex which aggregates, precipitates and elicits the feeling of astringency. As the degree of polymerization of tannins increases, their affinity for proteins also increases. This is due to the multi-dentate structure of tannins which allows for additional bonding sites between the protein and tannin. It also explains why tightly coiled globular proteins, with their hydrophobic residues hidden and a corresponding reduced surface area, have a lower affinity for tannins than proteins which have a looser conformation (Hagerman & Butler, 1981).

Aside from its organoleptic advantages of astringency, these tannins have other implications for cider as well. The tendency of tannins to complex with proteins can cause protein-polyphenol hazes that are typically undesirable in beverages, with pectin exacerbating the problem (Siebert, Carrasco, & Lynn, 1996). More often than not, however, tannins will form insoluble complexes which will precipitate out of solution and can be racked off or filtered resulting in a brilliant (clear) cider.

Other apple phenolics such as chlorogenic acid and catechin, as well as tannins, play an important role in the color of cider through enzymatic and non-enzymatic reactions. When apples are crushed and exposed to oxygen, this brings into contact the previously segregated enzyme polyphenol oxidase (PPO) and its preferred substrates chlorogenic acid and catechin. PPO oxidizes these phenolics into *o*-quinones that are highly reactive species that can then polymerize or react with other molecules to produce the yellow-orange-brown colors of cider. Malec et al. (2014) found that the color saturation, hue, and lightness of cider were most significantly influenced by oxidation of apple phenolics, the pH of the juice, and the interaction between the two.

The oxidation of procyanidins, along with the potential color changes, can result in decreased astringency in cider. As a result, all steps in the cider making process must be carefully controlled against unnecessary exposure to oxygen post-milling.

There are 2 primary classes of tannins: condensed and hydrolysable. The condensed tannins are polymerized flavan units, which in the case of apples, is primarily the class procyanidins. Thus, often when dealing with apples, 'procyanidins' and 'condensed tannins' will be used synonymously. The other class of tannins, hydrolysable (or 'gallo') tannins, yield gallic acid upon acid hydrolysis. This class of tannins, however, is not found in apples, it being more common in the stems and ligneous material of plants. Overall, the tannins in apples tend to be less complex than grapes, with lower average degrees of polymerization and lacking gallic acid esters on flavans (epicatechin-3-O-gallate) and trihydroxylation of the B-ring (prodelphinidins) (Symoneaux, Baron, Marnet, Bauduin, & Chollet, 2014).

The concentration of condensed tannins in the final juice varies based on a number of factors including but not limited to the ripeness of the fruit (Guyot, Marnet,

Sanoner, & Drilleau, 2003), harvesting method (Martinez-Romero et al., 2004), age of orchard and orchard management (Lea & Beech, 1978; Peck, Merwin, Watkins, Chapman, & Padilla-Zakour, 2009), weather and stress conditions (Petkovšek, Stampar, & Veberic, 2008), year to year variability (Guyot et al., 2003), duration of storage (Boyer & Liu, 2004), cultivar (Guyot et al., 2003), cropload (Peck, McGuire, Boudreau, & Stewart, 2016), post-harvest treatments such as sweating (Merwin et al., 2008), oxidation during processing (Lea & Timberlake, 1978), rootstock (Kviklys et al., 2014), region, within-tree variability, sun exposure, within-fruit variability (Awad, de Jager, & van Westing, 2000), addition of sulfites or enzymes, fining (Lea & Timberlake, 1978), and pressure applied at pressing (Prasad, Yang, Yi, Zhao, & Jiang, 2009).

Motivation

Non-astringent apples are often referred to as dessert and culinary apples (those commonly found in supermarkets). They have a long history of consistent demand from both consumers and processors and are the primary apples that NYS produces (National Agricultural Statistics Service, 2016). The production of cider apples is significantly more limited and absent data from USDA it is difficult to estimate current shortages. Data collected by Western Washington University showed that in 2015 there were 256 acres under cider apple production in Washington, the largest apple growing state, in comparison to the 149,500 total acres of dessert apples in production (Galinato, Tozer, Miles, & Coffey, 2015). In addition, they found in a survey of 9 cider producers that a lack of available desired cider apple varieties was the primary barrier to start, maintain, or expand hard cider production (Galinato, Gallardo, & Miles, 2014).

Since the recent growth of the cider industry, several studies have been published analyzing the economic feasibility of cider production (Becot, Bradshaw, & Conner, 2016; Farris, Peck, & Groover, 2013; Galinato et al., 2014; Matson Consulting, 2012). There are several advantages and opportunities in establishing a cider orchard. Chief among them may be the price premium for traditional cider apples of $15/bushel in comparison to juicing apples at around $8/bushel (2012-2013 prices) (Matson Consulting, 2012). This may be short lived as more cider apples become available since the sale price would then be expected to decline. Cider fruit can also be mechanically harvested as is done in the United Kingdom because cosmetically perfect fruit (for fresh fruit sales) is not necessary if the end result is juice. For many orchards, labor represents the highest variable cost of production and significant savings can be had through mechanical harvesting. Current research into exploring the viability of mechanical harvesting in the United States has found that labor cost could be reduced by a factor of four, that juice quality is not negatively affected, and that yields are comparable to hand harvesting (Miles & King, 2014).

The eventual price equilibrium achieved however should still exceed the market price for more conventional varieties if cider apple orchards are to be profitable. Several elements of growing these varieties make it more challenging and costly to produce than conventional varieties such as biennial-, uneven-, or non-bearing, as well as irregular or problematic blooming and ripening patterns (Merwin et al., 2008).

During the production of apple juice, after milling and pressing, the remaining material is referred to as the apple pomace. Pomace represents around 25-30% of the original weight of the whole apples, but this can vary based on press efficiency and the moisture content of the apple (Vendruscolo, Albuquerque, Streit, Esposito, & Ninow, 2008). The pomace is typically regarded as a waste product and used as either feed for

livestock or as fertilizer. Its use as either is limited due its relatively poor nitrogen content, high level of acidity, and, with a moisture content around 75%, propensity to ferment and spoil rapidly (Shalini & Gupta, 2010). Some producers will take an additional step of dehydrating the pomace to extend its shelf life and lower transportation costs, but this requires additional energy expense and investment in capital resources. The drying process, if done at elevated drying temperatures, can potentially degrade existing heat-sensitive vitamins and antioxidants that contribute to its value (Yan & Kerr, 2013).

Other uses of pomace that have been explored and some of which are currently in practice are the production of cellulase (Sun, Ge, Hao, & Peng, 2010), pectin esterase (Joshi, Parmar, & Rana, 2006), ethanol (Hang, Lee, & Woodams, 1982), natural gas, citric acid, charcoal, pectin, fiber, and many others (Kennedy et al., 1999).

Pomace consists of around 1.5% fat, 38.8% fermentable sugars, 2.1% malic acid, 1.4% sorbitol, 55.9% dietary fiber, and 0.3% polyphenols on a dry weight basis (Kolodziejczyk, Markowski, Kosmala, Król, & Plocharski, 2007). While decidedly heterogeneous, the average composition for pomace has been reported as 54% pulp, 34% peel, 7% seeds, 4% seed core, and 2% stalk (Kolodziejczyk et al., 2007).

With the paucity of available cider apples, for NYS cider producers looking to take advantage of the increased popularity in cider, another option is to fortify cider with added tannins either in powdered or liquid form. The most popular sources for these tannin additions, which are primarily used in wine, are grape skins and seeds, arboreal tannins from varieties of tree bark, and 'nut' tannins such as chestnut and gall 'nut.' It should also be mentioned that the juice of wild crab apples (*Malus sylvestris*) is occasionally added to craft ciders to increase astringency. There are, however, no apple-derived tannins for the cider maker looking to increase the astringency in their cider aside from growing or sourcing the cider apples.

Apple pomace, with its high phenolic content, represents an extremely valuable source of phenolics. Estimates based on USDA statistics of total U.S. apple utilization and tonnage of apple fresh weight used for juice and cider yielded a value of 339 million pounds of pomace generated annually (National Agricultural Statistics Service, 2016). Based on the compositional data of pomace, this represents approximately 250,000 pounds of potentially usable phenolics. Predominant among those phenolics are tannins (procyanidins) which represent an unutilized, endogenous source of apple tannins for use in hard cider.

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CHAPTER 2

EVALUATION OF ENDOGENOUS AND EXOGENOUS TANNIN ADDITIONS TO IMPROVE THE QUALITY OF FERMENTED CIDER

Abstract

Recent growth in the fermented cider market has accelerated interest in consumer preferences for premium products. The astringency of cider due to tannins represents a significant factor in determining the flavor profile of these products. We sought to determine the acceptability of endogenous and commercial exogenous tannins in fermented cider. Fermented ciders (standardized to 3% residual sugar) were produced from low-tannin ("dessert") and high-tannin ("cider") apple cultivars. The dessert blend (control) was then enriched with 150 ppm total phenolics (TP) as mg gallic acid equivalent (GAE) per liter using three treatments: 20% high-tannin cider (endogenous), and two commercially available tannins from grape skins and gall nuts (exogenous). Sensory evaluation to assess acceptability was conducted on the 4 treatments against a commercial hard cider sample. Sensory trials used a mixed design of a randomized monadic blind taste test of five samples with hedonic and just-about-right evaluation. Ciders were analyzed for titratable acidity, Lab color, TP, pH, percent alcohol, volumes of CO_2, total soluble solids, turbidity, and specific gravity. Chemical parameters and sensory results were subjected to one-way analysis of variance (ANOVA) and significant differences were analyzed using Tukey's HSD at the 0.05 level. Total phenols in ciders ranged from 275 to 814 ppm GAE. No significant differences in overall liking were observed between the four samples with endogenous and exogenous tannins, however all received positive hedonic ratings (6.1

on 9-point scale) significantly higher than the commercial cider (5.5). Astringency was just-about-right for all samples tested. Color and turbidity of samples were two significant factors in determining overall liking of ciders. The average willingness to pay (WTP) for the most preferred cider with the knowledge that it was produced locally from NY state apples was $12.74. Cider drinkers (consumed cider at least once a month) tended to have a significantly higher WTP than non-cider drinkers. Results indicated positive consumer preferences for tannin fortification of ciders.

Introduction

Cider, the fermented apple juice beverage, has been growing in popularity throughout the United States. According to data released by the partnership of the United States Association of Cider Makers and the Neilsen Corporation, total volume of sales in the cider segment has gone from approximately $150 million in 2012 to $475 million in 2016, which is still only 1.3% of total annual beer sales in the United States (Brager & Crompton, 2017). While this accelerating growth slowed from 2015 to 2016 in the large cider segment, craft and local cider continued to see a 39% increase in annual dollar sales.

Similar to the growth of the craft beer industry, the relatively recent growth of cider has encouraged innovation and diversification of styles. Unlike more traditional markets such as the United Kingdom, Spain, or France, consumer preferences in the United States remain relatively unknown and the full market potential for the segment has yet to be fully explored. As a gluten-free alternative to beer with a fresher, fruit-flavored profile, and as a low-alcohol alternative to wine, cider serves many market niches. In addition, apples are rich in polyphenols, compounds that have been associated with many positive health outcomes such as lowered risk of coronary heart disease (Liu, Eberhardt, & Lee, 2001).

In making cider, a blend of apples is necessary because each apple only contains some of the essential qualities for a premium final product. The widely-used categorization of apples by the Long Ashton Research Station (LARS) serves as a useful framework for understanding the choices cider producers make in choosing apple varieties. In this classification there are four types of apples: sharp, sweet, bittersharp, and bittersweet. They are distinguished by two factors with two levels each: acidity (high/low) as malic acid (w/v), and tannins (high/low) as polyphenolics (w/v). The resulting categories are: sharps (>0.45% acidity, <0.2% tannin), sweets (<0.45% acidity, <0.2% tannin), bittersharps (>0.45 % acidity, >0.2% tannin), and bittersweets (<0.45% acidity, >0.2% tannin).

Due to the continued growth of the cider segment, there is and will continue to be a demand for each of these types of apples. New York and Washington states are the two leading apple producers in the United States which makes them uniquely positioned to take advantage of this growing trend. However, the vast majority of the apples grown are sharps and sweets which are often referred to collectively as 'culinary,' 'dessert,' or 'processing apples,' depending on their final intended use. The 'bitter' apples are not intended for fresh eating due to their high levels of tannin which give the apples an unpalatable astringency.

Due to the high tannin content (and other beneficial characteristics such as aroma and flavor precursors), 'bitter' apples are important elements of traditional cider blends in places such as France and the United Kingdom where they represent at least 20% of the final blend (Merwin, Valois, & Padilla-Zakour, 2008). At present, there is a shortage of these varieties of apples planted in New York, Washington, and elsewhere in the United States. Apples used for juice and cider have much less stringent cosmetic requirements than out-of-hand eating apples, and as such, can even

be mechanically harvested leading to considerable cost savings in labor (Miles & King, 2014).

Not all successful ciders require the addition of tannins, in fact many popular commercial ciders in the United States attempt to avoid astringency, focusing instead on the sweetness, acidity, and aroma of cider. Traditionalists and many craft producers, however, view astringency in cider as an important component that creates balance and see modern ciders as insipid without it.

The role of tannins in grapes and wine has long been a popular area of research, but the research into cider has remained limited. In the making of red wine, tannin is usually added by choosing varieties that are higher in tannin, just as in traditional cider making. However, additional sources of tannins in winemaking are more commonplace than they are in cider including oak barrel aging, skin contact maceration, and powdered/liquid tannin additions. These tannin additions, if not arboreal, are derived from grape skins and seeds or other high-tannin plant materials such as gall nuts. And alongside their contributions to astringency, tannins also contribute to color through polymeric pigmentation and oxidation, wine clarity through precipitation, and others (Singleton, Orthofer, & Lamuela-Raventós, 1999).

In contrast to wine, no such endogenous, apple-derived tannins exist for addition to cider, apart from the juice itself. Many studies have shown strong correlations between the quantity of tannins in a wine and its market value (Fanzone et al., 2012; Gómez-Plaza, Olmos, & Bautista-Ortín, 2016; Mercurio, Dambergs, Cozzolino, Herderich, & Smith, 2010). Tozer, Galinato, Ross, Miles, & McCluskey (2015) found a strong relationship between higher percentages of tannin and increased willingness to pay for local craft cider.

It has been observed that tannin additions at manufacturer recommended rates achieved low retention in wine and did not achieve significant measurable effects

(Harbertson, Parpinello, Heymann, & Downey, 2012). Large additions were necessary to achieve significant effects, but these effects were deleterious to overall wine acceptability. The ultimate role of astringency is to bring balance between the other flavor components, but in excess, the tannins can be detracting. Impurities in tannin concentrates and proteins in grape juice, which are bound by tannins and precipitate out of solution, are two common reasons that the ineffectiveness of tannin additions has been noted (Springer, Sherwood, & Sacks, 2016). The association of tannins with proteins is one of the primary defining characteristic of tannins and is believed to be responsible for the astringency of tannins through their complexation with salivary proline-rich proteins (Luck et al., 1994). Similarly, with ciders, it has been shown that components of apple cell walls (cellulose, xylose, and pectin) also bind to tannins, limiting their extractability and thus role in final cider astringency (Renard, Baron, Guyot, & Drilleau, 2001).

Of what potential benefit tannins added to cider could be has received little attention. One experiment by Valois showed, using various exogenous tannin additions to cider, that clear flavor profiles can emerge and as such, differentiation based on consumer preferences would be possible (2007). Due to the commercial profile of ciders in the United States as sweeter and more fresh-apple flavored, consumer expectations as dictated by previous experience would suggest that ciders with enhanced astringency might receive depressed hedonic responses.

Addition of oenological tannins to wine has received scarce more attention, with most emphasis being on the potential role of tannins in enhancing or stabilizing color intensity (Parker et al., 2007). Manufacturers also market tannins as a protective measure against oxidation (Vivas & Glories, 1996). Similarly, it has been observed that native tannins (procyanidins) have inhibitory effects against polyphenol oxidase which is responsible for the browning in cider (Le Bourvellec, Le Quéré, Sanoner,

Drilleau, & Guyot, 2004). We were interested in assessing the acceptability of the addition of endogenous and exogenous tannins to cider made from dessert apples, to increase the astringency and complexity of the final cider.

Materials and Methods

Chemicals. Folin & Ciocalteu's phenol reagent and gallic acid standard (>97% purity) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Solvents for sample preparation and extraction were of ACS grade or better (Thermo Fisher Scientific; Pittsburgh, PA, USA or VWR; Radnor, PA, USA). Sodium hydroxide and sodium carbonate were purchased from VWR. Potassium metabisulfite and Clinitest tablets (Bayer; Pittsburgh, PA, USA) were obtained from Thermo Fisher Scientific. Go-Ferm, Fermaid K, DV10 yeast, diammonium phosphate, Scott'Tan Uva'Tan Soft, and Scott'Tan FT Blanc were acquired from Scott Laboratories (Petaluma, CA, USA).

Fermentation. Ciders for sensory analysis were prepared from the juice of four dessert apple cultivars that are commonly grown in New York State: Empire, Jonagold, Ida Red, and MacIntosh. Two additional samples of juice from high-tannin cider apples, Dabinettt and Harry Masters Jersey, were graciously provided by Dr. Susan Brown of the Cornell Apple Breeding Program (Geneva, NY, USA). All juice samples were fermented separately in duplicate with Lalvin DV-10 yeast and supplemented with a mixture of organic (Go-Ferm, Fermaid-K) and inorganic nitrogen (diammonium phosphate). DV10 was selected due to its ability to ferment to completion and at a rapid rate with low formation of off-flavors and aromas (Downing, 1989).

Fermentation was conducted at ambient temperature for 21-28 days until no residual sugar remained (verified with the use of Clinitest tablets). The finished ciders were moved to cold storage and remained there until preparation for sensory analysis,

with sulfite levels maintained by the addition of potassium metabisulfite in the range of 30-50 ppm free SO_2.

Sample Preparation. The four ciders from the dessert apples were blended as is standard commercial practice to produce a base blend which served as the first control sample. This blend was then tannin enriched by 150 ppm total phenolics (TP) as mg gallic acid equivalent (GAE)/L (determined using the Folin-Ciocalteu (FC) assay) to produce three treatments. The quantity of tannins added to each finished cider was corrected for percent purity of the commercial tannin by measuring actual TP at 100 ppm and 150 ppm. For the first treatment, the base cider was supplemented with 20% fermented cider from a blend of the two high-tannin apple ciders Dabinett and Harry Masters Jersey.

A commercially available grape tannin recommended for white wines, Uva'Tan Soft, was added at 278 ppm (purity of 54% TP) to produce the third treatment and a gall nut tannin recommended for cider and white wines, FT Blanc, was added at 179 ppm (purity of 84% TP). The two powdered tannins were selected based on manufacturers' recommendations and informal evaluation of the tannins dosed into commercial ciders. The samples were normalized to 3% residual sugar with sucrose. Prepared ciders were filled into 750 mL wine bottles, force-carbonated with two volumes of carbonation using a Steinfurth carbonator model LCS710P (Essen, Germany) and immediately capped. In previous studies, we had identified a commercial cider that received high consumer acceptability and this was used as an additional control to be evaluated against the four treatments (Gerling et al., 2016).

Sample Analysis. The samples were analyzed for titratable acidity (TA), color, total phenolic content (TPC), pH, percent ethanol, CO_2 volumes, total soluble solids, turbidity, and specific gravity (SG).

Total Phenolics. For each sample, the 280 nm absorbance was read and using this absorbance, the sample was diluted with water to yield an approximate $OD_{280\ nm}$ of 1 AU (absorbance unit). The diluted samples were then analyzed for TP using the FC assay (Agbor, Vinson, & Donnelly, 2014). The reaction mixture was prepared by mixing 40 µL diluted sample with 520 µL water and 40 µL of FC phenol reagent in a cuvette (10 mm pathlength) and vortexed. The mixture was allowed to stand for 8 min at room temperature followed by an addition of 400 µL of 7% (w/v) sodium carbonate. After a 1.5 h incubation, the absorbance of the blue solution was measured at 765 nm on a Genesys UV-visible Spectrophotometer, model 10S (Thermo Fisher Scientific) and results were expressed in mg gallic acid equivalent (GAE)/L. Measurements were performed in triplicate. The standard curve was generated using gallic acid from 0 mg/L to 200 mg/L in steps of 20 mg/L.

Soluble Solids. Undiluted ciders were also measured for the amount of total soluble solids reported as °Brix, measured using a Leica Auto ABBE digital refractometer model 10500B, temperature compensated (Leica Inc; Buffalo, NY, USA).

Acidity. Titratable acidity was measured using a G20 Compact Titrator (Mettler Toledo; Columbus, OH) by titration of 5 g of sample, diluted to 35 mL with deionized water, using 0.1 N sodium hydroxide until an end point of pH 8.2 was achieved. Results were expressed as percent malic acid (w/w). The pH was measured using a ThermoOrion 3 Star (Thermo Fisher Scientific) equipped with a Thermo 8172 BNWP electrode.

Turbidity, Specific Gravity, and Color. Samples were de-carbonated by placing them in an ultrasonicator filled with ice to prevent ethanol evaporation and sonicated for 30 min. The turbidity of the de-carbonated samples was measured by a Hach portable turbidimeter model 2100P (Hach Portable Turbidimeter; Loveland, CO,

USA). Measurements were reported in Nephelos Turbidity Units (NTU). Hunter color components were measured with a Hunter UltraScan VIS colorimeter (Reston, VA, USA). The specific gravity of the de-carbonated samples was also measured with a hydrometer with 0.002 divisions. The temperature of the sample was recorded and used to standardize specific gravity measurements.

Alcohol. The percent alcohol was determined using a Dujardin Salleron ebulliometer model 360 (Noizay, France). The instrument was calibrated by loading the sample chamber with 50 mL of deionized water and heating until steam was visible. The temperature of the water was recorded once the reading had stabilized. The sample chamber was emptied and rinsed with sample. Afterwards, 50 mL of sample were added to the chamber and the process was repeated. Once steam was visible and the temperature had stabilized, the temperature was recorded and compared to alcohol-temperature correction tables using the previously recorded boiling point of water as a zero point. The results were expressed in percent alcohol (v/v).

Carbon Dioxide. Total volumes of CO_2 were determined by the method of the American Society of Brewing (ASBC Methods of Analysis, 2011). Each sample bottle cap was punctured with an Omega handheld digital manometer model HHP-90 (Norwalk, CT) fitted with a rubber stopper to facilitate a tight seal. The pressure reading and temperature were recorded and were compared to carbon dioxide solubility tables to determine total volumes of CO_2.

Sensory Analysis. From the Cornell Sensory Center database, 193 participants were recruited. The study was conducted following all requirements of the Institutional Review Board of Cornell University regarding beverage samples for consumption. The research was conducted over three days at the Sensory Evaluation Center at Cornell University.

Sensory trials used a mixed model of a randomized complete block design with monadic blind taste testing of 5 samples. For each sample, the participants evaluated appearance, color, aroma, flavor, carbonation, and overall liking on a 9-point hedonic scale (from "Dislike extremely" to "Like extremely"). They also evaluated the qualities of sweetness, acidity, astringency, carbonation, and apple flavor on a 5-point Just-About-Right (JAR) scale (1 – "Not Enough", 3 – "Just-about-right", 5- "Too Much").

Samples (30 mL) were served in 4-oz clear plastic containers with tight-fitting lids. Each sample was poured à la minute, immediately capped, and served. Cider was kept refrigerated prior to serving. Participants were requested to consume an unsalted cracker and water between samples and prior to starting analysis.

Willingness to Pay. After completing the survey, sensory panelists were asked to assess their willingness to pay for a 750 mL bottle of the cider they selected as being the most liked with the post-hoc revelation that it was locally produced from a blend of NY state apples. A picture of a 750 mL bottle and its equivalent in ounces was provided to assist in making this comparison.

Statistical Analysis. Chemical parameters and sensory results were subjected to analysis of variance (ANOVA) and significant differences among treatments were analyzed using Tukey-Kramer HSD at the 0.05 level. Analyses were conducted in R (3.3.2) and R Studio (1.0.136).

Results and Discussion

There were no significant differences in overall liking between the four samples with endogenous and exogenous tannins; however, all received positive hedonic ratings, significantly higher than the commercial cider (5.5) (Figure 4). For the appearance and color attributes, the treatment with endogenous tannin was rated

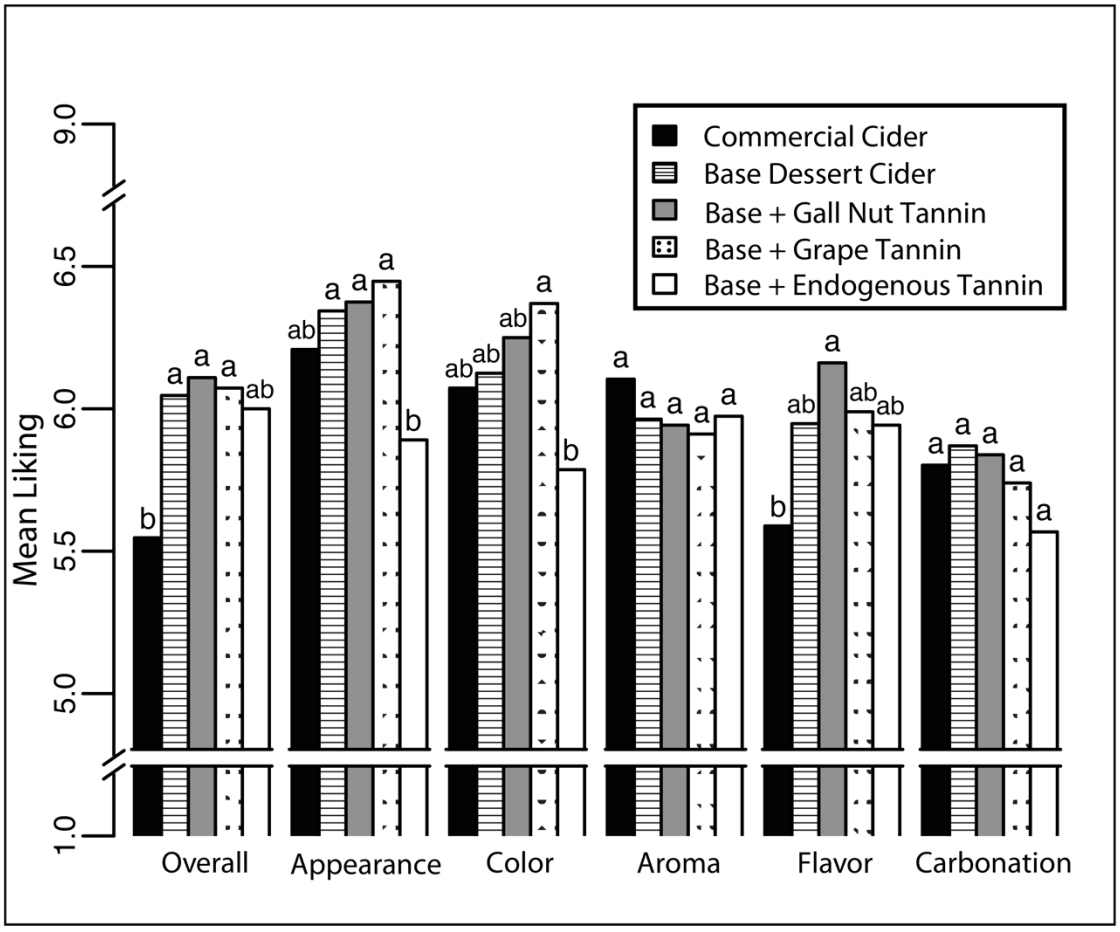

Figure 4. Sensory evaluation scores of mean liking of six cider attributes. Scores are reported using a 9-point hedonic scale. Difference values between liking scores within an attribute are significant at the .05 level (Tukey's HSD).

significantly below other treatment means (5.88 and 5.77 respectively), but still with positive mean liking. Chemical analysis of the cider samples (Table 2) revealed a significant increase in turbidity in this sample (16.63 NTU). Additionally, color measurements of the five ciders showed that the endogenous tannin sample was both darker and greener than other treatment samples (Table 33). These factors taken together offer a likely explanation for the diminished liking scores for the attributes of color and appearance in the endogenous tannin sample.

Table 2. Chemical composition of ciders used in sensory evaluation.

	Total Phenols (ppm GAE)	pH	Acidity (malic) g/L	CO_2 Vol.[1]	Brix°	Turbidity (NTU)	Ethanol (v/v)[2]	Specific Gravity
Commercial Cider	814 ± 50 a	3.70 ± 0.01 a	5.36 ± 0.03 b	3.30	8.57 ± 0.02 ab	1.1 ± 0.3 b	6.60% a	1.018 ± 0.001 ab
Base Dessert Cider	275 ± 2 b	3.59 ± 0.02 ab	6.84 ± 0.04 a	2.34	8.20 ± 0.01 ab	4.8 ± 0.3 ab	6.60% a	1.017 ± 0.001 ab
Base + Endogenous Tannin	540 ± 41 ab	3.58 ± 0.01 ab	5.77 ± 0.02 ab	1.83	8.08 ± 0.03 b	16.6 ± 1.5 a	6.60% a	1.016 ± 0.001 b
Base + Grape Tannin	478 ± 13 ab	3.67 ± 0.01 ab	5.90 ± 0.01 ab	1.60	9.93 ± 0.03 a	6.7 ± 1.1 ab	6.30% b	1.024 ± 0.001 b
Base + Gall Nut Tannin	471 ± 6 ab	3.54 ± 0.02 b	6.03 ± 0.02 ab	1.85	8.32 ± 0.02 ab	5.4 ± 1.1 ab	6.30% b	1.018 ± 0.001 ab

Data represents measurements done in triplicate and reported as mean ± standard deviation. Means within a column followed by different letters are significant at the 0.05 level (Tukey's HSD). [1]Data was sampled only once due to destructive nature of test. [2]Two replicates were performed and each replicate showed no deviation in measurement.

Table 3. CIELAB color coordinates for cider treatments.

Treatments	L	a	b[1]
Commercial Cider	93.27 ± 0.05 b	-3.58 ± 0.04 b	26.29 ± 0.7
Base Dessert Cider	94.92 ± 0.03 ab	-2.48 ± 0.03 ab	11.53 ± 0.6
Base + Endogenous Tannin	93.34 ± 0.05 b	-3.33 ± 0.03 b	27.26 ± 0.3
Base + Grape Tannin	93.57 ± 0.03 ab	-0.65 ± 0.02 a	18.23 ± 0.1
Base + Gall Nut Tannin	95.89 ± 0.06 a	-2.46 ± 0.02 ab	11.98 ± 0.2

Data represents measurements done in triplicate and reported as mean ± standard deviation. Means within a column followed by different letters are significant at the 0.05 level (Tukey's HSD). [1] No significant difference at the 0.05 level.

One challenge of tannin additions is that they often cause slight changes in color and turbidity which are effects that diminish over time. This is one reason why tannin additions are often done prior to fermentations, or, if done during blending, are matured prior to release. Most medium and large-scale commercial producers will also use a coarse and polishing filter to obtain a brilliant (clear) product, however the ciders that were prepared for this study were not filtered, only racked (siphoned/decanted off the lees and sediment) numerous times.

Many large-scale producers will use caramel coloring to reduce color variability between batches and to give the cider a pleasant reddish-brownish color that is typical of oxidized unfermented, unfiltered apple cider. The expectations suggested by this study were that customers are accustomed to and anticipate clear ciders without haze or sedimentation and without green (unripe) colors. Being a non-cider drinker (drinks cider less than once a month) had a significant effect of lowering mean liking scores for the appearance and color attribute in the endogenous tannin cider (2-sample t-test, $p < 0.01$; data not shown). Cider drinkers, on the other hand,

are likely more accustomed to 'craft' or local cider products which can often be turbid with sedimentation and show greater vintage variability.

The mean liking for the flavor attribute for all four prepared samples (including the base dessert cider), was higher than that of the commercial control. The penalty analysis showed that panelists penalized the commercial sample most heavily and for attributes of "lack of sweetness" and "lack of apple flavor" (Figure 5). However, these same deficiencies were also penalized heavily in all five samples, suggesting that perhaps another factor may be responsible for the significant difference in flavor liking results between the control and the prepared samples. Astringency was found to be just-about-right for all prepared samples which demonstrated a wide range of astringency acceptability of tannins from three different sources. However, for the commercial sample, over 30% of panelists found the sample to be overly astringent. In the chemical analysis (Table 2), the concentration of total phenols was the largest variable that separated the commercial cider from the other samples. In addition, the correlation between astringency in cider and total phenolic content is significant. While astringency liking was not evaluated in the hedonic portion of the study explicitly, it is implicitly involved in both the flavor and overall attributes and scores for astringency may have been 'dumped' into these two categories (Symoneaux, Guichard, Le Quéré, Baron, & Chollet, 2015). Taken together – the chemical analysis, the JAR scores, and the hedonic results – it can be inferred that the substantial astringency in the commercial cider sample contributed significantly to the disliking of that cider.

No significant differences were observed in the aroma or carbonation between the five samples. The sample with added grape tannin had the highest color and appearance scores and correspondingly was significantly less green and more red than the other samples from CIELAB color coordinates (Table 33). Often producers are

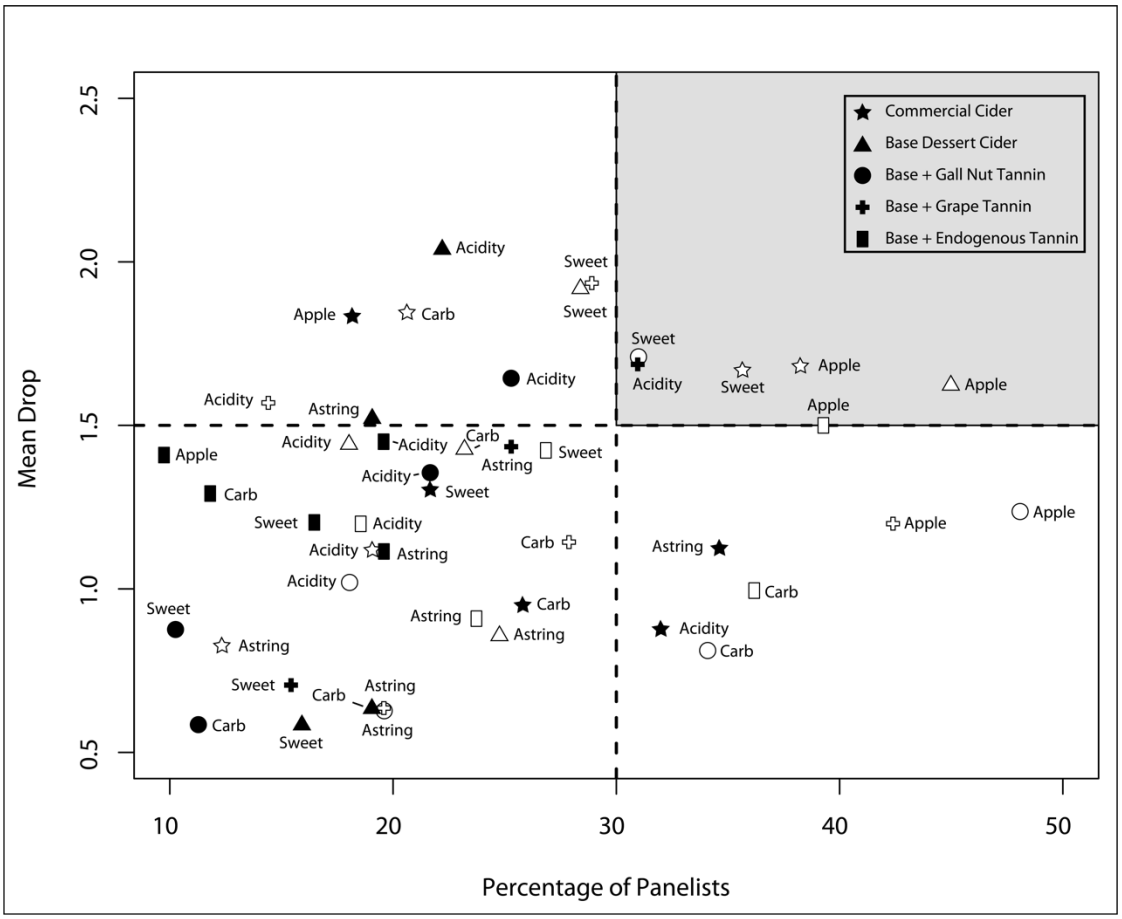

Figure 5. Penalty analysis of five cider samples from just-about-right scaling. Attributes were: Acidity, Sweetness ("Sweet"), Astringency ("Astring"), Carbonation ("Carb"), and Apple Flavor ("Apple"). The shaded portion represents attributes which should receive the most attention when considering reformulation. Open symbols denote "too little" of an attribute and closed symbols denote "too much."

concerned about the risk that color changes from tannin additions will be perceived negatively (and this seems to be the case in the endogenous tannin sample), but from the sensory results presented, it appears there can also be a positive effect.

The sample with the most preferred flavor was the gall nut tannin cider. Gall nuts contain a different type of tannin than grape skins or apples called hydrolysable tannins (or as it is also known, 'gall'otannin). These are the same class of tannins that are found in oak used for aging wine and cider. They impart a different astringent profile than condensed tannins (those found in grapes and apples).

Specific gravity to TA ratios would suggest that the commercial cider should be perceived as the sweetest and the base dessert cider as the least sweet (Table 4). From the penalty analysis, the lack of sweetness for the dessert cider is clustered around the other samples for the same attribute without any noticeable differences. The commercial sample was the most heavily penalized for insufficient sweetness. This would imply that the relationship of sweetness is more complex in cider than a simple ratio between TA and SG can predict. Notably, the significant levels of astringency

Table 4. Calculated ratio of specific gravity of ciders to titratable acidity.

Treatments	**SG to TA Ratio**
Commercial Cider	0.190 ± 0.001 a
Base Dessert Cider	0.149 ± 0.001 b
Base + Endogenous Tannin	0.176 ± 0.001 ab
Base + Grape Tannin	0.174 ± 0.001 ab
Base + Gall Nut Tannin	0.169 ± 0.001 ab

Data represents measurements done in triplicate and reported as mean ± standard deviation. Means within a column followed by different letters are significant at the 0.05 level (Tukey's HSD).

in the commercial sample likely modified sweetness perception for that sample (Fontoin, Saucier, Teissedre, & Glories, 2008).

After completing the study, sensory panelists were asked to assess their willingness to pay for a 750 mL bottle of the cider they selected as being the most liked now knowing that it was locally produced from a blend of NY state apples. A 750 mL bottle was chosen due to its popularity among local craft ciders. In contrast to wine and beer which come in predictable volumes of a 12-oz can or a 750 mL bottle, cost comparisons for cider can be challenging due to its variable packaging options. As such a picture of a 750 mL bottle and its equivalent in ounces was provided to assist in making this comparison.

The initial bid given to participants was $11.00 followed by a second bid of $13.00 if the initial bid was affirmed and $9.00 if it was denied. This partitioned the total willingness to pay into four intervals: (1) less than $9.00 (8% of panelists), (2) between $9.00 and $11.00 (15% of panelists), (3) between $11.00 and $13.00 (32% of panelists), (4) at least $13.00 (45% of panelists). From this information, the average willingness to pay for a cider drinker was calculated at $12.74, which for a bottle of craft cider in New York State is very typical. It also agrees well with a previous willingness to pay assessment for local craft cider at $12.82 for a 750 mL bottle (Tozer et al., 2015).

This number may be underestimated for two reasons. First, a plurality of respondents was in the highest bid category suggesting that an even higher bid range may have been suitable. Secondly, a majority of the panelists indicated that they were students (76%) and as such, had below average incomes (Table 5) thereby depressing their ability and possibly willingness to pay. Cider drinkers (consumed cider at least

once a month) tended to have a significantly higher willingness to pay than non-cider drinkers (chi-square independence test, $p < 0.001$).

Table 5. Summary of sensory panel demographics (n=193).

Variable	Description	Frequency (%)
Gender	Male	29
	Female	71
Age	21-34	85
	35-44	10
	45-54	3
	55+	2
Ethnicity	White or Caucasian	50
	Hispanic or Latino	8
	Black or African American	3
	Native American	0
	Asian	34
	Other	3
	Prefer not to answer	2
Income	<$20,000	24
	$20,000-$29,999	15
	$30,000-$39,999	18
	$40,000-$60,000	10
	$60,000-$100,000	10
	>$100,000	6
	Prefer not to answer	17
Student	Yes	76
	No	24
Education	Some High school	0
	High school degree	4
	Some college	21
	Associates degree	3
	Bachelor's degree	22
	Some graduate school	20
	Graduate degree	30

Conclusion

Ciders with additions of exogenous and endogenous tannins received positive overall likings, and increased the overall preference against a commercial control. The control with no tannins added also received high overall liking suggesting the potential for producer-differentiated products to meet diverse customer demands. Sweetness and apple flavor were the two most common attributes found lacking in all five ciders which are consistent with historical consumption patterns. Consumers' willingness to pay was highest among cider drinkers and consistent with typical market prices for premium ciders. The demonstrated interest in ciders with added tannins should support growers' decisions to grow more high-tannin 'cider apples' for use in fermented cider. Gallotannins may represent an additional opportunity for cider producers to enhance cider quality and differentiate products.

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CHAPTER 3

OPTIMIZATION OF AN ENDOGENOUS AQUEOUS TANNIN CONCENTRATE FROM APPLE POMACE TO ENHANCE ACCEPTABILITY OF FERMENTED CIDERS

Abstract

Commercially available tannins to increase astringency in fermented cider are all non-apple derived, however there is an opportunity to utilize apple pomace as a source of endogenous tannins. Aqueous extraction of apple tannins from dessert pomace was optimized for time, temperature, number of extractions, solvent to mass ratio, enzyme treatment, and concentration ratio. Extraction results were repeated in triplicate, results were analyzed by ANOVA, and significant differences among means determined by Tukey-Kramer HSD at the 0.05 level. Optimized extraction parameters were a 0.1% (w/w) protease pre-treatment followed by an extraction at 100°C for 30 min with 3 successive extractions at a 15:1 water to pomace ratio yielding an extract with 0.87 °Brix and 599 mg gallic acid equivalent (GAE)/kg of fresh pomace. To determine commercial viability, optimized extractions were prepared from Red Delicious and Dabinett apple varieties. Extracts were thermally pre-concentrated under vacuum to 11.2 °Brix, fermented to dryness, clarified with pectic enzymes, filtered, and concentrated to 41.0 °Brix. Concentrates were analyzed for total soluble solids, acidity, condensed tannins, Lab color, and percent ethanol. Total phenolics (TP) were characterized by reversed-phase HPLC and the Folin-Ciocalteu assay. Sensory analysis was conducted to determine acceptability of ciders with added endogenous tannins. A commercial cider was enriched with finished concentrates at a rate of 150 ppm TP as mg GAE/L. Sensory trials used a mixed design of a

randomized monadic blind taste test of 3 samples with hedonic and just-about-right evaluation. Addition of Red Delicious tannin concentrate at 1.14% (v/v) or Dabinett tannin concentrate at 0.62% (v/v) results in 150 ppm added apple tannins. Condensed tannins in the Red Delicious and Dabinett concentrates were 1.32 and 3.12 g catechin equivalent per liter with mean degree polymerizations of 3.32 and 2.25, respectively. No significant differences in overall liking were observed between the three samples, however all received positive hedonic ratings (6.3 on 9-point scale). Addition of tannin concentrate to ciders resulted in no significant chemical changes to ciders aside from TP. Mean liking of appearance for the Red Delicious sample (6.19) was rated below the control sample (6.68), with corresponding alterations to turbidity (5.09 and 3.07 NTU respectively). Just-about-right analysis showed sweetness lacking in all three samples, however lack of "apple flavor" in control sample was the most penalized.

Introduction

After the pressing of apples for juice and cider, the remaining material, the pomace, is typically discarded, used as fertilizer or given to livestock as fodder. Due to the increasing popularity of cider, and the historical popularity of apple juice, the amount of pomace generated has continued to rise. Estimates based by the USDA National Agricultural Statistics Service (2016) on total U.S. apple utilization for use in juice and cider amounted to an annual production of 339 million pounds of pomace.

Apple pomace contains approximately 38.8% fermentable sugars, 2.1% malic acid, 55.9% dietary fiber, and 0.3% polyphenols on a dry weight basis (Kolodziejczyk, Markowski, Kosmala, Król, & Plocharski, 2007). It is a heterogeneous mixture of pulp, peel, seeds, core, and stalks. Due to the large volume of pomace generated annually and its rich chemical composition, much research has been conducted in

search of strategies for valorizing this waste product. Options that have been explored or that are currently being manufactured are: cellulase (Sun, Ge, Hao, & Peng, 2010), pectin esterase (Joshi, Parmar, & Rana, 2006), ethanol (Hang, Lee, & Woodams, 1982), natural gas, citric acid, charcoal, pectin, fiber, and many others (Kennedy et al., 1999).

Its usefulness as a source of polyphenols has also drawn much attention. Polyphenols are potent antioxidants and free radical scavengers and as such have been demonstrated to reduce the risk and incidence of cardiovascular disease and is associated with other positive health outcomes (Boyer & Liu, 2004). As such, many papers have explored various methods of extracting polyphenols from apple pomace using different mixtures of traditional solvents such as acetone, methanol, ethyl acetate, and ethanol (Alberti et al., 2014; Kolodziejczyk et al., 2007). Non-thermal and green extraction technologies have also been employed to extract phenolics from apple pomace and other polyphenolic rich substrates. Some of those technologies include microwave (Gerard & Roberts, 2004), ultrasound assisted (Pingret, Fabiano-Tixier, Le Bourvellec, Renard, & Chemat, 2012), accelerated assisted solvent (Nayak et al., 2015), pressurized liquid (Prasad, Yang, Yi, Zhao, & Jiang, 2009), and supercritical CO_2 extractions (Sanjaya et al., 2014).

The predominant phenolics in apple pomace belong to four classes: phenolic acids (chlorogenic acid), flavan-3-ols ((-)-epicatechin, (+)-catechin, and their polymeric products, procyanidins) dihydrochalcones (phloridzin and phloretin xyloglycosides), and flavonols (quercetin and quercetin glycosides). Some measure of anthocyanins also exist in the apple peel of some varieties (Mazza & Velioglu, 1992). Procyanidins are of particular interest to many cider makers because these compounds are responsible for the astringency in cider. And yet, after pressing, only between 20-40% of the procyanidins found in an apple end up in the juice (Renard et al., 2011).

This inefficiency is true of all chemical components, but is especially true of procyanidins.

Procyanidins are highly reactive, polymerized flavan-3-ols that, along with the astringency in cider, also play a role in the clarity of finished cider and the stabilization of color. All of these properties are a consequence of procyanidin's ability to bind to and precipitate proteins, which is why this class of compounds is lumped under the more common name, 'tannins.' Following pressing these previously sequestered compounds come into contact with cellular components such as pectin, protein, enzymes, and cellulose which bind to and/or oxidize these compounds, leading to their inactivity or precipitation (Guyot, Marnet, Sanoner, & Drilleau, 2003).

Procyanidins are water-soluble and increased temperatures promote their extraction which is why practices such as heating the pomace during pressing is one strategy cider makers use to increase the astringency in their cider (Gerard & Roberts, 2004; Valois, 2007). Aside from using apples that are already high in tannins, referred to as 'bitters,' there are no options on the market for endogenous apple tannins to be added as a supplement to low-tannin juice. Higher tannin ciders are of interest to cider makers because often these blends are associated with better quality attributes and higher price premiums (Gómez-Plaza, Olmos, & Bautista-Ortín, 2016; Tozer, Galinato, Ross, Miles, & McCluskey, 2015).

Apple pomace, with its high phenolic content, represents a logical starting material for producing a high-tannin concentrate for addition to ciders. Aqueous extraction is appealing because it is regarded as a safe and sustainable extraction technology suitable for the food and nutraceutical industry (Lea & Timberlake, 1974). It avoids the use of harsh solvents and time intensive methods such as absorbent polymers (Saleh, Wibisono, & Lober, 2008).

Studies have optimized aqueous extractions for the purpose of maximizing polyphenols, potentially as nutraceuticals, but not as an endogenous source of tannins for addition to cider (Saleh et al., 2008). When extracting phenolics from apples with water, a loss in selectivity in comparison to organic solvents is expected. Along with pectin and organic acids, sugars are extracted in this process and are in much higher concentration than phenolics (Marcon, Vriesmann, Wosiacki, Beleski-Carneiro, & Petkowicz, 2005). The objective of this research was to develop a sustainable and cost-effective solution to address these challenges.

Materials and Methods

Chemicals. Folin & Ciocalteu's phenol reagent, albumin from bovine serum, sodium chloride, L-(-)-malic acid, acetic acid, sodium dodecyl sulfate (SDS), and ethanol (reagent grade) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Phenolic compound standards (>97% purity): gallic acid, (-)-epicatechin, (+)-catechin, procyanidin B2, phloridzin, and chlorogenic acid were also purchased from Sigma-Aldrich. Phenolic compound standards: hyperoside and procyanidin B1 were purchased from Extrasynthese (Genay, France). Solvents for sample preparation and extraction were of ACS grade or better; methanol, acetonitrile, and acetone were of HPLC grade (Thermo Fisher Scientific; Pittsburgh, PA, USA or VWR; Radnor, PA, USA). Hydrochloric acid, potassium hydroxide, sodium hydroxide, and sodium carbonate were obtained from VWR. Triethanolamine was sourced from Oakwood Chemicals (Estill, SC, USA). Ascorbic acid, phloroglucinol, potassium metabisulfite, ferric chloride, formic acid, phosphoric acid, and clinitest tablets (Bayer; Pittsburgh, PA, USA) were purchased from Thermo Fisher Scientific. Go-Ferm, Fermaid K, DV10 yeast, diammonium phosphate were acquired from Scott Laboratories (Petaluma, CA, USA). The pectolytic, proteolytic, and cellulolytic enyzmes: MaxiPro

AFP, Rapidase Power, Validase TRL, and Rapidase Fiber were kindly supplied by DSM Food Specialties (South Bend, IN, US). 3-(trimethylsilyl)-2,2,3,3,-tetradeuteropropionic acid sodium salt (0.018% w/v TMSP) and D_2O were obtained from Cambridge Isotope Laboratories (Tewksbury, MA, USA).

Fruit and Pomace. Previously frozen pomace from a blend of dessert apples and a separate blend of cider apples for extraction optimization experiments was provided by the New York State Agricultural Experiment Station, Geneva, NY, USA. For sensory samples, Red Delicious fruit was acquired from a local grocery chain and Dabinett apples were kindly supplied by Peckham's Orchard (Upper Moutere, New Zealand). Whole fruit was weighed, washed, milled with a Robocoupe food processor model R302V (Ridgeland, MS, USA), and pressed with a Vigo 4.5L Worktop screw press (Honiton, Devon, UK). Pressure was applied until pomace moisture content was 75%, comparable to commercial pomace (Ćetković et al., 2008). The pomace was retained along with 50 mL of expressed juice which was analyzed for total soluble solids (TSS) as °Brix and titratable acidity (TA) (expressed as mg malic acid/L).

Total Extractable Phenolics (TEP). For each of the four pomace samples, a portion of the pomace was weighed, freeze dried (Harvest Right; North Salt Lake, UT, USA), ground (mortar/pestle) and analyzed for total phenolics (TP) using the ultrasound method (Kim & Lee, 2002). Briefly, 1 g of lyophilized apple pomace was placed into a 15 mL conical centrifuge tube with 10 mL of 80% aqueous methanol and the headspace was flushed with nitrogen. The samples were sonicated for 20 min with a Branson 2200 sonicator (Thermo Fisher Scientific). Afterwards, the samples were centrifuged at 10,000 x g for 20 min in a Beckman Coulter centrifuge, model Avanti J-25 (Palo Alto, CA, US). The vials were then decanted and the process was repeated once more. Extracts were pooled and made up to 25 mL with 80% aqueous methanol. Samples were analyzed for TP by the Folin- Ciocalteu (FC) assay and expressed as mg

gallic acid equivalent (GAE)/kg fresh pomace based on yield of freeze dried pomace from fresh pomace.

Screening Experiments. Consistent with a technique employed by Çam and Aaby (2010), initial screening experiments were conducted to assess the relevant and significant factors influencing the extraction of phenolics from apple pomace. Factors investigated were agitation, successive extractions, pre-treatment with acidified water, and pressurized extraction. The extracts were filtered after extraction through Whatman No. 1 filter paper (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA) under vacuum using a Büchner funnel and analyzed immediately for TP. Extraction experiments were repeated in triplicate.

a) *Agitation*. A sample of 5 grams of apple pomace was added to a 50 mL beaker and 20 mL of water (20°C) was added into the beaker. The treatment samples were agitated every minute for 30 min while the control samples received no agitation.

b) *Successive extractions*. A sample of 5 grams of apple pomace was added to a 100 mL beaker and 75 mL of water (100°C) was added and the total weight recorded. The samples were maintained at 100°C on a stirring ceramic hot plate for 30 min. Afterwards, water at 100°C was added to the beaker to obtain the original weight and compensate for steam loss. The samples were then filtered as before, the pomace was discarded, and the extract was returned to the beaker with an additional 5 grams of apple pomace. The process was repeated four times.

c) *Pre-treatment with acidified water*. A sample of 5 grams of apple pomace was added to a 50 mL beaker and 20 mL of acidified water (citric acid, 0.01% w/v) at 0.0°C was added into the beaker. The samples were filtered, the pomace was retained and an additional 20 mL of acidified water was added. This process was repeated a total of three times.

d) *Pressurized extraction*. A sample of 5 grams of apple pomace was added to a 50 mL beaker, 20 mL of water (100°C) was added into the beaker, and the original weight was recorded. The beaker was placed into water (100°C) inside of a W.P. Applicances (Hollywood, FL, USA) pressure cooker (model BPCR0175) and the lid was affixed. After a come-up time of 3 min, the pressure was maintained at 15 psi (103 kPa) for 30 min at which point the pressure was immediately released. The samples were then weighed and water at 100°C was added to the beaker to obtain the original weight. The samples were covered and placed on ice to return the samples to room temperature.

Phenolic Extraction. Extraction of apple tannins from thawed dessert apple pomace was optimized for temperature, solvent to mass ratio, time, enzyme treatment and concentration ratio. The effect of each parameter was determined by changing the levels of the factor and keeping the other variables constant.

a) *Temperature*. A sample of 5 grams of apple pomace was added to a 50 mL beaker and 20 mL of water (0, 20, 40, 60, 80, 90, and 100°C) was added into the beaker and placed into a water bath at the pertinent experimental condition for each treatment. Samples were agitated every minute for 30 min to remove agitation as a confounding factor with respect to the 100°C sample. The samples were then covered and placed on ice to return the samples to room temperature.

b) *Solvent to mass ratio*. A sample of 5 grams of apple pomace was added to a beaker and a volume of water (20, 50, 75, 100, and 200 mL) at 100°C was added for each treatment. The samples were maintained at 100°C on a stirring ceramic hot plate for 30 min.

c) *Time*. A sample of 5 grams of apple pomace was added to a beaker and 75 mL of water at 100°C was added with the total weight recorded for each treatment. The samples were then maintained at 100°C on a stirring ceramic hot plate.

Measurements of TP were taken every 30 min (0.0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3 h). Prior to each measurement, water at 100°C was added to the beaker to obtain the original weight and compensate for steam loss. The samples were then covered and placed on ice to return the samples to room temperature.

d) *Enzyme Pre-treatment*. A sample of 5 grams of apple pomace was added to a 50 mL beaker for each treatment. Each beaker received one of four enzyme treatments (Rapidase Fiber pectinase, Rapidase Power pectinase, Validase cellulase, Maxipro protease) at one of four concentrations (0.05%, 0.10%, 0.20%, 0.40% w/w of pomace) for a total of 16 treatments and an additional no-treatment control. Enzyme concentrations were based on manufacturer recommendations. Afterwards, 20 mL of water at 20°C was added to the beakers and the mixtures remained at room temperature for one hour with agitation every 10 min.

A subsequent experiment was conducted to test the potential for possible synergistic effects among enzymes. Similarly, a sample of 5 grams of apple pomace was added to a 50 mL beaker. Each beaker received one of three enzyme treatments (Maxipro, Maxipro x Validase, Maxipro x Rapidase) at one of three concentrations (0.05%, 0.10%, 0.20% w/w of pomace), for a total of 9 treatments. Then 20 mL of water at 20°C was added to the beakers and the mixture remained at room temperature for one hour with agitation every 10 min.

Validation. Extraction of pomace from both dessert apples and cider apples was conducted using the optimized temperature, solvent to mass ratio, time, and enzyme pre-treatments. Samples were then analyzed for TSS, TA, and TP.

Concentration. Extraction of obtained pomace (as described previously) from Red Delicious apples and Dabinett apples was conducted using the optimized temperature, solvent to mass ratio, time, and enzyme pre-treatments with three successive extractions. The resulting extracts were then concentrated at 80°C to 11.2

°Brix with rotary evaporation. The concentrated extracts were then placed in laboratory flasks along with pectinase (0.01% v/v), yeast nutrients (0.4 g/L Go-Ferm, 0.4 g/L Ferm-K, 0.35 g/L DAP) and rehydrated yeast (0.25 g/L DV10). The concentrates were fermented until no residual sugar remained (verified with the use of Clinitest tablets). The resulting concentrates were centrifuged for 10 min at 10,000 x g in a Beckman Coulter centrifuge, model Avanti J-25 (Palo Alto, CA, US). They were then decanted and concentrated to 41 °Brix with rotary evaporation at 80°C.

Analysis of Concentrate. For analysis, four sampling points in the concentration process were identified: (1) juice obtained during the original pressing of Red Delicious and Dabinett apples; (2) extract obtained following the extraction optimization process prior to fermentation; (3) extract obtained following the fermentation of the extract; and (4) the final concentrate at 41 °Brix. These samples were analyzed for TSS, TA, pH, TP, and condensed tannin (CT). In addition, the final extracts were also analyzed for CIELAB color, HPLC profile, and percent ethanol.

a) *Total Soluble Solids.* Undiluted samples were measured for the amount of total soluble solids reported as °Brix as measured using a Leica Auto ABBE digital refractometer model 10500B, temperature compensated (Leica Inc; Buffalo, NY, USA).

b) *Acidity*. Titratable acidity was measured using a G20 Compact Titrator (Mettler Toledo; Columbus, OH, USA) by titration of 5 g of sample, diluted to 35 mL with deionized water, using 0.1 N sodium hydroxide until an end point of pH 8.2 was achieved. Results were expressed as percent malic acid (w/w). The pH was measured using a ThermoOrion 3 Star (Thermo Fisher Scientific) equipped with a Thermo 8172 BNWP electrode.

c) *Total Phenolic Content and Condensed Tannin*. For each sample, the 280 nm absorbance was recorded, and using this absorbance, the sample was diluted with

water to yield an approximate $OD_{280\ nm}$ of 1 AU (absorbance unit). The diluted samples were then analyzed for TP using the FC assay (Singleton, Orthofer, & Lamuela-Raventós, 1999). The reaction mixture was prepared by mixing 40 µL diluted sample with 520 µL water and 40 µL of FC's phenol reagent in a cuvette (10 mm path length) and vortexed. The mixture was allowed to stand for 8 min at room temperature followed by an addition of 400 µL of 7% (w/v) sodium carbonate. After a 1.5 h incubation, the absorbance of the blue solution was measured at 765 nm on a Genesys UV-visible Spectrophotometer, model 10S (Thermo Fisher Scientific) and results were expressed in mg GAE/L. Measurements were performed in triplicate. The standard curve was generated using gallic acid from 0 mg/L to 200 mg/L in steps of 20 mg/L. Condensed tannins (CT) were determined using the protein precipitation assay developed by Adams and Harbertson as described elsewhere (Harbertson, Kennedy, & Adams, 2002). Results were expressed in mg catechin equivalent (CE)/L.

d) *Color*. Hunter color components were measured with a Hunter UltraScan VIS colorimeter (Reston, VA, USA) and reported in the CIE L*, a*, b* color scale.

e) *HPLC with DAD Detection*. Chromatographic separations of phenolics in the final extracts were performed as described previously with minor modifications (Manns & Mansfield, 2012). All analyses were carried out with a C18 Agilent Zorbax Eclipse Column (Agilent Technologies; Santa Clara, CA, USA; 150 mm x 4.6 mm i.d., 5µm particle size). The system was an Agilent 1100 series equipped with a G1322 inline degassing unit, a G1312A binary pump, a G1313A autosampler, a G1316A thermostated column compartment, and a G1315A diode array detector. Preliminary data processing and system control was conducted with Agilent Chemstation software version B.04.03. Samples were diluted by a factor of four prior to sample preparation. The HPLC operating conditions were as described. Molar concentration of condensed tannin subunits following phloroglucinolysis was

determined using reported response factor conversions (Kennedy & Jones, 2001). Phenolics were quantified based upon the four main classes in apples: dihydrochalcones, hydroxycinnamic acids, flavonols, and flavan-3-ols as in Guyot et al. (1998). The molar sum of the terminal and extension subunits was divided by the molar sum of the terminal units to obtain the mean degree of polymerization (DP*n*) for the procyanidins (Kennedy & Jones, 2001).

f) *Ethanol.* Percent alcohol content in final extracts was evaluated using ^{1}H NMR spectrometry as in Zuriarrain et al. (2015) with modification. To an NMR tube was added 0.2 mL of 0.018% w/v of TMSP (internal standard) in D_2O, 20 μL of sample, and sufficient D_2O as needed to obtain a sample depth of 5 cm. The 600 MHz spectra was recorded using a Varian Inova spectrometer. Operating parameters were 64 scans of 32k data points were acquired with a spectral width of 16 ppm (-2 ppm to 14 ppm), acquisition time of 1.708 s, a 1.0 s relaxation delay, and a 90° pulse angle. Signals were Fourier transformed, manually phased, baseline corrected, and spectra horizontally shifted as needed to align the TMSP reference signal. Data analysis was achieved with MestReNova 11.0.2-18153 software package. A standard solution of 0.005% ethanol in water (v/v) served as a qualitative reference.

Sensory Sample Preparation. Samples for sensory analysis were prepared from a commercially available carbonated base cider which served as the control. The control cider was uncapped and tannin enriched by 150 ppm total phenolics (TP) with the Red Delicious concentrate (1.14% v/v) and the Dabinett concentrate (0.61% v/v). The samples were then immediately capped and refrigerated until the sensory experiment. Control samples were also briefly uncapped then resealed to remove this confounding factor. An additional sample was prepared from the Dabinett sample by normalizing TA (with the Red Delicious sample as the reference value) by addition of malic acid (0.3 g/L).

Cider Analysis. Cider samples for sensory analysis were analyzed for color, pH, TA, TSS, TP, and CT as before. In addition, samples were de-carbonated by placing them in an ultrasonicator filled with ice to prevent ethanol evaporation and sonicated for 30 minutes. The specific gravity was then measured with a hydrometer with 0.002 divisions. Temperature of the samples were recorded and used to standardize specific gravity measurements. The turbidity of the de-carbonated samples was also measured with a Hach portable turbidimeter model 2100P (Hach Portable Turbidimeter; Loveland, CO, USA). Measurements were reported in Nephelos Turbidity Units (NTU). Total volumes of CO_2 were determined by the method of the American Society of Brewing (ASBC Methods of Analysis, 2011). Each sample bottle cap was punctured with an Omega handheld digital manometer model HHP-90 (Norwalk, CT) fitted with a rubber stopper to facilitate a tight seal. The pressure reading and temperature were recorded and were compared to carbon dioxide solubility tables to determine total volumes of CO_2.

Sensory Analysis. From the Cornell Sensory Center database, 115 participants were recruited with emphasis on recruiting cider drinkers as defined as those who have a drink of cider "at least once a month." The research was conducted at the Sensory Evaluation Center at Cornell University. The study was conducted following all requirements of the Institutional Review Board of Cornell University regarding beverage samples for consumption. Sensory trials used a mixed model of a randomized complete block design with monadic blind taste testing of 3 samples: control, control with Red Delicious tannin concentrate (150 ppm), and control with Dabinett tannin concentrate (150 ppm). For each sample, the participants evaluated appearance, color, aroma, flavor, and overall liking on a 9-point hedonic scale (from "Dislike extremely" to "Like extremely"). They also evaluated the qualities of

sweetness, acidity, astringency, carbonation, and apple flavor on a 5-point Just-About-Right (JAR) scale (1 – "Not Enough", 3 – "Just-about-right", 5- "Too Much").

Following this analysis, a preference test was conducted with two additional samples: the control with Red Delicious tannin concentrate (as before) and the control with Dabinett tannin concentrate with normalized acidity. Additionally, panelists were asked if they perceived a difference in the samples and, if so, which sample they perceived as being higher in intensity for the attributes of sweetness, acidity, astringency, and bitterness.

Statistical Analysis. Chemical parameters and sensory results were subjected to analysis of variance (ANOVA) and significant differences among treatments were analyzed using Tukey-Kramer HSD at the 0.05 level. Analyses were conducted in R (3.3.2) and R Studio (1.0.136).

Results and Discussion

Screening Experiments. Agitation increased extractable phenolics by 62%, and a student's t-test showed it was significantly different than without agitation ($p < 0.001$). The effect of agitation on increasing the rate of mass transfer is well-established (Bellassouad, Feki, & Ayadi, 2015; Brodkey & Hershey, 2003). Increased movement of the extraction mixture continuously brings unsaturated solvent in contact with unsolvated phenolics remaining in the pomace. Agitation, through mixing, maintains an even heating profile throughout the media increasing extraction efficiency. With regard to pomace extractions in particular, hot water is effective at extracting pectin which is abundant in apple pomace and can increase mixture viscosity (Marcon et al., 2005). Agitation of pectin gels (or treatment with enzyme) will decrease viscosity thereby increasing efficiency. This effect of agitation is more significant at reduced temperature extractions because increased temperature rapidly

degrades pectin (Renard, 2005; Taylor, 2012). Based on this screening experiment, the remaining extractions were agitated either through manual stirring or naturally occurring mixing from extractions at rapid boil.

To mimic commercial counter-current extractions, extractions were repeated with recycled extract and fresh pomace to determine optimum number of successive extractions. It was anticipated that with successive extractions of new pomace material that the concentration of total phenolics would increase, but unknown was at what point extraction solute would reach practicable saturation.

The fourth extraction (Table 6) removed 60% as much TP as did the first extraction. The saturation of solute, affinity of tannins for cell wall material in pomace substrate, and polyphenolic degradation through oxidative and thermal effects have all been shown to contribute to diminishing extraction yields (Renard, Baron, Guyot, & Drilleau, 2001). Due to the diminishing extraction efficiency, the operational limit was chosen as three successive extractions.

Table 6. Optimization of number of successive aqueous extractions of dessert apple pomace for total phenolics.

Treatment	Total Phenolics (mg GAE/kg fresh pomace)
First Extraction	133.1 ± 2.5
Second Extraction	116.2 ± 3.8
Third Extraction	97.1 ± 7.5
Fourth Extraction	79.4 ± 7.2

Data represents measurements done in triplicate and reported as mean ± standard deviation.

Washes of pomace with acidified water (citric acid) prior to extraction reduced TSS by 12.1% (from 0.76 °Brix to 0.68 °Brix) and TP by 17.8% (from 14.5 mg

GAE/L to 11.9 mg GAE/L) in the final extracts. Since sugars are one of the primary soluble components of apple pomace, it was hypothesized that acidified washes with cold water would selectively reduce the concentration of sugars while minimizing phenolic loss. The percentage reduction in polyphenolic content of the pomace due to citric washes was greater than that of removed TSS. Thus, citric acid washing of pomace was not included as part of the optimized TP extraction process.

A growing trend in sustainable alternative extraction methods is subcritical water extraction or pressurized extraction (Prasad et al., 2009). As the pressure of water increases and approaches its critical point, the polarity of water begins to change dramatically. This property can be exploited to extract non-polar compounds that were previously un-extractable with water at atmospheric pressure. Typical pressure conditions for these extractions are not practical for most cider producers, however the levels of pressure in an at-home pressure cooker are achievable. Temperature is one of the most significant factors in extraction of phenolic compounds and the increase in achievable temperature at 15 psi (103 kPa) would be 20% (from 100°C at atmospheric pressure to 120°C).

At 15 psi (103 kPa) of pressure, pressurized extractions increased total phenolics in extracts from 139.4 to 227.7 mg GAE/kg fresh pomace, which represented an increase of 63.3% over conditions at 100°C. While not the focus of this work, this was an encouraging improvement over extractions at boiling temperatures and could be exploited if found to be an economically feasible method of phenolic extraction.

Phenolic Extraction. The temperature of the water had a positive relationship with the yield in extractable total phenolics (Figure 66). The largest increase was from 90°C to 100°C, the maximum temperature conditions tested (aside from pressurized screening experiments). Compared to extractions done at room temperature, this was

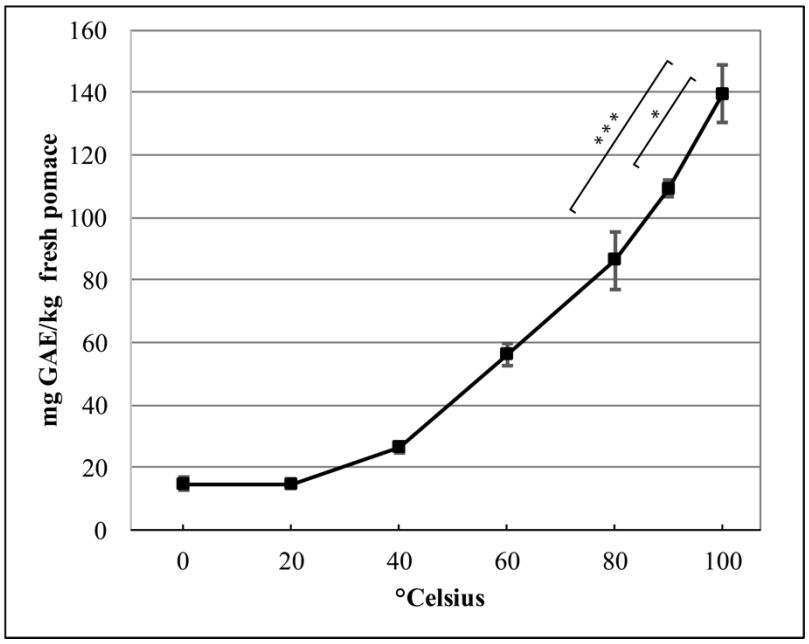


Figure 6. Optimization of temperature in aqueous extraction of dessert apple pomace for total phenolics. Data represents measurements done in triplicate and reported as mean ± standard error. Significant difference between means by Tukey-Kramer HSD in three leading treatments: * $p < 0.1$; *** $p < 0.01$.

a gain of over 955%. Treatments from 0°C to 90°C were manually agitated; however, the 100°C treatment was left to self-agitate at a rapid boil. Qualitative observations of pomace after extraction showed visible and significant degradation of pomace tissue for extractions at 80°C and above. Temperatures at or near boiling will result in loss of cell membrane integrity facilitating diffusion of smaller polyphenolic compounds (Renard, 2005). The significance of temperature in the extraction of phenolics and other compounds has been confirmed by similar studies (Çam & Aaby, 2010; Pinelo, Zornoza, & Meyer, 2008) where the optimal temperatures (90°C to 100°C) reported for aqueous phenolic extraction from apple pomace agree well with current research findings. Based on these optimization results, further extractions were conducted at 100°C.

The solvent-to-mass ratio achieved a maximum at 15 parts water to 1 part pomace (w/w) (Table 7). In previous studies of aqueous extraction of phenolics from

apple pomace, this value has ranged from 20:1 to 100:1 (water to mass), however, all such studies sought to optimize extraction yield without regard to tradeoffs in energy expenditure to remove additional water post-extraction (Çam & Aaby, 2010; Candrawinata, Golding, Roach, & Stathopoulos, 2014; Pinelo et al., 2008). Recommendations on optimized processing parameters should consider energy required for concentration as this is the largest variable expense in a commercial-scale concentration process (Eskew, Phillips, Homiller, Redfield, & Davis, 1951; Hu et al., 2016).

Table 7. Optimization of solvent-to-mass ratio in aqueous extraction of dessert apple pomace for total phenolics.

Solvent : Mass Ratio (w : w)	Total Phenolics[1] (mg GAE/kg fresh pomace)
4 : 1	147.0 ± 3.8 b
10 : 1	167 ± 14 ab
15 : 1	217.1 ± 8.1 a
20 : 1	189 ± 19 ab
40 : 1	177 ± 22 ab

Data represents measurements done in triplicate and reported as mean ± standard deviation. Difference values between treatments not connected by the same letter are significant ($p < 0.05$) by Tukey-Kramer HSD.

Inspection of optimal length of time also achieved a maximum within the range studied at one hour (Figure 7). However, the initial half hour of extraction removed 86.7% of the total phenolic concentration attained in a one-hour extraction. No significant differences ($p < 0.05$) between means in the leading treatments of 0.5 h, 1.0 h and 1.5 h were observed. After two hours, total phenolic concentration began to decline significantly suggesting that the rate of extracted phenolics was less than the

rate of thermal and oxidative degradation. This same effect was observed by previous studies examining pomace extractions and is consistent with current understanding of

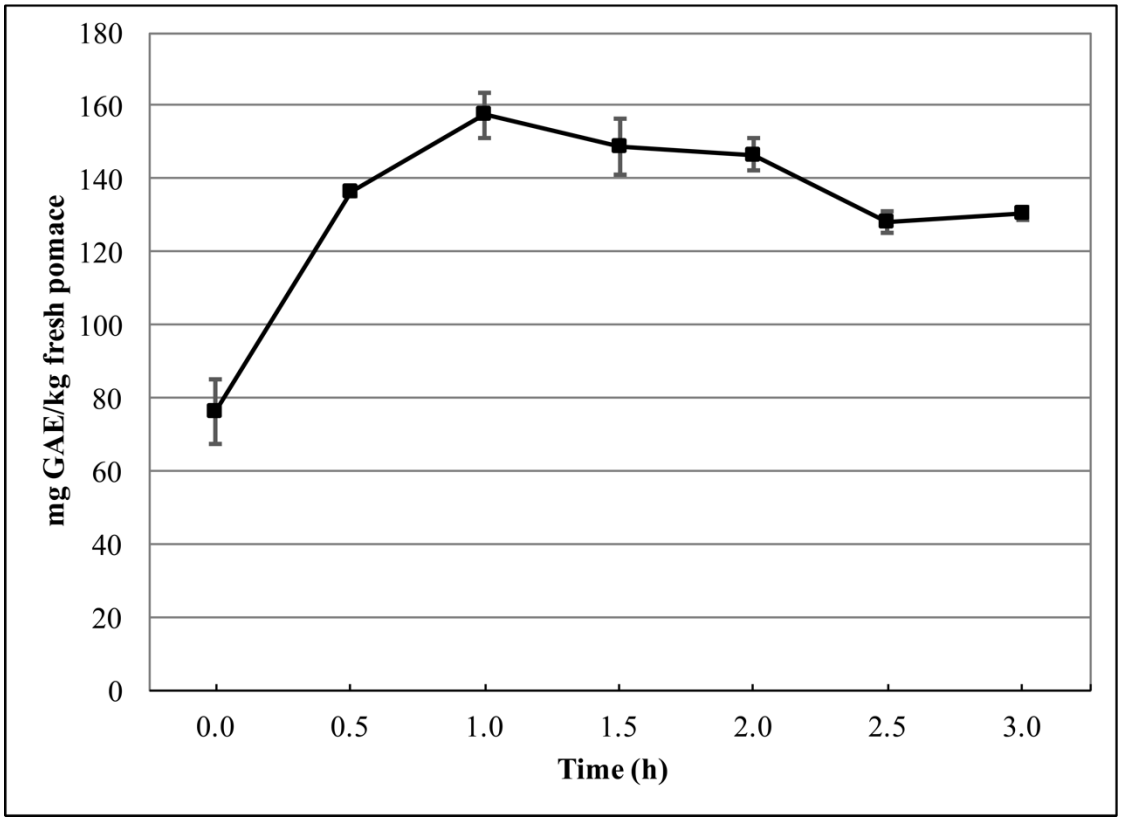


Figure 7. Optimization of length of time in aqueous extraction of dessert apple pomace for total phenolics. Data represents measurements done in triplicate and reported as mean ± standard error. No significant differences between means by Tukey HSD in leading treatments (0.5 h, 1.0 h, 1.5 h).

phenolic chemistry (Candrawinata et al., 2014; Renard, 2005).

The effects of oxidation and temperature affect procyanidins disproportionally whereas other phenolics in apples such as phloridzin, are more stable against such changes. Both phloridzin and quercetin (and their related conjugates) are more easily extracted than procyanidins, especially those of higher degree of polymerization. Due to these observed effects, the optimal time of extraction was found to be 30 minutes. With three successive extractions of pomace totaling 1.5 hours of extraction time, the dramatic losses in procyanidin and total phenolic content that started at 2 hours could

be avoided, while also minimizing processing time and associated processing expenses. Similar results were obtained from previously cited studies with optimized times between 15 minutes and 37 minutes (Candrawinata et al., 2014; Renard, 2005).

Finally, enzyme pre-treatments of pomace were evaluated for their effect on increasing TP yield in extracts. The temperature for enzyme maceration was maintained at 20°C to mimic likely operating conditions and because it was within the range of temperatures for which enzyme activity was recommended. Additionally, a solvent to mass ratio of 4:1 was used so that concentration prior to analysis was not necessary to obtain results within the limits of quantification.

From the first suite of enzymes to be tested (Figure 88), the protease (Maxipro) outperformed the other cellulase and pectinase enzymes. At a concentration of 0.05% and 0.10% w/w of fresh pomace, the protease enzyme achieved a significantly higher concentration of TP in the extract than all other treatments. Protease treatment of pomace for the enhanced extraction of phenolics has received little attention. Most studies that examine enzyme-assisted extraction of phenolics do so by considering the role of pectinase in catalyzing the breakdown of cell wall material and in so doing increasing solvent penetration and solute dissolution (Ajila et al., 2011; Oszmiański, Wojdyło, & Kolniak, 2011). All such studies have shown positive effects from the use of pectinase for this purpose.

The intended effect of proteases is similar. Proteolytic enzymes would target proteins in cell wall matrices to weaken the overall structure and potentially increase penetrability. The additional advantage of proteases is that the proteins which are most apt to bind to and precipitate procyanidins – large, loose, uncoiled, multidentate -- are also the ones that are most susceptible to proteolysis (Kato, Komatsu, Fujimoto, & Kobayashi, 1985; Mehansho, Butler, & Carlson, 1987). Other studies have

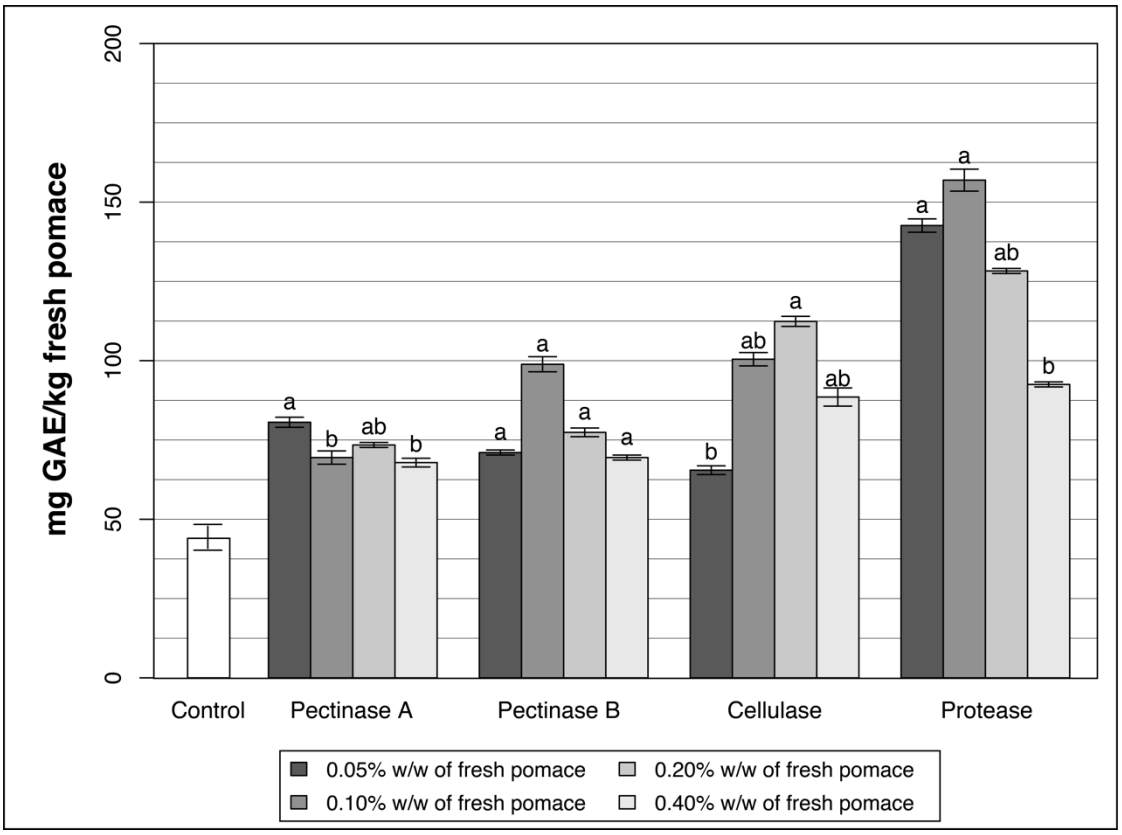


Figure 8. Optimization of enzyme type and enzyme concentration in aqueous extraction of dessert apple pomace for total phenolics. Data represents measurements done in triplicate and reported as mean ± standard error. Difference values between treatments not connected by the same letter are significant ($p < 0.05$) by Tukey-Kramer HSD.

investigated the interactions of condensed tannins and pathogenesis-related proteins in wine and have shown an increase in exogenous tannin retention with protein fining (Springer, Chen, Stahlecker, Cousins, & Sacks, 2016).

The few studies that have examined the role of proteases have yielded promising results, with mixed findings on the synergistic effects of proteases and pectinases for increasing total phenolics extracted (Landbo & Meyer, 2001; Pinelo et al., 2008). Our experiments comparing protease (Maxipro) at concentrations from 0.05 to 0.1 percent in combination with the same concentration of either cellulase or pectinase did increase total yields but the effect was not significant (data not shown), thus the optimized extraction process included only the protease as a pre-treatment.

Another method employed that is common in similar extraction experiments is to lyophilize and decrease the particle size of the material to be extracted. From first principles, increased surface area and decreased mean free path would result in significantly increased extraction efficiency. An additional benefit of this type of analysis is uniformity due to the removal of moisture and a more uniform particle size distribution. The current optimization experiments were conducted to mimic anticipated operating conditions in a cidery without access to equipment necessary for lyophilization and fine particle size reduction. Some of the variability in the extraction experiments for this research can be expected to be due to the variability in pomace as a heterogeneous mixture of stems, seeds, peel, core, and flesh.

Validation. Final optimized extraction parameters of one hour of 0.01% (w/w) protease pre-treatment followed by extraction at 100°C for 30 min, at a 15:1 water-to pomace ratio of dessert and hard cider pomaces yielded extracts with total phenolics of 76.1 and 39.9 ppm GAE/L respectively (Table 8). Expressed on a fresh weight basis, extracted phenolics were 599 mg GAE/kg of fresh Red Delicious pomace and 1,142 mg GAE/kg of fresh Dabinett pomace.

The total extractable phenolics (TEP) for hard cider and dessert apple pomace by way of 80% methanol extraction were 3,043 and 1,084 mg GAE/kg of fresh pomace respectively (Table 8). TEP numbers were calculated based on a yield of 1 g of lyophilized pomace from 3.75 g of fresh pomace, with mean moisture content of 73.33% ± 0.66. In terms of extraction efficiency, this then represents a 37.5% extraction of total extractable phenolics from hard cider pomace and 55.2% from dessert pomace. Similar extraction efficiencies and yields have been obtained in previous aqueous extractions of apple pomace (Candrawinata et al., 2014; Oszmiański et al., 2011).

Acidity for both samples represents a significant portion of the total soluble solids and would limit its usability in cider due to the simultaneous acidification of the cider. If tannin addition is the ultimate objective, the hard cider extract's acidity is 6.2 times the concentration of its TPC; for the dessert extract, the acid concentration is 19.0 times the concentration of its TPC. This significant difference is largely due to the initially high concentration of phenolics in hard cider apples and the low concentration in dessert varieties.

Concentration. To improve commercial viability as a tannin adjunct for cider, the extract was concentrated to minimize the volume of extract required for addition to cider. A summary of the optimized extraction and concentration process is given in Figure 9.

Concentrates were prepared from Red Delicious, the archetypal dessert apple variety, and Dabinett, a popular bittersweet variety. Red Delicious is notable for its high sugar content (14.17° Brix) and remarkably low acidity (1.76 g/L) (Table 10). Dabinett is also a very low acid fruit (1.09 g/L), being the hard cider apple with the lowest TA among 23 cider apple varieties surveyed (Valois, Merwin, & Padilla-Zakour, 2006). These qualities of sweetness and acidity are important because the fermentation process will convert the fermentable sugars in the apples to volatile products of approximately one volume of dissolved CO_2 and one percent ethanol per two degrees Brix. However, the level of acidity in the apples is mostly unchanged and will ultimately determine the level of acidity in the final extract. Alternatives to correct for this acidity are either chemically or procedurally intensive, or they impart undesired changes in flavor such as chalkiness ($CaCO_3$) or saltiness (NaOH).

Table 8. Final results from optimized aqueous extractions of dessert apple pomace and hard cider apple pomace.

	Total Phenolics (ppm GAE)	**Total Phenolics[1]**	**Total Extractable Phenolics[1]**	**Acidity (malic) g/L**	**Total Soluble Solids (°Brix)**
Hard Cider Extract	76.1 ± 1.8	1142 ± 27	3040 ± 170	0.47 ± 0.03	1.05 ± 0.03
Dessert Extract	39.9 ± 1.9	599 ± 29	1084 ± 80	0.76 ± 0.01	0.87 ± 0.01

Data represents measurements done in triplicate and reported as mean ± standard deviation. [1]Data is reported in mg GAE/kg of fresh pomace.

Final Red Delicious and Dabinett tannin concentrates at 41°Brix contained 13.16 g and 24.14 g TP as GAE/L, respectively (Table 9). For a cider maker looking to increase cider tannin by 100 ppm added apple tannins, this would be equivalent to a 0.76% and 0.41% (v/v) addition of concentrate to ciders. Condensed tannins as measured by the Adams-Harbertson (AH) protein precipitation assay, are nearly 2.5 times as concentrated in the Dabinett concentrate. The AH assay for condensed tannins has been repeatedly shown to have a high correlation with perceived astringency (Cáceres-Mella et al., 2013; Kennedy, Ferrier, Harbertson, & des Gachons, 2006). The proposed mechanistic model behind astringency relies on the precipitation of salivary proline-rich proteins with condensed tannins which is similar to the mechanism of the AH assay where precipitation occurs between the sample and bovine serum albumin. The implication is that the Dabinett tannin concentrate should contain three times the number of astringency-imparting compounds as the Red Delicious tannin concentrate.

There was good agreement between the measurement of phenolics with absorbance at 280 nm and the FC assay (sample size was insufficient to compute meaningful Pearson correlation coefficients). Acidity for the two concentrates was comparable, but higher in the Red Delicious concentrate which reflects initial juice chemistry (Table 10). The pH however follows the opposite trend; however, the poor correlation between TA and pH in fermented media is well-documented, and is further confounded for tannin concentrates where the high concentration of phenolic and other acids makes estimation of titratable acidity by malic acid imprecise. The low pH and high TSS content of the concentrate should contribute towards its long-term stability.

Extracts were standardized at 41°Brix for analysis which has the additional benefit of yielding a concentrate that is easily manipulated and homogenized into cider. Condensed tannins were measurable in samples except for Red Delicious juice, which was below levels of quantification. Total phenolic concentration in initial juice samples was nearly five times higher in Dabinett juice which is to be expected from a cider varietal. However, after extraction and prior to fermentation, Dabinett phenolics only exceeded those of Red Delicious by a factor of 1.5. Many of the aforementioned extraction kinetics are likely responsible for this difference such as preferential extraction of flavonols and dihydrochalcones, however results for condensed tannins in extracts are similar to levels found in juice at similar TSS. Oxidation of chlorogenic acid and flavan-3-ol monomers by polyphenol oxidase are also responsible for reductions in reported total phenolics.

The effect of fermentation did not significantly affect total phenolics for both concentrates, however in the Red Delicious sample a substantial increase in CT was observed from 9.8 mg/L to 54.4 mg/L CE (Table 10). Increases in condensed tannins of such a magnitude are rare, and the cause of this effect is unknown (Tan, 2005). In the Dabinett sample, there was some decrease in condensed tannins from 100.0 mg/L CE to 74.3 mg/L CE, but this degree of reduction in condensed tannins is not atypical (Guyot et al., 2003).

While prior to fermentation the two concentrates had been standardized to 11.2 °Brix, after fermentation, the Dabinett sample was lower in TSS. This may have been due to a larger percentage of fermentable sugars than in Red Delicious (which contains around 2% of total sugars as non-fermentable sorbitol (Richmond, Brandao, Gray, Markakis, & Stine, 1981)), but the differences in resultant acidity are also explanatory. Having a lower brix allowed for the Dabinett sample to be concentrated by a factor of 13.1 compared to the Red Delicious sample at 9.7 which allows for a more

Table 9. Chemical composition of Red Delicious and Dabinett apple pomace tannin concentrates

	Total Phenolics (g/L GAE) [1]	Total Phenolics (g/L GAE) [2]	Condensed Tannins (g/L CE)	pH	Acidity (malic) g/L	Total Soluble Solids (°Brix)
Red Delicious	13.16 ± 0.80	12.70 ± 0.33	1.32 ± 0.03	3.29 ± 0.02	47.06 ± 0.43	41.00 ± 0.04
Dabinett	24.14 ± 0.69	22.83 ± 0.59	3.12 ± 0.31	3.16 ± 0.01	39.52 ± 0.31	41.00 ± 0.04

[1]Data represents measurements done in triplicate and reported as mean ± standard deviation. [1]FC method was used . [2]Absorbance at 280 nm.

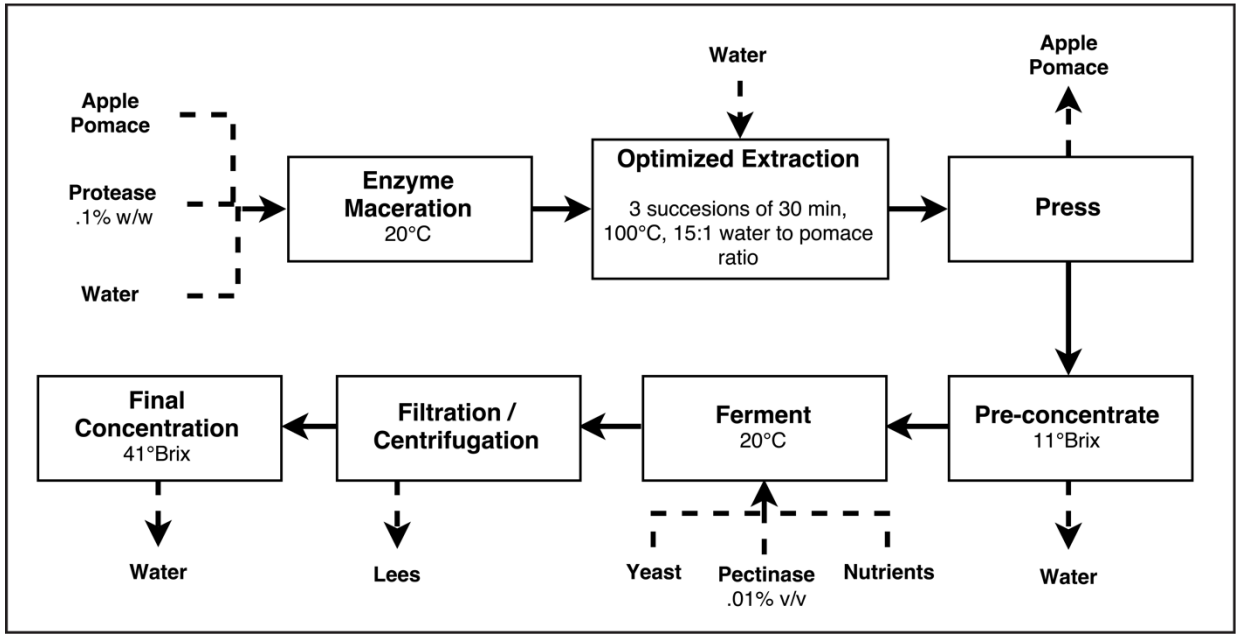


Figure 9. Process flow diagram for production of high tannin apple concentrate

Table 10. Chemical composition of Red Delicious and Dabinett apple pomace tannin concentrates throughout processing.

	Total Phenolics (g/L GAE) [1]	**Total Phenolics (g/L GAE)** [2]	**Condensed Tannins (mg/L CE)**	**pH**	**Acidity (malic) g/L**	**Total Soluble Solids (°Brix)**
Dabinett Juice	1.13 ± 0.11 ab	1.84 ± 0.04	105.7 ± 5.3 a	4.72 ± 0.01	1.09 ± 0.06 b	14.69 ± 0.04 a
R. Delicious Juice	0.23 ± 0.06 b	0.79 ± 0.02	*tr*[3]	4.27 ± 0.01	1.76 ± 0.02 ab	14.17 ± 0.03 a
Dabinett PF[4]	1.64 ± 0.17 a	1.65 ± 0.03	100.0 ± 5.0 a	4.72 ± 0.01	1.04 ± 0.05 b	11.22 ± 0.01 ab
Dabinett AF[5]	1.65 ± 0.28 a	1.56 ± 0.03	74.3 ± 0.5 a	3.72 ± 0.02	2.30 ± 0.05 ab	3.12 ± 0.01 b
R. Delicious PF[4]	1.09 ± 0.18 ab	1.00 ± 0.02	9.8 ± 1.6 b	4.45 ± 0.02	1.40 ± 0.02 ab	11.22 ± 0.02 ab
R. Delicious AF[5]	1.18 ± 0.13 ab	0.98 ± 0.02	54.4 ± 0.4 ab	3.43 ± 0.01	2.70 ± 0.02 a	4.23 ± 0.03 b

Data represents measurements done in triplicate and reported as mean ± standard deviation. [1]FC method was used . [2]Absorbance at 280 nm. [3]*tr* : trace. [4]PF: Prior to Fermentation [5]AF: After Fermentation. Difference values between treatments not connected by the same letter are significant ($p < 0.05$) by Tukey-Kramer HSD.

concentrated addition to cider. Making comparisons based on TSS, however, has its limitations at this point in the process due to dissolved ethanol and carbon dioxide which also affect the refractive index.

Differences were evidenced in the Lab scales of each concentrate. The Dabinett tannin concentrate was slightly darker, with more red color, and comparable levels of yellow color (Table 11).

Table 11. CIELAB color coordinates of Red Delicious and Dabinett apple pomace tannin concentrates

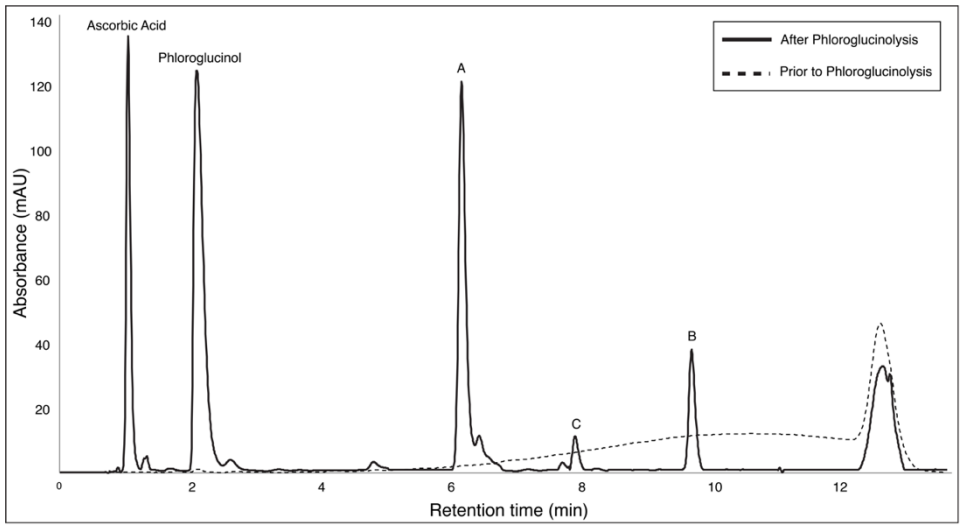
	L	**a**	**b**
Red Delicious	17.86 ± 0.03	31.33 ± 0.02	30.43 ± 0.43
Dabinett	17.52 ± 0.04	37.35 ± 0.05	30.15 ± 0.67

Data represents measurements done in triplicate and reported as mean ± standard deviation.

HPLC profiles of final concentrates show the high extractability of flavonols and polymerized flavan-3-ols (Table 12). No monomeric flavan-3-ols were observed which is consistent with the susceptibility of these compounds to thermal and oxidative degradation. Additionally, monomeric flavan-3-ols are more lipid soluble than polymerized flavan-3-ols making an aqueous extraction preferential against these compounds. Average degree of polymerization (DP*n*) for Dabinett was 2.25 and for Red Delicious was 3.33 (Table 13). Tannin mixtures with higher average degrees of polymerization are perceived as more astringent, while lower degree mixtures, similar to those observed in this study, are perceived as more bitter (Lea & Arnold, 1978).

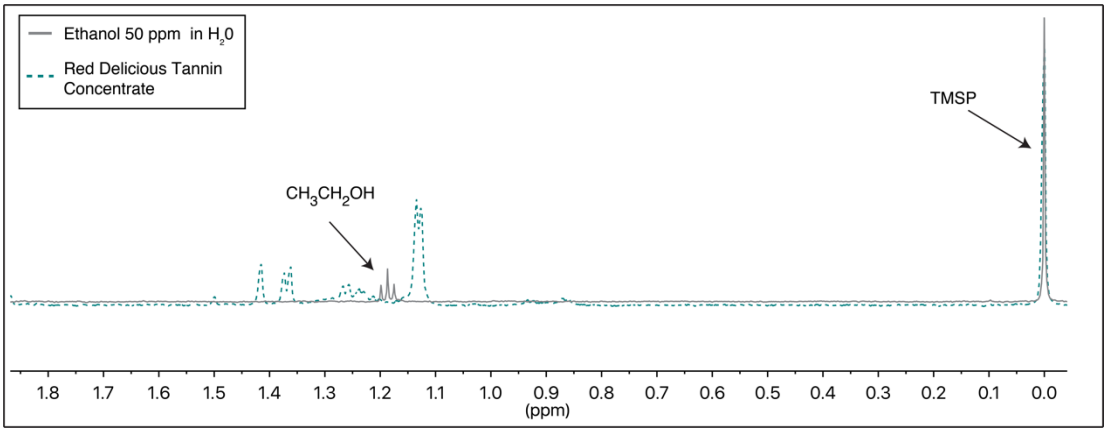
Epicatechin units were more predominant as terminal units, and they were the only extension unit observed for both concentrates (

Figure 10). Previous characterizations of Dabinett have found a DP*n* of 5.1 which demonstrates that some degradative changes during the extraction, fermentation, and concentration processes are occurring (Sanoner, Guyot, Marnet, Molle, & Drilleau, 1999). ^{1}H-NMR analysis of Red Delicious and Dabinett concentrates showed no quantifiable ethanol peaks at 1.19 ppm for a concentration of 0.005% ethanol (v/v) (Figure 11). Consequently, concentration processes can be said



to have removed ethanol in samples with up to 50 ppm remaining.

Figure 10. HPLC chromatogram of Red Delicious pomace tannin concentrate before and after phloroglucinolysis. Peak assignments were A) epicatechin-phloroglucinol



B) epicatechin C) catechin

Figure 11. ^{1}H-NMR spectrum of Red Delicious pomace tannin concentrate and ethanol spiked sample.

Table 12. HPLC phenolic profile (mg/L) of Red Delicious and Dabinett apple pomace tannin concentrates.

	Hydroxycinnamic Acids	Dihydrochalcones	Flavonols	Flavan-3-ols[1]	Sum of Total Phenolics
Red Delicious	211.9 ± 6.4	263.0 ± 10.0	1,209 ± 33	1,286 ± 12	2,970 ± 35
Dabinett	621 ± 31	320 ± 14	2,025 ± 61	2,700 ± 59	5,670 ± 120

Data represents measurements done in triplicate and reported as mean ± standard deviation. [1]No monomeric flavan-3-ols were detected in tannin concentrates.

Table 13. Characterization of condensed tannins in Red Delicious and Dabinett apple pomace tannin concentrates.

	Epicatechin Terminal Unit (%)	Catechin Terminal Unit (%)	Epicatechin Extension Unit (%)	DP*n*
Red Delicious	21.7 ± 0.8	8.3 ± 0.7	70.0 ± 0.6	3.33 ± 0.07
Dabinett	32.4 ± 0.2	12.0 ± 0.5	55.6 ± 0.6	2.25 ± 0.03

Data represents measurements done in triplicate and reported as mean ± standard deviation.

Sensory Analysis.

a) *Chemical Analysis.* As intended, finished ciders had statistically insignificant differences in all but TP (Table 14). TP in the commercial control were well within typical popular ciders found in the market (100 – 300 mg GAE/L) (Valois, 2007) whereas the two treatment samples had phenolic levels more typical of traditional high-tannin ciders (>300 mg GAE/L) (Gerling et al., 2016). Addition of Red Delicious tannin concentrate to the control at a dosage rate of 0.76% (v/v) resulted in an increase of titratable acidity of 0.34 g/L (10% increase). This effect was more modest (1.2% increase) with the Dabinett concentrate due to its lower TA and lower application rate of 0.31% (v/v).

A similar effect was evidenced in the turbidity of the three samples, with a greater, but still modest increase in turbidity from addition of the Red Delicious concentrate versus the Dabinett. The Color Lab values of these three ciders provides additional information as to their effect on the appearance of the samples (Table 15). There were no statistically significant differences in Lab values. However, addition of Red Delicious did darken the ciders somewhat, and addition of Dabinett did not. Analysis of the concentrates prior to addition (Table 11) showed that the Dabinett was slightly darker, but due to the rates of application, the Red Delicious had a more profound effect on treated samples. The ‘a’ component shows that the Red Delicious added a little more red color to the control and a little more yellow.

A better approximation for perceived sweetness than specific gravity alone is the ratio of the specific gravity to titratable acidity because it considers the effect of acidity on sweetness rather than sweetness in isolation. From this data (Table 16), no significant differences were seen between the three samples, but the control and the Dabinett fortified control were suggestive of being perceived as the sweetest.

Table 14. Chemical composition of ciders used in sensory evaluation.

	Total Phenols (ppm GAE)	pH	Acidity (malic) g/L	CO_2 Vol.[1]	Brix°	Turbidity (NTU)	Ethanol (v/v)[2]	Specific Gravity
Commercial Cider	265 ± 15 b	3.43 ± 0.01	3.34 ± 0.04	2.45	6.30 ± 0.02	3.07 ± 0.33	4.2%	1.016 ± 0.001
Red Delicious Fortified	423 ± 27 a	3.46 ± 0.01	3.68 ± 0.01	2.43	6.79 ± 0.03	5.09 ± 0.53	4.2%	1.019 ± 0.001
Dabinett Fortified	479 ± 62 a	3.51 ± 0.01	3.38 ± 0.02	2.41	6.35 ± 0.01	3.88 ± 0.31	4.2%	1.017 ± 0.001

Data represents measurements done in triplicate and reported as mean ± standard deviation. Means within a column followed by different letters are significant at the 0.05 level (Tukey's HSD). [1]Data was sampled only once due to the destructive nature of the test. [2]Data as provided by manufacturer.

Table 15. CIELAB color coordinates for cider treatments.

	L	a	b
Commercial Cider	90.24 ± 0.03	-1.10 ± 0.04	9.21 ± 0.25
Red Delicious Fortified	87.71 ± 0.04	-0.71 ± 0.02	10.51 ± 0.10
Dabinett Fortified	90.38 ± 0.05	-0.97 ± 0.02	9.67 ± 0.58

Data represents measurements done in triplicate and reported as mean ± standard deviation. No significant differences in L,a,b values were observed at the 0.05 level (Tukey's HSD).

Table 16. Calculated ratio of specific gravity of ciders to titratable acidity.

	SG to TA Ratio
Commercial Cider	0.304 ± 0.003
Red Delicious Fortified	0.277 ± 0.001
Dabinett Fortified	0.301 ± 0.002
Dabinett Fortified w/ malic	0.277 ± 0.001

Data represents measurements done in triplicate and reported as mean ± standard deviation. No significant differences between treatments at the 0.05 level (Tukey's HSD).

b) *Panel Analysis*. From the sequential monadic data, no statistically significant differences were evidenced in mean overall liking, or attributes of color, flavor, and aroma (Table 17). Means were identical for overall liking in both the commercial and Red Delicious fortified samples (6.30), but were higher in the Dabinett fortified samples (6.39).

With regard to the appearance of the samples, there was a statistically significant difference between the appearance of the control (mean liking of 6.68) and the Red Delicious sample (mean liking of 6.36). This is in keeping with the previous observations from the chemical analysis which suggested that the Red Delicious concentrate altered both the turbidity and the color of the control more significantly than did the Dabinett. This same trend is evidenced in the color, however the effect is not significant, suggesting turbidity also played a role in appearance liking, which was substantiated by elicited 'open comments.' A small increase in liking for aroma was observed with the Dabinett sample, however all were statistically similar. This is encouraging since the concentrate at application rate either does not significantly affect aroma, or does so in a minor but positive way.

In analyzing the penalty analysis, the control cider had three attributes requiring adjustment ("Not Sweet Enough", "Not Astringent Enough", "Not Acidic Enough"), and one requiring significant adjustment ("Not Enough Apple Flavor") (Figure 1212). The Red Delicious sample had no areas of improvement identified, and the Dabinett was penalized for the attribute of lack of sweetness.

Table 17. Mean overall liking and other attributes for control and treatment samples in sensory analysis (n=115).

	Overall	Appearance	Color	Flavor	Aroma
Commercial Cider	6.30 ± 0.16	6.68 ± 0.11 a	6.40 ± 0.14	6.32 ± 0.17	6.26 ± 0.13
Red Delicious Fortified	6.30 ± 0.13	6.19 ± 0.14 b	6.03 ± 0.16	6.24 ± 0.13	6.24 ± 0.13
Dabinett Fortified	6.39 ± 0.13	6.36 ± 0.12 ab	6.10 ± 0.15	6.38 ± 0.14	6.39 ± 0.13

Data is reported as mean ± standard error. Means within a column followed by different letters are significant at the 0.05 level (Tukey's HSD).

Chemical analysis of the three samples revealed very similar levels of carbonation, acidity, and sweetness. It was only astringency which varied significantly and this was one of the attributes panelists picked up on as lacking in the untreated sample. Astringency has been shown to give balance to ciders, and ciders lacking in astringency are often perceived as insipid.

In this experiment and previous sensory experiments, panelists emphasized apple flavor as being a key attribute in need of adjustment. It is possible that in calling attention to this attribute, additional significance was given to it than otherwise would have been. It may also be the case that consumers, with previous expectations, expect a strong apple flavor. Commercially, this is achieved through the addition of apple juice concentrate prior to bottling which also results in a sweeter cider, a quality perceived as lacking in two of the three ciders. The control cider contained 4.2% sugar (w/v) (per label declaration) which would put it in the category of a 'semi-sweet' wine. However, for cider, this amount of residual sugar was still lower than a majority of commercial ciders at 5 to 6% residual sugar (Valois, 2007).

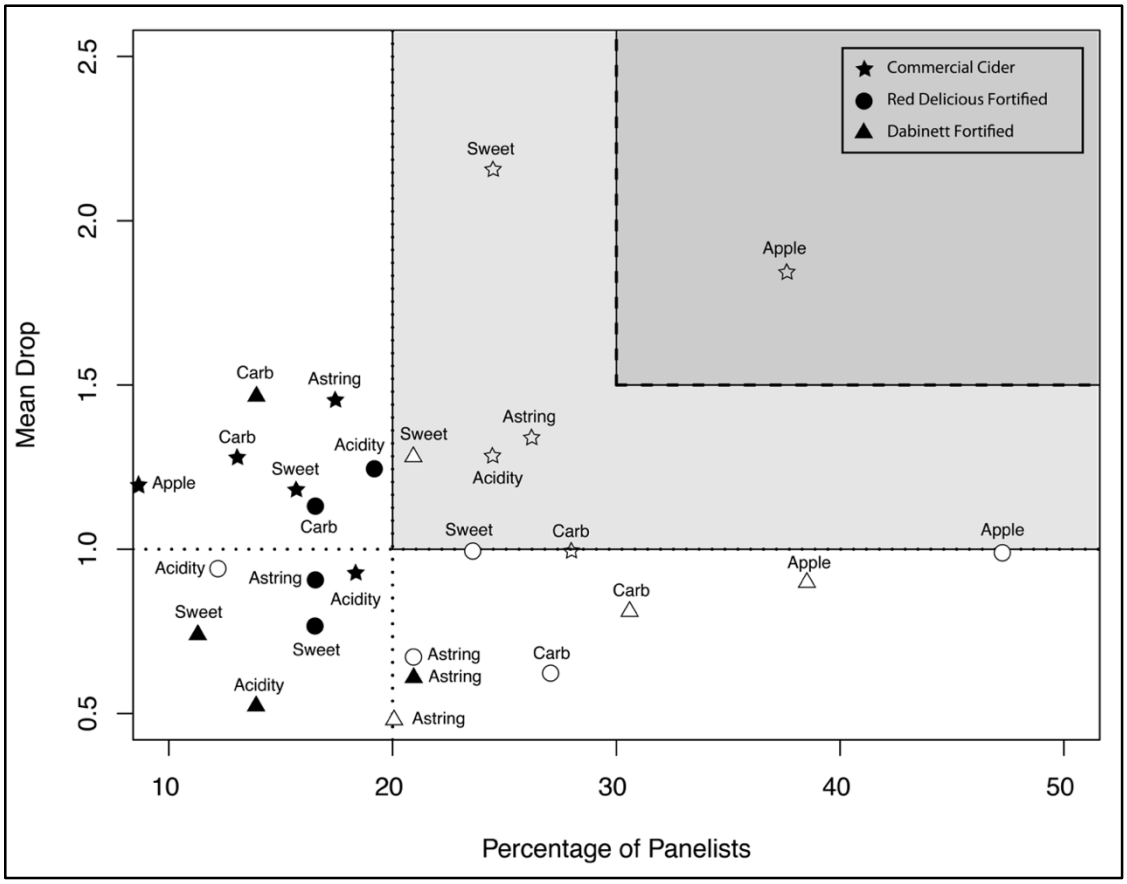


Figure 12. Penalty analysis of control and treatment cider samples from just-about-right scaling. Attributes were: Acidity, Sweetness ("Sweet"), Astringency ("Astring"), Carbonation ("Carb"), and Apple Flavor ("Apple"). Open symbols denote "too little" of an attribute and closed symbols denote "too much."

In the paired preference experiment for cider drinkers (those drinking at least one cider per month), 91.3% of individuals could perceive a difference between the two treatment samples, however, there was no statistically significant differences in overall preference, or difference attributes of sweetness, acidity, astringency, or bitterness with a 95% level of confidence. Panelists did prefer the acid adjusted Dabinett sample, but the difference was small with 53.6% preferring Dabinett and 46.6% preferring Red Delicious, which is in agreement with the monadic overall liking scores.

Table 18. Summary of sensory panel demographics (n=115).

Variable	Description	Frequency (%)
Gender	Male	41
	Female	59
Age	21-34	51
	35-44	20
	45-54	14
	55+	15
Education	Some High school	1
	High school degree	0
	Some college	5
	Associates degree	3
	Bachelor's degree	25
	Some graduate school	16
	Graduate degree	50

Conclusion

Apple pomace represents a substantial source of phenolics, and in particular, tannins. High-tannin concentrates from optimized aqueous extractions offer a green solution to the problem of pomace waste generation. From the two samples tested, Red Delicious and Dabinett pomace, representing two significantly different apple cultivars, concentrates were found to be acceptable as tannin additions for increasing astringency and quality in fermented cider. This gives cider producers and apple growers additional flexibility in which varieties of apple they choose to press into cider.

Cultivar tannin concentrates from bittersweet and sweet varieties should be tested to determine if significant differences in chemical profile and acceptability exist. While differences in color and turbidity were not significant from the sample size, panelists did penalize the Red Delicious sample for its color and appearance. Sweetness ratings did not conform to the specific gravity to titratable acidity ratio

suggesting additional confounding factors such as astringency should be taken into account.

HPLC profiles of final concentrates showed preferential extractability and stability of flavonols over other phenolics. Additional measures should be taken to limit oxidation of flavanols throughout the process. Equipment needed for manufacture of the tannin concentrate is within the scope of most cideries and should offer a viable alternative to other tannin sources. Pomace that is immediately processed into a tannin concentrate will likely have superior phenolic concentration than that demonstrated in this study. In formulating cider blends for a wide audience, care should be taken to differentiate product offerings since apple flavor and sweetness continue to be of importance to cider consumers.

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CHAPTER 4

ECONOMIC FEASIBILITY, CONCLUSIONS & FUTURE WORK

Economic Feasibility

The economic feasibility of producing high-tannin concentrates from Red Delicious and Dabinett pomaces was determined with final addition rates to cider of 100 and 150 ppm mg gallic acid equivalent per liter. In estimating the cost of

Table 19. Input assumptions for feasibility cost model of producing tannin concentrate from apple pomace.

Parameter	Value
Cider Producer Volumes	Small: Less than 20,000 gallons Medium: Between 20,000 and 100,000 gallons Large: More than 100,000 gallons
Cost of water	$2.865 per 1000 gallons
Cost of steam	$9.50 per 1000 pounds, includes boiler fuel cost (natural gas) and assumes 88% boiler efficiency
Labor	$10.90 per hour
Depreciation	Straight line, 10-year lifetime of equipment
Installation Cost	65% of equipment costs
Maintenance Cost	2% of equipment costs
Freight Cost	4% of equipment costs
Production Loss	10% of total production
Moisture of Pomace	75%
Yield of Juice from Apples	70% for small producers using rack and frame hydraulic press 75% for medium producers using a continuous belt press 88% for large producers using a Bucher-Vaslin (previously Bucher-Guyer) hydraulic piston press
Price of apples	$0.35 per pound for cider apples $0.30 per pound for mid-range $0.20 per pound for juicing apples
Production Days of Operation	250 days
Time to completion per batch	7.5 days
Phenolic Concentration at 41° Brix	Red Delicious: 13.16 g GAE/L ; Dabinett: 24.14 g GAE/L

production, several typical assumptions were required (Table 1918). *A priori,* production of tannin concentrate is assumed to take place at a currently operating cidery with implied equipment and facilities necessary to produce cider. Elements such as juice and pomace pumps, pumping lines, a boiler system, accumulation bins, clamps and miscellaneous accessories, filters, access to inert gas, and a refractometer are assumed pre-existing as they are integral to the concentration process, and additional capital expenditures are not required for these items.

Disposal of pomace has three financial outcomes: (1) no cost is incurred, often due to mutual agreements between growers and cider producers, (2) some cost is incurred through landfill, transport, and/or drying, (3) a credit is received from the sale of pomace for the production of secondary products such as pectin. Due to the unpredictability and incompatibility of these outcomes, the costs or benefits of disposing of pomace were disregarded in the model for simplicity.

Costs have been determined for three sizes of producers, and these and other such determinations such as percentage freight cost were made: (1) in conversation with local cider producers and manufacturers and (2) through extensive literature research. A small cidery is one that produces less than 20,000 gallons of finished cider per year; a medium cidery produces between 20,000 and 100,000 gallons; a large cidery produces at least 100,000 gallons. Reported costs for small producers will be based on an output of 20,000 gallons, thus cost estimates can be viewed as a maximum cost. Similarly, for large producers, estimates are based on a 100,000-gallon output, thus cost estimates are a minimum. Finally, for medium scale producers, estimates are based on an average output of 60,000 gallons, thus cost estimates are an average cost for this output range.

High-tannin concentrate is produced as in Figure 9. To summarize, milled apple pomace that has previously been pressed is conveyed, either manually or with a

conveyer, to an evaporating pan where it is treated with 0.1% protease and allowed to macerate for 1 h. The pomace is then submerged in 15 times its weight in water and heated to 100°C for 30 minutes. The resulting mixture is pumped to the juice press where the solids are separated and the extract reserved. After pressing, the extract is pumped back to the evaporating pan, and an equivalent weight of enzymatically pre-treated pomace is added to the extract and the process repeated twice more. The final extract is returned back to the evaporator where it is concentrated to 11.2 °Brix under

Table 20. Detailed equipment descriptions and cost for additional items involved in high-tannin concentrate production from apple pomace

Item	Cost
Jacketed vacuum pan with vacuum pump, condenser, and limited piping. (25, 50, 100 gallon)	$51,000; $56,000; $62,000
Variable capacity fermenting tank, sloped bottom, stainless steel with miscellaneous fittings, no jacketing. (100, 150, 250, 300, 400 liters)	$459; $489; $599; $629; $729
55-gal plastic food drum with air-tight lid.	$69

Table 21. Projected quantities of high-tannin concentrate produced from available pomace with concentrate-enriched cider equivalent.

		Yield of Concentrate from Pomace (gallons)		**Quantity of Cider Enriched by Concentrate** (gallons)			
Producer Size	**Pomace Generated** (pounds)	Red Delicious	Dabinett	Red Delicious (100 ppm)	Red Delicious (150 ppm)	Dabinett (100 ppm)	Dabinett (150 ppm)
Small	9,524	121	63	15,995	10,703	15,161	10,129
Medium	22,222	282	146	37,322	24,975	35,378	23,634
Large	25,253	320	166	42,411	28,381	40,202	26,856

vacuum. The concentrate is pumped to a stainless steel tank where pectinase (0.01% v/v), yeast nutrients and rehydrated yeast are added. The concentrate is fermented to completion (until no residual sugar remains) and is afterwards pumped through a coarse filter into the evaporating pan where it is reduced under vacuum to 41°Brix. The final concentrate is pumped into a food-grade plastic food drum with air-tight lid, topped with inert gas, and is ready for application.

Based on this process, the equipment and ancillary materials needed to produce the concentrate in addition to pre-existing equipment are listed in Table 2019. These quantities are based on projected quantities of concentrate production which are themselves based on the amount of pomace a producer would generate given finished product volume (Table 2120). Using this data, it is possible to then determine how much cider could be enriched with the concentrate.

For example, a small producer generates approximately 9,520 pounds of pomace in the process of producing 20,000 gallons of cider. Following extraction, fermentation, and concentration as outlined in the production process, anticipated volumes of tannin concentrate for Red Delicious pomace would be 121 gallons, and for Dabinett, 63 gallons. Concentrates are standardized based on °Brix and thus the difference between these two volumes is due to a number of factors including primarily the concentration of total soluble solids, phenolics, and acidity in the initial pomace. Given these volumes, with the Red Delicious tannin concentrate, a small producer would be able to enrich 15,994 gallons of cider at 100 ppm total phenolics (TP), and 10,703 gallons of cider at 150 ppm TP. For both Red Delicious and Dabinett tannin concentrates, the quantity of cider enriched approaches but is below total anticipated cider production. Therefore, a producer would be able to utilize all of its spent pomace for the production of an endogenous tannin concentrate to replace a substantial portion of required exogenous tannins, if any. Larger producers, due to

Table 22. Capital expenditures for production of Red Delicious tannin concentrate from apple pomace.

Small (Less than 20,000 gal./yr)		**Medium** (20,000 to 100,000 gal./yr)		**Large** (More than 100,000 gal./yr)	
Description	Cost	Description	Cost	Description	Cost
25-gallon vacuum pan	$51,000	50-gallon vacuum pan	$56,000	100-gallon vacuum pan	$62,000
150L tank x 2	$978	400L tank x 2	$1,458	400L tank x 2	$1,458
Storage food drum x 3	$207	Storage food drum x 6	$414	Storage food drum x 6	$414
Equipment Cost	**$52,185**	**Equipment Cost**	**$57,872**	**Equipment Cost**	**$63,872**
Installation	$33,920	Installation	$37,617	Installation	$41,517
Freight	$2,087	Freight	$2,315	Freight	$2,555
Total Fixed Capital	**$88,192**	**Total Fixed Capital**	**$97,804**	**Total Fixed Capital**	**$107,944**

Table 23. Capital expenditures for production of Dabinett tannin concentrate from apple pomace.

Small (Less than 20,000 gal./yr)		**Medium** (20,000 to 100,000 gal./yr)		**Large** (More than 100,000 gal./yr)	
Description	Cost	Description	Cost	Description	Cost
25-gallon vacuum pan	$51,000	50-gallon vacuum pan	$56,000	100-gallon vacuum pan	$62,000
100L tank x 2	$918	250L tank x 2	$1198	300L tank	$1,258
Storage food drum x 2	$138	Storage food drum x 3	$207	Storage food drum x 4	$276
Equipment Cost	**$52,056**	**Equipment Cost**	**$57,405**	**Equipment Cost**	**$63,534**
Installation	$33,836	Installation	$36,924	Installation	$41,297
Freight	$2,082	Freight	$2,272	Freight	$2,541
Total Fixed Capital	**$87,974**	**Total Fixed Capital**	**$96,601**	**Total Fixed Capital**	**$107,372**

Table 24. Unit cost of materials for producing tannin concentrate from pomace compared to commercially available tannin and high-tannin juice.

	Material Cost of Concentrate (per 750 mL)				Commercial Tannin Addition		High-Tannin Juice	
Producer Size	Red Delicious (100 ppm)	Red Delicious (150 ppm)	Dabinett (100 ppm)	Dabinett (150 ppm)	100 ppm	150 ppm	100 ppm	150 ppm
Small	$0.0116	$0.0173	$0.0116	$0.0174				
Medium	$0.0110	$0.0164	$0.0110	$0.0165	$0.0306	$0.0459	$0.0056	$0.0083
Large	$0.0107	$0.0160	$0.0107	$0.0161				

Table 25. Total cost of producing tannin concentrate per 750 mL unit of final cider.

	Total Cost of Concentrate (per 750 mL of cider)			
Producer Size	Red Delicious (100 ppm)	Red Delicious (150 ppm)	Dabinett (100 ppm)	Dabinett (150 ppm)
Small	$0.157	$0.234	$0.164	$0.246
Medium	$0.080	$0.119	$0.083	$0.125
Large	$0.078	$0.116	$0.081	$0.122

press and other operational efficiencies, produce less pomace per gallon of cider generated. Thus, the potential quantity of cider enriched by tannin concentrate is a smaller percentage of the total volume of cider produced.

From the determination of appropriate equipment sizing for necessary throughput, Table 2221 and Table 2322 list the capital expenditures in the production process. It is useful to understand the cost comparison of the tannin concentrate against the conventional tannin enrichment options on a per unit basis. Table 2423 provides the material cost comparison based on a 750 mL bottle of cider. Cost savings are realized for increased volumes of production, but these material savings are moderate because volume discounts for manufacturer quoted supplies and public utilities was not significant at the volumes of total production analyzed. Taking a medium-sized producer as a case study and comparing (1) Red Delicious, (2) Dabinett, (3) high-tannin juice, and (4) commercial tannin addition at 100 ppm, the high-tannin concentrate is almost three times less expensive than the commercial option and nearly twice as expensive as the high-tannin juice option.

Furthermore, if labor and factory overhead are included in the total cost calculations (Table 2625), then a complete picture of cost to producer per unit is established (Table 2524). With this information, producers can determine based on their own product margins whether on-site production of tannin concentrate from expended pomace is feasible. As would be expected, the savings in total cost of production per unit for increased volumes of cider production is more significant than for material cost alone. By way of example, a medium-scale producer would add an additional cost of eight cents to a 750 mL bottle of cider if enriched with 100 ppm TP from a Red Delicious concentrate. It is thus concluded that, with premium ciders selling in the

Table 26. Total cost of producing tannin concentrates from pomace based on producer volumes.

	Red Delicious Concentrate			**Dabinett Concentrate**		
	Small Producer	Medium Producer	Large Producer	Small Producer	Medium Producer	Large Producer
Materials						
Yeast	62.79	146.50	166.48	44.11	102.91	116.95
Enzymes	146.12	340.94	387.44	141.89	331.07	376.21
Yeast Nutrients	82.73	193.03	219.35	58.11	135.60	154.09
Water	16.35	38.15	43.35	16.35	38.15	43.35
Steam	625.52	1,350.90	1,474.40	630.97	1,362.16	1,487.54
Total Material Cost	**$933.51**	**$2,069.43**	**$2,291.02**	**$891.43**	**$1,969.89**	**$2,178.15**
Factory Overhead						
Maintenance	1,043.70	1,157.44	1,277.44	1,041.12	1,148.10	1,270.68
Depreciation	5,218.50	5,787.20	6,387.20	5,205.60	5,740.50	6,353.40
Total Overhead	6,262.20	6,944.64	7,664.64	6,246.72	6,888.60	7,624.08
Direct Labor	5,450.00	6,042.84	6,681.44	5,450.00	6,042.84	6,681.44
Total Cost	**$12,645.71**	**$15,056.91**	**$16,637.10**	**$12,588.15**	**$14,901.33**	**$16,483.67**
Projected Volume of Concentrate (gal)	**121**	**282**	**320**	**63**	**146**	**166**

range of $12 - $18 per 750 mL bottle, the cost of producing tannin concentrate is economically feasible. Ciders sold as a mass-market cider are typically sold in 12-oz units and bring an average price on a 750 mL basis of $3.17 - $3.52. Thus, with the addition of tannin concentrate from apple pomace, it is likely that cider producers could substantially increase total revenues by marketing the tannin-enriched cider as a premium cider.

Conclusions and Future Work

There is a preponderance of low-tannin dessert apples available for cider producers to use, but a growing shortage of high-tannin cider apples. The astringency these cider apples bring to cider is essential to premium products through addition of complexity and balance. As previous studies have shown and our own research demonstrates, this increase in quality is associated with an increased market value and consequently improved incomes for cider producers. The addition of tannin offers another way to differentiate product offerings in an increasingly crowded market.

Publicly available research demonstrating consumers' preferences for different styles is very limited. Sensory experiments from this research have demonstrated the existence of market opportunities for ciders with tannic profiles. However, the raw materials necessary to take advantage of this opportunity remain limited.

One proposed alternative is to produce a tannin-rich concentrate on premise from spent apple pomace using almost entirely existing equipment with minimal additional per unit cost. The concentrate is also produced with water which is renewable and natural, and ensures the safety and quality of the final product. In conducting research to optimize this extraction, costs can be minimized, and sustainability is enhanced. It also offers an environmentally friendly alternative to the

destructive process of producing commercial tannins through the felling of trees and use of harsh chemicals and solvents.

Final tannin-rich concentrates were produced at reduced temperatures using a moderate vacuum. The quality of the final product could potentially be enhanced by increasing the amount of vacuum and thus lowering the concentrate's boiling point. Other concentration processes such as forward osmosis and reverse osmosis were not explored and offer a non-thermal alternative that could enhance product quality. Commercial tannins are also manufactured as a powder. Lyophilization of the tannin concentrate could potentially ease application and diminish ancillary and unintended changes to treated cider. Economic analyses of all of these methods would be necessary along with quality and sensory tests to determine the best alternative. One advantage of the current process is that it can be implemented at the cidery with equipment that would not require additional training or large capital expenditures.

Fermentation of the tannin concentrate sought to mimic solubility conditions of final cider with regard to percent ethanol and tannin chemistry. Tannin additions to ciders for all sensory trials in this research were conducted shortly before the sensory trials. In practical application, tannins are often, but not always, added at the beginning of the fermentation process. It remains to study the effect of time, aging, and shelf stability of the concentrate and ciders with the concentrate added. It is expected that significant changes will occur due to the highly reactive nature and observed behavior of tannins in cider and wine.

Tannin concentrates were produced primarily from Red Delicious and Dabinett apple varieties from one growing season. Repetition over the course of several seasons with many different cultivars would substantiate these findings. In addition, this would allow for exploration of differentiated concentrates and thus differentiated product lines.

The production process from apple to pomace to tannin concentrate, through milling, extraction, concentration, fermentation, and filtering sought to limit exposure to oxygen however further opportunities for minimization exist. HPLC characterization of concentrate throughout the production process would be useful in understanding final concentrate chemistry and how the process could be further optimized for intended sensory characteristics. In addition to the enhanced quality of cider through addition of tannin concentrate, cider makers could potentially also market the increased antioxidant and polyphenolic content of their ciders.