OPTIMIZATION OF HARD CIDER PROCESSING TO MAXIMIZE TANNIN EXTRACTION: EXPLORING THE APPLICATION OF HEAT AND PULSED ELECTRIC FIELD TO APPLE POMACE AND MASH

A Thesis

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by

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ABSTRACT

The production of hard cider in the US grew almost 400% between 2011 and 2017 (ATTTB, 2011; ATTTB 2017), with both the largest US producer and the largest number of producers based in NY state. This growth exposed a dearth of "cider" apples, considered a necessary minority component of cider for their contribution of tannins. While orchardists scramble to plant trees to meet demand, cidermakers have tools at their disposal to maximize the extraction of tannins from their limited supply. The skins of many apple varieties contain a reservoir of unextracted tannin lost at pressing. Heat and the application of pulsed electric fields (PEF) can increase extraction into juice, creating more tannic cider. This study was divided into two parts: (1) the treatment of pomace to recover unextracted tannins for addition to cider; and (2) the treatment of apple mash prior to pressing to maximize extraction into juice.

Both PEF and heat treatment successfully extracted polyphenols from Red Delicious pomace, with maximum yields of 525 and 1209 GAE per kilogram of pomace, respectively. Pre-press heat and PEF treatment on cider apple mash yielded maximum juice phenolics of 72% and 28% higher than standard processing. Sensory evaluation of these treated tannic ciders blended 30:70 into standard-processed dessert apple ciders found that both were acceptable to consumers, suggesting that either process would be appropriate for processing tannic apples for use in blended ciders.

BIOGRAPHICAL SKETCH

Kate Pinsley received a B.A. in Middle Eastern Studies and History from New York University in 2005. It was during those studies, while very much underage in Manhattan, that Kate first began her journey with hard cider in an Irish pub in the East Village.

Over the next decade, Kate sold circus equipment in NY and heavily dabbled in flying trapeze, expanded her cider tastes far beyond Strongbow and Magners, and co-founded the Red Eye Cafe in Montclair, NJ, where she worked as the baker. Kate then returned to school for a B.S. in Biochemistry from Montclair State University in 2017.

In the Summer of 2016, Kate combined her culinary skills with her scientific curiosity as a summer scholar at Cornell University, where she developed a sports rehydration beverage from acid whey. Kate returned to Cornell in 2017 to pursue her Master's degree in Dr. Olga Padilla-Zakour's lab.

While at Cornell, Kate dove headfirst into hard cider, focusing on the valorization of pomace and maximizing the extraction of tannins both from pomace and apple mash at pressing. She studied winemaking and chemistry, pestering her professors with apple-specific questions, and produced, analyzed, and drank quite a bit of cider over the course of her studies. She served as teaching assistant for two classes, Chemistry & Functional Properties of Food and Cider Production. Kate presented her research at the 2019 CiderCon in Chicago, at the 2019 NY Cider Association annual meeting, and to interested cidermakers wherever she encountered them. Kate plans to take her cider experience and food chemistry skills to a cidery where she can help produce exceptional cider.

In loving memory of my father, Elliot Pinsley

and my grandmother, Helen Pinsley.

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Chapter 1

INTRODUCTION

A note on terminology: “cider” is used to describe two very different apple-derived beverages in the US: (1) unfiltered and occasionally unpasteurized apple juice primarily consumed in the Autumn during apple harvest season, also known as “sweet cider”; and (2) fermented (alcoholic) apple juice, also known as “hard cider.” For the purposes of this paper, the term “cider” will exclusively refer to the fermented product.

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1.1 History of Cider in America

The sudden exponential growth of the American cider industry in the second decade of the 21st century belies cider’s long history in America. Indeed, the bulk of apples grown in the Plymouth colony, and in the first 300 years of American history, would have been fermented into cider (Thacher, 1822; Janik, 2011). Thanks to the efforts of Johnny Appleseed (né John Chapman), and the motivation provided by land grant ordinances mandating that western settlers improve their land via the planting of at least 50 apple trees, apples spread west across the young nation (Lindley et al., 1937). These trees, grown from seed, were as unpredictable as the West itself, and certainly less palatable for fresh consumption than contemporary “dessert” apples bred for sweetness and hardiness. Without modern preservation technologies, there would have been little distinction between “hard” and “sweet” cider – any juices not consumed immediately would have inevitably fermented.

So, Americans made cider. Lots of it. George Washington made cider from apples grown in his Mount Vernon orchards, landowners included cider as payment to field workers, and William Henry Harrison won the 1840 presidential election as the "hard cider and log cabin" candidate, using cider to underscore his kinship with the common American man (Merwin, Valois, & Padilla-Zakour, 2008). An American family went through a barrel of cider a week, and even children drank "ciderkin", a watered-down version of the adult beverage (Williams, 1990).

But then industrialization moved most Americans off family farms and into cities and suburbs. Refrigeration made possible the regular consumption of unfermented apple juice. Waves of German and Irish immigrants brought their affinity for beer, which also happened to be cheaper to produce (Cumo, 2015). Finally, Prohibition changed Americans' relationship with alcohol and with apples. Apple orchards were a casualty of the temperance movement of the late 19th and early 20th centuries, chopped or burned down and eventually replaced with trees of sweeter, milder dessert apples that would be innocently consumed fresh (Pollan, 2002). By the end of Prohibition in 1933, American drinkers had firmly shifted their allegiance to beer.

The cider renaissance of the 2010s was a long time coming. While orchardists may have never truly ceased fermenting their own apples, it wasn't until Vermont-based Woodchuck launched in 1991 that cider found its place on the American bar scene; and while craft cideries proliferated in the early 2000s, it was not until Boston Beer Co. debuted Angry Orchard in 2012 that cider cracked 1% of the beer and malt beverage market (Keri, 2015). Between 2011 and 2017, the volume of bottled cider grew almost 400%, from 9.3 to 45.8

million gallons (ATTB 2011 & 2017). This growth in production occurred much too rapidly for orchardists to adjust, meaning that most American cider today is made from dessert apples.

1.2 Cider Apples

Among the four categories of apples described by the Long Ashton Research Station (LARS) in the UK, "sweet" apples are those low in acid and tannin, "sharp" apples are those high in acid and low in tannin, "bittersweet" apples are those low in acid and high in tannin, and "bittersharp" apples are those high in acid and tannin (Lea & Drilleau, 2003). "Dessert" or "culinary" apples – those used for fresh consumption or baking – fall almost exclusively into the first two categories, with tannin concentrations between 0.02-0.2% (Valois, 2007). This is not incidental: high concentrations of tannins render an apple nearly inedible with bitterness and astringency, which is why some of those bittersweet and bittersharp apples, with up to 1% tannins, are colloquially described as "spitters" for the reaction induced in unsuspecting mouths (Merwin et al.2008).

Table 1.1: LARS Classification of Apple Varieties

	Acid[1] (%)	**Tannin[2] (%)**
Sweet	Low < 0.45	Low < 0.2
Sharp	High > 0.45	Low < 0.2
Bittersweet	Low < 0.45	High > 0.2
Bittersharp	High > 0.45	High > 0.2

[1] as Malic Acid (w/v), [2] as Tannic Acid (w/v)

These same characteristics, while a flaw in dessert apples, are valued in cider apples. Fermentation "softens" the bitterness of tannins, markedly changing the sensation experienced from direct consumption of the bitter apple, while the lingering astringency provides mouthfeel and body to the cider, and the oxidation of tannins contributes to the archetypal golden-brown color of cider (Lea, 1990). Ciders made with only dessert apples are generally thin in body and insipid in flavor, relying on the post-fermentation addition of apple juice concentrate for taste and color. Few varieties of apple, cider or dessert, contain all the characteristics for fermentation into an ideal cider, so even when cider apples are cheap and abundant (as in the UK), they are typically blended as a minority component (20-40%) into a base of sweet and sharp apples (Merwin et al., 2008).

1.3 Standard Cidermaking Practices

The process of making cider (Figure 1.1) is, in its purest form, much like winemaking: (1) mechanically damage fruit, (2) press the damaged fruit into juice, (3) ferment the juice with native or added yeast. In practice, there are typically many additional steps, with decisions to be made based on budget, quality of the fruit, equipment, number and skill of cellar workers, and the style of cider being made. A cider's quality – the synthesis of flavor, aroma, texture, color, clarity and stability – is the sum of those decisions.

Most apples are laboriously hand-harvested in American orchards, unlike English cider-specific orchards where machine harvesting is standard. This is in part a residual effect of cider's nearly century-long disappearance in the US: because the overwhelming majority of American apples are dessert apples which have their highest value sold in supermarkets

for fresh consumption, the economic imperative to deliver pristine fruit effectively prohibits the use of mechanical harvesters, which may bruise up to 100% of apples (Galinato, Alexander, & Miles, 2016). Additionally, the US has stricter regulations regarding the use of dropped fruit, which is another side-effect (or feature, depending on the unit) of mechanical harvesting (Ewing & Rasco, 2018). The intact skin of hand-harvested apples does give American cidermakers one advantage, in that such fruit is suitable for long-term storage, allowing flexibility for year-round pressing.

Assuming that apples are intact following harvest, cidermakers will typically "sweat" the apples for seven to ten days after harvest, allowing them to ripen additionally off the tree (where droppage is no longer a concern) at ambient temperature. This extra ripening involves moisture loss, which effectively increases the concentration of sugar and flavor, and the hydrolysis of starch, which eases pressing (Miles & King, 2014). Apples are then washed before grinding, with many cideries taking advantage of the fact that apples float to wash and transport apples to the mill simultaneously via a water-based handling system (Mitchell, 2016). The apples are ground into mash via a hammer or grating mill, with finer grinding providing greater juice yield (Bump, 1989). Pectolytic enzymes (pectinases) are often added here to aid pressing by loosening the jelly-like structure of the mash (Heena, Lokesh, & Jyoti, 2018). Mills typically feed mash directly into the press via pumping or gravity.

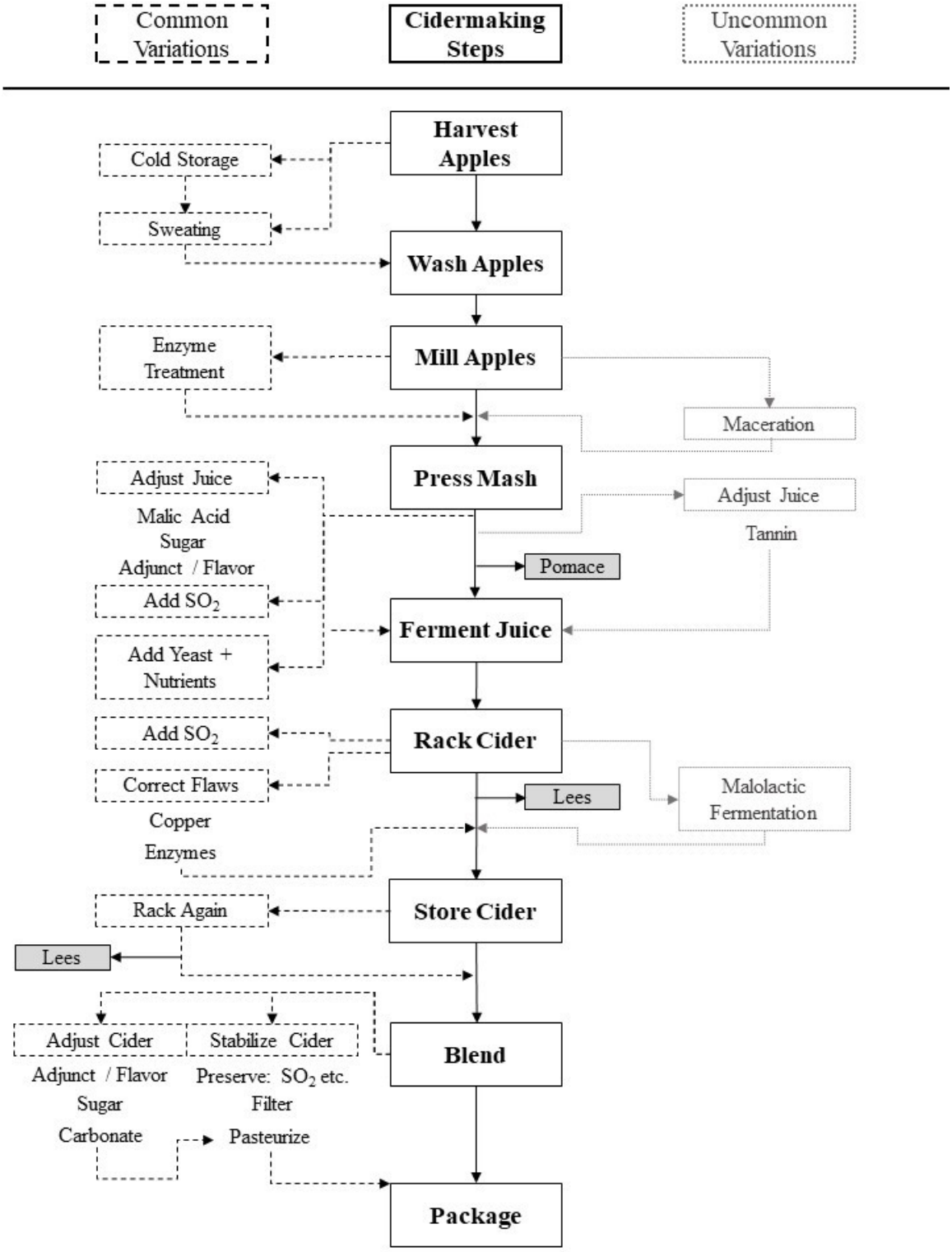

Figure 1.1: Cidermaking Process

It is during this period, from the moment of grinding through the end of pressing, when the physical damage of the apple's tissue permits contact between previously segregated polyphenols, oxygen, and polyphenol oxidase (PPO). PPO oxidizes polyphenols, resulting in both browning of the mash and loss of astringency. Any decisions that minimize the duration of milling and pressing (and thus limit oxygen exposure) or that otherwise limit the oxidation of the mash (i.e. the addition of ascorbic acid or potassium metabisulfite) may preserve some of the polyphenols lost to processing (Merwin et al., 2008).

Cidermakers have a range of presses to choose from, including crank or hydraulic rack-and-frame presses for small-scale batch processing, horizontal cage presses for large-scale batch processing, and continuous belt presses for mid- and large-scale processing (Mitchell, 2016). With any press, the juice is expelled from the mash and collected in a secondary container and the remaining solid cake (pomace) is typically discarded. In some instances, the pomace may be mixed with warm or cold water and re-pressed to recover additional dilute juice (Bump, 1989). Very rarely, some of the pomace or mash will be retained for fermentation in the juice (Merwin et al., 2008).

For many large commercial cideries and hobbyist cidermakers, the entirety of apple processing is outsourced, and their cidermaking begins with the receipt of apple juice or apple juice concentrate (Popa & Harte, 2002). Cidermakers make arguably the most significant decisions at this critical juncture. Most will add sulfites in the form of potassium metabisulfite to reduce the microbial load of the juice before the addition of commercial yeast, unless the cider is to undergo a "natural" or "wild" fermentation, using, respectively, only or some of the native yeasts present on the apples. Some cidermakers co-ferment other

fruit juices with the apple, and in that case would add the other juice (i.e. cranberry or mango juice) at this stage.

Juices will be tested, at a minimum, for total soluble solids (sugar as °Brix) and pH and adjusted accordingly. If the sugar is below the level needed for a target alcohol content, it may be increased via chaptalization (the addition of sucrose or corn syrup) or adjusted via blending with a higher °Brix juice. If the pH is above 3.8 and thus in danger of sustaining spoilage organisms post-fermentation, the acidity may be increased via the addition of malic acid or via blending with a more acidic juice. The thorough cidermaker will additionally test for titratable acidity (TA, as malic acid), which, while not wholly independent of pH, corresponds much better with the taste of acidity, and can similarly be adjusted with blending. Experienced cidermakers, and frequently orchardist-cidermakers, may forgo chemical testing and instead blend juices by taste or by their knowledge and experience of each variety's character. Such cidermakers may have a ratio in mind of sweet, sharp, bittersweet and bittersharp apples that they prefer to ferment together. If bittersweet and bittersharp apples are unavailable, the tannins may be adjusted here or post-fermentation with the addition of tannic apple juice concentrate imported from the UK or France, or of oak or grape-derived tannin powders (Martin, 2017).

Once the juice has been adjusted for quality, it is transferred to the fermentation vessel, which may be anything from a one-gallon glass jug fitted with an airlock to a 10,000 gallon stainless steel tank hooked to a glycol chiller system. Most commonly, a commercial strain of *Saccharomyces cerevisiae*, often as reconstituted dry yeast, is pitched into the juice

along with yeast nutrients. Cider-specific strains have recently become available, but both white wine and ale yeasts may be used.

If the juice has been sulfited and the cidery is diligent with hygiene, this pitched yeast should be the only organism participating in the fermentation. If the cidermaker chose not to sulfite the juice, the cider will contain a mixture of wild yeasts and bacteria which can, in the right ratios and given enough time, complete fermentation with minimal intervention from the cidermaker, resulting in more aromatically complex ciders than those fermented with a single commercial strain. This practice is common in France and Spain, where ciders are known for their smoky, medicinal, and "horsey" character (from *Brettanomyces bruxellensis* producing volatile phenols*)* and vinegary tang (from *Acetobacter* producing acetic acid). This, however, is a risky proposition, as these aromas in the wrong ratios are broadly perceived as flaws (Buron et al., 2011). American cidermakers desiring wild character in their cider may allow native yeasts a day or two head start before pitching commercial yeasts, which then easily dominate the remainder of fermentation. The more cautious route is to sulfite and then intentionally inoculate cider with a lab-isolated strain of the aroma-producing alternate yeast of choice. In any of these cases, whether a pitched commercial strain or part of the native microflora, *Saccharomyces cerevisiae* yeast will typically out-compete other species within 48 hours of the start of fermentation (Ribéreau-Gayon et al., 2006).

The type and amount of yeast nutrients needed are points of contention among cidermakers, but as most apples contain only low levels of yeast-assimilable nitrogen (YAN), some supplemental nitrogen is considered necessary for the yeast to successfully complete fermentation. Cidermakers may test the YAN levels of their juice, but many, assuming

negligible levels, add a standard amount in the range of 100-200 mg N/L. This nitrogen may be in the form of ammonium (inorganic nitrogen) or primary amino acids (organic nitrogen), with most cidermakers defaulting to commercial blends of the two types of nitrogen with vitamins and sterols. Symptoms of insufficient YAN include stuck fermentations, in which the yeast ceases fermentation before conversion of all sugar, and the formation of sulfuric off-odors (i.e. rotten eggs) from nitrogen-starved yeast degrading sulfur-containing amino acids (Jiranek, Langridge, & Henschke, 1995). Sterols are essential to the alcohol tolerance of yeast; fermentations that slow in the second half of fermentation may be suffering from either nitrogen or sterol deficiencies. Regular monitoring of the sugar depletion during fermentation can be vital, as rapid supplementation at the first signs of struggle may prevent lingering issues. Standard protocol is to stagger nutrient additions, with one addition high in inorganic nitrogen and vitamins at the onset of fermentation and a second addition high in organic nitrogen and sterols at 1/3 sugar depletion (deKramer et al., 2018).

After nutrition, temperature is the greatest determinate of fermentation speed. Yeast strains will have ranges in which they can survive, typically between 10-30°C. Within that range, a fermentation will progress more rapidly at the high end, and more slowly at the low end. A warm, nitrogen-rich fermentation can complete in as little as three to four days; a cool fermentation with low nitrogen may take three to four months to complete. Fermenting hot and fast risks the development of off odors in cider, while slower fermentations have a reputation for producing cider with fruitier aroma (Merwin et al., 2008). In practice, most cider is fermented for 2-3 weeks between 15-20°C to balance efficiency and quality. Ciders are usually fermented to dryness (no residual sugar), with the sweetness of the final product a

function of secondary processing; rare craft cidermakers will monitor sugar depletion and end fermentation at a target sugar level by “cold-crashing” the cider – rapidly dropping the tank temperature below the functioning temperature of the yeast.

Following the completion of fermentation, ciders will almost always be racked, or transferred to a fresh vessel in order to separate the cider from the lees (residual and dead yeast which has settled to the bottom of the tank). If the cider is hazy or the lees fail to settle, clarifying enzymes and cold storage can be used to facilitate the settling and removal of solids. Racking can be critical to the removal of sulfuric off-odors that developed during fermentation, and to preventing new off-odors from appearing in storage. Craft cidermakers may age the cider for months on the lees to reduce the astringency of the cider and extract nutty aromas from the yeast, but this technique is only appropriate for acidic cider produced from tannic (cider) apples and devoid of sulfuric aromas (Pucci, 2017). If racking fails to remove sulfuric odors, the cider may be treated with a copper sulfate solution and re-racked. The cider may be coarse filtered at this point to ensure removal of all solid material. If the cider is to be aged, it will be treated with an additional dose of sulfites and stored in air-tight and full vessels to limit the potential for oxidation or microbial growth. The cider may then spend as little as a few days or as long as a few years maturing in storage. As with lees aging, long maturation primarily benefits tannic ciders.

The final processing steps for cider are optional and may occur in varied order. Cider may be adjusted for taste via blending, the addition of adjunct flavors, or sweetening via table sugar, corn syrup, synthetic sweeteners, unfermented apple juice, or apple juice concentrate. The cider may be diluted with water or unfermented juice to reduce the alcohol

content. Most ciders will be carbonated in brite tanks, or less often, via secondary fermentation in bottle. Critically, all ciders will be stabilized in some fashion before bottling. In the case of a dry cider (no residual or added sugar), this may be as simple as sterile bottling. Ciders containing fermentable sugar must be more rigorously stabilized to prevent unwanted bottle fermentation, typically through a combination of methods that may include sterile filtration, thermal pasteurization, a final sulfite addition, or the addition of other preservatives.

1.4 Motivation

> 'Apples of a small size are always, if equal in quality, to be preferred to those of a larger size, in order that the rind and the kernel may bear the greatest proportion to the pulp, which affords the weakest and most watery juice.' To prove this, Dr. Symonds of Hereford, about the year 1800, made one hogshead of cider entirely from the rinds and cores of apples, and another from the pulp only, when the first was found of extraordinary strength and flavour; while the latter was sweet and insipid." (*Herefordshire Report*, 18??, as cited by Loudon, 1844)

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If American cidermakers are to make quality, balanced cider from a dearth of cider apples, they must use those rare apples efficiently. Standard cidermaking practices result in massive losses of tannins. With as much as 90% of an apple's tannins lost between milling and pressing, minor processing changes have the potential to dramatically improve tannin extraction and retention in cider (van der Sluis et al., 2002). Here we approached this problem from two angles: (1) water-based extraction methods to recover tannins from pomace, and (2) pre-press treatments to maximize the extraction of tannins from apple mash. If more of an apple's tannins can be transferred into the cider, cidermakers won't need to

look to imported juice concentrates or exogenous tannin powders to supplement their cider, and tannic ciders can be produced from American apples.

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Chapter 2

EXTRACTION OF PHENOLIC COMPOUNDS FROM APPLE POMACE: HOT WATER VS PULSED ELECTRIC FIELD

Abstract

Modern American cider is made primarily from fermented dessert apples, frequently with bittersweet cider apples as a minority component to enhance body and mouthfeel. With these cider apples in chronically short supply in the US, there is substantial motivation to maximize the efficiency of extraction of the chemical components of those apples – polyphenols – that define much of their value in cider. As much as 90% of the polyphenols in apples are lost at pressing, whether due to incomplete extraction or adsorption onto solid material (pomace). A series of experiments was conducted to extract polyphenols from apple pomace via the application of heat (40°C and 100°C) and pulsed electric field (E = 1.07 and 3.0 kV/cm). All treatments had a positive effect on total phenolics, with the greatest effect (12-fold increase over the control) from 100°C heat treatment, compared to a 4.8-fold increase from 100 pulses of PEF at 1.07 kV/cm. However, all treatments required the addition of large volumes of water for extraction, necessitating additional processing for use as a tannin supplement in cider.

2.1 Introduction

Hard cider – fermented (alcoholic) apple juice, or just "cider" – is in the midst of a decade-long renaissance in America. US cider revenue grew by over 600% between 2008-

2017, with an average annual growth of 33.9% between 2012-2017 (Hurley, 2017). Such rapid growth poses a unique agricultural problem: while any apples can be fermented into cider, bittersweet and bittersharp apples are most prized as "cider apples," and are rare in the US.

While the UK and France's history of uninterrupted cider production ensures them a sufficient supply of cider apples from centuries-old orchards, US apple-growers, focused primarily on such dessert apples as Red Delicious or Honeycrisp, are scrambling to keep up with demand for these specialized apples. The solution is not as simple as planting more trees, as cider apple trees take on average five years to reach full production, and even at full production have lower volume yields than typical apple trees (Galinato, Gallardo, & Miles, 2014). Even with an increase in cider tree plantings, cider apples will fail to meet US demand for the foreseeable future and will continue to command 3-4 times the price of dessert apples (Seetin, 2018; Galinato et al., 2014).

Not only are high-tannin apples expensive, but tannins are poorly extracted into juice during the normal cidermaking process. This is further complicated by the practicality of measuring tannins – most assays available to cidermakers are in fact measuring reduction (antioxidant) capacity, under the assumption that the primary antioxidants in apple juice are polyphenols (ignoring the presence of ascorbic acid and sulfur dioxide) and that the primary polyphenols in apples are tannins (Lea & Timberlake, 1974). While using an approximation of polyphenol content as a stand-in for tannins is not ideal, no current method both selectively and completely quantifies tannins (Ma et al., 2019). Since the apple categories devised at LARS are based on a method that measures antioxidant activity, and because most

cidermakers, if they do "measure" tannins do so via antioxidant activity, for cidermakers and for much of cider research, the terms "polyphenols," "phenolics," and "tannins" are effectively interchangeable.

Depending on the apple variety, processing conditions, and method of measurement, anywhere from 40-90% of an apple's total tannins are lost during processing (van der Sluis et al., 2002). Several phenomena are responsible for this inefficiency: (1) loss to oxidation following tissue damage from grinding, (2) incomplete extraction of tannins from the skin and seeds, which contain the majority of the tannins, and (3) non-covalent interactions between tannins and cell wall material, resulting in the trapping of tannins in pomace (Renard et al., 2011).

There is a pressing need to efficiently utilize the available cider apples in the US. One approach is to recover unextracted or bound tannins from pomace. Researchers have tried with varying degrees of efficiency to extract polyphenols out of pomace with maceration (Cristina-Gabriel et al., 2012; Valois, 2007), solid-state fermentation (Ajila et al., 2011), ultrasound-assisted extraction (Pingret et al., 2012; Martin, 2017), microwave heating (Perussello et al., 2017; Bai et al., 2010; Valois, 2007), and pressure-assisted extraction (Wjingaard & Brunton, 2009), utilizing water, methanol, and/or acetate in varying concentrations.

While solvent-based methods extract the highest yield of polyphenols, only food-safe processes and solvents are appropriate for cidermaking. Maceration is the most theoretically appealing to cidermakers, as it follows basic winemaking principles, but no scientific paper has confirmed its efficacy with apples. Those water-based extraction methods described by

Pingret et al. (2012) and Martin (2017) require multiple pieces of specialized (expensive) equipment and several rounds of concentration to achieve a usable extract, making their industrial feasibility a challenging proposition.

PEF is an emerging technology in which short high voltage pulses are applied to a substance (i.e. apple mash) in order to quickly and non-thermally damage cell tissue via electroporation. At higher levels, this electroporation creates pores large enough to kill a bacterial cell as a replacement for thermal pasteurization; at lower levels, this can be used to enhance mass transfer as a replacement for pectolytic enzymes (Barba et al., 2015). Some preliminary research has shown PEF to outperform enzymatic maceration with regards to polyphenol extraction when applied to crushed grapes or grape skins (Bousetta et al., 2009; Puértolas et al., 2010), but there have been mixed results from apple mash: 80% increase in total polyphenols found by Schilling et al. (2008), approximately 20% increase found by Grimi et al., (2011), and a lack of significant improvement found by Töpfl (2006). The body of research on PEF-assisted extraction is young, and confounded by equipment differences and varied process parameters, yet PEF remains an intriguing option for a simpler and greener method to enhance extraction from apple pomace.

More work is needed to develop a green and energy-efficient method for the extraction of polyphenols from apple pomace. The objective of this research was to build upon the most direct, non-solvent methods – maceration, heating, and PEF – optimizing and removing processing steps while evaluating alternate strategies to reduce the equipment and energy demands of polyphenol extraction.

2.2 Materials and Methods

2.2.1 Apples

Red Delicious apples were used for small-scale and off-season experiments due to their year-round availability and high levels of skin tannins; these apples (RD) were sourced from Wegman's supermarkets (Ithaca, NY) on the day prior to processing. Pomace of mixed (unknown variety) dessert apples (RJ) was provided fresh by commercial juice company, Red Jacket Orchards (Geneva, NY), following their first morning pressing, with experiments run on the same day.

2.2.2 Chemicals

Folin-Ciocalteu's Phenol Reagent and Methanol were purchased from Sigma-Aldrich (St. Louis, MO). Gallic Acid was purchased from Chem-Impex (Wood Dale, IL), Sodium Carbonate was purchased from Oakwood Chemical (Estill, SC), Ammonium Sulfate was purchased from VWR (Radnor, PA), food-grade Ascorbic Acid was purchased from Pure Bulk (Roseburg, OR). Potassium Metabisulfite, Clinitest tablets, Go-Ferm, Fermaid K, DAP, and DV10 yeast were purchased from Scott Laboratories (Petaluma, CA). HotHead ale yeast was provided by Omega Yeast Labs, Chicago, IL. Pectolytic enzyme Rapidase Pro L and proteolytic enzyme Maxipro AFP were provided by DSM Food Specialties, South Bend, IN.

2.2.3 Fermentation

All fermentations followed the same yeast and nutrient regiment, except where noted. Dry yeast was reconstituted in warm water with Go-Ferm rehydration nutrient and added to

fermentations at concentrations of 250 mg/L and 400 mg/L, respectively. Yeast nutrients were added in the form of 125 mg/L each of DAP and Fermaid K twice, once at the onset of fermentation and once at ⅓ sugar depletion. Fermentation progress was monitored daily via digital density meter (Anton Paar, Graz, Austria), with dryness confirmed via Clinitest tablet. All samples were racked off the lees immediately following the end of fermentation.

2.2.4 Hot Water Extraction of Phenolics from Pomace: Concentrated via FO and Fermented with Wine and Ale Yeasts at 22°C and 37°C

The first trials for pomace extraction were a modification of the optimized hot water extraction used by Martin (2017), without the addition of enzymes and with forward osmosis (FO) used to concentrate the extract. In brief, Red Delicious apples were ground in a small fruit chopper/slicer (Orchard Equipment Co., Conway, MA) with 0.1% Rapidase Press L enzyme and pressed in a small hydraulic rack and frame press (Orchard Equipment Co., Conway, MA) with press cloth at 8.3 MPa (1200 psi); the resulting pomace (RD) was frozen at -20°C.

On the day of treatment, RD pomace was defrosted and combined with DI water in a 1:16 ratio, heated to 100°C (covered) on a hot plate with stirring (Thermo Fisher Scientific, Waltham, MA) for 30 min, and vacuum filtered through Whatman Grade 1 filter paper (Whatman plc, Maidstone, UK). This process was repeated twice with new aliquots of pomace added to the filtrate at the same 1:16 ratio, and fresh DI water added to compensate for any evaporative losses. The extract was thus made, in quadruplicate, from a total of 600 g pomace in 3.2 L of water.

The resulting extracts were pooled, with 12.4 L of this 2.4° Brix extract concentrated via forward osmosis (FO) with a bench-scale unit (Evapeos – Ederna, Toulouse, France) to a final volume of 1.83 L. The FO unit was equipped with a spiral-wound cellulose triacetate membrane (63 mm outside diameter, 530 mm length, 0.5 m^2 filtration area) and a countercurrent plate heat exchanger (PROO13, AGC Engineering, U.S.) to maintain a temperature of 20°C. The concentration was conducted over 140 min in batch mode using a 60° Brix osmotic agent composed of 60% potassium lactate in water (Ultralac KL 60, Hawkins, Roseville, MN), until the target Brix of 11.5° was reached.

The extract was then divided into three groups (each 200 mL in triplicate, for a total of nine samples) in order to ferment off the sugars and investigate if fermentation conditions would have an impact on the resulting polyphenol content. One group was fermented under common commercial cidery conditions with the white wine yeast DV10 at 22°C. The second two groups were fermented with an ale yeast, HotHead, selected for its tolerance of higher temperature fermentations. Of these, one group was fermented with HotHead at the standard temperature of 22°C, the other fermented at 37°C to evaluate how a warmer and likely faster fermentation would affect polyphenol extraction. For this and experiment 2.2.5, yeasts were grown overnight in YPD and diluted in sterile water to achieve a density of $4x10^6$ cells/ml. Fermentation temperatures were kept steady within 1°C via shaking water baths. Nutrients were added as described above, with an additional dose of 125 mg/L of DAP added to both HotHead samples after an apparently rapid consumption of initial nutrients. Following the end of fermentation, the fermented extracts were racked off the lees and refrigerated at 3°C for next-day analysis.

2.2.5 Fermentation of Heat-Concentrated Pomace Purees for Phenolic Extraction

A second experiment was conducted with the RD pomace, in which the pomace was homogenized in a Ninja Blender (SharkNinja, Needham, MA) with water in a 1:7 ratio, heated to 100°C on a hot plate with stirring (Thermo Fisher Scientific, Waltham, MA) for 30 min and boiled uncovered until concentrated to a Brix over 5.0°. This semi-concentrated pomace puree was then fermented in two groups (each 200 g in triplicate): DV10 at 22°C as the standard condition, and HotHead at 37°C to evaluate if the warmer fermentation would extract more polyphenols from the pomace purees. Fermentation temperatures were kept steady within 1°C via shaking water baths. Nutrients were added as described above, with an additional 125 mg/L of DAP added to the HotHead samples at the onset of fermentation. Following the end of fermentation, the fermented purees were vacuum filtered through Whatman Grade 1 filter paper and refrigerated at 3°C for next-day analysis.

2.2.6 Pulsed Electric Field

Here we investigated the application of PEF to fresh apple pomace. All experiments were run using a CellCrack UL Batch Pulsed Electric Field System (Elea, Quackenbruck, Germany) at a voltage (*U)* of 30 kV. Field strength (*E)* is dependent on the chamber length (*L)*, $E = U/L$. With chambers of 28 and 10 cm, the applied field strengths were 1.07 and 3.00 kV/cm, respectively. Unless otherwise noted, all experiments were done in the 28 cm chamber with 1.07 kV/cm applied field strength. The intensity of treatment is best described by specific energy (*W)*, which is dependent on the voltage (U), the capacitance of the PEF

unit (*C*), mass of substance in the treatment chamber (*m)*, and number of pulses (*n)* (Equation 1, Ostermeier et al., 2018; Donsì et al., 2010).

$$W = \frac{U^2 C}{2m} * n$$

Equation 1. Specific Energy

2.2.6.1 PEF and Protease-Assisted Extraction of Polyphenols from Pomace

Fresh pomace of an unknown apple blend (RJ) was homogenized using a high shear mixer (ROSS, Hauppauge, NY) in water in either 1:7 or 1:1 ratio with and without 0.1% Maxipro AFP. The enzyme was selected based on its success in Martin's trials (2017); here we investigated its efficacy compared against PEF and combined with PEF treatment. All samples (800 g) were treated with 400 ppm food-grade ascorbic acid to limit oxidation during processing and allowed to sit for 30 min at room temperature before PEF treatment for 0, 100, or 400 pulses. Each treatment was performed in triplicate, for a total of 36 samples.

Table 2.1: Conditions for PEF and Protease-Assisted Extraction of Polyphenols from Red Delicious Pomace

Mass (g)	Dilution[1]	Protease	PEF Pulses	Specific Energy (kJ/kg)
800	7:1	0	0	0
	1:1	0.1% Maxipro AFP	100	56.25
			400	225.00

[1] Water to Pomace

Conductivity was recorded before treatment using Conductivity Meter Type 700 (Chemtrix, Hillsboro, OR); temperature was recorded before and after treatment with a waterproof thermocouple (Cooper-Atkins, Middlefield, CT). Following treatment, samples were immediately pressed in a small hydraulic rack-and-frame press (Orchard Equipment Co., Conway, MA) with press cloth at 8.3 MPa (1200 psi) and stored at 3°C for next-day analysis.

2.2.6.2 Effect of Pulse Number on PEF-Assisted Extraction of Polyphenols from Pomace

The previous PEF trial was repeated in triplicate with fresh RJ pomace at the 7:1 water dilution with a wider range of pulse numbers to gauge the effect of pulse number and specific energy on polyphenol extraction. Following PEF treatment, samples were centrifuged for 5 min at 3247xg in a Labnet Hermle Z4000 tabletop centrifuge (Gosheim, Germany) and decanted to remove pomace solids. Samples were stored at 3°C for next-day analysis.

Table 2.2: Conditions for PEF-Assisted Extraction of Polyphenols from Red Delicious Pomace, 2nd Trial

Mass (g)	Dilution[1]	PEF Pulses	Specific Energy (kJ/kg)
800	7:1	0	0
		100	56.25
		200	112.50
		400	225.00
		800	450.00

[1] Water to Pomace

2.2.6.3 PEF Pretreatment on Heat-Concentrated & Fermented Pomace Puree for Polyphenol Extraction

RD apples were ground and pressed in a Norwalk Hydraulic Juicer (Bentonville, AR). Pomace was immediately homogenized in water 1:7 as in the earlier experiment; three batches of 2400 g were subjected to 800 pulses (150 kJ/kg) of PEF treatment, and three batches were untreated. All samples were then left as pomace purees and heated uncovered as described in 2.2.5, to raise their Brix from 1.9 to 6.6°. These semi-concentrated purees were fermented at 22°C with DV10 yeast and the standard nutrient regiment. Following the end of fermentation, the fermented purees were vacuum filtered through Whatman Grade 1 filter paper and stored at 3°C for next-day analysis.

Table 2.3: Conditions for PEF Pretreatment on Heat-Concentrated & Fermented Red Delicious Pomace Puree for Polyphenol Extraction

Mass (g)	Dilution[1]	PEF Pulses	Specific Energy (kJ/kg)
2400	7:1	0	0
		800	150.00

[1] Water to Pomace

2.2.6.4 Effect of Sample Conductivity and Extended Pomace Maceration on PEF-Assisted Extraction of Polyphenols from Pomace

RD pomace was prepared as in 2.2.6.3, and suspended (without homogenization) in salted water (NaCl) 1:7 to achieve a range of conductivities in triplicate prior to PEF treatment in the 10 cm chamber for an applied field strength of 3.0 kV/cm. Fifty mL aliquots of 3 each of the treated and untreated pomace mixtures were reserved to test the effect of extended pomace maceration; the remaining slurries were centrifuged same day for 5 min at 3247xg, decanted to remove pomace solids and stored at 3°C for next-day analysis.

Table 2.4: Conditions for PEF-Assisted Extraction of Polyphenols from Red Delicious Pomace, with Varied Conductivity and Maceration Time

Mass (g)	Dilution[1]	PEF Pulses	Specific Energy (kJ/kg)	Conductivity (uS)	Maceration (days)
200	7:1	0	0	250 - 499	0
		50	112.50	500 - 999	7
		100	225.00	1000 - 1999	
				2000 - 4000	

[1] Water to Pomace

2.2.6.5 Comparison of PEF vs Simplified Hot Water Phenolic Extraction from Pomace

RD pomace was prepared as in 2.2.6.3 and suspended without homogenization in water 1:7. Pomace mixtures were divided into five groups, prepared in triplicate: control (no treatment), heat (40°C or 100°C) on a hot plate with stirring (Thermo Fisher Scientific, Waltham, MA) for 30 min, and PEF (50 or 100 pulses in the 10 cm chamber). Heat and PEF treatments were performed simultaneously, while controls sat for the duration of those treatments to standardize time before centrifugation and decanting. Centrifuged samples were stored at 3°C for next-day analysis.

Table 2.5: Conditions for PEF vs Simplified Hot Water Extraction of Polyphenols from Red Delicious Pomace

Mass (g)	Dilution[1]	PEF Pulses	Specific Energy (kJ/kg)	Heat (°C)
200	7:1	0	0	0
		50	112.50	40
		100	225.00	100

[1] Water to Pomace

2.2.7 Chemical Analysis

Total Soluble Solids (°Brix) was monitored prior to fermentation and then daily throughout fermentation with a digital density meter (DMA 35, Anton Paar, Graz, Austria). When °Brix fell below zero, Clinitest tablets were used daily to check for end of fermentation.

Total Phenolics (TP) were measured using the Folin-Ciocalteu colorimetric assay described by Singleton with minor modifications (Manns, 2014), in which solutions turn blue in linear proportion to phenolic content. The reaction for each sample was prepared in triplicate by mixing 25 uL of sample with 1375 uL of DI water and 100 uL of Folin-Ciocalteu reagent, combined in a cuvette via pipetting up and down. After standing for 7 min, the mixture was quenched with 1 mL of 7% (w/v) sodium carbonate. The solutions were then kept in the dark for 90 min before absorbance was measured at 765 nm on a Genesys UV-visible Spectrophotometer (10S, Thermo Fisher Scientific, Waltham, MA). Gallic Acid was used to prepare an 8-point standard curve from 0 to 1000 mg/L, with results expressed as gallic acid equivalents (GAE) mg/L.

2.2.8 Statistical Methods

Total phenolics were subjected to one-way analysis of variance (ANOVA). Significant differences were analyzed using Tukey-Kramer HSD with a 0.05 significance level. Analyses were conducted using JMP Pro (14.0.0).

2.3 Results and Discussion

2.3.1 Hot Water Extraction of Phenolics from Pomace: Effect of Forward Osmosis (FO), Yeast Selection, and Fermentation Temperature

The hot water extracted pomace, before any concentration steps, contained phenolics at a concentration of 320 mg/L gallic acid equivalents (GAE), 25% more than the juice from the initial pressing and equivalent to 2028 mg/kg pomace (see Figure 2.1). That this initial yield is more than three-fold higher than Martin's of 599 mg/kg dessert pomace (2017) is likely a combined function of the varied levels of polyphenols in the pomace (itself a function of the apples in the pomace and of the extent of pomace oxidation before treatment), and of potential oxidative losses in the initial processing of the pomace and during Martin's enzymatic maceration step.

After 6.78x concentration via forward osmosis, the extract concentrate contained 1413 mg/L GAE (1320 mg/kg pomace), representing a 19% loss from the process. The yeast choice appears to have had no effect, as the total phenolics of concentrates fermented with DV10 yeast at 22°C (A) were indistinguishable from those fermented with HotHead yeast at 22°C (B), at 1134 and 1178 mg/L GAE respectively. While yeast selection has been shown to affect tannin levels in red wine, it is unsurprising here that a white wine yeast (DV10) and an ale yeast (HotHead) are undifferentiated with respect to phenolics, as neither would have been bred or commonly used in a fermentation in which an increase in phenolics was desirable (Smith, McRae, & Bindon, 2015).

Losses were seen in the higher temperature fermentations, with 17% lower total phenolics in the 37°C HotHead (C) compared to the 22°C HotHead (B), though it is unclear

if these losses were due to the higher temperature or the associated increase in fermentation rate.

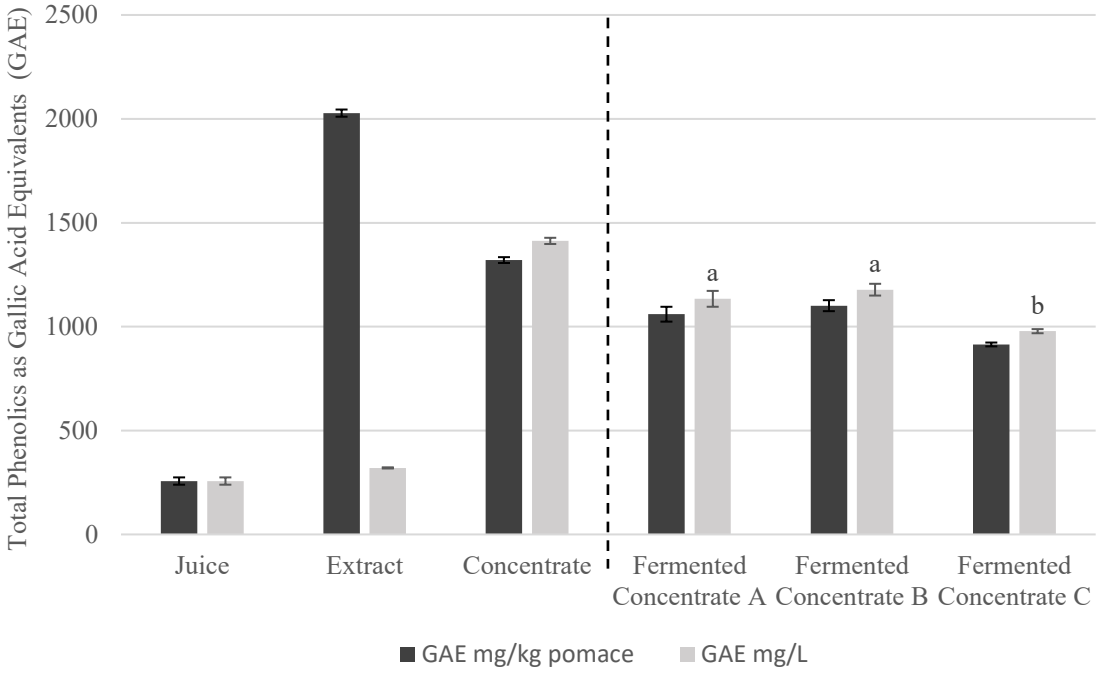

Figure 2.1: Hot-Water Extraction of Phenolics from Pomace: Effect of Forward Osmosis (FO) Concentration, Yeast Selection, and Fermentation Temperature. FO-concentrated extracts were fermented under the following conditions: DV10 yeast at 22°C (A), HotHead yeast at 22°C (B), or HotHead yeast at 37°C (C). Data represents triplicate measurements for three experimental samples per condition, reported as mean GAE (gallic acid equivalents) mg/L (pale gray) and mg/kg pomace (dark gray) ± standard deviation. Difference values between fermentation treatments not connected by the same letter are significant ($p < 0.05$) by Tukey-Kramer HSD.

2.3.2 Fermentation of Heat-Concentrated Pomace Purees for Polyphenol Extraction

The fermented purees performed slightly better than the fermented concentrates in GAE in mg/kg pomace – 1148 and 1073 mg/kg fermented with DV10 yeast at 22°C (A) and HotHead yeast at 37°C (C) for the purees, compared to 1060 and 914 mg/kg for the

concentrates fermented under the same conditions (Figure 2.2). However, no improvement was seen from fermenting the pomace, as the pre-fermentation polyphenol concentration was 409 mg/L, compared to post-fermentation concentrations of 377 and 352 mg/L for fermentation conditions A and B. This negative effect from maceration is supported by Renard et al.'s studies on the adsorption of apple polyphenols to cell wall material (2001, 2011). Nogueira et al. (2008) reported variety-based variability in the effect of mash fermentation on polyphenolics, with some apples gaining phenolics while other apples lost phenolics. The varied susceptibility of apple tannins to adsorptive loss is likely a consequence of the significant variability of polyphenol composition between apple varieties (Guyot et al., 2003). This phenomenon may not be unusual to apple polyphenols, as recent wines studies show that counter to conventional winemaking wisdom, fermenting on pomace can (for some grape varieties) reduce tannin levels, again due to binding to cell wall material (Nicolle et al., 2019).

Once again, the higher temperature fermentation resulted in losses, rather than increased extraction. These results are counter to expectations based on conventional red winemaking practice, in which fermentation is conducted on the mash and higher fermentation temperatures are the most common method for enhancing phenolic extraction by both speeding diffusion and increasing the water-solubility of tannins (Sacchi, Bisson, & Adams, 2005; Valois, 2007). However, any heat-associated gains may have been maximized by the earlier concentration of the pomace puree at 100°C, so the general value of warm-fermented mash cannot be ruled out.

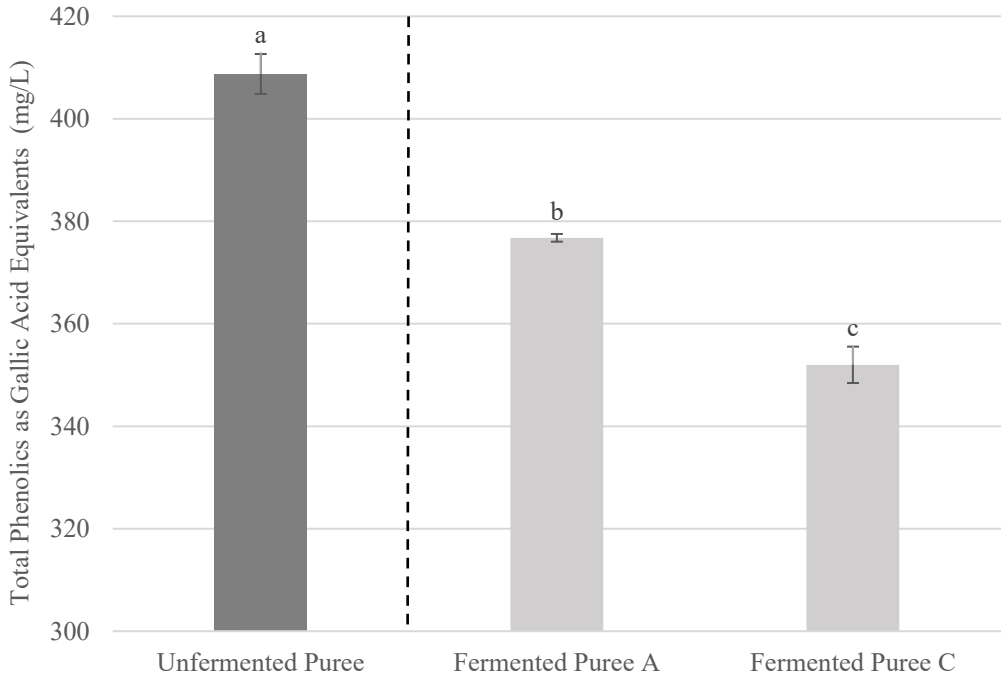

Figure 2.2: Effect of Fermenting Heat-concentrated Pomace Purees on Total Phenolics. Pomace homogenized 1:7 in water and concentrated 2.63x and fermented: DV10 yeast at 22°C (A) or with HotHead yeast at 37°C (C). Data represents triplicate measurements for three experimental samples per condition, reported as mean GAE (gallic acid equivalents) mg/L (pale gray) and mg/kg pomace (dark gray) ± standard deviation. Difference values between treatments not connected by the same letter are significant ($p < 0.05$) by Tukey-Kramer HSD.

2.3.3 PEF and Protease-Assisted Extraction of Polyphenols from Pomace

The chosen protease had no effect on the extraction of polyphenols from pomace, with or without PEF treatment (see Figure 2.3). This discrepancy between Martin's data (2017) again may be a result of the initial composition and treatment of the pomace: Martin's apples may have contained more highly polymerized polyphenols, which are more susceptible to protein binding, or higher levels of cell wall proteins to bind his polyphenols – either case would potentially increase the value of protease application (Renard et al., 2001; Guyot et al., 2003). That his pomace was provided to him frozen also allows for the potential

of greater air exposure prior to his processing, with oxidation having been shown to promote polyphenol polymerization reactions (Smith, et al., 2015), which would again increase the value of protease application. Alternatively, the reduced maceration time here (30 min vs Martin's 60 min) may have been insufficient for optimal enzymatic activity.

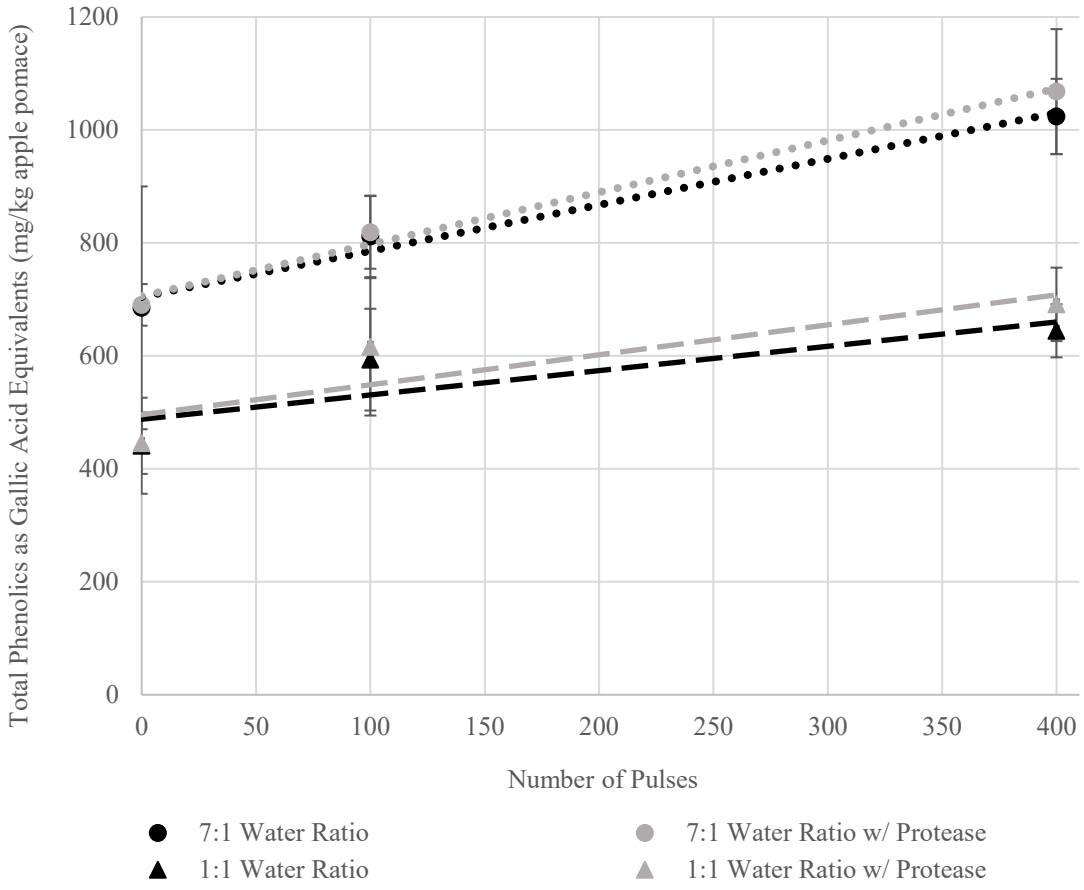

Figure 2.3: Effect of Water to Pomace Ratio, Pulse Number, and Protease Pretreatment on PEF-Assisted Phenolic Extraction. Pomace homogenized 1:7 and 1:1 in water with ascorbic acid and subjected to 0, 50, or 100 pulses. Data represents triplicate measurements for three experimental samples per condition, reported as mean GAE (gallic acid equivalents) mg/kg pomace ± standard deviation.

PEF treatment increased polyphenol content in the pomace purees, insignificantly at 100 pulses, but by 49% and 46% at 400 pulses for the 7:1 and 1:1 water ratio. The specific

energies for 100 pulses and 400 pulses are 56.25 and 225.0 kJ/kg, respectively, indicating that the lower value is insufficient intensity for permeabilization under these conditions (Donsì, Ferrari, & Pataro, 2010).

The 7:1 water ratio resulted in higher polyphenols than 1:1 for both treated and untreated pomace, suggesting that the enhanced conductivity of the more concentrated puree was less important than the enhanced oxidation protection provided by the higher ratio of water.

2.3.4 Effect of Pulse Number on PEF-Assisted Phenolic Extraction from Pomace.

Based upon the greater increases from more intense treatment seen in the previous experiment, the experiment was repeated with the 1:7 water ratio for a wider range of pulses. All treated samples had significantly higher concentrations of polyphenols than the controls, but only the 800-pulse samples saw the level of improvement of the previous experiment – at 878 GAE mg/kg apple pomace, a 45% improvement over 605 mg/kg for the control (Figure 2.4). This more intense treatment (W = 450 kJ/kg) increased the temperature of the samples dramatically, to an average temperature of 95°C. The effect from PEF here may be a result of the inadvertent thermal treatment. This is further complicated by the synergistic effect of heat on PEF efficacy – not only does heat enhance mass transfer, but higher temperatures change cell membrane fluidity, increasing their susceptibility to PEF-stimulated permeabilization (Puértolas et al., 2012).

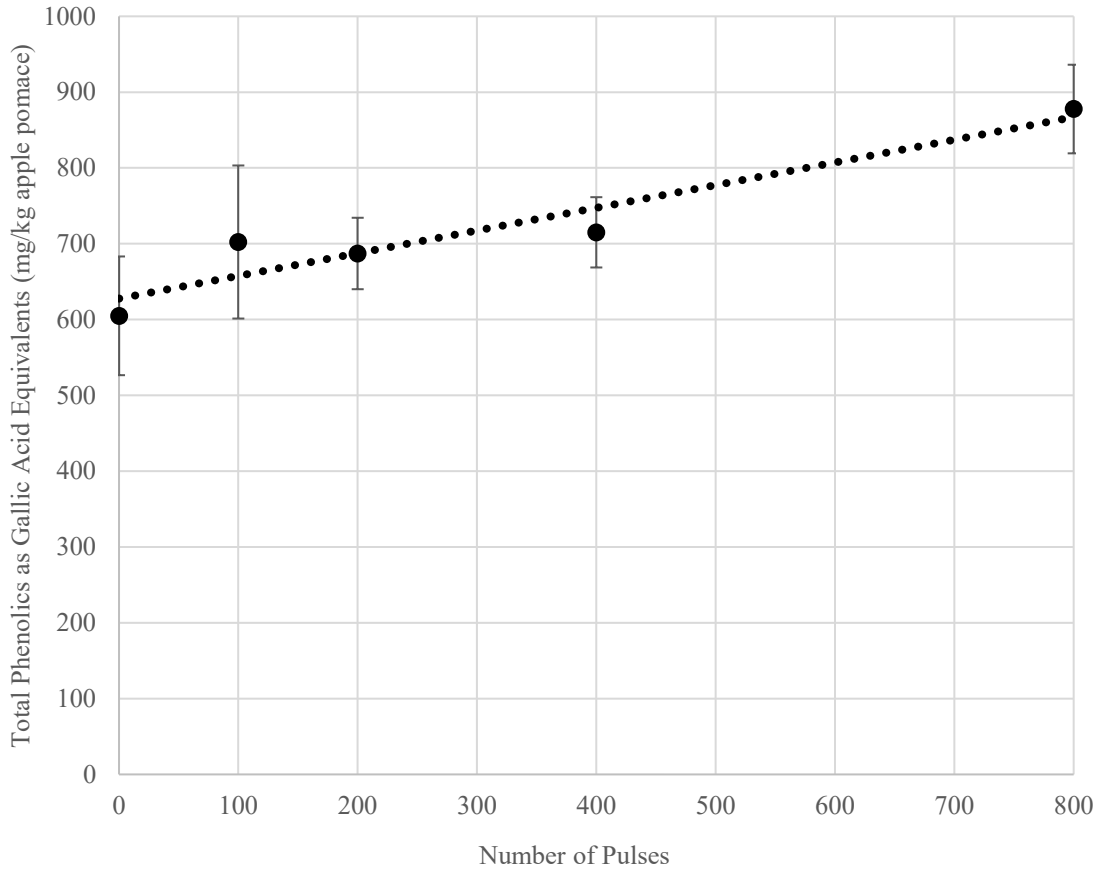

Figure 2.4: Effect of Pulse Number on PEF-Assisted Phenolic Extraction from Pomace. Pomace homogenized 1:7 in water with ascorbic acid and subjected to 0, 100, 200, 400, or 800 pulses. Data represents triplicate measurements for three experimental samples per condition, reported as mean GAE (gallic acid equivalents) mg/kg pomace ± standard deviation.

2.3.5 Effect of PEF Pretreatment on Phenolic Extraction from Heat-Concentrated and Fermented Pomace Puree

PEF pretreatment had no effect on the total phenolics of fermented pomace purees, with 662 and 670 GAE mg/kg apple pomace for the control and treatment group (Figure 2.5). This suggests that the homogenization and subsequent heating of the purees to 100°C caused sufficient tissue damage to render PEF treatment superfluous, which is supported by the

observation of Fincan, DeVito and Dejmek (2004) that the tissue damage effect of PEF was similar to that of fine mechanical grinding or freeze/thaw cycles.

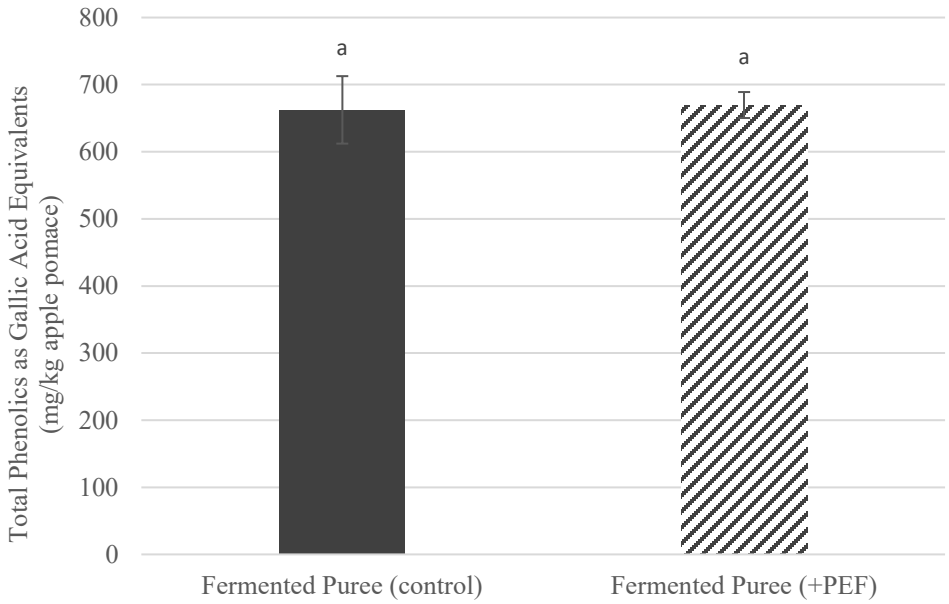

Figure 2.5: Effect of PEF Pretreatment on Phenolic Extraction from Heat-Concentrated and Fermented Pomace Puree. Data represents triplicate measurements for three experimental samples per condition, reported as mean GAE (gallic acid equivalents) mg/kg pomace ± standard deviation. Difference values between treatments not connected by the same letter are significant ($p < 0.05$) by Tukey-Kramer HSD.

2.3.6 Effect of Sample Conductivity and Extended Pomace Maceration Time on PEF-assisted Extraction of Polyphenols from Pomace

Sample conductivity had no impact on the efficacy of PEF treatment on pomace slurries (Figure 2.6). While a minimum sample conductivity is critical to the application of PEF treatment, it appears here that the natural conductivity of the pomace is sufficient for permeabilization, and that increases in conductivity beyond a minimum threshold provide no additional benefits (Barba et al., 2015).

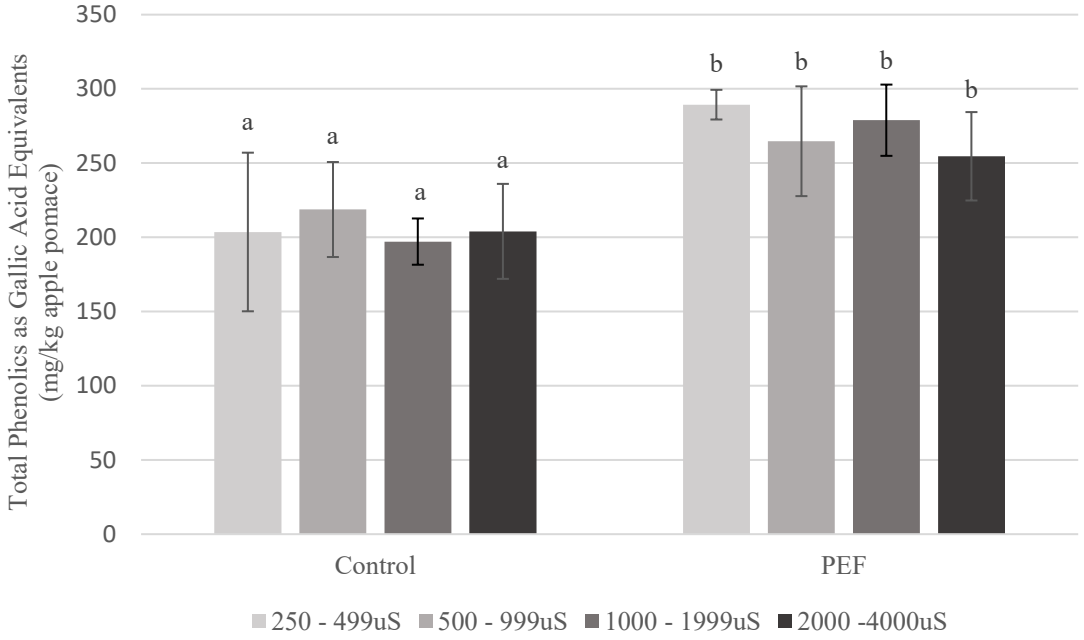

Figure 2.6: Effect of Conductivity on PEF-assisted Phenolic Extraction from Pomace. Data represents triplicate measurements for three experimental samples per condition, reported as mean GAE (gallic acid equivalents) mg/kg pomace ± standard deviation. Difference values between treatments not connected by the same letter are significant ($p < 0.05$) by Tukey-Kramer HSD.

Extended pomace maceration negatively affected the extraction of phenolics from pomace slurries (Figure 2.7). With immediate centrifugation and decanting, the PEF-treated samples had 53% higher total phenolics than the controls, 3357 compared to 2180 GAE mg/kg pomace. After seven days, both samples had decreased, lowering the treated sample 35% to 2175 mg/kg and hiding any treatment effect. As with the fermented pomace studies, this would suggest polyphenol losses to cell wall adsorption as described by Renard (2001, 2011).

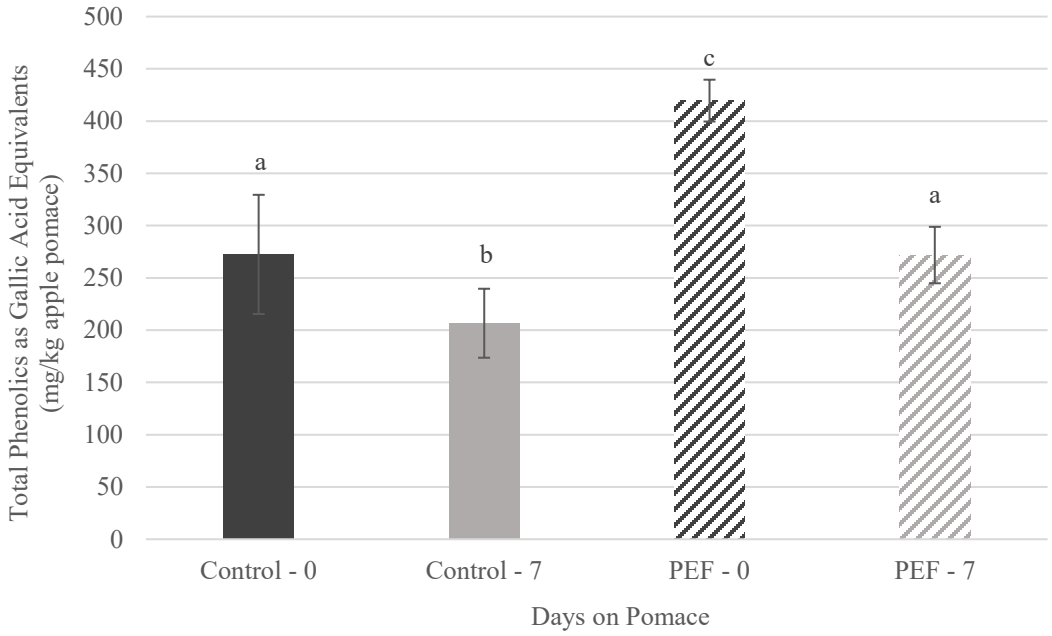

Figure 2.7: Effect of Extended Pomace Maceration following PEF Treatment on Phenolic Extraction. Following PEF Treatment. Data represents triplicate measurements for three experimental samples per condition, reported as mean GAE (gallic acid equivalents) mg/kg pomace ± standard deviation. Difference values between treatments not connected by the same letter are significant ($p < 0.05$) by Tukey-Kramer HSD.

2.3.7 Comparison of PEF vs Simplified Hot Water Phenolic Extraction from Pomace

The conditions in this final experiment were selected to control for the temperature increase with PEF treatment. With 50 pulses (W = 112.5 kJ/kg) the temperature was estimated to reach approximately 40°C, while at 100 pulses (W = 225 kJ/kg) the temperature was estimated to reach approximately 60°C. If the effect was primarily thermal, the 50-pulse PEF treatment should have similar phenolic content to pomace heated to 40°C. All treatments resulted in significant phenolic improvements over the control of 91 GAE mg/kg pomace. As shown in Figure 2.8, treatment of 50 pulses (where the actual maximum temperature was 43.6°C) resulted in a total phenolics of 441 GAE mg/kg pomace, compared

to a not-statistically-different 320 GAE mg/kg pomace for the 40°C heat-treated pomace. The 100-pulse sample (actual maximum temperature 62.5°C) performed marginally but not significantly better than the 50-pulse sample, at 525 GAE mg/kg pomace. The pomace heated to 100°C easily outperformed all other treatments with 1209 GAE mg/kg pomace – an increase of 1231% over the control. This agrees broadly with the literature, in which Valois (2007) achieved 400% increases with 70°C treatment, Gerard and Roberts (2004) achieved 60% increases with 70°C treatment, and Martin achieved an approximate 700% increase with 100°C treatment.

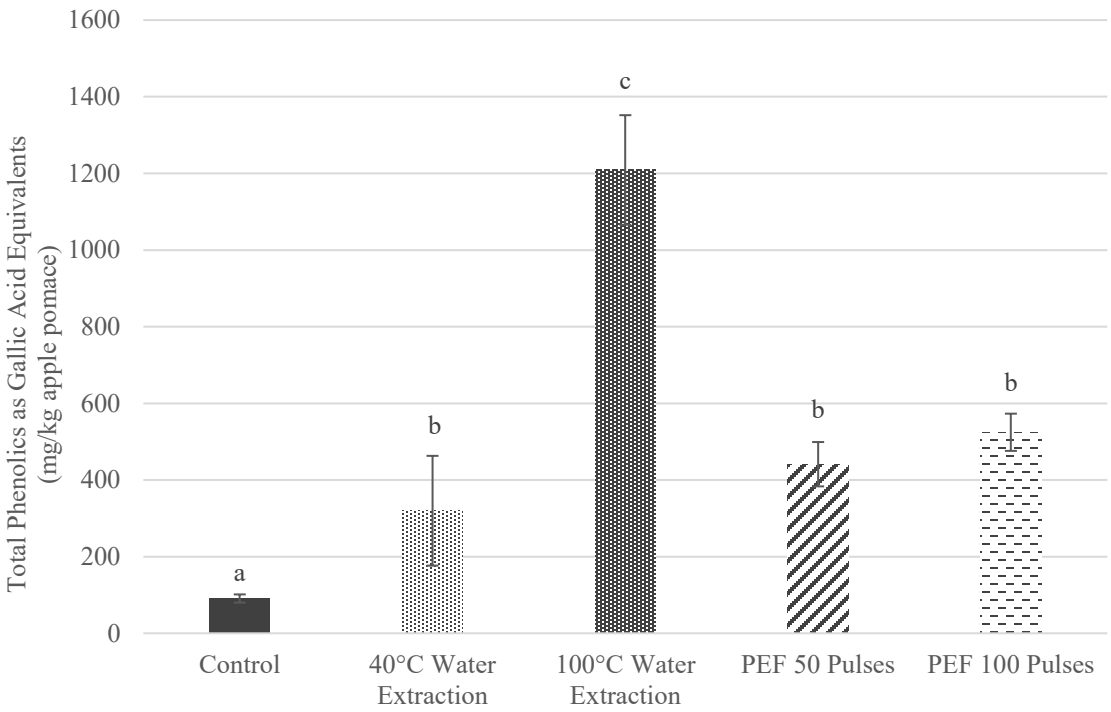

Figure 2.8: Comparison of PEF vs Simplified Hot Water Phenolic Extraction from Pomace. Control and all treatments conducted on pomace in 1:7 Pomace to Water Ratio. Data represents triplicate measurements for three experimental samples per condition, reported as mean GAE (gallic acid equivalents) mg/kg pomace ± standard deviation. Difference values between treatments not connected by the same letter are significant ($p < 0.05$) by Tukey-Kramer HSD.

The best PEF results here were seen at a high specific energy (225 kJ/kg) compared to 10 kJ/kg for Schilling et al. (2008) or 15 kJ/kg for Töpfl (2006). The applied energy of 1.07 kV/cm, however, fits right within the ideal range for electroporation of plant material described by Puértolas et al. (2012) as 0.1 – 5 kV/cm. Without knowing further details about their processing conditions, it is difficult to ascribe a cause for the specific energy discrepancies.

2.4 Conclusion

This series of pomace experiments clarified the advantages and pitfalls of various approaches available to cidermakers. Apple pomace tannins can be recovered for addition to cider without the use of organic solvents, but not all approaches are equally successful. Maceration, whether part of an enzymatic treatment prior to fermentation or with solids included in the fermentation, consistently failed to improve the polyphenolic content of cider, and more often resulted in losses. It remains to be seen if there are certain conditions under which maceration is a successful technique.

PEF treatment of pomace resulted in gains from 35-478% above control pomace, depending on the apples and processing parameters. Neither supplemented conductivity, pre-treatment with enzymes, nor solid fermentation exceeded the effect of PEF treatment, suggesting that any effect of those treatments is redundant with the application of PEF. PEF treatment is a viable option for enhancing polyphenol extraction, faster and more effective than maceration. However, the high variability in PEF unit operating parameters and the

inconsistent reporting of such variables (batch versus continuous processing, chamber dimensions, mass of sample, pulse shape, pulse duration, pulse frequency), means that conclusions are difficult to draw from study comparisons, and work must still be done to identify optimal operating conditions.

Heat treatment was the most consistently successful method, with a maximum efficiency of 12-fold increase over the control achieved when pomace was extracted 1:7 in water heated to 100°C. Even mild heating to 40°C increased polyphenol extraction from pomace 2.5-fold, suggesting that cidermakers have room to tailor the heating process to fit their capabilities and needs. All of these methods required the pomace to be suspended in water, resulting in a fairly dilute extract. Future work will have to optimize the concentration or incorporation of such extracts.

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Chapter 3

EXTRACTION OF PHENOLIC COMPOUNDS FROM APPLE MASH: HEAT VS PULSED ELECTRIC FIELD

Abstract

Quality cider is made from a blend of apples selected to balance sugar, acid, and tannins. High tannin cider apples are rare and expensive in the United States, and the efficiency of tannin extraction into juice is low. We investigated potential process improvements by applying treatments to apple mash prior to pressing, and then measuring the phenolics and tannins in the juice and cider. In pilot studies looking at changes in total phenolics (TP), apple mash was subjected to pulsed electric field (PEF) or to heating up to 70°C, followed by pasteurization either with standard thermal application or high pressure processing (HPP). With processing conditions optimized, apple mash made from cider apples was treated with either microwave heating to 70°C or 400 pulses of 1 kV/cm PEF. These mashes were then pressed, and the juice fermented following standard procedure. The resulting ciders were analyzed for TP, tannins, color, and turbidity. Both heat and PEF treatment significantly increased TP in cider (by 72% and 28%, respectively), and more modestly increased tannins (by 39% and 4%, respectively). Heat treatment resulted in significant changes to color and turbidity, while PEF had no effect on either. A sensory evaluation (100 panelists) showed the PEF-treated cider to be slightly preferred over the control, and significantly preferred over the heat-treated cider, which was ranked last with minor penalties for texture and taste. PEF improves phenolic extraction modestly without any

associated decrease in quality. Heat treatment improved phenolic extraction most effectively, with minor detriment to quality that would be inconsequential with blending.

3.1 Introduction

3.1.1 Significance of Tannins in Cider

Fermented or hard cider ("cider") is traditionally and most commonly made from a blend of several varieties of apples in order to achieve a "balanced" beverage. While most apples contain sufficient sugar for alcoholic fermentation, other conditions must be met to produce a quality cider. The juice must contain an adequate concentration of acid to provide tartness and protection against microbial spoilage, and adequate tannins to provide "mouthfeel," color, and additional protection against unwanted microbial growth (Lea & Drilleau, 2003). The Long Ashton Research Station (LARS) in the UK categorized apples thusly based on % acid, and % tannin: apples with > 0.45% acid and < 0.2% tannin were considered "sharp," apples with < 0.45% acid and < 0.2% tannin were considered "sweet," apples with > 0.45% acid and > 0.45% tannin were considered "bittersharp," and apples with < 0.45% acid and > 0.2% tannin were considered "bittersweet." The most popular apples in the US (Gala, Red Delicious, Fuji, Granny Smith, Golden Delicious and Honeycrisp) fall into the first two categories; no bittersweet or bittersharp is grown in sufficient quantity to warrant national tracking by the USDA, and the total production of these apples represents less than 1% of US apple production (Seetin, 2018).

While sweet and sharp apples are majority components of most ciders, their ubiquity outside of cider for fresh consumption, baking, or unfermented apple juice makes them

distinctly *not* cider apples. Such apples are referred to as "eating," "dessert," or "culinary" apples – though distinctions exist here, as dessert apples may be considered best for fresh consumption, while culinary apples refer to apples best baked into pies, such distinctions are a matter of taste and heated debates among apple enthusiasts and piemakers alike. Bittersweet and bittersharp apples, on the other hand, are desirable almost solely for cider, as their bitterness and astringency render them nearly inedible. Thus, here the term "dessert apple" will be used to apply to all sweet and sharp varieties, and "cider apple" will be used to describe all bittersweet and bittersharp apples.

Most modern American ciders are made from cull dessert apples – apples not attractive enough for the fresh market, with an average cost of $0.10/lb, compared to as high as $0.30-40/lb for cider apples (Seetin, 2018; Galinato, Gallardo, & Miles, 2014). Ciders made with *only* dessert apples have been derided for centuries for being "insipid," "treacly," and lacking "body." (Knight, 1801; Crowden, 1999). With cider apples expensive and in high demand (Merwin, 2014), there is a clear incentive to maximize the extraction of their most distinct feature: tannins.

Tannins present a challenge not only because of scarcity, but because they can be difficult to measure. The method used by researchers at the Long Ashton Research Station (Lowenthal Permanganate titration) does not measure tannins specifically, but rather phenolic compounds, of which tannins are just one type. The most common method used by cidermakers (Folin-Ciocalteu assay) similarly measures phenolics broadly. Both methods thus overestimate tannins, though typically one can assume that tannin extraction mirrors total phenolic extraction, at least in trends. University researchers have access to more high-

tech lab equipment, and more ease with chemicals, and thus have a wider range of options for measurement, each with their own drawbacks. More precise methods like HPLC can identify specific phenolic compounds, including some tannins, but the process is laborious and likely excludes large classes of tannins. More recently, the Australian Wine Research Institute has popularized tannin precipitation assays, which quantify only those compounds that would elicit the sensation of astringency on the tongue. They are therefore much more specifically relevant to the sensory experience of tannins than general phenolic assays, but exclude the smaller tannins often associated with bitterness. Whenever treatments and ciders are compared, the method of measurement must be considered, as the values may vary 10-fold between methods. Here, "phenolics" will be used when either the method is unknown or the method quantifies total phenolics; "tannins" will only be used when the method is specific to tannins, or when discussing the sensory experiences of bitterness and astringency.

3.1.2 Tannin Extraction

Whether cidermakers start with high tannin cider apples or low tannin dessert apples, the same phenomenon is observed: the juice and eventual cider have substantially lower levels of tannins than the apples themselves. The observed tannin deficit in juice is the result of three overlapping issues during processing: (1) incomplete extraction of tannins, (2) loss via adsorption of these extracted tannins onto apple solids (pomace), and (3) oxidative loss of tannins (Renard et al., 2011). All three issues are of primary significance in the brief window between milling and pressing, which can be addressed with both physical and chemical interventions.

One thoroughly investigated, yet ambiguously useful, method is the addition of pectolytic enzymes. Enzymes that degrade pectin are a common addition during cider processing, either to the mash to increase juice yield at pressing, or to the juice to improve clarity (Kashyap et al., 2001). In either case, enzyme application typically involves an incubation time of at least one hour and may require mild heating for maximum efficacy. The potential mechanism behind their application for phenolic extraction is the same as that for enhancing juice yield: by physically damaging apple tissue, they accelerate mass transfer. While some studies have shown as much as 200% increases in juice phenolics, others have seen no gains at all, perhaps due to oxidative losses during enzyme incubation (Will et al., 2002; Pinelo, Zornoza, & Meyer, 2008; Mihalev et al., 2004; Cliff, Devery, & Gayton, 1991; Schilling et al., 2008).

Heating apple mash is another extensively investigated method, with the potential to enhance extraction by (1) increasing the rate of diffusion, (2) increasing the water-solubility of tannins, and (3) inactivating the oxidation-catalyzing enzyme polyphenol oxidase (PPO). Thermovinification is a fairly common practice in red wine production, in which grape must is heated above 60°C for part of the fermentation process in order to extract more phenolic compounds including tannins and anthocyanins. Studies in which apple mash were heated to 50-70°C prior to or during pressing showed consistent increases in juice phenolics, by as much as 400% for some varieties (Lea & Timberlake, 1978; Will et al., 2002; Gerard & Roberts, 2004; Valois, 2007). However, with those studies that performed sensory analysis, heated ciders consistently rated lowest, usually noting increased bitterness and off-flavors and odors.

Limiting oxidation is the third common approach in retaining phenolic compounds in juice, either by physically limiting contact with oxygen or via the addition of antioxidants and inhibitors of PPO. However, the results of oxidation limitation experiments have been mixed. The addition of ascorbic acid as an antioxidant resulted in only very minor gains if any, while the addition of bisulfite (which inhibits PPO) performed only marginally better (Lea & Timberlake, 1978; Mihalev et al., 2004; Cliff, et al., 1991; Sayavedra-Soto & Montgomery, 1986). The complete elimination of oxygen by processing in a closed system blanketed with inert gas, while difficult to repeat in a commercial cellar, resulted in significant gains of over 200% (Renard et al., 2011). Cidermakers themselves may be resistant to recommendations to aggressively limit oxidation for the sake of phenolics, as the conventional wisdom of centuries of cidermakers is that oxygen is crucial to flavor and color development (Knight, 1801; Lea & Piggott, 2012; Merwin, Valois, & Padilla-Zakour, 2008).

With tannic apples in high demand, and no validated method that increases tannin extraction without sacrificing other metrics of quality, we sought to explore a new technology – pulsed electric field (PEF) – as an option for cidermakers to improve their cider quality. PEF treatment is the application of a series of short high voltage pulses through food items. These pulses create micropores in cell membranes, increasing permeability. While PEF's applications are still being investigated and optimized, it is particularly appealing as a nonthermal option to enhance mass transfer (Puértolas et al., 2012).

We investigated the potential of PEF treatment to enhance phenolic extraction from cider apples, thereby minimizing waste and reducing the volume of this more precious juice necessary for a balanced blend. PEF treatment was tested in several conditions with both

dessert and cider apples and compared against heat treatment for effect on total phenolics, tannins, and sensory experience.

3.2 Preliminary Trials

For the first trials, Red Delicious apples were selected due to their year-round availability and high levels of unextracted skin tannins (Lees et al., 1995). Trials run at 50 - 800 pulses (specific energies, *W,* from 14 – 450 kJ/kg), found that the more aggressive treatments (W >200 kJ/kg) raised mash temperatures above 70°C with decreasing phenolic gains. The optimal treatment conditions for enhancing polyphenol extraction without excessive heating were determined to be 400 pulses of 1 kV/cm for an applied specific energy of 112.5 kJ/kg.

The apples were ground in a CX5 Bertocchi extractor (Bertocchi SLR., Parma, Italy), with seeds and skin mixed back into the mash, and then divided into PEF-treated and control groups. Following PEF-treatment in the 30 cm chamber of CellCrack UL Batch Pulsed Electric Field System (Elea, Quackenbruck, Germany), half of each group was combined with 30 ml/ton of ColorPro macerating enzyme (Scott Labs, Petaluma, CA) and allowed to sit for 30 min at room temperature before pressing in a small hydraulic rack and frame press (Orchard Equipment Co., Conway, MA) with press cloth at 8.3 MPa (1200 psi).

After pressing, samples were again divided to be either thermally pasteurized at 71°C for 10 s, or nonthermally pasteurized via high pressure processing (HPP) at 600 MPa for 2 min. The juice was analyzed for total phenolics (TP) and showed a 34% increase in TP from

PEF vs control (Figure 3.1), 24% increase in TP for thermal pasteurization vs HPP pasteurization, and no effect from the enzyme treatment.

A second trial was conducted to determine if the same trends would be seen with other apple varieties. The same conditions were repeated on four dessert apple varieties (Fuji, Jonagold, McIntosh and Mutsu) and one bittersweet cider variety (Binet Rouge), omitting the ineffective enzyme and HPP steps. The results showed a statistically significant improvement in TP, ranging from 20-34% for the PEF-treated Binet Rouge, Fuji, Mutsu, and Red Delicious apples (Figure 3.1). These results were encouraging enough to warrant a more in-depth trial with sensory analysis to identify potential quality defects from the treatment.

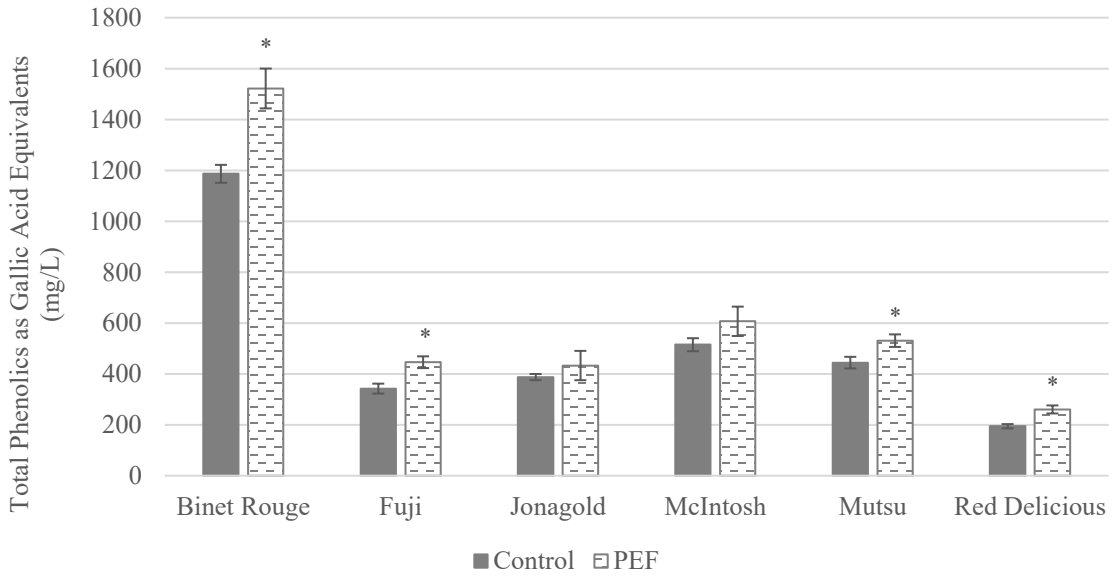

Figure 3.1: Effect of PEF Treatment of Apple Mash Before Pressing on Total Phenolics of Apple Juice from Six Varieties. Data represents triplicate measurements for three experimental samples per condition, reported as mean GAE (gallic acid equivalents) mg/L ± standard deviation. PEF-treated columns marked '*' are significantly different from the control ($p < 0.05$) by Tukey-Kramer HSD.

3.3 Cider Sensory Trial

3.3.1 Materials and Methods

3.3.1.1 Apples

Apples were divided into one of two blends for processing through the end of fermentation: dessert blend (DB; 64.5% McIntosh and 24.5% Mutsu for sugar and aromatics, 11% Gold Rush for acidity), and cider blend (CB; 55% Porter's Perfection for tannins, 45% Golden Russet for sugar and aromatics). All apples were harvested in the fall of 2018. The McIntosh and Gold Rush apples were sourced from Cornell Orchards in Ithaca, NY; the remaining apples were supplied by Black Diamond Farm in Trumansburg, NY.

3.3.1.2 Chemicals

Folin-Ciocalteu's Phenol Reagent (Sigma-Aldrich, St. Louis, MO), Gallic Acid (Chem-Impex, Wood Dale, IL), Epicatechin (Alfa Aesar, Havervill, MA), Sodium Carbonate (Oakwood Chemical, Estill, SC), Methyl Cellulose and Ammonium Sulfate (VWR, Radnor, PA), Methanol (Sigma-Aldrich, St. Louis, MO), Potassium Metabisulfite (LD Carlson, Kent, OH), Clinitest tablets (Bayer, Leverkausen, Germany). Go-Ferm, Fermaid O, and DV10 yeast were sourced from Lallemand, Montreal, Canada).

3.3.1.3 Juice Processing

Seventeen kilograms of CB apples were ground in a CX5 Bertocchi extractor (Bertocchi SLR., Parma, Italy), with seeds and skin mixed back into the mash, then divided into 9 batches of 1.7 kg: three each of the treatment groups: control (C1, C2, C3), heat (H1,

H2, H3), and pulsed electric field (P1, P2, P3). H1-H3 were heated to 70°C via microwave heating (Model KSA-8225A, Montgomery Ward, Chicago, IL) at the same time that P1-P3 received 400 pulses of 1 kV/cm in the 30 cm chamber of CellCrack UL Batch Pulsed Electric Field System (Elea, Quackenbruck, Germany). The conductivity was measured for all PEF-treated samples with a Chemtrix Type 700 Conductivity Meter. Initial temperature and maximum temperature (immediately following treatment) were measured for all samples using a handheld digital thermocouple (Atkins AquaTuff 351, Cooper-Atkins Corp., Middlefield, CT). From the milled apples, 1 kg was immediately vacuum-packaged (Multivac Type AGW, Allgau, Germany) in food-grade polymeric barrier bags and frozen at -20°C for later analysis.

The pressing of the control group mash (C1-C3) was staggered so that the average time between grinding and pressing was the same for each treatment group. Each treatment group (C1-P3) was batch pressed in a GoodNature X1 mini press (GoodNature, Buffalo, NY). After two 50 mL aliquots were reserved for juice analysis, the remaining juice from each batch (approximately 1.3 L) was immediately transferred into 1.9 L glass carboys. Potassium metabisulfite was added to achieve a final concentration of 50 ppm as free sulfur dioxide.

Separately, and as one batch, 89 kg of DB apples were ground and pressed without any additional treatment, in a GoodNature grinder and accordion press (X-6 Coldpress Juicer, GoodNature, Buffalo, NY). After two 50 mL aliquots were reserved for juice analysis, the remaining juice (49 L) was immediately distributed between two 22.7 L and two 3.8 L glass carboys and treated with Potassium metabisulfite to achieve a final concentration of 50 ppm

as free sulfur dioxide. The 50 ml aliquots from each pressing were frozen at -20°C until analysis.

3.3.1.4 Fermentation

All carboys were stored in a 1°C cold room for 40 hr before being removed from the cold room to warm to 20°C prior to inoculation. Each juice was inoculated with 25 g/hL of dried yeast (DV10, rehydrated with 30 g/hL Go-Ferm) and kept at 12°C for the duration of fermentation. Soluble solids measured as °Brix was monitored daily with a digital density meter (Anton Paar, Graz, Austria). Organic nitrogen (25 g/hL Ferm-O) was added after 1° Brix depletion, and then again at ⅓ Brix depletion. All fermentations reached dryness between days 14-17, confirmed by Clinitest tablet. Following completion of fermentation, two 30 mL aliquots of each cider were frozen for later analysis. The remaining ciders were racked, treated with 50 ppm sulfur dioxide, pooled by treatment group into sanitized 3.8 L glass carboys, and transferred to the 1°C cold room. Nitrogen gas was sparged into each carboy to fill headspace during storage.

3.3.1.5 Chemical Analysis

Prior to analysis, all CB samples were centrifuged for 20 min at 4000xg in a Dupont Instruments Sorvall RC-58 Refrigerated Superspeed Centrifuge (Midland, MI) and decanted to remove particulate. Juice samples were analyzed for titratable acidity (TA), pH, total soluble solids (°Brix), yeast assimilable nitrogen (YAN), total phenolics (TP). Cider samples were analyzed for TA, pH, °Brix, TP, tannins, color, turbidity, volatile acidity (VA), and

percent alcohol by volume (ABV). The frozen milled apples were lyophilized, ground to a fine powder, and extracted with 80% aqueous methanol according to the ultra-sound assisted method described by Kim and Lee (2002) as an additional comparison and theoretical maximum extractable tannins.

Total Soluble Solids (°Brix) was monitored prior to fermentation and then daily throughout fermentation with a digital density meter (DMA 35, Anton Paar, Graz, Austria). When °Brix fell below zero, Clinitest tablets were used daily to check for end of fermentation.

TA, YAN, VA and ABV were measured by the NY State Wine Analytical Lab (Geneva, NY). TA was measured via autotitrator with 8.2 endpoint (Metrohm, 848 Titrino plus, Herisau, Switzerland). YAN (as primary amino acids, as ammonium was not detected) and VA (as acetic acid) were measured by enzymatic assay in the Randox RX Monaco (Crumlin, UK).

TP was determined using the Folin-Ciocalteu colorimetric assay described by Singleton with minor modifications (Manns, 2014), in which solutions turn blue in linear proportion to phenolic content. The reaction for each sample was prepared in triplicate by mixing 25 uL of sample with 1375 uL of DI water and 100 uL of Folin-Ciocalteu reagent, combined in a cuvette via pipetting up and down. After standing for 7 min, the mixture was quenched with 1 mL of 7% (w/v) sodium carbonate. The solutions were then kept in the dark for 90 min before absorbance was measured at 765 nm on a Genesys UV-visible Spectrophotometer (10S, Thermo Fisher Scientific, Waltham, MA). Gallic Acid was used to

prepare an eight-point standard curve from 0 to 1000 mg/L, with results expressed as gallic acid equivalents (GAE) mg/L.

Turbidity was measured via handheld turbidimeter (HI 98703, Hanna Instruments, Woonsocket, RI) and expressed as NTU. CIELAB color components were measured using the Konica Minolta CR-400 handheld colorimeter (Chiyoda, Japan).

Tannins were measured following the Australian Wine Research Institute's protocol for methyl cellulose precipitation (Smith, 2015) using the 10 mL assay format. In brief, 1 mL of cider was combined with 3 mL of 0.04% methyl cellulose solution for the reaction flask, and after 2 min, combined with 2 mL of saturated ammonium sulfate solution and 5 mL of DI water. After 10 min, mixtures were centrifuged for 5 min at 3247xg in a Labnet Hermle Z4000 tabletop centrifuge (Gosheim, Germany). The same process was repeated for controls, with the methyl cellulose replaced with DI water. The absorbances were read at 280 nm for both the control and reaction flasks, and the difference between the two was presumed to be precipitated tannins. A seven-point standard curve was made from 7 to 250 mg/L of epicatechin and used to quantify the results of the assay.

3.3.1.6 Sensory Evaluation

Prior to sensory evaluation, CB ciders were blended 30:70 with DB cider plus 3% sucrose to approximate typical commercial cider recipes. Sensory evaluation was conducted at the Cornell Sensory Center with 100 untrained panelists recruited from the alcoholic beverage panel, all of whom reported consumption of cider at least once a month. The study

was conducted following all requirements of the Institutional Review Board of Cornell University regarding beverage samples for consumption.

Sensory trials were conducted over one day using a mixed model randomized block design with monadic blind taste testing of three samples. The ciders were kept refrigerated at 4°C and poured immediately prior to serving in 25 mL portions in 4 oz clear plastic cups with lids. Participants were requested to consume unsalted crackers and room temperature water between samples to avoid sensory overlap and limit tongue fatigue.

For each sample, participants evaluated appearance, aroma, taste, texture, and overall liking on a nine-point hedonic scale (from "Dislike Extremely" to "Like Extremely), and to rate visual cloudiness, sweetness, tartness, bitterness, and astringency on a 5-point Just-About-Right (JAR) scale (in which 1 = "Not Enough," 3 = "Just About Right," and 5 = "Too Much"). For each cider they were asked to rate their interest in purchasing the product on a seven-point scale (from "Definitely Would Not Purchase" to "Definitely Would Purchase"), and then finally to rank the samples from most to least preferred.

3.3.1.7 Statistical Methods

Chemical parameters were subjected to one-way analysis of variance (ANOVA), while sensory results were subjected to mixed model ANOVA with the panelist as the random effect factor and treatment as the fixed effect factor. Sensory data was collected using RedJade Sensory Software; analyses were conducted using JMP Pro (14.0.0). Significant differences were analyzed using Tukey-Kramer HSD with a 0.05 significance level.

3.3.2 Results and Discussion

The treatment and control juices had no significant differences in juice yield, pH, or titratable acidity (TA as malic acid). The juice from heat-treated mash did have higher soluble solids (°Brix) and yeast assimilable nitrogen (YAN), as seen in Table 3.1. The observed increases match those of thermovinification (heating mashed grapes during wine fermentation) on juice soluble solids and YAN, which are well-documented in wine literature, and unsurprising here, as heat facilitates tissue breakdown and mass transfer in apples as well as grapes (Geffroy et al., 2017).

Table 3.1: Effect of Heat or PEF Pre-Press Treatment of Cider Apple Mash on Apple Juice Composition

	Juice Yield[1] (ml)	TSS (°Brix)	pH	TA (g/L Malic Acid)	YAN (mg N/L)
Control	1107 ± 70	14.0 ± 0.4 a	3.48 ± 0.01	5.20 ± 0.20	38.6 ± 4.1 a
Heat	1217 ± 93	15.3 ± 0.2 b	3.50 ± 0.01	5.56 ± 0.07	47.3 ± 2.1 b
PEF	1240 ± 69	14.2 ± 0.2 a	3.50 ± 0.01	5.44 ± 0.05	38.7 ± 3.5 a

Data represents single measurements for three experimental samples per condition, reported as mean ± standard deviation. Significant differences ($p < 0.05$) by Tukey-Kramer HSD are indicated with different letters. No significant difference in measured values for juice yield, pH, or TA. [1] from 1.7 kg apples

The total phenolics from the three sets of juices were compared to those of the apples extracted with aqueous methanol (to approximate maximum total extractable tannins). The heat-treated juices had the highest overall TP with a 60% increase over the control, higher even than the methanol-extracted apples and with a very low standard deviation between samples, implying maximum extraction (Figure 3.2). This increase was anticipated based on the premise of thermovinification, and the efficiency perfectly matches that of Gerard & Roberts (2004), who treated Fuji and McIntosh mash to 70°C heat treatment.

PEF-treated juice had a more modest 25% higher TP than the control. This extraction improvement fits within the published range for PEF-treated apple mash, with an approximately 20% increase found by Grimi et al., (2011) in the treatment of Golden Delicious apples and an 80% increase in total polyphenols found by Schilling et al. (2008) in the treatment of a high-tannin cider apple blend.

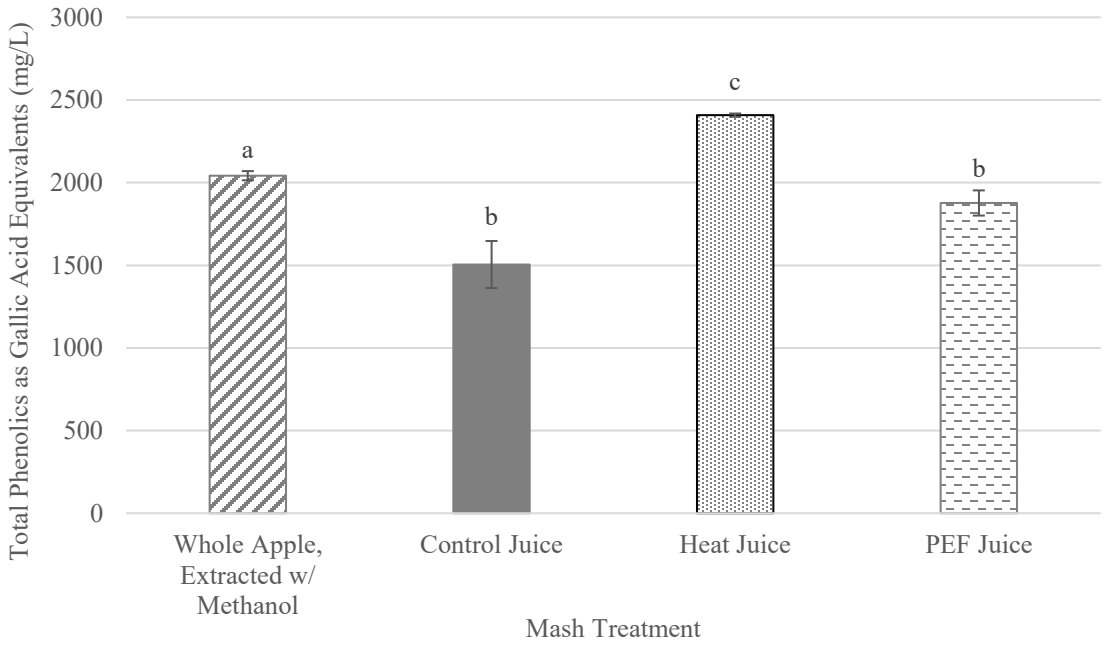

Figure 3.2: Effect of Heat or PEF Treatment of Apple Mash Before Pressing on Total Phenolics of Apple Juice. Data represents triplicate measurements for three experimental samples per condition, reported as mean GAE (gallic acid equivalents) mg/L ± standard deviation. Difference values between treatments not connected by the same letter are significant ($p < 0.05$) by Tukey-Kramer HSD. The first (diagonal-striped) bar represents the maximum methanol-extractable tannins of the apples, an approximation of total extractable tannins.

Once fermented, the ciders deviated more from each other by various physiochemical parameters (Table 3.2). While TA was still indistinguishable between samples, both treated ciders contained higher levels of volatile acidity (VA) than the control, though still below the

odor threshold. The heated sample had a predictably (based on its higher initial level of sugar) higher ABV by approximately 0.2%. Both the higher VA and ABV of the heated cider are again common side effects of thermovinification (Geffroy et al., 2017).

Table 3.2: Effect of Heat or PEF Pre-Press Treatment of Cider Apple Mash on Cider Composition

	pH	TA (g/L Malic Acid)	VA (g/L Acetic Acid	ABV (% v/v)
Control	3.55 ± 0.01 a	5.84 ± 0.10	0.26 ± 0.03 a	8.89 ± 0.07 ab
Heat	3.58 ± 0.02 b	5.93 ± 0.07	0.36 ± 0.02 b	9.09 ± 0.08 a
PEF	3.57 ± 0.01 ab	5.85 ± 0.07	0.35 ± 0.03 b	8.80 ± 0.02 b

Data represents single measurements for three experimental samples per condition, reported as mean ± standard deviation. Difference values between treatments not connected by the same letter are significant ($p < 0.05$) by Tukey-Kramer HSD.

The greatest differences can be seen in the color and turbidity, where heat treated ciders were significantly darker (lower L), redder (higher a), bluer (lower b), and dramatically more turbid, even after centrifugation (Table 3.3). These effects have been documented in the brief literature on heating cider mash, with the color change the result of greater anthocyanin extraction from the skin, and the increased turbidity a result of increased interactions between haze-active proteins and haze-active polyphenols (Gerard & Roberts, 2004; Valois, 2007). This suggests that treatment to reduce haze would reduce the gains in polyphenols.

Table 3.3: Effect of Heat or PEF Pre-Press Treatment of Cider Apple Mash on Cider Color and Turbidity

	L	a	b	Turbidity (NTU)
Control	39.5 ± 1.8 a	2.1 ± 0.8 a	20.4 ± 1.3 a	32 ± 18 a
Heat	28.5 ± 3.0 b	7.6 ± 1.8 b	11.6 ± 2.9 b	164 ± 52 b
PEF	38.2 ± 3.9 a	4.9 ± 2.2 a	20.2 ± 2.2 a	23 ± 20 a

Data represents single measurements of CIELAB color coordinates for three experimental samples per condition, reported as mean ± standard deviation. Difference values between treatments not connected by the same letter are significant ($p < 0.05$) by Tukey-Kramer HSD.

Total phenolics of the ciders were similar in trend to the TP of the juice, with losses between 20-25% after fermentation (Figure 3.3). Loss of polyphenols during fermentation is typical of cider fermentations (Valois, 2007; Ye, Yue, Yuan, 2014). Again, the heat-treated cider had significantly higher levels of phenolics than other ciders, at 1928 mg/L a 72% increase over the control of 1118 mg/L; PEF-treated ciders again showed a more modest advantage over the control with its 1428 mg/L representing a 28% improvement.

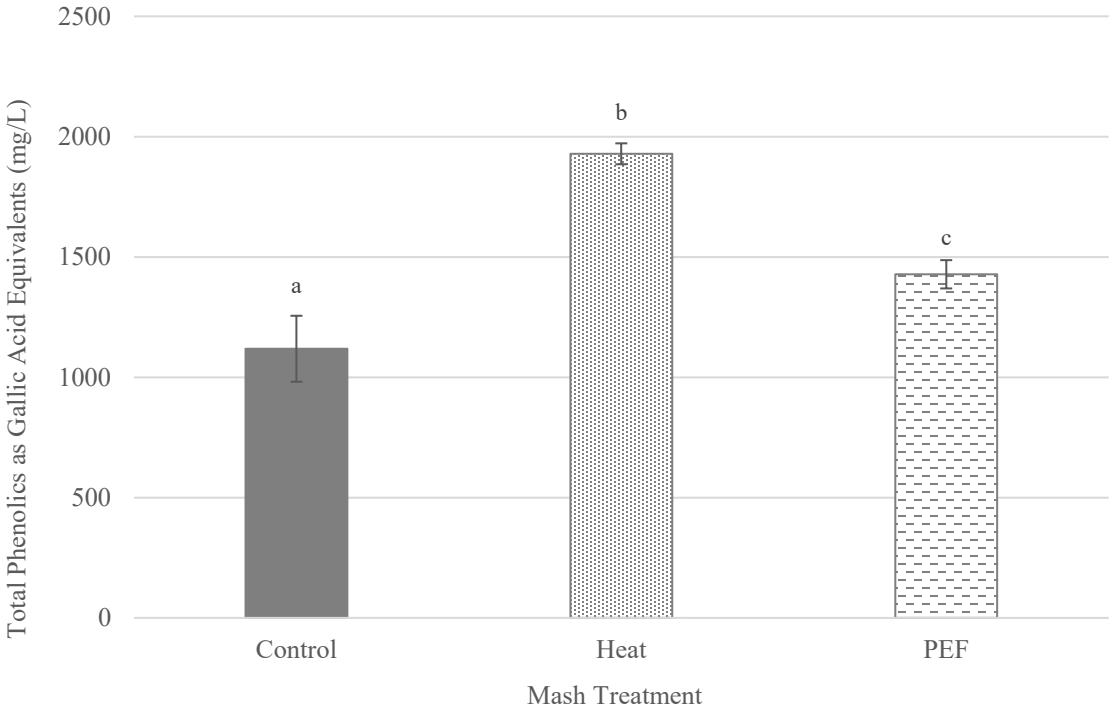

Figure 3.3: Effect of Heat or PEF Treatment of Apple Mash Before Pressing on Total Phenolics of Cider. Data represents triplicate measurements for three experimental samples per condition, reported as mean GAE (gallic acid equivalents) mg/L ± standard deviation. Difference values between treatments not connected by the same letter are significant ($p < 0.05$) by Tukey-Kramer HSD.

Tannin as measured by methyl cellulose precipitation in hard ciders showed similar trends, though as expected, at a lower scale. Heat treatment resulted in an average tannin level of 1076 mg/L, providing a 39% improvement over the 772 mg/L of the control; PEF-treated cider averaged 801 mg/L tannin, providing only a 4% improvement over the control (Figure 3.4). It must be reiterated that this assay, while well-correlated to the perception of astringency in wine, has not been studied or optimized in cider (Mercurio & Smith, 2008). It is unknown how differences between cider and wine tannins and cider and wine matrices may affect the assay, and thus whether these values reflect the cider tannins as perceived by cider drinkers.

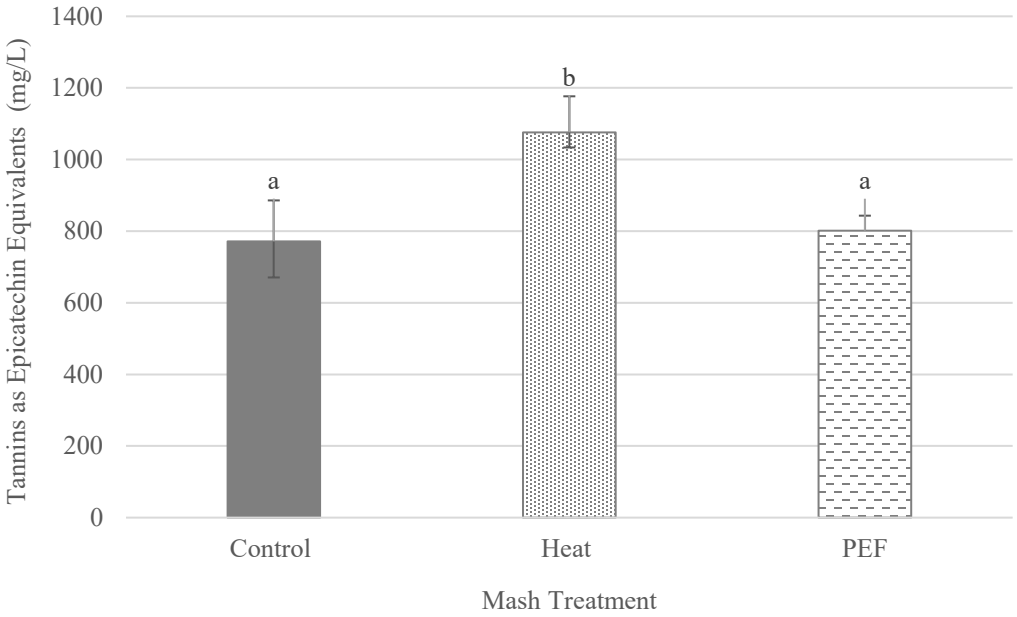

Figure 3.4: Effect of Heat or PEF Treatment of Apple Mash Before Pressing on Tannins of Cider. Data represents triplicate measurements for three experimental samples per condition, reported as mean tannins mg/L (epicatechin equivalents) ± standard deviation. Difference values between treatments not connected by the same letter are significant ($p < 0.05$) by Tukey-Kramer HSD.

The sensory evaluation was conducted with servings of the experimental CB ciders, pooled by treatment group and blended 30:70 with untreated DB (dessert blend) cider. All blends were sweetened to approximately 3% sugar with sucrose, and the resulting ciders were functionally identical in composition except for TP and tannins (Table 3.4).

Table 3.4: Composition of Blended Ciders Used for Sensory Evaluation

	Sugar (mg/L)	TA[1] (g/L)	ABV (% v/v)	TP[2] (mg/L)	Tannins[3] (mg/L)
Control	29.5	6.2	7.66	516 ± 11 a	230 ± 13 a
Heat	28.4	6.3	7.66	698 ± 9 c	465 ± 14 b
PEF	28.7	6.3	7.56	558 ± 2 b	267 ± 18 a

Data represents single measurements for experimental samples pooled by condition, except for TP and tannins, which represent triplicate measurements, reported as mean ± standard deviation. Difference values between treatments not connected by the same letter are significant ($p < 0.05$) by Tukey-Kramer HSD. [1] as Malic Acid, [2] as Gallic Acid, [3] as Epicatechin equivalents.

The 100 panelists for the sensory evaluation were 73% female, ranged in age from 21-60+, and were entirely composed of consumers who reported consuming cider at least once a month (Table 3.5).

Table 3.5: Sensory Panel Demographics (n = 100)

Variable	Description	Frequency (%)
Gender	Female	73
	Male	27
Age	21-30	70
	31-40	16
	41-50	6
	51-60	7
	60+	1
Alcoholic Beverage Preference	Cider as First Choice	14
	Cider as Second Choice after Wine	34
	Cider as Second Choice after Beer	12
	Cider as Second Choice after Mixed Drinks	14
	Cider Only as a Seasonal Beverage	24
	Cider if Only Option	2

Panelists rated all samples favorably, with the PEF-treated cider slightly favored in all categories except texture, where its rating of 6.4 out of 9 exactly matched the rating for the control (Figure 3.5). The preference for PEF-treated cider over the control was not statistically significant for any ratings. However, the heat-treated sample was rated statistically lower than the PEF-treated for most metrics, including appearance liking, taste liking, texture liking, bitterness, cloudiness, overall liking and overall ranking. These penalties were largely expected, and in fact more minor than anticipated, as heat-treated ciders are often rejected by sensory panelists for their bitterness, astringency, cloudiness and perceived “off-odors” (Lea & Timberlake, 1978; Valois, 2007). That these treated ciders were blended with untreated cider made from low-tannin apples at typical commercial ratios,

and were *liked* by panelists, suggests that thermal processing may have been unfairly maligned.

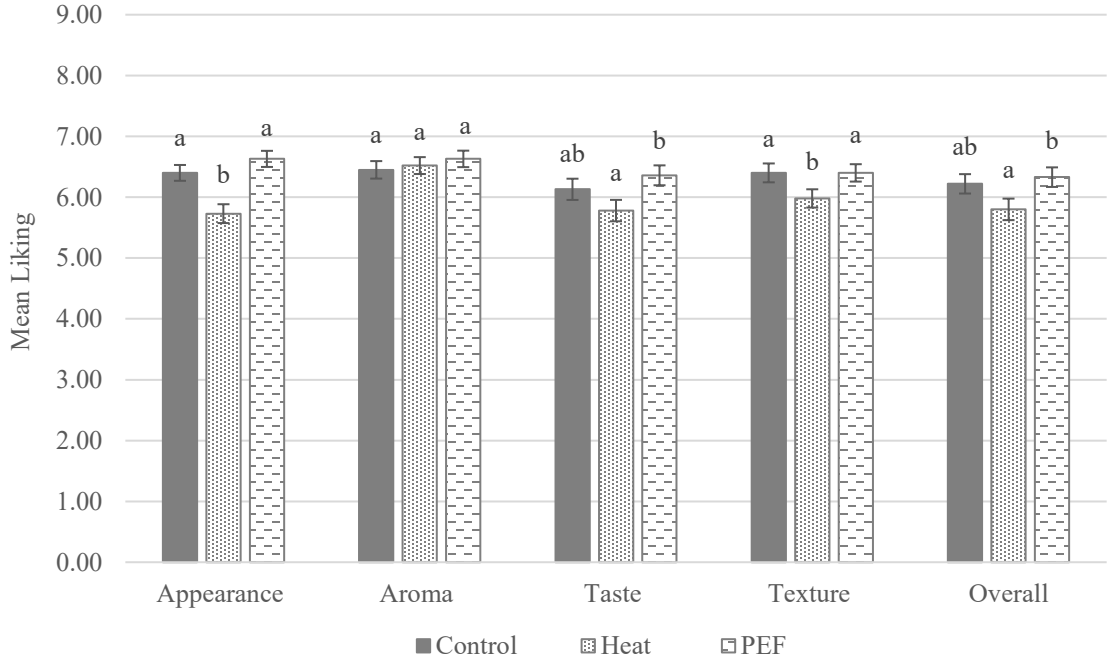

Figure 3.5: Sensory Evaluation of Quality Liking on 9-point Hedonic Scale.
Data represents responses of 100 panelists, reported as mean ± standard error. Difference values between treatments not connected by the same letter are significant ($p < 0.05$) by Tukey-Kramer HSD.

Bitterness ratings on the Just-About-Right (JAR) scale and taste ratings on the Hedonic scale were most predictive of overall liking (Table 3.6). It is notable that neither astringency nor serving position were correlated with each other, bitterness, or overall liking. This indicates, at the least, that the panelists did not suffer from tongue fatigue (tannin saturation) during testing. It may also unfortunately indicate a general inability of the panelists to recognize or identify astringency, as this is a notorious challenge in the field of

sensory science (Colonna, Adams, & Noble, 2004; Villamor, Evans, & Ross, 2014; Symoneaux et al., 2015).

Table 3.6: Sensory Evaluation Just-About-Right Rankings

	Cloudiness	Sweetness	Tartness	Apple Flavor	Bitterness	Astringency
Control	0.3 ± 0.6 a	-0.2 ± 0.8	-0.1 ± 0.8	-0.4 ± 0.8	0.1 ± 0.8 ab	0.0 ± 0.6
Heat	0.8 ± 0.8 c	-0.2 ± 0.7	0.1 ± 0.9	-0.4 ± 0.7	0.2 ± 0.7 b	0.2 ± 0.8
PEF	0.1 ± 0.7 b	0.0 ± 0.7	-0.1 ± 0.7	-0.4 ± 0.7	0.0 ± 0.8 a	0.1 ± 0.7

Too Little	Just-About-Right	Too Much

Data represents responses of 100 panelists, reported as mean deviation from "Just-About-Right" on a 5-point scale ± standard deviation. Ratings ≥0.2 below "Just-About-Right" are unshaded to represent "Too Little" of that component; ratings ≥0.2 above "Just-About-Right" are shaded dark gray to represent "Too Much" of that component; ratings within 0.1 of "Just-About-Right" are shaded light gray to represent "Just-About-Right". Difference values between treatments not connected by the same letter are significant ($p < 0.05$) by Tukey-Kramer HSD

3.4 Conclusion

Both the application of PEF and heat to apple mash prior to pressing resulted in increased phenolic levels in cider. While heating the mash to 70°C enhanced phenolic levels to the greatest extent, PEF-treated cider, with its minor phenolic improvement but otherwise unchanged character, was preferred in sensory analysis over both the control and heat-treated ciders. These trials make it clear that there is no loss in quality from PEF treatment, but also that more research is needed to determine if PEF treatment can extract statistically and practically significant amounts of polyphenols from apple mash.

Perhaps of greater interest to the cidermaker is the high acceptability of the heat-treated cider blend. Specifically, the heat-treated cider was only mildly penalized for its

faults, two of which – bitterness and texture – are evidence of the desired increased extraction, while a third – turbidity – is easily rectified with the addition of clarifying enzymes. The heat treatment may thus be the greatest success, as the polyphenol content increased by 72% over the control, with its main fault being the bitterness it imparted in a 30-70 blend with low tannin cider. These too-bitter cider blends would likely have been improved by blending in a lower ratio, in which case the intent of this research – more efficient utilization of high-price cider apples – would be fulfilled.

3.5 References

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Chapter 4

DISCUSSION AND FUTURE WORK

Both heat and PEF can successfully and significantly extract polyphenols from apple pomace and apple mash that would otherwise be lost. Each of these approaches has benefits and challenges.

PEF treatment has the benefit of being effective at low energy input levels, with or without the heaving of the apple pomace or mash. However, as this research was conducted using a batch system, and operating parameters vary between units and labs, future studies will have to investigate continuous PEF systems for apple pomace or mash treatment.

As to whether cidermakers should focus their efforts on pomace tannin recovery or mash tannin extraction, this will depend on their equipment set up and their processing priorities and capabilities. Processing pomace is appealing for its independence from the processing of the primary juice. Any oxidation from the additional processing time, or aroma changes from heat, will affect only the pomace extract without any risk to the costly cider apple juice. However, the pomace itself must then be protected from oxidation and processed quickly to maximize its value, and cidermakers may balk at an added time-sensitive task during pressing season. Additionally, cidermakers must decide how to incorporate dilute pomace extracts into the cider, with three distinct routes available: (1) concentration via heat, heat under vacuum, freezing, forward osmosis, or freeze-drying in order to add small doses of concentrated tannin; (2) direction addition of dilute pomace extract in place of added water

(appropriate only for ciders that would be diluted); or (3) suspension and treatment of the pomace in a low-polyphenol dessert apple juice instead of water.

On the other hand, treatment of apple mash has the clear benefit of elegance and easy integration, with the addition of one processing step (whether PEF or heat) to the standard process. In particular, the heat treatment of high tannin cider apples or high skin-tannin dessert apples (i.e. Red Delicious) to maximize tannin extraction may allow for tannic ciders to be produced using lower blending ratios of expensive cider apples. While thermal pasteurization is common at bottling to prevent refermentation in ciders with residual or added sugar, the heating of apple mash or unfermented apple juice is viewed skeptically by many cidermakers who fear undesirable changes to the apple aroma. Future work should investigate the industrial-scale feasibility, cost savings potential, and consumer acceptance of cider produced with lower-ratio blends of juice from heat-treated tannic mash and traditionally processed juice. If it can be shown that fewer cider apples can be used to achieve the same quality cider, cidermakers may be cured of their aversion to heat.

Lastly, it must be emphasized that when treating apples or pomace to maximize polyphenol extraction, the apple variety is a critical variable, as there must be a substantial reservoir of unextracted tannins for any improvement to be achieved. Cider apples or dessert apples with high levels of skin tannins are ideal for heat or PEF-assisted extraction and blending into cider, while dessert apples with low levels of skin tannins would yield little from the most aggressive treatment. Further research should be done to identify the apple varieties that would benefit most from these treatment methods.