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An Investigation of the Shelf Life of Cold Brew Coffee and the Influence of Extraction Temperature Using Chemical Microbial and Sensory Analysis

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AN INVESTIGATION OF THE SHELF LIFE OF COLD BREW COFFEE AND
THE INFLUENCE OF EXTRACTION TEMPERATURE USING CHEMICAL,
MICROBIAL AND SENSORY ANALYSIS

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by
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ABSTRACT

Coffee is a globally popular beverage – it is the second most consumed beverage in the world after tea (Petracco, 2001). According to a report from the National Coffee Association (NCA), in 2018, 64% of US consumers drink coffee daily. The same NCA report in 2017 shows that 59% of all coffee consumed in the US is specialty or gourmet coffee. Within this trend of specialty coffee, a product with rapidly growing market share is cold brew coffee. Cold brew coffee can offer the potential for a unique flavor profile, a position within the growing specialty coffee world, and RTD (ready-to-drink) convenience (Sisel, 2016). Products touted as cold brew coffee are coffee beverages that are extracted using low temperature and longer time than traditional hot brewed drip coffee or espresso (Hwang, and others, 2014). This can give cold water extracted (CWE) coffee an entirely different extraction profile, as different compounds are extracted at different rates, and many coffee constituents have temperature dependent solubility (Bladyka, 2014). Many producers are now selling cold brew coffee in a canned or bottled RTD (ready-to-drink) format to be consumed at a later date (Sisel, 2016). This presents the potential problem of microbial and sensorial deterioration. However, there is little published information around the exact chemical characteristics of cold brew to verify producers' claims and allow for prediction of shelf life. In this study, the shelf-life of refrigerated cold and hot brewed coffees were investigated based on sensory and chemical profile and microbial growth, while also examining the influence of extraction temperature on the chemical and sensorial profile of cold water extraction coffee. Based on the results, the refrigerated shelf life of bottled hot and cold brewed coffee is limited

not by microbial stability, but rather by deterioration in sensory attributes. Further work is recommended to elucidate the mechanisms of coffee staling in a refrigerated environment, with particular interest in the degradation products of chlorogenic acid, as a significant decline in chlorogenic acid concentration was found over the storage period. Cold extracted coffees were found to be chemically and organoleptically different beverages from coffees extracted at high temperature, specifically, the cold brewed coffees had higher sweetness and lower bitterness than the hot extracted coffee, supporting claims made by producers of cold brew. Additionally, the cold brewed coffees had greater flavor stability over the storage time than the hot brewed treatment.

DEDICATION

I dedicate this thesis to my parents, Frank and Heather Lopane. My supporters throughout everything, this would not have been possible without them.

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CHAPTER ONE

LITERATURE REVIEW

Introduction

Coffee is a globally popular beverage – it is the second most consumed beverage in the world after tea (Petracco, 2001). According to a report from the National Coffee Association (NCA), in 2018, 64% of US consumers drink coffee daily. An increasing amount of this coffee consumption in the US is specialty coffee, which can have varying definitions. The NCA defines gourmet or specialty coffee as "coffee drunk hot or iced that is brewed from premium whole bean or ground varieties. This includes espresso based beverages, iced/frozen blended coffee, cold brew, and iced coffee infused with nitrogen." The same NCA report in 2017 shows that 59% of all coffee consumed in the US is specialty or gourmet coffee. The growth of specialty coffee shops in the United States, as well as the acquisition of specialty cafes by large food companies, make it clear that there is an increasing appreciation, interest, and demand from consumers for high quality coffees with unique flavors (Lynley, 2017).

The dark, aromatic beverage that most consumers are familiar with actually begins as the bright red fruit of the *Coffea* tree, often grown far away from where it is finally consumed. The life cycle of coffee, the journey from seed to cup, is governed by six main stages that determine the final quality of the cup: growth, harvest, processing, roasting, grinding, and extraction.

Growth

The first step begins on a farm where coffee trees, a species of plant from the genus *Coffea*, are grown, and its fruit eventually harvested. The genus *Coffea* has its origin in tropical Africa, and the discovery of the unique properties of its fruit and its subsequent cultivation and domestication has been subject to many stories and legends over the span of history (Illy, 2005; Flament and Bessière-Thomas, 2002; Petracco, 2001). In modern times, two species of *Coffea* are used to produce coffee – *C. arabica* (Arabica) and *C. canephora* (Robusta). Robusta is less flavorful and complex than Arabica, but is hardier and easier to grow (Illy, 2002). Much of the world prefers the superior flavor of Arabica coffee, and the majority of coffee production is still *C. arabica* (Uman and others, 2016). What is known colloquially as a coffee "bean" is actually the seed of the fruit of the coffee tree.

Within the category of growth, there are a number of environmental influences such as rainfall, sunlight, temperature, and altitude, as well as the specific variety of coffee that is grown (Catuai, Bourbon, etc), that have an influence on the flavor of the cup of coffee (Uman and others, 2016). Much like wine, the overall terroir and origin of the coffee are paramount in determining its unique flavor and quality characteristics (Avelino et al. 2005). A high quality cup of coffee begins with careful cultivation and harvest practices. Changes that seem minute, such as percentage of shade provided by intercropped trees, can completely modulate the flavor of a coffee. "Once a coffee bean is grown, nothing can be added or removed: the quality must already be present." (Illy, 2002).

Harvest

When the coffee plant is fruiting, the bright red coffee cherries must then be harvested. Only ripe coffee cherries can be used, normally determined by color, so either selective hand picking or mechanical harvesting with post-harvest separation is employed to ensure high-quality (Illy and Viani, 2005).

Processing

The final step that occurs at the coffee farm or nearby processing center is the processing and drying of coffee, which must occur immediately after harvest to prevent fermentation and creation of unwanted flavors (Illy and Viani, 2005). In this step, whole coffee cherries are processed into dry green coffee for export. From the outside in, coffee cherries consist of skin, pulp, mucilage, parchment, silverskin, and finally, the seed (Heeger, Kosińska-Cagnazzo, Cantergiani, and Andlauer 2017). In order to make green coffee, all of the layers above the seed must be removed and the seed must be dried to a water content of about 9-13% (Illy and Viani, 2005, Flament I and Bessière-Thomas, 2002) This is typically done through one of two main methods: the natural (dry) process or the washed (wet) process. This selection is typically based on the country of origin and farmer's preference.

 Dry processing begins with sun-drying of the coffee cherries on open patios, with both fruit and seed intact, and concludes with dehulling, removing skin, pulp and parchment and leaving the seed free (Flament I and Bessière-Thomas, 2002). In wet processing of coffee, the cherries are first pulped, then fermented to remove the mucilage, then washed, and finally dried, either using the sun, or mechanical driers (Osorio-

Hernandez, Guerra-Garcia, Ferreira-Tinôco, Osorio-Saraz, and Aristizábal-Torres 2015).

Whatever method is used, at the end of the process, the coffee cherries must be dried

from 55-60% water content to 9-13%, which will reduce all metabolic changes to the

green coffee. This drying step has a crucial impact on the final flavor of the coffee – a dry

processed coffee will taste distinctly different from the same coffee processed using the

wet processing method. Chemically, the difference lies in the soluble solids content.

Because of the osmotic phenomena occurring during drying, natural processed coffees

will have a higher soluble solids content than the equivalent wet processed coffee,

resulting in a noticeably different mouthfeel (Illy and Viani, 2005.)

Roasting

After the green coffee is dried, milled, and sorted, it is packaged and shipped to

the roaster. Roasting is a vital chemical step in the coffee journey. Unlike the later steps,

roasting of coffee is a flavor creation step. When coffee is roasted, green coffee, a

chemically complex agricultural product, is exposed to high temperatures (170-230ºC),

leading to vast chemical, physical, and thermodynamic changes. Coffee is roasted in a

specialized piece of equipment known generically as a coffee roaster; the basic principle

of this technology is "forced convective flow of hot gases passes through a moving bed

of coffee beans (Illy and Viani, 2005). These roasters transfer heat by a mixture of

convection, conduction, and radiation, but because of the shape of the bean and the

temperature gradient within the bean, convection is the preferred mode of heat transfer,

as it ensures even heating of the bean and prevents scorching of the exterior (Rao, 2014).

When roasting coffee, the most obvious changes are the physical ones. Roasting begins

with the drying phase, when the high temperatures cause free water to be driven off and the bean temperature begins to rise. The color begins to change from green to yellow, and once the bean dries to about 6% moisture, exothermic reactions and the actual roasting begin. Here, the bean changes in color from yellow to brown to black, and large amounts of CO_2 are generating, swelling the bean in volume and decreasing its density. This creates high pressures within the microstructure of the bean, eventually leading to the cells exploding from internal pressure, which can be heard as an audible crack (Yeretzian, Jordan, and Lindinger, 2002). However drastic the physical changes, there are even more chemical changes to the constituents of the coffee bean. Sucrose pyrolyzes into reducing sugars, which then react with amino acids and free amino groups in proteins and peptides in the Maillard reaction, forming more than 1000 volatile organic compounds. These compounds are responsible for the aroma of coffee, and nonvolatiles such as the melanoidins, responsible for the color change in coffee. Additionally, almost all of the protein in the coffee bean is denatured. Chlorogenic acids, the most important group of acids in green coffee, are broken down, mostly through hydrolysis, and some become bound to melanoidins. At the end of roasting, green coffee has been both physically and chemically changed. The flavor and potential of the bean has been transformed – however, the exact way in which the coffee is roasted will dramatically change the flavor. Yeretzian and others, describe coffee roasting as a path-dependent function – meaning that the exact profile of how the coffee is roasted determines the flavor that is developed. Flavor development during roasting is not solely based on

getting the coffee from temperature X to temperature Y, but rather the "time-temperature history to which the beans are subjected." (Yeretzian and others, 2002).

Coffee Extraction

Extraction is the final step in coffee preparation, thus accurate and precise extractions are crucial to creating a pleasing beverage. Extracting coffee is the aqueous solvation of the flavorsome, soluble compounds in coffee which are mainly aprotic charge neutral organic compounds, organic acids, and conjugate salts (Hendon, Colonna-Dashwood, and Colonna-Dashwood, 2014). Extracting, or brewing coffee is the attempt to pull as many pleasing flavors from the bean into the cup in order to create a balanced and tasty beverage.

There are countless methods of preparation of coffee, some with long and rich histories, and others that are newer techniques based on recent technological innovation. They have various names and different technological principles at work, from immersion brewing (French Press), percolation brewing (drip coffee) to pressurized percolation brewing (espresso), but the unifying theme is that they are all methods of extracting soluble solids from a ground coffee particle into a liquid solution, and they are all characterized by the intimate contact of water with roasted coffee solids (Petracco, 2005). This is a mass transfer unit-operation, a solid-liquid extraction where the solvent is water and the solid is a mass of coffee ground into particulates.

The precise details vary between methods of extraction, but all coffee brewing relies on the same chemical principles. The polarity of water allows it to solvate the polar constituents of a coffee bean through either molecular or ionic dissolution (Bladyka,

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2014; Wellinger, Smrke, and Yeretzian, 2017). Chemically, the specific intermolecular forces in H_2O are what allow solvation to occur; many of the organoleptic compounds in coffee have both hydrophilic and hydrophobic regions "that interact with the water through hydrogen bonding, Coulombic interactions and through the formation of ordered hydrate cages" (Hendon, Colonna-Dashwood, and Colonna-Dashwood, 2014). In addition to solvation of soluble compounds, hydrolysis of larger and less soluble compounds also occurs and is dependent on the temperature and pressure of the extraction system (Cammenga and others, 1997). Nonpolar (hydrophobic) molecules will dissolve in water at low levels through the formation of the aforementioned cages. (Hendon and Colonna-Dashwood, 2015). The primary mechanism of the transport of coffee constituents into brewing water extraction is the diffusion of soluble solids. This extraction is governed by Fick's law of diffusion, which describes the quantity of a solute diffusing from a solid particle surrounded by a liquid (Cammenga and others, 1997; Petracco, 2001).

In order to facilitate extraction of organic compounds from the coffee into the water, coffee must be ground from its "whole bean" form into ground particulates. When coffee is ground, the cellular structure (largely a polysaccharide matrix) of the coffee bean is ruptured, increasing the surface area available for solvent interaction and releasing the contents of the plant cells (Cammenga, Eggers, Hinz, Steer, and Waldmann, 1997). This is a vital quality control step in coffee extraction, and it is essential to have a small grind size distribution in order to facilitate the even transfer of soluble compounds into the brew (Petracco, 2005).

For pressureless coffee extraction (not espresso), the mass transport can be explained in seven steps.

"(1) penetration of water into the coffee bed, displacement of gases (air, roast gases), moisturing and whirling-up of particles. (2) washing of material off the surface off the coffee particles, which were ruptured by grinding (3) penetration of water into the pores of the particles, (4) swelling of the particles, (5) solubilization of water-soluble substances, possibly hydrolysis of non-water-soluble substances (6) diffusion of dissolved substances to the particle surface (rate determining step), (7) convective mass transfer into the surrounding solution." (Cammenga and others, 1997).

These seven steps can be summarized in two basic stages; first the water contacts the ground coffee, displacing gases, and dissolving the solids that are on the surface of the grounds. While this is occurring, a second, slower process is occurring – the coffee particles absorb the brewing water, causing them to swell as the water-soluble substances within the particles solubilize and then diffuse to the surface of the particle and into the coffee solution.

In other industries, it may seem that the best way to insure a quality product is to increase the extraction percentage as high as possible. However, for coffee this is not necessarily desirable because the soluble substances present in the roasted coffee seed vary widely, and not all compounds within coffee have desirable flavors. There are at least 1800 chemical constituents in coffee, many of which have different extraction rates and different flavors that they will contribute to the cup (Lee, and others, 1992). Thus the

percentage of soluble solids extracted and method of extraction will change not just the concentration of soluble substances (total dissolved solids, TDS) in the cup, but it will control which soluble compounds from the coffee bean end up in the cup, ultimately modulating the flavor of the final product. This means that extraction controls not just the concentration of the beverage, but also the chemical identity. For instance, if only a small percentage of a coffee's dry mass is extracted, just the most soluble substances, which are generally acidic and sweet, will end up in the resulting brew; referred to as underextraction, this is generally considered organoleptically undesirable. Conversely, higher extraction yields will force slower extracting compounds, which are generally bitter and astringent, into the beverage; this is known as overextraction (Petracco, 2001). The current thinking within the specialty coffee industry is that an extraction of 18-22% of the roasted coffee seed by weight will result in an ideal beverage from a flavor quality standpoint (Lingle, 2011). This extraction range generally balances sweetness, acidity, and bitterness and results in the highest quality beverage a coffee can offer (Rao, 2010). No matter what coffee is used, extraction is the final control over the flavor and overall quality of the coffee beverage (Petracco, 2005).

The extraction percentage of coffee is determined by the rate and quality of an extraction, which is in turn subject to a number of variables. The extraction rate is dependent on the specific extraction surface (particle/grind size), the temperature of the solvent/solid interaction (known as the slurry), the agitation of the brewing matrix, and the solid/solvent ratio (Rao, 2010; Uman and others, 2016). Using a finer grind, or increasing the agitation or temperature will increase the extraction rate – the rate at which

compounds solubilize into the brewing water. The final solute concentration is then determined by the extraction rate and the extraction time (time of contact between solute and solvent) (Rao, 2010). The quality of the extraction (the efficiency and balance of compounds extracted) is dependent on the slurry temperature, the distribution of the particle size, the contact size, the specific water chemistry, the extraction time, and the uniformity of the extraction. (Rao, 2010)

Role of Dissolved Minerals in Coffee Extraction

The composition and concentration of the minerals in the water used for coffee extraction will dictate the dissolution and extraction of the flavor compounds from the ground coffee bean into the resulting coffee solution. Early work from Pangborn, Trabue, and Little demonstrated the sensory effects of different levels of minerals on the extraction of coffee and tea, and more recent work from Hendon, Colonna-Dashwood, and Colonna-Dashwood, used density functional theory to further investigate the effects that cation species have on coffee extraction (Pangborn, Trabue, and Little 1971; Hendon, Colonna-Dashwood, and Colonna-Dashwood, 2014). Water takes a long and varied path before it is used to brew coffee, and along the way it often dissolves salts, resulting in water that contains positively charged ionic species and their dissociated anions. These cations then increase the ionic strength of H_2O. As Hendon explains it: "Water is more polar with the inclusion of dissolved point charges. These dissolved point charges orientate in such a way that they minimise their charge locality by surrounding themselves with their highly polarized materials of opposite polarity." (Hendon and Colonna-Dashwood, 2015). Thus cations strongly bind to electron-rich flavor compounds

in coffee, and facilitate their extraction into water. Hendon's work in 2014 demonstrated that the two cations that are the most important in this regard are magnesium and calcium. Another "impurity" that strongly affects the flavor of coffee is bicarbonate (HCO_3^-, IUPAC hydrogencarbonate). Bicarbonate, a buffer, interacts with the solvated molecules in a coffee solution and can alter the protonation of acids in the brew, depending on the pKa. The concentration of bicarbonate in brewing water is important: in too high concentrations it can remove the pleasurable and desirable acidity of a coffee and result in chalky or flat flavors, at low levels it will prevent the presentation of vinegary or sour acidity (Hendon and Colonna-Dashwood, 2015). Overall, water used for coffee extraction must be a balance of general hardness (cationic concentration) which will facilitate extraction, and carbonate hardness (buffer concentration) which will control the acidity of the cup.

Cold Brew Extraction

Cold brew coffee is a product with growing market share – it can offer the potential for a unique flavor profile, a position within the growing specialty coffee world, and RTD (ready-to-drink) convenience (Sisel, 2016). Products touted as cold brew coffee, also known in various places as "Dutch coffee", are coffee beverages that are extracted using low temperature and longer time than traditional hot brewed drip coffee or espresso (Hwang, Kim, Kang, Kim, and Kim 2014). These beverages will be referred to as cold water extraction (CWE) coffee. As previously discussed in the extraction section, the different temperature of water gives CWE coffee an entirely different extraction profile, because different compounds are extracted at different rates, and many

coffee constituents have temperature dependent solubility (Bladyka, 2014). Many producers of CWE coffee claim that it has lower acidity, improved taste and increased smoothness when compared to traditional hot-brewed coffee (Sisel, 2016; Starbucks, 2018, Stumptown Coffee Roasters, 2018). However, there is little published data around the exact chemical characteristics of CWE coffee to verify these claims (Hwang, 2016).

Cold brew coffee is most often produced either by dripping (percolation) or steeping (decoction) (Kim and Kim, 2014). Generally, these extractions can be carried out at different temperatures and still be considered CWE – some producers extract at ambient temperatures, and some at refrigeration (Kim and Kim, 2014). These extractions can be carried out over different periods of time – often ranging anywhere from 3 to 24 hours (Daeschel and others, 2014; Hwang and others, 2014). Another benefit of CWE coffee is that it can be canned or bottled in a RTD format to be consumed at a later date (Sisel, 2016). This presents the potential problem of microbial and sensorial shelf life.

Summary of previous research

Coffee is a complex field that has been studied frequently – however because of the nature of cold-brew as an emerging product, there is still much to be researched. Kim and Kim (2014) used HPLC to study the flavor-contributing non-volatile chemical components (caffeine, chlorogenic acid, and trigonelline) of CWE-based coffee depending on extraction time (3, 6, 9, 18 h) and extraction method (dripping vs steeping). These researchers also investigated the flavor characteristics of the coffee samples by using quantitative descriptive analysis (QDA). Based on the sensory data, there was a significant difference ($p<0.05$) in the flavor characteristics between the two extraction

methods, showing that the extraction method does create a different tasting product. Furthermore, the sensory analysis via QDA also revealed that 18 hours was the preferred extraction time for the panel. The HPLC data demonstrates that the flavor contributing non-volatile components were significantly affected by extraction time and method (Kim and Kim, 2014). Hwang and others (2014) investigated the changes in the flavor compounds of CWE-based coffee resulting from different extraction times and storage days. Fifty-six flavor compounds in cold coffee extracts were identified using headspace GC/MS. One of the most significant findings from this study was that acetic acid, which is a major contributor to typical coffee flavor, was not found in the analysis of the CWE coffee, which supports the current thinking that CWE coffee has lower acidity than hot brewed coffee. However, the researchers did not do a side-by-side comparison of HWE and CWE coffee.

Daeschel, Armbrust, and Vieru (2017) conducted a challenge study on the survival of vegetative foodborne pathogens in CWE coffee, concluding that cold brew does not favor the survival or growth of vegetative bacterial pathogens, most likely due to the lack of microbial nutrients and the presence of antimicrobial factors within the coffee. However, there is still work to be done to assess the risk of spore-forming pathogens in CWE coffee, and to assess the shelf-life of cold brew based on sensorial quality (Daeschel, Armbrust, and Vietru, 2017).

Pérez-Martínez and others (2008) studied the effects of refrigerated storage on HWE coffee on the basis of pH change. The article also develops a theory of the increase of acidity of stored coffee brews over time. In 2013, Sopelana and others studied the

effect of UHT pasteurization on hot brewed, bottle, refrigerated coffees. The authors used a variety of tests including pH, microbiological colony count, HPLC, volatile analysis via HPLC, antioxidant potential, and sensory analysis, to generate a large amount of data about the two treatments. This group of researchers concluded that UHT pasteurization results in a 60-day proposed shelf life for UHT pasteurized refrigerated coffee brews, and a 20-day shelf life for untreated refrigerated coffee brews. However, there was no cold water extraction coffee included in this study, and many manufacturers are wary of pasteurizing cold brew, as there is potential for flavor degradation during heating (Sopelana, Pérez-Martínez, López-Galilea, de Peña, and Cid, 2013).

One continuing area of research that still has much room to be explored is the mechanism by which brewed coffee staling occurs. On the shelf life of desirable coffee aroma, Hofmann and Schieberle demonstrated that pyrazinium compounds formed as oxidation products of radical cations present in coffee can cause the covalent binding of odor active thiols to melanoidins. This covalent binding ultimately has the effect of decreasing certain sulfury-roasty aromas, which explains one mechanism of aroma staling of brewed coffee (Hofmann and Schieberle, 2002). In 2013, Sopelana and others noted that some have suggested that the development of sourness in stored coffee is due to the hydrolysis of quinic and chlorogenic acid lactones, or the breakdown of chlorogenic acid into caffeic and quinic acid (Sopelana and others, 2013, Lingle, 2011). However, Pérez-Martínez and others (2008), note that many conclusions have been drawn based on results from accelerated ageing studies that use unrealistic high temperatures that do not accurately simulate the storage of coffee under refrigeration;

they then conclude that "…further investigations should be carried out in order to know more deeply which are the factors involved in the staling of this type of product stored at room or refrigeration temperatures and with or without oxygen" (Pérez-Martínez and others, 2008).

Conclusion

Overall, the literature is inconclusive in establishing an exact mechanism by which cold coffee stales, pointing to the fact that coffee is biochemically complex and active, and there is much more work to be done to understand the changes in brewed coffee during storage. Further investigation is also needed to clarify the exact chemical characteristics of cold brew to verify producers' claims and allow for prediction of shelf life. A deeper understanding of the influence of extraction temperature would also allow for producers to make data-driven decisions when selecting brewing parameters.

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CHAPTER TWO

AN INVESTIGATION OF THE SHELF LIFE OF COLD BREW COFFEE AND THE

INFLUENCE OF EXTRACTION TEMPERATURE USING CHEMICAL,

MICROBIAL, AND SENSORY ANALYSIS

Abstract

Cold brew coffee is a product with rapidly growing market share – it offers the
potential for high quality, a unique flavor profile, a position within the growing specialty
coffee world, and RTD (ready-to-drink) convenience. However, there is little published
information around the exact chemical and microbial characteristics of cold brew to
verify producers' claims and allow for an accurate prediction of shelf life. This study
examined the shelf-life of cold and hot water extraction coffees based on sensory and
chemical profile and microbial growth, while also investigating the influence of
extraction temperature on the chemical and sensorial profile of cold water extraction
coffee. Based on the findings of this study, the shelf life of refrigerated cold and hot
brewed coffee is limited not by microbial stability, but rather by deterioration in sensory
attributes. Further work is recommended to elucidate the mechanisms of coffee staling in
a refrigerated environment, with particular interest in the degradation products of
chlorogenic acid, as a significant decline in chlorogenic acid concentration was found
over the storage period. Cold extracted coffees were found to be chemically and
organoleptically different beverages from coffees extracted at high temperatures;
specifically, the cold brewed coffees had higher sweetness and lower bitterness than the
hot extracted coffee, supporting claims made by producers of cold brew. Additionally,

the cold brewed coffees had greater flavor stability over the storage time than the hot brewed treatment.

Introduction

Coffee is a globally popular beverage – it is the second most consumed beverage in the world after tea (Petracco, 2001). According to a report from the National Coffee Association (NCA), in 2018, 64% of US consumers drink coffee daily. An increasing amount of this coffee consumption in the US is specialty coffee, which can have varying definitions. The NCA defines gourmet or specialty coffee as "coffee drunk hot or iced that is brewed from premium whole bean or ground varieties. This includes espresso based beverages, iced/frozen blended coffee, cold brew, and iced coffee infused with nitrogen." The same NCA report in 2017 shows that 59% of all coffee consumed in the US is specialty or gourmet coffee. The growth of specialty coffee shops in the United States, as well as the acquisition of specialty cafes by large food companies, make it clear that there is an increasing appreciation, interest, and demand from consumers for high quality coffees with unique flavors (Lynley, 2017).

In coffee's often global journey from seed to cup, the first three main steps, growth, harvest, processing, occur in the country of origin of coffee, at the coffee farm or nearby processing center. The final three steps, roasting, grinding, and extraction take place in the country of coffee consumption. This study will focus on the extraction of coffee, but it is important to emphasize that all of the previous steps in the coffee journey govern the chemical and physical components of the coffee bean that will be extracted into the final beverage.

Extraction Details

Extracting coffee is the aqueous solvation of the flavorsome, soluble compounds in coffee which are mainly aprotic charge neutral organic compounds, organic acids, and conjugate salts (Hendon, Colonna-Dashwood, and Colonna-Dashwood, 2014). Extracting, or brewing coffee is the attempt to dissolve as many pleasing flavors from the bean into the cup to create a balanced and tasty beverage, and this happens through the intimate contact of water with roasted coffee solids (Petracco, 2005).

Chemically, the polarity of water allows it to solvate the polar constituents of a coffee bean through either molecular or ionic dissolution (Bladyka, 2014; Wellinger and others, 2017). In addition to solvation of soluble compounds, hydrolysis of larger and less soluble compounds also occurs and is dependent on the temperature and pressure of the extraction system (Cammenga and others, 1997). Nonpolar (hydrophobic) molecules will dissolve in water at low levels through the formation of hydrate cages. (Hendon and Colonna-Dashwood, 2015).

Physically, this can be understood in two basic stages; first the water contacts the ground coffee, displacing gases, and dissolving the solids that are on the surface of the grounds. While this is occurring, a second, slower process is occurring – the coffee particles absorb the brewing water, causing them to swell as the water-soluble substances within the particles solubilize and then diffuse to the surface of the particle and into the coffee solution (Cammenga and others, 1997).

The soluble substances present in the roasted coffee seed vary widely, and not all compounds within coffee have desirable flavors. There are at least 1800 chemical

constituents in coffee, many of which have different extraction rates and different flavors that they will contribute to the cup (Lee, and others, 1992). Thus extraction controls not just the concentration of the beverage, but also the chemical identity. In order to avoid both underextraction, resulting in an overly acidic and sweet brew, and overextraction, resulting in bitterness and astringency, the current thinking within the specialty coffee industry is that an extraction of 18-22% of the roasted coffee seed by weight is ideal (Lingle, 2011). This extraction range generally balances sweetness, acidity, and bitterness and results in the highest quality beverage a coffee can offer (Rao, 2010).

The extraction percentage of coffee is determined by the rate and quality of an extraction. The extraction rate is dependent on the specific extraction surface (particle size), the temperature of the solvent/solid interaction, the agitation of the brewing matrix, and the solid/solvent ratio (Rao, 2010; Uman and others, 2016). The final solute concentration is then determined by the extraction rate and the extraction time (Rao, 2010). The quality of the extraction is dependent on the slurry temperature, the distribution of the particle size, the contact size, the specific water chemistry, the extraction time, and the uniformity of the extraction. (Rao, 2010)

Cold brew extraction

Cold brew coffee is a product with growing market share – it can offer the potential for a unique flavor profile, a position within the growing specialty coffee world, and RTD (ready-to-drink) convenience (Sisel, 2016). Products touted as cold brew coffee, also known in various places as "Dutch coffee", are coffee beverages that are extracted using low temperature and longer time than traditional hot brewed drip coffee

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or espresso (Hwang, and others, 2014). The different temperature of water gives cold water extracted (CWE) coffee an entirely different extraction profile, as different compounds are extracted at different rates, and many coffee constituents have temperature dependent solubility (Bladyka, 2014). Many producers of CWE coffee claim that it has lower acidity, improved taste and increased smoothness when compared to traditional hot-brewed coffee (Sisel, 2016; Starbucks, 2018, Stumptown Coffee Roasters, 2018). However, there is little published data around the exact chemical characteristics of CWE coffee to verify these claims (Hwang, 2016).

Cold brew coffee is most often produced either by dripping (percolation) or steeping (decoction) (Kim and Kim, 2014). Generally, these extractions can be carried out at different temperatures and still be considered CWE – some producers extract at ambient temperatures, and some at refrigeration (Kim and Kim, 2014). These extractions can be carried out over different periods of time – often ranging anywhere from 3 to 24 hours (Daeschel and others, 2014; Hwang and others, 2014). Another benefit of CWE coffee is that it can be canned or bottled in a RTD format to be consumed at a later date (Sisel, 2016). This presents the potential problem of microbial and sensorial shelf life. Coffee is a complex field that has been studied frequently – however because of the nature of cold-brew as an emerging product, there is still much to be researched, especially regarding shelf-life and the influence of extraction method and temperature.

In this study, three extraction treatments of coffee were brewed, bottled, and analyzed over a period of 42 days. The shelf-life of refrigerated cold and hot brewed coffees were investigated based on sensory and chemical profile and microbial growth,

while also examining the influence of extraction temperature on the chemical and sensorial profile of cold water extraction coffee. Because of the dual purpose of this study, the experiment was structured as a split-plot design: there were multiple independent variables (time and extraction temperature) that affected a large number of dependent variables that were measured. This experimental design, as well as the statistical techniques used, allowed the study of both the effect of time and extraction method on the coffee beverages.

Materials and Methods

1. *Sample Preparation*

This study was divided into three treatment groups based on the extraction method of the same roasted and ground coffee. All three treatments used the same coffee, which was obtained from Due South Coffee in Greenville. SC. All coffee used for the three treatments was of the same geographic origin, processing method, roast level and grind size. Specifically, it was Due South's Brazilian Black Diamond coffee, a natural-processed coffee from the farm Vista Elegre in the Cerrado Mineiro region of Brazil. This region has altitudes varying between 800 and 1000 meters above sea level. The Arabica cultivar of the coffee used was Acaiá Cerrado, and it was imported to the US by Ally Coffee. The tasting notes from the roaster were "Brown Sugar, Chocolate, and Smooth." The coffee was roasted 6 days before extraction and the roast degree was quantified with a Javalytics roast analyzer as Gourmet 72.9.

The coffee used for all treatments was freshly ground using a Mahlkönig EK 43 (Mahlkönig, Hamburg, Germany) with a grinder burr aperture of 8, and the particle size

was quantified using a standard set of coffee sieves, as shown in **Table 2.1**. 50% of the

ground particles were 850-710μm. In order to ensure a consistent extraction, all of the

water for extracting each treatment of coffee was standardized by adding the same ratio

of minerals to distilled water using Third Wave Water mineralization packets (Third

Wave Water, Cedarville, OH), (**Table 2**). All treatments were prepared in triplicate and

assigned a randomized three-digit code, resulting in nine groups of samples.

Table 2.1: Particle Size of Ground Coffee

μm	% particles
1,180	5
850	35
710	50
600	5

Table 2.2: Mineral Content of Water for Extraction

Mineral	Quantity
$[Mg^{2+}]$	85 ppm
$[Ca^{2+}]$	45 ppm
$[HCO_3^-]$	20 ppm

2. *Cold Water Extraction*

Cold Water Extraction (CWE) coffee was prepared using an infusion method (full

immersion). The coffee was ground to the particle size specified in Table 2.2, and filled

into a Toddy cold brew commercial filter bag (Toddy LLC, Fort Collins, CO). This bag

was then placed in a 3-gallon Cambro brewing vessel and mineral standardized water

(Table 2.1) at 4ºC was added at a coffee to water ratio of 1:14. After an extraction time of

4 hours in a refrigerator set to 4ºC, the filter bag was removed and the resulting coffee

solution from each replicate was bottled.

3. *Ambient Water Extraction*

The Ambient Water Extraction (AWE) coffee was prepared in the same way as the CWE,

but at ambient (25ºC) temperatures.

4. *Hot Water Extraction*

Hot Water Extraction coffee was prepared by a hot drip method in a Bonavita BV1900TS

(Bonavita, Seattle, WA) at a coffee-to-water ratio of 1:18 and a water temperature of

~97ºC. Immediately after brewing, the coffee was flash chilled by filling of a retort bag

and immersion into an ice bath (coffee chilled to 50ºF in ~10 minutes).

5. *Bottling*

All three treatments were performed in triplicate, and the samples from each group were

bottled into fifty 120mL amber glass bottles, sealed with a plastic screw cap, labeled, and

stored at 7ºC. A randomization plan was created that determined which tests would be

administered to each bottle at each date. All of the treatment groups were analyzed at 0,

7, 14, 21, 28, and 42 days by the following analytical tests. An outline of the tests

performed is in **Table 2.3.**

Table 2.3: Schedule of analytical tests performed on coffee treatments

Day	Analysis Performed
0	Refractive Index/TDS, pH and titratable acidity, GC/MS, Sensory Analysis, and Microbiological Analysis
7	pH and Titratable acidity, Sensory Analysis, and Microbiological Analysis
14	pH and Titratable Acidity, HPLC, Sensory Analysis, and Microbiological Analysis
21	Refractive Index/TDS, pH and Titratable acidity, HPLC, GC/MS, and Microbiological Analysis
28	pH and Titratable acidity, Sensory Analysis, and Microbiological Analysis
42	Refractive Index/TDS, pH and titratable acidity, HPLC, GC/MS Sensory Analysis, Microbiological Analysis

Analytical Techniques

1. *Refractive Index / Total Dissolved Solids*

The percentage of total dissolved solids (%TDS) in each of the samples was measured using a specialized coffee refractometer (VST, Inc). First, the refractometer was calibrated using distilled water, then a clean plastic pipette was used to transfer 3 drops of the sample to the sample well of the refractometer. The sample was allowed to equilibrate, and then the %TDS obtained and recorded.

2. *HPLC/Non-volatile Analysis*

The concentration of a chlorogenic acid, 3-O-caffeoylquinic acid (3-CQA), and caffeine in the brewed coffees in each of the sample groups were measured at day 21 and 42 using high performance liquid chromatography (HPLC) connected with a UV detector. Sample preparation was performed following the methods in Gloess and others,

2013. Briefly, 2g of the brewed coffee sample was added to 500µl Carrez I (30% $ZnSO_4$ aqueous solution), 500µl Carrez II (15% potassium hexacyano ferrate trihydrate), 500µl methanol, and then diluted with 25 ml H_2O and filtered with a syringe filter. The samples were then analyzed in triplicate using a Waters 1525 binary HPLC pump with a Waters 717+ autosampler, degasser and thermostat, and a Waters 2487 dual absorbance detector. Mobile phase A was H_2O with 0.1% formic acid, and mobile phase B was acetonitrile with 0.1% formic acid. The flow rate was 0.5 ml / minute in isocratic conditions of 30% in 40 minutes. The column used was a Gemini 5u C18 110A with a size of 150 mm x 4.60 mm x 5 microns. For the analysis of caffeine, the detector was set to 272nm in single wavelength mode, and for 3-O-caffeoylquinic acid (3CQA, a chlorogenic acid) the detector was set to 325nm. The compounds were identified and quantified using commercially available HPLC-grade standards.

3. pH and Titratable Acidity

The pH and titratable acidity (TA) in the brewed coffees in each of the sample groups was measured at 0, 7, 14, 21, 28, and 42 days. The pH was measured in duplicate at 25ºC using an Orion 420A pH meter, calibrated using a two-point calibration. For the titratable acidity, also measured in duplicate, 40mL of each sample was titrated with 0.1M NaOH to a pH of 8.0, and the volume of NaOH recorded.

4. Headspace Analysis – GC/MS

The headspace intensity of each of the sample groups was measured at 0, 21 and 42 days using gas chromatography/mass spectrometry (GC/MS). Ten mL of each sample was placed into clean 15 ml GC-MS vials, capped with Teflon™-lined septa, sealed, then

analyzed using a gas chromatograph/mass selective detector system (Hewlett Packard 7694 headspace sampler, Hewlett Packard HP 6890 series gas chromatograph, and Hewlett Packard HP 5973 mass selective detector (Hewlett Packard, Wilmington, DE). The sample was equilibrated in the autosampler for 30 minutes at 90ºC, with a loop/valve temperature of 110ºC and a transfer line temperature of 115ºC. The gas headspace was then automatically injected onto a 30-meter-long HP 5 MS capillary column with a 0.25 μm internal diameter. The GC oven was programmed for a time-temperature profile of: 35ºC for 1 min, followed by a 3ºC/min ramp to 100ºC, then 5ºC/min to 220ºC, with a total runtime of 46.67 min. The data analysis was performed with HP Chem Chemstation Integrator, and the identification of volatiles was based on the comparison of retention times to authentic standards and computer matching of mass spectra to a reference library (Hewlett Packard). Although many compounds were identified, 11 compounds that have a majority contribution to typical coffee flavor, based on Flament and Bessière-Thomas's work, were chosen and summed in order to study the headspace of the samples (Flament and Bessière-Thomas 2002). The integrated intensities of the compounds chosen were then summed to give a headspace intensity (Gloess and others, 2013).

5. *Microbiological Growth – APC and Psychrotrophic Count*

All of the brewed coffees in each of the sample groups were tested for microbiological growth at 0, 7, 14, 21, 28, and 42 days. Enumeration of microbiological growth was achieved with a total aerobic plate count using standard plate count procedures as well as a psychotropic count. All plate counts were performed with 3M

Petrifilm and followed procedures as outlined in 3M Petrifilm Aerobic Count Plate

Interpretation Guide and AOAC official method 990.12. Aseptic technique was followed,

and all inoculation and preparation of plates was performed under a laminar air flow

hood. Briefly, 1 ml of each sample was placed onto the medium using a sterile pipette tip,

and the inoculum was evenly distributed over a circular area using the 3M Petrifilm

spreader. The aerobic plate count was performed in duplicate, and two dilutions were

used: 10^0 and 10^{-1}. For the aerobic plate count, the plates were then incubated for 48

hours at 35ºC and any microbiological growth was enumerated on a colony counter

following the counting guidelines in AOAC 990.12 (AOAC, 1994). For the

psychrotrophic count, the plates were incubated for 10 days at 7ºC and counted in the

same manner.

6. *Sensory Descriptive Analysis*

The sensorial quality shelf life of the coffee extracts was determined by use of a

blind sensory descriptive analysis, using trained panel of five coffee professionals

evaluating the sensory attributes of the coffees over a period of 42 days (adapted from

methods in Gloess and others, 2013, and Pérez-Martínez and others, 2008, Stokes and

others, 2017). The panel was made up of pre-trained coffee professionals from green

coffee importer Ally Coffee and roaster Due South Coffee that are engaged in cupping

coffee at least once a week. Using pre-trained professionals reduced the amount of pre-

training that needed to be administered and allowed for a smaller panel to be used.

This sensory panel made extensive use of the World Coffee Research (WCR)

Sensory Lexicon, "a universal language of coffee's sensory qualities, and tool for

measuring them." (World Coffee Research, 2016; Edgar and others, 2016). This sensory lexicon is based upon hundreds of attributes with definitions, and commercially available reference samples that are scaled with intensities. Before the descriptive analysis began, the authors conducted a calibration session that consisted of tasting references according to the coffee lexicon, and discussing and standardizing terms used in the panel, and tasting commercially available cold brews.

The members of the panel evaluated all three treatment groups (extraction methods) in triplicate. The evaluations all took place in Ally Coffee's cupping room. The samples were presented monadically, in a randomized order, in 120ml amber glass bottles, labeled with 3-digit randomly coded numbers, and poured into a white porcelain cupping bowl. The serving temperature was 45ºF. The panelists evaluated each sample for the intensity of thirteen sensory attributes in six categories (Table 2.4) on a scale from 0 to 15 (0 = none, 2 = barely detectable, 6 = slightly intense, 8 = moderately intense, 10 = intense, 12-15 = very intense). Each of the judges' scores was compiled and statistically analyzed to create a sensory score for each sample. The sensory form that was used to evaluate the coffees can be found in Appendix A1.

Table 2.4: Guide to Sensory Attributes (adapted from WCR Sensory Lexicon)

Attribute	Definition
Overall Acceptability	Overall acceptance of the sample
Acidity	The fundamental taste factor associated with a citric acid solution.
Sweetness	Fundamental taste sensation of which sucrose is typical. Generally associated with sweet aroma descriptors such as fruity, chocolate and caramel.
Bitterness	The fundamental taste factor associated with a caffeine solution
Astringency / Mouth Drying	Characteristic of an after-taste sensation consistent of a drying effect in the mouth. A drying, puckering, or tingling sensation on the surface and/or edge of the tongue and mouth.
Longevity	Persistence of flavor in the mouth; the time that the full, integrated sensory experience sustains itself in the mouth and after swallowing.
Stale	The flavor characterized by a lack of freshness
Papery	The aromatic associated with white paper cups.
Musty/Earthy	The somewhat sweet, heavy aromatic associated with decaying vegetation and damp, black soil.
Fermented	The pungent, sweet, slightly sour, sometimes yeasty, alcohol-like aromatic characteristic of fermented fruits or sugar or over-proofed dough.
Over-Ripe	The sweet, slightly sour, damp, musty/earthy aromatic characteristic of fruit or vegetable past their optimum ripeness.
Sour	Excessively sharp, biting, unpleasant acidity
Winey	The sharp, pungent, somewhat fruity, alcohol-like aromatic associated with wine.
Medicinal	A clean, sterile aromatic characteristic of antiseptic-like products such as Band-Aids, alcohol, and iodine.
Rubber	A dark, heavy, slightly sharp, and pungent aromatic associated with rubber.
Skunky	A combination of aromatic associated with skunks
Hay-like	The lightly sweet, dry, dusty aromatic with slight green character associated with dry grasses.
Herb-like	The aromatic commonly associated with green herbs that may be characterized as sweet, slightly pungent, and slightly bitter.
Under-Ripe	An aromatic found in green/under-ripe fruit.
Aroma Intensity	Strength of the aroma – evaluated by smell as well as intensity of volatile flavor when drinking
Overall Acceptability	Overall acceptance of the sample
Mouthfeel / Thickness	The thickness of a sample in the mouth
Amplitude	Overall impression and impact of the product longevity, blending of sensory experience, longevity, body, fullness, flavor and aroma blending together.

7. *Statistical Analysis*

A randomized design was used for the sampling and assigning of each of the 50 bottles of the three treatments in triplicate to each test on each sampling date. The MIXED procedure from Statistical Analysis Software was used to analyze the data, and comparison of means was done using Fisher's LSD method at a significance level of 0.05.

Results

The purpose of this study was to examine the shelf-life of cold water extraction coffees based on sensory and chemical profile and microbial growth, while also investigating the influence of extraction temperature on the chemical and sensorial profile of cold water extraction coffee. Because of the dual purpose of this study, the experiment was structured as a split-plot design – meaning there were multiple independent variables (time and extraction temperature) that affected a large number of dependent variables that were measured. This experimental design, as well as the statistical techniques used, allowed the study of both the effect of time and extraction method on the coffee beverages. For the purposes of this study, shelf life was examined by studying the effect of time – the changes occurring from day 0 to day 42 – whereas the influence of extraction temperature and method was primarily determined by examining the differences between the treatments at day 0.

1. Total Dissolved Solids

The AWE treatment group had the highest mean %TDS, followed by CWE, with HWE at the lowest. As expected, the mean %TDS did not change significantly over time (Figure 2.1).
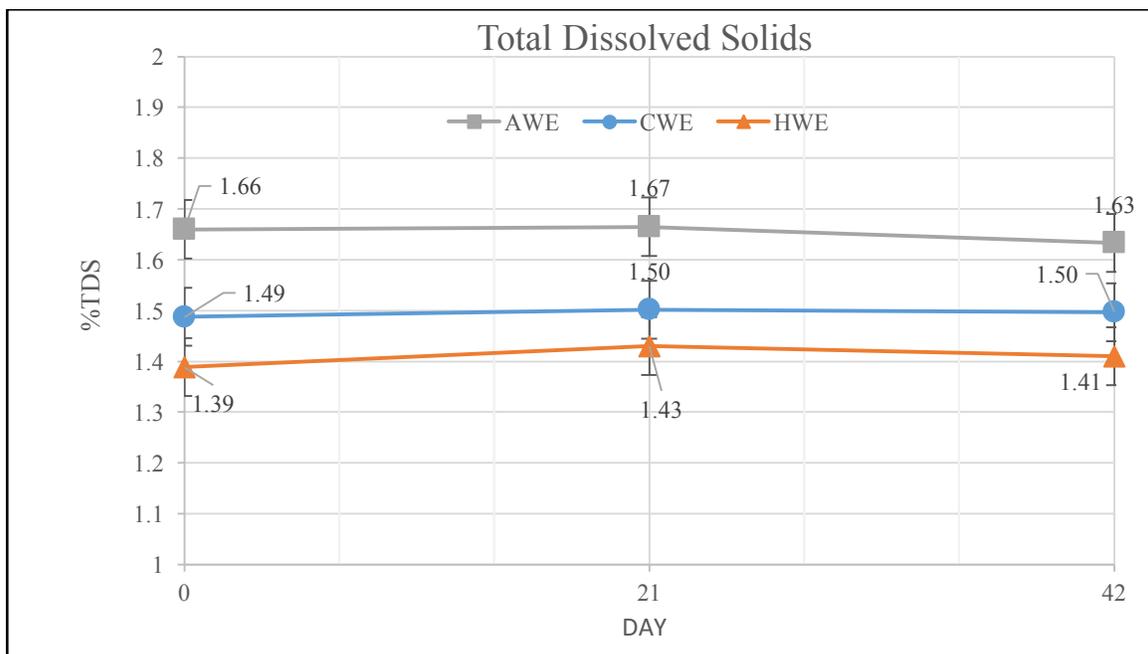

Figure 2.1: Graph of the concentration of total dissolved solids in each extraction treatment at days 0, 21, and 42, expressed in %TDS.

2. Caffeine and CQA Analysis

At day 21, when the first caffeine analysis was performed, there was not a statistically significant difference in the mean concentration of caffeine between the three treatment groups. Interestingly, the CWE and HWE treatment groups did exhibit a significant decrease in the mean caffeine concentration from day 21 to day 42. However,

AWE did not exhibit this significant decrease. As Figure 2.2 shows, there is not a clear

trend in the caffeine concentration; based on these data, neither time nor extraction

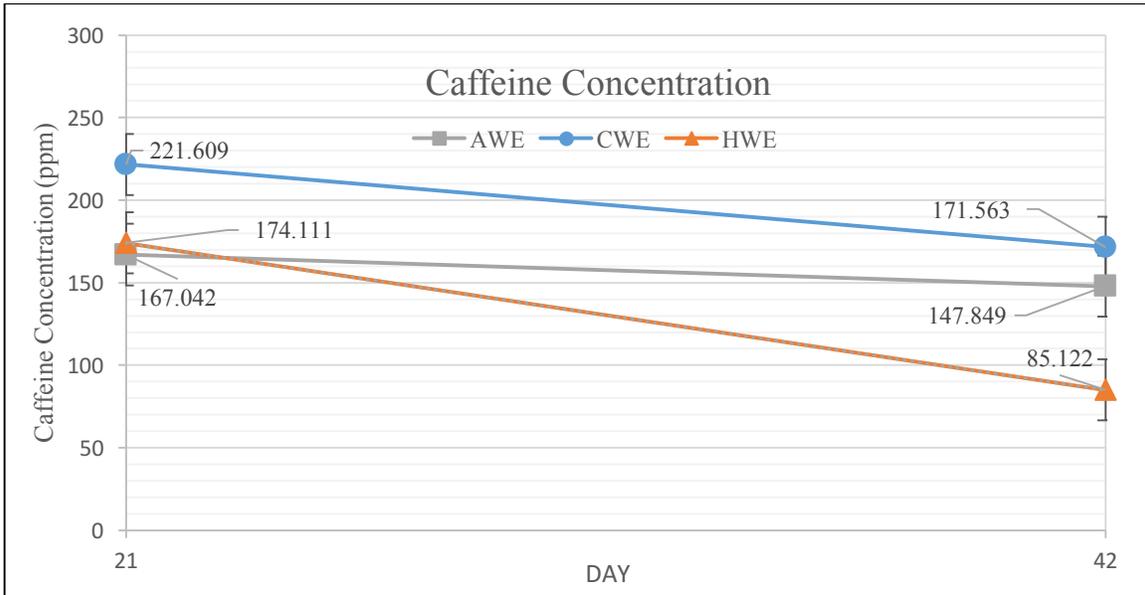method time had a clear effect on caffeine concentration in the coffee beverages.

Figure 2.2: Graph of the concentration of caffeine in each extraction treatment at days 21 and 42, expressed in ppm.

The mean CQA concentration between the three treatment groups was not statistically

different at either sampling date. However, there was a significant decrease in the mean

concentration of CQA in all three treatment groups from day 21 to 42. As Figure 2.3

indicates, this is a clear trend across all three groups. Thus time of storage had a clear

effect on chlorogenic acid concentration in the coffee beverages.
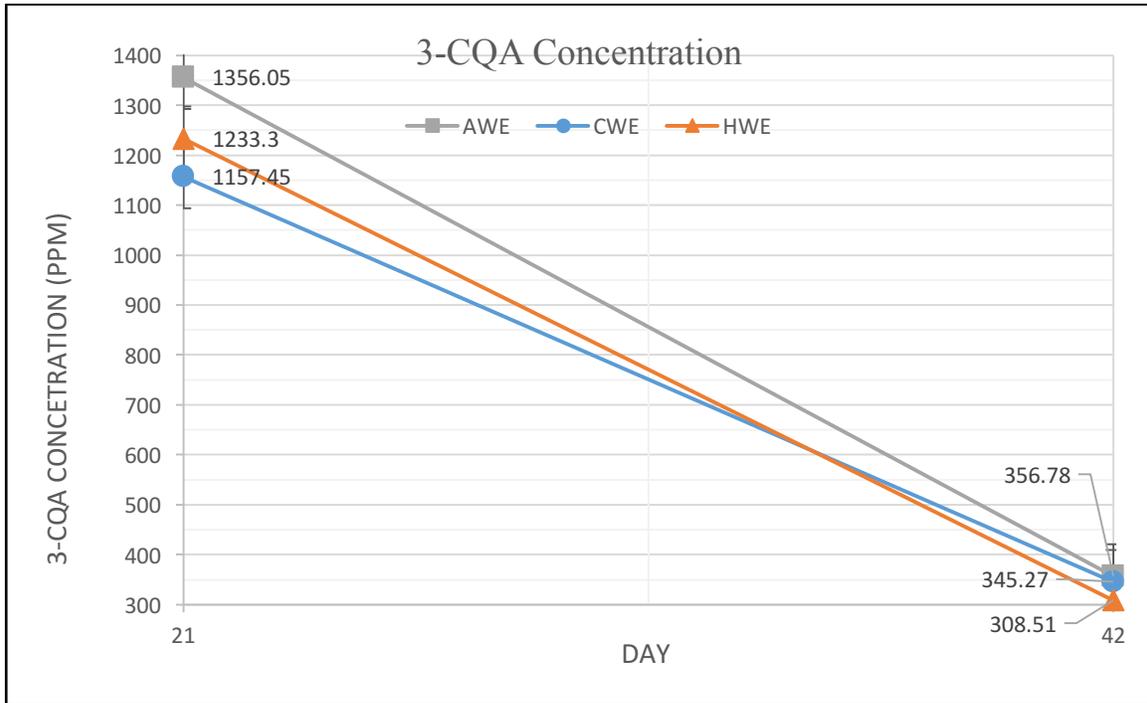
Figure 2.3: Graph of the concentration of 3-CQA in each extraction treatment at days 21 and 42, expressed in ppm.

3. pH and Titratable Acidity

The extraction treatment did affect the pH of the coffee beverages. As Figure 2.4 shows, at day 0, HWE had the lowest pH value, followed by AWE and then CWE. The CWE and AWE did have significantly lower hydrogen ion concentration (measured via pH) than the hot brewed treatment (HWE). This suggests that extraction temperature had a negative correlation with pH: the higher the temperature the coffee was extracted at, the lower the pH. By day 42, the pH of the AWE and CWE were not significantly different from each other, but the mean pH of HWE was still significantly lower than the other two treatments.

There was a statistically significant decrease in the mean pH of all three treatment groups from day 0 to day 42. This indicates that over the 42-day testing period, the concentration of H+ ions increased with time in all of the coffee beverages.
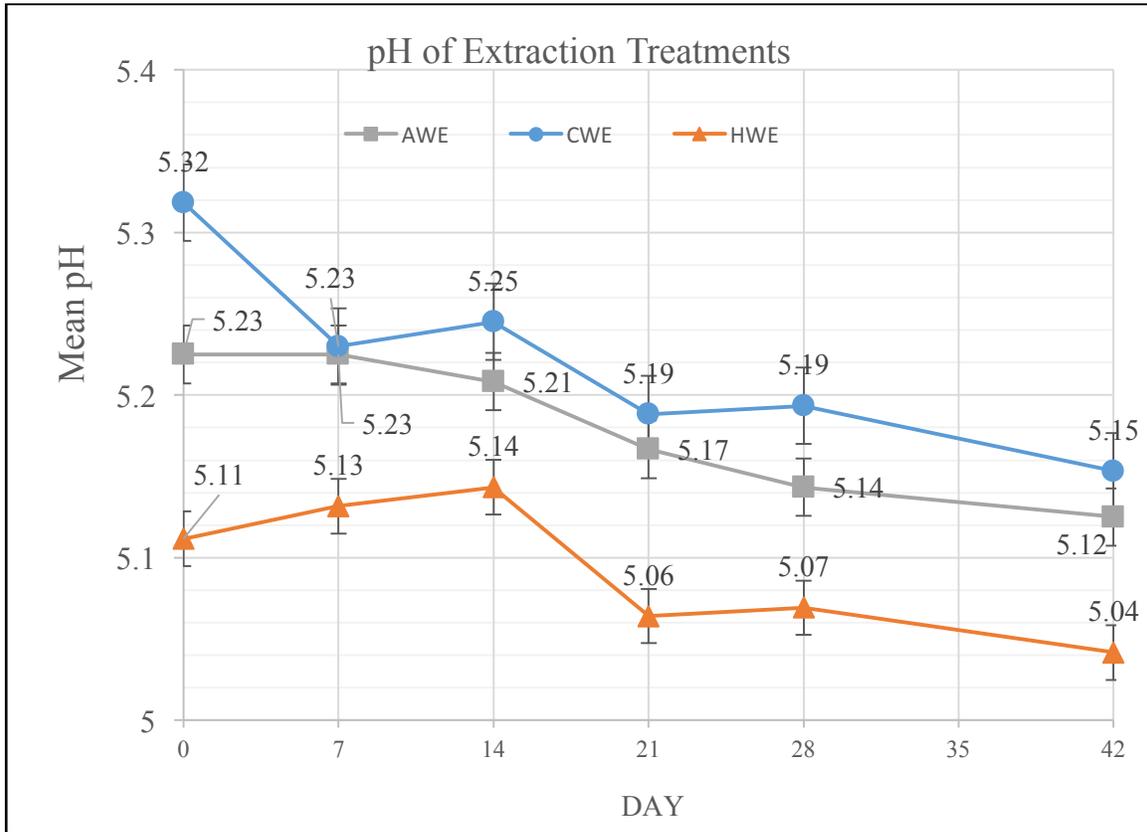

Figure 2.4: Graph of the pH of each extraction treatment at days 0, 7, 14, 21, 28, and 42.

Extraction temperature also had an effect on titratable acidity, however, the trend was not the same as pH. At days 0, 14, 28, and 42 all three treatment groups had significantly different TA (Figure 2.5) Overall HWE had the lowest TA, followed by CWE and then AWE. Thus the coffees that would be considered cold brewed (AWE and CWE) had higher titratable acidity than the hot extracted coffee.

The mean titratable acidity, measured in mL of 0.1M NaOH per 40mL of coffee, increased over the 42-day period in all three treatments, however the increase was only statistically significant for the CWE treatment group (Figure 2.5). This trend agrees with the decrease in pH over the 42 days.
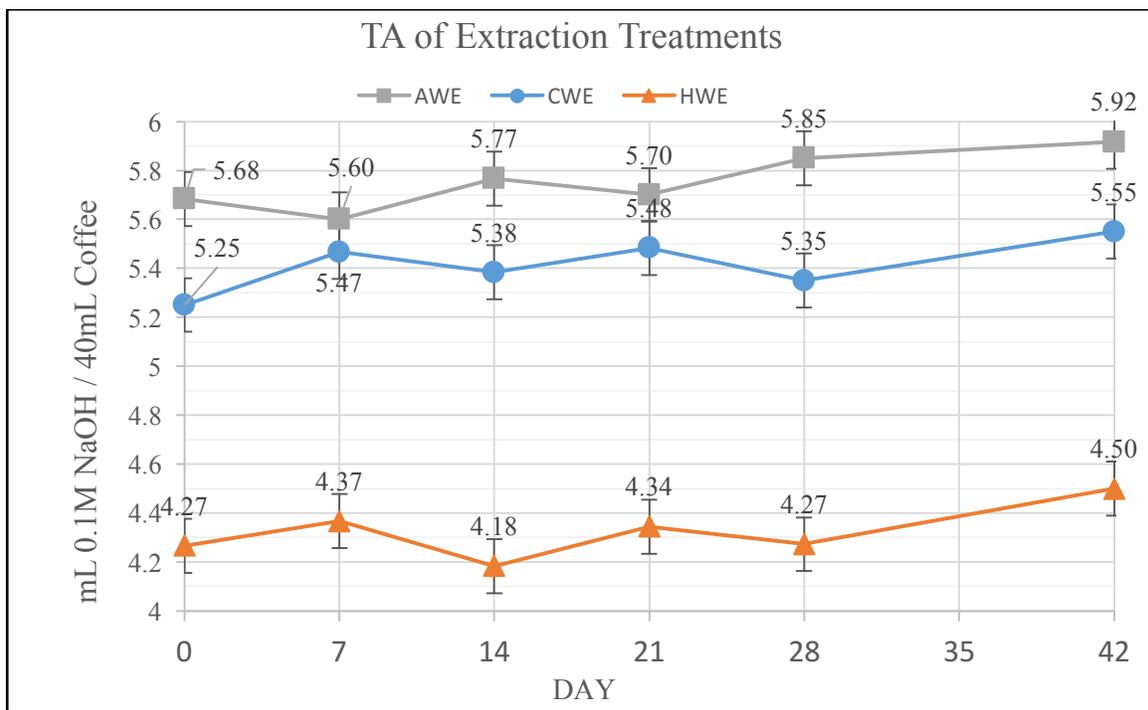

Figure 2.5: Graph of the TA of each extraction treatment at days 0, 7, 14, 21, 28, and 42, expressed in mL of 0.1M NaOH added to 40mL of sample.

4. GC/MS Headspace Analysis

The mean headspace intensity, measured in intensity counts, was highest in the CWE coffee for all three sampling dates (Figure 2.6). At days 0 and 42, the mean headspace intensity of CWE and HWE treatment groups were statistically different from

each other, but not from AWE. Each treatment group did not exhibit a significant change across the three sampling dates – meaning time did not have an effect on the headspace intensity, but the extraction treatment did.
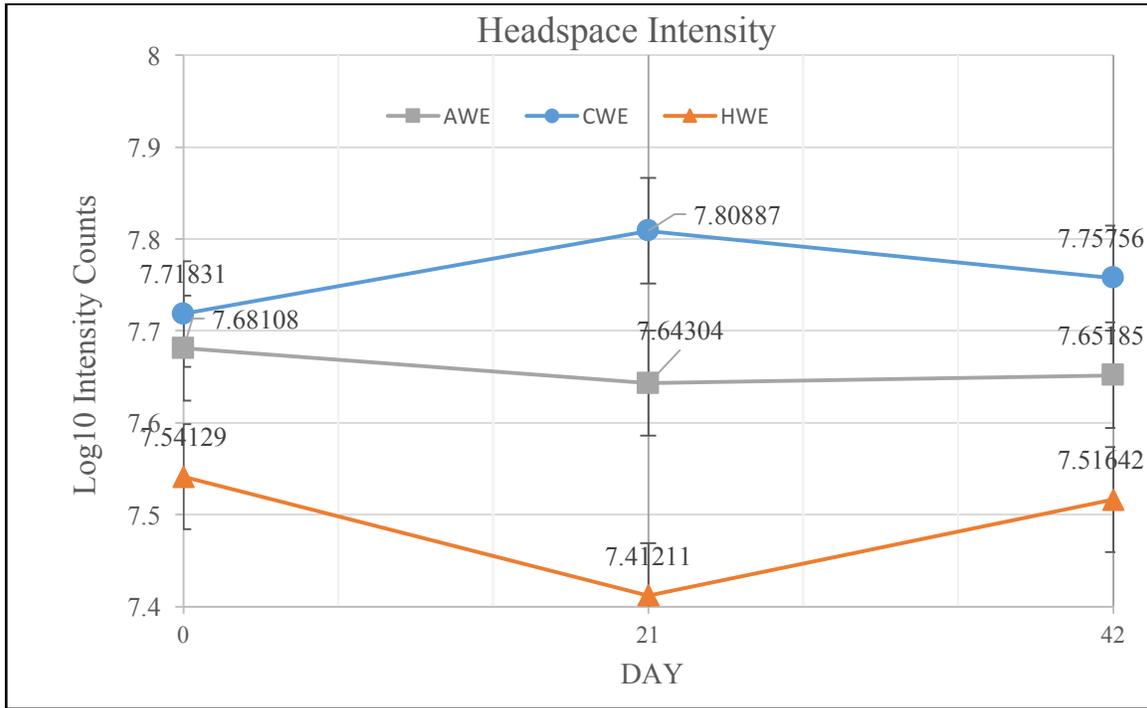

Figure 2.6: Graph of the headspace intensity of each extraction treatment at days 0, 21, 42, expressed in $\log_{10}$ intensity counts.

5. Microbiological Growth (TPC and Psychrotrophic count)

For all sampling dates, (0, 7, 14, 21, 28, and 42 days) all samples were <25 CFU/mL for both the total aerobic count, and for the psychrotrophic count. As all samples were below the countable range, no statistical analysis was performed. However, it can be assumed that there was no detectable aerobic or psychrotrophic growth over the 42-day period.

6. Sensory Descriptive Analysis

6.1 Aroma

Storage time did not have an effect on aroma of the coffee beverages: the mean aroma intensity score did not significantly change over the 42-day period for any of the treatment groups (Figure 2.7). The only significant difference in aroma intensity score between the three treatments was at day 0, when the CWE scored significantly lower than AWE and HWE.
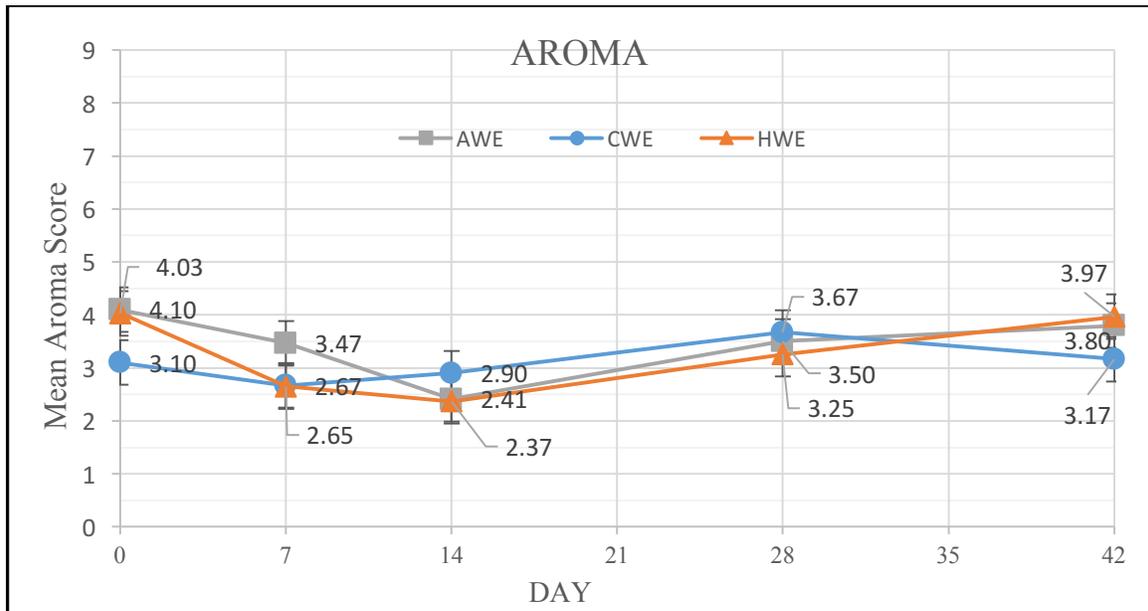

Figure 2.7: Graph of the mean aroma score for each extraction treatment at days 0, 7, 14, 28, and 42 days. Samples were scored on a scale from 0 to 15.

6.2 Texture

Extraction method and storage time had little effect on mouthfeel. The mean mouthfeel score also did not significantly change over the 42-day period for any of the

treatment groups. The only significant difference in mouthfeel between the three treatments was at day 0, when the CWE scored significantly lower than AWE and HWE.
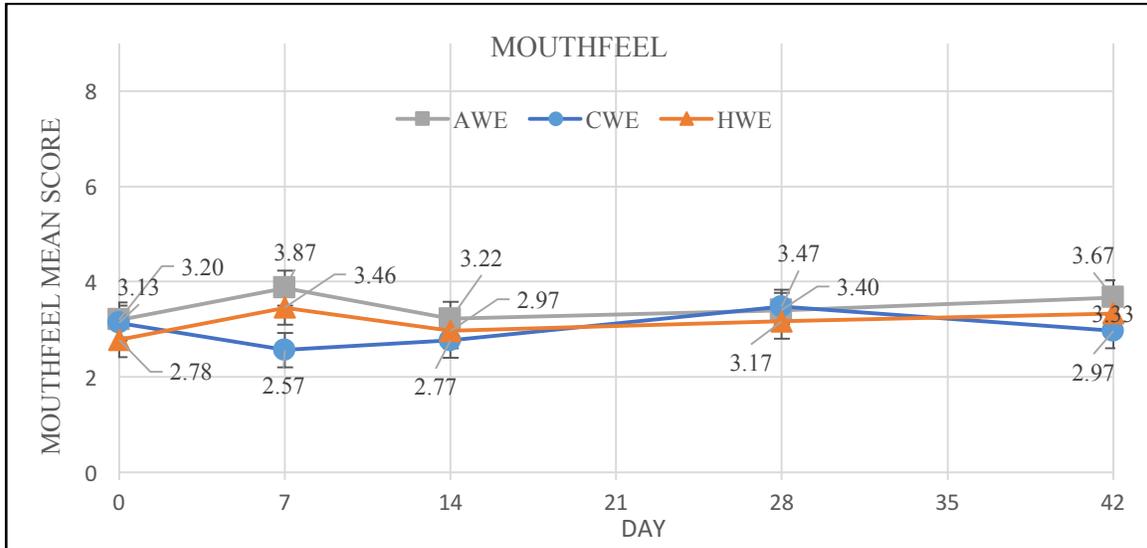

Figure 2.8: Graph of the mean mouthfeel score for each extraction treatment at days 0, 7, 14, 28, and 42 days. Samples were scored on a scale from 0 to 15.

6.3 Flavor Spectrum

The extraction treatment did have an effect on the bitterness perceived in the coffee beverages, as shown in Figure 2.9. At days 0 and 42, the mean bitterness score was significantly higher in the HWE treatment than the other two treatment groups. However, the differences in bitterness between the treatments were nonsignificant on all the other sampling dates. When looking at the effect of time over the 42-day period, there was a significant decrease in bitterness for all treatment groups.
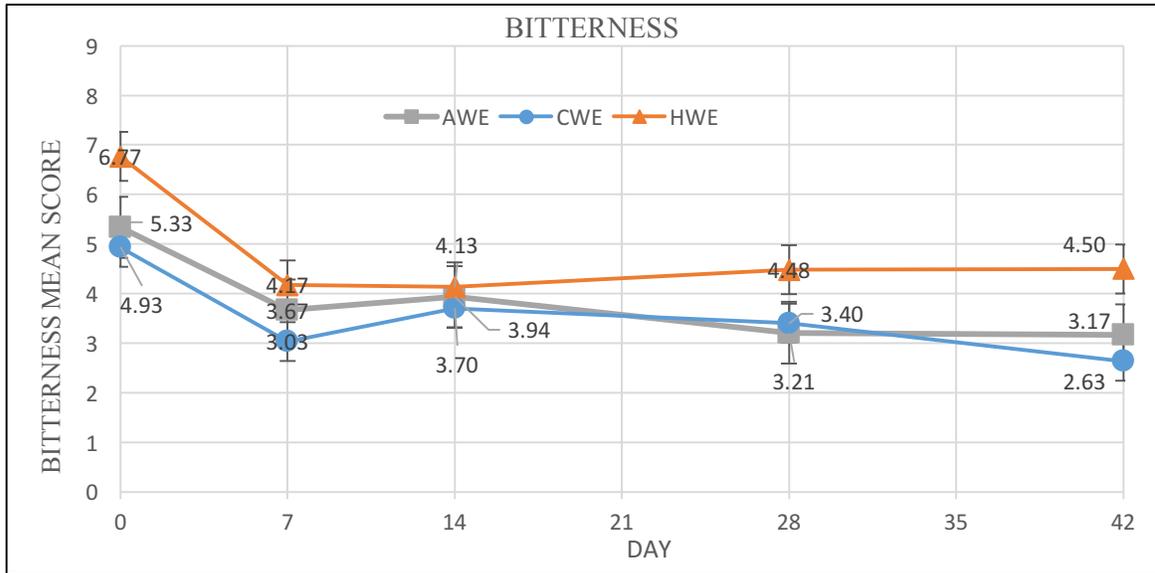
Figure 2.9: Graph of the mean bitterness score for each extraction treatment at days 0, 7, 14, 28, and 42 days. Samples were scored on a scale from 0 to 15.

The only significant differences in acidity between the three treatments were at days 28 and 42 (Figure 2.10). HWE had significantly lower mean acidity scores than CWE and AWE at day 28, and significantly lower scores than AWE at day 42. However, this difference did not appear until the last two sampling dates, and was not apparent at the beginning of the study. Overall, all of the treatments exhibited a nonsignificant increase in mean acidity score over the 42 days.
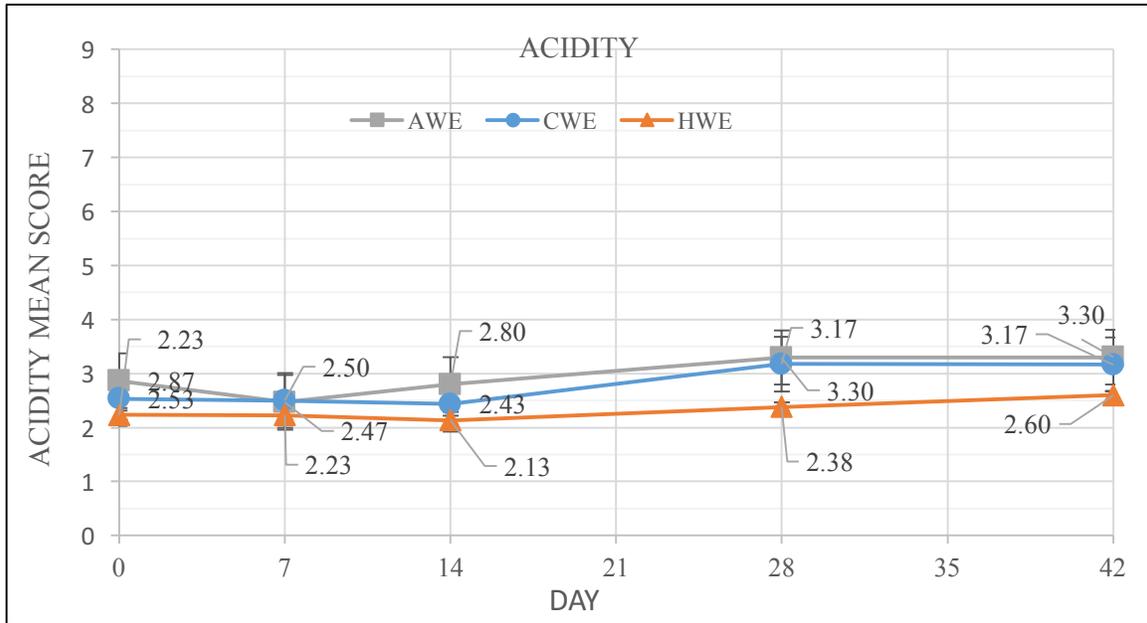
Figure 2.10: Graph of the mean acidity score for each extraction treatment at days 0, 7, 14, 28, and 42 days. Samples were scored on a scale from 0 to 15.

For the effect of time, the mean sweetness score did not significantly change over the 42-day period for CWE and AWE (Figure 2.11). HWE did have an increase in mean sweetness score at day 7, but the score then returned to its prior baseline for the rest of the sampling dates. Between the extraction treatments, HWE exhibited a lower mean sweetness score than both the AWE and CWE treatments across all sampling dates. This difference was statistically significant at days 0 and 42.
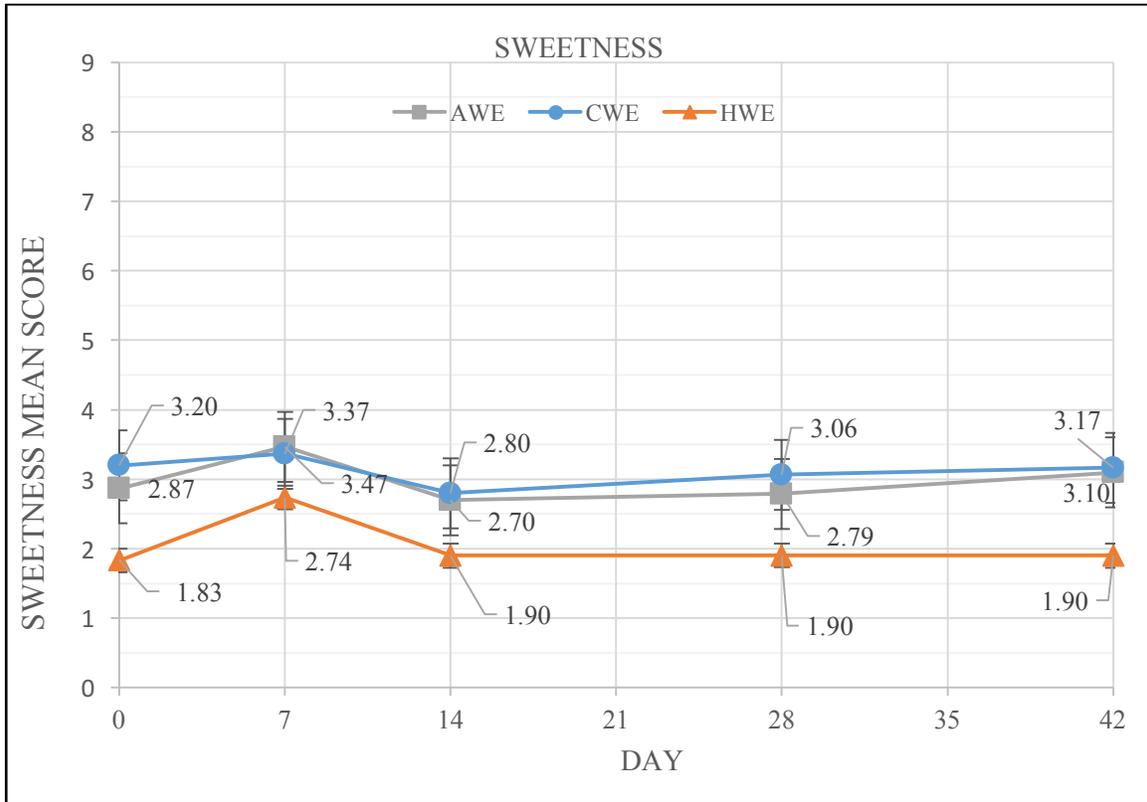
Figure 2.11: Graph of the mean acidity score for each extraction treatment at days 0, 7, 14, 28, and 42 days. Samples were scored on a scale from 0 to 15.

Over the 42-day period, astringency fluctuated in the samples, but comparing the mean astringency score of day 0 and 42, there were no significant changes for all three treatments (Figure 2.12). For the effect of extraction method, there was only a difference at day 14, when CWE had significantly higher mean astringency score. Overall, astringency was not very effected by extraction method or time in this study.
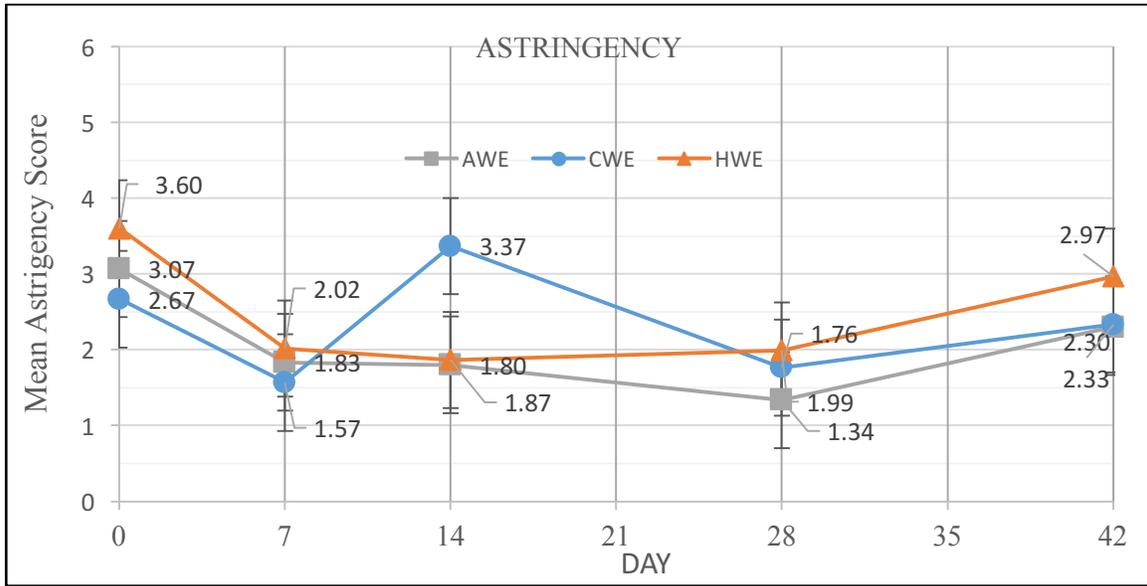
Figure 2.12: Graph of the mean astringency score for each extraction treatment at days 0, 7, 14, 28, and 42 days. Samples were scored on a scale from 0 to 15.

Similarly, the mean longevity score fluctuated in the samples over the 42-day sampling period, but the data do not show any clear trend (Figure 2.12). All three treatment groups had similar longevity scores for all of the sampling dates except for day 42, where HWE was scored significantly higher than CWE, but not AWE.
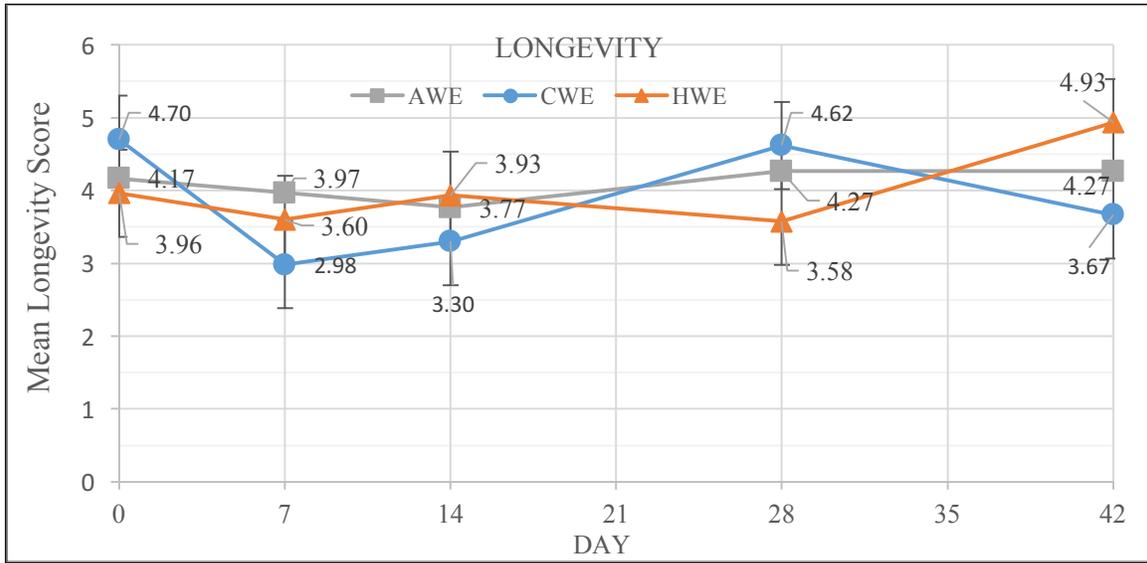
Figure 2.13: Graph of the mean longevity score for each extraction treatment at days 0, 7, 14, 28, and 42 days. Samples were scored on a scale from 0 to 15.

In summary of the changes in sensory scores for flavor profile, at day 0, considered the best case to compare the effect extraction method had on immediate flavor profile, the cold brew treatments had significantly lower mean bitterness scores and higher mean sweetness scores (Figure 2.9 and 2.11). Differences between extraction method in mean acidity, astringency, and longevity were nonsignificant at day 0 (Figures 2.10, 2.12, and 2.13). Figure 2.14 also summarizes these flavor profile changes. The storage time also had significant effects on the flavor profile. Across all treatment groups, bitterness significantly decreased, and acidity nonsignificantly increased (Figure 2.9 and 2.10). Sweetness, astringency, and longevity mean scores fluctuated but did not have any clear trends over time (Figure 2.11, 2.12, and 2.13).
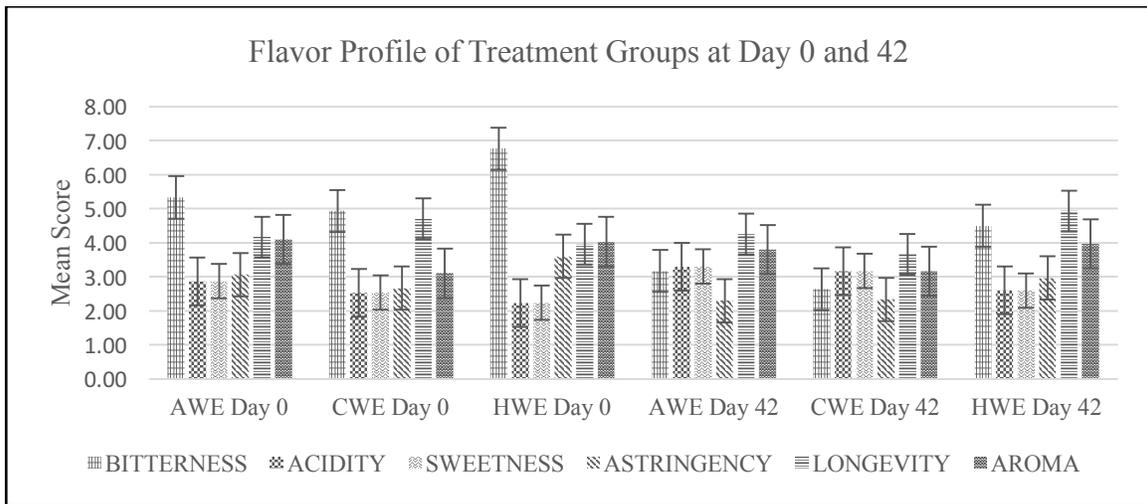
Figure 2.14: Graph of the combined flavor profile of each extraction treatment at days 0 and 42. Samples were scored on a scale from 0 to 15.

6.4 Off-Flavors

An area of particular interest in this study was the development of off-flavors in the stored coffee beverages. The four main categories of off-flavors were sour-fermented-winey-overripe, papery-musty-stale-earthy, chemical-rubber-skunky-medicinal, and vegetative-hay-herb.

For the mean sour-fermented-winey-overripe score, AWE and CWE showed significant increases from day 0 to day 42, and HWE showed significant increases from day 7 to day 42 (Figure 2.15). Treatment group did not have much effect on the mean sour-fermented-winey-overripe score; the only significant difference between treatment groups was at day 28, when HWE was significantly lower than AWE, but not CWE.

Figure 2.15: Graph of the mean sour-fermented-winey-overripe score for each extraction treatment at days 0, 7, 14, 28, and 42 days. Samples were scored on a scale from 0 to 15.

All three treatment groups showed increases in mean papery-musty-stale-earthy score from day 0 to day 42. As shown in Figure 2.16, this trend was clear and consistent. There was not a significant difference in papery score between treatments until days 28 and 42. HWE had a significantly higher papery score than AWE on day 28, and a significantly higher score than CWE on day 42.
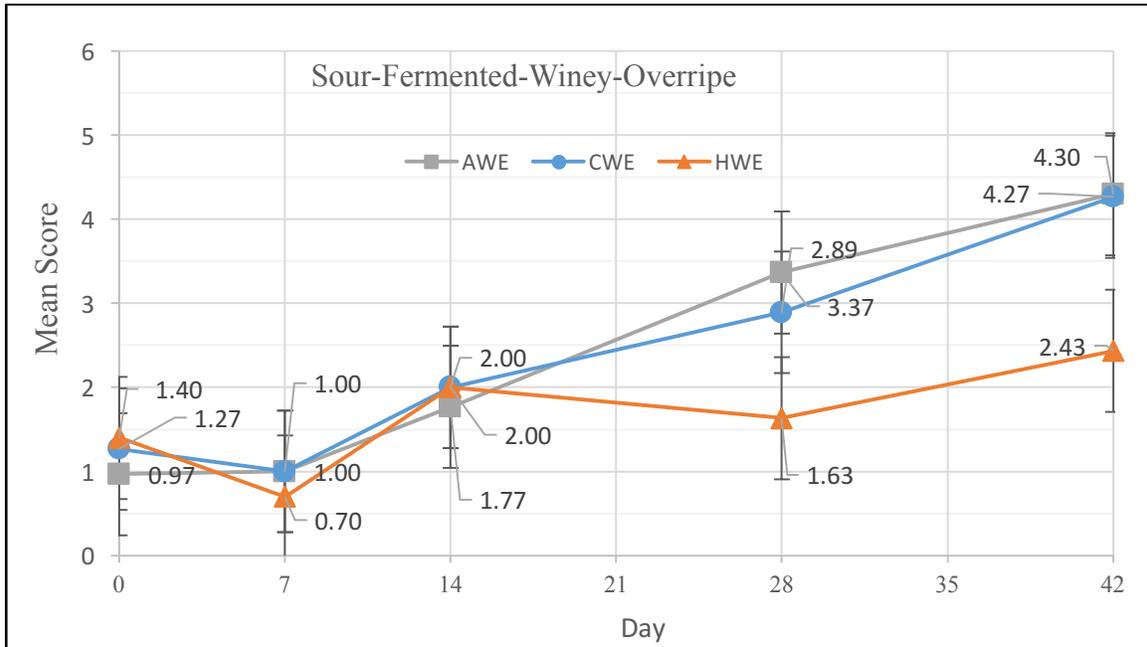
Figure 2.16: Graph of the mean papery-musty-stale-earthy score for each extraction treatment at days 0, 7, 14, 28, and 42 days. Samples were scored on a scale from 0 to 15.

For the mean chemical-rubber-skunky-medicinal score, there was significant increase from day 0 to 42 for treatment groups HWE and AWE (Figure 2.17). HWE was higher than the other treatments in mean chemical score on all sampling dates – this difference was significant on days 0, 14, and 28.

Figure 2.17: Graph of the mean chemical-rubbery-skunky-medicinal score for each extraction treatment at days 0, 7, 14, 28, and 42 days. Samples were scored on a scale from 0 to 15.

All mean vegetative-hay-herb scores were low (Figure 2.18). The highest mean score was for HWE at day 0. There was a significant drop in vegetative score for HWE after day 0. All other changes were nonsignificant. The low scores and nonsignificant changes indicate that vegetative defect is not of concern in the products being tested.
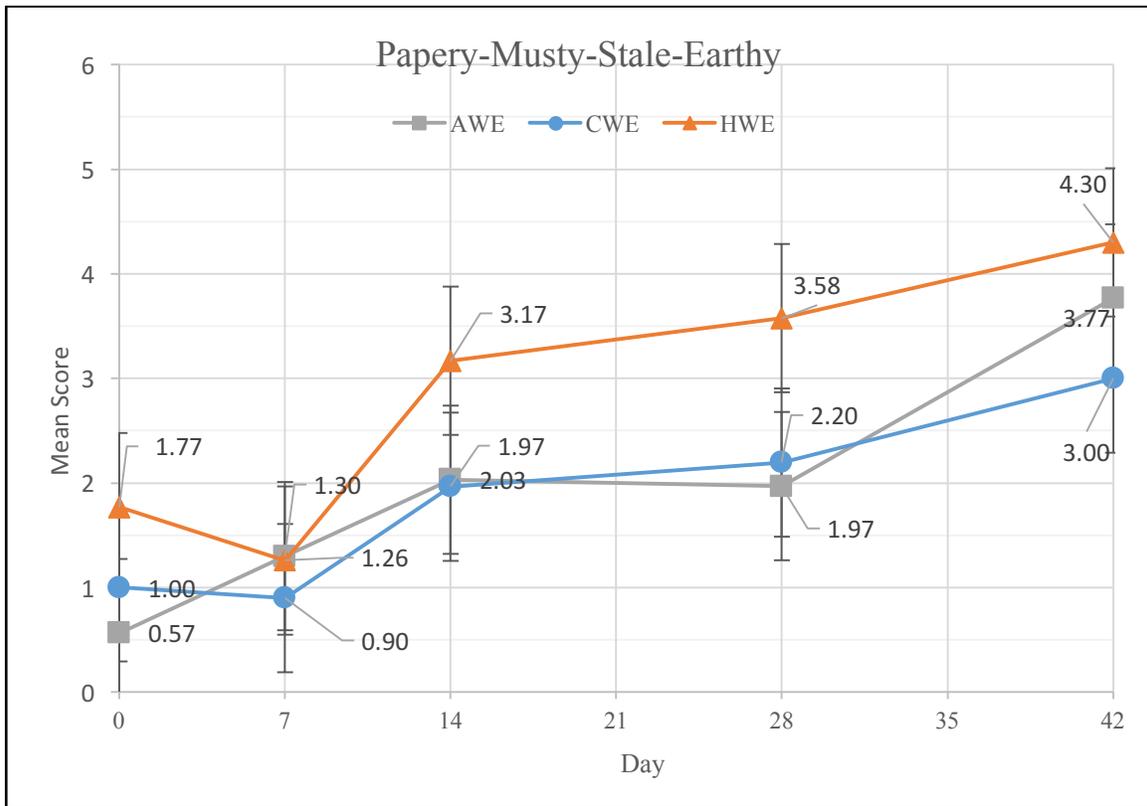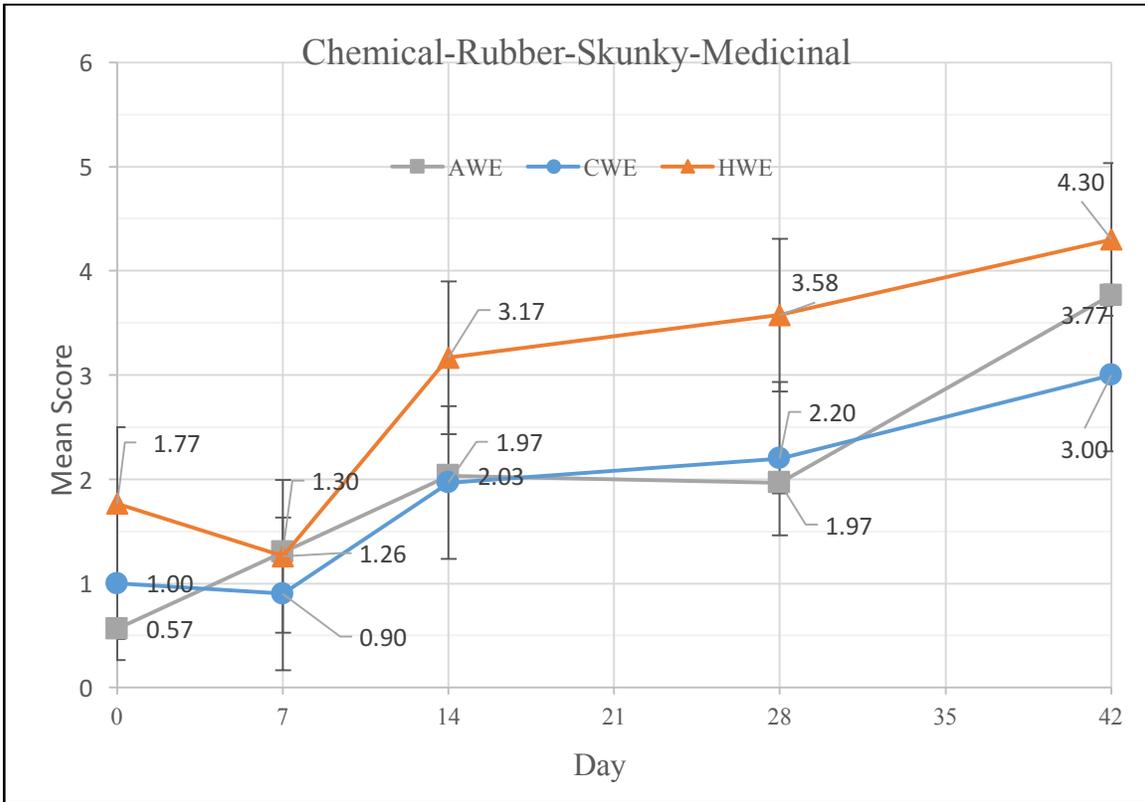
Figure 2.18: Graph of the mean vegetative-hay-herb score for each extraction treatment at days 0, 7, 14, 28, and 42 days. Samples were scored on a scale from 0 to 15.

Overall, for the defects, vegetative flavor did not play much of a role, but for all the other defects, there were significant increases in mean score over time. Sour and papery defect categories increased in all treatments (Figure 2.15 and 2.16), whereas the chemical defect increased steadily and significantly in the HWE treatment group, and significantly for AWE after day 28 (Figure 2.17). Figure 2.19 summarizes the off-flavor scores for the samples at day 42.
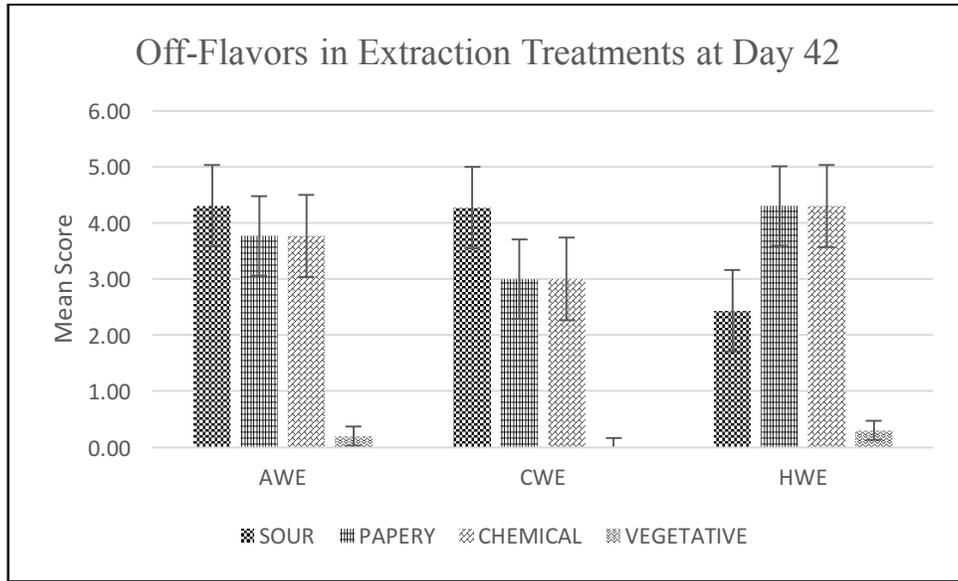
Figure 2.19: Graph of the mean off-flavor scores for each extraction treatment at days 42 days. Samples were scored on a scale from 0 to 15.

6.5 Amplitude

The only significant change over time in mean amplitude score was for the CWE treatment group, which showed a decrease in amplitude after day 0 that was significant for all sampling dates except day 28. At day 0, HWE was significantly lower than AWE and CWE in mean amplitude score, but otherwise there were no significant differences in amplitude across treatment groups (Figure 2.20).
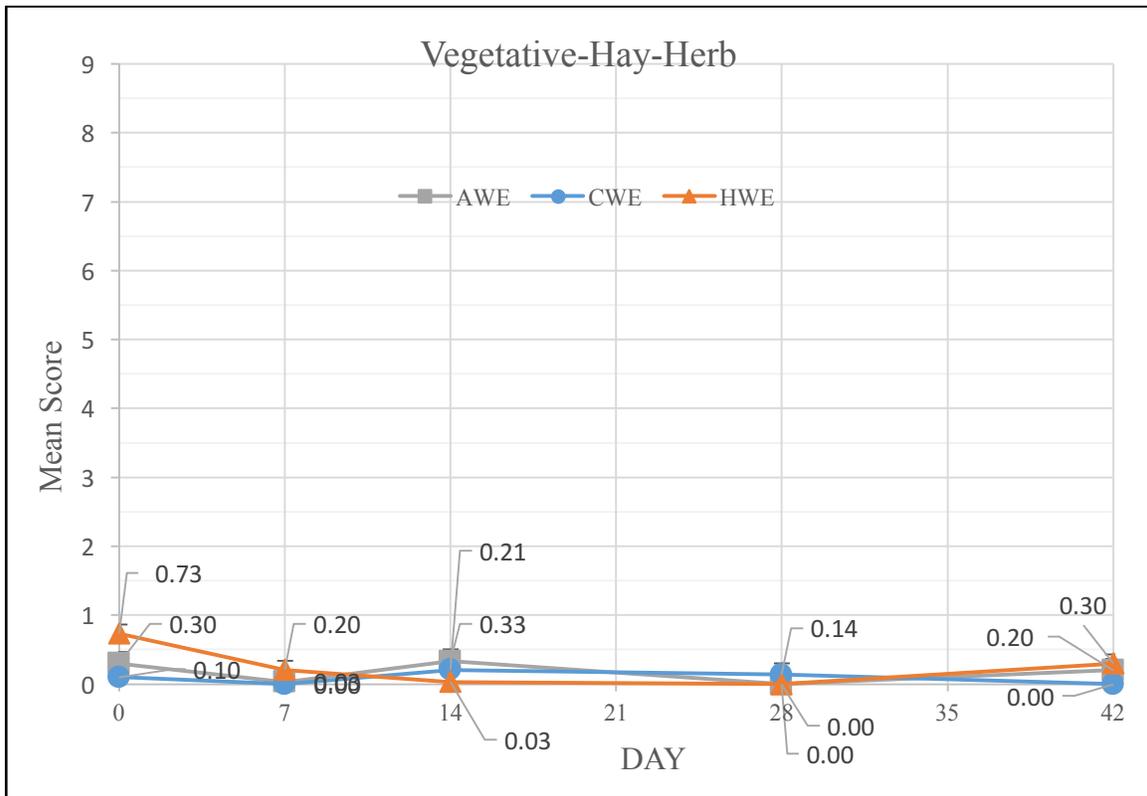
Figure 2.20: Graph of the mean amplitude score for each extraction treatment at days 0, 7, 14, 28, and 42 days. Samples were scored on a scale from 0 to 15.

6.5 Acceptability

When examining the effect of extraction method on acceptability, there was not a significant difference for the mean acceptability scores between the different extraction methods at days 0 and 42, but there were differences at the other sampling dates, mainly due to the rapid decline in acceptability of the HWE treatment.

For mean acceptability score, all three treatment groups showed a clear decreasing trend that was accompanied by a statistically significant decrease from day 0 to 42. This decrease was most marked in HWE.
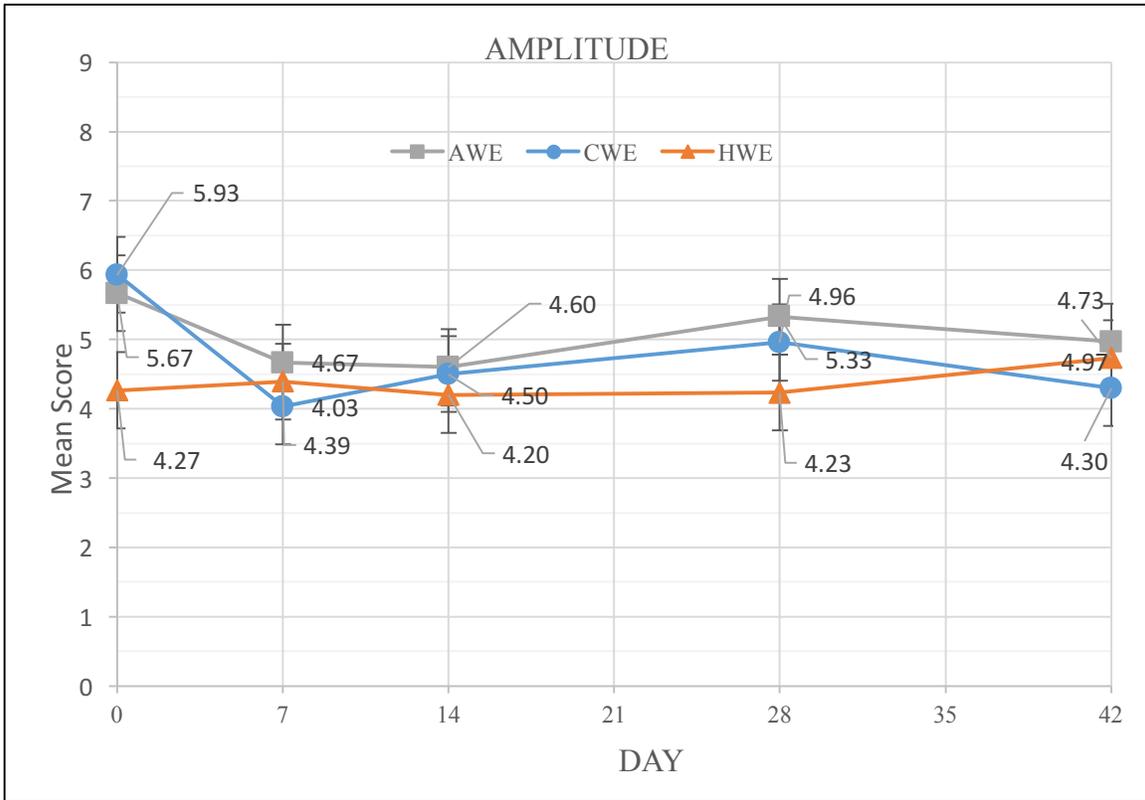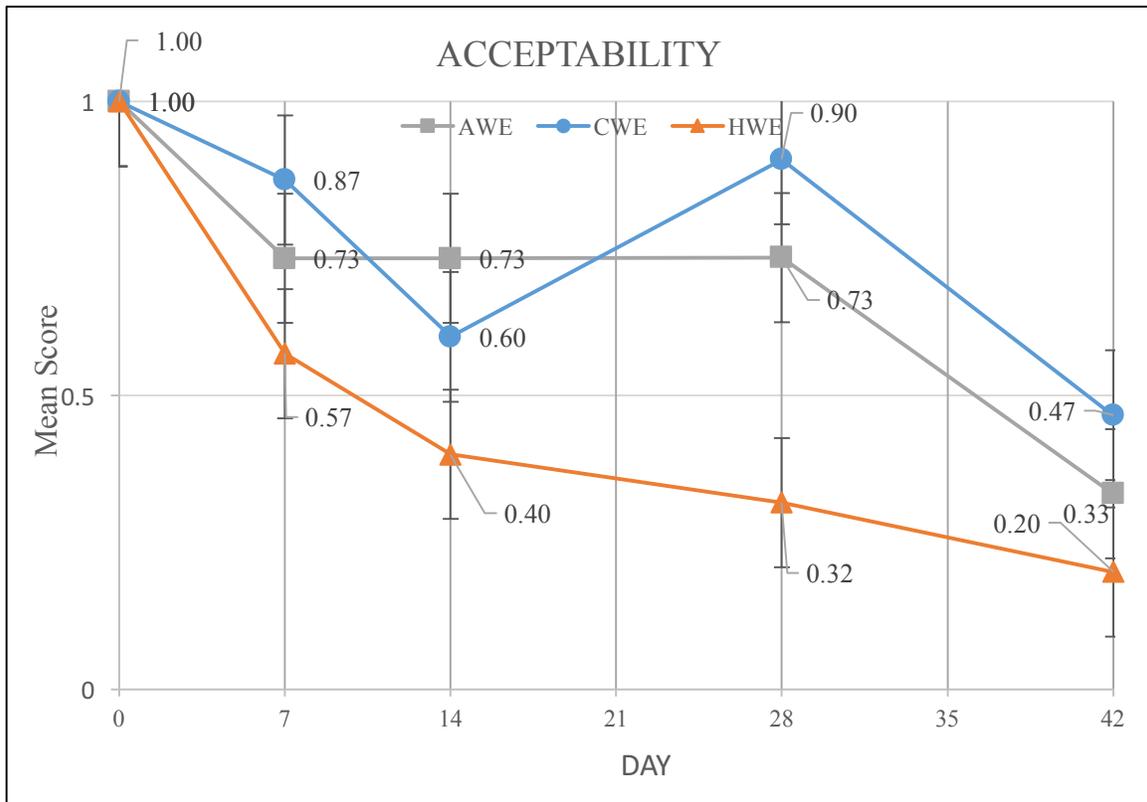
Figure 2.21: Graph of the mean acceptability score for each extraction treatment at days 0, 7, 14, 28, and 42 days. Samples were scored either acceptable (1) or unacceptable (0).

Discussion

1. *Shelf Life – Significant Microbial Changes*

One of the primary goals of this study was to investigate the shelf-life of cold coffee by tracking chemical, organoleptic, and microbial changes. In the evaluation of the shelf life of a product, the safety of the product and limitation of microbial growth is paramount. In this study, there was no detectable bacterial growth in any of the samples. This agrees with other established work on refrigerated coffee brews, but is the first known work on storage of cold brew. This lack of microbial growth in coffee extracts stored at refrigeration temperatures is likely due to the multiple hurdles presented:

scarcity of microbial nutrients, low pH, and other antimicrobial factors present within the coffee (Daeschel, and others, 2017). All bottles and equipment for brewing in this study were sanitized with a sodium hypochlorite solution before brewing and bottling. Thus the microbial growth results here may not necessarily be representative of all producers of cold brew. In addition to large volume bottlers, many independent, small cafés are now producing cold brew in-house, and this could lead to considerable variance in sanitation practices and consequently, a range in microbial stability. Although Daeschel's challenge study demonstrated that the cold brew tested (Stumptown) did not favor the growth of microorganisms when large numbers of pathogenic microorganisms are introduced, more testing is needed, and it is recommended that all producers of cold brew follow good manufacturing practices and put preventative controls in place to prevent contamination of their product (Daeschel, and others, 2017). Many bottled cold brews have ingredients added to them, such as sweeteners or dairy, that would remove the hurdle of the lack of microbial nutrients. This further reinforces the importance of good manufacturing practices while making cold brew – no matter the size of the producer.

Finally, this study, and the one by Daeschel and others, only tested for vegetative growth, which are the primary concern for products that are held constantly at refrigeration, but are not the only concern for products being sold unrefrigerated. In September of 2017, Death Wish Coffee issued a recall for its Nitro Cold Brew Cans due to concerns about the growth of *Clostridium botulinum*, a spore-forming, anaerobic pathogen (FDA, 2017). Any cold brew being sold as a shelf-stable canned product is classified as a low-acid product, thus it must be processed to prevent growth of *C.*

botulinum. Further work is recommended to study the effect of processing on bottled or canned cold brew coffee.

2. *Shelf Life – Significant Chemical Changes*

The lack of microbial growth over 42 days means that the shelf life evaluation must depend on the chemical and sensorial changes in the coffee extracts. As coffee is an incredibly complex chemical solution, this study was far from being able to analyze all chemical changes that occurred over the 42-day period. The chemical parameters studied were %TDS, caffeine concentration, 3-CQA concentration, headspace intensity, pH, and titratable acidity. Of these chemical parameters, the most significant changes over the storage time that were consistent across treatments were the decrease in 3-CQA concentration and the decrease in pH.

The decrease in pH for the coffee brews in this study (Figure 2.4) agrees with previous work on aging of coffee brews under refrigerated storage (Pérez-Martínez and other, 2008), however, this is the first known study to establish this decrease in pH for coffee extracted at low temperatures. Other researchers (Dalla Rosa) have proposed a certain pH (4.8) as a limit of acceptance for shelf-life, however, more recent work from Perez-Martinez and others on stored coffee brews has suggested that this pH limit is not suitable to be applied to all coffees and all brewing methods, and "pH is not the only factor which determines the limit of acceptance of coffee brews." (Perez-Martinez and others, 2008; Dalla Rosa and others, 1990). The results presented here agree with Perez-Martinez's conclusions, as the coffees never reached the limit of acceptance proposed by Dalla Rosa, but they all had significantly lowered sensory acceptability scores by the end

of the testing period (Figure 2.21). Chemical analysis alone is not the best way to determine shelf-life of brewed coffees, and the results of this study validate the importance of sensory evaluation. Furthermore, when comparing pH values from this study to pH values from Perez-Martinez's work with Colombian Arabica coffee, it is clear that there is considerable variance in pH values based on extraction method and coffee origin. The titratable acidity, which is a better measure of acid's contribution to flavor profile of a beverage, also experienced an increase for all three treatments over the 42 days, however, the increase was only significant for the CWE treatment (Figure 2.5).

One of the other significant chemical evolutions over the 42-day period was the decrease in 3-CQA concentration (Figure 2.3). The thermal degradation of chlorogenic acid into caffeic and quinic acids is known to cause development of sourness (Clarke and Vitzthum, 2008). This effect has mainly been demonstrated at elevated temperatures, and some have suggested that this would not occur at refrigeration temperatures (Perez-Martinez and others, 2008). The effect does increase with time, and the length of storage may make up for the lack of elevated storage temperatures in this study. One possibility for further investigation is that the decrease of 3-CQA observed in this study was due to a low temperature hydrolysis of CQA into caffeic and quinic acids, which contributed to the staling and loss of quality in the beverages over time. Increase in quinic acid has been indicated to be the compound primarily responsible for increasing sour perception in brewed coffee (van der Stegen and van Duijn, 1987). However, this study was limited in its scope and without analyzing the degradation products of 3-CQA, it is impossible to

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know for certain if this decrease in concentration of chlorogenic acid was the cause of the decline in sensory acceptability.

3. *Shelf Life – Significant Sensory Changes*

Of the sensory attributes evaluated in this study, the most significant changes over the storage time that were consistent across treatments were the decrease in mean bitterness score, and the increase in mean score for sour-fermented-winey-overripe, and papery-musty-stale-earthy (Figures 2.9, 2.15, 2.16). There was a clear relationship between storage time and decreasing bitterness score, as well as sour and papery defect score for all treatments. It is quite possible that the decrease in mean bitterness score was caused by the interaction of bitterness and sour perception – meaning the increase in sour off-flavor dulled the perception of bitterness. There were other significant changes from storage time, but these are the changes that were universal to all three treatments.

In addition to defect scores, the mean acceptability was scored by the panelists. Time of storage did have a clear negative effect on mean acceptability score for all samples (Figure 2.21). There was a clear negative trend of acceptability over time with one main exception: the CWE score on day 28. The mean acceptability score was defined as "the overall acceptance of the sample" and was intended to be used to indicate if the product is acceptable to be sold and consumed from an organoleptic perspective. The panelists were forced to make a binary choice, acceptable (1), or unacceptable (0), and the mean score was tabulated. This was the only sensory attribute that was not based on quantifiable, fixed reference points from the WCR Sensory Lexicon. Consequently, this attribute was the most subjective to panelist bias and opinion. The panelists, who were all

trained cuppers (specialized coffee sensory evaluators) often have to evaluate acceptance of hot coffee samples, but as the cold brew industry is still developing, there may not be uniformity of opinion on what is acceptable or unacceptable. This lack of uniformity likely led to confusion among the panelists on the term "acceptability" which lead to the outlier at day 28 for the CWE treatment that did not agree with the general trend of the other treatments. Based on the accompanying defect scores for CWE, which rose at day 28, it is clear that this score is not indicative of the true quality of the sample at that point in time. However, this does suggest that as the cold brew industry grows, if quality standards are to be maintained, a level of standardization is needed in a manner similar to hot brewed coffee. This study's application of the WCR Sensory Lexicon to cold brew was the first known of its kind, and many of the reference standards and intensity scales were well suited to evaluating cold coffee. It is recommended that there be further official adaptation of the lexicon to cold coffee, as well as further standardization of overall quality of cold brew coffee across the sector. Green coffee quality is scored using a cupping form that sums many sensory attributes into an overall quality score. A similar method could be developed to evaluate cold brew. Overall, this would greatly benefit the cold coffee industry, and would help ensure that every consumer that tries a cold brew product for the first time has a high quality product.

Although there was an outlier in the acceptability scores, the defect scores, which are based on quantifiable standards, still support the trends that acceptability scores show. The decline in acceptability over time was most exaggerated on the HWE coffees, as HWE had a lower mean acceptability score on all sampling dates after day 0. The

development of defects and the decline in acceptability observed in the samples over time was clear, and lines up with previous work on stored coffee brews. Storage effects are well documented in hot brewed coffees, and these results confirm that the sensory deterioration of cold brew with storage time is similar, but not identical, to hot brewed coffees. As discussed in the previous section, it is unclear what the exact chemical cause of these sensory changes is, but these results are helpful, as they show the development of these defects over time, even at refrigerated storage.

Another insight that can be gleaned from these results is that the cold brew treatments did not deteriorate in the exact same manner or time as the hot brewed treatment. This is demonstrated in the fact that the extraction method did influence the defect scores: there were some notable differences in the staling of the HWE vs. AWE and CWE. Notably, HWE had significantly higher chemical scores overall, and sour scores were significantly higher for the cold brew extractions at the last sampling date, perhaps suggesting that the chemical-rubber-skunky-medicinal flavor is more likely to develop in hot brewed coffees, whereas sour-fermented-winey-overripe is more likely to develop in colder brewed coffees (Figure 2.15 and 2.17). Furthermore, the HWE decreased more quickly than the CWE and AWE in the mean score for overall acceptability (Figure 2.21). This suggests that coffees brewed at low temperatures may have a longer organoleptic shelf life than coffees that have been brewed at high temperatures and then cooled. This may be due to the multitude of staling reactions that increase with rising extraction temperature (Clarke and Vitzthum, 2008).

4. *Influence of extraction temperature and method – Chemical profile*

The other purpose of this study was to investigate the influence of extraction temperature on the chemical and sensorial profile of cold water extraction coffee. Chemically, there were a number of differences between the hot brewed coffee and the two cold brewed coffees.

One of the primary differences in this study was the strength of the beverages. The AWE had the highest %TDS, followed by CWE and then HWE (Figure 2.1). %TDS is the measure of how many solids are actually dissolved in the aqueous solution, thus many other chemical properties are highly correlated with it. All groups used the same grind size, but the HWE treatment group used a lower water-to-coffee ratio than the two immersion methods, so the difference in %TDS between the hot brew and the cold brew cannot be entirely attributed to the influence of extraction temperature. However, all of the brewing parameters were identical between AWE and CWE in order to isolate the effect of the temperature of the brewing. The higher concentration of soluble solids in the AWE coffees, brewed at 25ºC, than the CWE coffees, brewed at 4ºC, agrees with previous literature that higher temperatures increase the extraction rate (Rao, 2010, Uman and others, 2016).

The extraction temperature also significantly influenced the pH and TA of the coffee extracts (Figure 2.4 and 2.5). The HWE had significantly lower pH than the two cold brewed coffee extracts. The CWE had the highest pH, with AWE in the middle, so for these extractions, the higher the temperature of the extraction, the lower the pH of the resulting extract. The titratable acidity reversed the trend of pH though, as the HWE had the lowest titratable acidity, followed by the CWE and then AWE. This means that

although the HWE had greater free H+ concentration, it had lower total acidity, as measured by TA, than the two cold brewed extracts. Titratable acidity is "a better predictor of acid's impact on the on flavor than pH" so it follows that the two cold brewed treatments, with higher TA, ended the study with a significantly higher mean sour sensory score than the HWE group (Nielsen, 2003). This reversal of pH and TA results is worth considering – the cold brew treatments had higher TA, but a lower hydrogen ion concentration, and inversely, the hot brewed treatment had lower TA and higher H+ concentration. From these two data points, it seems that the higher brewing temperature of the HWE extracted more completely dissociated acids (leading to a low pH, low acidic flavor impact), whereas the cold brew extracted more weak acids, which have lower levels of completely dissociated ions, leading to a higher pH, and high TA, with more higher flavor impact.

The low H+ concentration, high TA of the AWE and CWE could also be a function of the concentration of total dissolved solids in each of the coffee extracts. The TDS decreased from AWE, to CWE, to HWE, and similarly, the TA decreased from AWE, to CWE, to HWE. The higher TA in CWE and AWE could have simply been because those two solutions had more compounds solvated per mL of water. Results from Gloess and others comparison of extraction methods in 2013 support this: "In this study (based on one single type of coffee), a positive correlation of the refractive index (°Brix) with the following attributes was observed: concentration of total solids, headspace intensity, concentration of caffeine, 3-CQA and 5-CQA, and titratable acidity." When

comparing extraction methods, it is important to consider that the total concentration of a coffee solution will dictate many of the other chemical parameters.

Interestingly, the extraction temperature also influenced the headspace intensity of the coffee extracts (Figure 2.6). The CWE and AWE had higher mean headspace intensity on all three sampling dates than the HWE, and for the CWE, this difference was significant. Much of the appreciated flavor of coffee is present in the aroma/headspace of the brew, so it is interesting to consider that the lower temperature/longer time extractions actually had a higher concentration of volatiles. Further work needs to be done to elucidate these differences, as HS intensity is a rough measure of volatile concentration, and is heavily influenced by the strength of the solution, and the selection of the compounds for sampling.

5. *Influence of extraction temperature and method – Sensory profile*

Overall, the extraction method of the coffee did affect the flavor spectrum of the coffee beverages resulting in three products with distinct organoleptic characteristics. CWE and AWE, had significantly higher mean sweetness scores and significantly lower mean bitterness scores than the traditionally extracted HWE at days 0 and 42 (Figure 2.14). These sensory data suggest that cold brewing does create a beverage distinct from traditional hot brewed coffee that is consumed immediately or after cold storage for extended times. Some producers of cold brew claim that it is sweeter than hot brewed coffee, and these data from this study support this claim (Stumptown Coffee Roasters, 2018; Starbucks, 2018).

Another attribute of the coffee extracts that extraction temperature seemed to influence were the off-flavors at the end of the 42-day period (Figure 2.19). Based on the higher chemical scores for the HWE treatment, and the significantly higher sour scores for CWE and AWE, the data suggest that the chemical-rubber-skunky-medicinal defect was more likely to develop in hot brewed coffees, whereas the sour-fermented-winey-overripe is more likely to develop in colder brewed coffees. Further investigation is needed, but the sensory results also may point to low temperature extraction having greater flavor stability. At the end of the 42-day period, all treatments had significantly lower acceptability scores, but the cold extraction coffee still had the highest mean acceptability score, followed by the ambient extraction, with the hot extraction at the lowest. Based on these results, it seems that the colder extraction reduced the flavor deterioration of the brewed coffee. For example, at day 42, for both chemical, and papery defects, the CWE mean is significantly lower than the HWE, but the AWE mean is not significantly different than either. In many cases, the means for flavor profile attributes between CWE and AWE could not be separated, meaning the flavor profile between the different methods of making cold brew was very similar. However, the largest difference between the two temperatures of extraction that are both considered cold brew may be this difference in deterioration.

6. *Considerations – Panel Size*

One consideration and limitation to the results presented here is that the sensory descriptive analysis was performed with a panel limited to five members. However, these members were professionals in the coffee industry who are engaged in tasting coffee in

order to make purchasing decisions at least once a week. In this way, the panelists were experts whose experience goes beyond those of a typical trained panel. The use of a randomization plan, blinding codes, and samples in triplicate further reinforces the reliability of the sensory data.

7. *Considerations – Caffeine Concentration Decrease*

One unexpected result from this experiment was the decrease in caffeine concentration from day 21 to 42. This decrease was significant for the CWE and HWE treatment groups, but not for AWE. Caffeine is a purine alkaloid with a high boiling point, and it is stable during the intense thermodynamic environment of coffee roasting (Ludwig and others, 2014; National Center for Biotechnology Information, 2018). Previous work on caffeine concentration in coffee has not shown any significant changes due to storage time (Perez and others, 2008; Sopelana and others 2013). It is surprising that a decrease in concentration from day 21 to 42 was shown here. However, it could have been due to human experimenter error and inexperience with the HPLC system that the caffeine was analyzed with, or stratification of the coffee bottles and incorrect sampling leading to bottles with lower concentrations of caffeine. The difference in concentration could also be attributable to caffeine's tendency to undergo association in aqueous solutions (Guttman and Higuchi, 1957). Caffeine often exists as a monomer, dimer, or tetramer, and this aggregation could have caused a shift in absorbance that caused the detector, which was set to a single wavelength, to not detect all of the caffeine present. Further tests are needed to confirm the results shown here.

Conclusions

Based on the data presented here, the refrigerated shelf life of hot and cold brewed coffee is limited not by microbial stability, but rather by deterioration in sensory attributes, reinforcing the importance of sensory evaluation in quality control. Further work is recommended to elucidate the mechanisms of coffee staling in a refrigerated environment, with particular interest in the degradation products of chlorogenic acid, as a significant decline in 3-CQA concentration was found over the storage period. In order to ensure safety, good manufacturing practices, preventative controls, and maintenance of the cold chain are recommended for all producers of cold brew coffee.

Cold extracted coffees were found to be chemically and organoleptically different beverages from hot brewed coffees. For flavor profile the cold extracted coffees had higher sweetness and lower bitterness than the hot extracted coffee, supporting claims made by producers of cold brew. The cold extracted coffees also had greater flavor stability, as they exhibited a more gradual increase in off-flavor scores, and slower decrease in acceptability.

Based on the chemical and sensory differences between hot and cold extracted coffees, as well as the defect and acceptability scores over time, it is clear that if quality standards in cold extracted coffee are to be maintained, a level of standardization is needed in a manner similar to hot brewed coffee. This study's application of the WCR Sensory Lexicon to cold brew was the first known of its kind, and many of the reference standards and intensity scales were well suited to evaluating cold coffee. It is recommended that there be further official adaptation of the lexicon to cold coffee, as

well as further standardization of overall quality of cold brew coffee across the sector.

Green coffee quality is scored using a cupping form that sums many sensory attributes

into an overall quality score. A similar method could be developed to evaluate cold brew.

Overall, this would greatly benefit the cold coffee industry, and would help ensure that

every consumer that tries a cold brew product for the first time has a high quality product.

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APPENDICES

Sensory Evaluation Form for Cold Brew Coffee

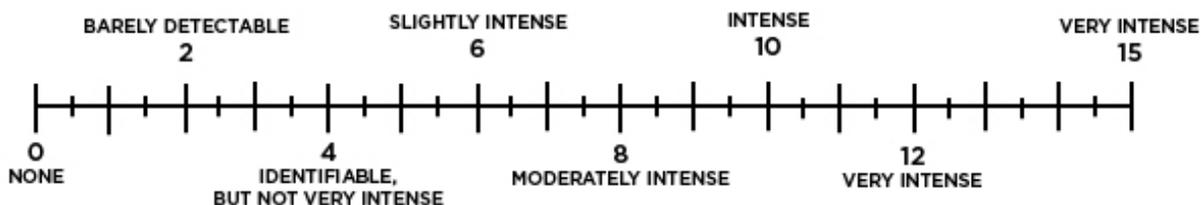**Sensory Evaluation Form – Cold Brew Coffee**

DATE _____

SAMPLE CODE _____

PANELIST NUMBER_____

Please evaluate each coffee sample on the intensity of the twelve sensory attributes, using a scale from 0 to 15. Please circle the line that represents the score you would like to give the intensity of the attribute. Between samples, please cleanse your palate.
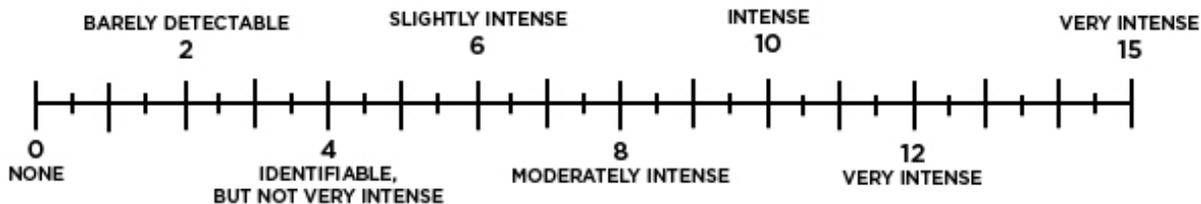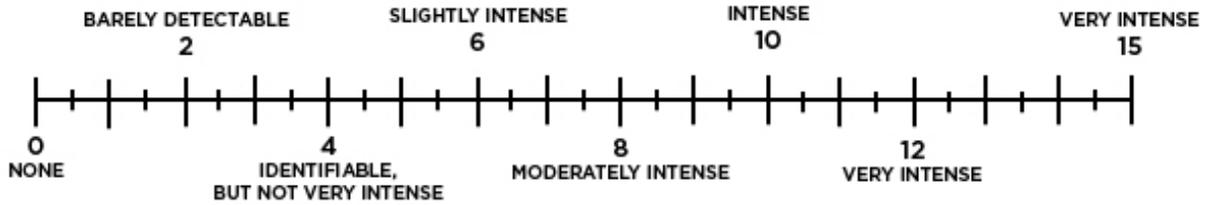
AROMA

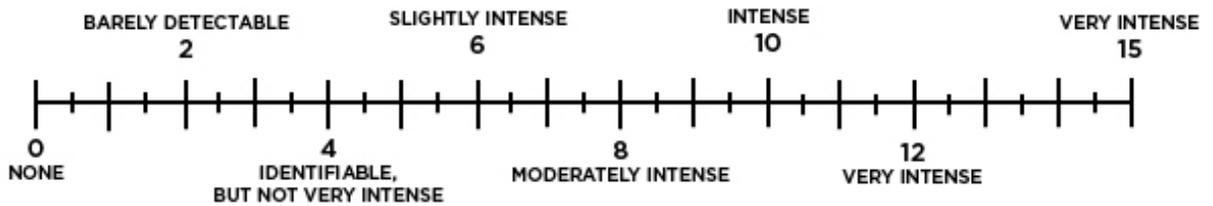### Overall Aroma Intensity

TEXTURE

Mouthfeel / Thickness

74

TASTE / FLAVOR SPECTRUM

Bitterness

BARELY DETECTABLE
2

SLIGHTLY INTENSE
6

INTENSE
10

VERY INTENSE
15

0
NONE

4
IDENTIFIABLE,
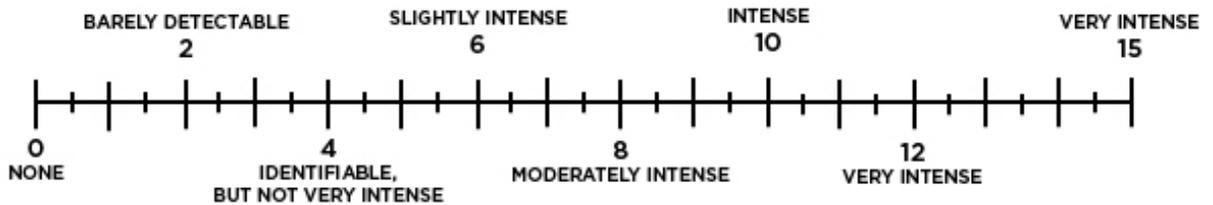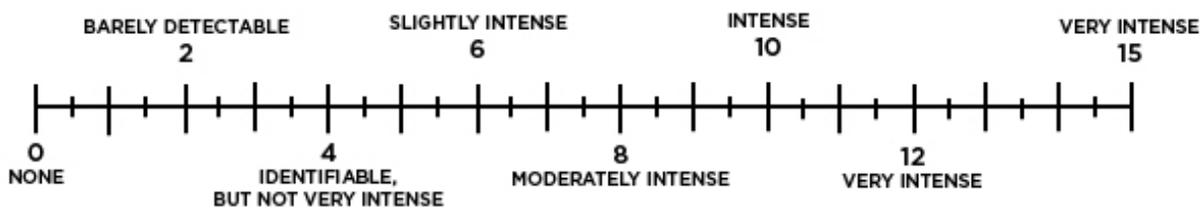BUT NOT VERY INTENSE

8
MODERATELY INTENSE

12
VERY INTENSE

Acidity

BARELY DETECTABLE
2

SLIGHTLY INTENSE
6

INTENSE
10

VERY INTENSE
15

0
NONE

4
IDENTIFIABLE,
BUT NOT VERY INTENSE

8
MODERATELY INTENSE

12
VERY INTENSE

Sweetness

BARELY DETECTABLE
2

SLIGHTLY INTENSE
6

INTENSE
10

VERY INTENSE
15

0
NONE

4
IDENTIFIABLE,
BUT NOT VERY INTENSE

8
MODERATELY INTENSE

12
VERY INTENSE

Astringency / Mouth Drying

BARELY DETECTABLE
2

SLIGHTLY INTENSE
6

INTENSE
10

VERY INTENSE
15

0
NONE

4
IDENTIFIABLE,
BUT NOT VERY INTENSE

8
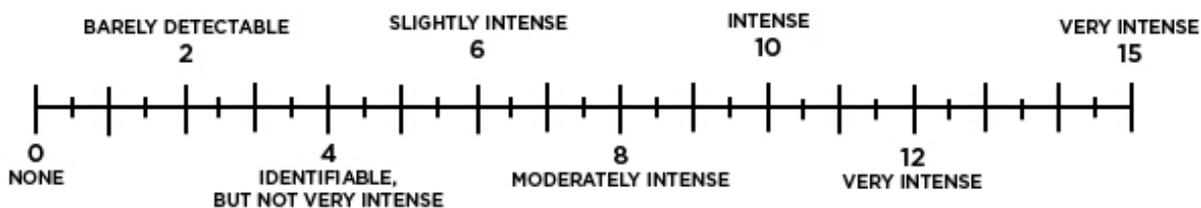MODERATELY INTENSE

12
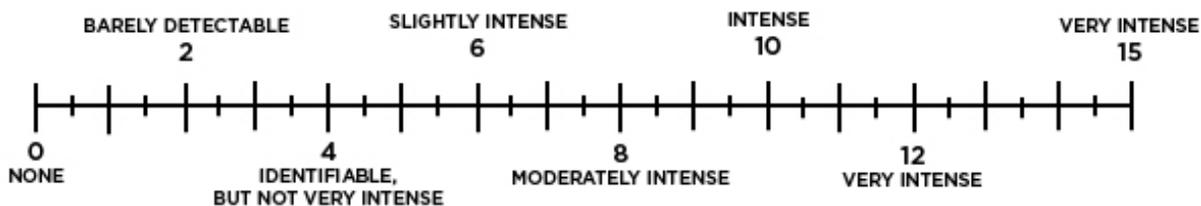VERY INTENSE

Longevity

Off-Flavors
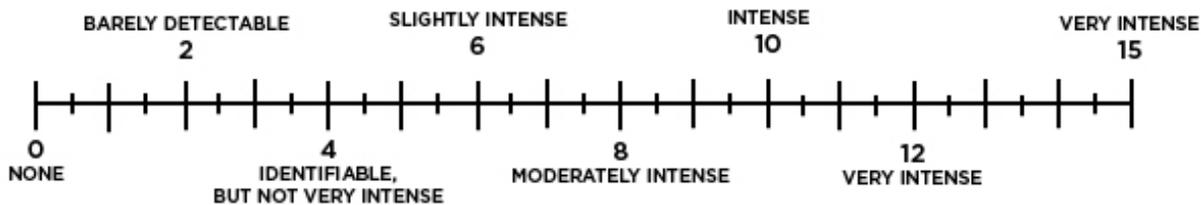
Sour/Fermented/Winey/Over-Ripe
Identify:_____

Papery/Musty/Stale/Earthy
Identify:_____

Chemical/Rubber/Skunky/Medicinal
Identify:_____

Vegetative/Hay/Herb
Identify:_____

| BARELY DETECTABLE | SLIGHTLY INTENSE | INTENSE | VERY INTENSE |
| 2 | 6 | 10 | 15 |

| 0 | 4 | 8 | 12 | |
| NONE | IDENTIFIABLE, BUT NOT VERY INTENSE | MODERATELY INTENSE | VERY INTENSE | |

CONGRUENCE

Amplitude
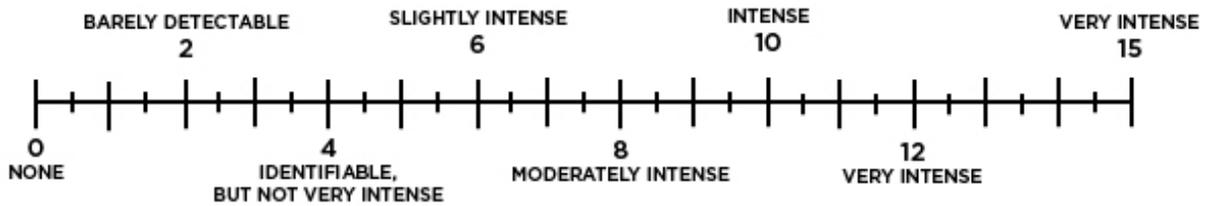
| BARELY DETECTABLE | SLIGHTLY INTENSE | INTENSE | VERY INTENSE |
| 2 | 6 | 10 | 15 |

| 0 | 4 | 8 | 12 | |
| NONE | IDENTIFIABLE, BUT NOT VERY INTENSE | MODERATELY INTENSE | VERY INTENSE | |
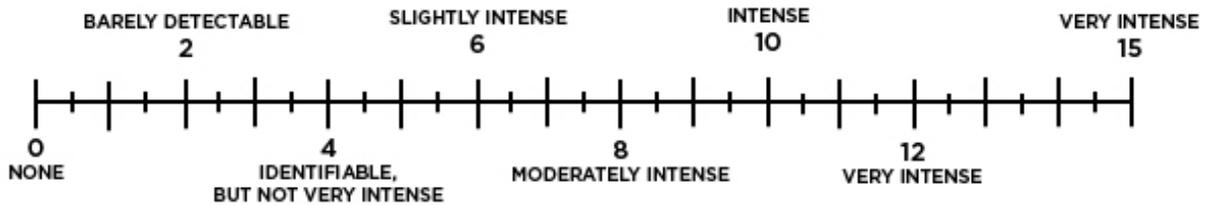
Overall Acceptability (please only mark acceptable or unacceptable)

ACCEPTABLE

UNACCEPTABLE

Guide to Sensory Attributes (adapted from WCR Sensory Lexicon).

Attribute	Definition
Overall Acceptability	Overall acceptance of the sample
Acidity	The fundamental taste factor associated with a citric acid solution.
Sweetness	Fundamental taste sensation of which sucrose is typical. Generally associated with sweet aroma descriptors such as fruity, chocolate and caramel.
Bitterness	The fundamental taste factor associated with a caffeine solution
Astringency / Mouth Drying	Characteristic of an after-taste sensation consistent of a drying effect in the mouth. A drying, puckering, or tingling sensation on the surface and/or edge of the tongue and mouth.
Longevity	Persistence of flavor in the mouth, The time that the full, integrated sensory experience sustains itself in the mouth and after swallowing.
Stale	The flavor characterized by a lack of freshness
Papery	The aromatic associated with white paper cups.
Musty/Earthy	The somewhat sweet, heavy aromatic associated with decaying vegetation and damp, black soil.
Fermented	The pungent, sweet, slightly sour, sometimes yeasty, alcohol-like aromatic characteristic of fermented fruits or sugar or over-proofed dough.
Over-Ripe	The sweet, slightly sour, damp, musty/earthy aromatic characteristic of fruit or vegetable past their optimum ripeness.
Winey	The sharp, pungent, somewhat fruity, alcohol-like aromatic associated with wine.
Medicinal	A clean, sterile aromatic characteristic of antiseptic-like products such as Band-Aids, alcohol, and iodine.
Rubber	A dark, heavy, slightly sharp, and pungent aromatic associated with rubber.
Skunky	A combination of aromatic associated with skunks
Hay-like	The lightly sweet, dry, dusty aromatic with slight green character associated with dry grasses.
Herb-like	The aromatic commonly associated with green herbs that may be characterized as sweet, slightly pungent, and slightly bitter. May or may not include green or brown notes.
Under-Ripe	An aromatic found in green/under-ripe fruit.
Aroma Intensity	Strength of the aroma – evaluated by smell as well as intensity of volatile flavor when drinking
Blended	The melding of individual sensory notes such that the products present a unified overall sensory experience as opposed to spikes or individual notes.
Amplitude	Overall impression and impact of the product longevity, blending of sensory experience, longevity, body, fullness, flavor and aroma blending together.