CAUDILL, MORGAN. The Effect of Accelerated Processing Conditions on Cold Brew Coffee. (Under the direction of Dr. Gabriel Harris).

Consumer demand for cold brew (CB) coffee has significantly increased over the last few years. To meet future production requirements, understanding of CB coffee must also improve. CB allows ground coffee to infuse in cold water for extended periods of time, which can increase production costs and may affect beverage safety. Sales of ready-to-drink (RTD) beverages have also risen over recent years. RTD beverages are convenient for consumers and could either be refrigerated or shelf stable. As industry capitalizes on interest in CB coffee and RTD beverages, it will become increasingly necessary to define CB coffee as a beverage and to improve processing capabilities.

This research compared CB coffee to commercial and homemade beverages to help inform industry on current standards. Hot brew and CB coffees were purchased from restaurants, grocery stores, and also made in the lab. The hot brew coffee was made at coffee to water ratios of 27.5 g/L, 55 g/L (the standard ratio), and 110 g/L while the CB coffee was made at 55 g/L to determine which concentration was most similar to CB. Commercial CB coffees varied widely in their sensory (standard sourness, bitterness units), physical (total dissolved solids (TDS), color measured by L* value), and chemical (caffeine, chlorogenic acid) characteristics. Sourness ranged from 0.78-3.72, bitterness 4.55-6.95, TDS 0.76-3.07%, L* 1.83-29.74, caffeine and chlorogenic acid (3-CQA) from 30.35-77.98 and 5.59-85.43 mg/100 mL, respectively. In-house CB coffee (55 g/L) characteristics were most similar to the 27.5 g/L hot brew coffee, which brewed at a rate over 200 times faster than the CB coffee. This suggests that CB coffee uses an unnecessary amount of coffee beans and time.
To improve processing conditions of CB coffee, an initial study of the various ways to produce coffee was conducted. CB coffee was compared to hot brew coffee which is produced much more quickly. CB was also compared to coffee which received a brief heat treatment, from either hot water or microwave heating, by analyzing the following attributes: sourness, bitterness, TDS, L*, caffeine, and 3-CQA. Steeping CB samples for longer (5 h vs 3 h) did not result in statistical differences (sour, 1.58-1.63; bitter, 4.19-4.53; TDS, 0.84-0.93; L*, 40.15-38.78; caffeine, 55.81-74.99; 3-CQA, 44.11-47.05). The microwave treated samples were extracted much more quickly and only had a few differences when compared to CB samples.

To further determine the effect of temperature combinations on CB coffee, this study analyzed hot brewed coffee (HB), CB, heat-treated CB coffee (H-CB), and microwave treated CB coffee (M-CB) over time in terms of four attributes: L*, TDS, and mg/100 g caffeine and 3-CQA. L* decreased over time for both HB and CB (34.98-17.19 vs 64.62-43.00), while TDS (0.89-1.39 vs 0.29-0.85), caffeine (48.90-84.39 vs 15.23-61.42) and 3-CQA (33.60-62.85 vs 5.55-44.82) increased. H-CB and M-CB attributes remained constant after the heat treatment (L*, 34.46-35.33 vs 29.23-29.29; TDS, 0.80-1.03 vs 1.00-0.94; caffeine, 56.10-62.21 vs 60.88-69.85; 3-CQA, 39.17-46.00 vs 41.39-49.95), were similar to CB samples, but required less preparation time. All HB values fell within reported ranges. A brief heat treatment prior to cold infusion accelerates CB production, allowing industry to develop faster, less costly processing methods.

In conclusion, more research is needed to further profile CB coffees and determine how the process can be optimized and defined. This study analyzed the physical, chemical, and some sensory properties but did not include volatiles. Therefore, it could be beneficial to investigate the volatile compounds associated with these processing methods and with commercial coffees. The
results of this research indicate that it may be possible for industry to develop faster, less costly CB processing methods.
The Effect of Accelerated Processing Conditions on Cold Brew Coffee

by
Morgan Frances Caudill

A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

Food Science

Raleigh, North Carolina
2020

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DEDICATION

To those that fill my life with love and happiness.
BIOGRAPHY

Morgan Caudill was raised on a small family beef farm in North Carolina. By high school she was responsible for operating and managing the daily tasks and long-term goals of the farm in order to optimize profitability. Activities on the farm included hay production, maintenance of farm equipment, managing the health of animals, and cost assessment of daily operations. During Morgan’s time on the farm, significant changes in climate resulted in many challenges such as decreased hay production. These challenges were overcome by experimenting with the use of drought resistant grass varieties within plots of the pasture. It was also necessary to develop creative solutions for mechanical issues that arose. These experiences influenced Morgan’s decision to pursue a science and engineering career. After graduating as salutatorian from East Montgomery High School, Morgan used the profits from the farm to pay for college. The management of farm operations was then turned over to her younger sister.

Morgan went on to complete a Bachelor’s degree in Bioprocessing Science at NC State University. As an undergraduate student Morgan was very involved in student organizations, such as the Food Science club, and participated in numerous leadership activities as an executive board member and committee leader. The opportunity to complete an undergraduate research project with Dr. Josip Simunovic helped to further increase Morgan’s interest in science and contributed to her decision to pursue a Master’s degree. Morgan graduated summa cum laude from the NC State University honors program in 2015.

After receiving her bachelors, Morgan began a Master’s degree in Food Science at NC State University. As part of the sweetpotato lab, Morgan’s research involved the development of a small-scale modular microwave processing system for sweetpotato puree. This technology has the potential to aid in food processing in developing countries, to provide small businesses with
affordable processing options, or to be used by research and development groups. The modular microwave processing system had a high retention rate of nutrients, which are typically destroyed by other common processing techniques. As a Master’s student Morgan continued her involvement with the Food Science club and also became an executive board member of the International Society for Pharmaceutical Engineering (ISPE). Morgan received her Master’s degree in 2017.

Continuing her pursuit of knowledge, Morgan began working on a doctorate degree in Food Science. By applying engineering principles to cold brew coffee processing, Morgan’s research aims to reduce the extraction time required to brew cold brew coffee. This research would help increase the sustainability of cold brew coffee. In conjunction with her research, Morgan continued her involvement in ISPE as vice president and has worked to connect the national chapter with the university.
ACKNOWLEDGMENTS

It has been an honor to have been mentored by such amazing committee members. A sincere thanks to Dr. Harris, Dr. Sandeep, Dr. Simunovic, and Dr. Cheng for their time, wisdom, and guidance.

Many others in the department were instrumental in my research and volunteered countless hours to lend an extra pair of hands, a sympathetic ear, creative ideas, and even their taste buds. Trisha, Ruth, Lina, Matt, Corrine, Wendy, Paul, Katharine, Jake, Mike, Rachel, and Sarah contributed hundreds of hours in the name of research to help with this project. Every minute of your time is appreciated, and your friendship is cherished!

There are so many amazing people in Schaub Hall whom I’m proud to count as friends. Thanks to those individuals, my time as a graduate student was made immeasurably better than anything I could have hoped for. It has been a privilege and a pleasure to work with the people in Schaub Hall who make our department a family.
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1. Engineering

1.1 Brewing Methods

1.1.1 Hot Brewed Coffee

The popularity of coffee in the United States has continued to grow in recent years as evidenced by increased consumption. The number of people consuming coffee on a daily basis in 2019 was about 63 percent, which is a 6 percent increase over 2016 (Brown 2019a). The number of Americans drinking specialty coffee has also increased from 9 percent in 1999 to 41 percent in 2017 (Ward 2017). Specialty coffee is a phrase commonly used when refereeing to the highest grade of coffee available which encompass the quality of the entire production process from farming to roasting to brewing. This has led to a greater number of coffee shops. With an estimated $47.5 billion market in the U.S., coffee shops showed a 3.3 percent increase from 2018 to 2019 to just over 37,000 coffee shops (Brown 2019b). Overall, coffee has been and will likely continue to be a popular beverage in the U.S.

Since the discovery of coffee in Ethiopia, sometime in the 1400-1500’s, methods of preparing and processing coffee have continued to evolve (Morris and Thurston 2013). While coffee shops are currently enjoying a healthy rate of expansion, coffee as a beverage has had a long history. During this history, advancing brewing methods contributed to increased demand and consumption of coffee. For a majority of history, coffee was traditionally made by consumers in their own homes. Over time, new brewing methods were developed. These methods can be broken into three categories: decoction, infusion and pressure methods (Cordoba and others 2020; Petracco 2008). Each of these categories involves the use of hot water to extract solutes from ground coffee. A description of the brewing methods is displayed in Figure 1.1. The decoction
method includes boiled coffee, Turkish coffee, percolator coffee, and vacuum coffee. Decoction allows water and ground coffee to be in contact for extended periods of time. For most of coffee’s history the Turkish method or boiled coffee were the main brewing methods. Coffee brewed using the infusion method includes filter coffee and Napoletana coffee. Infusion methods reduce the amount of time water is in contact with the ground coffee by allowing the water to slowly flow through the coffee grounds. Filter coffee, also known as drip coffee, has become a popular infusion brewing method. The final category, pressure methods, includes the plunger, Moka, and espresso methods. Moka and espresso methods use pressure to force hot water through the ground coffee and therefore further reduces the amount of time hot water is in contact with the coffee. The plunger method uses pressure to separate the water from the coffee grounds but first allows the water to steep with the ground coffee for a few minutes. These three categories of brewing methods show how society has continued to innovate the way in which coffee is consumed and enjoyed.

Consumer demand for convenience drove the creation of other types of brewed coffee, such as instant and ready-to-drink (RTD) beverages. Instant coffee allows consumers to save time preparing their coffee. Rather than taking time to brew a cup of coffee at home, the coffee is brewed elsewhere, and spray dried or freeze dried into a powder. This powder can then be mixed into a cup of water to make an ‘instant’ cup of coffee. Instant coffee made up more than 34 percent of brewed coffee consumed in the world in 2013 and was worth almost $31 billion. While instant coffee is popular in many parts of the world, due to the ease of storage, transportation, and preparation for the consumer, it has failed to grow in popularity in the U.S. (Ferdman 2014). RTD bottled beverages have found a market in convenience and grocery stores in recent years. Many companies have begun to develop premade coffee beverages, which include black coffee as well
as coffees with added flavorings, milk, and sugar. This allows a wide variety of consumers to enjoy coffee beverages on-the-go without the need to brew the coffee themselves or visit a coffee shop.

In the process of developing different ways to brew and consume coffee, other aspects of the production process have also been altered. For example, coffee grounds could be ground to varying sizes, roasted to varying degrees, or be a mix of varieties, such as Arabica and Robusta. These are all variables that contribute to the coffee’s flavor, color, aroma, and character. However, what is common among these different methods is the use of hot water as a solvent. For example, boiled and Turkish coffee utilizes hot water at 100°C, espresso coffee is made with water at about 90°C, and water for drip brewed coffee ranges from 80°C to 90°C. Variations in temperature affect the solvent properties of water, with higher temperature water allowing for the extraction of less polar compounds from coffee grounds. As coffee continues to evolve, these are just a few variables that will likely be adapted to improve coffee and develop new, interesting variations on the beverage.
### Decoction Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>In use since</th>
<th>Procedure</th>
<th>Contact Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boiling</td>
<td>Discovery of coffee</td>
<td>Coffee grounds placed in water and brought to a boil.</td>
<td>Extended</td>
</tr>
<tr>
<td>Turkish</td>
<td>Discovery of coffee</td>
<td>Finely ground coffee is placed in an ibrik over an open flame.</td>
<td>Extended</td>
</tr>
<tr>
<td>Percolator</td>
<td>1880</td>
<td>Steam continually condenses over a strainer filled with coffee grounds.</td>
<td>Extended</td>
</tr>
<tr>
<td>Vacuum</td>
<td>1830</td>
<td>Pressure differential pushes water into a vessel containing coffee grounds. When the pressure differential is removed, a vacuum pulls the coffee into a lower flask</td>
<td>Extended</td>
</tr>
</tbody>
</table>

### Infusion Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>In use since</th>
<th>Procedure</th>
<th>Contact Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filter/Drip</td>
<td>1908</td>
<td>Hot water flows over ground coffee placed in a paper filter.</td>
<td>Minutes</td>
</tr>
<tr>
<td>Napoleotana</td>
<td>1800s</td>
<td>Hot water flows from above through coffee grounds in a bushel into a bottom pot.</td>
<td>Minutes</td>
</tr>
</tbody>
</table>

### Pressure Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>In use since</th>
<th>Procedure</th>
<th>Contact Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plunger</td>
<td>1929</td>
<td>Boiling water is added to ground coffee in a beaker. A plunger pushes the coffee grounds to the bottom of the beaker after steeping is complete.</td>
<td>Many min</td>
</tr>
<tr>
<td>Moka</td>
<td>1933</td>
<td>Boiling water forces steam through a coffee cake in a basket into an upper vessel.</td>
<td>Seconds</td>
</tr>
<tr>
<td>Espresso</td>
<td>1905</td>
<td>Boiling water under pressure is forced through a coffee cake</td>
<td>Few sec</td>
</tr>
</tbody>
</table>

### Other Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>In use since</th>
<th>Procedure</th>
<th>Contact Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instant</td>
<td>1938</td>
<td>Coffee is industrially brewed and dried into a powder. Consumers mix the powder into water.</td>
<td>n/a</td>
</tr>
<tr>
<td>Cold Brew</td>
<td>1600s</td>
<td>Ground coffee and water is steeped at refrigeration temperatures for an extended amount of time.</td>
<td>Hours</td>
</tr>
</tbody>
</table>

Figure 1.1: Brewing Methods
1.1.2 Cold Brewed Coffee

RTD beverages have become popular with many food products such as smoothies, energy
drinks, tea, yogurt, protein shakes etc. The objective is to provide consumers with convenient
beverages to drink at any point in the day without the need for preparation. A description of how
the market has begun to utilize a variety of brewing methods and sales options is displayed in
figure 1.2. The cold brew coffee industry has also begun to take advantage of this area of the
market. In July of 2018, Mintel reported that RTD coffee beverages account for 22% of sales in
the coffee market. Growth in RTD coffee is expected to continue to increase while other portions
of the coffee market remain stagnant (Mintel, 2018). Younger generations have also demonstrated
enthusiasm for new products and are more likely to drink cold brew coffee than other generations.
These trends indicate that the interest in cold brew coffee as an RTD beverage will continue to
rise. Therefore, as the demand for RTD beverages increases, research into enhancing production
capacity of cold brew coffee and other coffee products will be essential.

There is no standard of identity associated with cold brew coffee, and methods used for
brewing cold brew coffee vary greatly. A common setup used by consumers to make cold brew
coffee involves a metal filter that can be filled with the desired amount of ground coffee and
submerged in a glass pitcher filled with water. The glass pitcher is then kept at refrigeration
temperatures for up to 24 hours, or as little as 5 hours, depending on the preferred strength of the
coffee. Some ready-to-drink cold brew coffee products advertise a brewing time of 10 hours while
others market a 6-hour brew time. This wide variation of time used to brew the coffee highlights
the fact that there is no defined means of preparation.

The temperature at which cold brew coffee is brewed is also debatable. Most home recipes
for cold brew coffees recommend brewing at refrigeration temperatures. However, some studies
and recipes have prepared “cold brew” coffee at room temperature (Fuller and Rao 2017; Rao and Fuller 2018). For commercially sold cold brew coffee, in both coffee shops and grocery stores, it is unclear what temperature is used. Some may be brewed exclusively with cold water while others are brewed using hot water and then chilled. When brewing hot coffee, the temperature of the water helps to ensure that possible microbial contamination is reduced. However, brewing coffee at cold temperatures does not provide that benefit and it is important to ensure cleanliness and a cold temperature to reduce the risk of microbial contamination. Brewing coffee at room temperatures, therefore, could present a greater chance of microbial contamination. There are also some who consider hot coffee poured over ice to be cold brewed coffee, although the term “iced coffee” more accurately describes this type of beverage. To further complicate the issue, these commercial beverages most likely receive some form of a heat treatment in order to inactivate any harmful microbes. This wide range of temperatures used to cold brew coffee is another source of variability when it comes to defining the beverage. For the purposes of this discussion, cold brew will be considered as coffee that is at some point brewed at refrigeration temperatures.
1.1.3 Safety of Cold Brewed Coffee

The rise in popularity of cold brew coffee has led to safety concerns as the beverage enters the RTD market. Strategies to eliminate growth of pathogens in cold brew coffee must be in place due to the low acidity of coffee and, in many cases, the lack of a heat treatment. These strategies may include a heat treatment, strict temperature control over refrigeration, and a focus on sanitation. In order to produce a shelf stable product, a low acid product must receive an extensive heat treatment or be acidified. An acidic product requires a slightly less extensive heat treatment. Acidic products are considered as naturally having a pH of 4.6 or less (US Food and Drug Administration). At this pH, pathogenic spores such as Clostridium botulinum cannot grow. Coffee is not considered to be an acidic product due to its pH of around 5.0-6.0 (Mazzafera 1999; Angeloni and others 2019). Therefore, in order to be shelf stable, products such as coffee, which are above
this pH must undergo a heat treatment during processing to ensure that there are no viable C.
botulinum spores in the product. Unfortunately, there have been recalls of shelf stable RTD cold
brew coffee beverages because the product was canned without a heat treatment leaving it at risk
of microbial contamination (US Food and Drug Administration, 2017). Other RTD cold brew
coffees are sold as refrigerated products, which are not required to have a heat treatment. While a
heat treatment may not be mandatory, some form of a preventive control for microbial
contamination is required. One focus could be on sanitation to ensure the number of microbes
being introduced into the system is kept to a minimum (Puro 2016). However, this method of
control relies heavily on refrigeration throughout the product’s life. In summary, while there are
ways to limit the growth of pathogens in cold brewed coffee, it can be difficult and expensive.

1.1.4 Guidelines for Brewing Conditions

The process of making a cup of coffee has been described by some as an art form due to
the variety of variables involved with coffee production. Defining the steps involved with making
a cup of coffee becomes even more complex due to the many personal preferences of consumers.
For example, consumers may prefer a particular roast, brewing method, or brew strength. Some
consumers who consider themselves to be coffee connoisseurs may be critical of many more
attributes than a more casual consumer. These consumers, who are more critical of the coffee they
drink, may seek to optimize other variables such as the temperature used to roast the beans, the
grind size, the mineral content of water used to brew the coffee, etc. For many of these variables
there are no set definitions. For instance, there are no parameters regarding roast color or any other
measurement to indicate what constitutes a light, medium, or dark roast. Some organizations have
attempted to address this lack of standardization. The Specialty Coffee Association (SCA) has
defined standards for some variables in order to produce a high-quality cup of coffee. For example, the SCA indicates that water used for brewing should target a pH of 7.0, a calcium content of 150 mg/L, a sodium content of 10 mg/L, etc (Specialty Coffee Association, 2018). The SCA also developed a brewing control chart to indicate the optimal ratio of coffee to water in order to get the best yield and strength. This chart recommends that the optimal brew strength measured by total dissolved solids is between 1.15% and 1.35% and the solubles extraction yield is 18% to 22%. In order to achieve this golden cup standard, which is traditionally defined as the optimal cup of coffee, it is recommended that 55 g/L of ground coffee in water is used for brewing (SCAA Standard Golden Cup).

While the standards outlined by the SCA help to ensure consistency, they may not be representative of what constitutes an optimal cup of coffee. For example, the brewing control chart was developed in the late 1950’s by Ernest Lockhart (Sage 2013). Lockhart’s chart was created at a time when percolator coffee was the primary method of brewing (Shinsuke). Since then, many new methods have become more popular such as drip brew, espresso, or instant coffee. Therefore, the brewing control chart may not reflect modern preferences for ‘good’ coffee. There have been more recent attempts to develop new models to estimate coffee strength and yield (Moroney, K. M. and others 2015; Moroney, Kevin and others 2016; Melrose and others 2018; Frost and others 2020). These papers mathematically analyzed the brewing process and developed equations to estimate yield. However, there are flaws with this approach such as the need for different models in different brewing situations or the fact that the models did not take into consideration sensory preferences of consumers. Therefore, the brewing control chart remains the industry standard due to wide acceptance and ease of use.
1.2 Microwave Processing

1.2.1 Advantages and Disadvantages of Microwave processing

As previously discussed, there are many methods available for brewing coffee. However, it is advantageous for industry to develop faster more efficient processing methods in order to reduce the amount of time required to produce a product. One possible technology that could reduce production times is microwave processing. However, there are advantages and disadvantages associated with microwave technology which are summarized in table 1.1.

There are some disadvantages of microwaves, such as non-uniform heating. In industrial systems, this problem required extensive research to ensure that the entire product received adequate heating from microwaves. For pumpable products, some solutions were to introduce static mixers in the system, or to use simulated particles to monitor the heat treatment received by particles (Jasrotia and others 2008). Another disadvantage to microwaves is that certain products do not absorb microwave energy and therefore cannot be heated using microwaves. One way to determine the severity of this issue for a particular product is to measure its dielectric properties. This helps to predict how a product will absorb microwave energy.

There are many benefits associated with microwave processing of food and beverages. The most prominent advantage of microwaves is the rapid heating of products (Salazar-González and others 2012). Even among household appliances, it is common knowledge that microwaving food requires far less time than heating food in a conventional oven. The same is true for commercially processed food. Electromagnetic waves from microwaves interact with water molecules and ions throughout a product which allows the product to rapidly heat virtually all at once, or "volumetrically". This minimizes the products exposure to heat. When using any form of heat treatment, many heat labile compounds such as carotenoids, anthocyanins, phenols, etc. are lost
due to extended periods of exposure to heat. Therefore, the rapid heating from microwaves drastically reduces the loss of these compounds and results in a higher value product (Steed and others 2008). Many products are amenable to microwave heating due to the ability of electromagnetic waves to interact with water molecules. Some products already benefit from the rapid heating such as soups and juices, however it may also be possible to utilize microwave technology with products such as coffee.

Microwaves are also able to rapidly heat certain products that are difficult to process with other methods, such as sweetpotato puree. Due to the viscous consistency of sweetpotato purees, conventional heating methods which rely on conduction of heat from the outside of the product to the inside, can be very slow and result in a low-quality product. Microwaves, on the other hand, rapidly heat products and produce a higher quality product (Coronel and others 2005). Another benefit is that it is possible to immediately stop or start microwave heating unlike conventional methods that require hot water or steam to heat the product. These are some capabilities of microwaves which could help industry to process various food products more efficiently.

**Table 1.1: Advantages and Disadvantages of Microwave Technology**

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Volumetric heating</td>
<td>• Non-uniform heating</td>
</tr>
<tr>
<td>o Rapid heating</td>
<td>o Potential cold spots and safety concerns</td>
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<tr>
<td>- Minimizes the products exposure to heat</td>
<td>• Inability to heat products that do not absorb microwave energy</td>
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<td>- Reduces loss of heat labile compounds</td>
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<td>- Reduced processing time</td>
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<td>o Able to heat products that</td>
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<td>conventional methods may not heat as</td>
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<td>efficiently</td>
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<td>• No need to maintain a heat source (such as</td>
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<td>steam or hot water)</td>
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<tr>
<td>• Electromagnetic waves do not lose</td>
<td></td>
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<td>power over distances</td>
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1.2.2 Dielectric Properties

Microwave heating relies on the interaction between electromagnetic waves and charged material within a product. This material could be polar molecules or ions. An example of a polar molecule is water, which has a partial negative charge due to the oxygen molecule and a partial positive charge due to the hydrogen molecules. Ions, such as dissociated salts and acids possess full charges. These charged particles interact with the oscillating electromagnetic field created by the waves. The interactions result in rapid movement of the particles as they realign themselves with the electric field and generates volumetric heating of food (Vadivambal and Jayas 2010). The interaction of these molecules with electromagnetic waves allows microwaves to heat in a unique way and is responsible for many of the benefits associated with microwave heating.

Determining the dielectric properties is one method for measuring the ability of a material to interact with microwaves. The dielectric constant, $\varepsilon'$, represents the ability of microwaves to penetrate a product while the loss factor, $\varepsilon''$, represents a material’s ability to convert microwaves into heat. The loss tangent is a ratio of these two terms and “indicates the ability of a product to absorb microwave energy.” (Zhu and others 2007) Together these dielectric properties help to better predict how products will respond to microwave energy.

Microwave heating is typically done at two different frequencies, 2450 MHz for household microwaves and 915 MHz for industry. The dielectric properties are affected by these frequencies and also by the state of a material. (Venkatesh and Raghavan 2004). Thus, the product’s dielectric properties may change depending on temperature, moisture content, ionic content, viscosity, microwave frequency, and other factors. Determining the dielectric properties of a material over a range of temperatures and frequencies is important if the objective is to estimate how well a material will interact with microwave energy.
1.2.3 Microwave Systems

Microwaves became a popular household appliance in the late 1960’s (Osepchuk Jun 2009). Many studies have worked to improve the heating within these microwaves. Specifically, research has been focused on studying ways to increase the uniformity of microwave heating. Some suggestions to improve microwave heating related to the size, shape, and placement of the food, others proposed using microwaves in conjunction with other forms of heating, and some recommended pulsing the microwave rather than continuously heating food (Vadivambal and Jayas 2010). Due to dielectric properties, the effectiveness of microwave heating is specific to individual products. Therefore, a universal solution to the nonuniformity of microwave heating is not likely.

Microwave technology has also been useful for industrial processing of foods. Large scale systems have been used in several different ways such as for drying food, in pumpable food, or in pre-packaged foods (Koskiniemi and others 2011). When used in conjunction with other drying methods, microwaves were shown to significantly reduce the drying time of fruits and vegetables. In most cases, this improved the quality of the product (Zhang and others 2006). For pumpable foods, microwave technology has been very successful. There are currently large-scale continuous flow systems for purees, soups, juice, etc. (Salazar-González and others 2012). These systems allow product to flow through pipes made of materials which are transparent to microwaves, such as Teflon. The microwave energy is applied around that pipe (Steed and others 2008). Some systems involve a very small area of heating in which an applicator focuses the microwave energy on a small section of pipe. Other setups apply the microwave energy over a longer length of pipe. These innovations and other future improvements of microwave technology will allow the industry to continue to become more versatile.
2. Chemistry

2.1 Variability of Coffee

2.1.1 Natural Variability

There are many variables that could impact the final sensory, chemical, and physical properties of a cup of coffee. One of these possible variables is the variety of coffee. There are two varieties of coffee that are typically used in very different products due to the disparity in quality, Robusta and Arabica. Robusta coffee is considered to be inferior in quality. However, Robust is cheaper to produce because it grows more easily, is less susceptible to disease, and is able to grow in more locations. Robusta is commonly used for lower quality products such as instant coffee. Arabica is widely considered to be a high-quality coffee and is often sold as whole roasted coffee beans. Arabica is also used to produce specialty coffee.

Coffee is grown in many different countries around the world, but some of the prominent growers are Brazil, Vietnam, Central America, Indonesia, India, Uganda, etc (United States Department of Agriculture, 2018). These different geographical growing locations of coffee trees are another variable affecting the coffee bean (Komes and Vojvadić 2014). Growing conditions in these locations also impact the development of the plant. Effects of climate change is another factor that affects growing conditions by creating unpredictable weather patterns resulting in variation of rainfall and temperature. These natural variations in climate and their effect on the development of the coffee plant may affect the timing of the harvest, and composition of the bean.

2.1.2 Post Harvest Variability

Once the coffee beans have ripened, the method used to harvest the beans adds further variability to the quality of the beans. Most harvesting is done manually in which ripe cherries are
either handpicked, or all the cherries are stripped from the branch. Some producers utilize mechanical harvesting especially in areas in which labor costs are high. However, mechanical equipment can only be used in areas with moderate slopes, require a high capital investment, and risk damaging the trees. Depending on the harvesting method, beans at different stages of maturation could be collected. These beans may require different processing methods depending on what stage of ripeness they were picked.

After harvesting, the cherries must be further processed before they are ready for shipping. One of the first steps includes sorting the cherries to remove impurities and defective cherries. The cherries are then either dried, semi-dried, or wet processed. Depending on the process used, the cherries may then need to be pulped in order to remove the pulp and mucilage. The beans are then dried before being cleaned, destoned, husked, and polished. The coffee is graded by size to aid in uniform roasting and undergoes various steps to remove defective coffee. To achieve a more homogeneous product, coffees of different qualities or of the same quality from different growers may be combined. Finally, the coffee can be weighed, packaged, and shipped to processors. The shipping and storage conditions could also introduce inconsistencies to the supply chain of coffee beans (Brando 2004). These many processing methods could contribute to the final composition of the coffee. Therefore, research is ongoing to improve the processing conditions and to determine the best ways to obtain a consistent product.

2.1.3 Processing Variability

Once the coffee beans have been received by processors, the sources of variability continue when considering the degree to which the coffee is roasted (Bhumiratana and others 2011). Traditionally, as normally determined by a visual color inspection, coffee beans are considered to
be either a light, medium, or dark roast. However, the color of roasted coffee is not always consistent with the time or temperature involved in roasting the coffee (Franca and others 2009). This may be due to the chemistry (e.g. sugar content) of the coffee beans, the geometry of the roasting equipment, and the amount of coffee being roasted, relative to the size of the coffee roaster. After the coffee beans are roasted, they are ground before being brewed, which can be another source of variability in coffee. Depending on the brewing method used, different particle sizes may be desired. But even when one size is selected, there is typically a range of particle sizes created by the grinding process. The size of the particles could impact the extraction efficiency due to surface area exposed, packing of the grounds, etc. As discussed previously, the brewing method could also impact the coffee’s properties. Derossi and others found that different brewing methods affect the phenolic content of coffee (2017). Different brewing methods may utilize different temperatures, times, and water to coffee ratios, which could affect the final cup of coffee. Other variables to consider include the storage conditions of the coffee throughout the production process and water quality of the water used in the brewing process. These many variables contribute to the properties of the final product.
Figure 1.3: Potential Sources of Variation in Coffee Composition
A summary of types of variability that could affect coffee composition: Natural, Post-Harvest, and Processing variability.

2.2 Composition of Coffee Beans and Beverages

It has been estimated that there are approximately 2,000 compounds found in coffee (Fischer and others 2019). To make coffee even more complex, the compounds may be found in different ratios and combinations at different stages of the coffee production process. The water content of a whole ripe fresh cherry is about 65% and, after being processed, the green coffee beans should have a water content of approximately 10-13% (Brando 2004; Clifford, Ludwig, and Crozier 2018). Green coffee contains approximately 13% lipids, 9% carbohydrates, 2% caffeine, and 9% chlorogenic acid among other acids and compounds (Mussatto and others 2011).
The process of roasting green coffee may change the composition of the beans. For example, the moisture content of green coffee samples in one study was reported as 9 g/100 g, after being roasted, the moisture content decreased to 1.5 g/100 g (Franca and others 2005). The protein, lipid, carbohydrate, and caffeine content of roasted coffee has been reported to be approximately 9-14%, 10-14%, 62% and 0.68-1.5% respectively (Franca and others 2005; Muzaifa and others 2020; Trigg 1922). Some compounds may degrade due to the roasting process. Depending on the quality of the initial coffee beans, one study estimated that about one third of caffeine and over 90% of chlorogenic acid was degraded due to roasting (Franca and others 2005). During the roasting process it is also possible for coffee to develop potentially harmful compounds such as acrylamide.

Of the many compounds found in coffee, the caffeine content has been of particular interest due to the wide consumption of coffee as a stimulant. Many consumers intentionally drink coffee specifically to receive a high dose of caffeine. Therefore, caffeine is a commonly analyzed component of coffee. However, many consumers also place high importance on the flavor of their coffee. This has led many researchers to analyze the flavor components in the liquid coffee and the various volatile compounds release from brewed coffee in order to better characterize flavor (Mestdagh and others 2014; Jeon and others 2017). More recently, as the focus on health has increased, the phenolic content of coffee has also been analyzed due to the antioxidant potential. One such compound that contributes to the phenolic content of coffee is chlorogenic acid (Fuller and Rao 2017). While there are thousands of compounds found in coffee, caffeine, chlorogenic acid, and various flavor components, have been heavily researched due to their effect on the final beverage.
2.3 Differences Between Hot and Cold Brew Coffee

Depending on the brewing method, coffee varies widely in terms of its chemical composition, physical properties, and flavor. Regular drip brewed coffee contains approximately 95-330 mg of caffeine in one 8 oz serving while instant coffee contains approximately 30-70 mg and Espresso contains about 400-1200 mg (Mejia and Ramirez-Mares 2014). In cold brew coffee which has been brewed for about 6 hours, there was between 930 and 1130 mg/L of caffeine (Fuller and Rao 2017). This is equivalent to about 220-267 mg/8oz cup. However, this cold brew coffee was brewed at room temperature, which is not recommended, due to safety concerns. Another study, which brewed cold brew coffee at 5°C found that there was 0.893 mg/ml of caffeine in the coffee (Angeloni and others 2018). This is equivalent to about 211 mg/8oz. Espresso coffee contains a much higher amount of caffeine per serving than most other types of coffee. However, when comparing to cold brew coffee to regular drip brewed coffee, the caffeine content is similar.

Together, aroma and taste are the essential components that create a flavor. Different brewing methods yield differences in aroma and taste. This can result in the development of very different flavor profiles in a cup of coffee. Aroma has been shown to be affected by every part of the coffee bean supply chain. However, different roasting and extractions techniques may be utilized to achieve the desired composition of the final cup of coffee. The reason that brewing techniques are so crucial to aroma may be due to the fact that polarity of the odorants plays a major role in how they are extracted (Mestdagh and others 2014). When comparing hot and cold brewed coffee, the difference in temperature could create variations in the flavor profile of the coffee. Angeloni found that cold brewed coffee tended to be more sweet and less intense than coffees brewed at a higher temperature (Angeloni and others 2018). Similarly, a study performed on hot brewed coffees and cold brewed coffees brewed at room temperature, indicated that the cold
brewed coffee was less intense and less acidic than hot brewed coffee (Cordoba and others 2019). These studies indicate that the different temperatures used to brew coffee could impact the final flavor of the coffee.

2.4 Sensory

2.4.1 Attributes Associated with Coffee

Coffee is a complex product with many compounds contributing to its aroma, taste, and flavor. Many studies have worked to develop a lexicon to describe the attributes associated with coffee. One such study identified 92 terms with a nearly even split of those terms being assigned to aroma and flavor (Di Donfrancesco and others 2014). Another study defined a total of 110 terms but narrowed the list to 11 terms for the sake of efficient sample analysis. The terms were as follows: coffee impact (fullness), woody, roasted, sour (taste), sweet (taste), sweet aromatics, green, bitter, nutty, fruity, and floral (Chambers and others 2016). It has been suggested that as coffee continues to evolve as a beverage, the lexicon should be expanded.

There are many tools available to define the quality of a cup of coffee such as physical, chemical, or sensory analysis. In order to develop the coffee lexicon, a combination of these analyses may be used. However, the predominant way in which coffee is analyzed around the world is with coffee cupping. This process utilizes highly trained cuppers who are specially trained to identify the physical and sensory criteria as outlined by the Specialty Coffee Association of America (SCAA 2015). The cuppers analyze coffee prepared based on a specific set of procedures over a range of 0 to 10 points. The aroma, flavor, aftertaste, acidity, body, balance, sweetness, uniformity, and cleanliness are examples of the attributes being analyzed. However, there is some uncertainty as to the reproducibility of the cupping procedure (Worku and others 2016). Therefore,
while the cupping process provides producers with an estimate of the quality, it would be good practice to use a supplementary method of analysis if the objective is to study a particular attribute of the coffee. This can be accomplished by chemical analysis or trained sensory panels. In summary, the process of cupping coffee yields information about many aspects of the coffee but may not be as reproducible as standard chemical analyses.

2.4.2 Chemical Analysis Compared with a Trained Panel

Trained panels have long been used to perform descriptive analysis on samples or to give quantitative analysis regarding specific attributes of samples. However, with advancements in technology, arguments have been made that chemical analysis of samples could result in data similar to that collected from trained panels. The benefit of using lab techniques to analyze samples is that chemical analyses could be more repeatable, less prone to bias, and human subjects that require extensive training are not required. Despite those benefits, laboratory methods can be time consuming to develop and may not accurately depict what a human might experience. Therefore, trained sensory panels remain a common method of analysis.

2.4.3 Sensory Panel

A sensory panel trained in attribute rating is led by a panel leader who guides the panel through standards and samples. Typically, a standard is used to help define a particular attribute. In the case of descriptive panels, it is common to have both chemical and commercially available standards. For example, a descriptive panel considering butter flavor could be given Diacetyl as the chemical standard and popcorn as the commercial standard. If a panel’s objective is to score the intensity of an attribute, they may be given increasing levels of concentration of a chemical.
For example, to measure sourness, the panel could be given increasing concentrations of citric acid in water to demonstrate different values of sourness. Some methodologies require that the panel assigns their own values to different concentrations of citric acid in water, while other methods have predefined values for certain concentrations. Depending on the number and complexity of attributes being analyzed the panel could take ten hours to train or hundreds of hours. It may also be necessary to retrain the panel at certain points during the test or to train an individual panelist more than the other panelists (Anonymous 1981).

2.5 Analyses

2.5.1 Particle Size

While a number of studies have analyzed and discussed particle size relative to espresso, few studies have reported size related to drip brew and other methods. The studies that analyzed particle size and espresso examined a range of approximately 100 µm to 700 µm (Andueza and others 2003; Severini and others 2015). Although there may be a lack of specifically defined recommendations for particle size of ground coffee, many brewing techniques do recommend different grind sizes. These recommendations however are very general and only refer to fine, medium, or coarse grinds rather than specific particle sizes.

The particle size of ground coffee has long been known to greatly affect the contents of the final brewed coffee. Particle size is so critical that different brewing techniques recommend different grind sizes in order to modify extraction and achieve distinctive flavors. For example, espresso coffee generally uses finely ground coffee while filter coffee uses medium ground coffee (Petracco 2008). Another study found that finely ground coffee beans increased the intensity of six attributes when compared to coarsely ground coffee: brown roast flavor, burnt wood/ash flavor,
dark green flavor, dried fruit flavor, hay like flavor, and smoke aroma (Frost and others 2019). This difference in extraction is due to the change in surface area and the ability of the water to come into contact with the coffee. Many studies have attempted to analyze and model the effect of particle size on brewing coffee (Kuhn and others 2017). Research has been done to determine the optimal grind size for brewing espresso coffee (Andueza and others 2003). Andueza (2003) found that a fine particle size produced the most desirable sensory and chemical characteristics in espresso coffee but noted that medium to coarse grinds are usually required for other types of brewing methods such as filter coffee. In order to regulate the extraction of coffee from grounds it is important to control the grind size of the coffee grounds.

To measure particle size there are a few different methods available. The most basic method is to utilize different sized sieves to determine the particle size distribution of a sample. This method can be done by hand or by using a mechanical shaker. Some possible problems with this method are that the ground coffee becomes packed together so that it does not fall through the sieves. A solution to this problem is to include balls within each layer of the sieves to keep the coffee grounds from compacting (Kuhn and others 2017). However, with more coarsely ground coffee this may not be a significant problem. Regardless of the possible problems associated with sieves, they are an extremely simple and widespread method of measuring the particle size of coffee grounds.

More advanced methods have also been developed to analyze the average spherical shape of particles in a sample. These methods use laser diffraction to analyze the particle size of individual particles. The angle at which particles scatter a single laser beam is used to calculate the size of the particle. However, it is important to ensure that only one particle is being measured at a time. This is done by slowly introducing a stream of powder into the system under laminar
flow. This method has successfully been used to analyze the particle size of ground coffee (Khamitova and others 2020). While these advanced methods may provide more information than the commonly used sieves, the cost of the equipment may impede the use of these methods.

2.5.2 Caffeine and Chlorogenic Acid

To further analyze coffee, high pressure liquid chromatography (HPLC) is commonly used to determine the caffeine and chlorogenic acid content of the samples. A standard curve is required to quantify the concentrations of each compound. Figure 1.4 and 1.5 depict the chromatogram that might be produced from one point in a standard curve for caffeine and chlorogenic acid. There are many isomers of chlorogenic acid that could be analyzed from three main subgroups; 3-, 4-, and 5- caffeoylquinic acids (CGA); 3,4-, 3,5-, and 4,5- dicafeoylquinic acids; and 3-, 4-, and 5-feruloylquinic acids (Jeon and others 2017). Different studies quantify various isomers when reporting the chlorogenic acid content of coffee. However, usually only one of the isomers is used to represent the overall chlorogenic acid content of a sample. The mobile phase has also been shown to affect the retention time of different chlorogenic isomers, therefore, it is important to ensure that the correct combination of mobile phases is used. Usually a gradient is used to better control elution times of the compounds. Samples are often extracted and then clarified before being loaded onto an HPLC system. In the case of coffee, water or methanol can be used to extract dried coffee or ground coffee.
Figure 1.4: Example Chromatogram of Caffeine
Caffeine is measured at a wavelength of 276 nm and had a retention time of approximately 11.9 min.
Figure 1.5: Example Chromatogram of Chlorogenic Acid (3-CQA)
3-CQA is measured at a wavelength of 325 nm and had a retention time of approximately 11.2 min
2.5.3 Total Dissolved Solids

Total dissolved solids (TDS) is a measurement that reflects the amount of dissolved coffee solids in a cup of coffee. In industry, it is often used as an indicator of coffee strength and is typically reported as a percentage (Kingston 2015). There are many aspects of the brewing process that could contribute to TDS of the final brewed coffee including the quality of the beans, the roasting process, and the extraction method (Cordoba and others 2020). The Brewing Control Chart of the Specialty Coffee Association indicates that the TDS of coffee should be 1.15%–1.35% in order to obtain the optimal coffee balance (SCAA Standard Golden Cup).

A handheld refractometer is a commonly used device to determine the TDS of coffee. The refractometer measures the angle at which light passes through a small sample in order to determine the dissolved solids of the sample (Geake and Smalley 1983). Refractometers are used with a variety of other food products such as fruit juices, milk, as well as non-food materials, like coolants, sea water, etc. It is important to use a refractometer that is calibrated specifically for a certain product. This ensures that the device will accurately measure the correct range of the sample.

2.5.4 Color

The color of coffee beans (green and roasted) and of the final brewed coffee is a commonly measured attribute. A colorimeter is a widely used instrument used to determine the L*, a*, and b* values of the coffee (HunterLab 2008). L* represents the lightness or darkness of a sample with a value of 0 being black and 100 being white. The a* indicates green or red in a sample, negative values indicate green color and positive values represent red color. The b* measures the blue and yellow color in a sample, blue would result in more negative values and yellow in more positive.
Colorimeters utilize a source of light which is then reflected by the sample, the light is then filtered by a tristimulus absorption filter before entering a detector (Pathare and others 2013). The colorimeter requires calibration standards in order to accurately analyze samples. The measurements provided by the colorimeter allows for a comparison between samples and studies.

One study reported the color of green coffee beans as an L* of 46-48, an a* of 2.53-3.12, and an b* of 7.68-10.12 (Tripetch and Borompichaichartkul 2019). The color of roasted coffee has been reported as having an L* of approximately 20, an a* of approximately 5, and an b* of approximately 5 (Pramudita and others 2017). For roasted coffee beans the L* value was seen as the more significant value to report (Wang and Lim 2012).

3. Summary

Coffee has had a 15-century long history and undergone numerous innovations to the way in which it is consumed. There are many coffee brewing methods which utilize hot water to extract coffee from grounds. The use of cold water to brew coffee has been increasing in popularity and is being researched to better determine its sensory, chemical, and physical characteristics. However, there is some debate surrounding the definition of cold brew coffee in which the temperature of the water and length of time used to brew the coffee may differ. Another trend that is becoming popular is the consumption of ready-to-drink (RTD) coffee beverages. This creates the need for research into processing methods that might improve the production of coffee which could then be sold as a RTD beverage. One technology that might be beneficial to the process of brewing coffee is microwave technology. Microwaves are able to heat products rapidly and aid in the extraction of compounds. However, coffee is a complex product composed of over 2000 compounds which could be altered by many aspects of the coffee supply chain. This nearly
unlimited source of variation in coffee will likely lead to continued innovations as coffee evolves to meet the demand of each generation.

4. Objectives

Based on the current literature, it is apparent that preparation methods for and the resulting chemistry and sensory properties of cold brew coffee have not been fully characterized. While much progress has been made in the analysis of cold brew coffee, there are missing components. This research attempted to better define cold brew coffee as it is made commercially and how it is produced at home by consumers. A further goal of the research was to analyze the effect of water temperature on the extraction of coffee beverage components from ground coffee.

Our hypotheses were:

1. The changes in solvent properties of water at hot vs cold temperatures would result in differential extraction of coffee compounds into hot or cold brewed beverages.
2. Delivering a brief heat treatment to a coffee water mixture prior to steeping at cold temperatures would accelerate the rate of extraction of cold brew coffee.
3. Microwave heating will act differently on coffee than hot water heating alone due to the ability of microwaves to heat non-water components in coffee.

The core objectives were as follows:

1. Evaluate various commercial cold brew products to determine similarities and patterns.
2. Analyze home brewing methods of hot brewed and cold brewed coffee.
3. Study the relationship between water temperature and contact time of water with ground coffee on extraction efficiency of coffee components.

4. Investigate the effect of brief heat treatment prior to cold steeping on extraction rates, utilizing pre-heated water added to coffee or microwave-heated, coffee-containing water.
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CHAPTER 2: Chemical, Physical, and Sensory Attributes of Commercial Hot and Cold Brew Coffee Compared with Cold Brew Produced In-House

Abstract

While cold brew (CB) coffee is increasing in popularity, brewing methods are not clearly defined resulting in a wide range of beverage quality and sensory profiles. In this study, commercial CB were compared to each other and to hot brew (HB) coffee. Two CB and three HB coffees were purchased from restaurants. Four bottled CB coffees were purchased from grocery stores. CB coffee was produced in-house at a ratio of 55 g/L coffee/water. Three HB coffees were produced in-house at 27.5 g/L, 55 g/L, and 110 g/L to determine which concentration was most similar to CB. Commercial CB coffees varied widely in their sensory (standard sourness, bitterness units), physical (total dissolved solids (TDS), color measured by L* values), and chemical (caffeine, chlorogenic acid) characteristics. Sourness ranged from 0.78-3.72, bitterness 4.55-6.95, TDS 0.76-3.07%, L* 1.83-29.74, caffeine and chlorogenic acid 30.35-77.98 and 5.59-85.43 mg/100 mL. In-house CB coffee (55 g/L) characteristics were most similar to 27.5 g/L HB coffee, which brewed over 200 times faster than the CB coffee. This suggests that CB coffee uses an unnecessary amounts of coffee beans and time. More research is needed to further profile CB coffees and determine how the process can be optimized and defined.

Introduction

The popularity of coffee in the United States has increased in recent years. The number of people who consume coffee on a daily basis was about 63 percent in 2019 which is a 6 percent increase from 2016 (Brown 2019). Cold brew, especially, has enjoyed a surge in demand. In 2015 cold brew was worth $8.1 million in sales but had increased to $38.1 million by 2017 (Maynard
This expansion in the cold brew market has led to a multitude of interpretations for how to produce an authentic cold brew coffee. The process of producing cold brew requires extended amounts of time to allow water to infuse with coffee grounds; the amount of time varies depending on the recipe or company. Many methods of making cold brew coffee recommend that the coffee infuse under refrigerated conditions. However, some recipes and researchers have allowed the coffee to infuse at room temperature (Fuller and Rao 2017). Several cold brew coffees that are sold commercially in stores are bottled and kept refrigerated; others are shelf stable and do not require refrigeration. Normally, shelf stable products require aseptic processing conditions, preservatives, or heat treatments in order to minimize microbial contamination. If a heat treatment is applied, this would be seemingly contrary to the idea of cold brew coffee. With these various interpretations of the appropriate time and temperature required to produce cold brew coffee, it is clear that more research is needed to define the beverage.

In an attempt to establish a standard definition for cold brew coffee, based on certain attributes, this study compared commercially purchased products of hot brewed and cold brewed coffees. For the same reason, these attributes were then analyzed in “lab made samples” of cold brew, weak drip brew, regular drip brew, and strong drip brew. These lab-made samples were prepared using controlled procedures in order to minimize variation and focus on the processing differences. The attributes analyzed were sourness, bitterness, TDS, color (L* value), caffeine (mg/100 mL) and chlorogenic acid (3-CQA as mg/100mL).
Materials and Methods

Experimental Setup

The commercially purchased samples were broken into three groups. Group one was hot brew coffee purchased from restaurants: McDonald’s, Dunkin, and Starbucks. Group two was cold brew coffee purchased from a restaurant: Dunkin and Panera Bread. Group three was bottled cold brew coffee. The following brands were purchased at a grocery store: Gevalia Concentrate, SlingShot, Starbucks, Califia Farms. Of the bottled cold brews, SlingShot is made by a North Carolina company, Gevalia is a shelf stable cold brew, and Starbucks and Califia Farms are widely available. These samples were selected in order to observe a range of products that are sold as cold brew as compared with popular hot brew coffees.

Laboratory (lab-) made samples were produced using the following ratios of coffee in water: 27.5 g/L for the weak drip brew, 55 g/L for the cold brew and regular drip brew and, 110 g/L for the strong drip brew. The same brand of ground coffee was used for all three ratios of coffee in water; it was also purchased and ground at the same time. The cold brew coffee was allowed to infuse for 20 hours in the refrigerator before being served to a trained panel. The drip brew coffees were made the morning of the day they were evaluated by the sensory panel. All of the lab made samples were allowed to come to room temperature before being tasted.

Sample Preparation

The in-lab hot coffee samples were prepared using a twelve cup Mr. Coffee coffee maker on the morning of the sensory panel evaluations. The lab made cold coffee samples were prepared using a Gourmia cold brew coffee maker with a removable steeping column (Brooklyn, NY). For the cold brew coffee, the coffee grounds and water were allowed to infuse for 20 hours prior to the
sensory panel evaluations. In order to minimize the variables associated with growing conditions, harvesting, and processing, the coffee beans used to make the hot and cold coffee was purchased at one time as whole coffee beans (Big Trouble Blend, Counter Culture, Durham NC). To reduce variability due to particle size, the coffee beans were ground in the store on the medium grind setting. The overall particle size distributing ranged from 250-2000 µm, with over 95% of the particles ranging from 500-2000 µm (figure 2.1). Coffees purchased from a restaurant were purchased on the morning of the sensory panel evaluations. The bottled cold brew coffees were purchased no more than two days prior to the sensory panel evaluations. Each of the samples were allowed to reach room temperature before being evaluated by the sensory panel.

![Figure 2.1: Average particle size distribution of ground coffee](image)

**Sensory Analysis**

The sensory analysis of bitter and sour intensity was performed by a panel of 10 trained tasters. The panel was trained for at least 10 hours over a period of 9 weeks. Panelists were trained to score bitter and sour intensity based on the World Coffee Research Sensory Lexicon values (2017). The lexicon utilizes a scale of 0-15 in order to score the intensity of samples. A score of 0
indicates that the attribute is less intense and a score of 15 represents a higher intensity. Training was performed by providing each panelist with a set of known standards for each attribute which were then used to score an unknown sample. The various intensities of the bitter standard were prepared by mixing increasing quantities of caffeine into deionized water. Similarly, different levels of the sour standard were prepared using citric acid. Table 2.1 indicates the concentration of each compound and the corresponding score. The same set of standards were provided during all testing sessions in which samples were analyzed. No more than five samples were analyzed during a session. Samples were assigned a random three-digit code and provided to each panelist in a random order at room temperature. Each of the samples and standards were served to the panelists at in 59 ml lidded, clear plastic soufflé cups. The soufflé cups were filled with approximately 30 ml of sample or standard. Unsalted crackers and deionized water were served to panelists to cleanse the palate between samples. Panelists waited at least 2 minutes between each sample in order to further cleanse the palate. Samples were purchased or prepared the day before or the same day as each test. Each sample was tested in triplicate.

Table 2.1: Citric Acid and Caffeine Solution Concentrations Correlated to Corresponding Score

<table>
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<tr>
<th>Concentration</th>
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<tr>
<td>0.015% Citric Acid Solution</td>
<td>1.5 Sour Standard</td>
</tr>
<tr>
<td>0.033% Citric Acid Solution</td>
<td>2.5 Sour Standard</td>
</tr>
<tr>
<td>0.05% Citric Acid Solution</td>
<td>3.5 Sour Standard</td>
</tr>
<tr>
<td>0.01% Caffeine Solution</td>
<td>2.0 Bitter Standard</td>
</tr>
<tr>
<td>0.02% Caffeine Solution</td>
<td>3.5 Bitter Standard</td>
</tr>
<tr>
<td>0.035% Caffeine Solution</td>
<td>5.0 Bitter Standard</td>
</tr>
<tr>
<td>0.05% Caffeine Solution</td>
<td>6.5 Bitter Standard</td>
</tr>
</tbody>
</table>
Physical Analysis

Total dissolved solids (TDS) were measured using a digital pocket refractometer (ATAGO pal-coffee digital pocket coffee refractometer, Tokyo, Japan). Color was measured using a Colorflex EZ Spectrophotometer (HunterLab, Reston, VA). The color is expressed using the “LAB” color model in which L* represents the lightness or darkness of a sample with a value of 0 being black and 100 being white. The a* indicates green or red in a sample, negative values indicate green color and positive values represent red color. The b* measures the blue and yellow color in a sample, blue would result in more negative values and yellow in more positive. The L* value was reported for these samples.

Chemical Analysis

Caffeine and 3-CQA were measured using an adapted methodology reported by Fujioka and Shibamoto (2008). High pressure liquid chromatography (HPLC) is commonly used to determine the caffeine and chlorogenic acid content of the samples. A Waters X-Bridge C-18, 3.5 μm column (100 mm x 4.6 mm) (Waters Corporation, Millford, MA) was run at ambient temperature. A gradient of two mobile phases were used during the study. Mobile phase A consisted of 90% 20 mM citric acid monohydrate and 10% methanol. Mobile phase B was 100% methanol. The gradient began at 100% A and 0% B and increased to 100% B. The flow rate was 0.7 mL/min with an injection volume of 10 μL. A Waters software system was utilized to run the HPLC and autosampler (Waters Corporation, Millford, MA).

Standard curve solutions for caffeine and 3-CQA were made from chemicals purchased from Sigma-Aldrich (St. Louis, MO). The chromatogram of one point in the caffeine and 3-CQA standard curve is shown in figure 2.2. A chromatogram depicting an example of one sample in
this study is shown in figure 2.3. HPLC grade methanol and water and citric acid monohydrate were purchased from Fisher Scientific (Hampton, NH).
Figure 2.2: Example Chromatograms of One Caffeine and 3-CQA Standard
In this figure, the A) caffeine and B) 3-CQA chromatogram for the 0.625 mM standard is shown. 
Caffeine is measured at a wavelength of 276 nm and had a retention time of approximately 11.9 min.
3-CQA is measured at a wavelength of 325 nm and had a retention time of approximately 11.2 min.
Figure 2.3: Example Chromatograms of One Sample
In this figure, the A) caffeine and B) 3-CQA chromatogram for a lab made cold brew sample is shown.
Statistical Analysis

A one-way analysis of variance (ANOVA) and Tukey-Kramer HSD was used to determine differences and similarities between samples for sourness, bitterness, TDS, L* value, caffeine, and 3-CQA. Commercial products and lab made samples were analyzed separately. Values were reported as means ± standard error of the mean. Statistically significant differences were determined by a p < 0.05. SAS JMP 14 statistical software (Cary, NC) was used to analyze the data.

Results and Discussion

Commercial Coffee Sample Analysis Summary

The values of the commercial samples varied widely. As seen in table 2.2, the Panera Bread cold brew coffee had the highest sour and bitter scores (3.72 and 6.95 respectively), the highest TDS (3.07), the lowest L* value (1.83), and the most caffeine (77.98 mg/100 ml). However, SlingShot bottled cold brew had the highest 3-CQA content (85.43 mg/100 ml) at just over 2.5 times higher than the sample with the second highest 3-CQA content, which was Dunkin hot brew (24.87 mg/100 ml). Dunkin hot brew also had the lightest colored coffee with an L* value of 29.74. Starbucks bottled cold brew had the lowest sour and bitter scores (0.78 and 4.55 respectively), McDonalds had the lowest TDS (0.76), and Gevalia bottled cold brew at 50% had the lowest caffeine and 3-CQA content (30.35 mg/100 ml and 5.59 mg/100ml respectively).

The results for caffeine and TDS of commercially purchased samples in this study were examined for their correlation to values found in literature. Published caffeine values for cold brew coffee (infused for about 6 hours) reported that is contained 93-113 mg/100 ml of caffeine, while regular drip brew coffee contained 40-139 mg/100 ml of caffeine (Mejia and Ramirez-Mares 2014;
Fuller and Rao 2017). Caffeine values for the cold brew samples analyzed in this study ranged from 30.35-77.98 mg/100 ml, which is lower than those found in literature. This could be due to the use of room temperature water in previous studies, rather than the 4°C water used to produce cold brew samples in this study. The caffeine content of the hot brew samples in this study ranged from 43.47-79.36 mg/100 ml and were comparable to those found in the literature. The Brewing Control Chart of the Specialty Coffee Association indicates that the TDS of coffee should be 1.15%-1.35% in order to obtain the optimal coffee balance (SCAA Standard Golden Cup). Each of the commercial samples in this study were either above or below the recommended TDS range. However, most of the samples were not far outside of the range and were within 0.2%. The Panera bread was the sample that was most different from the recommended TDS values.

Analysis of Sensory, Physical, and Chemical Attributes

The sour score of Panera Bread cold brew coffee (3.72) was significantly different from all commercial samples other than SlingShot (3.44) bottled cold brew. No other statistically significant patterns for sourness were observed between the remaining commercial samples. These commercial samples had an average sour score ranging from 0.78-2.62. An analysis of color showed that Panera Bread cold brew coffee was significantly darker than all other commercial samples with an L* value of 1.83. Results showed that the 3-CQA content of SlingShot bottled cold brew coffee was significantly higher (85.43) than the other commercial samples. Among the remaining commercial samples there were no statistically significant differences observed for L* value and 3-CQA with ranges of 10.04-29.74 and 5.59-52.65 mg/100 ml, respectively. Statistically significant differences were also not observed for bitterness, TDS, or caffeine, which ranged from 4.55-6.95, 0.76-3.07, and 30.35-79.36 mg/100 ml, respectively.
Table 2.2: Comparison of Sensory, Physical, and Chemical Values of Commercial Samples

Samples with the highest value for a particular attribute are highlighted in gray and samples with the lowest value for a particular attribute are bolded. However, other samples in each column may not be statistically different from the highlighted or bolded values. An L* value of 0 represents a darker material and a value of 100 represents a lighter material.

<table>
<thead>
<tr>
<th>Hot Brew from Restaurant</th>
<th>Sour</th>
<th>Bitter</th>
<th>TDS</th>
<th>L*</th>
<th>Caffeine (mg/100ml)</th>
<th>3-CQA (mg/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>McDonald’s</td>
<td>2.01 ± 0.15 cd</td>
<td>4.99 ± 0.35 bc</td>
<td><strong>0.76 ± 0.03 a</strong></td>
<td>25.01 ± 1.22 bc</td>
<td>56.51 ± 5.68 bcd</td>
<td>32.96 ± 3.872 c</td>
</tr>
<tr>
<td>Dunkin Donuts HB</td>
<td>2.22 ± 0.12 c</td>
<td>4.73 ± 0.48 c</td>
<td>0.88 ± 0.02 a</td>
<td>29.74 ± 2.21 ab</td>
<td>43.47 ± 1.01 cd</td>
<td>31.66 ± 1.289 c</td>
</tr>
<tr>
<td>Starbucks HB</td>
<td>1.59 ± 0.18 cde</td>
<td>6.56 ± 0.27 ab</td>
<td>1.01 ± 0.04 a</td>
<td>21.11 ± 0.80 bcd</td>
<td>53.77 ± 3.68 bcd</td>
<td>5.67 ± 0.107 f</td>
</tr>
<tr>
<td>Cold Brew from Restaurant</td>
<td>Dunkin Donuts CB</td>
<td>1.61 ± 0.12 cde</td>
<td>6.12 ± 0.57 abc</td>
<td>1.46 ± 0.20 a</td>
<td>23.22 ± 2.46 bc</td>
<td>67.57 ± 10.43 bc</td>
</tr>
<tr>
<td>Panera Bread</td>
<td>3.72 ± 0.17 a</td>
<td>6.95 ± 0.24 a</td>
<td>3.07 ± 1.12 a</td>
<td><strong>1.83 ± 0.77 f</strong></td>
<td>77.98 ± 29.11 a</td>
<td>27.21 ± 11 bc</td>
</tr>
<tr>
<td>Bottled Cold Brew from Store</td>
<td>Gevalia Concentrate 100%</td>
<td>1.58 ± 0.16 cde</td>
<td>6.45 ± 0.23 ab</td>
<td>1.94 ± 0.02 a</td>
<td>10.04 ± 2.24 e</td>
<td>57.04 ± 1.29 bcd</td>
</tr>
<tr>
<td>Gevalia Concentrate 50%</td>
<td>1.06 ± 0.43 de</td>
<td>5.09 ± 0.09 bc</td>
<td>1.04 ± 0.07 a</td>
<td>10.76 ± 2.53 e</td>
<td><strong>30.35 ± 2.83 d</strong></td>
<td><strong>5.59 ± 0.975 f</strong></td>
</tr>
<tr>
<td>SlingShot</td>
<td>3.44 ± 0.14 ab</td>
<td>4.63 ± 0.12 c</td>
<td>1.75 ± 0.02 a</td>
<td>18.57 ± 1.07 cde</td>
<td>74.02 ± 1.47 bc</td>
<td>85.43 ± 1.954 a</td>
</tr>
<tr>
<td>Starbucks Bottled CB</td>
<td><strong>0.78 ± 0.15 e</strong></td>
<td><strong>4.55 ± 0.23 c</strong></td>
<td>1.13 ± 0.01 a</td>
<td>20.90 ± 0.51 cd</td>
<td>54.78 ± 1.21 bcd</td>
<td>12.35 ± 0.415 ef</td>
</tr>
<tr>
<td>Califia Farms</td>
<td>2.62 ± 0.14 bc</td>
<td>5.02 ± 0.30 bc</td>
<td>1.37 ± 0.01 a</td>
<td>14.12 ± 1.50 de</td>
<td>75.86 ± 1.68 bc</td>
<td>28.07 ± 1.488 cd</td>
</tr>
</tbody>
</table>
Lab-Made Samples

The weak drip hot brew coffee was made using half the amount of ground coffee (27.5 g/L) as was used in the lab-made cold brew coffee. Despite this difference in coffee concentration, there was no statistically significant difference between the lab-made cold brew and the weak drip brew coffee. This pattern, which is illustrated in table 2.3, indicates that for sour, bitter, TDS, L* value, caffeine, and 3-CQA, the cold brew coffee was not different from the weak drip brew.

The only statistically significant difference between the regular drip brew and the lab made cold brew was for color, for which the regular drip brew was darker. The regular drip brew had an L* value of 18.22 while the lab made cold brew was 34.37. In this case, the regular drip brew and the lab made cold brew were made using the same amount of ground coffee. Therefore, it was expected that the regular drip brew and lab made cold brew would have similar values for all measured attributes.

There was a statistically significant difference between the strong drip brew and the lab-made cold brew for bitterness and color (L*Value). The strong drip brew had a higher bitter value when compared to the lab made cold brew (6.95 and 4.97, respectively). Additionally, the strong drip brew had a lower L* value when compared to the lab made cold brew (10.36 and 34.37, respectively). There was not a statistically significant difference between the strong drip brew and the lab made cold brew for sourness, TDS, caffeine content, or 3-CQA content. The strong drip brew was made with twice the amount of ground coffee as the lab made cold brew, therefore, it was expected to be a more concentrated coffee exhibiting higher sour and bitter values as well as higher TDS, caffeine, and 3CGA, and lower an L* value. Figure 2.4 uses TDS to display the similarity of the lab-made cold brew when compared to the weak, regular, and strong drip brew coffee.
Table 2.3: Sensory, Physical, and Chemical Values of Lab-Made Samples

<table>
<thead>
<tr>
<th></th>
<th>Sour</th>
<th>Bitter</th>
<th>TDS</th>
<th>L*</th>
<th>Caffeine (mg/100ml)</th>
<th>3-CQA (mg/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>27.5 g/L ‘Weak’ Drip Brew</td>
<td>1.44 ± 0.13 b</td>
<td>5.02 ± 0.38 b</td>
<td>0.57 ± 0.03 b</td>
<td>32.72 ± 2.74 a</td>
<td>43.72 ± 3.04 b</td>
<td>23.96 ± 1.85 b</td>
</tr>
<tr>
<td>55 g/L ‘Regular’ Drip Brew</td>
<td>2.67 ± 0.38 a</td>
<td>6.06 ± 0.26 ab</td>
<td>1.07 ± 0.15 ab</td>
<td>18.22 ± 3.43 b</td>
<td>93.53 ± 9.98 a</td>
<td>58.51 ± 7.12 a</td>
</tr>
<tr>
<td>110 g/L ‘Strong’ Drip Brew</td>
<td>2.95 ± 0.19 a</td>
<td>6.95 ± 0.09 a</td>
<td>1.76 ± 0.34 a</td>
<td>10.36 ± 1.88 b</td>
<td>121.19 ± 15.03 a</td>
<td>80.36 ± 12.17 a</td>
</tr>
<tr>
<td>Lab Made Cold Brew (55 g/L)</td>
<td>1.98 ± 0.28 ab</td>
<td>4.97 ± 0.38 b</td>
<td>0.98 ± 0.02 ab</td>
<td>34.37 ± 2.01 a</td>
<td>79.36 ± 4.79 ab</td>
<td>52.65 ± 3.80 ab</td>
</tr>
</tbody>
</table>

Figure 2.4: Comparison of CB coffee to HB coffee of Differing Strengths

Conclusion

The analysis performed on commercial samples in this study highlights the fact that cold brew coffee does not have a standard brewing method. For example, the time, temperature, and apparatus used to brew cold brew coffee are different depending on the recipe. Inconsistencies in the definition of cold brew coffee is seen not only in the processing methods, but also in sensory, physical, and chemical attributes. Possible discrepancies in processing techniques arise from the wide variations of time and temperature used to cold brew coffee. Other inconsistencies stem from
the way in which coffee is packaged and stored which also affect the attributes of the final product. Though a clear definition of cold brew coffee is desirable, it remains elusive.

Some recipes indicate that cold brew coffee should infuse for up to 24 hours or as little as 5 hours. Different brands of bottled cold brew coffee also advertise various lengths of time for which the ground coffee is infused in water. It is common for cold brew coffee recipes to suggest that the ground coffee and water be infused at refrigeration temperatures. However, there have been some studies and recipes that have suggested brewing cold brew coffee at room temperature (Fuller and Rao 2017; Rao and Fuller 2018). Others have used a heat treatment to make it shelf stable.

Demand for convenient, shelf stable packaging of coffee products also drives processing variability in cold brew coffee. An example of this is the Gevalia cold brew concentrate which is a shelf stable product. In order to be shelf stable, the coffee would have required some form of microbial mitigation. Consequently, an assumption could be made that Gevalia cold brew concentrate underwent an extensive heat treatment to make it shelf stable. This further complicates the definition of cold brew coffee since a heat treatment of cold brew coffee is seemingly contradictory. Not only did Gevalia likely include a heat treatment in their process, but the time and temperature at which their ground coffee was infused with water is also unknown. As shown by this one example, the definition of cold brew coffee is open to interpretation.

Sensory (sourness and bitterness), physical (TDS and color as L* value), and chemical (caffeine and 3-CQA) attributes are additional sources of variation when defining cold brew coffee. Some consumers believe that cold brew coffee is less sour and has a higher caffeine content than hot brew coffee (Strand 2017; Maynard 2018). Based on the attributes measured in this study, no significant patterns were identified among the commercial cold brew coffees analyzed. The
same was true when comparing the commercial cold brew to the commercial hot brew. Consequently, the lack of similarities between commercial cold brews and the lack of difference when comparing cold and hot brews, further demonstrated a need for an industry standard regarding what constitutes a cold brew coffee.

Due to the unknown variables associated with purchased samples, cold and hot brew samples were made in the lab using controlled procedures. This made it possible to explore their differences in a more structured way. The objective was to determine if the cold and hot brew was the same or if the cold brew adhered to the popular belief of having more caffeine while also being less sour. The results of this study contradicted popular belief by showing that, for caffeine content, and sourness there was no difference between the lab made cold brew and any of the hot brews. Further analysis of all the attributes measured in this study indicated that the strong drip brew was different from the lab made cold brew for a total of two out of six attributes: bitterness and L* value. The regular drip brew was only different from the lab made cold brew in L* value and the weak drip brew did not differ in any attribute.

While few differences were observed between the lab made cold and hot brew coffees for the attributes measured, the striking similarities were meaningful. Most importantly, the cold brew coffee was no different than the weak drip brew coffee. It is crucial to note that less ground coffee and less time was required to produce the weak drip brew coffee. The weak drip brew was made using half the amount of ground coffee and was over 200 times faster to brew than the cold brew coffee. This creates the argument that cold brew coffee is using an unnecessary amount of coffee beans and time, both of which are costly to consumers and industry and decrease sustainability of the coffee supply chain. Given that cost and sustainability are vital factors in the food industry, continuing consumer demand for cold brew should drive innovations in brewing efficiency. The
attributes measured in this study may not completely characterize the complexities of coffee. There are many more attributes that could further characterize coffee such as volatile compounds, other attributes on the sensory lexicon, and other chemical compounds. Therefore, more research is needed to further profile cold brew coffees and determine how they compare to hot brew coffee. A better understanding of cold brew coffee would create a definition that industry could work towards when developing new beverages. This would also give assurance to the consumers that a beverage labeled as cold brew coffee is authentic.
REFERENCES


Strand O. 2017. How Cold Brew Changed the Coffee Business. NYTimes.com Feed
CHAPTER 3: A Comparison of Chemical, Physical, and Sensory Attributes of
Conventional vs. Accelerated Cold Brew Coffee Processing Methods

Abstract

Demand for cold brew (CB) coffee is increasing with rapid expansion into the ready-to-drink beverage industry. Process improvements would be beneficial to reduce extraction times and decrease production costs. This study compared CB coffee to hot brew coffee which requires less time (minutes vs hours) to produce. CB was also compared to coffee which received a brief heat treatment from hot water or from microwave technology using the following attributes: sourness, bitterness, total dissolved solids (TDS), color measured by L*, caffeine, and chlorogenic acid (3-CQA). Steeping CB samples for longer did not result in statistical differences (sour, 1.58-1.63; bitter, 4.19-4.53; TDS, 0.84-0.93; L*, 40.15-38.78; caffeine, 55.81-74.99; 3-CQA, 44.11-47.05). The microwave treated samples also were extracted much more quickly with only a few differences when compared to CB samples. These results indicate that it may be possible for industry to dramatically decrease the amount of time used to cold brew coffee.

Introduction

Preparing a cup of coffee has been described by some as an art form due to the variety of variables involved with coffee production. The processing of coffee is an involved operation with numerous requirements: starting with how the trees are grown, continuing through harvesting the coffee cherries, roasting the coffee beans, brewing the ground coffee, and, in some cases, packaging the final beverage. Coffee flavor develops and can be altered throughout the different stages of the process. But for many consumers, the brewing method is the most accessible step to improve. Petracco (2008) breaks the brewing methods into three categories: decoction, infusion
and pressure methods. Each of these categories involves the use of hot water to extract solutes from ground coffee. Although hot brew coffee has a long history, recently there has been increasing interest in cold brew coffee. Cold brew coffee is produced by allowing ground coffee and water to steep at refrigerated temperatures for extended amounts of time. Essentially, cold brew coffee replaces the heat used to brew traditional, hot coffee, with time.

As cold brew coffee has continued to increase in popularity, more research has been done in order to improve the industry’s understanding of the beverage. Different stages of the production process have been analyzed as potential sources of variation or improvement. One study analyzed the effect of medium roasted and dark roasted coffee beans on the effect of caffeine and chlorogenic acid concentrations in the final cold brew coffee. The study found that medium roasted coffee beans resulted in higher concentration of 3-CQA and caffeine (Fuller and Rao 2017). The same study also analyzed the effect of grind size and reported no significant effect on the extraction of caffeine and 3-CQA. However, a second study determined that a course grind size resulted in higher extractions of total dissolved solids, phenols, and overall extraction yield (Cordoba and others 2019). The time required for extraction has also been analyzed by multiple studies with mixed results. Some have reported that 14 hours was the optimal steeping time, others concluded that an equilibrium was reached after 6 to 7 hours, while some commercial coffees advertise a 12-hour brewing process (Fuller and Rao 2017; Coffee-Mate Natural Bliss 2018; Cordoba and others 2019). Another area that has been inconclusive is the temperature at which the coffee should be steeped. Some research used multiple temperatures (5°C and 22°C) while others used room temperature water (Angeloni and others 2017; Cordoba and others 2019). With this limited amount of information, it is evident that more research would be helpful to better understand how cold brew coffee might be improved.
Some cold brew enthusiasts claim that cold brew coffee is less acidic and contains more caffeine than traditional hot brewed coffee (Strand 2017; Maynard 2018). However, research has only begun to analyze the sensory characteristics of cold brew coffee. One study narrowed the language surrounding cold brew coffee from 108 terms to one group of 17 terms and a second group of 48 terms (Heo and others 2019). Another area that has not been researched heavily is possible technical improvements to increase the extraction rate of the steeping process. Although cold brew coffee is gaining in popularity, the extended amount of time required to steep cold brew coffee (hours versus minutes for hot brew methods) makes it more costly to produce commercially; therefore, it would be desirable to industry to accelerate the extraction process. Microwave technology has been used successfully in many different areas to decrease extraction times of various products (Tsubaki and others 2010; Mustapa and others 2015; Belwal and others 2018). Therefore, microwave processing may also be capable of accelerating commercial production of cold brew coffee.

Microwave processing, however, inevitably leads to a temperature increase which could result in a coffee that is very different from cold brewed coffee. Therefore, two variations of microwave treated samples were used for this study, “half water” samples and “full water” samples. The half water samples were made by microwaving half of the total water with coffee grounds and then adding the remaining water, which had been chilled, to the mixture. It was hoped that the addition of chilled water would rapidly decrease the temperature of the mixture and therefore minimize potential differences from a traditional cold brew coffee. To determine if this step did in fact decrease difference compared to cold brew coffee, the full water samples were also studied. Two different temperatures were also analyzed, 50°C and 80°C. It was expected that microwaving the mixtures to 50°C would minimize differences when compared to cold brew
coffee while still achieving an accelerated extraction. However, if temperatures of 80°C were used, the safety of the coffee would be improved. Therefore, both temperatures were studied in order to determine if either temperature was comparable to a cold brew coffee.

Each of the samples in this study were analyzed for sourness, bitterness, total dissolved solids (TDS), color (L* value), caffeine content, and chlorogenic acid (3-CQA) content in an attempt to better understand the effect of the different processing parameters. These parameters describe the coffee in three different ways: the sensory characteristics (sour and bitter), the physical characteristics (TDS and color), and the chemical characteristics (caffeine and 3-CQA). An inspection of these parameters facilitated an overall comparison between the cold and hot brew samples and between the cold only (CB) and microwave-treated cold brew (M-CB) samples.

In summary, to decrease the time needed for cold brew extraction while minimizing the impact to the final cold brew coffee product, one objective of this study was to investigate the effect of microwave assisted extraction on cold brew coffee. Another goal of this study was to gain a better understanding of the extraction of coffee compounds in cold and hot brew coffee steeped for different lengths of time.

Materials and Methods

Sample Preparation

Cold Brew Samples

This study analyzed three groups of samples, cold brew samples (CB), hot brew samples (HB), and microwave treated cold brew samples (M-CB).

The CB samples were prepared in 50 ml tubes and refrigerated in a cold-water bath to maintain a constant temperature of 4°C. Pre-measured water (40 ml) equilibrated to 4°C was
poured into tubes containing 2.2 g pre-weighed coffee grounds. This helped to ensure the extraction took place at refrigeration temperatures while the coffee grounds and water steeped. After steeping, each of the samples was filtered using a regular basket style coffee filter. Three steeping times were tested in this study (1 hour, 3 hours, and 5 hours) which resulted in the following sample designations: 1 Hour Cold Brew, 3 Hour Cold Brew, and 5 Hour Cold Brew.

*Hot Brew Samples*

HB samples were prepared in 50 ml tubes. A hot-water bath was used to maintain a constant temperature of 80°C. Pre-measured water (40 ml) equilibrated to 80°C the hot water bath temperature was poured into the 50 ml tubes containing 2.2 g pre-weighed coffee grounds. Again, this helped to ensure the extraction took place at a constant temperature. After steeping, each of the samples was filtered using a regular basket style coffee filter. Five steeping times were tested for hot brewed samples, resulting in the following sample designations: 2 Min Hot Brew, 4 Min Hot Brew, 6 Min Hot Brew, 8 Min Hot Brew, and 10 Min Hot Brew.

*Microwave-Treated Cold Brew Samples*

Figure 3.1 summarizes the combinations used to prepare the microwave-treated cold brew (M-CB) samples. Two variations of M-CB samples were created for this study, “half water” samples and “full water” samples. These variations were made using two different amounts of water combined with the coffee grounds before being microwaved. The half water samples had 50% of the total amount of water added to the coffee grounds before microwaving, whereas the full water samples had 100% of the water added to the coffee grounds before microwaving. After being microwaved, the remaining water, which had been chilled, was added to the half water
samples. The objective of the half water samples was to facilitate rapid cooling after the samples were microwaved while maintaining a constant water to coffee ratio. These two combinations of water, half water and full water, could serve as a model of a rapid cooling process and a slow cooling process.

Once the appropriate volume of deionized water (100 or 200 ml) was combined with the pre-weighed coffee grounds (11 g) in a beaker, the mixture was microwave heated to either 50°C or 80°C. The coffee-water mixture was then poured into a bottle and, once the total amount of water was added, the bottle was placed in a refrigerated water bath to steep for 0 min, 10 min, 1 h, or 3 h. A water bath was used in order to maintain a more constant temperature while the samples were in the refrigerator.

A 2,450 MHz microwave oven (Panasonic, Osaka, Japan) was used to heat the samples. A Fiso temperature measuring software (Fiso Technologies Inc., Quebec, Canada) was used to monitor the temperature in the beakers during microwaving. When the desired temperature was reached, the microwave was stopped, and the beaker was removed and either mixed with additional water before being placed in a cold-water bath or immediately placed in a cold water bath.

To prepare the half water M-CB samples, the 50% microwave-treated coffee-water mixture was prepared as described above. The remaining 50% of the total water was allowed to equilibrate with a refrigerated water bath. After reaching the appropriate temperature, the 50% microwave-treated coffee-water mixture was poured into a bottle, followed immediately by the 50% equilibrated refrigerated water. Similarly, the full water M-CB samples were prepared as described above; however, these only required pouring the microwave-treated coffee-water mixture into a bottle. Since 100% of the total volume of water was microwave-treated for the full water samples, they did not receive the addition of equilibrated refrigerated water.
The bottles of M-CB samples were allowed to steep in a refrigerated cold-water bath for the following times: 0 min, 10 min, 1 hour, and 3 hours. The M-CB samples receiving 0 min of steeping time were not placed in the cold-water bath at all and were filtered immediately. The other samples were filtered after steeping. Each of the samples were filtered using a regular basket style coffee filter.

Figure 3.1: Microwave-Treated Cold Brew Samples Preparation
Italicized text indicates a portion of the method that differed depending on the sample being made
Non-italicized text indicates a portion of the method that was the same for all samples

Sensory Analysis

The sensory analysis was conducted in accordance with the North Carolina State University Institutional Review Board for the Protection of Human Subjects in Research regulations. A panel of 10 trained tasters performed the sensory analysis for bitter and sour intensity. The panel received training over a period of 9 weeks for a total of at least 10 hours of training. The World Coffee Research Sensory Lexicon values were used when training the panelists to score bitter and sour intensity. This lexicon uses a scale of 0-15 to evaluate attributes
with 0 being less intense and 15 being more intense. During training, each panelist was provided a set of known standards for each attribute. The various intensities of the bitter standard were prepared by mixing increasing quantities of caffeine into deionized water. Similarly, different levels of the sour standard were prepared using citric acid. Table 3.1 indicates the concentration of each compound and the corresponding score. These standards were then used by each panelist to score unknown samples, thus improving the panelists’ sensory skills for each attribute. After training was completed, the same standards were used during all test sessions in which samples were analyzed.

Table 3.1: Citric Acid and Caffeine Solution Concentrations Correlated to Corresponding Scores

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Standards</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.015% Citric Acid Solution</td>
<td>1.5 Sour Standard</td>
</tr>
<tr>
<td>0.033% Citric Acid Solution</td>
<td>2.5 Sour Standard</td>
</tr>
<tr>
<td>0.05% Citric Acid Solution</td>
<td>3.5 Sour Standard</td>
</tr>
<tr>
<td>0.01% Caffeine Solution</td>
<td>2.0 Bitter Standard</td>
</tr>
<tr>
<td>0.02% Caffeine Solution</td>
<td>3.5 Bitter Standard</td>
</tr>
<tr>
<td>0.035% Caffeine Solution</td>
<td>5.0 Bitter Standard</td>
</tr>
<tr>
<td>0.05% Caffeine Solution</td>
<td>6.5 Bitter Standard</td>
</tr>
</tbody>
</table>

In order to minimize fatigue, no more than five samples were analyzed by the panel during a given session. To prevent bias, each sample was assigned a random three-digit number. Each of the samples and standards were served to the panelists at room temperature in 59 ml lidded, clear plastic soufflé cups. The soufflé cups were filled with approximately 30 ml of sample or standard. The samples were made the day before being served to the panel and stored at room temperature overnight. Unsalted crackers and deionized water were served to panelists to cleanse the palate between samples. Panelists waited at least 2 minutes between each sample in order to further cleanse the palate. Each sample was analyzed on three different days.
Physical Analysis

Color was measured using a Colorflex EZ Spectrophotometer (HunterLab, Reston, VA). The LAB color model was used to report color. L* represents the lightness or darkness of a sample with a value of 0 being black and 100 being white. The a* indicates green or red in a sample, negative values indicate green color and positive values represent red color. The b* measures the blue and yellow color in a sample, blue would result in more negative values and yellow in more positive. For this study, the L* value was reported as an indication of the degree to which the coffee samples were dark or light. A digital pocket refractometer (ATAGO pal-coffee digital pocket coffee refractometer, Tokyo, Japan) was used to analyze TDS.

Chemical Analysis

Caffeine and 3-CQA were measured using an adapted methodology reported by Fujioka and Shibamoto (2008). High pressure liquid chromatography (HPLC) is commonly used to determine the caffeine and chlorogenic acid content of the samples. A Waters X-Bridge C-18, 3.5 μm column (100 mm x 4.6 mm) (Waters Corporation, Millford, MA) was run at ambient temperature. A gradient of two mobile phases were used during the study. Mobile phase A consisted of 90% 20 mM citric acid monohydrate and 10% methanol. Mobile phase B was 100% methanol. The gradient began at 100% A and 0% B and increased to 100% B. The flow rate was 0.7 mL/min with an injection volume of 10 μL. A Waters software system was utilized to run the HPLC and autosampler (Waters Corporation, Millford, MA).

Standard curve solutions for caffeine and 3-CQA were made from chemicals purchased from Sigma-Aldrich (St. Louis, MO). A chromatogram depicting one point on the caffeine and 3-CQA standard curve is shown in figure 3.2 while figure 3.3 shows an example chromatogram of a
coffee sample. HPLC grade methanol and water and citric acid monohydrate were purchased from Fisher Scientific (Hampton, NH).
Figure 3.2: Example Chromatograms of a Point on the Caffeine and 3-CQA Standard Curve
In this figure, the A) caffeine and B) 3-CQA chromatogram for the 0.625 mM standard is shown. Caffeine is measured at a wavelength of 276 nm and had a retention time of approximately 11.9 min. 3-CQA is measured at a wavelength of 325 nm and had a retention time of approximately 11.2 min.
Figure 3.3: Caffeine and 3-CQA Chromatograms of a Sample
The A) caffeine and B) 3-CQA chromatogram for the following sample is shown: full water, M-CB, microwaved to 50°C, and steeped for 1 hour in a cold water bath.
Statistical Analysis

A one-way analysis of variance (ANOVA) and Tukey-Kramer HSD was used to determine differences and similarities between samples for sourness, bitterness, TDS, L* value, caffeine, and 3-CQA. Statistically significant differences were determined by a p < 0.05. SAS JMP 14 statistical software (Cary, NC) was used to analyze the data. Values were reported as means ± standard error of the mean.

Results and Discussion

Hot vs Cold Brew

There were two main observations displayed in the data (table 3.2). The first notable observation was regarding color (L* value), which demonstrated a clear pattern between the hot and cold coffee. For each of the hot brew (HB) samples, the L* value had a statistically significant lower value; indicating that they were darker in color than any of the CB samples. The CB samples had an L* value that ranged from 38.78 to 40.15, while the HB samples ranged from 19.04 to 24.32. The second notable observation was a lack of statistically significant differences between the three CB samples. This was true for each of the measured attributes: sourness, bitterness, TDS, L* value, caffeine content, and 3-CQA content. The minimal differences between the three CB samples indicated that the brewing time only had a small impact on the extraction of the measured attributes. Extraction of both HB and CB indicates that extraction takes place more quickly than previously thought. However, it would be useful to further study the kinetics of CB and HB extraction. Although these two observations were notable, they did not help define cold brew coffee. Future studies should examine likeability to determine if the differences between attributes contribute significantly to the consumer preference.
When comparing attributes of the CB samples to the HB samples, once again, the noted differences did not identify any patterns that would improve or clarify the definition of cold brew coffee. However, a few differences were evident in the data. A comparison of the 1 Hour Cold Brew with the HB samples showed statistically significant differences in the following samples and attributes: the 4 Min Hot Brew in sourness, all of the HB samples (except the 2 Min Hot Brew) in bitterness, all of the HB samples in TDS, the 8 Min and 10 Min Hot Brew in caffeine content and 3-CQA content, and all of the HB samples in color (L*value). The 3 Hour Cold Brew had a statistically significant difference from the 10 Min Hot Brew in bitterness, from all of the HB samples in TDS, and all of the HB samples in color (L*value). The 5 Hour Cold Brew had a statistically significant difference from: the 4 Min Hot Brew in sourness, the 10 Min Hot Brew in bitterness and TDS, and all of the HB sample in color (L* value). In summary, the CB sample that steeped longer was more similar to the HB samples and the one with the least amount of time to steep was the least similar.

It was expected that the longer cold brew coffee steeps, it will extract more compounds from the coffee grounds and become more similar to hot brew coffee. This was illustrated by the number of differences observed between the three CB samples compared to the HB samples. As shown in table 3.3, there were a total of 30 possible differences that could have been observed when comparing just one of the CB samples to all of the HB samples (6 attributes x 5 hot brew samples). This study looked at cold brews steeped over three different time intervals: 1 hour, 3 hours, and 5 hours. Therefore, there was a total of 90 possible differences that could have been observed for the CB samples over all three time intervals. The 1 Hour Cold Brew was most different from the HB samples while the 5 Hour Cold Brew was most similar. Of the possible differences that could have been observed when comparing the CB samples to the HB samples for
each attribute, the 1 Hour Cold Brew had 19 observed differences, the 3 Hour Cold Brew had 11 observed differences, and the 5 Hour Cold Brew had 8 observed differences. In total, the CB samples were 42% different from the HB samples with 38 total observed differences out of a possible 90. Analyzing the CB samples individually, the 1 Hour Cold Brew was 63% different from the HB samples with 19 total observed differences out of a possible 30. The 3 Hour Cold Brew was 37% different and the 5 Hour Cold Brew was 27% different. Figure 3.2 depicts the way in which the extraction occurred over time. Although the differences were minimal, as expected, the longer the cold brew coffee steeps, the more similar to hot brew coffee it becomes.

Comparing the CB samples, collectively, to the different brewing times of the HB samples showed that there were 5 differences between the CB samples and the 2 Min Hot Brew, 8 differences between the CB samples and the 4 Min Hot Brew, 6 differences compared to the 6 Min Hot Brew, 8 compared to the 8 Min Hot Brew, and 11 compared to the 10 Min Hot Brew. These results show that the CB samples were most similar to the HB samples with the least brewing time and became more dissimilar as brewing time increased.

As shown in table 3.2, the sourness for the CB samples ranged from 1.58-1.69 while the HB samples were 1.91-2.43. The CB samples had a bitter value of 4.19-4.53 and the HB samples ranged from 4.99-5.96. TDS ranged from 0.84-0.93 in the CB samples and from 1.09-1.32 in the HB samples. It was noted that the TDS values for the HB samples in this study were close to the TDS range recommended by the Specialty Coffee Association’s Brewing Control Chart of 1.15%-1.35% (SCAA Standard Golden Cup). This suggests that the HB samples in this study serve as a useful reference point of comparison to the CB samples. The L* color values ranged from 37.78-40.15 in the CB samples and 21.50-24.32 in the HB samples. The CB samples had a caffeine and 3-CQA content of 55.81-74.99 mg/100 ml and 32.46-47.05 mg/100 ml, respectively. The HB
samples ranged from 70.35-84.72 mg/100 ml for caffeine and 38.81-52.86 mg/100 ml for 3-CQA content. Other sources have found that cold brew coffee, that had been steeped for about 6 hours, contained approximately 93-113 mg/100 ml of caffeine while regular drip brew coffee contained approximately 40-139 mg/100 ml of caffeine (Fuller and Rao 2017; Mejia and Ramirez-Mares 2014).
Table 3.2: CB Samples Compared to HB samples
Two means in the same column with the same letter do not differ significantly.
Each sample was measured in triplicate

<table>
<thead>
<tr>
<th></th>
<th>Sour</th>
<th>Bitter</th>
<th>TDS</th>
<th>L*</th>
<th>Caffeine (mg/100ml)</th>
<th>3-CQA (mg/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HB @ 2 min</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.21 ± 0.10 ab</td>
<td>4.99 ± 0.15 abc</td>
<td>1.09 ± 0.11 ab</td>
<td>24.32 ± 0.19 b</td>
<td>70.35 ± 0.88 ab</td>
<td>44.11 ± 2.31 ab</td>
</tr>
<tr>
<td><strong>HB @ 4 min</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>2.43 ± 0.15 a</td>
<td>5.74 ± 0.12 ab</td>
<td>1.16 ± 0.01 ab</td>
<td>23.04 ± 0.49 b</td>
<td>64.61 ± 5.59 ab</td>
<td>38.81 ± 2.50 ab</td>
</tr>
<tr>
<td><strong>HB @ 6 min</strong></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>2.13 ± 0.24 ab</td>
<td>5.72 ± 0.45 ab</td>
<td>1.13 ± 0.01 ab</td>
<td>21.68 ± 0.87 b</td>
<td>71.74 ± 0.53 ab</td>
<td>46.03 ± 2.08 ab</td>
</tr>
<tr>
<td><strong>HB @ 8 min</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.29 ± 0.10 ab</td>
<td>5.65 ± 0.26 ab</td>
<td>1.14 ± 0.02 ab</td>
<td>21.50 ± 1.02 b</td>
<td>83.48 ± 5.86 a</td>
<td>52.26 ± 4.34 a</td>
</tr>
<tr>
<td><strong>HB @ 10 min</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.91 ± 0.06 ab</td>
<td>5.96 ± 0.20 a</td>
<td>1.32 ± 0.01 a</td>
<td>19.04 ± 2.67 b</td>
<td>84.72 ± 2.17 a</td>
<td>52.86 ± 5.01 a</td>
</tr>
<tr>
<td><strong>CB @ 1 h</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.58 ± 0.23 b</td>
<td>4.19 ± 0.50 c</td>
<td>0.84 ± 0.06 c</td>
<td>40.15 ± 0.98 a</td>
<td>55.81 ± 6.53 b</td>
<td>32.46 ± 4.84 b</td>
</tr>
<tr>
<td><strong>CB @ 3 h</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.69 ± 0.15 ab</td>
<td>4.44 ± 0.12 bc</td>
<td>0.85 ± 0.04 c</td>
<td>42.84 ± 2.50 a</td>
<td>70.57 ± 3.78 ab</td>
<td>43.26 ± 1.57 ab</td>
</tr>
<tr>
<td><strong>CB @ 5 h</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.63 ± 0.03 b</td>
<td>4.53 ± 0.24 bc</td>
<td>0.93 ± 0.02 bc</td>
<td>38.78 ± 3.33 a</td>
<td>74.99 ± 2.86 ab</td>
<td>47.05 ± 3.43 ab</td>
</tr>
</tbody>
</table>
Figure 3.4: Demonstration of Extraction Over Time

Graph C, TDS, and graph D, Caffeine, represent a physical and chemical attribute respectively. These two attributes in combination with the sensory characteristics (graph A Sour and graph B Bitter) help to show how the extraction progressed over time. The CB sample correspond to the topmost x-axis while the HB samples correspond to the bottom most x-axis. Error bars represent standard error.
Microwave vs Cold Brew

There were very few differences between the CB samples and those that were microwaved before being cold brewed (M-CB) as shown in table 3.3 and table 3.4. No statistically significant difference for sourness was observed between any of the CB samples compared to M-CB samples. The 1 Hour Cold Brew was different from two M-CB samples for bitterness, 6 for TDS, 5 for color, and 8 for both caffeine and 3-CQA. The 3 Hour Cold Brew was not statistically different from any of the M-CB samples in bitterness or sourness and was only different from one sample in 3-CQA, from 4 in TDS, 7 in color, and 3 in caffeine. Even fewer differences were observed when comparing the 5 Hour Cold Brew to the M-CB samples. There were no statistical differences between the 5 Hour Cold Brew and the M-CB samples for bitterness, sourness, or TDS; however, differences were noted for 4 samples in color, 5 samples in caffeine, and 1 sample in 3-CQA.

Table 3.3 shows that, when comparing the CB samples to the M-CB samples for each attribute, there were a total of 96 possible differences that can be observed for each cold brewed sample (6 attributes x 16 variations of microwaved samples). This brings the total number of possible differences for all three CB samples to 288 (3 x 96). The 1 Hour Cold Brew had the most observed differences with a total of 29, followed by the 3 Hour Cold Brew with 16 observed differences, and the 5 Hour Cold Brew with 10 observed differences. In total, the three variations of CB samples were only 19% different from all of the variations of M-CB samples, with 55 total differences out of a possible 288. Of the 55 observed differences, 71% were in samples microwaved to 80°C (both the half water and the full water). However, the processing method that created the most differences overall, was microwaved with full water to 80°C. This accounted for 51% of the total differences.
Sourness for the CB samples ranged from 1.58-1.69 while the M-CB samples ranged from 1.60-2.27. The CB samples had a bitter value of 4.19-4.53 and the M-CB samples ranged from 4.56-5.83. TDS ranged from 0.84-0.93 in the CB samples and from 0.88-1.08 in the M-CB samples. These TDS values are below the 1.15%-1.35% values recommended by the Brewing Control Chart. Color (L*) values were between 38.78-42.84 in the CB samples and 23.99-35.78 in the M-CB samples. The CB samples had a caffeine and 3-CQA content of 55.81-74.99 mg/100 ml and 32.46-47.05 mg/100 ml, respectively, while the M-CB samples ranged from 48.72-100.00 mg/100 ml and 29.98-62.64 mg/100 ml for caffeine and 3-CQA content, respectively.

When comparing the 1 Hour and 3 Hour Cold Brew samples to the M-CB samples, the following was the order of least number of differences to most: half water microwaved to 50°C, full water microwaved to 50°C, half water microwaved to 80°C, and full water microwaved to 80°C. When comparing the 5 Hour Cold Brew to the M-CB samples, the order of least number of differences to most was, half water microwaved to 80°C, full water microwaved to 50°C, full water microwaved to 80°C, and half water microwaved to 50°C. The 5 Hour Cold Brew and M-CB samples microwaved to 80°C may have yielded a more complete extraction and, therefore, were more similar than the cold brew samples steeped for less time.
Table 3.3: Statistically Significant Difference Between the CB Samples and Other Samples

A “−” indicates that the CB sample had a lower value than the sample its being compared to. A “+” indicates that the CB sample had a higher value than the sample its being compared to. An L* value of 0 represents a darker material and a value of 100 represents a lighter material.

<table>
<thead>
<tr>
<th>Steeping Time</th>
<th>Hot Brewed Samples</th>
<th>Cold Brew 1 hr</th>
<th>Cold Brew 3 hr</th>
<th>Cold Brew 5 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 min</td>
<td>-</td>
<td>- +</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4 min</td>
<td>- -</td>
<td>- +</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6 min</td>
<td>- -</td>
<td>- +</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>8 min</td>
<td>- -</td>
<td>- +</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>10 min</td>
<td>- -</td>
<td>- +</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>0 min</td>
<td>- -</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>10 min</td>
<td>-</td>
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<td>-</td>
<td>+</td>
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<tr>
<td>1 hr</td>
<td>-</td>
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<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3 hr</td>
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<td>+</td>
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<tr>
<td>0 min</td>
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<tr>
<td>10 min</td>
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<td>+</td>
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<tr>
<td>1 hr</td>
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<td>+</td>
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<tr>
<td>3 hr</td>
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<td>0 min</td>
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<td>10 min</td>
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<td>1 hr</td>
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<td>3 hr</td>
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<td>10 min</td>
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<tr>
<td>1 hr</td>
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<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3 hr</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Hot Brewed Samples

Cold Brew 1 hr

Cold Brew 3 hr

Cold Brew 5 hr

80C

50C

Half Water (50/50)

Cold Brew 1 hr

Cold Brew 3 hr

Cold Brew 5 hr

Microwaved then Cold Brewed Samples

80C

50C

Full Water (100/0)

Cold Brew 1 hr

Cold Brew 3 hr

Cold Brew 5 hr
Table 3.4: CB Samples Compared to M-CB Samples
Two means in the same column with the same letter do not differ significantly
Each sample was measured in triplicate

<table>
<thead>
<tr>
<th></th>
<th>Sour</th>
<th>Bitter</th>
<th>TDS</th>
<th>L*</th>
<th>Caffeine (mg/100ml)</th>
<th>3-CQA (mg/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold Brew @ 1 h</td>
<td>1.58 ± 0.23 a</td>
<td>4.19 ± 0.50 b</td>
<td>0.84 ± 0.06 d</td>
<td>40.15 ± 0.98 ab</td>
<td>55.81 ± 6.53 fghi</td>
<td>32.46 ± 4.84 ef</td>
</tr>
<tr>
<td>Cold Brew @ 3 h</td>
<td>1.69 ± 0.15 a</td>
<td>4.44 ± 0.12 ab</td>
<td>0.85 ± 0.04 cd</td>
<td>42.84 ± 2.50 a</td>
<td>70.57 ± 3.78 cdefgh</td>
<td>43.26 ± 1.57 bcdef</td>
</tr>
<tr>
<td>Cold Brew @ 5 h</td>
<td>1.63 ± 0.03 a</td>
<td>4.53 ± 0.24 ab</td>
<td>0.93 ± 0.02 abcd</td>
<td>38.78 ± 3.33 abc</td>
<td>74.99 ± 2.86 bcdef</td>
<td>47.05 ± 3.43 abcdef</td>
</tr>
<tr>
<td>50/50 to 80°C, 0 h</td>
<td>2.26 ± 0.12 a</td>
<td>5.78 ± 0.23 a</td>
<td>1.04 ± 0.01 ab</td>
<td>27.64 ± 1.43 cde</td>
<td>62.82 ± 2.52 defghi</td>
<td>39.15 ± 3.55 cdef</td>
</tr>
<tr>
<td>50/50 to 80°C, 10 min</td>
<td>1.89 ± 0.25 a</td>
<td>5.29 ± 0.27 ab</td>
<td>1.02 ± 0.03 abc</td>
<td>30.05 ± 2.30 bcde</td>
<td>71.92 ± 5.89 bcdefgh</td>
<td>46.19 ± 3.20 bcde</td>
</tr>
<tr>
<td>50/50 to 80°C, 1 h</td>
<td>1.96 ± 0.20 a</td>
<td>5.61 ± 0.29 ab</td>
<td>0.98 ± 0.04 abcd</td>
<td>30.64 ± 0.93 bcde</td>
<td>73.05 ± 7.02 bcdefg</td>
<td>45.87 ± 3.72 bcdef</td>
</tr>
<tr>
<td>50/50 to 80°C, 3 h</td>
<td>1.90 ± 0.12 a</td>
<td>5.83 ± 0.12 a</td>
<td>0.98 ± 0.02 abcd</td>
<td>26.41 ± 1.03 abcde</td>
<td>78.85 ± 7.48 bcd</td>
<td>51.93 ± 5.76 abcd</td>
</tr>
<tr>
<td>50/50 to 50°C, 0 h</td>
<td>1.84 ± 0.15 a</td>
<td>4.79 ± 0.60 ab</td>
<td>0.90 ± 0.01 abcd</td>
<td>33.05 ± 3.15 abcde</td>
<td>53.96 ± 0.41 gh</td>
<td>33.14 ± 1.29 ef</td>
</tr>
<tr>
<td>50/50 to 50°C, 10 min</td>
<td>1.60 ± 0.10 a</td>
<td>5.13 ± 0.17 ab</td>
<td>0.88 ± 0.01 abcd</td>
<td>35.78 ± 2.19 abcd</td>
<td>48.72 ± 3.42 i</td>
<td>29.98 ± 2.90 f</td>
</tr>
<tr>
<td>50/50 to 50°C, 1 h</td>
<td>1.81 ± 0.03 a</td>
<td>4.56 ± 0.49 ab</td>
<td>0.88 ± 0.03 abcd</td>
<td>31.64 ± 2.70 abcde</td>
<td>51.93 ± 1.61 hi</td>
<td>32.66 ± 1.02 ef</td>
</tr>
<tr>
<td>50/50 to 50°C, 3 h</td>
<td>1.96 ± 0.13 a</td>
<td>4.56 ± 0.23 ab</td>
<td>0.89 ± 0.02 abcd</td>
<td>35.57 ± 1.93 abcde</td>
<td>52.37 ± 0.53 hi</td>
<td>33.29 ± 0.75 ef</td>
</tr>
<tr>
<td>100/0 to 80°C, 0 h</td>
<td>2.07 ± 0.06 a</td>
<td>5.36 ± 0.38 ab</td>
<td>1.04 ± 0.04 ab</td>
<td>27.35 ± 3.43 de</td>
<td>78.24 ± 3.55 bcdce</td>
<td>49.73 ± 3.04 abcd</td>
</tr>
<tr>
<td>100/0 to 80°C, 10 min</td>
<td>2.27 ± 0.07 a</td>
<td>5.38 ± 0.29 ab</td>
<td>1.08 ± 0.02 a</td>
<td>23.99 ± 1.47 e</td>
<td>87.20 ± 3.15 abcde</td>
<td>58.56 ± 2.94 ab</td>
</tr>
<tr>
<td>100/0 to 80°C, 1 h</td>
<td>2.18 ± 0.13 a</td>
<td>5.63 ± 0.12 ab</td>
<td>1.08 ± 0.02 a</td>
<td>25.97 ± 1.53 de</td>
<td>79.33 ± 1.15 bcd</td>
<td>53.70 ± 1.60 abc</td>
</tr>
<tr>
<td>100/0 to 80°C, 3 h</td>
<td>1.92 ± 0.22 a</td>
<td>5.20 ± 0.18 ab</td>
<td>1.05 ± 0.08 ab</td>
<td>26.95 ± 2.45 de</td>
<td>79.97 ± 4.62 abcd</td>
<td>53.40 ± 5.13 abc</td>
</tr>
<tr>
<td>100/0 to 50°C, 0 h</td>
<td>1.95 ± 0.15 a</td>
<td>4.74 ± 0.03 ab</td>
<td>0.92 ± 0.03 bcd</td>
<td>34.00 ± 1.20 abcde</td>
<td>91.42 ± 2.34 ab</td>
<td>55.79 ± 1.88 ab</td>
</tr>
<tr>
<td>100/0 to 50°C, 10 min</td>
<td>1.81 ± 0.21 a</td>
<td>5.33 ± 0.22 ab</td>
<td>0.97 ± 0.02 bcd</td>
<td>32.22 ± 0.73 abcde</td>
<td>100.00 ± 1.31 a</td>
<td>62.64 ± 0.94 a</td>
</tr>
<tr>
<td>100/0 to 50°C, 1 h</td>
<td>1.93 ± 0.13 a</td>
<td>5.52 ± 0.26 ab</td>
<td>0.97 ± 0.02 bcd</td>
<td>32.75 ± 2.09 abcde</td>
<td>89.58 ± 0.56 abc</td>
<td>57.78 ± 1.11 ab</td>
</tr>
<tr>
<td>100/0 to 50°C, 3 h</td>
<td>1.94 ± 0.07 a</td>
<td>5.07 ± 0.03 ab</td>
<td>0.99 ± 0.03 bcd</td>
<td>31.79 ± 1.63 abcde</td>
<td>58.24 ± 1.11 efghi</td>
<td>36.51 ± 0.97 def</td>
</tr>
</tbody>
</table>
Conclusion

The CB samples which steeped for 1 hour, 3 hours, and 5 hours were compared; there were no statistically significant differences among the measured attributes. This indicates that it may be unnecessary to steep cold brew coffee for more than an hour, contrary to what is recommended by many recipes or advertised by many commercially sold products. Notably, other studies have also reported a slower rate of extraction after 3 hours of cold brewing which supports the results of this study: steeping for more than an hour may be unnecessary (Fuller and Rao 2017).

Some similarities and differences were observed between the CB samples and the HB samples. As expected, the 5 Hour Cold Brew was most similar to the HB samples and the 1 Hour Cold Brew was most different from the HB samples. Another expectation, which was also supported by the data, was that the CB samples would be most similar to the 2 Min Hot Brew coffee and different from the 10 Min Hot Brew coffee.

When comparing all of the CB samples to the M-CB samples, it is important to note that there were no differences in sour taste and only two differences in bitter taste. Most of the differences that did occur were because the M-CB samples extracted a higher concentration of caffeine and 3-CQA. Therefore, adding a microwave processing step prior to steeping at refrigerated temperatures causes only a slight difference to traditional cold brew coffee. A beverage with a higher concentration of caffeine, similar to traditional cold brew, and produced in less time would be desirable to industry.
REFERENCES


Strand O. 2017. How Cold Brew Changed the Coffee Business. NYTimes.com Feed

CHAPTER 4: Viability of Microwave Technology for Accelerated Cold Brew Coffee

Processing vs Conventional Brewing Methods

Abstract

Production of popular cold brew (CB) coffee beverages requires 10-24 h of cold-water infusion. Accelerating this process would facilitate its production on a large scale. This study compared hot brewed coffee (HB), CB, heat-treated CB coffee (H-CB), and microwave treated CB coffee (M-CB) over time in terms of four attributes: color measured by L* values, total dissolved solids (TDS), and mg/100 g caffeine and chlorogenic acid (3-CQA). L* decreased over time for both HB and CB (34.98-17.19 vs 64.62-43.00), while TDS (0.89-1.39 vs 0.29-0.85), caffeine (48.90-84.39 vs 15.23-61.42) and 3-CQA (33.60-62.85 vs 5.55-44.82) increased. H-CB and M-CB attributes remained constant after the heat treatment (L*, 34.46-35.33 vs 29.23-29.29; TDS, 0.80-1.03 vs 1.00-0.94; caffeine, 56.10-62.21 vs 60.88-69.85; 3-CQA, 39.17-46.00 vs 41.39-49.95), were similar to CB samples, but required less preparation time. A brief heat treatment prior to cold infusion accelerates CB production, allowing industry to develop faster, less costly processing methods.

Introduction

Water is a universally used solvent, however, it is well known that temperature impacts the effectiveness of water’s solvent properties; an increase in temperature usually accelerates the ability of water to dissolve or extract compounds from a solute material. The same principle is true when using water to brew coffee. Hot water has been used to brew coffee for much of the world's history. Throughout the years, innovations to the brewing process have occurred, resulting in the development of methods such as boiled coffee, espresso, instant, drip brew, etc. For example,
Turkish coffee, which was developed sometime in the 1500s and continues to be a common brewing method throughout countries comprising the former Ottoman Empire, involves briefly boiling very finely ground coffee in water (Pendergast, 1999). Recently, there have even been advances in the production of ready-to-drink coffee beverages. These beverages are fully prepared and may be either refrigerated or shelf stable. The convenience of ready to drink coffee does not require brewing by the consumer or a long wait time in a coffee shop queue. Ready-to-drink (RTD) beverages have become a well-known product to 89 percent of consumers (National Coffee Association 2019).

A common trait among most coffee brewing methods is the use of hot water, at 80-95°C, to rapidly (seconds to minutes) extract compounds from ground coffee. This is true for coffee made by consumers as well as for coffee produced commercially on a small or large scale (Petracco 2008). However, a new method of brewing coffee has quickly become popular, cold brewed coffee. This coffee is commonly brewed by allowing ground coffee to infuse in cold water for extended periods of time (hrs to days). Interest in cold brew coffee has been increasing which has led to consumer behaviors that are creating a coffee trend that is evident by the rise in cold brew coffee sales. In 2017, $38.1 million in coffee sales were attributed to cold brew coffee, while in 2015, cold brew was worth $8.1 million in sales (Maynard 2018). The number of restaurant menus mentioning cold brew coffee has also increased by 36% in recent years (Cobe and Nash 2020). These consumer trends are shaping beverage industry decisions concerning product development.

The increasing demand for cold brew coffee will place a burden on both industry and on the sustainability of the supply chain of cold brew coffee beverages to consumers. Cold brew coffee requires long infusion time which slows the production process for industry when producing RTD beverages. It is also cumbersome to ensure safety of the coffee which must be kept at
refrigerated temperatures throughout the supply chain as the RTD cold brew coffee is shipped from the processing facility to grocery stores and into consumers’ homes. Because cold brew coffee is not an acidic beverage (pH above 4.6) and does not receive a heat treatment, there is no mitigating step to control for microbial contamination. Companies must therefore rely on their sanitation efforts and refrigeration to ensure both shelf life and safety. The introduction of a heating step into the cold brewing process could minimize these concerns over safety. Some RTD cold brew coffees are advertised as shelf stable and may have undergone a heat step. However, this would only be desirable if the heat treatment does not impact the sensory characteristics of the final beverage. This heat step may also improve extraction rates and therefore decrease the required infusion time.

One technology that has been used to achieve rapid heating and accelerated extraction rates is microwave heating. Microwave technology successfully increased extraction rates of various products such as phenolic compounds from tea, and medicinal plants and extraction of many compounds from botanicals (Tsubaki and others 2010; Mustapa and others 2015; Belwal and others 2018). It is hoped that it is possible to utilize microwave processing to also accelerate the commercial production of RTD cold brew coffee to help meet increased consumer demands.

Studying the effect of microwave technology on cold brew coffee would be beneficial to the beverage industry if it helps to reduce infusion times and increase processing speeds. Understanding how cold brew coffee is affected by different temperature treatments would also be helpful when attempting to improve processing methods. Therefore, this study will investigate the differences in coffee extraction by performing an analysis of extracted attributes for cold brewed coffee, hot brewed coffee, hot water treated cold brewed coffee, and microwave treated cold brewed coffee.
Materials and Methods

Sample Preparation

Each sample was prepared using deionized water and a single source of ground coffee. Medium roast, whole coffee beans were purchased (Big Trouble Blend, Counter Culture, Durham NC) and ground in the store on a medium grind setting. The overall particle size distribution ranged from 200-1400 µm with over 65% of the particles ranging from 710-1000 um. A ratio of 55 g/L of ground coffee to water was used for each sample. This ratio was selected based on the recommendations of the brewing control chart developed by the Specialty Coffee Association (SCAA Standard Golden Cup). Four different brewing methods (described below) were used: cold brew, hot brew, heat-treated cold brew, and microwave-treated cold brew. To maintain a constant temperature, samples were placed in a water bath of the appropriate temperature for each treatment. A completely randomized split-plot design was employed. A complete set of samples was prepared across three different days for each brewing method. Within each day, samples were prepared in triplicate across nine time points (4 brewing methods x 3 days per brewing method x 9 time points x 3 replications). Only one brewing method (either CB, HB, H-CB, or M-CB) was prepared each day for a total experiment time of 12 days. The day on which each brewing method was prepared was also randomized. Samples measured at the 0 min time point never underwent infusion in a water bath. Samples were infused for 2 min, 4 min, 8 min, 30 min, 1 h, 2 h, and 3 h. All other infusion times were based on the amount of time the sample was exposed to the water bath; measurement of infusion time was initiated upon placement of the samples in the water bath. After reaching each time point, extraction was halted by filtering samples into a beaker using 8-inch, natural unbleached paper filters (Food Lion brand, Salisbury NC). Following filtration, samples were transferred to 50 ml conical tubes and stored at -50°C.
Cold Brew Samples (CB)

The cold brew samples were prepared using a water bath placed in a refrigerator (cold water bath). The water bath was used to create a stable temperature while the CB samples were in the refrigerator (4°C). Two 50 ml conical tubes were used to prepare each sample. In one tube, 40 ml of deionized water was pre-measured and placed into the cold-water bath to equilibrate. The second tube contained 2.2 g of pre-measured ground coffee. Once the temperature of the deionized water had equilibrated to 4°C, the water was poured into the tube containing ground coffee. The tube containing the coffee-water mixture was then placed in the cold-water bath for the desired infusion time. To ensure there was no effect on the samples due to location within the water bath, they were randomly placed in the cold-water bath on each day of preparation.

Hot Brew Samples (HB)

A hot water bath was used to prepare the hot brewed samples. A sous vide precision cooker (Anova, San Francisco, CA) was used to maintain the temperature of the water bath at 80°C; insulation around the water bath also helped to maintain a stable temperature. Samples were prepared in 50 ml conical tubes in a similar manner to that used during preparation of the cold brew samples. Ground coffee and deionized water was pre-measured and placed into separate tubes, the water was allowed to equilibrate to 80°C, after which the contents of the two tubes were combined to create the samples. The 0 min samples were measured at this time, while the rest of the samples were placed in the hot water bath for the desired infusion time. The position of the HB samples in the hot water bath was also randomized each day.
Hot Water Treated Cold Brew Samples (H-CB)

A process utilizing a combination of steps involving a hot water bath and a cold-water bath was used to prepare the H-CB samples. Similar to the CB and HB samples, the water and ground coffee were pre-measured into separate 50 ml conical tubes for each sample. However, for the H-CB samples, the tube containing water was placed in the hot water bath to equilibrate. Once the water in the tube had equilibrated to 80°C, it was poured into the tube containing ground coffee. The tube containing the coffee-water mixture was then placed in the cold-water bath (4°C) for the desired infusion time. This process simulated a rapid heat treatment followed by a period of cold infusion. The sample placement in the cold-water bath was randomized each day.

Microwave Treated Cold Brew Samples (M-CB)

A 1250 MHz microwave oven (Panasonic, Osaka, Japan) was used to apply a microwave treatment to the M-CB samples. A Fiso temperature measuring device and software (Fiso Technologies Inc., Quebec, Canada) was used to constantly monitor the temperature of the samples while they were microwaved up to 80°C. The ground coffee (11 g) and water (200 ml) were pre-measured and combined in a beaker immediately before the microwave treatment. Upon reaching the desired temperature, the microwave was stopped, and the beaker was removed. The 0 min M-CB sample was filtered immediately after completing the microwave treatment. All other samples of the microwaved coffee-water mixture were poured into bottles and placed in a cold-water bath in the refrigerator (4°C) for the desired infusion time. Measurement of infusion time was initiated upon placement of these samples in the cold-water bath; the amount of time the mixture spent in the microwave was not included in the infusion time.
Physical Analysis

Total Dissolved Solids (TDS) were measured using a digital pocket refractometer (ATAGO pal-coffee digital pocket coffee refractometer, Tokyo Japan). Color was measured using a Colorflex EZ Spectrophotometer (HunterLab, Reston, VA). The color is expressed using the LAB color model in which L* represents the lightness or darkness of a sample with a value of 0 being black and 100 being white. The a* indicates green or red in a sample, negative values indicate green color and positive values represent red color. The b* measures the blue and yellow color in a sample, blue would result in more negative values and yellow in more positive. For this study, the L* value was reported as an indication of the degree to which the coffee samples were dark or light.

Chemical Analysis

Caffeine and 3-CQA were measured using an adapted methodology reported by Fujioka and Shibamoto (2008). High pressure liquid chromatography (HPLC) is commonly used to determine the caffeine and chlorogenic acid content of the samples. A Waters X-Bridge C-18, 3.5 μm column (100 mm x 4.6 mm) (Waters Corporation, Millford, MA) was run at ambient temperature. A gradient of two mobile phases was used during the study. Mobile phase A consisted of 90% 20 mM citric acid monohydrate and 10% methanol. Mobile phase B was 100% methanol. The gradient began at 100% A and 0% B and increased to 100% B. The flow rate was 0.7 mL/min with an injection volume of 10 μL. A Waters software system was utilized to run the HPLC and autosampler (Waters Corporation, Millford, MA).

Standard curve solutions for caffeine and 3-CQA were made from chemicals purchased from Sigma-Aldrich (St. Louis, MO). An example of a chromatogram that might be produced
when preparing the caffeine and 3-CQA standard curves is shown in figure 4.1. HPLC grade methanol and water and citric acid monohydrate were purchased from Fisher Scientific (Hampton, NH).
Figure 4.1: Example Chromatograms of a Point on the Caffeine and 3-CQA Standard Curve
In this figure, the A) caffeine and B) 3-CQA chromatogram for the 0.625 mM standard is shown. Caffeine is measured at a wavelength of 276 nm and had a retention time of approximately 11.9 min. 3-CQA is measured at a wavelength of 325 nm and had a retention time of approximately 11.2 min.
Figure 4.2: Example Chromatograms of Caffeine and 3-CQA for a Sample

The A) caffeine and B) 3-CQA chromatogram for an M-CB sample which was allowed to infuse for 6 min in a cold water bath is shown.
Statistical Analysis

To determine the combined effect of time and temperature on coffee extraction, a fixed effect model was used. SAS JMP 14 statistical software (Cary, NC) was used to generate the model. In order to determine individual differences and similarities between samples for TDS, L* value, caffeine, and 3-CQA, a one-way analysis of variance (ANOVA) and Tukey-Kramer Honestly Significant Difference means comparison were used. Statistically significant differences were determined by a p < 0.05. SAS JMP 14 statistical software (Cary, NC) was used to analyze the data. Values were reported as means ± standard error of the mean. A P-value of < 0.05 was considered to be statistically significant across all models.

Results and Discussion

Statistical Analysis

Coffee extraction is a process that utilizes water to dissolve soluble compounds from ground coffee. This study aimed to determine the effect of time and temperature on the extraction of coffee. A completely randomized split-plot design allowed for an analysis of the effect of these two factors on the extraction of coffee. In order to quantify the extraction, the following attributes were measured: TDS, color, caffeine, and 3-CQA. This statistical model describes this analysis:

\[ Y_{ijkl} = \mu + \alpha_i + D_{k(i)} + \tau_j + (\alpha\tau)_{it} + R_{jk(i)} + E_{l(ijk)} \]

Where \( i = 1, 2, 3, 4 \), \( j = 1, \ldots, 9 \), \( k = 1, \ldots, 4 \), \( l = 1, 2, 3 \) index the brewing method, time points, days, and technical replicates, respectively. The terms denoted with upper case letters are random effects, assumed independent and normally distributed, for day, day-by-time (to accommodate the technical replication) and experimental error. The terms denoted with Greek symbols are the fixed factorial effects of interest.
A fixed effect model was used to determine both the individual and combined effect of time and temperature on coffee extraction (table 4.1). Figure 4.3 demonstrates the code used to fit the model in JMP. Analysis using the model indicated that the observed effects of time and temperature on each of the four responses were significant (p<0.05). This model also showed that the effect of temperature on color was particularly strong. The combination of time and temperature also was statistically meaningful and displayed that TDS, color, caffeine, and 3-CQA were affected by the combination both factors.

```
Fit Model( 
  Y( :Name( "measured attribute" ) ), 
  Effects( :Time, :Temp, :Time * :Temp ), 
  Random Effects( :Day[:Temp], :Day * :Time[:Temp] ), 
  NoBounds( 1 ), 
  Personality( "Mixed Model" ), 
  Run( Repeated Effects Covariance Parameter Estimates( 0 ) ), 
  SendToReport( 
    Dispatch( {}, 
    "Fixed Effects Parameter Estimates", 
    OutlineBox, 
  ) 
)
```

**Figure 4.3: JMP Code to Model Fixed Effect of Time and Temperature**

<table>
<thead>
<tr>
<th>Source</th>
<th>Nparm</th>
<th>DFNum</th>
<th>DFDen</th>
<th>F Ratio</th>
<th>P value</th>
<th>F Ratio</th>
<th>P value</th>
<th>F Ratio</th>
<th>P value</th>
<th>F Ratio</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>8</td>
<td>8</td>
<td>56</td>
<td>30.19</td>
<td>&lt; 0.0001</td>
<td>16.10</td>
<td>&lt; 0.0001</td>
<td>11.11</td>
<td>&lt; 0.0001</td>
<td>14.22</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Temp</td>
<td>3</td>
<td>3</td>
<td>7</td>
<td>12.32</td>
<td>&lt; 0.0035</td>
<td>140.66</td>
<td>&lt; 0.0001</td>
<td>6.12</td>
<td>&lt; 0.0228</td>
<td>5.64</td>
<td>&lt; 0.0277</td>
</tr>
<tr>
<td>Time*Temp</td>
<td>24</td>
<td>24</td>
<td>56</td>
<td>8.83</td>
<td>&lt; 0.0001</td>
<td>6.21</td>
<td>&lt; 0.0001</td>
<td>3.83</td>
<td>&lt; 0.0001</td>
<td>3.97</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

**Table 4.1: Time and Temperature Fixed Effect Test**

Differences Between Processing Methods

*Cold Brew (CB) Compared to the Other Treatments (H-CB, M-CB, & HB)*

As expected, of the four temperature treatments, the most differences occurred between the CB samples and HB samples. The CB and HB samples were statistically different in all of the
attributes measured over all of the time points, except at the 2 h time point for the caffeine and 3-CQA measurements. Another interesting observation was noted when comparing the CB samples to the samples that underwent a temperature treatment involving heat (HB, M-CB, and H-CB); the color of the CB samples were statistically different at every time point regardless of temperature treatment. The values of the CB samples (table 4.2) ranged from 0.29-0.94 in TDS, 43.00-64.62 in L* value, 15.23-66.08 mg/100 ml in caffeine, and 5.55-44.82 mg/100 ml in 3-CQA. While the values of the HB samples (table 4.2) ranged from 0.89-1.39 in TDS, 17.19-34.98 in L* value, 48.90-84.39 mg/100 ml in caffeine, and 33.60-62.85 mg/100 ml in 3-CQA. In summary, the CB samples were very different from the HB samples for every attribute and were lighter in color than all of the samples that received a heat treatment.

The effects of each temperature treatment used in this study were evaluated; an analysis of each attribute, measured over time, for each temperature treatment was performed. The results of each temperature treatment were then compared to the results of the CB. A comparison of just one temperature treatment to the CB resulted in 36 measurements that could possibly demonstrate a difference; the four attributes were measured over nine time points. There were 26 statistically significant differences noted when comparing samples of the H-CB and CB: at the 0-8 min time points for every attribute, the 30 min and 1 h for color, and the 2 h and 3 h for both color and TDS. Additionally, the comparison of H-CB and CB showed no statistically significant differences at the 30 min and 1 h time points for TDS, and the 30 min through 3 h time points for caffeine and 3-CQA. For the comparison of M-CB and CB, there were 29 statistically significant differences noted between samples: at the 0-30 min time points for every attribute, the 1 h for color and caffeine, the 2 h for color, and the 3 h for color and TDS. Additionally, there were no statistically significant differences noted between M-CB and CB at the 1 h time point for TDS and 3-CQA, the
2 h for TDS, caffeine and 3-CQA, and the 3 h for caffeine and 3-CQA. When comparing the HB and CB, 34 statistically significant differences were noted: at all time points for both TDS and color, and all time points (except the 2 hour) for both caffeine and 3-CQA. The 2 h time point was the only time point where no statistically significant difference was noted between the HB and CB (for caffeine and 3-CQA). This analysis of the effects of temperature treatments compared to the CB method indicated the temperature treatment with the least statistically significant differences in attributes measured over time; the temperature treatment with the least number of differences, H-CB, was most similar to CB.

The temperature treatments most similar to the CB were the H-CB followed by the M-CB. The H-CB samples had the least number of attributes demonstrating statistically significant differences when compared to the CB samples; statistically significant differences were noted in 26 out of 36 measurements that could possibly demonstrate a difference, and no statistically significant differences were noted for 10 of the 36. A comparison of the M-CB temperature treatment with the CB, showed statistically significant differences in 29 of the possible 36 measurements, and no statistically significant differences for 7. The temperature treatment that was least similar to CB was the HB; statistically significant differences were noted for 34 of the possible 36 measurements and only 2 measurements showed no statistically significant difference.

Comparison of Hot Water Treated Cold Brew (H-CB) and Microwave Treated Cold Brew (M-CB)

The H-CB and M-CB were expected to be similar to each other because both involved a brief heat treatment followed by infusion at cold temperatures; the attributes measured in this study appeared to support this expectation. When looking at the overall number of differences that occurred
between each of the temperature treatments, the comparison with the least differences was the H-CB compared to the M-CB. However, when looking at individual differences of attributes between time points, there are no clear patterns. There were a few overall observations noted when an analysis was performed on a single attribute. For example, the M-CB samples were darker in color except for the 2 min and 8 min time points and extracted more caffeine and 3-CQA for the 30 min, 1 h, and 2 h time points. The values of the H-CB samples (table 4.2) ranged from 0.8-1.08 in TDS, 31.96-35.51 in L* value, 53.20-66.26 mg/100 ml in caffeine, and 38.46-47.08 mg/100 ml in 3-CQA. While the values of the M-CB samples (table 4.2) ranged from 0.94-1.08 in TDS, 24.19-30.32 in L* value, 60.03-73.17 mg/100 ml in caffeine, and 41.39-51.66 mg/100 ml in 3-CQA. In summary, as indicated by the measured attributes, the H-CB and M-CB treatment performed similar extractions.
### Table 4.2: TDS of Each Processing Method

All values represent means ± standard error

Two means in the same row with the same lower case letter do not differ significantly

Two means in the same column with the same upper case letter do not differ significantly

<table>
<thead>
<tr>
<th>Time</th>
<th>M-CB</th>
<th>HB</th>
<th>CB</th>
<th>H-CB</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>1.00 ± 0.05</td>
<td>0.89 ± 0.02</td>
<td>0.29 ± 0.02</td>
<td>0.80 ± 0.02</td>
</tr>
<tr>
<td>2 min</td>
<td>0.99 ± 0.05</td>
<td>1.13 ± 0.04</td>
<td>0.37 ± 0.02</td>
<td>0.94 ± 0.02</td>
</tr>
<tr>
<td>4 min</td>
<td>1.08 ± 0.04</td>
<td>1.12 ± 0.02</td>
<td>0.45 ± 0.02</td>
<td>0.91 ± 0.01</td>
</tr>
<tr>
<td>6 min</td>
<td>0.98 ± 0.04</td>
<td>1.21 ± 0.03</td>
<td>0.52 ± 0.02</td>
<td>0.95 ± 0.02</td>
</tr>
<tr>
<td>8 min</td>
<td>1.02 ± 0.05</td>
<td>1.18 ± 0.03</td>
<td>0.54 ± 0.01</td>
<td>0.91 ± 0.02</td>
</tr>
<tr>
<td>30 min</td>
<td>1.01 ± 0.03</td>
<td>1.32 ± 0.03</td>
<td>0.79 ± 0.02</td>
<td>0.93 ± 0.02</td>
</tr>
<tr>
<td>1 h</td>
<td>0.97 ± 0.02</td>
<td>1.28 ± 0.02</td>
<td>0.92 ± 0.04</td>
<td>0.93 ± 0.03</td>
</tr>
<tr>
<td>2 h</td>
<td>1.01 ± 0.02</td>
<td>1.30 ± 0.03</td>
<td>0.94 ± 0.04</td>
<td>1.08 ± 0.03</td>
</tr>
<tr>
<td>3 h</td>
<td>0.94 ± 0.02</td>
<td>1.39 ± 0.02</td>
<td>0.85 ± 0.02</td>
<td>1.03 ± 0.03</td>
</tr>
</tbody>
</table>

### Table 4.3: L* of Each Processing Method

All values represent means ± standard error

Two means in the same row with the same lower case letter do not differ significantly

Two means in the same column with the same upper case letter do not differ significantly

<table>
<thead>
<tr>
<th>Time</th>
<th>M-CB</th>
<th>HB</th>
<th>CB</th>
<th>H-CB</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>29.23 ± 1.17</td>
<td>34.98 ± 0.56</td>
<td>64.62 ± 1.65</td>
<td>34.46 ± 0.54</td>
</tr>
<tr>
<td>2 min</td>
<td>30.32 ± 1.12</td>
<td>29.67 ± 1.16</td>
<td>60.34 ± 0.87</td>
<td>31.96 ± 0.28</td>
</tr>
<tr>
<td>4 min</td>
<td>27.17 ± 1.37</td>
<td>29.23 ± 0.71</td>
<td>59.58 ± 0.65</td>
<td>32.20 ± 0.58</td>
</tr>
<tr>
<td>6 min</td>
<td>27.58 ± 1.43</td>
<td>26.02 ± 1.16</td>
<td>56.51 ± 1.16</td>
<td>34.56 ± 0.60</td>
</tr>
<tr>
<td>8 min</td>
<td>28.84 ± 1.12</td>
<td>25.19 ± 1.07</td>
<td>55.78 ± 0.82</td>
<td>32.21 ± 0.60</td>
</tr>
<tr>
<td>30 min</td>
<td>27.40 ± 1.30</td>
<td>20.97 ± 0.73</td>
<td>49.28 ± 0.91</td>
<td>35.51 ± 0.78</td>
</tr>
<tr>
<td>1 h</td>
<td>24.19 ± 0.80</td>
<td>21.02 ± 0.69</td>
<td>49.89 ± 0.88</td>
<td>35.10 ± 0.63</td>
</tr>
<tr>
<td>2 h</td>
<td>27.94 ± 1.47</td>
<td>18.55 ± 0.57</td>
<td>45.98 ± 0.50</td>
<td>33.61 ± 0.81</td>
</tr>
<tr>
<td>3 h</td>
<td>29.29 ± 1.34</td>
<td>17.19 ± 0.60</td>
<td>43.00 ± 0.87</td>
<td>35.33 ± 0.68</td>
</tr>
</tbody>
</table>
Table 4.4: Caffeine Content Resulting from Each Processing Method
Caffeine concentration was measured in mg/100 ml
All values represent means ± standard error
Two means in the same row with the same lower case letter do not differ significantly
Two means in the same column with the same upper case letter do not differ significantly

<table>
<thead>
<tr>
<th>Time</th>
<th>M-CB</th>
<th>HB</th>
<th>CB</th>
<th>H-CB</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>60.88 ± 2.16 AB a</td>
<td>48.90 ± 2.34 A b</td>
<td>15.23 ± 1.32 A c</td>
<td>56.10 ± 2.14 AB b</td>
</tr>
<tr>
<td>2 min</td>
<td>60.03 ± 1.54 A a</td>
<td>66.43 ± 3.27 B a</td>
<td>29.15 ± 0.84 AB b</td>
<td>66.26 ± 2.63 A a</td>
</tr>
<tr>
<td>4 min</td>
<td>63.87 ± 2.10 AB a</td>
<td>77.62 ± 3.26 B ab</td>
<td>30.58 ± 1.65 BC c</td>
<td>64.24 ± 3.09 A b</td>
</tr>
<tr>
<td>6 min</td>
<td>66.24 ± 1.48 AB a</td>
<td>78.56 ± 2.84 BC a</td>
<td>31.38 ± 1.46 BC b</td>
<td>62.14 ± 2.86 AB c</td>
</tr>
<tr>
<td>8 min</td>
<td>67.81 ± 1.88 AB a</td>
<td>76.69 ± 2.75 C b</td>
<td>35.43 ± 1.02 C c</td>
<td>59.50 ± 2.52 A a</td>
</tr>
<tr>
<td>30 min</td>
<td>67.07 ± 2.44 AB a</td>
<td>77.32 ± 2.97 D b</td>
<td>49.10 ± 1.00 D c</td>
<td>56.00 ± 2.46 B d</td>
</tr>
<tr>
<td>1 h</td>
<td>67.11 ± 2.04 B a</td>
<td>75.38 ± 2.79 D b</td>
<td>55.33 ± 3.03 D c</td>
<td>53.20 ± 2.55 B d</td>
</tr>
<tr>
<td>2 h</td>
<td>73.17 ± 4.02 AB a</td>
<td>74.46 ± 2.38 DE b</td>
<td>66.08 ± 0.69 DE c</td>
<td>59.18 ± 1.71 AB d</td>
</tr>
<tr>
<td>3 h</td>
<td>69.85 ± 3.86 AB a</td>
<td>84.39 ± 2.29 E b</td>
<td>61.42 ± 1.23 E c</td>
<td>62.21 ± 2.43 B d</td>
</tr>
</tbody>
</table>

Table 4.5: 3-CQA Content Resulting from Each Processing Method
Chlorogenic Acid (3-CQA) concentration was measured in mg/100 ml
All values represent means ± standard error
Two means in the same row with the same lower case letter do not differ significantly
Two means in the same column with the same upper case letter do not differ significantly

<table>
<thead>
<tr>
<th>Time</th>
<th>M-CB</th>
<th>HB</th>
<th>CB</th>
<th>H-CB</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>41.39 ± 1.67 AB a</td>
<td>33.60 ± 2.01 A b</td>
<td>5.55 ± 0.93 A c</td>
<td>39.17 ± 1.67 AB b</td>
</tr>
<tr>
<td>2 min</td>
<td>41.51 ± 1.27 A a</td>
<td>47.12 ± 2.68 B a</td>
<td>11.22 ± 0.78 AB b</td>
<td>47.08 ± 2.08 A a</td>
</tr>
<tr>
<td>4 min</td>
<td>44.73 ± 1.76 AB a</td>
<td>55.54 ± 2.40 B ab</td>
<td>16.26 ± 1.29 BC c</td>
<td>46.12 ± 2.46 A b</td>
</tr>
<tr>
<td>6 min</td>
<td>46.37 ± 1.05 AB a</td>
<td>57.02 ± 2.13 BC a</td>
<td>16.93 ± 1.04 BC b</td>
<td>44.73 ± 2.32 AB c</td>
</tr>
<tr>
<td>8 min</td>
<td>48.00 ± 1.31 AB a</td>
<td>56.12 ± 2.09 C b</td>
<td>18.31 ± 0.74 C c</td>
<td>42.64 ± 2.01 A a</td>
</tr>
<tr>
<td>30 min</td>
<td>47.95 ± 1.87 AB a</td>
<td>58.24 ± 2.40 D b</td>
<td>26.25 ± 0.71 D c</td>
<td>40.15 ± 1.99 B d</td>
</tr>
<tr>
<td>1 h</td>
<td>47.12 ± 1.59 B a</td>
<td>56.44 ± 2.25 D b</td>
<td>30.58 ± 2.32 D c</td>
<td>38.46 ± 2.08 B d</td>
</tr>
<tr>
<td>2 h</td>
<td>51.66 ± 2.64 AB a</td>
<td>54.78 ± 1.94 DE b</td>
<td>43.42 ± 0.56 DE c</td>
<td>43.85 ± 1.48 AB d</td>
</tr>
<tr>
<td>3 h</td>
<td>49.95 ± 2.82 AB a</td>
<td>62.85 ± 1.88 E b</td>
<td>44.82 ± 1.02 E c</td>
<td>46.00 ± 2.04 B d</td>
</tr>
</tbody>
</table>

Differences Over Time
Many of the traditional coffee brewing methods utilize high temperatures which normally results in a rapid extraction. This extraction process can range from a few seconds to a few minutes depending on the method. However, cold brew coffee is frequently brewed using low temperatures...
and extended amounts of time (hours). This drastic difference in time used to brew coffee, rapid versus extended, has not been directly compared. Therefore, one of the objectives of this study was to examine how time affected the extraction of coffee (table 4.2). A depiction of the extraction of time is shown in Figure 4.4.

**Cold Brew (CB)**

The CB samples changed significantly over time; as infusion time increased, extraction of the measured attributes increased and became more statistically different. The 0 min CB only differed in 2 attributes when compared to the 2 min; however, as infusion time increased beyond two minutes, the 0 min differed in all attributes. A similar pattern was noted for all infusion times; each infusion time was most similar to samples measured at times directly before and after it, however, the further the time varied from the measured infusion time, more differences were noted. For example, the 4 min CB was most similar to samples measured at times directly before and after it; no differences were noted between the 4 min and either the 2 min or the 6 min. The further the time varied from the 4 min time point, more differences were noted: all attributes were different compared to the 0 min and also when the infusion time reached 30 min and beyond. This pattern of differences between time points continued, however, less differences were noted between time points after reaching the 30 min time point (even though there was a greater amount of time between the time points after 30 minutes). The 30 min CB differed from the 1 h in only one attribute. Even the 1 h CB only differed from the 2 h and 3 h in two attributes each. Most notably, the 2 h CB was not statistically different from the 3 h for any of the measured attributes: after reaching two hours, providing more infusion time for extraction did not result in statistically significant changes in the measured attributes. This pattern illustrates that as the CB samples were
infused, the samples were able to extract more compounds and became more different from the previous time point until reaching about two hours, after which, extraction leveled off.

*Hot Brew (HB)*

When analyzing the extraction over time of the HB samples, the pattern was not as clearly defined as that of the CB samples. Although the pattern was less clear, some similarities to the CB samples were noted; as the HB samples were infused, more material was extracted. Extraction of TDS and color in the HB samples had a pattern similar to that in the CB samples: as infusion time increased, extraction of the measured attributes increased and became more statistically different. However, after reaching 30 minutes, the measured extraction of TDS and color in the HB samples reached a level that did not show a statistically significant difference from samples measured at greater infusion times; infusing longer than 30 minutes did not change the extraction of TDS or color. Extraction of caffeine in the HB samples was less defined. After the HB samples reached the 4 min time point, the amount of caffeine measured did not significantly change when compared to samples at any of the longer infusion times; providing more than 4 minutes of infusion time had no statistically significant effect on the amount of caffeine extraction. No clear pattern was noted for 3-CQA extraction over time in the HB samples; although the amount of 3-CQA measured at 0 min and 2 min was significantly different when compared to the 3 h samples. Overall, the 0 min HB was statistically different from every time point in each of the four attributes. The differences between the 2 min, 4 min, 6 min, and 8 min HB samples were mostly due to color and TDS. The least number of differences between infusion times occurred after reaching the 30 min time; the 30 min HB and 1 h HB were only different from the 3 h HB in color, and the 2 h HB was not different from the 3 h HB in any attribute. Again, this showed that as the hot brew coffee was
allowed to infuse, the samples extracted more compounds and became more different from the
previous time points until the infusion time reached about 30 minutes, after which extraction
leveled off.

*Hot Water Treated Cold Brew (H-CB)*

The analysis of extraction over time for the H-CB samples did not show a pattern. The
initial pre-treatment appeared to extract most of the material from the ground coffee prior to
infusion. Therefore, there were few patterns exhibited over time as the coffee was infused. Pre-
treatment of H-CB samples consisted of exposing ground coffee to a rapid heat treatment with
equilibrated 80°C water, followed by a cold water bath infusion phase over time. Measurement of
extraction began when the cold water bath infusion phase was initiated. Differences observed in
the measured attributes were primarily seen in TDS and color. Analysis of TDS extraction over
time from H-CB samples showed the amount of TDS measured at 0 min was significantly different
from the amount measured in samples at any other infusion time. However, starting at the 2 min
infusion time and continuing through 1 h, the extraction of TDS did not change significantly over
time. Additionally, all of the H-CB infusion times, except the 3 h, showed a difference in the
extraction of TDS when compared to the 2 h infusion time, and most of the infusion times showed
a difference when compared to the 3 h. It was noted that the extraction of TDS from the H-CB
samples reached a level that did not change significantly after infusion for 2 hours; the amount of
TDS measured in the 2 h and 3 h samples did not show a statistically significant difference.
Analysis of color extraction from H-CB samples measured over time showed inconsistencies. The
amount of color extracted at 2 min, 4 min, and 8 min was different from the amount extracted after
infusion 30 min, 1 h, and 3 h, however, there was no difference in color between the shortest and
longest infusion times; the 0 min and 3 h samples had no statistically significant differences. Furthermore, samples measured at the 0 min infusion time showed no difference in color extraction when compared to any samples at any infusion time. Extraction of caffeine and 3-CQA from the H-CB samples measured over time had the least number of differences noted; only one difference was noted for caffeine extraction (between the 2 min and 1 h samples), and there were no differences noted for the extraction of 3-CQA. Because there were few differences in extraction between the samples over time, this would indicate that the rapid pre-treatment of heat resulted in an almost complete extraction, nearly instantaneously. The lack of a clear pattern and inconsistencies in extraction over time indicate there was minimal additional extraction as a result of infusion in the cold water bath.

**Microwave Treated Cold Brew (M-CB)**

There were no patterns observed in extraction when comparing the M-CB samples over time. Similar to the H-CB samples, after the microwave pre-treatment, TDS, color, caffeine, and 3-CQA did not change over time as the coffee was infused. The M-CB sample preparation involved using a microwave pre-treatment of samples to achieve a temperature of 80°C, followed immediately by the infusion phase in a cold water bath. Measurement of extraction began when the cold water infusion phase was initiated. The only observed differences between M-CB samples were noted when comparing the 0 min and 2 min M-CB samples to the 1 h, 2 h, or 3 h samples. Samples measured at 0 min and 2 min were different from the 2 h sample for caffeine, and from both the 2 h and 3 h samples for 3-CQA. Additionally, the 2 min M-CB sample was different from the 1 h sample in color. It is important to note that after the M-CB samples reached the 4 min infusion time, no additional extraction occurred; there were no statistically significant differences
for any of the attributes when comparing samples that had been infused between 4 min to 3 h. This indicates that a microwave pre-treatment accelerates the extraction of the coffee and reduces the length of time needed for infusion at cold temperatures.
Figure 4.4: Extraction Over Time of the Four Processing Methods
Graph A) depicts the TDS of each processing method over time.
Graph B) depicts the L* of each processing method over time.
Graph C) depicts the caffeine content of each processing method over time.
Graph D) depicts the 3-CQA content of each processing method over time.
Error bars represent the standard error.
Conclusion

Cold brew coffee is a lucrative and rapidly growing market. Increasing demand for cold brew coffee has captured the attention of industry and created research opportunities aimed at developing better, faster, and more cost-effective methods of production. One opportunity for research is to determine whether it is possible to accelerate the extraction process of cold brew coffee through the use of a brief heat treatment prior to infusion at refrigerated temperatures. Microwave heating in particular would be well suited to quickly delivering a brief heat treatment. However, for this to be a feasible contribution to the industry, it would also have to establish that the cold brew coffee was not negatively impacted by the new process. The objective of this study was to determine the effect of time and temperature on the extraction of coffee, with the main focus being the effect in regards to cold brew coffee. The expected effect of time was that as water and ground coffee have more time to infuse, more of each attribute would be extracted. Moreover, higher temperatures were expected to result in more complete extractions. Hence, the combination of both time and temperature also were expected to increase the extraction of coffee. A statistical analysis of each measured attribute (TDS, color, caffeine, and 3-CQA) showed that time and temperature, individually and combined, affected the extraction of coffee.

An analysis of the data supported the assumptions that more time and higher temperatures would result in more material being extracted from ground coffee. The effect of time was apparent for both the CB and HB treatments. More material was extracted from the coffee grounds as the infusion time increased for both the CB and HB samples; samples measured at shorter infusion times had less TDS, caffeine, and 3-CQA and were lighter in color than samples measured at longer infusion times. In regard to the effect of temperature on extraction, it was observed that the HB samples had higher concentrations of TDS, caffeine, and 3-CQA and were darker in color than
the other samples which underwent a cold water infusion phase. Therefore, the treatment that utilizes heat during the entire brewing process extracted more material than the treatments that involved the use of cold water for infusion.

Another objective of this study was to examine whether a heat treatment could be advantageous in the production of cold brew coffee. It was unknown whether the addition of a brief heat treatment prior to infusion at cold temperatures could potentially accelerate extraction rates of cold brew coffee. Two pre-treatment methods of applying rapid heat were analyzed: microwaving (M-CB) and hot water (H-CB). Microwaves have been known to accelerate the extraction of compounds (Veggi and others 2013). The data indicated that the two sample groups that underwent a brief heat pre-treatment, M-CB and H-CB, extracted more material more quickly than the CB samples, which only infused at cold temperatures. The M-CB and H-CB samples were primarily extracted during the pre-treatment prior to the cold infusion phase. Additional extraction of the M-CB and H-CB samples during the cold infusion phase was significantly reduced; most of the extraction had already occurred, almost instantaneously, during the brief heat pre-treatment. This contrasted with the much slower extraction of the CB samples. Although extraction of CB samples was slower, after infusion approximately 30 minutes to two hours, the extraction values of the CB samples became similar to the M-CB and H-CB samples. It is important to note that during the rapid heat pre-treatment or, at most, within the first few minutes of cold infusion, the M-CB and H-CB samples reached the same level of extraction of the CB samples which was infused for 30 minutes to two hours at refrigeration temperature. This is a possible indication that the M-CB and H-CB treatments performed a similar extraction to the CB treatment in less time. Therefore, a rapid heat treatment followed by infusion at refrigeration temperatures may be a possible method for improving extraction rates and accelerating the production of cold brew
coffee. This result would be very advantageous to industry. However, this study analyzed only four of the many attributes that contribute to coffee’s flavor, texture, appearance, aroma, etc. More research is needed to ensure that a rapid heat treatment would not alter the overall characteristics of a cold brew coffee.
REFERENCES


APPENDICES
Appendix A: HPLC Method

Preparation of Mobile Phase

Mobile Phase A

- The molecular weight of citric acid monohydrate is 210.4 g/M; to prepare a 20 mM solution, weigh 4.208g of citric acid monohydrate and add to a 1L volumetric flask. Bring to volume using HPLC grade water.
- Measure (graduated cylinder) 900 mL of 20 mM citric acid solution and pour into a 1L screw capped bottle.
- Measure (graduated cylinder) 100 mL methanol (HPLC grade) and add to the bottle containing the 900 mL 20 mM citric acid.
- Gently mix.
- Filter using a 0.45 um nylon membrane filter.

Mobile Phase B

- Filter HPLC grade methanol using a 0.45um membrane filter.
Preparation of Standards

Stock Solution

- Prepare a series of dilutions from a stock of 20 mM caffeine and 20 mM 3-caffeylquinic acid (3-CQA).
- The molecular weight of 3-CQA is 354.30872 g/M; for a 20mM solution, dissolve 0.17715 g of 3-CQA into 25 mL methanol.
- The molecular weight of caffeine is 194.19 g/M; for a 20mM solution, dissolve 0.097095 g of caffeine into 25 mL methanol
- Both of these stock solutions may need to be sonicated to get them into solution.

Retention Time Identification:

- Create independent 1.0 mM solutions of caffeine and 3-CQA. 0.1 mL of 20 mM stock solution can be diluted with 1.9 mL methanol (2.0 mL total volume), mix well.
- Into a fresh vial, pipet 1 mL of 1.0 mM caffeine and 1 mL of 1.0 mM 3-CQA. This yields a 0.5 mM mix of both standards to use daily for retention time confirmation.
  *Note: record the weight of each standard and make adjustments to the molar content as needed. The exact weight of the standards should be close to the weight needed- but minor adjustments can be made to the molarity to account for the weight difference.
- The retention time of caffeine was approximately 11.9 min while chlorogenic acid was approximately 11.2 min.
Standard Curve

Note* For the standard curve, the two standards may be mixed together. An example of one point on the standard curve is depicted in Figure 1A.

- Beginning with the two 20 mM stock solutions, prepare the dilutions for the standard curve as indicated in Table 1A. Mix each dilution well before proceeding to the next.
- Filter the following dilutions through a 0.45um syringe filter into an HPLC vial: 0.08 mM, 0.15 mM, 0.31 mM, 0.625 mM, 1.25 mM, and 2.5 mM. These dilutions will be used to create the standard curve.

Table 1A: Caffeine and 3-CQA Standard Curve Dilutions

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Solution Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mM</td>
<td>1 mL Caffeine Stock Solution + 1 ml 3-CQA Stock Solution</td>
</tr>
<tr>
<td>5 mM</td>
<td>2 mL of 10 mM mix + 2 ml Methanol</td>
</tr>
<tr>
<td>2.5 mM</td>
<td>2 mL of 5 mM mix + 2 ml Methanol</td>
</tr>
<tr>
<td>1.25 mM</td>
<td>2 mL of 2.5 mM mix + 2 ml Methanol</td>
</tr>
<tr>
<td>0.625 mM</td>
<td>2 mL of 1.25 mM mix + 2 ml Methanol</td>
</tr>
<tr>
<td>0.31 mM</td>
<td>2 mL of 0.625 mM mix + 2 ml Methanol</td>
</tr>
<tr>
<td>0.15 mM</td>
<td>2 mL of 0.31 mM mix + 2 ml Methanol</td>
</tr>
<tr>
<td>0.08 mM</td>
<td>2 mL of 0.15 mM mix + 2 ml Methanol</td>
</tr>
</tbody>
</table>
Figure 1A: Chromatograms of the 0.625 mM Caffeine and 3-CQA Standard Solution
In this figure, the A) caffeine and B) 3-CQA chromatogram for the 0.625 mM standard is shown
Preparation of Samples

- Samples were diluted using deionized water until the measured absorbances were within
  the standard curve range. Each of the samples in this study were diluted at a ratio of 1:1
  (1ml sample + 1 ml of water).
- Samples were filtered into HPLC vials using a 0.45um syringe filter

HPLC Settings

- 10 uL is injected from each vial
- Allow for a 25 min run time for equilibration
- Caffeine is measured at a wavelength of 276 nm while Chlorogenic Acid is measured at a
  wavelength of 325 nm.
- Table 2A Indicates the gradient utilized for the mobile phase solutions

<table>
<thead>
<tr>
<th>Table 2A: Mobile Phase Gradient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (min)</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
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</tr>
<tr>
<td>17:00</td>
</tr>
<tr>
<td>20:00</td>
</tr>
<tr>
<td>21:00</td>
</tr>
<tr>
<td>24:00</td>
</tr>
</tbody>
</table>
Appendix B: Processing Methods

The hot water bath used to provide the heat treatment to samples in this study consisted of a large metal pan filled with water. This pan was then insulated on all sides in order to better maintain temperature. A sous vide precision cooker (Anova, San Francisco, CA) heated the water to the desired temperature. Figure 1B displays the insulated pan with sous vide.

![Figure 1B: Hot Water Bath](image)

The cold water used to provide the cold treatment to samples in this study consisted of a large plastic pan filled with water. This pan of water was placed in a walk in refrigerator overnight before the study in order to allow for temperature equilibration.

50 ml tube racks were placed in the water baths. The tube holes were numbered 1-30, samples were assigned a randomized number in advance of the test and placed in the appropriate location. This accounted for any temperature variations within the water bath. Figure 2B indicates the flow of sample preparation.
A similar sample preparation method was used to prepare the microwave treated cold brew samples. However, these samples were mixed in a beaker and stored in a 1 L bottle while in the cold water bath. Figure 3B demonstrates this flow path and the microwave system used to heat the samples to the appropriate temperature.