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# The Effect of Grind Size and Brew Time upon Antioxidant Potential, Sensory Profile, and Consumer Likability of Cold Brew Coffee

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**Abstract:**

Coffee is one of the most widely consumed beverages in the world. Cold brew coffee has grown in popularity among the recent years. **PURPOSE:** To determine the effects of grind size and extraction time on the antioxidant potential, sensory profile, and consumer likability of cold brew coffee. **MATERIALS AND METHODS:** 138 adults participated in randomized sample consumer assessment of the likeability of 18 different cold brew coffee samples based on appearance, aroma, flavor, and overall. The samples were made with 2 different types of beans, 3 different grind sizes (0.65mm, 1.15mm, and 1.65mm), and 3 different brewing times (18h, 24h, and 30h). Additionally, twelve trained panelists participated in a descriptive analysis of these 18 cold brew coffee samples. These samples were assessed on 19 selected attributes. Antioxidant potential was also assessed using Trolox Equivalent Antioxidant Capacity (TEAC) assay for 9 samples (1 bean type: Los Santos, 3 grind sizes (0.65mm, 1.15mm, and 1.65mm), and 3 brew times (18h, 24h, 30h)). Multivariable modeling was performed to quantify the effects of the processing factors on each of the assessment outcomes. **RESULTS:** For Consumer Assessment, samples brewed at 24 hours consistently scored lower than 18 and 30 hours. The sample with a grind size of 1.65 mm brewed for 30 hours scored the highest. For Descriptive Analysis, 24 hours consistently produced the lowest scores across all attributes. For grind size, it was found that 0.65mm had significantly higher antioxidant potential than 1.15mm and 1.65mm ($p<.001$ and $p<.001$). For brew time, 24 hours had the lowest antioxidant potential of 0.81. 18 hours had an antioxidant potential of 1.4. 30 hours had the highest antioxidant potential of 2.86. **CONCLUSIONS:** Cold brew coffee brewed for 18 or 30 hours had higher magnitudes of descriptive traits, higher acceptability and antioxidant potential than 24 hours. Further research into the compounds that are dissolving between the hours of 18 and 24 would be warranted to

identify what is mitigating flavor attributes and perceived quality as well as the antioxidant potential. To produce cold brew coffee considering its overall flavor, likability and antioxidant potential, it is recommended to brew cold brew coffee for 30 hours with a coarser grind size (1.65mm).

Montclair State University

# The effect of grind size and brew time upon antioxidant potential, sensory profile, and consumer likability of cold brew coffee

By

**Angela Morresi**

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2019

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# CHAPTER 1: INTRODUCTION

## 1.1 COFFEE BACKGROUND

Coffee is one of the most widely consumed beverages in the world. Coffee is an infusion of ground, roasted coffee beans. It appeals to many for its aroma, flavor, and caffeine content (Esquivel & Jiménez, 2012). Coffee is produced in approximately 60 tropical and subtropical countries, being the main agricultural export for some (Esquivel & Jiménez, 2012).

Brazil is the largest producer and exporter of coffee making up for a third of the world's coffee. Coffee exists as two main species, *Coffea arabica*, Arabica, and *Coffea canephora*, Robusta. Arabica is more valuable because its beans produce a higher quality beverage, which is therefore more expensive than the Robusta coffee (Bröhan, Huybrighs, Wouters, & Van der Bruggen, 2009).

## 1.2 COFFEE COMPOSITION

Coffee beans grow within the coffee fruit, or commonly referred to as a "coffee cherry". The coffee fruit is composed of the pericarp and the seed. The pericarp is the walls of a ripened fruit consisting out the outermost layer, epicarp, middle layer, mesocarp, and the innermost layer. The pericarp is made up of the skin, which is a smooth protective covering that turns deep red/violet when ripe (Esquivel & Jiménez, 2012). The mesocarp consists of the pulp and the mucilage. The pulp is sweet, yellowish, soft and fibrous which is then followed by the mucilage, the pectin layer. The mucilage is a thin, colorless, highly hydrated layer (Esquivel & Jiménez, 2012). Just underneath lies the endocarp, the thin and yellow cover that surrounds the seed, the parchment. Finally, the silverskin covers each half of the coffee bean, which is the fruits endosperm.

## 1.3 PREPARATION AND PROCESSING

Removal of the beans consists of elimination of the coffee fruit. The beans inside are referred to as green beans, or “green coffee”. They are removed from the cherry and roasted or exported globally (Esquivel & Jiménez, 2012). The removal of the green beans happens in two main ways; dry/natural processing, and wet processing. In dry processing, harvested coffee fruits are dried in the sun and then the husks (skin, pulp, mucilage, parchment, and as much of the silverskin as possible) are mechanically hulled (Esquivel & Jiménez, 2012). In wet processing, the coffee berries are put into water and separated based on ripeness; ripe berries float at the top and unripe or damaged berries sink to the bottom (Esquivel & Jiménez, 2012). The skin and most of the pulp from the sunken berries are mechanically removed by pressing the fruit in water through a screen (Esquivel & Jiménez, 2012). This is done by using a pulper. Pulp remnants and the mucilage layer are subsequently removed through mechanical scrubbing or by “controlled” fermentation for 12–48 h (Esquivel & Jiménez, 2012). In the fermentation step, the mucilage is hydrolyzed by enzymes from both the coffee tissues and from microorganisms found on the fruit skins. The population of microorganisms has a direct influence on the final quality of the coffee beans. Mechanical scrubbing reduces the amount of water used and waste water produced. At this point the beans are still covered by the parchment, which is removed after drying and hulling steps (Esquivel & Jiménez, 2012). The silverskin can be optionally removed by a polishing machine to produce premium-priced coffee beans (Esquivel & Jiménez, 2012).

The processing method used to obtain the green coffee has an influence on the sensory properties of the coffee brew produced afterwards (Mussatto et al., 2011). The drying processing method is complex as it includes respiration and transpiration of the green coffee beans which can lead to oxidation and off flavor notes (Rendón, de Jesus Garcia Salva, & Bragagnolo, 2014). It is generally assumed that wet-processed coffee has superior aroma and, therefore, higher acceptance (Esquivel & Jiménez, 2012). After processing, the flavor of the green coffee beans changes during prolonged storage, even under optimal conditions, and becomes slightly woody (Rendón et al., 2014). During storage, the green color of the bean becomes fainter, and the market value of the product decreases (Rendón et al., 2014).

## 1.4 ROASTING

During roasting, the green coffee beans go through a number of pyrolytic reactions forming aroma compounds (Bröhan et al., 2009). Different degrees of roasting (light, medium, dark) produce various aroma profiles (Bröhan et al., 2009). Medium roast is usually the most preferable based on its sensory qualities (Esquivel & Jiménez, 2012). The roasting time varies between 90 seconds and 40 minutes which greatly influences the reactions within the beans and thus the aroma characteristics of the coffee brew (Bröhan et al., 2009). Longer roasting times lead to a coffee with a bitter taste, whereas shorter periods produce a coffee with an underdeveloped aroma because not all pyrolytic reactions can be completed (Buffo & Cardelli-Freire, 2004). Dark roasts result in more burnt and bitter flavor notes (Masi, Dinnella, Monteleone, & Prescott, 2015).

Carbon dioxide is formed during the roasting process and assists in the Maillard, Strecker, and pyrolysis reactions (Wang & Lim, 2014). Carbon dioxide and other volatile compounds increase the internal pressure of the beans, causing them to expand and eventually crack (Wang & Lim, 2014). After roasting, the roasted coffee beans need to have a degassing period. Carbon dioxide not lost during roasting remains trapped in the beans, which will slowly diffuse out during subsequent storage (Wang & Lim, 2014). Coffees are usually partially degassed to minimize aroma loss (Wang & Lim, 2014).

## 1.5 PACKAGING AND STORAGE

Roasted coffee needs to be tempered to adequately remove the entrapped carbon dioxide before packaging to prevent package swelling which can lead to leaking or bursting (Wang & Lim, 2014). Active packaging systems are equipped with a vent valve to allow the release of CO2 during storage (Wang & Lim, 2014). Once the roasted coffee beans are degassed, they should be stored in an airtight container made of either glass, ceramic, or non-reactive metal.

Flavor notes in coffee beans can be altered by oxygen, light, heat, and moisture. Oils on the surface of the coffee beans can oxidize when in contact with oxygen. When this happens, it can result in off flavor notes. The sensory changes of the coffee brew during storage have been associated with lipid oxidation. Active lipases, free unsaturated fatty acids and hydroperoxides are found in stored raw coffees (Rendón et al., 2014). The secondary products of lipid oxidation can react with other coffee bean components, such as proteins, which may also contribute to sensory changes. (Rendón et al., 2014). Proper packaging and storage of green coffee beans as well as roasted coffee is extremely important to maintaining quality of the beans.

## 1.6 GRIND SIZE

The grind size of coffee is characterized into three main categories; fine, medium, and coarse. Different grind sizes are generally used for specific brewing methods. The optimal combination of grind size and brewing method allows maximum surface area to be exposed to water to obtain a high-quality coffee brew (Andueza, De Pena, & Cid, 2003). A grind that is too fine could decrease extraction, yielding low volume of a bitter, over extracted coffee due to agglomeration and insufficient wetting of particles (Andueza et al., 2003). On the other hand, a grind that is too coarse could also decrease extraction, yielding under extracted coffee due to small surface area which would be too small to retain water and allow coffee compounds solubilization and emulsification (Andueza et al., 2003). Medium-coarse grinds are generally required for boiled coffee and filter coffee, whereas fine grinds are needed for espresso coffee, and extremely fine grinds are required for Turkish coffee (Andueza et al., 2003).

There are few research studies investigating the effect of grind size and extraction of soluble molecules. Research done by Bell et al. (1997) states that the finely ground coffee yielded a significantly higher caffeine content while the coarsely ground coffee yielded significantly lower caffeine values. These results are similar to the findings of Andueza et al. (2003) which explained that finer grinds leads to a higher extraction of soluble and volatile compounds. This difference in caffeine content between finely and coarsely ground coffee is expected because more finely ground coffee would have a larger surface area, allowing for greater caffeine extraction (Bell, Wetzel, & Grand, 1997). The finer the grind also led to more total solids being extracted from the coffee grounds leading to significantly higher caffeine contents in filtered coffee (Bell et al., 1997). However, in cold brew samples, grind size did not impact 3-chlorogenic acid (3-CGA) and caffeine concentrations significantly, indicating that the rate determining step in extraction for these compounds did not depend on surface area (Fuller & Rao, 2017).

### 1.7 EXTRACTION METHODS

Brewing coffee is an extraction process dependent on a multitude of variables such as water volume, water temperature, grind size, the porosity of the coffee grind matrix, the pore network between coffee grind particles, and brewing time (Fuller & Rao, 2017). Temperature often significantly influences compounds aqueous solubility, so differences in brewing temperatures may result in significantly different compositions in hot brew and cold brew coffees (Fuller & Rao, 2017). Most traditionally, coffee is brewed using a hot water brewing method. The water is heated to just below boiling, around 98°C, is incorporated with the grounded coffee beans then filtered.

Research by Gloess et al. reports there is no correlation between pH and perceived acidity in the favor of coffees. Fuller and Rao (2017) found that pH is comparable in both hot and cold water extracted coffees and ranged from 5.40 to 5.63. It has also been speculated that cold brew coffee has more caffeine than other brewing methods, as the heat does not degrade the caffeine molecules (Fuller & Rao, 2017).

## 1.8 FLAVOR PROFILE

Coffee contains over 800 volatiles that belong to different chemical families, including acids, alcohols, aldehydes, anisoles, esters, furans, ketones, pyrazines, pyridines, pyrroles, thiazoles and thiophenes, as well as phenolic and sulphur compounds (Buffo & Cardelli-Freire, 2004). The basic taste sensation of coffee is given by non-volatile compounds (e.g. caffeine, polysaccharides and chlorogenic acids), which determine bitterness, sourness and astringency (Bröhan et al., 2009). Key odorants are responsible for the coffee aroma (Bröhan et al., 2009). There are two ways one can experience aroma, nasally or retronasally. Nasally is when key odorants are sensed directly through the nose. Retronasally is when flavor is obtained when the coffee is present in the mouth or has been swallowed, and the aroma compounds drift upward into the nasal passage (Bröhan et al., 2009).

There are hundreds of compounds that have been reported as constituents of coffee aroma (Maeztu et al., 2001). Although the volatile fraction in coffee is very complex, only the bioactive substances, key odorants, are responsible for coffee flavor (Maeztu et al., 2001). Twenty-eight volatile compounds have been identified as important contributors to the flavor (Maeztu et al., 2001).

Chlorogenic acid compounds convey bitterness to coffee (Fuller & Rao, 2017). The longer steeping times associated with cold brew coffee may result in increased extraction of catechol oligomers, which are characterized by harsh bitter-tasting properties (Fuller & Rao, 2017). Over brewing cold brew coffee may result in unpalatable extracts due to these and other relatively slow extracting compounds (Fuller & Rao, 2017).

## 1.9 CAFFEINE

One of the major compounds found in coffee that makes it so appealing to many is its caffeine content. Caffeine is bitter, white, crystalline purine, meaning it has a ring structure and contains a nitrogen molecule (Higdon & Frei, 2007). It is a central nervous stimulant naturally found in over 60 plants. Caffeine blocks the inhibitory neurotransmitter, adenosine, which in turn prevents one from feeling tired. When this happens, the amount of other neurotransmitters, like dopamine and norepinephrine, increase and there is more neuron activity. Caffeine is the only legal psychoactive drug and is used as a performance enhancer. In a regular cup of coffee, there is about 94 mg of caffeine. It is possible to get addicted to caffeine, and experience withdrawal without it. Withdrawal symptoms include headache, fatigue, and irritability. Toxicity can occur at over 10 g a day but these levels are much higher than the recommended limit of 400 mg a day, or at most four cups of coffee (Higdon & Frei, 2006).

In research done by Fuller & Rao (2017), it was found that medium roast samples showed higher concentrations of caffeine than dark roast samples. When looking into caffeine content of robusta and arabica coffees, it was found that there was a larger caffeine content in robusta coffee and resulted in greater antioxidant activity (Vignoli, Bassoli, & Benassi, 2011). Research by Fuller and Rao (2017) found that caffeine was at a higher concentration in cold brew coffee as opposed to coffee made with hot brewing methods. It was also found that cold brew made with medium roast coffees, rather than dark roast, had a higher caffeine content suggesting that higher roasting temperatures decrease the concentration of caffeine (Fuller & Rao, 2017).

## 1.10 ANTIOXIDANTS

Coffee is considered one of the best sources of dietary antioxidants due to the large number of the world's population consuming coffee daily (Higdon & Frei, 2006). An antioxidant is a substance that may prevent or delay some types of cell damage by neutralizing free radicals. Free radicals are unstable molecules with an unpaired electron. These unstable molecules can do damage to cells and cause unwanted oxidation. Free radicals can form from regular cellular metabolism and consumption of certain foods. Antioxidants are able to donate an election to the free radical without becoming damaged. This stabilizes the free radical and prevents damage to cells.

Green coffee beans contain anthocyanins which are antioxidants that are beneficial to human health (Oroian & Escriche, 2015). Anthocyanins exhibit anticarcinogenic activity (Hui et al., 2010) and play a vital role in the prevention of neuronal and cardiovascular disease, cancer, and diabetes (Oroian & Escriche, 2015). Green coffee beans also contain phenolic acids which demonstrate antioxidant, antibacterial, antiviral, anticarcinogenic, and anti-inflammatory and vasodilatory actions (Mudnic et al., 2010). Tannins are also present in green coffee beans, exhibiting antioxidant, anti-thrombotic, anti-atherogenic, anti-mutagenic, anti-diabetic and antiproliferative effects, anti-carcinogenic, anti-inflammatory, antiviral and antibacterial properties (Oroian & Escriche, 2015). Antioxidant compounds commonly found in coffee contribute to its astringent, bitter, and acidic flavor notes (Aguiar et al., 2016).

Coffee contains a large number of antioxidants including hydrocinnamic acids and polyphenols. Hydrocinnamic acids are very effective in neutralizing free radicals and preventing oxidative stress. Polyphenols also act as an antioxidant and may prevent against diseases like heart disease, cancer, and type 2 diabetes (Higdon & Frei, 2006). Chlorogenic acid compounds are known to be active antioxidants that may cause health benefits in coffee drinkers (Fuller & Rao, 2017).

Aguiar et al. (2016) found that medium-roast coffee has more antioxidant effects than other roasts. In both hot and cold brew extractions, chlorogenic acid was found in higher concentrations in medium roasts than in darker roasts, suggests that higher roasting temperatures decomposes chlorogenic acid and results in lower extraction concentrations (Fuller & Rao, 2017). Multiple studies have found that Robusta coffee had higher antioxidative effects than Arabica coffee beans (Esquivel & Jiménez, 2012; Vignoli et al., 2011). It was also found that boiling ground coffee beans under elevated pressure was the most efficient method for extraction of antioxidants (Esquivel & Jiménez, 2012). Research is needed on the antioxidant activity of cold brew coffee.

### 1.11 HUMAN HEALTH BENEFITS

Coffee contains many nutrients, including B Vitamins and minerals. One cup of coffee includes 11% of the RDA of riboflavin (Vitamin B2), 3% of the RDA of manganese and potassium, 2% of the RDA of magnesium and niacin (Vitamin B3) and 6% of the RDA of pantothenic acid (Vitamin B5) (Higdon & Frei, 2006).

Caffeine not only prevents tiredness, it can also increase brain function. When the adenosine receptor is blocked, other neurotransmitters increase leading to enhanced firing of neurons (Higdon & Frei, 2006). Many studies have shown that coffee can actually improve brain function like memory, mood, reaction time, and general cognitive function (Higdon & Frei, 2006). Caffeine can also aid in fat burning by boosting the metabolic rate by 3-11%. It stimulates the central nervous system and sends signals to fat cells to break down body fat. Physical performance can improve as well. Caffeine increase epinephrine, also known as adrenaline, levels in the blood. This hormone allows our bodies to be ready for intense physical exertion. It does this by sending a message to fat cells to break down body fat and release it into the blood as free fatty acids to be used as fuel (Higdon & Frei, 2006).

People who consume coffee regularly may have a decreased risk of many different diseases. Studies have shown the decreased development of Type II Diabetes, Alzheimer's, and Parkinson's in coffee drinkers (Higdon & Frei, 2006). Coffee also has shown to have protective effects on the liver and can reduce the risk of developing liver cancer (Higdon & Frei, 2006).

Although coffee has been linked to an increase in blood pressure, it is minimal. Studies have shown it does not contribute to heart disease. There is some evidence that shows people who drink coffee regularly have a decreased risk of heart disease and a 20% lower risk of stroke (Higdon & Frei, 2006).

### 1.12 STUDY OVERVIEW AND OBJECTIVES

Cold brew coffee, not to be confused with iced coffee (which is hot brewed coffee served over ice), is brewed in colder temperatures over a longer time period than traditional hot brewing methods (typically steeping times range from 8 to 24 hours; Fuller & Rao, 2017). In 2016, hot coffee sales fell 3% while cold brew coffee sales rose nearly 80% (Fuller & Rao, 2017). There was a reported 460% increase in retail sales of refrigerated cold brew coffee from 2015 to 2017, generating $38 million in 2017 alone (Fuller & Rao, 2017). Cold brew coffee has become extremely popular as it has a smoother profile and tastes less acidic as there is no heat to dissolve these molecules (Fuller & Rao, 2017).

Although hot coffee has been researched extensively, there is not much current research on cold brew coffee. Research is needed to examine the effects of different brewing methods on the properties of cold brewed coffee. The main objective of this research is to determine the effects of grind size and extraction time on the antioxidant potential, sensory profile, and consumer likability of cold brew coffee.

## 2. Manuscript I

Sensory Evaluation of Cold Brew Coffee

Based on

Consumer Assessment and Descriptive Analysis

**2.1 ABSTRACT**

While hot coffee has been studied extensively, there is much less known about the sensory qualities of cold brew coffee. **PURPOSE:** To determine the effects of grind size and extraction time on the sensory profile and consumer likability of cold brew coffee. **MATERIALS AND METHODS:** 138 adults participated in randomized sample consumer assessment of the likeability of 18 different cold brew coffee samples based on appearance, aroma, flavor, and overall. The samples were made with 2 different types of beans, 3 different grind sizes (0.65mm, 1.15mm, and 1.65mm), and 3 different brewing times (18h, 24h, and 30h). Additionally, twelve trained panelists participated in a descriptive analysis of these 18 cold brew coffee samples. These samples were assessed on 19 selected attributes. Multivariable modeling was performed to quantify the effects of the processing factors on each of the assessment outcomes. **RESULTS:** For Consumer Assessment, samples brewed at 24 hours is consistently scored lower than 18 and 30 hours. The sample with a grind size of 1.65 mm brewed for 30 hours scored the highest for measures of flavor and overall likability. For Descriptive Analysis, 24 hours consistently produced the lowest scores across all attributes. **CONCLUSIONS:** This data suggests that time was the most important factor for both consumer acceptability and magnitude of descriptive traits. This data showed that coffee brewed for 18 or 30 hours rather than 24 hours had higher magnitudes of descriptive traits and higher acceptability. It would be recommended to brew cold brew coffee either longer or shorter than 24 hours.

## 2.2 INTRODUCTION

Sensory evaluation is the scientific discipline which looks at how characteristics of food and materials are perceived by senses (Lazim & Suriani, 2009). Human judges are used to measure and evaluate sensory characteristics of food including flavor, color, smell, taste, and mouthfeel (Lazim & Suriani, 2009). Sensory data is obtained through subjective evaluation, then analyzed statistically (Lazim & Suriani, 2009). In statistical analysis of the sensory evaluation data, average scores of attributes are generally calculated and compared with a certain significance level among the samples (Lazim & Suriani, 2009).

Sensory evaluation is very important when finding the market acceptability of a product (Lazim & Suriani, 2009). When looking at food products, it has been found that consumer behaviors can be affected by its sensory properties as well as contextual factors including environment, ambiance, consumption motivation, etc. (Kim, Lee, & Kim, 2016). Influential factors are essential for consumers to get the best product and for manufacturers to develop and sell the best product (Lazim & Suriani, 2009). Sensory evaluation is also necessary to ensure that their products will be succeeding in the marketplace (Lazim & Suriani, 2009). Without appropriate sensory analysis, there is a high risk of market failure (Lazim & Suriani, 2009).

There have been many studies looking at general preferences of coffee using hot brewing methods. There is not much research available on the sensory evaluation of cold brew coffee. Since cold brew coffee has increased in popularity so rapidly, it is important to understand why.

## 2.3 Materials and Methods

*Coffee Sample Preparation*

All samples were prepared in triplicate. All coffee in this study has been obtained from 1000 Faces Coffee located in Athens, GA. Two types of coffee beans were used; a washed Guatemalan coffee and a naturally processed Brazilian coffee. Both are medium-roast. Once received, the bags of coffee sat in ambient temperature to degas for three days. Once residual carbon dioxide was released, the bags of the same type of coffee were mixed together. Individual portions were taken out and vacuum sealed. The coffee was then stored at -81°C until use. When needed, individual coffee packages were removed from the deep freezer and placed in refrigeration at 4°C for 24 hours.

Both types of coffee were grinded and sieved to three different sizes 0.65mm, 1.15mm, and 1.65mm. Each were brewed for a variety of times; 18, 24, and 30 hours to produce 18 distinct samples (see Table 1). The Capresso Infinity Conical Burr Grinder was used, then the coffee grounds were sieved using the Kruve sieves. We have set up a three-tiered sieving system using 1400mm, 900mm, and 400mm screens to ensure standardized grind sizes.

The coffee samples were prepared as shown in **Table 1.** Cold water extracted (CWE) coffee were brewed using an immersion method at various times. Coffee grounds were immersed in filtered water in a 33.8 x 29.0 x 29.0 cm glass container. CWE coffee steeped for 18, 24, or 30 hours at 4°C. The mass ratio of coffee to water was 1:16. The grinds were then filtered out and stored at 4°C until use.

### 2.3.1 Consumer Acceptability

*Study Population*

All procedures in this study were approved by the Institutional Review Board of Montclair State University. Consumer acceptability was tested with an untrained panel (n=40 panelist's responses per sample). A total of 138 participants signed informed consent forms and participated in the consumer assessment profiling protocol. Simultaneous to the assessment of the coffee, the panelists had the option to also be assessed by face reader technology. Participation requirements included being 18 years or older, able to consume caffeine, and all participants must enrolled in graduate or undergraduate programs as a student or an employee at Montclair State University.

*Consumer Assessment Protocol*

In this test the general public analyzed the likability of each of the 18 cold water extraction coffee samples. Each panelist was recruited through various methods; flyers, in person plea, email plea, and general announcement. Panelists were given 5 samples of cold water extracted coffee. Panelists were asked to rate the samples based on flavor, aroma, overall evaluation, and willingness to purchase on a 7-point hedonic scale. They were also given a short survey to complete prior to the consumption of the coffee (See Figure 1 in the Appendix of this Manuscript).

Each sample of coffee was given a random 3 digit number and given to the participant in counterbalanced orders. Panelists were asked to test the coffee left to right. The number on the cups corresponded with the number on their scorecards (See Figure 2 in the Appendix of this Manuscript). Participants were given water and asked to cleanse their pallet in between each sample. This was achieved by asking participants to take a sip of water and wait approximately 30 seconds before tasting the next sample.

### *FaceReader Assessment*

With the permission of the participant, their facial expressions were recorded with the intent of analyzing it for their emotional response. It was our goal to see if their written responses correlate with their emotional response recorded with FaceReader. Participants were instructed to look directly into the camera while consuming the coffee samples. Analysis of emotional response began as the participant began consumption of the sample, and ended when finished with the sample. Participants were also advised to provide their most honest response.

FaceReader technology uses Noldus Software and analyses seven key emotions: neutral, angry, happy, sad, scared, surprised, and disgusted. Videos were recorded using the program, Media Recorder 3.0, and uploaded into FaceReader to be analyzed. FaceReader analysis is hindered if a participant is wearing thick glasses, or anything that could obstruct the view of their face including hats, thick facial hair, or if they put their hand in front of their face while sampling. Participants were advised to keep their hands away from their face while sampling, and to remove any hats or glasses with thick frames.

The FaceReader data collected was not analyzed for the results of this thesis, but are available for further research to be done.

### 2.3.2 Descriptive Analysis

*Study Population*

All procedures in this study were approved by the Institutional Review Board of Montclair State University. A total of 12 participants signed informed consent forms and participated in the descriptive analysis profiling protocol. Participation requirements included being 18 years or older, able to consume caffeine, and all participants must enrolled in graduate or undergraduate programs as a student or an employee at Montclair State University. Current students of Dr. Kerrihard were not eligible to participate.

*Descriptive Analysis Protocol*

In this test the general public analyzed the flavor profile of eighteen samples of cold water extracted coffee. The purpose of this test was to analyze the coffees based on different flavor notes. Each panelist was recruited through various methods; flyers, in person plea, email plea, and general announcement. Descriptive analysis was performed by a trained panel (n=12). Participants met a total of 11 times. There were 3 training sessions. Training was provided on site by investigators prior to assessment. Training included the assessment of 19 flavor standards selected from the World Coffee Research Lexicon. Training sessions were followed by 8 testing sessions. Participants tested a total of 18 cold brew coffee samples. Each participant tested 3 samples per testing session and were asked to rate the intensity of each attribute on a scale of 0-15, 0 being not present, and 15 being very intense. For each flavor note, participants were asked to draw a line representing the intensity of each flavor. Participants were directed not to discuss during the testing sessions.

*Sensory Characteristics*

Samples were assessed for the magnitude of the following attributes: Sweet, sour, bitter, fruity, citrus fruit, musty/earthy, woody, ashy, nutty, chocolate, caramelized, molasses, honey, vanilla, floral, overall impact, overall aroma, mouth drying, and metallic. These attributes have been selected from the World Coffee Research Sensory Lexicon. Each attribute had a reference, or standard, for the participants to either taste or smell. Participants were calibrated on the level of intensity of each standard. A discussion was had after tasting each standard to ensure each participant recorded a similar intensity score. These standards have been given an intensity score on a scale from 1-15. Participants will then determine the amount of each attribute in each sample of coffee in comparison to the standard.

Sweet was made by creating a 1% sugar solution. The sweet standard had an intensity of 1 out of 15. Sour was made by making a .05% citric acid solution. The sour standard had an intensity of 3.5 out of 15. Bitter was achieved by having participants eat instant coffee. They were directed to focus on how the instant coffee felt on their tongue. This had an intensity of 12 out of 15. Fruity was made by mixing one-part Juicy Juice 100% Juice Kimi Strawberry, one-part water. This had an intensity of 4 out of 15. Citrus Fruit was made by mixing 96% Grapefruit and Tangerine Juice, 2% Lemon Juice, and 2% Lime Juice. This had an intensity of 6.5 out of 15. Participants were given Miracle-Gro Potting Soil to smell for the musty-earthy standard. This had an intensity of 9 out of 15. Participants were given broken wooden Popsicle sticks to smell for the woods standard. This had an intensity of 7.5 out of 15. Participants were given burnt paper to smell for the ashy standard. This had an intensity of 4 out of 15. Participants were given raw peanuts to smell for the nutty standard. This had an intensity of 7.5 out of 15. Participants were given Nestle Toll House Chocolate Chips to eat as the chocolate standard. This had an intensity of 7.5 out of 15.

Caramelized was made by mixing 60g of brown sugar in a liter of water. This had an intensity of 4.5 out of 15. Molasses was made by mixing 4 teaspoons of molasses in 500 milliliters of water. This had an intensity of 6.5 out of 15. Honey was made by mixing 2 teaspoons of honey into 500mL of hot water. This had an intensity of 6.5 out of 15. Vanilla was made by mixing ¼ teaspoons of vanilla extract into 1 cup of whole milk. This had an intensity of 3 out of 15. Floral was made by mixing one-part grape juice to one-part water. This has an intensity of 5 out of 15. Overall impact and overall aroma was made by mixing 30g instant coffee into 40 oz of water at 165°F. This had an intensity of 12 out of 15. Mouth drying was achieved by making a .07% alum solution. This had an intensity of 3.5 out of 15. Metallic was achieved by making a .1% potassium chloride solution. This had an intensity of 1.5 out of 15.

*Statistical Analysis*

Three-way ANOVA was used to assess significant differences within each factor. Multivariable modeling was performed to quantify the effects of the processing factors on each of the assessment outcomes.

## 2.4 Results and Discussions

### 2.4.1 Consumer Assessment

Consumer Assessment results for overall of all samples are shown in Figure 1. All samples were not significantly different from each other. However, there is a clear pattern that samples brewed at 24 hours consistently scored lower than 18 and 30 hours. For consumer assessment overall, the sample with a grind size of 1.65 mm brewed for 30 hours scored the highest.

*Grind Size*

Results for Consumer Assessment by grind size can be seen in Table 2. For aroma, samples made with 1.65 mm grind size scored significantly higher than 0.65mm ($p<.001$). For aroma, the mean scores were 4.12, 4.31, and 4.57 for grind sizes 0.65, 1.15, and 1.65 respectively. While not significant, 1.65mm scored higher than 0.65mm and 1.15mm in flavor and overall. For flavor, the mean scores were 3.47, 3.45, and 3.63 for grind sizes 0.65, 1.15, and 1.65 respectively. For overall, the mean scores were 3.59, 3.68, and 3.80 for grind sizes 0.65, 1.15, and 1.65 respectively.

For appearance, flavor and overall, there was no significant difference according to grind size. For appearance, the mean scores were 4.37, 4.32, and 4.23 for grind sizes 0.65, 1.15, and 1.65 respectively. This suggests that larger grind size is preferable in regards to aroma and perhaps the other assessed attributes.

*Brew Time*

Results for Consumer Assessment by brew time can be seen in Table 3. For flavor, samples brewed for 18 and 30 hours scored significantly higher than 24 hours ($p=.046$ and $p=.038$ respectively). For flavor, the mean scores were 3.65, 3.25, 3.65 for 18, 24, and 30 hours respectively. For overall, the mean scores were 3.76, 3.44, 3.86 for 18, 24, and 30 hours respectively.

There was no significant difference in appearance and aroma according to brew time. For appearance, the mean scores were 4.25, 4.37, 4.31 for 18, 24, and 30 hours respectively. For aroma, the mean scores were 4.46, 4.22, 4.31 for 18, 24, and 30 hours respectively.

*Bean Type*

There were no significant differences in appearance, aroma, flavor, or overall between the two bean types.

*Predictive Linear Modeling of Consumer Assessment Output*

Predictive linear modeling of Consumer Assessment output were assessed with each variable (see Table 3). For grind size, it was shown to be the best predictive linear model for aroma. For every millimeter larger the grind size increases within the range assessed, it could be predicted that the aroma score would increase by .485.

It was found that time was a negative predictor of aroma. For every additional hour of brew time within the range assessed, it could be predicted that the aroma score would decrease by .023.

When analyzing the data, scores were frequently lower at the 24 hour mark when compared to 18 and 30 hours. For this reason, a new variable was added to the model parameters; time away from 24 hours. This variable was the sole significant predictor for flavor and overall. It was found that with brew time away from 24, in either direction within the range assessed, it could be predicted that scores for flavor and overall would increase by .058 and .045 respectively.

While this model is substantiating the significance of this variable, the correlation coefficients were not high enough to be strong predictive models ($r^2_{adj}$ = .032 for aroma, $r^2_{adj}$ = .008 for flavor, and $r^2_{adj}$ = .005 for overall).

*Effect of Coffee Drinking Preference on Assessment*

Survey responses were factored into statistical analysis. It was found that participants who responded that they drink coffee with "nothing added" scored higher than those who responded adding cream, sugar, and/or flavor into their coffee. For flavor, those who add nothing into their coffee reported significantly higher responses ($p$= .041). The mean value for those who add cream, sugar, and/or flavor to coffee was 3.37, while those who do not add anything to coffee had a mean value of 3.72. For overall, those who add nothing into their coffee reported significantly higher responses ($p$= .008). The mean value for those who add cream, sugar, and/or flavor to coffee was 3.53, while those who do not add anything to coffee had a mean value of 3.89.

### 2.4.2 Descriptive Analysis

*Grind Size*

Descriptive Analysis results based on grind size are shown in Table 5. Grind size had no significant differences in the assessed descriptive attributes. There were no clear patterns in descriptive analysis according to grind size.

*Brew Time*

Descriptive Analysis results based on brew time are shown in Table 6. Graphical depictions of the attributes with significant differences are shown in Figures 2-8. Brew time has significant differences in some of the assessed descriptive attributes. For sweet, 24 hours was significantly lower than 30 hours ($p$=.034). For bitter, 24 hours was significantly lower than 18 and 30 hours ($p$<.001). For musty/earthy, 24 hours was significantly lower than 18 and 30 hours ($p$<0.001 and

$p$=.001 respectively). For caramelized, 24 and 18 hours were significantly lower than 30 hours ($p$=0.008 and $p$=.044 respectively). For honey, 24 hours was significantly lower than 18 and 30 hours ($p$=0.044 and $p$=.011 respectively). For overall aroma, 24 hours was significantly lower than 18 and 30 hours ($p$=0.001 and $p$=.007 respectively). For mouth drying, 24 and 30 hours were significantly lower than 18 hours ($p$=0.001 and $p$=.028 respectively).

While some aren't significant, 24 hours produced the lowest scores across all attributes. The different dissolution rates of flavor active compounds within each sample may be mitigating certain flavors, making the samples less flavorful. This corresponds to our consumer assessment data which also showed lower hedonic responses at 24 hours. This is interesting because industry standard of making cold brew is 24 hours, yet this data is suggesting that it is not producing the best tasting coffee.

*Predictive Linear Modeling of Descriptive Analysis Output*

Predictive linear modeling of Descriptive Analysis Output were assessed with each variable and are shown in Table 7. For sweet, time produced the best predictive linear model. For every additional hour, it could be predicted that the sweet score would increase by .102. For bitter, time from 24 produced the best predictive linear model. For every hour away from 24 hours within the range assessed, it could be predicted that the bitter score would increase by .293. For fruity, time from 24 produced the best predictive linear model. For every hour away from 24 hours within the range assessed, it could be predicted that the fruity score would increase by .116. For citrus fruit, time from 24 produced the best predictive linear model. For every hour away from 24 hours within the range assessed, it could be predicted that the citrus fruit score would increase by .175. For musty/earthy, time from 24 produced the best predictive linear model. For every hour away from 24 hours within the range assessed, it could be predicted that the musty/earthy score would increase by .296. For woody, time from 24 produced the best predictive linear model. For every hour away from 24 hours within the range assessed, it could be predicted that the woody score would increase by .111. For ashy, time from 24 produced the best predictive linear model. For every hour away from 24 hours within the range assessed, it could be predicted that the ashy score would increase by .118. For nutty, time from 24 produced the best predictive linear model. For every hour away from 24 hours within the range assessed, it could be predicted that the nutty score would increase by .117. For caramelized, grind size was a negative predictor. For every millimeter larger, the caramelized score would decrease by .723. Time from 24 produced the best predictive linear model. For every hour away from 24 hours within the range assessed, it could be predicted that the caramelized score would increase by .098. For molasses, time from 24 produced the best predictive linear model. For every hour away from 24 hours within the range assessed, it could be predicted that the molasses score would increase by .125. For honey, time from 24 produced the best predictive linear model. For every hour away from 24 hours within the range assessed, it could be predicted that the honey score would increase by .208. For floral, time from 24 produced the best predictive linear model. For every hour away from 24 hours within the range assessed, it could be predicted that the floral score would increase by .106. For overall impact, time from 24 produced the best predictive linear model. For every hour away from 24 hours within the range assessed, it could be predicted that the overall impact score would increase by .238. For overall aroma, bean type and time from 24 produced the best predictive linear models. The predicted overall aroma score would be higher for Los Santos than Fazenda Primavera. For every hour away from 24 hours within the range assessed, it could be predicted that the woodiness score would increase by .305. For mouth drying, time was a negative predictor. For every hour more of brew time, the mouth drying score would decrease by .107. Time from 24 produced the best predictive linear model. For every hour away from 24 hours within the range assessed, it could be predicted that the caramelized score would increase by .211. For metallic, time from 24 produced the best predictive linear model. For every hour away from 24 hours within the range assessed, it could be predicted that the metallic score would increase by .172.

Consistently, time from 24 was the best predictor of the magnitude of the assessed flavor attributes. In all cases, time from 24 hours was a positive predictor. This data is consistent with consumer assessment data as well as the multiple comparisons test of the consumer assessment. Not being brewed for 24 hours showed higher scores.

## 2.5 Tables and Figures

**Table 1: Coffee Samples**

| **Fazenda Primavera** | | **Processing Time (hours)** | | |
|---|---|---|---|---|
| | | **18** | **24** | **30** |
| **Grind Size (mm)** | **0.65** | Sample 1 | Sample 2 | Sample 3 |
| | **1.15** | Sample 4 | Sample 5 | Sample 6 |
| | **1.65** | Sample 7 | Sample 8 | Sample 9 |

| **Los Santos** | | **Processing Time (hours)** | | |
|---|---|---|---|---|
| | | **18** | **24** | **30** |
| **Grind Size (mm)** | **0.65** | Sample 10 | Sample 11 | Sample 12 |
| | **1.15** | Sample 13 | Sample 14 | Sample 15 |
| | **1.65** | Sample 16 | Sample 17 | Sample 18 |

**Table 2: Consumer Assessment Results by Grind Size[a]**

| | Grind Size (mm) | | |
|---|---|---|---|
| | **0.65** | **1.15** | **1.65** |
| Appearance | 4.37A | 4.32A | 4.23A |
| Aroma | 4.12A | 4.31AB | 4.57B |
| Flavor | 3.47A | 3.45A | 3.63A |
| Overall | 3.59A | 3.68A | 3.80A |

[a](n=240 assessments per reported mean) assessment on 7-point hedonic scale; 1=dislike extremely, 7=like extremely

Samples in rows without the same letter are significantly different ($\alpha = 0.05$). Significance determined with 3-way ANOVA with a consideration of grind size (n=3), brew time (n=3), and bean type (n=2).

**Table 3: Consumer Assessment Results by Brew Time[a]**

| | Brew Time (h) | | |
|---|---|---|---|
| | **18** | **24** | **30** |
| Appearance | 4.25A | 4.37A | 4.31A |
| Aroma | 4.46A | 4.22A | 4.31A |
| Flavor | 3.65B | 3.25A | 3.65B |
| Overall | 3.76AB | 3.44A | 3.86B |

[a](n=240 assessments per reported mean) assessment on 7-point hedonic scale; 1=dislike extremely, 7=like extremely

Samples in rows without the same letter are significantly different ($\alpha = 0.05$). Significance determined with 3-way ANOVA with a consideration of grind size (n=3), brew time (n=3), and bean type (n=2).

**Table 4–Predictive modeling of consumer assessment results[ab]**

| **Attribute** | **Bean Type** | **Grind Size (mm)** | **Time (h)** | **Time from 24 Hours (h)** |
|---|---|---|---|---|
| **Appearance** | *n.s.* | *n.s.* | *n.s.* | *n.s.* |
| **Aroma** | *n.s* | **.485** | **-.023** | *n.s* |
| **Flavor** | *n.s.* | *n.s.* | *n.s.* | **.058** |
| **Overall** | *n.s.* | *n.s.* | *n.s.* | **.045** |

[a]Models based upon sensory scores determined by consumer assessment (n = 40 per sample).
[b]"n.s." denotes not significant ($\alpha = 0.05$)
Linear regression models made with SPSS software.

Table 5: Descriptive Analysis Results by Grind Size[a]

| | Grind Size (mm) | | |
|---|---|---|---|
| | **0.65** | **1.15** | **1.65** |
| Sweet | 2.03A | 2.72A | 2.49A |
| Sour | 2.82A | 3.07A | 3.08A |
| Bitter | 5.54A | 5.07A | 4.49A |
| Fruity | 2.43A | 2.57A | 2.58A |
| Citrus Fruit | 2.61A | 2.71A | 2.82A |
| Musty/Earthy | 4.47A | 3.43A | 4.11A |
| Woody | 4.66A | 4.02A | 4.22A |
| Ashy | 2.64A | 3.06A | 2.97A |
| Nutty | 3.46A | 3.38A | 3.06A |
| Chocolate | 2.90A | 2.74A | 2.38A |
| Caramelized | 2.97A | 2.80A | 2.23A |
| Molasses | 3.35A | 3.34A | 3.23A |
| Honey | 3.14A | 2.63A | 2.90A |
| Vanilla | 1.76A | 1.86A | 1.68A |
| Floral | 2.15A | 2.35A | 2.32A |
| Overall Impact | 5.45A | 5.62A | 5.03A |
| Overall Aroma | 4.86A | 4.29A | 4.28A |
| Mouth Drying | 3.07A | 3.47A | 3.30A |
| Metallic | 2.91A | 3.07A | 2.97A |

[a](n=72 assessments per reported mean) assessment on a 15-point scale using trained panelists (0= minimum, 15= maximum).

Samples in rows without the same letter are significantly different ($\alpha = 0.05$). Significance determined with 3-way ANOVA with a consideration of grind size (n=3), brew time (n=3), and bean type (n=2).

Table 6: Descriptive Analysis Results by Brew Time[a]

| | Brew Time (h) | | |
|---|---|---|---|
| | **18** | **24** | **30** |
| Sweet | 2.06AB | 1.86A | 3.13B |
| Sour | 3.28A | 2.48A | 3.17A |
| Bitter | 6.22B | 3.76A | 5.18B |
| Fruity | 2.67A | 1.87A | 2.94A |
| Citrus Fruit | 2.89A | 2.11A | 3.07A |
| Musty/Earthy | 4.79B | 2.70A | 4.48B |
| Woody | 4.38A | 3.75A | 4.71A |
| Ashy | 2.63A | 2.49A | 3.41A |
| Nutty | 3.61A | 2.72A | 3.54A |
| Chocolate | 2.83A | 2.14A | 3.01A |
| Caramelized | 2.38A | 2.14A | 3.35B |
| Molasses | 3.25A | 2.84A | 3.73A |
| Honey | 3.20B | 2.02A | 3.37B |
| Vanilla | 1.58A | 1.55A | 2.09A |
| Floral | 2.48A | 1.94A | 2.38A |
| Overall Impact | 5.49A | 4.58A | 5.92A |
| Overall Aroma | 5.28B | 3.24A | 4.88B |
| Mouth Drying | 4.28B | 2.51A | 3.13A |
| Metallic | 3.25A | 2.41A | 3.24A |

[a](n=72 assessments per reported mean) assessment on a 15-point scale using trained panelists (0= minimum, 15= maximum).

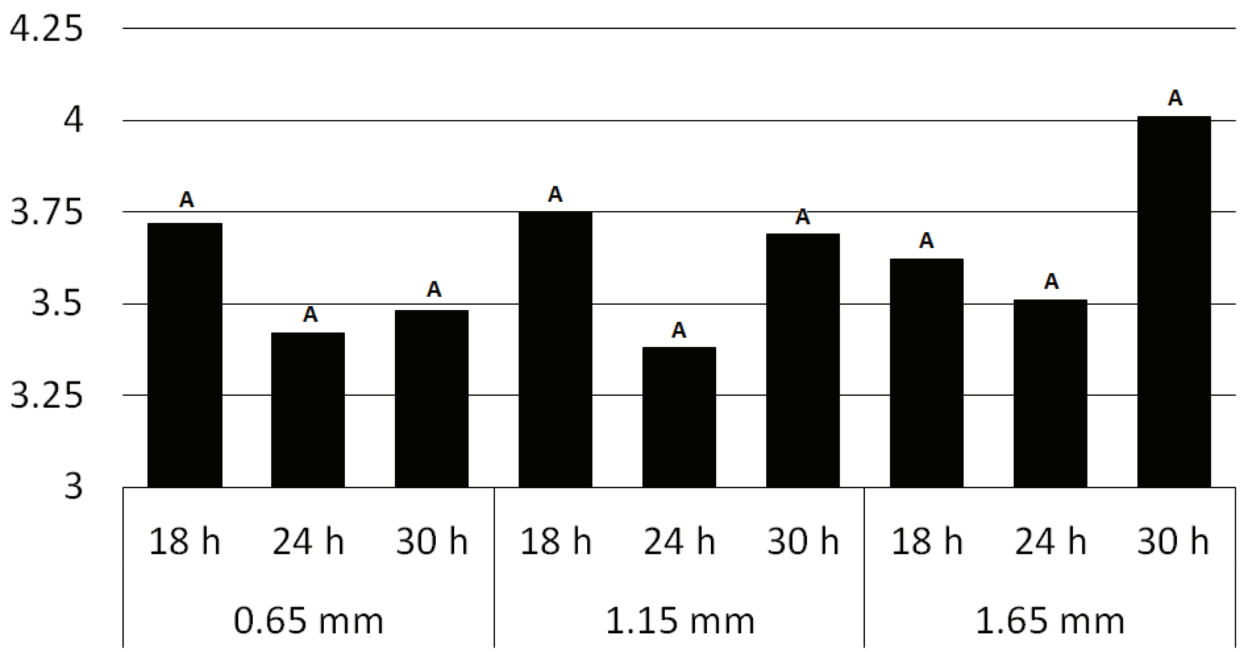
Samples in rows without the same letter are significantly different ($\alpha = 0.05$). Significance determined with 3-way ANOVA with a consideration of grind size (n=3), brew time (n=3), and bean type (n=2).

**Table 7–Predictive Modeling of Descriptive Assessment Results[ab]**

| Attribute | Bean Type[c] | Grind Size (mm) | Time (h) | Time from 24 Hours (h) |
|---|---|---|---|---|
| **Sweet** | *n.s.* | *n.s.* | **.102** | *n.s.* |
| **Sour** | *n.s* | *n.s* | *n.s* | *n.s* |
| **Bitter** | *n.s* | *n.s* | *n.s* | **.293** |
| **Fruity** | *n.s* | *n.s* | *n.s* | **.116** |
| **Citrus Fruit** | *n.s* | *n.s* | *n.s* | **.175** |
| **Musty/Earthy** | *n.s* | *n.s* | *n.s* | **.296** |
| **Woody** | *n.s* | *n.s* | *n.s* | **.111** |
| **Ashy** | *n.s* | *n.s* | *n.s* | **.118** |
| **Nutty** | *n.s* | *n.s* | *n.s* | **.117** |
| **Chocolate** | *n.s* | *n.s* | *n.s* | *n.s* |
| **Caramelized** | *n.s* | **-.723** | *n.s* | **.098** |
| **Molasses** | *n.s* | *n.s* | *n.s* | **.125** |
| **Honey** | *n.s* | *n.s* | *n.s* | **.208** |
| **Vanilla** | *n.s* | *n.s* | *n.s* | *n.s* |
| **Floral** | *n.s* | *n.s* | *n.s* | **.106** |
| **Overall Impact** | *n.s* | *n.s* | *n.s* | **.238** |
| **Overall Aroma** | **.941** | *n.s* | *n.s* | **.305** |
| **Mouth Drying** | *n.s* | *n.s* | **-.107** | **.211** |
| **Metallic** | *n.s* | *n.s* | *n.s* | **.172** |

[a]Models based upon sensory scores determined by consumer assessment (n = 40 per sample).
[b]"n.s." denotes not significant ($\alpha = 0.05$)
[c]Bean Type is a binary term (1=Fazenda Primavera, 2=Los Santos).
Linear regression models made with SPSS software.

Figure 1: Consumer Assessment: Overall[a]




[a](n=80 assessments per reported mean) assessment on 7-point hedonic scale; 1=dislike extremely, 7=like extremely
Samples in rows without the same letter are significantly different (α = 0.05). Significance determined with 3-way ANOVA with a consideration of grind size (n=3), brew time (n=3), and bean type (n=2).

*Graphs of Descriptive Assessments (Significant Differences Only)*

Figure 2: Means for Sweet by Grind Size and Brew Time

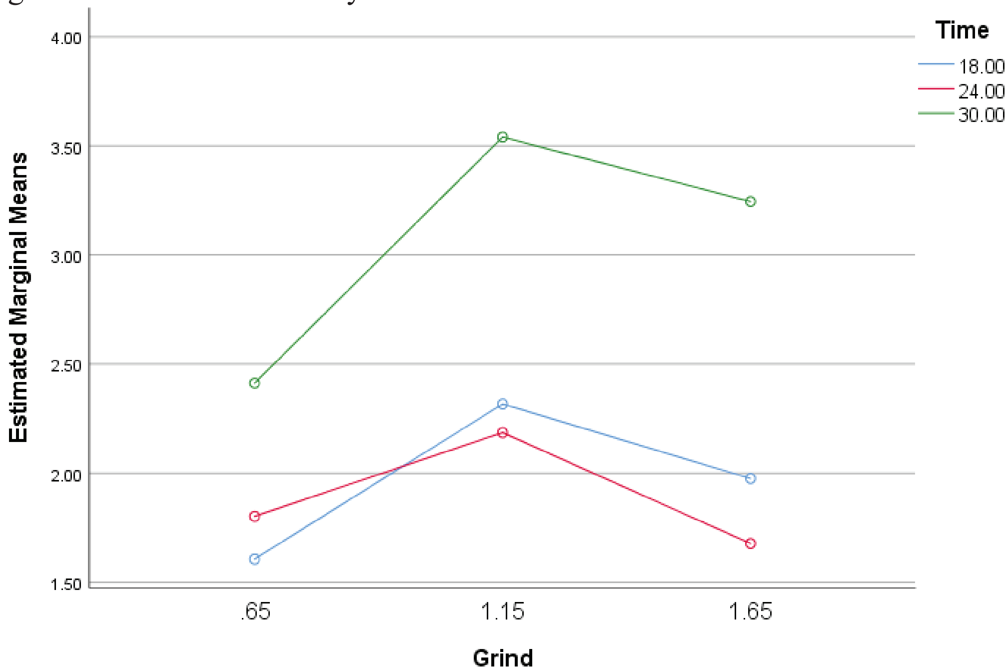



Figure 3: Means for Bitter by Grind Size and Brew Time

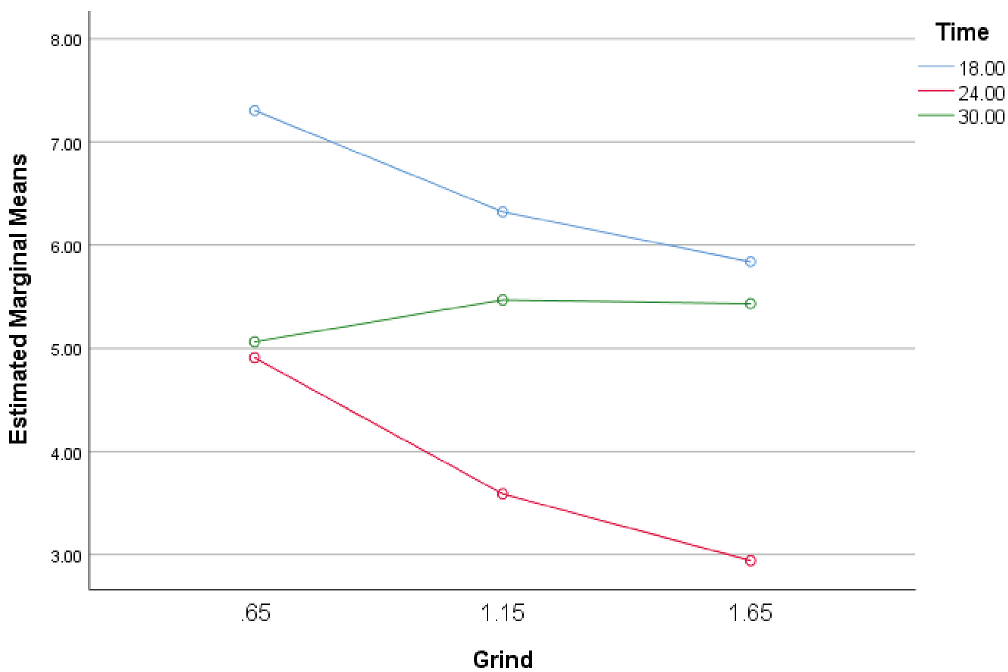


Figure 4: Means for Musty/Earthy by Grind Size and Brew Time

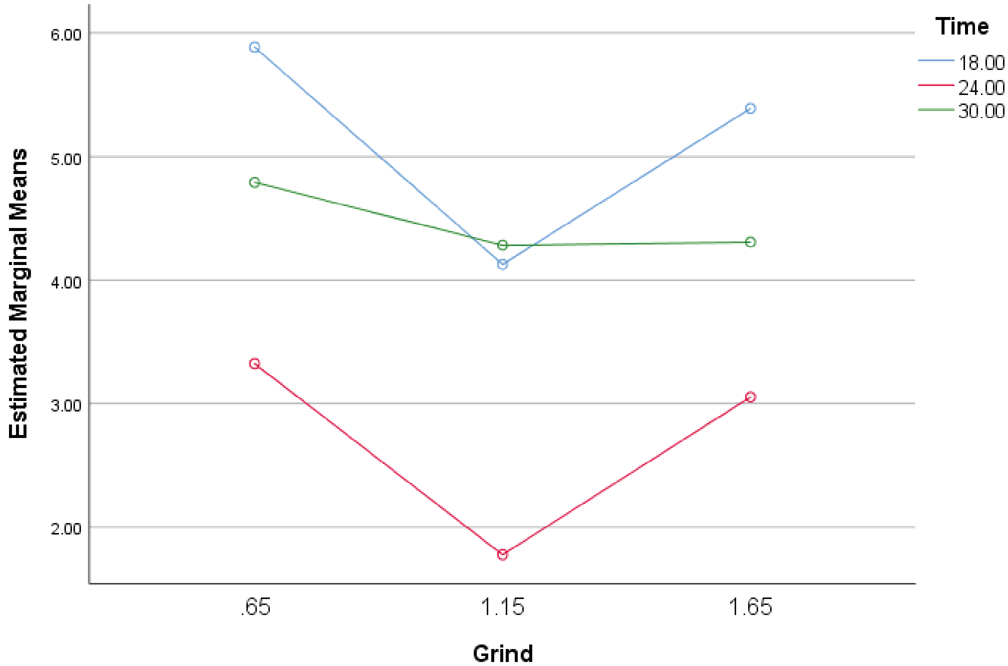



Figure 5: Means for Caramelized by Grind Size and Brew Time

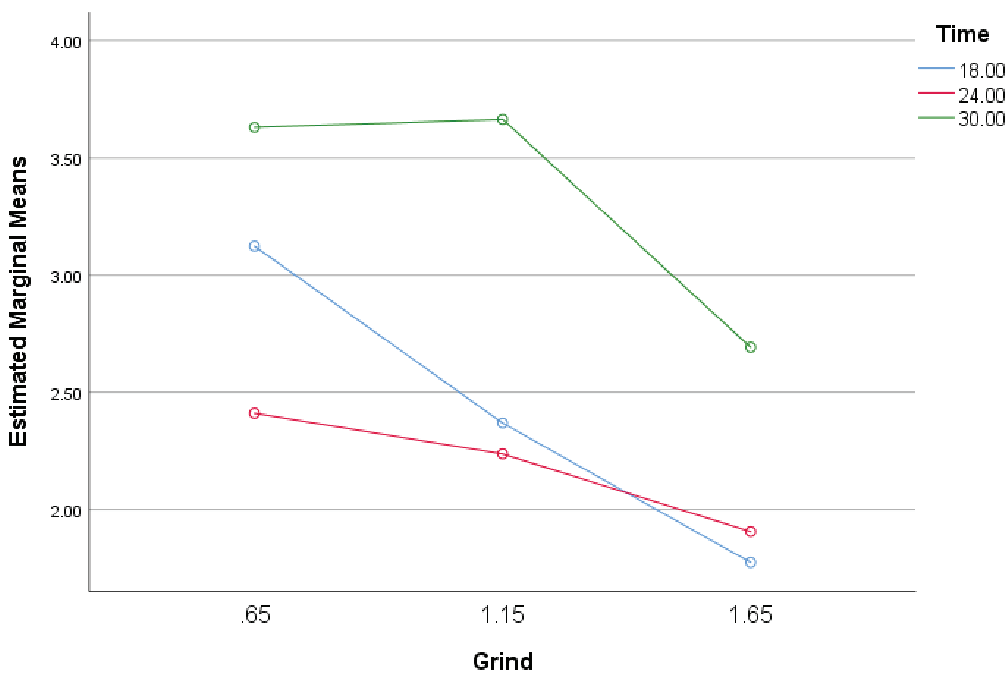


Figure 6: Means for Honey by Grind Size and Brew Time

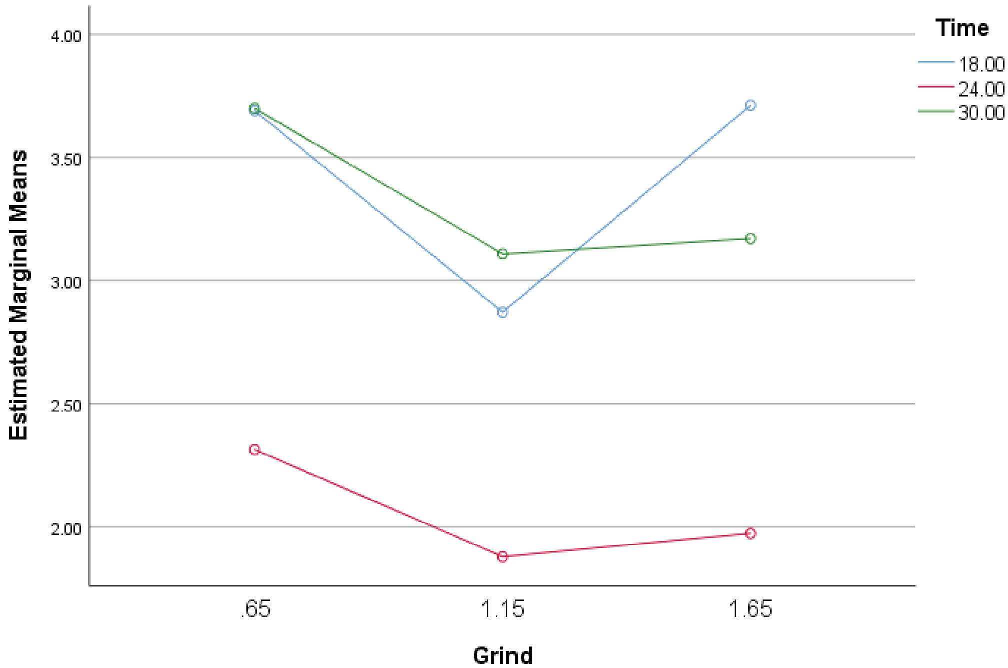



Figure 7: Means for Overall Aroma by Grind Size and Brew Time

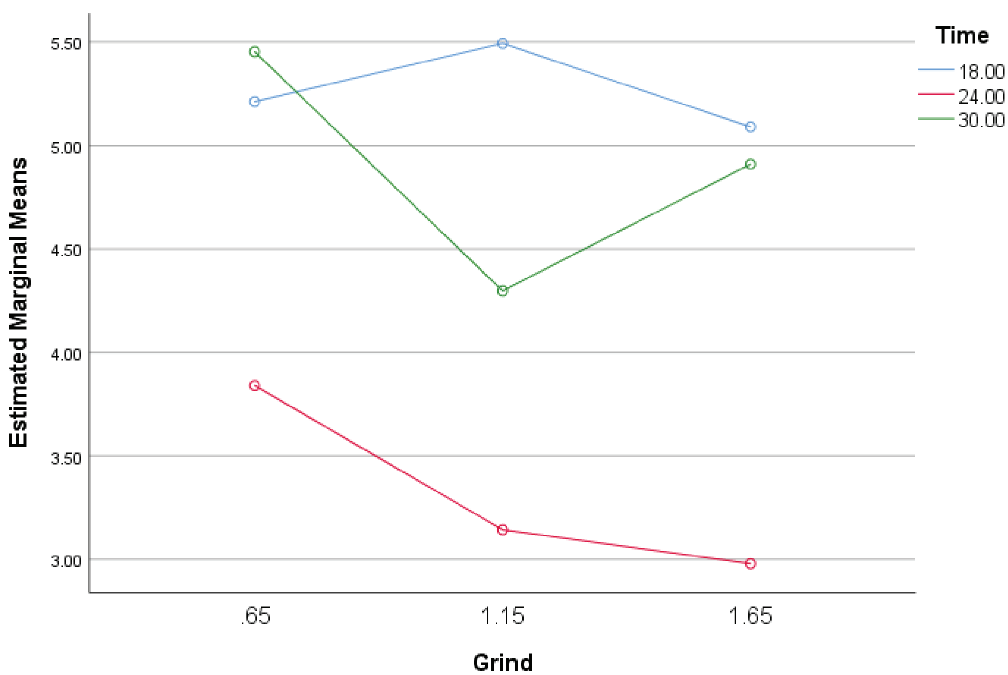
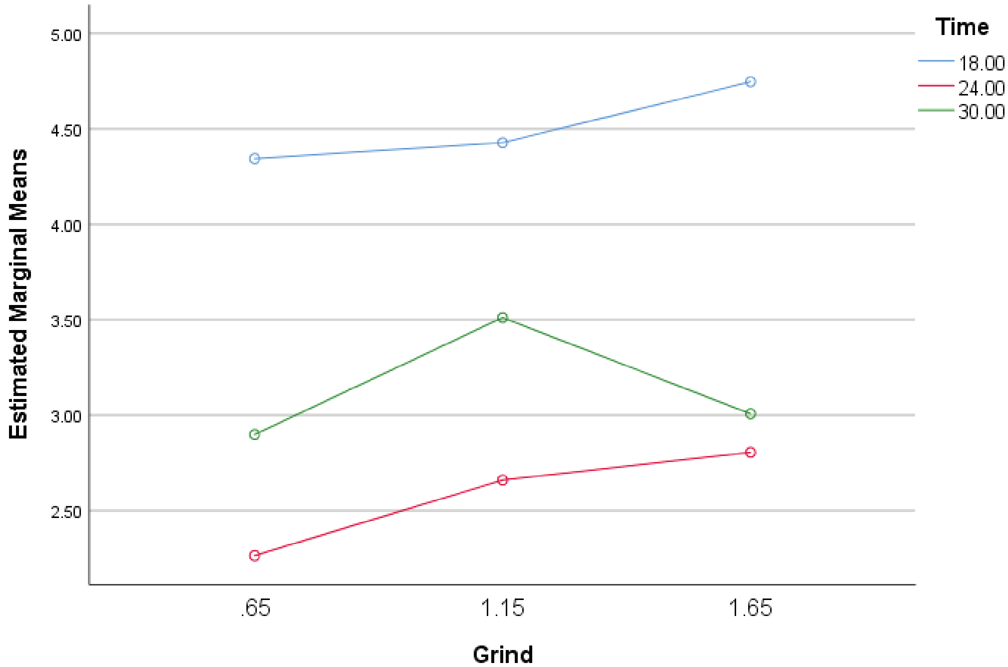


Figure 8: Means for Mouth Drying by Grind Size and Brew Time



## 2.6 Conclusion

This data suggests that time was the most important factor for both consumer acceptability and magnitude of descriptive traits. This data showed that coffee brewed for 18 or 30 hours rather than 24 hours had higher magnitudes of descriptive traits and higher acceptability. Grind size was generally not a significant factor when assessing descriptive traits or acceptability. Although not significant, coffee brewed with 1.65mm for 30 hours scored the highest in overall acceptability. For industry members, based on this study, it would be recommended to brew cold brew coffee either longer or shorter than 24 hours. Further research into the compounds that are dissolving between the hours of 18 and 24 would be warranted to identify what is mitigating the flavor attributes and perceived quality.

## 2.8 Appendix

Figure 1: Consumer Assessment Survey

# Coffee Scorecard

Panelist Number ____________________

Survey Questions

Please circle the best answer. If you prefer not to answer, leave the question blank.

1. On average, how many cups of coffee do you drink per day?
    a. 0
    b. 1
    c. 2
    d. 3
    e. 4+

2. What percentage of your coffee drinking is cold brew coffee?
    a. 0-25%
    b. 25-50%
    c. 50-75%
    d. 75-100%
    e. Never

3. Chose two that best describe what you look for in a cup of coffee.
    a. Bold
    b. Strong (Caffeine)
    c. Strong (Flavor)
    d. Smooth
    e. Dark
    f. Roasted
    g. Light Bodied
    h. Complex
    i. Sweet
    j. Decaffeinated

4. If you make coffee at home, which do you purchase?
    a. Buy whole
    b. Ground beans

5. Which would you be more inclined to purchase?
    a. Lower quality coffee at a low price
    b. Decent coffee at a moderate price
    c. High quality coffee at a high price
    d. Coffee defined by sociocultural/environmental factors (organic, Fair Trade, etc.), even for a premium cost

6. Where do you typically have/get coffee?
    a. At home (brewed at home)
    b. Starbucks
    c. Dunkin Donuts
    d. Green Mountain
    e. Other local Café

7. What kind of coffee beverage do you typically consume?
    a. Espresso
    b. Americano
    c. Immersion Methods (Drip Coffee, French Press)
    d. Latte
    e. Cappuccino
    f. Iced Coffee
    g. Cold Brew

8. What do you add to your coffee?
    a. Cream, no sugar
    b. Sugar, no cream
    c. Both cream and sugar
    d. Flavoring
    e. Nothing added

1

Figure 2: Consumer Assessment Scorecard

## Coffee Scorecard

Panelist Number ____________________

Sample Number ____________________

| | Dislike extremely | Dislike very much | Dislike slightly | Neither like nor dislike | Like slightly | Like very much | Like extremely |
|---|---|---|---|---|---|---|---|
| **Appearance** | | | | | | | |
| **Aroma** | | | | | | | |
| **Flavor** | | | | | | | |
| **Overall** | | | | | | | |

Sample Number ____________________

| | Dislike extremely | Dislike very much | Dislike slightly | Neither like nor dislike | Like slightly | Like very much | Like extremely |
|---|---|---|---|---|---|---|---|
| **Appearance** | | | | | | | |
| **Aroma** | | | | | | | |
| **Flavor** | | | | | | | |
| **Overall** | | | | | | | |

Sample Number ____________________

| | Dislike extremely | Dislike very much | Dislike slightly | Neither like nor dislike | Like slightly | Like very much | Like extremely |
|---|---|---|---|---|---|---|---|
| **Appearance** | | | | | | | |
| **Aroma** | | | | | | | |
| **Flavor** | | | | | | | |
| **Overall** | | | | | | | |

Sample Number ____________________

| | Dislike extremely | Dislike very much | Dislike slightly | Neither like nor dislike | Like slightly | Like very much | Like extremely |
|---|---|---|---|---|---|---|---|
| **Appearance** | | | | | | | |
| **Aroma** | | | | | | | |
| **Flavor** | | | | | | | |
| **Overall** | | | | | | | |

Sample Number ____________________

| | Dislike extremely | Dislike very much | Dislike slightly | Neither like nor dislike | Like slightly | Like very much | Like extremely |
|---|---|---|---|---|---|---|---|
| **Appearance** | | | | | | | |
| **Aroma** | | | | | | | |
| **Flavor** | | | | | | | |
| **Overall** | | | | | | | |

2

# 3. Manuscript II

TEAC Antioxidant Analysis of Cold Water Extracted Coffee

## 3.1 Abstract

Coffee is considered one of the best sources of dietary antioxidants due to the large number of the world's population consuming coffee daily. Antioxidant compounds commonly found in coffee contribute astringent, bitter, and acidic flavor notes to coffee. Studies have found that medium roast coffee has the highest antioxidant content. **PURPOSE:** To determine the effects of grind size and extraction time on the antioxidant potential, sensory profile, and consumer likability of cold brew coffee. **MATERIALS AND METHODS:** 18 cold brew coffee samples were made with 2 different bean types, 3 different grind sizes (0.65mm, 1.15mm, and 1.65mm), and 3 brew times (18h, 24h, 30h). Antioxidant potential was assessed using Trolox Equivalent Antioxidant Capacity (TEAC) assay for 9 samples (1 bean type: Los Santos, 3 grind sizes (0.65mm, 1.15mm, and 1.65mm), and 3 brew times (18h, 24h, 30h)). Multivariable modeling was performed to quantify the effects of the processing factors on each of the assessment outcomes. **RESULTS:** For grind size, it was found that 0.65mm had significantly higher antioxidant potential than 1.15mm and 1.65mm ($p$<.001 and $p$<.001). For brew time, 24 hours had the lowest antioxidant potential of 0.81. 18 hours had an antioxidant potential of 1.4. 30 hours had the highest antioxidant potential of 2.86. **CONCLUSIONS:** It is recommended to avoid a brew time of 24 hours when considering antioxidant potential. If brewing cold brew coffee for 18 hours, a smaller grind size (0.65mm) would yield significantly higher antioxidant potential than coarser grind sizes (1.15mm and 1.65mm).

## 3.2 INTRODUCTION

Coffee is considered one of the best sources of dietary antioxidants due to the large number of the world's population consuming coffee daily (Higdon & Frei, 2006). An antioxidant is a substance that may prevent or delay some types of cell damage by neutralizing free radicals. Free radicals are unstable molecules with an unpaired electron. These unstable molecules can do damage to cells and cause unwanted oxidation. Free radicals can form from regular cellular metabolism and consumption of certain foods. Antioxidants are able to donate an election to the free radical without becoming damaged. This stabilizes the free radical and prevents damage to cells.

Green coffee beans contain anthocyanins which are antioxidants that are beneficial to human health (Oroian & Escriche, 2015). Anthocyanins exhibit anticarcinogenic activity (Hui et al., 2010) and play a vital role in the prevention of neuronal and cardiovascular disease, cancer, and diabetes (Oroian & Escriche, 2015). Green coffee beans also contain phenolic acids which demonstrate antioxidant, antibacterial, antiviral, anticarcinogenic, and anti-inflammatory and vasodilatory actions (Mudnic et al., 2010). Tannins are also present in green coffee beans, exhibiting antioxidant, anti-thrombotic, anti-atherogenic, anti-mutagenic, anti-diabetic and antiproliferative effects, anti-carcinogenic, anti-inflammatory, antiviral and antibacterial properties (Oroian & Escriche, 2015). Antioxidant compounds commonly found in coffee contribute astringent, bitter, and acidic flavor notes to coffee (Aguiar et al., 2016).

Coffee contains a large number of antioxidants including hydrocinnamic acids and polyphenols. Hydrocinnamic acids are very effective in neutralizing free radicals and preventing oxidative stress. Polyphenols also act as an antioxidant and may prevent against diseases like heart disease, cancer, and type 2 diabetes (Higdon & Frei, 2006). Chlorogenic acid compounds are known to be active antioxidants that may cause health benefits in coffee drinkers (Fuller & Rao, 2017).

Aguiar et al. (2016) found that medium-roast coffee has more antioxidant effects than other roasts. In both hot and cold brew extractions, chlorogenic acid was found in higher concentrations in medium roasts than in darker roasts, suggests that higher roasting temperatures decomposes chlorogenic acid and results in lower extraction concentrations (Fuller & Rao, 2017). Multiple studies have found that Robusta coffee had higher antioxidative effects than Arabica coffee beans (Esquivel & Jiménez, 2012; Vignoli et al., 2011). It was also found that boiling ground coffee beans under elevated pressure was the most efficient method for extraction of antioxidants (Esquivel & Jiménez, 2012). Research is needed on the antioxidant activity of cold brew coffee.

## 3.3 Materials and Methods

*Sample Preparation*

Antioxidant potential was analyzed for the washed, Guatemalan coffee, Los Santos. All coffee used in this study was obtained by 1000 Faces Coffee located in Athens, GA. Los Santos is a medium-roast coffee from the Chimaltenango region of Guatemala. Please refer to Manuscript I for all coffee sample prep reparation. Coffee used for antioxidant testing was stored at -81°C until use. When needed, individual coffee packages were removed from the deep freezer and placed in refrigeration at 4°C for 24 hours.

The Los Santos coffee was grinded to three different sizes 0.65mm, 1.15mm, and 1.65mm. Each were brewed for a variety of times; 18, 24, and 30 hours to produce 9 distinct samples, as seen in Table 1. The Capresso Infinity Conical Burr Grinder was used, then the coffee grounds were sieved using the Kruve sieves. A three-tiered sieving system was used, with the 1400mm, 900mm, and 400mm screens to ensure standardized grind sizes.

The coffee samples prepared are shown in **Table 1.** Cold water extracted (CWE) coffee were brewed using an immersion method at various times. The mass ratio of coffee to water was 1:16. Coffee grounds were immersed in filtered water in a 33.8 x 29.0 x 29.0 cm glass container. CWE coffee steeped for 18, 24, or 30 hours at 4°C. The grinds were then filtered out and 50mL of CWE coffee was placed in freeze dryer flasks. Coffee samples were lyophilized for 24 hours and removed. The powdered coffee samples were then placed in airtight glass containers and stored at -81°C.

*Extraction Protocol*

Samples of coffee were selected randomly from storage. 100% of each lyophilized sample was used and mixed with 2.5 mL of a 4/1 acetone/water solution. Samples were then sonicated for 10 minutes and placed in the centrifuge at 1000g for 10 minutes. Once finished, the supernatant was removed and set aside. The samples were then mixed with another 2.5mL of the 4/1 acetone/water solution, sonicated for 10 minutes, and placed in the centrifuge for 10 minutes. The second supernatant was removed and combined with the first.

*Evaporation (Isolation) Protocol*

The combined supernatants were placed in different distilling spider flasks. The tub was set to 40°C, and the spider flask rotated at 20 rpm. The evaporation process began at approximately 307mBar for about 30 minutes, or until no more acetone was pulled off. The pressure was then decreased 20mBar every 5 minutes until reaching a final pressure of 100mBar. It was held there until no more acetone was being pulled off.

*A Trolox Equivalent Antioxidant Capacity (TEAC) assay*

This series of extraction steps is used to isolate phenolics, water based antioxidants from the lyophilized coffee. To analyze the antioxidant potential, a Trolox Equivalency Antioxidant Capacity (TEAC) analysis was performed on each sample. In this analysis, antioxidant potential was evaluated by comparing the effectiveness of each coffee sample in slowing oxidative reactions to that of the measured effects of Trolox, a known powerful antioxidant. The results of this assessment are described as a measure of "Trolox Equivalency". This test included DPPH as a radical standard and we have assessed the sample's capacity to neutralize the radical compared to a standard curve of Trolox. The loss of absorbance at 517 nm was measured in a microplate reader after 30 minutes of incubation at 27°C.

The first step in this assessment was creating a 99.7/0.3 water/ formic acid solution. This was done by adding 15 µL of formic acid to approximately 4mL of water. This solution was then diluted with water to 5mL. The next step in this assessment was sample dissolution. For each sample, the product from the previous extraction/evaporation step was combined with 0.3 mL of the 99.7/0.3 water/formic acid solution. The samples were diluted with the water/formic acid solution to a volume of 3mL. Next was preparing the DPPH solution (101.4405MM). This 101.4405 µM DPPH solution was made by mixing 1mg of DPPH in 25 mL of an 80/20 methanol/water solution then sonicated for 4 minutes. Finally the diluted Trolox solution was made by preparing 34mL of a 1:1 ratio of acetone to water solution. To prepare a 1.5 mM Trolox solution, 6mg of Trolox was added to 16mL of the 1:1 acetone/water solution. A series of Trolox solutions were made by diluting the previously described 1.5mM Trolox solution with the 1:1 acetone/water solution. To make the 0mM Trolox solution, 0mL of 1.5 mM Trolox solution was mixed with 5 mL of the 1:1 acetone water solution. To make the 0.3 mM Trolox solution, 1mL of 1.5 mM Trolox solution was mixed with 4 mL of the 1:1 acetone water solution. To make the 0.6 mM Trolox solution, 2mL of 1.5 mM Trolox solution was mixed with 3 mL of the 1:1 acetone water solution. To make the 0.9 mM Trolox solution, 3mL of 1.5 mM Trolox solution was mixed with 2 mL of the 1:1 acetone water solution. To make the 1.2 mM Trolox solution, 4 mL of 1.5 mM Trolox solution was mixed with 1 mL of the 1:1 acetone water solution.

*Microplate Analysis*

A VersaMax was used for the Microplate Analysis in this study. Absorbance was set to 517 nm and was set for a Kinetic measurement of 30 minutes at 27℃. In a microplate plate, the following combinations were prepared for assessment: sample blank was made by mixing 10 µL 99.7/0.3 water/formic acid solution with 290µL DPPH (101.4405µM), 0mM Trolox was made by mixing 10 µL 0mM Trolox Solution with 290µL DPPH (101.4405µM), 0.3 mM Trolox was made by mixing 10 µL 0.3 mM Trolox Solution with 290µL DPPH (101.4405µM), 0.6 mM Trolox was made by mixing 10 µL 0.6 mM Trolox Solution with 290µL DPPH (101.4405µM), 1.2 mM Trolox was made by mixing 10 µL 1.2 mM Trolox Solution with 290µL DPPH (101.4405µM), 1.5 mM Trolox was made by mixing 10 µL 1.5 mM Trolox Solution with 290µL DPPH (101.4405µM). The coffee samples were diluted to 20%, 10%, 5%, and 1% for assessment.

*Statistical Analysis*

Multivariable modeling was performed to quantify the effects of the processing factors on each of the assessment outcomes.

## 3.4 Results and Discussions

*TEAC Results*

TEAC results of all samples are shown in Figure 1. 0.65mm x 24 h, 1.15 mm x 18 h, 1.15 mm x 24 h, 1.65 mm x 18 h, and 1.65 mm x 24 h showed significantly lower antioxidant potential than 0.65mm x 18 h, 0.65mm x 30 h, 1.15mm x 30 h, and 1.65mm x 30h. 0.65mm x 30 h showed significantly higher antioxidant potential than 0.65mm x 18h, but was not significantly different than 1.15mm x 30 h and 1.65mm x 30h. 30 hour brew time showed the highest antioxidant potential. 18 hours showed the second highest antioxidant potential, followed by 24 hours showing the lowest antioxidant potential consistently.

*Grind Size*

Table 2 shows TEAC results of Los Santos by grind size. It was found that 0.65mm was significantly higher than 1.15mm and 1.65mm ($p<.001$ and $p<.001$). 1.15mm was not significantly different than 1.65mm ($p=.440$). Greater dissolution is expected with a finer grind size which explains the observed greater antioxidant potential in samples with a finer grind size. 1.15mm was not significantly better than 1.65mm which suggests to achieve greater antioxidant potential, there might a grind size threshold to observe the effect.

*Brew Time*

Table 3 shows TEAC results of Los Santos by brew time. It was found that each of the samples are significantly different from each other (in all cases $p<.001$). 24 hours had the lowest antioxidant potential of 0.81. 18 hours had an antioxidant potential of 1.4. 30 hours had the highest antioxidant potential of 2.86. The data suggests that compounds are dissolving between the hours of 18 and 24 to mitigate antioxidant potential. This corresponds to Manuscript I of this thesis, where flavor and desirability qualities are also lowest at 24 hrs.

Further research to identify the dissolved solids occurring between 18 and 24 hours would provide a more complete look at what is happening to mitigate some of the antioxidant potential. The relationship between our proposed antioxidant assessment method (*in vitro* colorimetric measures of antioxidant potential) and true *in vivo* health outcomes is not well understood. Further research is needed to see if these results can be replicated in vivo.

### *Predictive Modeling of TEAC Results*

Linear regression models were made to predict antioxidant potential according to grind size, brew time and time from 24 hours and are shown in Table 4. Grind size was a negative predictor, meaning for every mm larger the grind size, the TEAC output is predicted to decrease by .649. Time was a positive predictor; for every hour of brew time longer within the ranged assessed, the TEAC output is predicted to increase by .122. Time from 24 hours provided the best predictive linear model showing every hour away from 24 hours in either direction, within the range assessed, the TEAC output is predicted to increase .220. Each of the three factors were significant ($p=.001$, $p<.001$ and $p<.001$ respectively). The model was quite predictive, with a correlation coefficient of $R^2_{adj} = .864$. Figure 2 shows TEAC results with predictive outputs of the model.

Not being 24 hours in the most important factor when predicting antioxidant potential. Our research suggests that using a smaller grind size and brewing the coffee for longer will result in higher antioxidant potential. This data is consistent with the results in Manuscript I, where sampled brewed for 24 hours had the lowest scores.

### 3.5 Conclusion

This data found that samples brewed at 30 hours had significantly higher antioxidant potential than samples brewed at 18 hours, which in turn had significantly higher antioxidant potential than those brewed for 24 hours. It was also found that 0.65mm demonstrated higher antioxidant potential than 1.15mm and 1.65mm. Comparison of individual samples determined that the three samples brewed at 30 hours (0.65mm, 1.15mm, 1.65mm) showed the highest antioxidant potentials, and were not significantly different from each other. For industry members, it is recommended to avoid a brew time of 24 hours when considering antioxidant potential. If brewing cold brew coffee for 18 hours, a smaller grind size (0.65mm) would yield significantly higher antioxidant potential than coarser grind sizes (1.15mm and 1.65mm). Further research into the compounds that are dissolving between the hours of 18 and 24 would be warranted to identify what is mitigating the antioxidant potential.

## 3.6 Tables and Figures

**Table 1: Sample Preparation**

| Los Santos | | Processing Time (hours) | | |
|---|---|---|---|---|
| | | **18** | **24** | **30** |
| **Grind Size (mm)** | **0.65** | Sample 1 | Sample 2 | Sample 3 |
| | **1.15** | Sample 4 | Sample 5 | Sample 6 |
| | **1.65** | Sample 7 | Sample 8 | Sample 9 |

**Table 2: TEAC Results of Los Santos by Grind Size**

| Grind Size (mm) | TEAC (mMol Equivalence / 100g wet sample) |
|---|---|
| 0.65 | 2.17B |
| 1.15 | 1.39A |
| 1.65 | 1.52A |

Samples in columns without the same letter are significantly different ($\alpha = 0.05$). Significance determined with 2-way ANOVA with a consideration of grind size (n=3) and brew time (n=3). Samples were assessed in triplicate.

**Table 3: TEAC Results of Los Santos by Brew Time**

| Brew Time (h) | TEAC (mMol Equivalence / 100g wet sample) |
|---|---|
| 18 | 1.40B |
| 24 | 0.81A |
| 30 | 2.86C |

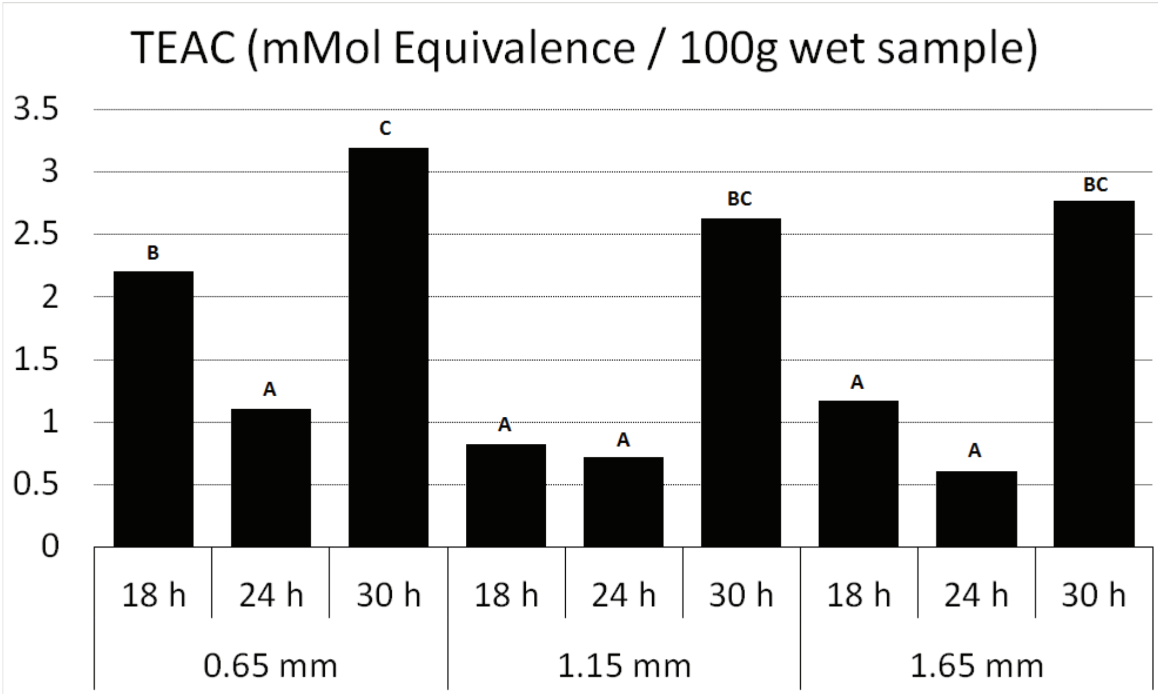
Samples in columns without the same letter are significantly different ($\alpha = 0.05$). Significance determined with 2-way ANOVA with a consideration of grind size (n=3) and brew time (n=3). Samples were assessed in triplicate.

**Table 4–Predictive modeling of TEAC results**

| | Grind Size (mm) | Time (h) | Time from 24 Hours (h) | $R^2$ |
|---|---|---|---|---|
| **TEAC** | -.649 | .122 | .220 | .864 |

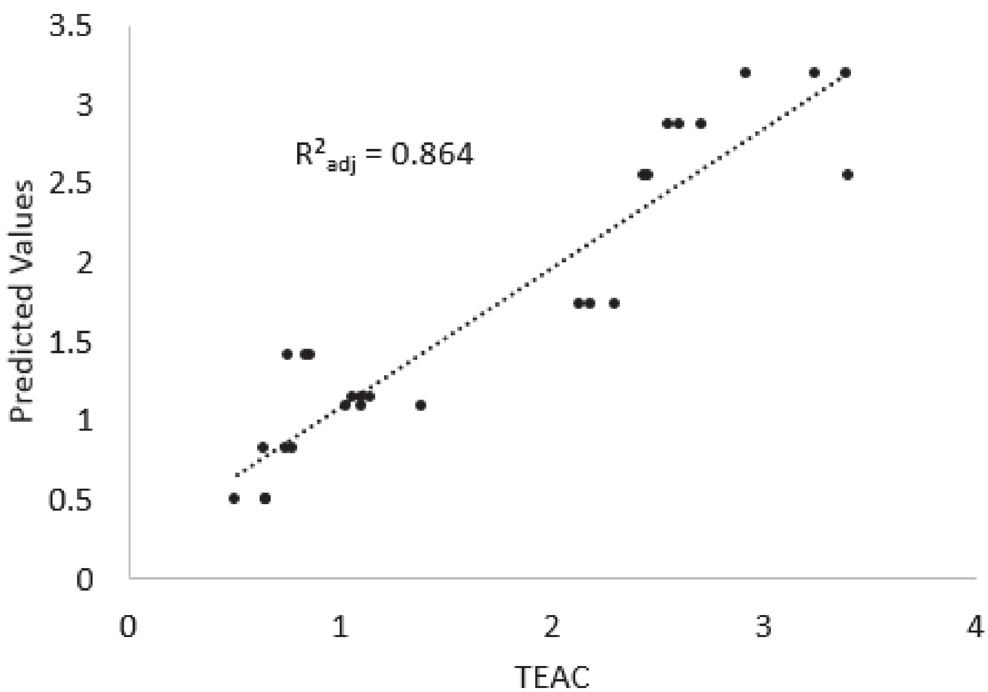
Linear regression models made with SPSS software ($\alpha = 0.05$).

**Figure 1: Samples Ordered by TEAC Output of Los Santos**




Samples in columns without the same letter are significantly different ($\alpha = 0.05$). Significance determined with 2-way ANOVA with a consideration of grind size (n=3) and brew time (n=3). Samples were assessed in triplicate.

**Figure 2: Comparison of TEAC Results with Outputs of Predictive Model**



# CHAPTER 4: CONCLUSION

This data showed that cold brew coffee brewed for 18 or 30 hours had higher magnitudes of descriptive traits, higher acceptability and antioxidant potential than coffee brewed for 24 hours. For consumer assessment, grind size was not a significant factor. Although not significant, the 1.65mm sample brewed for 30 hours had the highest overall consumer likability. For antioxidants, finer grind sizes resulted in higher antioxidant potential, although this effect was not significant for samples brewed at 30 hours. Further research into the compounds that are dissolving between the hours of 18 and 24 would be warranted to identify what is mitigating flavor attributes and perceived quality as well as the antioxidant potential. To produce cold brew coffee considering its overall flavor, likability and antioxidant potential, it is recommended to brew cold brew coffee for 30 hours with a coarser grind size (1.65mm).

## CHAPTER 5: REFERENCES

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