An Analysis of the Acid Profile of Coffee Brews: Caffeine and Chlorogenic Acid Concentrations in Different Forms of Coffee Brew

Jeffory Taylor Wallace
University of Mississippi. Sally McDonnell Barksdale Honors College

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An Analysis of the Acid Profile of Coffee Brews: Caffeine and Chlorogenic Acid Concentrations in Different Forms of Coffee Brew

By Jeffory Taylor Wallace

A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

Oxford April 2017

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Finally, to everyone who has been apart of my undergraduate studies in some way, thank you for your comradeship and support. I will look back fondly on the past four years, and I have the people of this great University to thank for that.
ABSTRACT
An Analysis of Acid Profile of Coffee Brews: Caffeine and Chlorogenic Acid Concentrations in Different Forms of Coffee Brew

In this project, different coffee brews were analyzed in order to determine the effects the brewing method had on the final product, particularly the acid profile of the final product. Our hypothesis is that the use of a cold brewing method will produce different amounts of caffeine and chlorogenic acids in the final brewed product compared to traditional hot brewing methods. There are many brewing methods available. For the purposes of this research, three were chosen and one was created. The three chosen methods were: a traditional drip brew, a cold brew, and a Pezzetti espresso brew. The final method was a pour over method and was adapted for the lab. It involved pouring hot water through coffee grounds. High-performance liquid chromatography was the method of choice used to test the amounts of caffeine and chlorogenic acids in each brew. Two separate methods were used, each adapted from their respective DIN (German Institute for Standardization) method.\textsuperscript{1,2} Caffeine and chlorogenic acid solutions were used to create a standard curve for concentrations of both, which in turn was used to determine the concentration of caffeine and chlorogenic acids in the coffee samples. Finally, it was determined that while regular drip and pour over brewing methods produced the most caffeine of the four, the Pezzetti espresso method produced the least. For chlorogenic acids, the cold brewing method produced the highest concentration, while the Pezzetti brew produced the smallest.
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<td>--------------</td>
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Introduction

Coffee may be one the most highly consumed beverages in the world. Most use it as a stimulant in the mornings, others drink it for the taste, and some drink it to cleanse the palate after a meal. Coffee is an interesting beverage because of how widely consumed it is. Across all countries and races, across all age groups, coffee is one beverage that is consumed almost as much as water. Some historians have even credited coffee as the fuel of the Industrial Revolution. The consumption of coffee as a drink dates back to the 15th century. Evidence has been found in scriptures and other historical documents claiming it was drank by the monks in their temples. Interestingly, it was said that the beverage allowed monks to stay up very late into the night translating scriptures. Other stories talk of wanderers coming across coffee trees in the wild and using the berries as a source of energy. Later these people would bring the berries back and make different drinks and foods out of them. After its discovery, coffee was first produced and traded in Yemen in the 15th century. By the 17th century, coffee had made its way to Europe, and from there, onto the Americas.

Coffee, as it is consumed today, is produced from coffee beans. Coffee beans are the seeds of a fruit known as coffee cherries, which are grown on coffee trees. Coffee plants are members of the genus *Coffea*. There are several plants of the genus that produce coffee berries and in turn coffee beans, but only two are commercially
cultivated. Those are species Robusta and Arabica.\textsuperscript{5,6} \textit{C. arabica} is known to be more refined whereas \textit{C. robusta} is a heartier species, typically containing more caffeine but producing lower quality coffee. Arabica coffee makes up the majority of the world coffee, but can be much more expensive than Robusta. Arabica coffee must be grown in mild temperatures, and typically on steep terrains. Arabica coffee is also more partial to diseases than Robusta. The fickle nature of Arabica coffee combined with the often times challenging harvesting conditions leads to the higher prices.\textsuperscript{5,6} Arabica coffee is typically grown in Latin America, Africa in particularly Ethiopia where it is native to, and several Asian countries. Robusta plants are heartier than their relative. They can be grown at lower altitudes, a wider range of temperatures, and are more resistant to pests. Robusta contains more caffeine than Arabica as well. Robusta coffee isn’t as highly produced as Arabica coffee, and often times is used as a substitute for the more refined Arabica coffee.\textsuperscript{5,6}

Once the coffee cherries are harvested, there are still several steps that must be taken to process them before they reach their final form that can be brewed into the beverage which we consume. This process involves drying the cherries and then milling them so that only the bean is left.

First, the cherries are picked. This can be done by hand or machine. The next step is to dry the cherries. This can either be done immediately following the harvest, or sometimes a method is used in which some of the skin and the pulp are removed from the cherries prior to drying.\textsuperscript{7} After the drying process, the cherries are then milled, polished, and sorted. In the milling/ polishing process, the husk of the cherry is removed and the cherries are now referred to as beans.\textsuperscript{7} The beans are sorted by weight and size and then
prepared for export. At this stage the coffee beans are known as green coffee. All that is left is to roast the beans so that they become the brown aromatic coffee beans that can then be ground up and transformed into a coffee brew.\(^7\)

Once the green coffee reaches its destination, it is roasted prior to brewing. The roasting usually occurs at about 550 degrees Fahrenheit, or 288 degrees Celsius. After the beans are roasted, they can then be ground up and brewed.\(^7\) Coffee beans are sold either whole or already ground, depending on the consumers’ desires. The final step is to get the coffee brewed.

Brewing coffee essentially involves combining the roasted and ground coffee beans and water. By doing so, the soluble parts of the bean are extracted into the water to make the coffee.\(^8\) Essentially, with respect to coffee, brewing is synonymous with extracting. In a coffee bean, soluble components make up ~28 % of the overall mass.\(^8\) While it is most common to use hot water in the brew, newer methods involve using cold or room temperature water.

Before the water can be added, the coffee beans must be ground up. This increases the surface area of the beans, allowing for quicker extraction of the soluble chemicals in the beans. If one were to combine whole beans with water, the brewing process would be much slower. So by increasing the surface area of the beans, the brewing time is significantly shortened. This is good for several reasons, namely that time is a valuable resource and that the longer the water is in contact with the beans, the more bitter components will be extracted into the brew as well.\(^8\) Optimal grind sizes depend on the method of brewing as well as the flavor that is desired. The same goes for
the amount of time the beans are in contact with the water as well as the temperature of the water.

The final step is to filter out the insoluble parts of the coffee brew. Approximately 70% of a coffee bean is insoluble. This portion is mostly made up of cellulose. The filtering is usually carried out in conjunction with the extraction process by coffee filters. While the filtration sorts the insoluble compounds out of the brew, it will inadvertently filter out some desired compounds as well. Thus, the mass of extracted material dissolved in solution can affect the overall quality of the brew, and can be varied in different brews. For example, in a small volume espresso brew there are almost twice the concentration of soluble compounds in solution as compared to a standard drip brewer. Different brewing methods have the ability to create different tasting cups of coffee because of changes in the temperature of the water used, the amount of time the water and grinds are in contact, and the amount of filtration used in the extraction/brewing process. An article at coffeechemistry.com details recommended levels.

For the tests performed here, several different brewing methods were used. The first three methods involved hot brewing the coffee, while the last method was a cold brewing technique. For each method, the same amount of coffee was used with equal amounts of water to make a coffee brew equal in strength if not slightly stronger than the average brew one would make at home. The first of the hot brewing methods was a standard drip brew. The next brewing method was adapted to the lab from a basic pour over method for the purpose of this research and utilized basic lab equipment and an understanding of how coffee is made. The third hot brew method was an espresso brew. The fourth brew method was a cold brew coffee. Cold brewing
coffee is a relatively new technique that involves soaking coffee grinds in water at room temperature or sub-room temperature for a time (varies from one day to several) and then filtering out the grinds to have a final coffee product. Finally, an instant coffee brew was made, in order to have a standard brewing method which would be equivalent to something that would be created exactly as it was at home.

High-Performance Liquid Chromatography (HPLC) is one of the most effective and commonly used chromatography techniques. Chromatography, in general, is a separation technique in which a mixture is separated into individual components through different interactions with a stationary phase. There are many different chromatography techniques used in labs today, with HPLC being one of the most common and easy to automate. In HPLC, the sample of interest is pushed through a chromatographic column under high pressure. The sample is carried through the column by a mobile phase and the column is packed with a stationary phase that separates the components of the sample.

Modern HPLC columns are packed with silica beads of diameters ~ 3.5- 5 µm. The extremely tight packing of the column requires a high pressure in order to achieve the desired flow rate of the mobile phase. This large surface area of the stationary phase allows for a high resolution in a smaller volume. The two main components at work are the mobile and stationary phases. The solvent that moves through the system is known as the mobile phase and the material in the column is known as the stationary phase. The sample partitions into both the stationary phase and mobile phase through various physical interactions, and thus elutes from the column at different rates. The interaction between sample components and mobile and stationary phases can be due to
Typically, the mobile phase introduces the sample to the stationary phase. Components of the sample then interact with the stationary phase for differing amounts of time until they are eluted through by the mobile phase. Here, and most often, these interactions stem from the polarity of the molecules and mobile and stationary phases. One of the most basic chemical concepts is utilized here, that molecules of similar polarity attract each other. Thus, polar molecules will interact with a polar column longer than nonpolar molecules will, causing different elution rates.

The use of a nonpolar stationary phase and polar mobile phase is known as reverse phase chromatography (RPC). In reverse phase chromatography, the nonpolar molecules in the sample will have a high affinity for the stationary phase and thus will take longer to elute. Typically, the stationary phase for RPC is a column packed with C-18 coated silica beads. The mobile phase is often a combination of a polar solvent (usually water) and a relatively polar organic solvent (such as methanol or acetonitrile). The mobile phase can be run with a changing concentration gradient over time, or an isocratic mixture (constant concentration) can be used.

In RPC with a gradient mobile phase, the sample is washed onto the column starting with a mobile phase that is very polar. The nonpolar molecules in the sample will bind to the nonpolar column, while the more polar molecules will elute faster through the column with the mobile phase. Over the course of the experiment, the concentration of the mobile phase changes to contain more and more of the less-polar organic solvent, and the nonpolar molecules begin to partition more from the stationary phase into the mobile
phase, thus eluting from the column. This is the basis of how the desired separation is created in HPLC.

The eluting compounds are measured by the detector, typically based on absorbance or fluorescence of the eluting compounds, as the compounds leave the column. The detection process is described below. The amount of time each compound takes to elute is the retention time for that compound. The retention time for a compound is a function of the strength of the interactions of the compound with the mobile and stationary phases. Because these interactions are in turn products of the physical nature of the molecule, the retention time for a given molecule will remain constant if the mobile and stationary phases remain the same. By comparing the retention times of known compounds to those found from the sample, the identities of each component in the sample can be determined. Thus, we can prepare standard solutions of the molecule of interest, and measure the retention time of standards to compare with experimental mixtures.

High-performance liquid chromatography can be further paired with other techniques to determine even more information about the sample. One such technique that was applied here is UV-Vis spectroscopy. Spectroscopy involves the interaction of electromagnetic radiation (light) and matter. In this case, molecules absorb some of the radiation that they are exposed to. This occurs when the energy of the radiation is similar to the electronic energy levels of the molecule. These levels are a function of the molecular structure and atoms that make the molecule up. Thus different molecules absorb radiation of different amounts of energy. By knowing what wavelength of light a molecule will absorb, we can set the UV-Vis spectrometer light source to this wavelength.
and see if any absorption occurs. The UV-Vis spectrometer will produce a signal when the radiation is absorbed that is a function of the concentration of the molecule being analyzed. The signal is quantified as the absorption of the molecule at the given wavelength. The relationship between the concentration and absorption is given in the Beer-Lambert law.¹⁴ For the scope of this experiment, it is important to know that Absorbance and Concentration are directly proportional. In the experiment performed here, a spectrum was produced following an analysis. On the x-axis was retention time, and on the y-axis was the absorption signal. Experimentally obtained spectrums can be seen in figures 9-14.

By using data obtained from the DIN procedure, it is possible to replicate their analysis and know what time the molecule of interest elutes at. This can also be checked using a pure standard that contains only the molecule of interest. Once the retention time is determined, it is then possible to use the signal at that time to determine the concentration of the molecule of interest in the sample. This is done by comparing the signal of the unknown sample to a standards curve of the signals given from solutions that have known concentrations of the molecule of interest.

The two molecules that we analyzed were caffeine and chlorogenic acid. The structures of these two molecules can be seen in figure 1.
Figures 1. This figure shows the molecular structure of both chlorogenic acid and caffeine. Chlorogenic acid is the larger molecule on the left, caffeine is on the right.\textsuperscript{15,16}

Caffeine, known by its IUPAC name 1,3,7-trimethylpurine-2,6-dione,\textsuperscript{16} is perhaps the most important chemical found in coffee and the one that typically leads to the consumption of coffee. Caffeine functions as a central nervous system stimulator in the human body, thus working to keep one awake and energetic.\textsuperscript{16,17} Caffeine is a derivative of the purine base methylxanthine. Other important properties of caffeine are its sublimation point of 178 °C and its bitter taste upon consumption.\textsuperscript{16} Caffeine is relatively stable in solution and has a high temperature required for it to sublime, or a low vapor pressure. Chlorogenic acid (CGA) was the other chemical found in coffee that was studied here. While the chlorogenic acid in figure 1 is a specific compound, chlorogenic acids actually make up a range of isomeric compounds with similar physical properties and flavor profiles.\textsuperscript{18,15} CGAs are polyphenolic acids, usually the ester derived from quinic acid and caffeic acid.\textsuperscript{15} The IUPAC name is given as (1S,3R,4R,5R)-3-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-1,4,5-trihydroxycyclohexane-1-carboxylic acid, obviously depending on which isomer one is referring to.\textsuperscript{15} The difference in naming depends on the location of the ester bond on the quinic ring.\textsuperscript{15} CGAs are antioxidants, and are usually responsible for the bitter/ metallic taste that coffee brews often have.\textsuperscript{18} CGAs
typically melt around 205 °C. Details regarding the presence of both of these molecules can be found in the data obtained in this experiment.
Experimental

Brewing Equipment.

coffeeAM Brazilian Santos Coffee\textsuperscript{9}. The coffee used here was a dark roast, coffeeAM Brazilian Santos gourmet coffee. The coffee was ground in two sizes, finely ground and coarsely ground. The finely ground coffee was used for the drip brew method, pour over brew method, and cold brew method. The coarse ground coffee was used for the espresso brew method. For each brew, 8.999 ± 0.004 g of ground coffee was used.

Folgers Classic Roast Instant Coffee. The Folgers Classic Roast Instant Coffee crystals were used as a standard coffee brew, one that could be simply prepared by anyone.

BLACK & DECKER 12-cup Programmable Coffee Maker (model # DLX1050B)\textsuperscript{19} This BLACK & DECKER coffee maker was a simple drip brew coffee maker, and was used to make all drip brewed coffee samples in this experiment. A photo of the coffee maker used here is seen in figure 2 below.

Pezzetti Moka-Pot Stove Top Espresso Maker. The Pezzetti espresso maker was used to make all espresso brews used in this experiment. The Pezzetti used here is seen in figure 2 below.
**Figure 2.** The image on the left is the *Black and Decker* 12-cup Programmable Coffee Maker (model # DLX1050B) used to make the regular drip brewed coffee. On the right is the *Pezzetti* Espresso stove-top moka pot, used to make the pezzetti espresso brew.

*Filters:* Several different filters were necessary in the sample preparation. The coffee filters used here were generic *CVS* basket style coffee filters. Also used in the sample preparation were *Fisherbrand* qualitative P5 medium porosity filter papers. Finally, before the samples could be injected into the HPLC column, they were filtered through *Fisherbrand* PTFE 0.45 μm syringe filter tips.

**Chemicals, Materials, and Instrumentation.**

*Caffeine Standard:* A lab grade finely powdered caffeine standard was obtained in the lab and used to create the standard caffeine solutions.
**Chlorogenic Acid Standard:** The chlorogenic acid standard used here was Chlorogenic acid, 95% titration, obtained from Sigma-Aldrich. The product identification number was C3878 Aldrich.

**HPLC Instrument:** The HPLC system used for both caffeine and chlorogenic acid determination was an *Agilent Series 1100 HPLC System*. The entire HPLC system can be seen in figure 3 below. The column, also used for both methods, was an *Agilent ZORBAX Eclipse Plus C18* column, with a particle size of 3.5 µm, diameter of 4.6 mm, and a length of 150 mm.

![Figure 3. HPLC instrument used in the experiment](image)
Sample Preparation

*Caffeine Standards*: Standards were made to have concentrations 0.80 mg/ml, 0.40 mg/ml, 0.20 mg/ml, 0.10 mg/ml, and 0.05 mg/ml. This was done using a method of serial dilutions. First, a concentrated stock solution was prepared. Using a volumetric flask in order to insure high accuracy, 0.400 g of pure caffeine was place in a 50.00 mL flask to make the 0.80 mg/ml solution. The flask was then filled to the mark using distilled water. The flask was then placed on a hot plate and heated with occasionally stirring in order to make sure the caffeine was fully dissolved.

The 0.40 mg/ml solution was made next. To do this, 25.00 ml of the 0.80 mg/ml stock solution was placed in a 50.00 ml volumetric flask using a volumetric pipette, and the flask was then filled to the mark with distilled water. The new solution was placed on a hot plate again in order to ensure that the caffeine had fully dissolved. Next, the 0.20 mg/ml solution was created using the 0.40 mg/ml solution. The method was the same as previously mentioned; only this time a 25.00 ml volumetric flask was used because no other 50.00 ml ones remained. So, 12.50 ml of the 0.40 mg/ml solution was placed in a 25.00 ml volumetric flask using a volumetric pipette and then diluted to the mark using distilled water. The new solution was again placed on a hot plate to ensure that the solute had fully dissolved. This process was repeated twice more in order to create solutions of concentration 0.10 mg/ml and 0.05 mg/ml.

*Chlorogenic Acid Standards*: Five standard solutions were created ranging in concentration from 2.00 mg/ml to 0.125 mg/ml, using serial dilutions with volumetric flasks as described above.
Drip Brew Coffee Sample: The first brew was done using a standard drip coffee maker. The coffee maker itself was a BLACK & DECKER 12-Cup Black Programmable Coffee Maker, product # DLX1050B (Figure 2). Two CVS Pharmacy coffee filters were placed in the top of the coffee maker. Then ~ 9.000 g of finely ground Dark Brazilian Santo coffee was placed in the filter. Then 150.00 mL of distilled water was measured into a graduated cylinder and poured into the back of the coffee maker. The coffee maker was then allowed to run and the coffee was collected in the pot below. Using a medium porosity Fisherbrand filter and a glass funnel, the coffee was further filtered and collected in a beaker. The additional filtering helped to ensure no particulates would make it into the HPLC machine, leading to clogged column. Then, using a BD 3 mL syringe and Fisherbrand PTFE 0.45 µm syringe filter tip, ~ 2.00 mL of the sample was placed into a labeled HPLC vial and set in the vial holder.

Pour Over Brew Coffee Sample: The next brewing method was one that was created in the lab, adapted from pour over methods found online. Two CVS Pharmacy coffee filters were placed in a glass funnel, in to which ~ 9.000 g of Brazilian Santos finely ground coffee was placed. An indention was made in the middle of the coffee, in order to help ensure an even pour of water. Then 150.00 mL of distilled water was brought to a temperature between 90-95 degrees Fahrenheit [32-35 degrees Celsius] in a beaker. The water was then slowly poured into the funnel containing the filter and coffee. Care was taken to make sure the water level in the funnel never reached the top of the filter paper. The sample was collected in a beaker and then filtered again through a medium porosity Fisherbrand filter, to ensure no particles would clog up the HPLC
column. Approximately 2.00 mL of the filtered sample was placed into a labeled HPLC vial using a syringe and syringe tip filter.

*Espresso Brew Coffee Sample*\(^{20}\): The final hot brewing method was an espresso brew. For this method a *Pezzetti* Stove-top Moka Pot Espresso Maker (figure 2) was used. Again 9.000 g of coffee and 150.00 mL of distilled water were used. The coffee was still Dark *Brazilian Santos* but this time it was coarsely ground, so that the espresso filter would not become clogged.\(^{20}\) The water was placed in the bottom of the pot and the coffee was placed above the metal filter. The pot was then placed on a hot plate on high and checked periodically until the coffee was made. Once the coffee was done, the heat was removed and the pot was allowed to cool. Then, as with the other brews, a medium *Fisherbrand* filter was used to further filter the brew. The final product was placed in an HPLC vial for testing, using a syringe with a filter tip as before.

*Cold Brew Coffee*\(^{11}\): The fourth brew method was a cold brew. For this method, 9.000 g of finely ground Dark *Brazilian Santos* coffee and 150.00 mL of distilled water were combined in an airtight jar and allowed to sit overnight in a refrigerator.\(^{11}\) Once this was done, the mixture was double filtered as with the other brews, first through a double coffee filter, and then through a medium *Fisherbrand* filter. The final filtrate was placed in a labeled HPLC vial, again using a syringe and syringe filter tip. After the coffee was brewed, the two tests were ready to be run.

*Instant Coffee Brew*: The final brew was an instant coffee. This brew was made using *Folgers Classic Roast Instant Coffee*, freeze dried chips that just needed water. The directions given on the back of the container were followed exactly in order to make this brew, in order to have a standard which would be prepared in the lab exactly as it would
have been prepared at home. For this method, 1 rounded teaspoon was combined with 1 cup of hot (not boiling) water. The chips were dissolved. The final brew was filtered through a syringe filter tip, and placed in an HPLC vial to be tested.

**Analytical Methods**

*Caffeine Determination:* The following method used for caffeine analysis was adapted from the DIN 20481\(^1\), one of many quality control tests for coffee products. Before the caffeine test could be run, the instrumental parameters had to be programmed into the computer. The solvents used were methanol and water, both of HPLC grade. The mobile phase was isocratic, 25% methanol and 75% water. The flow rate was set at 1.00 mL/minute. The injection volume of the sample was 10 \(\mu\)L, and a needle wash was done with methanol. The solvent timetable for caffeine is seen below. The stop time was set to stop at 15.20 minutes. The analyte was detected with an absorbance detector set at 272 nm with a bandwidth of 4 nm, the reference was set at 360 nm with a bandwidth of 16 nm.

After the method was set up, the caffeine standards were run first in order to create a standards curve. The labeled vials containing the standards were loaded into the HPLC instrument and the sample table was filled out. The method was then run and the results saved. Using the offline software mode, the results were checked to make sure they appeared correct, and then they were downloaded onto a flash drive and transferred to a computer with Microsoft Excel. Using Excel and the data, a standard curve was created which showed the area of each peak as a function of the concentration. This curve
is seen in figure 4. The retention time was also noted for each peak so that the correct peak could be determined from a coffee sample.

Next each coffee sample was run. By using the concentration curve (figure 4), the amount of caffeine in each sample could be calculated, and the differences could then be analyzed.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Solvent A (Water)</th>
<th>Solvent B (Methanol)</th>
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<tbody>
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<tr>
<td>15.20</td>
<td>75%</td>
<td>25%</td>
<td>Equilibrate Column</td>
</tr>
</tbody>
</table>

Table 1. Timetable for mobile phases in caffeine test. The method was isocratic.

![Caffeine Calibration Curve](image)

**Figure 4.** Calibration Curve used for determination of caffeine.
Chlorogenic Acid Determination: The test for chlorogenic acids was very similar to the one used for caffeine and was adapted from the DIN 10767\textsuperscript{2}, another of many quality control tests for coffee products. Similar to the caffeine test, the method information had to be entered in first. There were several differences in the two methods, starting with the mobile phases. Solvent A was a solution of water + 1\% phosphoric acid. This was made by combining 300.00 ml of HPLC grade water with 3.00 ml of phosphoric acid. Solvent B was Acetonitrile. The mobile phase timetable for the CGA test is seen in table 2 below. A flow rate of 1.00 ml/minute was used again. The injection volume was 10 µl and the needle wash was done with acetonitrile. The stop time was set to stop at 23.20 minutes, or as the pump stopped. The analyte was detected using an absorbance detector at 324 nm with a bandwidth of 8 nm and no reference was used.

Five standard solutions were created ranging in concentration from 2.000 mg/ml to 0.125 mg/ml, using serial dilutions with volumetric flasks as described above. These were analyzed as noted above. The calibration curve created from these solutions can be seen in figure 5 below. Next the four coffee samples were made and analyzed as noted above. The data for the samples was collected similarly to that of the caffeine method. All data was analyzed using Microsoft Excel.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Solvent A (H\textsubscript{2}O + 1% H\textsubscript{3}PO\textsubscript{4})</th>
<th>Solvent B (Acetonitrile)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>90%</td>
<td>10%</td>
<td>Sample Run</td>
</tr>
<tr>
<td>20.00</td>
<td>80%</td>
<td>20%</td>
<td>Sample Run</td>
</tr>
<tr>
<td>20.10</td>
<td>10%</td>
<td>90%</td>
<td>Column Wash</td>
</tr>
<tr>
<td>24.10</td>
<td>10%</td>
<td>90%</td>
<td>Column Wash</td>
</tr>
<tr>
<td>24.20</td>
<td>90%</td>
<td>10%</td>
<td>Equilibrate Column</td>
</tr>
<tr>
<td>25.20</td>
<td>90%</td>
<td>10%</td>
<td>Equilibrate Column</td>
</tr>
</tbody>
</table>

**Table 2.** Mobile phase timetable for the chlorogenic acid test.
Figure 5. Calibration curve used for the determination of Chlorogenic acids.

**Instant Coffee Analysis**: After the above work had been completed, the instant coffee sample was analyzed as well, according to the same methods as noted above. The results are detailed below.
Results/ Discussion

By analyzing each brew using the HPLC methods described above, it was possible to draw conclusions with regards to the caffeine and chlorogenic acid content of each brew type. Table 3 gives the averages and standard deviations for the concentration of caffeine and chlorogenic acids in each brew. The charts seen in figures 6 and 7 help to illustrate these results as well.

<table>
<thead>
<tr>
<th>Brew Type</th>
<th>Caffeine Concentration (mg/ml)</th>
<th>Chlorogenic Acid Concentration (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>Regular</td>
<td>0.809 ± 0.008</td>
<td></td>
</tr>
<tr>
<td>Pour Over</td>
<td>0.818 ± 0.017</td>
<td></td>
</tr>
<tr>
<td>Cold</td>
<td>0.775 ± 0.009</td>
<td></td>
</tr>
<tr>
<td>Pezzetti</td>
<td>0.675 ± 0.031</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. This table indicates the average and standard deviations from the mean for the concentration of caffeine and chlorogenic acids in each brew. Each brew was analyzed three times.

In the analysis of caffeine content, it was found that the regular and pour-over brews had relatively the same amount of caffeine. While the pour-over brew may have had slightly more, the amounts were very close. The large standard deviation associated with the caffeine concentration in the pour-over brew shows that this result may be less accurate than the average value predicts, and in fact may be much closer to the value obtained for the concentration found in a regular, drip brew. The cold brew method
produced the next highest amount of caffeine, followed by the Pezzetti brew producing the least. The fact that the Pezzetti espresso brew produced the least amount of caffeine was of particular interest, and will be addressed momentarily.

**Figure 6.** Analysis of caffeine content in each type of brew. The analysis was done three times, using new brews each time.

In the analysis of chlorogenic acid content found in the four coffee brews, it was found that the cold brew method had the highest concentration of CGAs, followed by the pour-over and regular brews, with the Pezzetti espresso brew again having the lowest concentration. These results can be visualized in figure 7. Again, the concentrations were extremely close for both the regular and mad scientist brews. One of the concentration values drives the average up for the pour over brew, but by looking at figure 7, it is clear
this value is an outlier. Again the Pezzetti brew had the smallest concentration of the four.

Figure 7. This figure shows the results of the analysis of the chlorogenic acid content of each brew. Each brew was analyzed three times.

The fact that the Pezzetti espresso brew has the lowest concentration of both caffeine and chlorogenic acids is strange. This seems to go against much of what is believed about coffee brewed in the espresso form. Typically, espresso coffee is known to be much stronger than other brewing types. In fact this strength, as does the strength of all coffee brews, typically stems from the concentration of soluble coffee compounds found in solution. In a regular brew, the ratio of water to soluble compounds is around
99% water to 1% soluble compounds. In espresso brew, the ratio is more like 98% water to 2% solubles. In addition, it is estimated that espresso coffee has \( \sim 2.5 \times \) more caffeine per ounce of water than does drip brewed coffee. The reason that the Pezzetti espresso brew contained the least amount of both caffeine and chlorogenic acids may result from the additional filtering done in the preparation of that brew. Because the HPLC column is susceptible to clogging from particles in the samples run through it, extra care must be used in order to ensure that solids do not make it into the column. Thus, it appears the extra filtering reduces the amount of caffeine that is characteristic of an espresso brew. The effects of the extra filtering can be seen in the photos of a Fisherbrand medium porosity filter that was used to filter the espresso brew, compared to one that was used to filter a regular brew, figure 8. The residue is clearly much thicker on the filter used to filter the espresso brew.

It appears that the most important aspect governing the brew strength is actually the size of the grind. As mentioned above, increasing the surface area of the coffee grinds by using a finer grind allows for a more thorough extraction. When learning how to use the Pezzetti moka pot, a source claimed that the best grind to use was a coarse grind because finer ground coffee could clog the Pezzetti. Thus for the analysis performed here, course ground coffee was used. Upon further research, it was determined that the preferred grind to use with espresso brewing methods is a fine grind, typically finer even than drip brew methods. An analysis was done using the same sample and caffeine determination method as all other samples, but this time finely ground coffee was used. It was found that the finer ground espresso brew had almost twice as much caffeine as the
other brews. Thus, it appears that the grind size, and thus the amount of extraction determines the amount of caffeine and other constituents in solution.

**Figure 8.** This figure shows a comparison of two medium porosity *Fisherbrand* filters, one used to filter the Pezzetti espresso brew (left), the other used to filter the regular drip brew (right).
Finally, the values obtained for each compound concentration in instant coffee are found in table 4. The values depicted here function as standard, controlled brew values. Instant coffee was prepared here just as depicted on the back of the container, as it would be prepared at home. The values obtained were very close to the values of caffeine and chlorogenic acids obtained from the other brewing methods, thus providing validity to the results obtained for those brews.

<table>
<thead>
<tr>
<th>Brew</th>
<th>Caffeine Concentration</th>
<th>Chlorogenic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instant Coffee</td>
<td>0.775 mg/mL</td>
<td>0.247 mg/mL</td>
</tr>
</tbody>
</table>

*Table 4. Caffeine and Chlorogenic acid found in instant coffee.*

The HPLC methods adapted from the DIN Standards institute worked really well here. Photos have been added of the chromatograms obtained for one of the standards for each molecule, as well as a spectrum for the regular brew under each analysis, and finally spectrums obtained from the analysis of instant coffee. One of the first things to notice is that the peaks do not match up exactly in time between the standards and the samples. This does not appear to affect the interpretation of the results though, as the peak can still be determined in the sample spectrum. Of note in the spectrum obtained from chlorogenic acid analysis of the drip brewed coffee are the two large peaks, one before and one immediately following the chlorogenic acid peak. If not for clear distinction given in the DIN method for chlorogenic acid determination, it would be easy to mistake one of these peaks for the one corresponding to chlorogenic acid. Instead, these are different forms of the acid, neo-chlorogenic acid and crypto-chlorogenic acid, respectively.²
Figure 9. Spectrum obtained following HPLC separation of standard 0.400 mg/ml caffeine. The peak at ~ 7.9 minutes corresponds to caffeine.

Figure 10. Spectrum obtained following HPLC separation of regular drip brewed coffee. The peak corresponding to caffeine can be seen at ~ 7.8 minutes.

Figure 11. Spectrum obtained following HPLC separation of 0.125 mg/ml CGA. The peak at ~ 7.3 minutes corresponds to the chlorogenic acid peak.

Figure 12. Spectrum obtained following HPLC separation of regular drip brewed coffee. The peak corresponding to chlorogenic acid can be seen at ~ 7.0 minutes.
Figure 13. Spectrum obtained following HPLC separation of instant coffee. The peak corresponding to caffeine can be seen at ~6.5 minutes.

Figure 14. Spectrum obtained following HPLC separation instant coffee. The peak corresponding to Chlorogenic Acid can be seen at ~7.1 minutes.
Conclusions

Today, even more so than oil, coffee may be considered the fuel that keeps the world running. This is why many studies have been done regarding the beverage, and why many more studies are to come. It is important that we understand the chemistry behind coffee because of the vastness of its consumption. Here, the caffeine content and chlorogenic acid content was analyzed among four different brews of coffee. Of particular interest was the content of both molecules in coffee brewed by a regular hot brewing methods compared to a cold brewed method. It was found that the cold brew produced less caffeine, yet had a higher concentration of chlorogenic acid. Since both of these molecules contribute a bitter taste to the final brew, and work in opposition to each other here, it is hard to comment on the effect of these differing concentrations on the final taste of the brewed product. It is possible that other molecules found in coffee brews could contribute to the overall taste profile as well. While this work provides insight on the effects the four brewing methods have on their respective brews, it is important to understand that there is still so much research that can be done on coffee. Because of the importance of this beverage in modern society, it becomes all the more important that we understand everything possible about that which we consume so heavily. I hope that while this work answers questions about different brewing techniques, it also leads to more questions as well.
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