DEVELOPING CHLOROGENIC ACID AS A COFFEE METRIC

by

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A THESIS

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Chlorogenic acids are an abundant family of organic compounds in coffee that have been associated with various aspects of coffee flavor, including sweetness, sourness, and astringency. When coffee is roasted, CGAs decompose into various non-volatile products including quinic and caffeic acids, suggesting that CGA content can be an indirect measure of the roast process. Our hypothesis is that electrochemical detection of chlorogenic acid in brewed coffee can be developed as a rapid compositional measurement. CGAs are phenolic, meaning they are readily oxidized and can be detected using electrochemical methods. We are exploring the use of voltammetry (applying voltage and measuring current) to directly assess the concentration of CGAs in coffee extracts obtained from the roasting and brewing processes. This presents our efforts to measure CGAs in coffee samples using electrochemical methods with the intention of correlating roast profile and potentially coffee quality with the trackable aspect of chlorogenic acid content in a brewed cup.

Acknowledgements

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Introduction

Coffee is remarkably chemically complex, though the specialty coffee industry has not put significant effort into compositional fluency as staple of quality control. A more thorough understanding of the behavior of common coffee compounds found in the brew can empirically evaluate dogmatic coffee industry practice. With over 1,000 reported organic compounds found in coffee, it is a contender for one of the most chemically diverse beverages.[1] The organic compounds in coffee can be classified as volatile or non-volatile. Volatile compounds are small molecules that are lost from coffee over time because their vapor pressure, both at room temperature during storage and grinding and at elevated temperatures during brewing, is sufficiently high to enable the molecules to escape into the atmosphere. Because they are lost over time, the depletion of volatile compounds is responsible for staling which contributes to the loss of complex flavors when brewed coffee sits for too long. Volatile compounds are just that – volatile. They are the first things to escape into the atmosphere, diminishing the aromatic intensity of the coffee forever. Conversely, non-volatile compounds common to coffee, such as caffeine and chlorogenic acids, are stable in beans and in brewed cups. Their vapor pressure is too low for a significant number of molecules to evaporate across temperatures encountered during storage and brewing. The most significant change to non-volatiles is when green coffee undergoes the roasting process, where temperatures exceed 200 °C and decomposition reactions become accessible. Green coffee has some of the highest chlorogenic acid content in any natural plant.[2] While a lot of chlorogenic acids are lost as coffee is roasted, they still make up the prevailing portion of detectible organic compounds in coffee, even after brewing.[3]

As specialty coffee gains traction with consumers, the science to back-up age-old coffee dogma is just blooming. Much of the current coffee-based science falls into two categories: food

science and fundamental chemistry. Food science is highly sensory based, which is valuable for the consumer impact side of science but has notable pitfalls in objectivity and reproducibility. The hardcore chemistry approach to coffee has yielded in-depth understanding of the incredible number and diversity of compounds packed within coffee beans. The methods and technology required, such as gas chromatography mass spectrometry (GCMS), liquid chromatography mass spectrometry (LCMS) and high-performance liquid chromatography (HPLC) are dependent on experience and high-quality technical training. Mass spectrometry uses the discrete mass-to-charge ratio for a molecule to isolate molecular weight.[4] GCMS heats up a solution to a vaporization point and introduces the sample to a mass spectrometer after the components of the solution are separated in a column to identify the molecules present in the sample.[5] This form of analysis is useful for detecting volatile compounds, because only molecules which enter the gas phase can be analyzed using GCMS. LCMS separates components in liquid phases before introducing them to the mass spectrometer which in turn identifies unknown compounds.[4] Like LCMS, HPLC uses liquid to separate out samples that are passed through a stationary phase column.[6] Eluted compounds are in turn passed through a detector which generates an electrical signal that can be read on a computer.[6] Liquid chromatography methods are more useful for non-volatile analysis because all compounds remain in solution during the procedure.

In contrast to these separative chromatographic methods, electrochemical techniques can measure specific compounds without requiring chemical separation. Every molecule has a specific redox potential that corresponds to the quantum mechanical energy gap between electronic energy levels in the compound. If there are multiple compounds in a solution with different energy gaps, they can be measured individually without the need for separation in an electrochemical cell by choosing the appropriate energy (voltage) to cause a movement of

electrons (current). The amount of current passed enables us to measure the concentrations of organic compounds in a solution. The characteristic voltage, or potential, at which a compound undergoes a reduction or oxidation within the electrochemical cell can give insight into the species present. Redox reactions involve either oxidation, the loss of electrons from the compound, or reduction, where the compound gains electrons (Figure 1). The overall transfer of electrons to or from the compounds in solution through the electrodes connected to the potentiostat give the resulting current output. Electrochemistry takes very rapid measurements, typically giving results within seconds or minutes, compared to chromatographic methods which can take up to an hour. Additionally, very minimal sample preparation is required to perform a typical electrochemistry experiment, compared to other forms of wet chemistry. This means that data on coffee can be collected incredibly quicky, especially compared to more involved and time-consuming processes such as HPLC and GCMS. This is an exciting way to approach coffee science because it lends itself to real-time measurements in café settings.

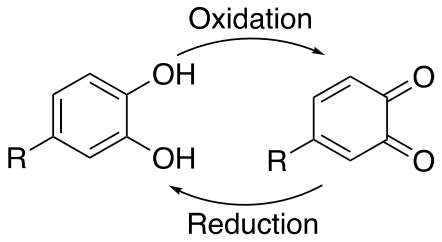

Figure 1

Simple depiction of oxidation (-2H, +2e$^-$) and reduction (+2H, -2e$^-$), aka a “redox” reaction on a model organic compound.

Chromatographic methods are invaluable in helping create a framework for the hundreds of compounds we know can be present in coffee. From this point on, however, the most useful data is that which is directly applicable in a café setting. Not only do LCMS, GCMS, and HPLC require significant technical training, but the physical machines are massive in comparison to the

set up required to perform electrochemistry in a cup of coffee. We use the Gamry potentiostat, which fits easily on a countertop and can be stored in any cabinet. The machines required for GCMS, LCMS, and HPLC themselves are extremely expensive, well beyond the means or practicality of any given coffee business. The required machinery is also complicated and bulky. By employing electrochemistry, the research conducted at the Hendon Lab walks a beautifully fine line of being fundamental chemistry, while still being extremely viable outside of a heavy-duty chemistry lab space.

Using electrochemistry as a rapid measurement technique to assess stable non-volatile compounds in coffee, such as chlorogenic acids, has a high potential to prove significant in the world of coffee. It is reasonable to expand the measurement technique to correlate to coffee quality, or to provide another coffee metric that is based in coffee's unique and complex chemistry. This innovation in the field of coffee science makes our work especially distinct, evident by our café-style research lab located in the middle of Willamette Hall on the University of Oregon campus. I have had the privilege of conducting my research on coffee in this lab space.

Background

Like other gourmet food niches such as wine and chocolate, coffee has a "Coffee Taster's Flavor Wheel" (Figure 2) that allows for a standardized discussion of particular flavors that might be found in a brewed coffee.[7,8]

Figure 2

The Specialty Coffee Association's Coffee Taster's Flavor Wheel. Created using the sensory lexicon developed by World Coffee Research, 2017.[7,8]

This standardization of coffee flavors indicates that there is a diverse range of flavor notes that can be detected in any specialty grade coffee. Flavor notes also apply to non-specialty grade coffee; however, they may be less nuanced, varied, and diverse. In my experience, non-specialty grade coffee is likely to be perceived as having low acidity, with notes of "brown, roast," "dark chocolate," "woody," "grain," etc. To be ranked as specialty coffee, a coffee is assessed against a grading form developed by the Specialty Coffee Association (Figure 3) and must score above 80.[9]

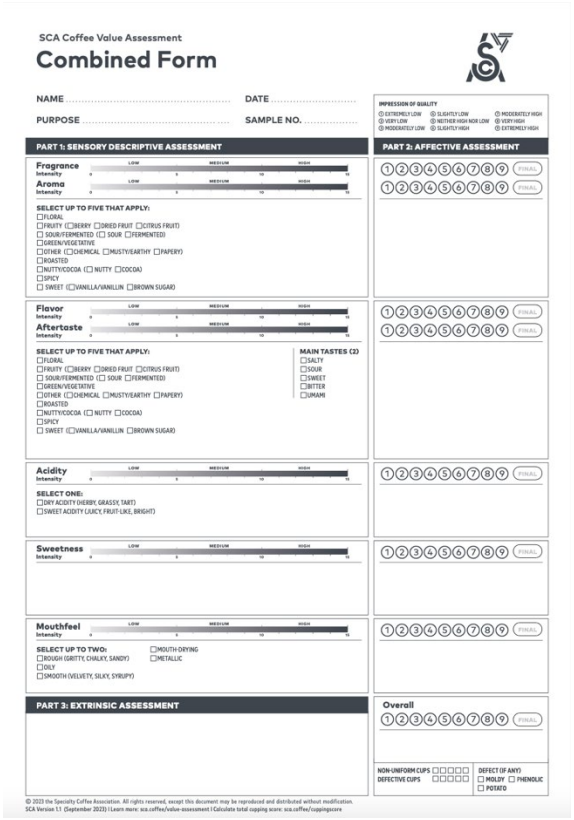

Figure 3

Example of a Specialty Coffee Association Value Assessment Grading Form, Version 1.1 updated September 2023. The Combined Form uses both sensory description and affective assessment to grade the quality of brewed coffee using the standard cupping method.[9]

In general, specialty coffee is also roasted more lightly than commodity coffee. Lighter coffee roasts retain higher acidity, more CGA, and can also produce a more "tea-like" product, as opposed to darker roasted coffee. Employing lighter roasts on coffee retains a given coffee's distinct characteristics, while darker roasts tend to move towards homogeneity in the flavor profile. Chlorogenic acids, the family of molecules I am using electrochemistry to detect in brewed coffee, are among the organic species understood to contribute to any given coffee's unique flavor profile.[3] Darker roasted coffees contain less chlorogenic acids, as the application of heat breaks down CGA into its subunits: caffeic acid and quinic acid (Figure 3).[3] Both caffeic

and quinic acid are known to contribute to coffee's distinguishable bitterness.[10] It is also important to note that there is a positive health correlation to high CGA content. CGAs are believed to fight damaged caused in the body from free radicals, which can in turn reduce inflammation and help routine bodily regulation.[11–17]

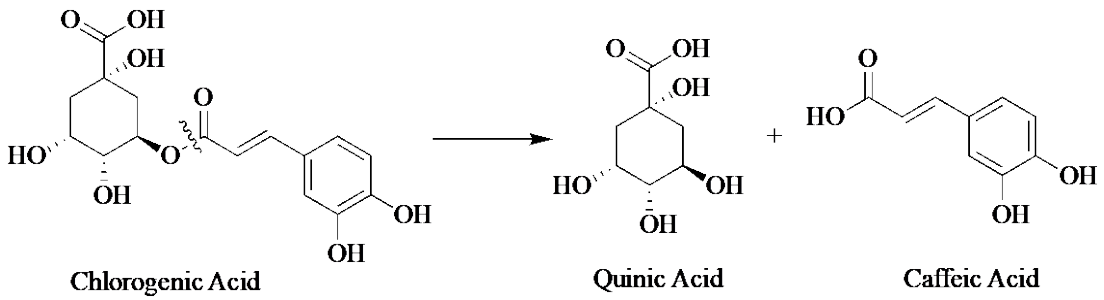

Figure 4

Chlorogenic acids decompose into subunits quinic acid and caffeic acid.

In addition to roast, coffee is subject to myriad variables that will influence the final product, such as species, varietal, growing conditions, country of origin, and processing methodology. Specialty coffee is nearly entirely Arabica coffee, but Robusta coffee represents a sizeable portion of production in the commodity sector. On the growing end, which happens in predominantly equatorial countries fondly referred to as the "bean belt," any environmentally suited variety of Coffea arabica can be selected for planting (Figure 4).[18]

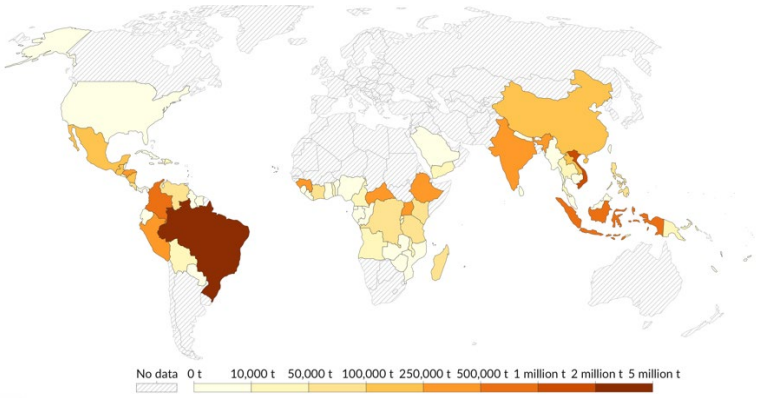

Figure 5

Geographical depiction of the coffee "bean belt" with coffee producing regions from around the world shaded in terms of production density (tones), data from the Food and Agriculture Organization of the United Nations (2023) – with major processing by Our World in data.[19]

Factors such as altitude, rainfall, soil acidity, etc. are important to track to understand how these factors can shape a farm's harvest and quality assessment. Occasionally, this information is included as supplemental information on specialty coffee bags for purchase or on coffee roaster's websites. Environmental factors are known to influence the amount of chlorogenic acids a particular coffee plant produces as well.[20,21] When a coffee cherry is ready to be picked for processing, there is a crucial decision whether to process the coffee "naturally" or using the "washed" method. The natural process for fermenting coffee involves allowing the whole cherry with the coffee seed inside of it to sun dry; the entire process takes three to six weeks.[22] While this method is more prone to bacterial issues or "bad batches," naturally processed coffee is sought after for its fruity, kombucha-like qualities. The washed process of coffee fermentation takes a picked coffee cherry still intact with seed, pulp, and skin, and removes all of the fruit skin and mucilage, leaving just the small, hard coffee seed to be fermented and dried.[22] It is after this processing that the specialty grade green coffee beans are exported from the equatorial growing regions to roasters in the United States and Europe. An experienced roaster will be able to roast a given coffee to maximize sweetness, acidity, and

flavor complexity, using metrics such as a coffee's growing conditions, processing method, variety, and country of origin. Utilizing a rapid measurement of chlorogenic acid content in a coffee has the potential to augment the determination of an optimal roast profile.

The central dogma in the Specialty Coffee industry is to first and foremost contextualize a coffee by its origin, that is, the country or region a coffee was grown in. When packaging a coffee, a specialty coffee roaster will likely include the coffee's country of origin, flavor notes, the roast date, and general degree to which the coffee was roasted (light, medium, dark). Specialty coffee is typically sold as single origin, which typically indicates a coffee from one farm/area in a country. This is small batch and homogeneous. Occasionally there will be blends, but it is common that a blend is detailed with where the various coffees are from and in what percent they compose of the blend. For example, a bag might be labeled as 45% washed Brazilian coffee, plus 55% natural Mexican coffee. For specialty coffee consumers, detailing where a coffee is sourced from indicates a set of preconceived expectations for their resultant cup of coffee. For example, washed Ethiopian coffee is well known for tasting like black tea. Rwandan coffee is often discussed as being "stew-like" or reminiscent of "stewed fruits." The potential to manipulate these qualities in a cup of coffee is on the horizon. Taking the first step of fully understanding the role chlorogenic acids in coffee will provide background for the future of reshaping the flavor producing compounds that are already present in coffee.

One of the more common assessment tools for brewed coffee is refractive index, which gives insight into the total dissolved solids (TDS) in a liquid like coffee.[23] This measurement is understood to elucidate differences between coffee factors, such as roast profile, but fundamentally it is a simple measurement of concentration using a polynomial.[24] Total dissolved solids via measurement of refractive index falls short of demonstrating anything about the

chemical character of the "coffee stuff" in the cup. We know that compounds exist, but we can't know what they are or in what proportions they exist in with the total dissolved solids reflective index metric alone. Through methods such as HPLC, we know that chlorogenic acids make up 5-10% of green coffee, and caffeine makes up 1-2% of green coffee, as a generalized metric.[10] It is subsequently asserted that CGA has a strong impact on coffee flavors, including key sensory components such as astringency, sweetness, and sourness.[3] As this is generally accepted to be true in the coffee industry, it would prove especially useful to be able to make measurements to specifically read these compounds in coffee, instead of the bare-bones metric of overall "coffee stuff" dissolved in water.

My research is developing the use of electrochemistry to make measurements correlating to the chlorogenic acid content in any given cup of coffee. This research uses techniques being pioneered in the Hendon Coffee Lab to assess brewed coffee. The lab I work in is developing the use of electrochemistry, specifically square wave voltammetry, to test the concentration of a family of molecules – chlorogenic acids (CGAs) – in coffee. My work aims to determine the chemical abundance of CGAs using methods that are accessible to coffee professionals in café settings. This method is distinct, unique, and important to the coffee industry because it provides an accurate chemical analysis of a cup of coffee without disturbing its inherent equilibrium. The electrochemical method we are developing in the lab is functionally a replacement for the refractometer which is currently the industry standard. The refractometer (in the Hendon Lab we use the VST Refractometer) uses a programmed polynomial calibration curve to convert the measured refractive index into the precent total dissolved solids (%TDS) in a solution. The VST is a handheld device that provides quick, real-time information on any coffee sample. This sort of measurement is quite useful for products such as alcohol, which have a known prevailing

group of compounds: ethanol (-OH). Coffee, however, is considerably more varied. It is an incredibly complex beverage. This measurement, while useful in determining a coffee's perceived intensity based on a simple polynomial, tell us no more than how much coffee "stuff" is in a cup. Coffee "stuff" has the potential to correlate to how strong/over/under extracted the sample is but gives no further insight into the chemistry of the cup. Extraction is one of the most important factors of brewing acceptable coffee, as illustrated the "Coffee Brewing Control Chart" (Figure 6), a ubiquitous figure in coffee.[25] Afterall, coffee is merely extracted bean juice. To this day, the Specialty Coffee Association touts this brew control chart as the gold standard.[26] Understanding extraction is extremely important in coffee quality control, but this metric is based entirely off of an inherently flawed measurement: %TDS. Our method can not only tell us how much, but what sort of compounds are in the sample as well.

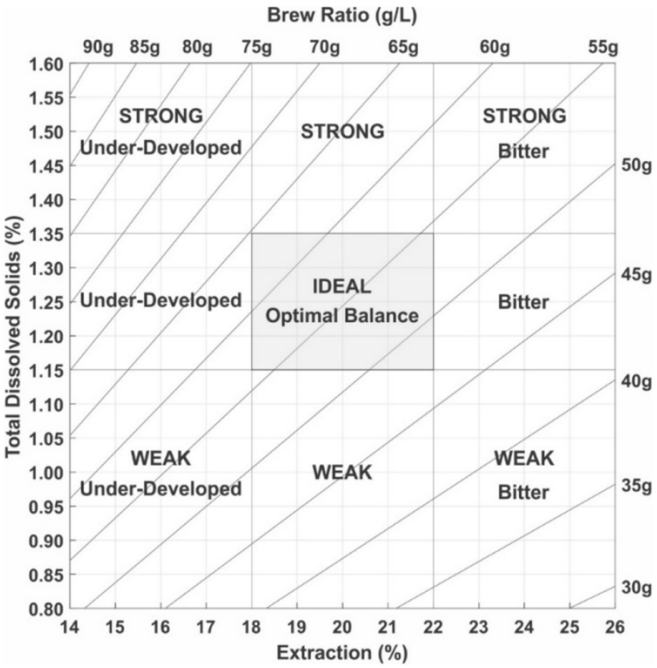

Figure 6

The classic Coffee Brewing Control Chart originally developed by Lockhart, 1957.[25] Reproduced from Lingle.[27] This remains an industry standard.[26]

Under the umbrella of electrochemistry there are a multitude of different experimental techniques that can be applied to make measurements. Each can reveal some distinct aspect of the sample, meaning each measurement technique has the potential to serve a different experimental purpose. Throughout my research I have employed a multitude of techniques, including cyclic voltammetry (CV), differential pulse voltammetry (DPV), square wave voltammetry (SWV), and chronoamperometry. Cyclic voltammetry is characterized by sweeping the voltage (x axis) back and forth across some defined window at a specified scan rate cyclically and measuring the current response (y axis). It is characterized by a "duck" shaped cyclic curve made up of reduction and oxidation peaks (Figure 7, Figure 8). Differential pulse voltammetry is characterized by sweeping voltage in just one direction, as opposed to back and forth coupled with jumping voltage up and holding it for a designated period of time, dropping it back down and holding, then repeating this stepwise process multiple times (Figure 7). This creates a stair step style application of voltage. Current difference is measured between the current immediately before the voltage jump is applied and the current at the end of the pulse. Whenever redox occurs, a Lorentzian peak is produced (Figure 9) as opposed to the "duck" produced by cyclic voltammetry (Figure 7, Figure 8). Square wave voltammetry is very similar to DPV but becomes "square" because an equal amount of time is applied at the pulses and the voltage difference is regular (Figure 7). Finally, chronoamperometry is characterized by holding the voltage at a set potential for a given amount of time and measuring the resultant current.

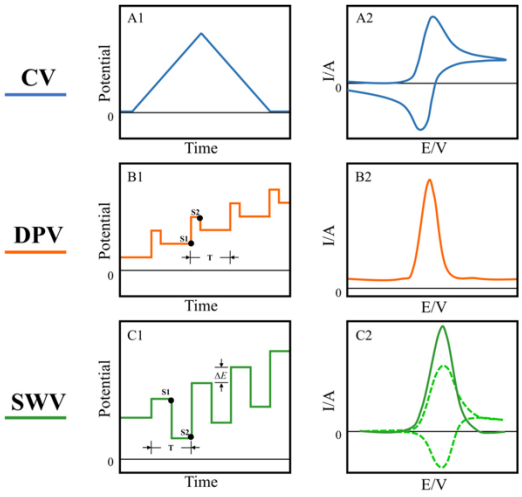

Figure 7

Graphical depiction of the electrochemical measurement techniques employed to read CGAs in coffee.[28]

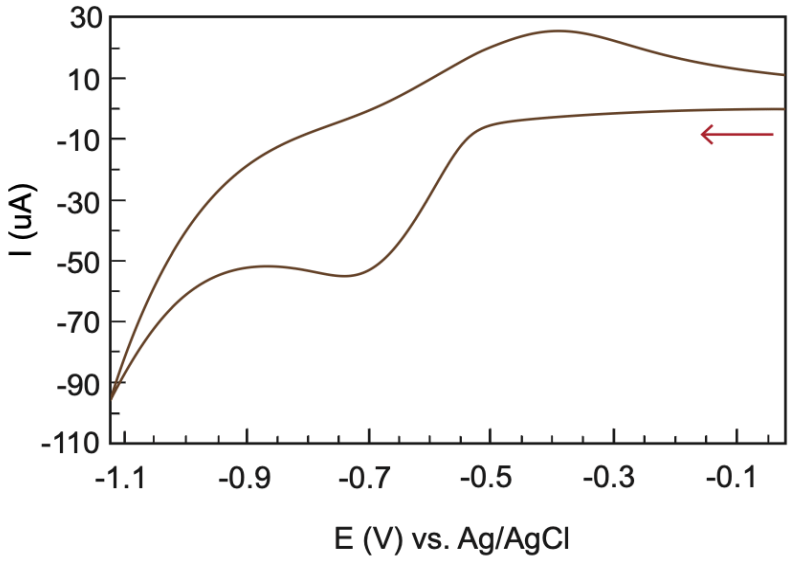

Figure 8

Typical cyclic voltammetry output showing oxidation (x = -0.4, y = 20) and reduction (x = -0.7, y = -60) peaks in coffee.

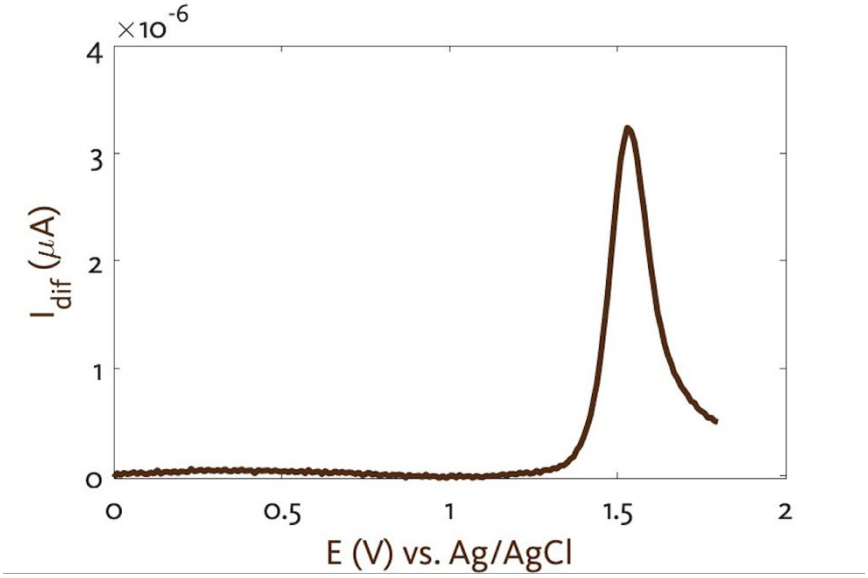

Figure 9

Lorentzian peak showing the presence of caffeine (~1.5 V) from square-wave voltammetry measurement, baseline subtracted.

This approach to making real time assessments of coffee concentration has exciting implications for the future of specialty coffee. My work will serve to contextualize and provide groundwork for the future database of coffee information that will continue to be developed in the Hendon Lab along with the introduction of chemistry-based coffee quality assessment technology into specialty cafes.

Methods

Electrochemistry works by sending a current through a closed system to cause the movement of electrons within and between chemical compounds. A three-electrode set up was used, consisting of a working electrode, a counter electrode, and a silver/silver chloride reference electrode (Ag/AgCl) all connected to the Gamry 1010B Potentiostat with electrical leads. These three elements are inserted into a small sample of coffee (the electrochemical cell) diluted in 18 $M\Omega \cdot cm$ water and 0.1 M sulfuric acid (H_2SO_4). An electrolyte, in this case sulfuric acid, is needed to close the circuit because pure water is very resistive and would not conduct electricity. The potentiostat controls the voltage of the working electrode and measures the current passed between the working electrode and the solution being analyzed. The electrical current counts the transfer of electrons between the working electrode and the organic molecules in the system (coffee), correlating to the number of molecules being electrochemically sensed, which then produces a graphical representation of organic compound concentrations in coffee. Current allows tracking of the number of molecules in the system, in turn extrapolating concentration.

Equipment

To make electrochemical measurements Gamry interface 1010B potentiostat was used. The lab space has been designed to mimic a café bar. The lab was equipped with coffee grinders (Mahlkonig EK43S and Nuova Simonelli Mythos) that ground the coffee used in experiments. To tamp espresso with reproducible force the PUQpress automatic tamper was used. Shots of espresso were pulled using the Victoria Arduino Black Eagle espresso machine. Each shot was weighed out with Acaia scales to 18 grams of coffee, with an aim of 45 grams of brewed espresso per shot. Before grinding, the beans are spritzed with water to reduce static charge during the grinding process.[29] Each aliquot of ground coffee was treated with a Weiss

distribution tool for consistency, ensuring de-clumping before being tamped. Ideal water temperature to brew coffee is between 90.5-96ºC.[30] We use a lab standard of 93ºC water for coffee brewing with an espresso machine pump pressure of 8 bars.

Experimental Design

The experimental parameters were adapted from a literature reference.[31] Glassy carbon (GC) and boron-doped diamond (BDD) working electrodes with a 3 mm diameter active surface were used to make the electrochemical measurements with the glassy carbon working electrode proving to be the most consistent. Future research will continue with the glassy carbon working electrode. For all experiment parameters I used a Ag/AgCl reference electrode filled with saturated KCl. A platinum wire electrode was most often the counter electrode of choice, but most recent success has been with a graphite counter electrode which has a greater surface area. Current measurements (GC working electrode, graphite counter electrode, Ag/AgCl reference electrode) show viable reproducibility and a linear calibration curve.

Procedure

The initial step was to ensure that an electrochemical measurement could be made on brewed coffee. The first electrochemical method explored to detect chlorogenic acid (CGA) in coffee was differential pulse voltammetry (DPV). To make this measurement, 1000 µL of brewed coffee was diluted in 50 mL of 0.1x phosphate buffer solution (PBS) as the supporting electrolyte. Between each measurement, the glassy carbon working electrode must be hand polished with a slurry of 0.3 µm alumina powder on micro cloth (aggressive surface) followed by a 0.05 µm alumina powder slurry on a less abrasive surface.[32] This is the accepted mechanical polishing process to prevent fouling, a phenomenon where the electrode surface becomes coated with contaminants that prevent the active surface of the working electrode from contacting the

solution. This means that the electrode is no longer able to make accurate, representative measurements on compounds present. The graphical effect of fouling is a much weaker peak signal, or no peak signal at all. Glassy carbon electrodes must be manually cleaned after each round of sampling, as opposed to platinum or boron doped diamond electrodes, which can withstand electrochemical polishing. For the initial CV of preliminary coffee samples, the potentiostat set up was as follows: Initial E (V) = 0; Scan limit 1 = 1.0 V; Scan limit 2 = -0.2 V; Final E (V) = 0; Scan rate = 50 mVs^{-1}. This measurement sweeps across a voltage range to measure the potentials where redox occurs and is used to confirm the presence of redox-active CGA. According to the literature, CGA can be detected in PBS buffer with a clean peak using differential pulse voltammetry (DPV) at the following settings: Initial E (V) = -0.1; Final E (V) = 1.0; Step size (mV) = 2; Pulse Size (mV) = 50; Sample Period (s) = 0.45; Pulse Time = 70 ms.[33,34] This measurement can be used to quantify the amount of CGA present. Each trial started with a freshly polished glassy carbon electrode surface, followed by 8 cycles of CV, and concluded with a DPV measurement, both at the previously detailed settings. After working with these experimental parameters and not achieving the desired results, new methods were introduced.

The next set of trials were run using square wave voltammetry (SWV) instead of DPV. Additionally, a chronoamperometry step for chemical electrode polishing, as opposed to mechanical electrode polishing with alumina powder, was included in the procedure. Both GC and BDD working electrodes were tested. When working with GC, mechanical polishing was used. When working with BDD mechanical polishing was only appropriate at the beginning of each data collection day because repeated mechanical polishing risks damaging the electrode surface over time. Instead, electrochemical polishing using chronoamperometry in 0.5 M H_2SO_4

was used between each trial to clean the BDD electrode surface and prevent fouling. Holding the voltage stable at the appropriate potential ensures that anything stuck to the electrode surface will be released, leaving the electrode clean to make new measurements. The initial parameters for chronoamperometry with the BDD electrode involved preparing the surface by polarizing the electrode to +2.0 V vs Ag/AgCl for 120 s in electrolyte followed by accumulating CGA at the surface of the electrode by holding the potential at open circuit for 60 seconds.[35] The SWV settings from the literature reference were pulse amplitude = 50 mV; frequency = 25-150 Hz; potential increment = 2 mV; scan rate = 50-300 mV s^{-1}.[33] The GC electrode was polished with 0.3 µm then 0.05 µm alumina powder and rinsed with 18 MΩ · cm water.[33] A cyclic voltammetry chemical cleaning step was also included before each measurement was taken, with parameters of: -0.2 to 1.0 V; scan rate = 50 mV s^{-1}; supporting electrolyte 0.1 mol L^{-1} PBS until the cycles were overlaying.[33] Both GC and BDD electrodes were tested to determine if either was capable of quantifying caffeine (CAF) and chlorogenic acid (CGA) content in a stock solution reproducibly and in line with the literature expected values using SWV. Additionally, a Britton-Robinson buffer, made from 0.1 M acetic acid; 0.1 M boric acid; 0.1 M phosphoric acid combined in 1 L deionized water and adjusted to pH 1.24 was explored in place of the previously used PBS buffer.

The final voltametric method explored was performing SWV with sulfuric acid (H_2SO_4) as an electrolyte, a platinum wire counter electrode and a GC working electrode. The 2018 paper by Redivo was stated to include CV and DPV, although the parameters provided in the publication led us to believe that SWV was used instead.[36] Basing the experimental parameters off this paper, our experimental setup was: Initial E (V) = 0.6; Final E (V) = 1.6; Pulse size peak (mV) = 50; Frequency (Hz) = 50.[36] Standard mechanical polishing was

performed with alumina powder to clean the GC electrode, followed by an electrochemical preconditioning of the electrode at +2.0 V for 30 s in 0.01 M sulfuric acid. The unique combination of mechanical cleaning with sulfuric acid chemical cleaning on GC proved to produce the best results for a reproducibly clean electrode surface. With these parameter settings

CAF in diluted espresso (Figure 15) as well as pure CAF (Figure 16) was measured. Figure 16 shows the determination of CAF using a graphite counter electrode and all other parameters held the same. Figure 17 shows the successful detection of CGA in 0.1 M sulfuric acid solution with these SWV parameters and electrode setup.

Data

Trial	Electrochemical Method	Electrolyte	Counter Electrode	Working electrode	Cleaning Procedure	Compound
1	DPV	PBS	Pt	GC	mechanical	CGA
2	DPV	PBS	Pt	GC	mechanical	CGA
3	DPV	PBS	Pt	GC	mechanical	CGA
4	DPV	PBS	Pt	GC	mechanical	CGA
5	DPV	PBS	Pt	GC	mechanical	CGA
6	SWV	PBS	Pt	GC	Mechanical	CGA
7	SWV	PBS	Pt	GC	Mechanical + Chemical	CGA
8	SWV	PBS	Pt	BDD	Chemical	CGA
9	SWV	Britton-Robinson	Pt	BDD	Chemical	CGA
10	SWV	Britton-Robinson	Pt	BDD	Chemical	CAF
11	SWV	PBS	Pt	GC	Mechanical	CAF
12	SWV	Britton-Robinson	Pt	GC	Mechanical	CAF
13	SWV	New Britton-Robinson	Pt	BDD	Chemical	CAF
14	SWV	Britton-Robinson (new)	Pt	BDD	Chemical	CAF
15	CV	Britton-Robinson (new)	Pt	BDD & GC	Chemical & Mechanical	CAF
16	SWV	Britton-Robinson (new)	Pt	BDD	Chemical, then mechanical once to show BDD gets fouled	CGA (new)
17	SWV	Britton-Robinson (new)	Pt	BDD	Start w 1 mechanical, then chemical	CAF
18	SWV	H2SO4	Pt then graphite	GC	Mechanical then Chemical @ 0.1 M H2SO4 each trial	CAF
19	SWV	H2SO4	Graphite	GC	Mechanical then Chemical @ 0.1 M H2SO4 each trial	CGA
20	SWV	H2SO4	Graphite	GC	Mechanical then Chemical @ 0.1 M H2SO4 each trial	CGA

Table 1

Overview of every distinct set of experiment trials I have performed working towards a viable measurement method. Ag/AgCl reference electrode is used in every trial as part of the 3-electrode setup.

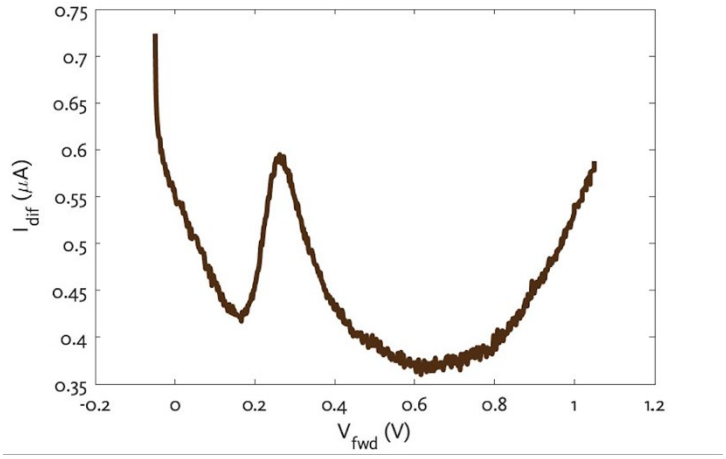

Figure 10

First experiment; demonstrated it is possible to see a CGA peak from espresso using DPV. Performed with GC working electrode, Pt counter electrode, Ag/AgCl reference electrode. 5mL espresso diluted in 50mL PBS.

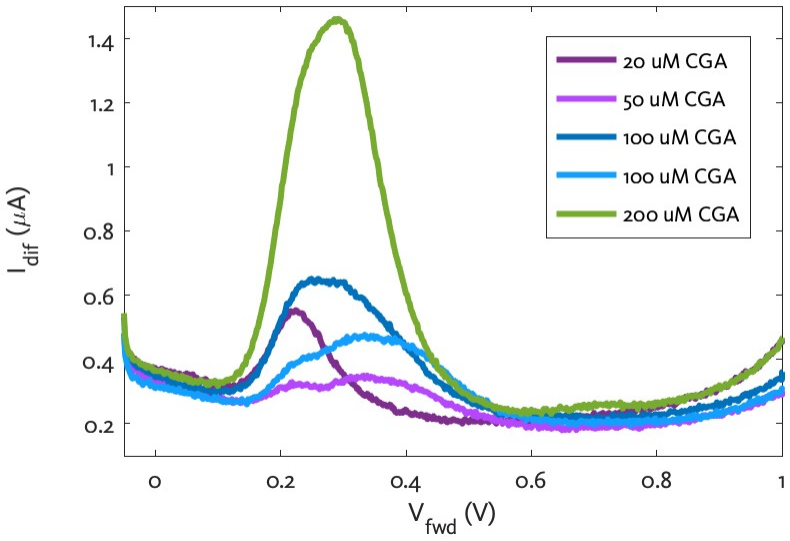

Figure 11

Irreproducibility between consecutive DPV trials, evidence of fouling present, especially in side by side 100 µM measurements of dark and light blue. Performed with GC working electrode, Pt counter electrode, Ag/AgCl reference electrode in PBS.

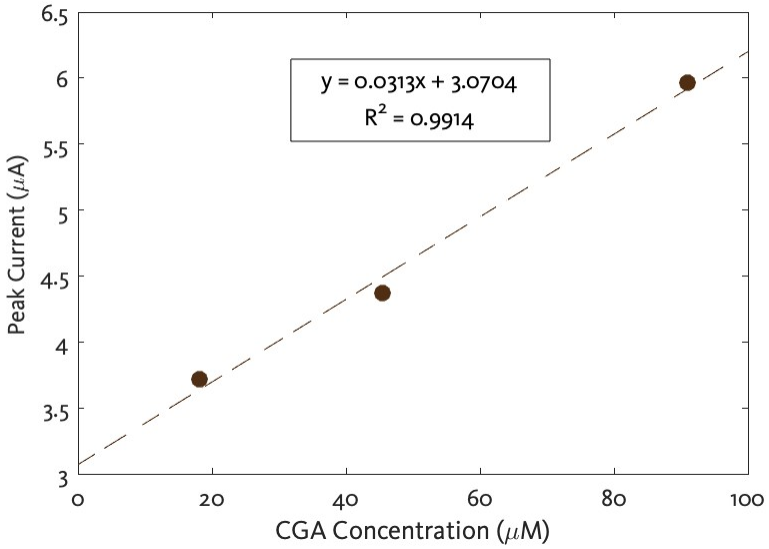

Figure 12

Calibration curve for CGA measured with DPV, provided evidence a calibration curve with strong R^2 is possible. Performed with GC working electrode, Pt counter electrode, Ag/AgCl reference electrode in PBS.

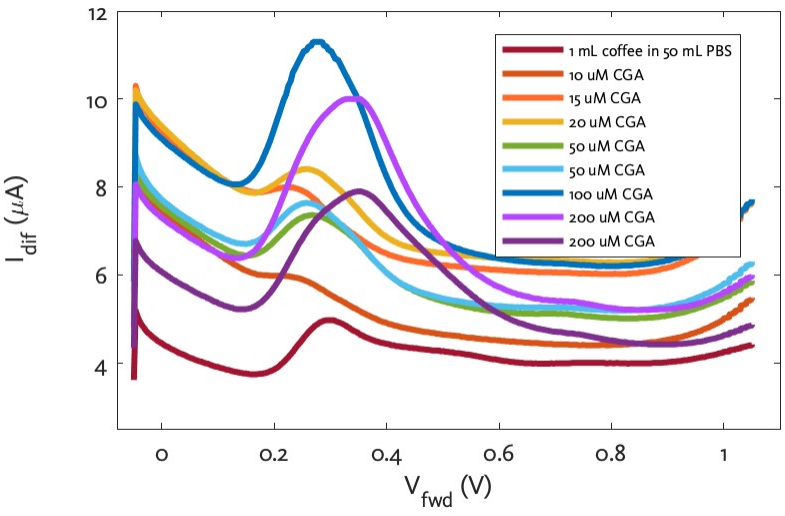

Figure 13

SWV with evidence of reproducibility (see light blue 50 µM and lime green 50 µM), but overall failure to find measurement consistency (yellow 20 µM vs. dark purple 200 µM 2) and prevent fouling (dark blue 100 µM vs. lilac 200 µM vs. dark purple 200 µM). Note irregularity of peak shape and position. PBS buffer, Pt counter electrode, GC working electrode, Ag/AgCl reference electrode.

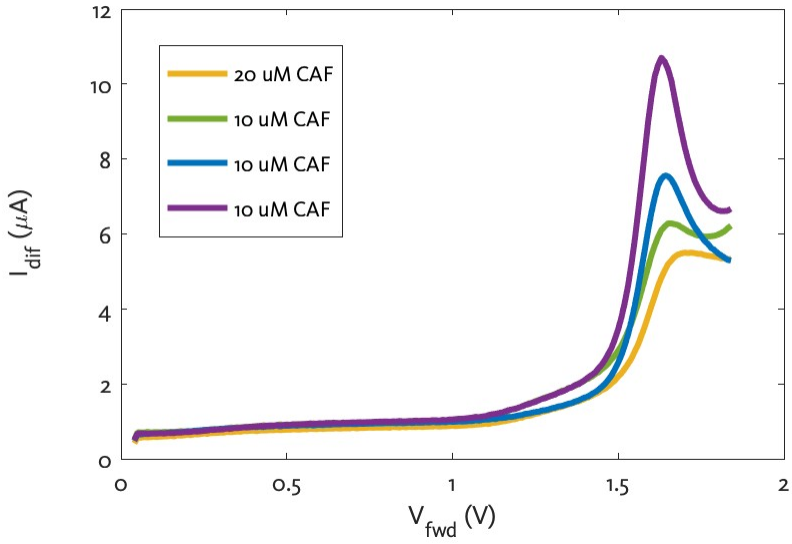

Figure 14

Detection of CAF in Britton-Robinson solution using SWV with BDD working electrode, Pt counter electrode. Error with fouling present, (first trial: purple 10 µM, second trial: blue 10 µM, third trial: green 10 µM, finally increase to yellow 20 µM) evidence that sulfuric acid cleaning step is not effective on BDD electrode. Scan is cut off just after known caffeine peak at ~1.6 V.

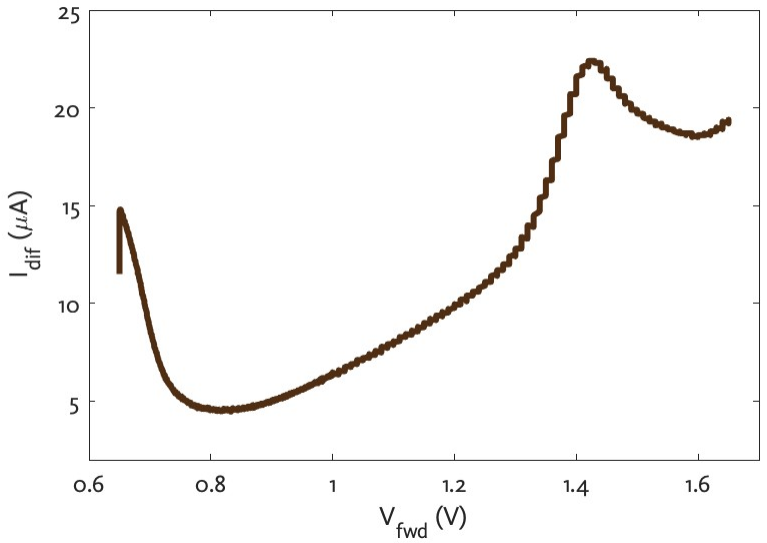

Figure 15

Success with SWV – GC working electrode, Pt counter electrode, 0.1 M sulfuric acid electrolyte demonstrating the ability to pick up CAF from a sample of 200 µL espresso in 40 mL electrolyte.

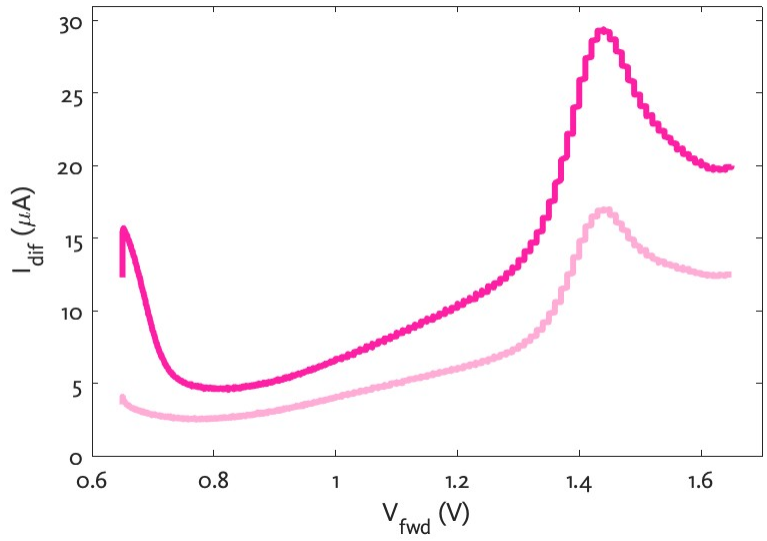

Figure 16

Success with SWV: GC working electrode, 0.1 M sulfuric acid electrolyte. Demonstrates the ability to produce a sharper CAF peak from sample of pure CAF with graphite counter electrode (hot pink) versus Pt counter electrode (light pink). 200 μL CAF in 20 mL electrolyte.

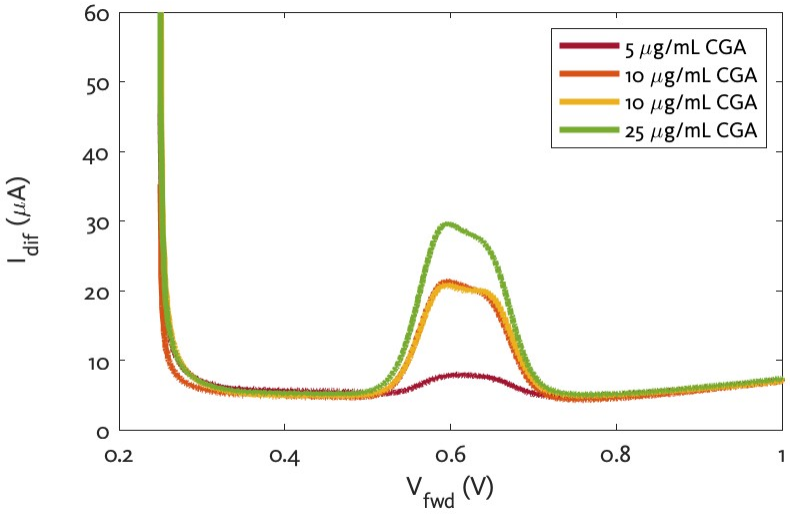

Figure 17

Strong reproducibility in CGA solutions of 5-25 µg/mL concentration with 0.1 M sulfuric acid electrolyte, GC working electrode, graphite counter electrode, Ag/AgCl reference electrode on pure CGA solution. Mechanical cleaning step followed by electrochemical cleaning step in 0.1 M sulfuric acid between each measurement.

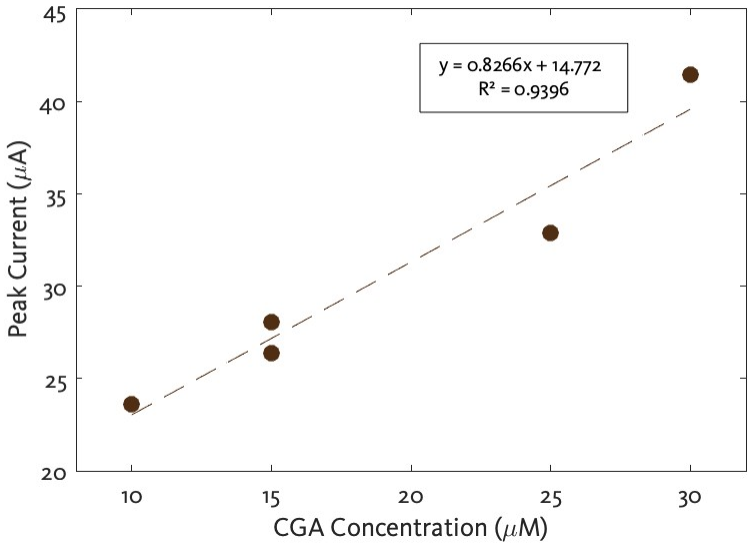

Figure 18

Strong positive correlation on calibration curve using SWV, GC working electrode, graphite counter electrode, 0.1 M sulfuric acid electrolyte, mechanical and electrochemical cleaning steps.

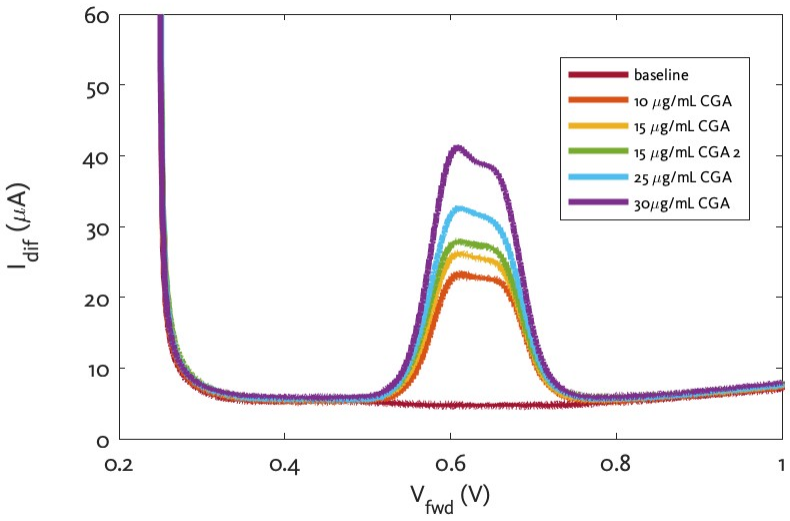

Figure 19

Discussion

Results

The result of the first test on espresso diluted in PBS was a small peak. While it did not match the literature reference, it was promising enough to suggest this model was viable. A second DPV scan was taken, this time spiked with a bit more CGA in solution (Figure 10). Because the peaks did not match the reported literature as cleanly as hoped, multiple trials were run in attempt to hone the electrochemical parameters using DPV with GC in PBS. Much of the process from this point on involved troubleshooting; making small changes to the procedure and potentiostat settings, finding literature to emulate that successfully measures CGA and CAF in coffee, and determining the reproducibility of measurements taken in succession. Many of these trials did not prove to be as reproducible as needed, nor did they produce results that matched their literature reference. One of the largest issues encountered was electrode fouling, which led to decreasing peak strengths between measurements (Figure 10). Despite cleaning efforts, the initial method did not behave reproducibly. It is best to avoid using electrochemical cleaning methods on GC electrodes because its risks damaging the electrode surface. However, hand polishing before each measurement means that each experiment run in a session sees a different electrode surface, making direct comparison of runs finnicky and unpredictable. Electrochemical electrode cleaning is more systemically reproducible, but the literature reference did not discuss using one, and we did not want to risk damage to our electrode surface.

To test many of these methods for creating a calibration curve I used caffeine (CAF) solutions instead of CGA solutions because pure CGA is expensive and susceptible to degradation, while CAF is less expensive and more stable. The standard reduction potentials of CGA and CAF are distinct but not far from one another, such that using CAF initially to test a

method was a viable model. Additionally, there was reasonable concern that some of the CGA I had been working with had degraded (oxidized) over time as a powder or after being added to a solvent to make a stock solution. To determine if CGA was the issue with experimental poor peak height and reproducibility or if it was an equipment issue, I tested CAF. I also used CV as a failsafe method to check if CGA was detectable in solution. This allowed me to determine that similar issues were coming up with the CAF measurements as were with the CGA measurements, indicating that it was an equipment issue rather than a chemical one.

In the second phase of determining electrochemical settings and experimental parameters that can reproducibly report CGA content in coffee, SWV was used (Figure 13).[33] This proved to be a more effective method than DPV showing a sharper peak produced at the expected CGA voltage, but issues with electrode surface quickly fouling were still present (Figure 13, Figure 14). Many trials were performed to explore the reproducibility of the measurement, with both GC and BDD electrodes. These methods, while making important contributions to the final procedure, also did not provide results consistent with literature expectations. Initially, the SWV was run was run with a GC working electrode, but fast fouling and irreproducible cleaning steps showed that this method was not viable (Figure 13). I then moved to a BDD working electrode, which allows for electrochemical instead of mechanical cleaning (Figure 14). This required performing chronoamperometry in a 0.5 M sulfuric acid as the cleaning step, and a daily mechanical polish in alumina slurries. Using a BDD working electrode also showed issues with decreasing peak height due to the electrode surface quickly fouling, despite multiple cleaning steps. I tried numerous different buffers, potentiostat settings, and combinations of electrodes and CGA concentrations in the attempt to mitigate the fouling, but nothing proved to be acceptably reproducible to use as a technical measurement.

One of the larger known sources of error for making electrochemical measurements in coffee is that CGA and CAF are known to combine to form a larger compound. This aggregate behaves differently in chemical analysis than the two compounds alone. Using very small amounts of coffee diluted into a supporting electrolyte mitigates the issue of the CGA/CAF aggregate forming. While this is a source of error in undiluted coffee, a small aliquot of coffee diluted in electrolyte yields a solution which is not concentrated enough for the CAF-CGA aggregate to form.

The final distinct method was a combination of my previous trials, inspired by "Bare carbon electrodes as simple and efficient sensors for the quantification of caffeine in commercial beverages" from the journal Royal Society Open Science. This procedure used both mechanical and chemical cleaning on a GC electrode.[36] Crucially, their chemical cleaning step was at a low molarity of sulfuric acid (0.1 M H2SO4) such that they did not see damage to the GC electrode surface, even when using this as an electrochemical cleaning solution. The combination of these cleaning procedures proved to be the most reproducible method overall, plus it produced a sharp peak for caffeine (Figure 15). During the initial testing I switched the Pt counter electrode for a graphite electrode and saw a sharper peak (Figure 16). The next step was to use this method with our shift from the Pt counter to the graphite counter electrode to test in the CGA voltage range at varying concentrations. The reproducibility of performing SVW with a GC working electrode, graphite counter electrode, Ag/AgCl reference electrode, and a combination of repeated mechanical and electrochemical cleaning steps was far stronger than any of the previous methods (Figure 17). This is evident in the consistency of CGA peak shape, relative peak height, and peak location. This data allowed for me to extrapolate a viable calibration curve with an R2 value of 0.9396 (Figure 18). This shows a strong positive linear correlation. Further trials using the same

method corroborated my initial success (Figure 19), showing a high degree of reproducibility. Critically, the peak heights for varying concentrations show up at the expected voltage and overlay in the expected manner when repeated – see 2 repeated trials of 15 µg/mL in Figure 19. The continuation of this project will include a refinement of this procedure for CGA detection in coffee. Once the method is honed, roasted coffees will be fit to the standard CGA calibration curve to test relative CGA content as a function of roast profile.

Significance

The work at the Hendon Coffee Lab at the University of Oregon is directed at contextualizing traditional coffee knowledge with chemistry. I plan on finishing this research and publishing a paper on the electrochemical methodology and calibration of CGA content in roasted coffee. This publication will serve as an important foundation for ongoing projects in the Hendon Lab and in the scientific community studying coffee. The development of a method to electrochemically alter flavor compounds in coffee is ongoing. Understanding CGA as a practical metric for coffee beyond the current rudimentary standards like TDS will advance the coffee industry. The vision for this is akin to a diabetic test strip – while it is fundamentally a potentiostat, it is not understood as such in practical use. While the work at the Hendon Lab is technical, there are far more accessible ways to do this work in a café setting. There is great potential for implementing potentiostats in cafes as "CGA detectors." I am working towards translating these analytical methods into something a café can use.

Next Steps

Work of the past six months has been dedicated to experimenting with and refining the parameters best suited for performing electrochemistry on coffee to measure CGA in solution. This review is intended to summarize the work that has gone into this project to get me where I

am now. I have settled upon the parameters best suited for making this measurement in terms of cost-effectiveness, reproducibility, and peak strength. I intend to continue work on this project for the next few months. I will begin with honing my procedure to ensure I have the best parameters and calibration curve. Next, I will roast a choice coffee to varying roast profiles (light, medium, dark) and measure the resultant CGA content using my developed electrochemical parameters. I expect to be able to fit this within my CGA calibration curve. Following this I will roast different green coffees to the same profile and measure their resulting CGA content. If afforded the time, I will roast different green coffees to multiple roast profiles and fit those to the CGA calibration curve as well. The outcome of this research will be a rapid measurement that can provide more chemical information on the composition of a brewed coffee beverage than is possible with the current industry-standard TDS measurement. I intend to finish and publish this research by the end of summer, 2024.

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