<u>An Analysis of Caffeine Content Based on the Region it was Grown</u>

IB Chemistry HL

Research Question:
How and why does the caffeine content in coffee differ based on the region it was grown?

Introduction:
Personal Engagement:

The idea for this paper started while I was working as a Starbucks barista. It just so happened that the specific store I was working at was called a "reserve store". What this meant was that the store was shipped specific small batches of coffee from around the world and brewed on a small scale brewing machine. As a highschooler sometimes trying to stay up to the late hours of the night, I wanted to know which type of coffee had the most caffeine in order to keep me awake to complete my work. This interest has continued and I now want to find out for myself which type of coffee has the highest caffeine content based on what region it was grown in.

Relevance:

This topic is relevant and significant because it applies to my individual life. I personally wish to see what type of coffee has the highest caffeine so that I can buy it. Of course there are other papers that have been written about how caffeine content differs based on region, but I wanted to take a holistic approach. I want to investigate *why* certain beans have higher caffeine content, whether that be climate, soil conditions, or others reasons unknown to me.

Environmental and Ethical Concerns:

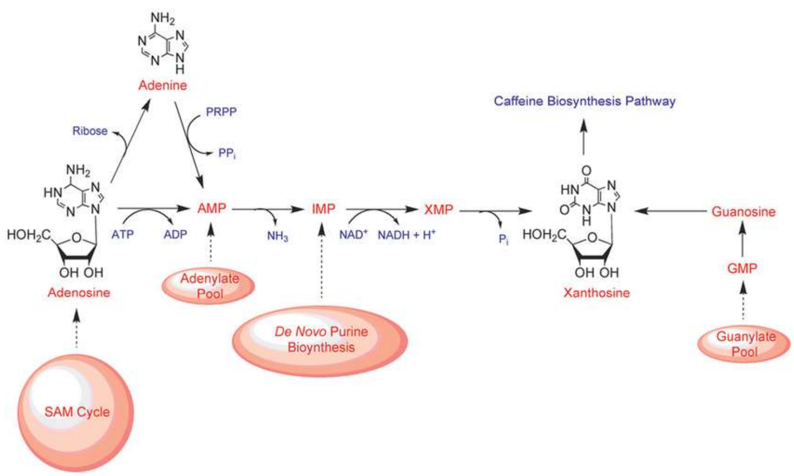
There are very few ethical or environmental concerns when it comes to my paper. However, just because those concerns are not in relation to me personally does not mean that they are irrelevant. For example, a large ethical issue that I would have doing this experiment would be the fact that the beans may have been harvested unethically. Many times in foreign countries locals can be pushed into jobs, such as coffee bean harvesting, for very little money in very poor conditions (Zamora). Thankfully I can avoid this concern as the beans harvested by Starbucks are all ethically sourced. If I were using beans from a company I did not know as well as Starbucks I would have done research into how the beans are harvested in each company to ensure people were being treated humanely.

Background: How is Caffeine taken in by the body

Before investigating which region produces more powerful coffee it's important to recognize the chain of events that occur for a plant to produce caffeine, then harvested and then eventually processed by the human body.

Obviously, caffeine is an integral part of Starbucks' business model, but many people don't realize how it is processed in the human body. Caffeine goes through the process of being grown as an espresso or coffee bean, brewed into coffee, and then processed by the human body.

The first thing that needs to be investigated is where the energy comes from in a coffee bean. A new study that has come out published by the New York Times has a new take on how the coffee plant creates caffeine in its beans. The study details how, “Caffeine starts out in coffee plants as a precursor compound called xanthosine. The coffee plant makes an enzyme that chops off a dangling arm of atoms from the xanthosine; a second enzyme adds a cluster of atoms at another spot. The plant then uses two additional enzymes to add two additional clusters. Once the process is complete, they’ve turned xanthosine into caffeine” (Zimmer). The figure below illustrates how this process occurs.



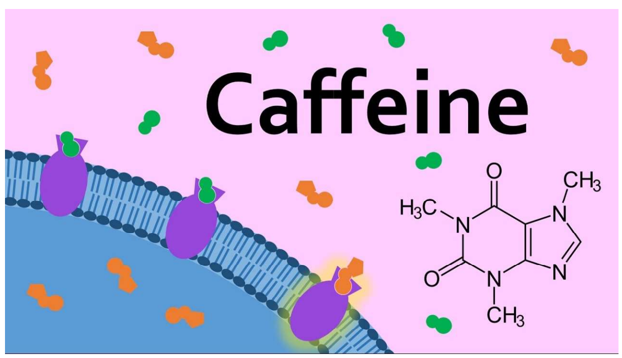
There are few different theories why the coffee plant turns xanthosine into caffeine. One potential reason is that the caffeine takes away competition. When the leaves fall off the plant to the ground and decompose, the caffeine kills any competition from other plants. Another is that the caffeine kills insects that try to eat the beans of the plant. Even though the caffeine is produced to deal with the coffee plants predators or competition, it is humans who reap the benefit of the energy.

However, before humans can drink the coffee, it has to be harvested and roasted to be palatable. Most coffee beans are harvested from different places which constitute different levels of caffeine, which will make up the majority of the analysis for this investigation.

Once the beans are sourced and harvested, they then have to be brewed by a barista . First the beans are ground-up to allow water to pass through more easily, and then they are put into a metal filter. Water is poured through at 195^{oF} over the grounds to brew the coffee. When the water passes through the grounds it absorbs the flavor and caffeine from the beans in order to form the cup of coffee. Even though this process brings caffeine into the coffee, it does not isolate the molecule. In order to get pure caffeine you have to use the process of, “direct organic solvent extraction, the water process method [or] supercritical carbon dioxide extraction” (Gillepsie). When coffee is brewed it still contains various other compounds besides caffeine, so the only way to get a true measure of caffeine content is to extract it. Once the caffeine is isolated, it can be deduced how much energy it provides to the body.

After the first sip of coffee, the caffeine immediately starts to absorb into the body through the mouth, throat and esophagus. Once it reaches the stomach, the caffeine then takes about 6 hours to fully dissolve into the body, which explains why people generally drink it in the morning to get through the work day. However, what I wanted to specifically examine was the energy produced when the caffeine was fully absorbed. The results that I found astonished me.

What I initially wanted to investigate in this experiment was the total energy produced by a cup of starbucks coffee, however I found that it actually produces no energy at all, but rather acts as an inhibitor in the brain. When the caffeine enters the body it travels to the brain and the caffeine molecules interact with the receptors that typically absorb adenosine. Adenosine is an inhibitory neurotransmitter which is the chemical that partly induces sleep. For the time that you are awake this chemical builds up until you fall asleep. Thus, the longer you stay awake, the more adenosine the brain produces. What the caffeine molecule does is work as an inhibitor for the inhibitor. Instead of adenosine falling into receptors to induce sleep and tiredness, the caffeine molecules take its place (as denoted in the figure below with the orange molecules showing caffeine and the green molecules representing adenosine).



When the caffeine takes its place, it inhibits the body from getting drowsy. Instead of actually producing energy for the body to burn, it simply inhibits a chemical from going into the brain. What I initially hypothesized was that it would produce energy, however my initial hypothesis was proven incorrect.

When I first started this investigation I thought that caffeine gave the body a jolt of energy. I figured that if people were using it to finish a paper late at night, or to wake themselves up in the morning, it had to be a supplier of energy to burn. However, I was mistaken. I predicted that part of my experiment would be to investigate where caffeine comes from and then calculate how much energy is produced from certain caffeine strains. Despite this, upon further examination I found that the relationship between the human body and caffeine is a biochemical one that occurs in the brain, not one that has to do with energy and the body.

Moving into the remainder of my investigation I then came to the updated question of, “how does the caffeine content in coffee differ based on the region it was grown?”. I built on this to include a background question which is “why” this difference in caffeine occurs.

Methodology:

When originally completing this paper the plan was to isolate the caffeine content from various starbucks coffee strains and have an independent table of data. However, due to the COVID-19 virus in the United States that was not possible due to all lab areas being closed, along with the starbucks reserve stores needed for the coffee to test.

With this in mind the investigation procedure and data below is the work of C. Campa, S. Doulbeau, S. Dussert, S. Hamon, and M. Noirot. For this reason some details are limited as to the precise step-by-step instructions. However, it gives a sufficient amount of information to make it valuable.

As described by these scientists in their investigation “Diversity in bean Caffeine content among wild *Coffea* species: Evidence of a discontinuous distribution” there was a distinct method used to extract caffeine for data. That procedure is as follows:

> *2.2 Sample Preparation:*
> Coffee cherries were harvested at full maturity and depulped using the wet processing method. After Desiccation on silicagel, 50 green beans per tree were frozen in liquid nitrogen before crushing in a ball mill (Dangoumill) for 2 min. The fine powder was split into six samples; three were used to estimate water content and the other three underwent further analysis.
>
> *2.3 Extraction, purification, and analytical HPLC:*
> Caffeine extraction was performed as previously described by Barre et al. (1998). Analyses were carried out on a HPLC system (Waters) consisting of a 250 mm x 4mm Merck LiChrospher 100 RP-18 column (5 µm particle size), a C_{18} guard column and a photodiode-array detector (Waters 996). The elution system (1Ml min^{-1}) consisted of two solvents solvents that were filtered (0.2 nm pore size filter), degassed and sonicated (Ney, 300 Ultrasonik): solvent A (10mM acetic acid/triethyl-amine 1000/1, pH 5.3) and solvent B (methanol). The gradient applied was: 0-15 min, 65% solvent A, isocratic; 15-20 min, 100% solvent B, linear; 20-26 min, 100% solvent B, isocratic; 26-30 min, 65% solvent A, linear. Identification and quantification were performed at room temperature (10 nl of sample) using a reference standard (Sigma Chemical Co.) at 273 nm. The calibration curve was plotted using three replicate points for a caffeine solution at 5, 10, 25, and 50 mg 1^{-1}.
> The processing order was fully randomised. Every 10 samples, a control was used to check the measurement stability. Caffeine content was expressed as a percentage on dry matter basis (% dmb).

Certain aspects of this procedure, specifically the simplified extraction method (Barre et al., 1998), made it possible to detect extremely small levels of caffeine after extraction. As shown in the analysis section below, data was able to be calculated extremely precisely.

Analysis:

The analysis of the separate investigation was also detailed as to how the scientists produced data to analyse. They describe how:

> *2.4 Statistical Analysis:*
> All results were analysed using the Statistica software package (5.1 version, 1997 Microsoft Windows).
> Each tree was represented by its mean caffeine content.The statistical analysis only concerned between-species variations which were tested using a one-way ANOVA. A Newan and Keul's test was carried out for multiple mean comparisons.

Data Collected:

Table 1
Geographical origin of the species and taxa

Species and taxa	Geographical origin
C. brevipes	Cameroon
C. canephora	Côte-d'Ivoire
C. congensis	Congo Democratic Republic
C. eugenioides	Kenya
C. heterocalyx	Cameroon
C. humblotiana	Comores
C. humilis	Côte-d'Ivoire
C. kapakata	Angola
C. liberica dewevrei	Central African Republic
C. liberica Koto	Cameroon
C. liberica liberica	Côte-d'Ivoire
C. pseudozanguebariae	Kenya
C. racemosa	Tanzania
C. salvatrix	Tanzania
C. pocsii	Tanzania
C. stenophylla	Côte-d'Ivoire
Coffea sp. Bakossi	Cameroon
Coffea sp. Congo	Congo Democratic Republic
Coffea sp. Ngongo 2	Congo Democratic Republic
Coffea sp. Moloundou	Congo Democratic Republic
Coffea sp. N'koumbala	Cameroon

Table 2
Caffeine content between and within species (the within-species range is in brackets)

Species and taxa	Mean	Range
C. brevipes	2.54	2.36–2.96
C. canephora	2.64	1.51–3.33
C. congensis	1.47	1.08–1.83
C. eugenioides	0.51	0.44–0.60
C. heterocalyx	0.92	0.86–0.99
C. humblotiana	0.00	0.00–0.01
C. humilis	1.93	1.67–2.27
C. kapakata	1.20	1.04–1.39
C. liberica dewevrei	0.94	0.81–1.10
C. liberica Koto	1.31	0.91–1.70
C. liberica liberica	1.24	1.12–1.39
C. pseudozanguebariae	0.00	0.00–0.00
C. racemosa	1.06	0.86–1.25
C. salvatrix	0.03	0.01–0.06
C. pocsii	1.27	1.04–1.71
C. stenophylla	2.27	2.05–2.43
Coffea sp. Bakossi	0.00	0.00–0.03
Coffea sp. Congo	2.27	2.11–2.37
Coffea sp. Ngongo 2	2.12	1.90–2.32
Coffea sp. Moloundou	0.58	0.52–0.61
Coffea sp. N'koumbala	2.36	1.89–2.89

Data Uncertainty

As detailed in the ethical concerns section above, it is important to identify any ethical reasons for not carrying out an experiment. When initially planning this investigation I knew that the beans being used would have been harvested ethically. However, because the data-set above was collected by a separate investigation it is not clear how the coffee cherries were harvested and if the location producing those beans treated their workers ethically.

A clear limitation for the data in relation to my investigation is that all samples were harvested on the continent of Africa. The initial retrieval of data was initially meant to come from coffee beans from different parts of the world, preferably from different continents. For example, one sample from: Hawaii, Sumatra, Guatemala, and Ethiopia. The goal of initial intention would have been to retrieve beans from areas of the world that are significantly separated, whereas the data used was only retrieved from one continent.

Evaluation:

Sources of error and improvement:

Despite the data uncertainty, certain conclusions can still be made about location's influence on caffeine content in coffee beans. As demonstrated in the data above, location may have played a role in the caffeine content however it is inconclusive to make a definitive conclusion based on the fact that there was not a demonstrated trend in relation to African countries. If a trend was presented where beans harvested closer to the equator or closer to the ocean had a disproportionate amount of caffeine it may prove a definitive conclusion. However, a trend such as that was not present in the data acquired.

Because many aspects of this investigation were cancelled due to the COVID-19 pandemic virus in the United States it is difficult to judge what improvements could have been made to improve the investigation. One possibility could have been using data from multiple different investigations to extract a better conclusion as to how the region impacts caffeine content. However, the reason this was not the approach I took was because it would make the data even less reliable. After researching and trusting that the investigation I used was reliable, I weighed that having consistent data was more important than many different regions. If many investigations were used it would make it exceedingly difficult to prove that the data was consistent and that it was reliable.

Conclusion:

The initial assumption that caffeine content was dependent on region was incorrect. Caffeine content varies based on Genus and evolution. These genes and evolution can sometimes be dependent on the region that the plants grow. The difference is that the environment is secondary to the caffeine content when compared to the coffee plant's genetics, the real reason for caffeine variations in *coffea* species.

The conclusion that can be made solely through the data provided is that location does not impact caffeine content in coffee beans significantly. This conclusion is limited in the fact that the data collected only included one continent with all countries being relatively close to one another. It can also be reasonably concluded that the significant influence factor for caffeine content in coffee beans is in relation to the species plant.

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