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Faculty of Tropical AgriSciences

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Caffeine content in coffee beverages on the campus of the Czech University of Life Sciences Prague

BACHELOR'S THESIS

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Author: **Eliška Jurošková**

Supervisor: Ing. Olga Leuner, Ph. D.

Declaration

I hereby declare that I have done this thesis entitled Caffeine content in coffee beverages on the campus of the Czech University of Life Sciences Prague independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague

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Eliška Jurošková

Acknowledgments

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Abstrakt

Kávový nápoj má již dlouholetou historii a je známý po celém světě. Nejhojněji zasoupenou látkou v kávě je kofein. Jeho psychosomatické účinky na lidský organismus mohou být pozitivní či negativní. Ve výzkumu této práce hraje kofein důležitou roli.

Literární část této bakalářské práce se zaměřuje na historii kávovníku, jeho podrobný botanický popis, způsoby sklizně kávových zrn. Také popisuje největší producenty kávy na celém světě a různé metody pražení kávových zrn. Dále se zaměřuje na již zmíněný kofein a jeho vliv na lidský organismus a také na to, jak působí na studenty.

V praktické části byl proveden výzkum zabývající se porovnáním obsahu kofeinu v kávových nápojích ze sedmi různých míst v areálu České zemědělské university v Praze. Kolekce vzorků byla prováděna ve třech opákováních v rozmezí půl roku. Pro vyhodnocení vzorků byla použita metoda vysoce účinného kapalinového chromatografu. Hodnoty objemu vzorků se pohybují v rozmezí 25 ml do 120 ml. Nejvyšší naměřená hodnota kofeinu v jedné dávce je 165,94 mg a pochází z podniku Club C a nejmenší 58,20 mg z AULA Café. Veliké množství studentů či profesorů pije denně ve škole kávu, či jiné nápoje s kofeinem. Je proto velice důležité kontrolovat a hlídat si hladinu zkonzumovaného kofeinu, aby se nepřekročila doporučená denní dávka.

Klíčová slova: Káva, Kofein, *Coffea arabica*, *Coffea canephora*, HPLC-DAD

Abstract

Coffee beverages have a long history and it is known all over the world. The most abundant substance in coffee is caffeine. Caffeine has psychosomatic effects on the human body that can be both positive and negative. In the research of this thesis, caffeine has an important role.

The literary part of this bachelor thesis focuses on the history of the coffee plant, the botanical description, and the methods of harvesting coffee beans. It also describes the biggest producer of coffee in the world and different ways of roasting coffee beans. Further, it focuses on the aforementioned caffeine and its effect on the human body and how it affects students.

In the practical part, research was conducted to compare the caffeine content of coffee drinks from seven different locations on the campus of the Czech University of Life Sciences Prague. The sample collection was carried out in three replications six months apart. To evaluate the samples was used method of high-performance liquid chromatography. Sample volume values range from 25 ml to 120 ml. The highest measured value of caffeine in a single dose is 165.94 mg and comes from Club C and the lowest is 58.20 mg from AULA Café. A large number of students or professors drink coffee or another caffeinated beverage on campus every day. And that is why it is very important to control and monitor caffeine intake to ensure that they do not exceed the recommended daily dose.

Keywords: Coffee, caffeine, *Coffea arabica*, *Coffea canephora*, HPLC-DAD

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List of the abbreviations used in the thesis

C. arabica – Coffea arabica

C. canephora – Coffea canephora

DAD – Diode array detector

HPLC – High-performance liquid chromatography

PTEE - Polytetrafluorethylene

FES – Faculty of Environmental Sciences

FAPPZ - Faculty of Agrobiology, Food, and Natural Resources

FEM – Faculty of Economics and Management

FTA – Faculty of Tropic AgriSciences

SIC – Study and Information center

1. Introduction

A cup of coffee is a daily necessity for most of the world´s population. It has been a part of our lives for many years for consumers, farmers, and traders. Coffee is a tropical crop, and it is one of the most traded crops in the world. One reason coffee is so popular is because of the interesting taste and chemical composition. Coffee beverages contain caffeine, which positively or negatively impacts human behavior or health. It is also popular because of easy preparation, people can easily prepare coffee at home or buy a coffee machine. When someone wants high-quality coffee, they seek out cafés with fine coffee and don´t mind paying extra for it.

Caffeine is commonly encountered in coffee, medications, tea, chocolate, and soft drinks. His popularity has grown over the last few years also in various forms not only in coffee for example energy drinks, sports drinks, and gels (Davis et al. n.d.). Caffeine is one of the most widely used psychoactive substances in the world.

In my bachelor thesis, I research caffeine content in coffee beverages on the campus of the Czech University of Life Sciences. When students or professors are on campus they often need to concentrate, be productive, and work even if they are tired, and caffeine can a little help them a little to do that. Especially students can be addicted to caffeine when trying to handle school, essays, exams, and sometimes work. Therefore, we should have places on campus where students can go for quality coffee. To cope with fatigue or just hang out with friends.

2. Literature Review

2.1. History of coffee

Over the course of many years, a lot of theories and legends have arisen about where and when coffee came from. Some of their dates are before Christ but most of the sources are unreliable. No one can guarantee what is true today so we will only look at the information that we can scientifically submit. Already before 1454, the people of Yemen started growing coffee plants and drinking coffee (Augustín 2016). It is common knowledge that the coffee plant and the fruit originated in present-day Ethiopia. The expansion into the world involved nomadic tribes, traders, aborigines, and foreigners. The scientific name *Coffea* was given by Linnaeus (Teketay 1999).

One of the mentions of coffee in Europe has been recorded in the travelogue of a German doctor and naturalist Leonhard Rauwolf in 1582. It includes notes from his travel from Levanta (Eastern Mediterranean) and describes rituals of drinking coffee. Also, Pietro della Valle around 1615 brought coffee beans from Istanbul to Europe. And last but not least botanic, pharmacist and doctor Prospero Alpini traveled to Egypt in 1850 and described the coffee plant in the writings of the Book of Egyptian Plants (Augustín, 2016).

Coffee has come to Europe in the 17^{th} century thanks to enterprising Dutch, English, and French who transported the seedlings to their colonies in Java, Sri Lanka, Martinique, India, Cuba, America (Colombia, Mexico, Nicaragua, Guatemala, Brazil, Ecuador), and back to Africa (Ivory Coast, Angola, Guinea). After that, the expansion stopped, and industrial cultivation began (Pössl 2010). A significant person in the history of coffee is Nicolass Witson, who was the mayor of Amsterdam and successfully started coffee farming in Java. After that, they extended cultivation to Sumatra and Celebes, making Indonesia the first and one of the world´s most important coffee exporters (Thorn 2000).

A professor from Northwestern University Charles B. Reed, claims that coffee drinks can be considered a cure and a type of substance that supports genius. Many historians say that it is possible to see which parts of the works Voltaire are from coffee´s inspiration. Because coffee supports creativity and concentration which is necessary to create masterpieces of literature and art. The most ardent coffee connoisseurs were Balzac and Voltaire, who drank fifty cups of coffee daily (Ukers 2012).

2.2. The coffee plant

The coffee plant is very extended and grows throughout the tropical and subtropical zones, around the equator. It is cultivated in about 70 countries around the world. Growers graft, prune, and cultivate coffee plants in various ways to make them hardy and fruitful (Veselá 2018). In Africa, especially Ethiopia and Yemen are the only ones where wild plants of *Coffea* grow in the understorey of tropical forests. The average harvesting period of the coffee plant is thirty years. After such a time the plant may still be alive and growing but no longer produces fruits or very little (Ferreira et al. 2019).

2.2.1. Botanical classification

The height of coffee plants is very varied. Some of them are large, robust trees about 10 m tall, and some of them are short like bushes. On the plantations, the plants do not reach such a height because the farmers would struggle with harvesting, so they are notching the plants (Veselá 2018). The botanical family to which coffee belongs is *Rubiaceae* which has 500 genera and over 6000 species. Also, coffee belongs to the genus *Coffea*, which is the most economically important member of the *Rubiaceae* family. The height and shape of a coffee tree are various and depend on the specific species. In general, the coffee tree consists of an upright main trunk (shoot) with hard, dense wood. They usually have horizontal or near horizontal primary, secondary, and tertiary lateral branches/stems (Davis et al. 2006). The leaves are shiny, a little wavy, and dark green with conspicuous veins. They are opposite decussate on suckers; each pair is cross positioned to the next leaf pair. Coffee tree leaves grow up to 15 cm. Coffee has a shallow root which consists of a main taproot and lateral small feeder roots (Herrera & Lambot 2017). Coffee *arabica* has feeder roots deep into the soil on the contrary coffee *canephora* have feeder roots close to the soil´s surface. Coffee is a short-day plant. (Farah & dos Santos 2015) For most species, the flowers are borne with the leaves, but sometimes are flowers on branches without leaves. The corolla is white or rarely pink, mostly they have

(4)-5 lobed corollas, sometimes have 6-9-(-12) lobed corollas. The tube is usually cylindrical, widening just below the throat. The stamens equal the corolla lobes in number. The cherries are the fruit is a drupe, which is fleshy, containing 2 or 1, more or less coriaceous, one-seeded pyrenes. The size varies, but the shape is always the same (Teketay 1999). As for reproduction, pollination runs until 6 hours after flowering. The fertilization process is finished 24-48 hours after pollination. After that is an emerging fruit and develops into a 10-15 mm long cherry which contains two seeds, the coffee beans. The fruit is consisting of the skin (epicarp or exocarp), which is a monocellular layer that covers the pulp and protects the fruit, it is normally red, dark pink, or yellow. Then is pulp (mesocarp), which consists of fleshy pulp and has a pectinaceous layer of mucilage adhering to parchment or parch (endocarp) which is a thin, crumbly-like paper cover (Davis et al. 2006). After that is silver skin, which is a seed coat from polysaccharides, cellulose, and hemicellulose. And the last two ellipticals or egg-shaped seeds consist of endosperm and embryos (Farah & dos Santos 2015).

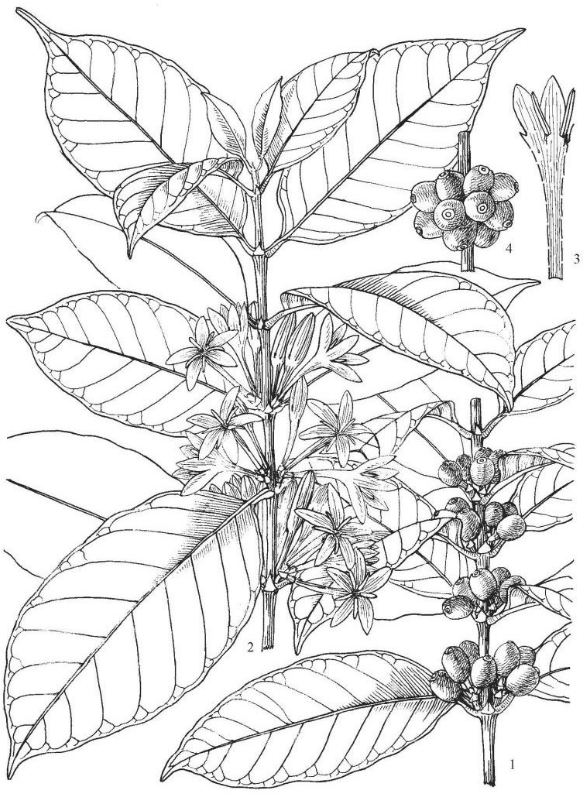


Figure 1. ***Coffea***

Author: Flora of China 2023.

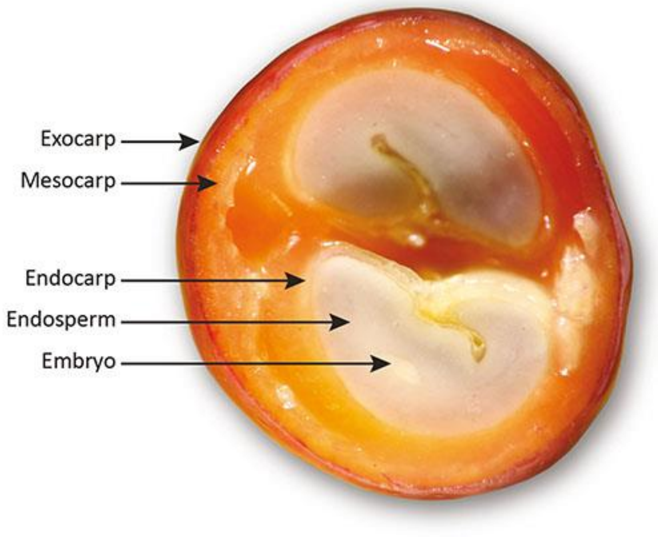


Figure 2. The transverse cut of the coffee fruit

Author: (Ferreira et al. 2019)

2.2.2. Coffea arabica

Originally *Coffea arabica* is from Ethiopia but has been cultivated in Arabia for over a thousand years. Nowadays it is commercially cultivated in tropical and subtropical regions over the world. Ordinarily, it is called Arabian coffee or simply arabica. It grows in height to 4 – 5 tall so it is a small tree or a shrub (Missouri botanical garden 2023). *C. arabica* is classified as one of the best and highest-quality coffees, which is why it accounts for 60-65% of world production. It thrives at higher altitudes between 600 – 2500 meters above sea level. This coffee plant is a little more demanding to grow, the first harvest can be after 5 years. It is less resistant to diseases and pests but doesn´t mind the change of weather. The ideal temperature for cultivation is 15 – 24 °C. (Veselá 2018)

As stated by van der Vossen et al. 2015 the basic species of arabica, from which plenty of other varieties have evolved, is called *Typica*. It originated in Ethiopia and it grew on the first coffee plantations in Yemen. *Typica* has a conical shape plant and a straight trunk, it grows to a height of 4 meters and its fruit has a red color. It has low production but excellent quality. The next is the variety *Bourbon*, which the French planted in the middle the of Indian Ocean on the island of Réunion in 1708. *Bourbon* has a higher yield and produces more than *Typica* by about 30% but is still less than the other varieties. It has small fruits that grow densely, close together, and quickly ripen. The quality of this coffee is similar to *Typica* (Anthony et al. 2001) The mutation of the variety *Bourbon* is *Caturra*. They bred it in Brazil in about the year 1930. It has high production and at the same time high quality. *Caturra* requires thorough care and fertilization. The taste has a distinct acidity and soft body. Nature hybrid between *Typica* and *Bourbon* was first discovered in Brazil and called *Mundo Nuovo*. It is a strong and resilient variety with great production. It matures a little later than the other varieties. Another hybrid bred by Brazilians is *Catuai*, which is a hybrid of *Caturra,* and *Mundo Nuovo*. It has lower growth but strong production (Ghosh et al. 2014). In the last years, it is one of the most desirable, popular, and most expensive varieties of *Geisha*. In 1931 it was discovered in woods in Southwest Ethiopia but mostly is cultivated in Panama and Costa Rica. Farmers cultivate *Geisha* at higher altitudes to have the most intense flavor. The taste is distinctive after flowers, especially jasmine, it is sweet, gentle a little bit like tropical fruit, and sometimes reminds of a tee. Of course, there are many other varieties of *C. arabica*. (Veselá 2018)

2.2.3. Coffea canephora

If you are buying coffee in the supermarket, it will usually be *C. canephora.* It is cheaper and much lower quality. It thrives at lower altitudes between 200 – 600 meters above sea level. This coffee tree can grow over 10 meters the ideal cultivation requires a warmer and more stable climate with temperatures of 24 – 29 °C (Herrera & Lambot 2017). It is less demanding than Arabica and the first harvest comes after 2-3 years. *C. canephora* does not suffer so much from disease and pests it is more resilient. It has more caffeine than Arabica, sometimes twice as much (Veselá 2018). The biggest producer of *C. canephora* is especially Vietnam, but rather worse quality. The taste is very bitter and, in the mouth, and tongue leaves a very strange tail, that´s because of the strong, earthy body. Chemically it is very different from Arabica, which means that the taste is also very different (Perrois et al. 2015). The big difference between *C. arabica* and *C. canephora* can be seen in the grain itself. *C. arabica* has flat and elongated grains with a curved groove in the middle, and soft green color sometimes with a bluish tone. Compared to *C. canephora* have more convex and rounded grains, the groove is straight, and the color is soft green with a brownish or greyish tone. Also, after the roasting, the grains are easily recognizable mainly by shape (Campuzano-Duque et al. 2021). *C. canephora* is bigger than *C. Arabica* it grows up to 8 – 12 m, and also have long flexible branches, shorter taproot, and shallower root. The leaves have 15 – 30 (40) cm in comparison to 5 – 15 cm long Arabica. The fruits are smaller about 8 – 16 mm long at *C. canephora*. In general *C. canephora* is more luxuriantly growing than *C. arabica* and has higher polymorphism (PlantUse English 2019).

2.3. The coffee harvest

The harvest season is different, it varies according to geographical location. For example in Central America, Ethiopia, and other countries that lie north of the equator harvesting is ongoing from September to December. Conversely, in countries south of the equator like Brazil, and Zimbabwe the main harvest is from April or May to August. States on the equator like Uganda and Columbia harvest coffee throughout the year. This is particularly the case for plantations that lie at different altitudes. The harvest begins in lower altitudes where coffee cherries ripen faster, and gatherers then move to higher altitudes (Thorn 2000). Harvesting can be carried out in several ways. A very important task is not to destroy the trees during the harvesting process. So, farmers should know what the most appropriate and gentle way for their crops is. Harvest has also a strong influence on the quality of the resulting drink (Toledo Castanheira 2020).

2.3.1. Manual harvesting (combing)

During the combing of coffee, cherries are all crops plucked from the branches in one pass through the plantation. It’s one of the manual harvesting methods and it’s called stripping (Vieira 2008). This is done in the following way: the comber takes a whole branch of the plant at the trunk of the bush, and up to the edge of the branch plucks all the fruit. But this method is not completely gentle to coffee plants, however, it’s quick and effective. In this method, there is a risk that unripe fruit will be picked, and they do not ripen further after picking. The collected fruits must be sorted after collection (Ramos Da Silva et al. 2018)

2.3.2. Selective combing

The second method of manual harvesting is selective combing. The combers carry out the harvesting of coffee several times in succession 8 to 10 days apart. This secures that only ripe cherries will be collected. This method is very gentle to coffee plants but also is much more laborious and more expensive. Mostly it is used for higher quality coffee, especially with *Coffea arabica* (Thorn 2000). The harvest is one of the most important works in coffee cultivation, so if it is handmade it represents 25 - 35 % of manufacturing costs (Vieira 2008).

2.3.3. Combing machines

The cost of harvesting is estimated to be half of all the money spent on farm and plantation resources, so the owners are looking for ways how to reduce these costs. One of the options is combing machines. The machines pass by the trees and shake the branches until the ripe fruit falls into a prepared container (Tongumpai 1994). After this, it is necessary that the fruits are sifted to remove debris in the form of leaves and branches. The plantation must be adapted to combing machines. The coffee plants must be grown in rows, the surface must be flat, and the soil soft. This method is as unfriendly to plants as hand combing but it's faster. That’s the reason why it is so popular. Of course, this method can be only used on flat fields. It’s most common in Brazil and Australia (Thorn 2000).

2.4. Coffee beans processing

Every cherry from the coffee plant must be further processed. It depends on what kind of coffee plant it is because each coffee bean had different methods of processing. Three primary methods are dry-process (natural), semi-washed-process, and wet-process. Each of these following methods immediately after harvest has different unique methods, but more importantly different processing difficulties (Kleinwächter et al. 2015). The process has a big impact on the final taste and the price of the coffee. In earlier times individual countries prefer specific methods of processing coffee beans. For example, in Central America and Rwanda, they preferred the wet process, but in Brazil, they tended to use the dry or honey process. But nowadays farmers are willing to try new methods and techniques of processing, which create new tastes and increase the value of their harvest. It is especially because of the high demand for fine coffee of the highest quality (Veselá 2018).

2.4.1. Dry-process

As Augustín 2016 is a dry – process is the oldest, simplest, and most inexpensive but requires the skills of the farmers. It is mostly used on plantations in water–scarce areas and used for lower-quality grains. Most often you will come across this process in Ethiopia and Brazil. The farmer's coffee cherries spread evenly on the ground, for example on concrete, stone floors, or mats. The fruits must be spread evenly so that air and sunlight can reach them all equally. Also, they must be in regular intervals turned and rummaged, so that they dry evenly. Care must also be taken to make cherries not dry out or start to ferment. At night and when it rains the fruits must be covered. This process takes up to one month (Ghosh et al. 2014). Sometimes, farmers speed up this process using mechanical dryers, but it's tricky because the beans can be damaged. After that, the beans are still stored in silos for some time to lose even more moisture. The dried beans go all over the world after one or two months. The beans from this method have a stronger and more pronounced body. Various substances from the skin and pulp during weeks penetrate the beans and that brings sweety, honey, nutty, and chocolatey flavor (Ghosh et al. 2014; Veselá 2018).

2.4.2. Honey process (semi-washed process)

It is called the honey process because the coffee beans are dried together with the mucilage and after being dried, they produce a substance like honey or sugar. The honey process of coffee cherries is ranked between the dry and wet processes and on every plantation, the farmers have different methods of this processing (Bastian et al. 2021). On some plantations, same as at the wet - process wander the cherries to a water bath, where a selection of ripe and unripe fruits takes place. Sorted fruits continue to peel machines, where they are stripped of almost all pulp and the upper skin. After that, the farmers leave the beans washed with water and in parchment with minimum rest of the pulp on the surface, which they dry in the sun as in dry coffee processing. This method is called *pulped-natural* and farmers use much less water. It is very popular in Brazil (Várady et al. 2022).

Other plantations choose the option where they consume almost no water. The cherries are straight peeled from the skin and the beans are dried with the parchment, especially with the pulp on the surface (Veselá 2018). The honey process is further divided according to how much pulp remains on the bean on the skin. *Black honey* has a large amount of pulp, *red honey* has a medium amount of pulp, and *yellow honey* has a small amount of pulp. The amount of pulp can be identified by the color of the dried pulp on the beans. The coffee from each variant tastes a little bit different. These methods use a lot in Central America (Cao et al. 2022).

As Cao et al. 2022 in this process, it needed to frequently rake and turn the coffee cherries. Especially the ones with more pulp, even several times a day, to prevent them from becoming moldy. On some plantations, they turn the cherries every 15 minutes. In this process, coffee beans have fine but distinctive body and do not include sour tones, often, on the contrary, sweet (Bastian et al. 2021).

2.4.3. Wet-process

The wet - process of coffee beans is more demanding than the previous ones. It is necessary to have enough water on the plantation because the consumption is about 120 to 160 liters of water. The modern farm is working with the system of recycling water and its return to process, which reduces costs plus it’s more environmentally friendly (Figueroa Campos et al. 2020).

In the first phase, the coffee cherries are washed with enough water in special tanks. This gets rid of all other unwanted particles such as leaves, and twigs, and mainly it separates ripe and unripe fruits. Immediately after washing and selection follow the removal of the upper skin and part of the pulp in machines (Cao et al. 2022). It is important that the peeling takes place within 24 hours of harvesting. Later, the process is more difficult, and the beans could become damaged. The ripe beans sink to the bottom and separate them from the unripe beans that´s what the wash channels are for. Loose skins are removed by a stream of water (Bastian et al. 2021). The second phase of the wet process is fermentation during which the mucilaginous sheath is separated around the bean. In the concrete tanks, the fermentation is carried out by *Lactobacillus acidophilus* for 12-72 hours. During fermentation, lactic acid is produced, which lowers the pH, and creates an acidic environment. The mucilaginous layer is separated by the enzymes present. If the fermentation process takes too long the beans could begin to rot, so there must be a regular check (Gebremariam Woldesenbet et al. 2014).

After this process, the drying of coffee beans begins because they contain about 50 % of humidity which needs to be reduced to be stored well and don´t become moldy. The drying time depends on the ambient temperature about 12 to 15 days (Augustín 2016). They dry them with hot air, in dryers, or naturally in the sun. In this case, the beans are spread over a large asphalt or concrete area and are necessary to turn them regularly and evenly. Depending on the surface on which the coffee beans are dried they acquire different flavors and aromas (Poltronieri & Rossi 2016). For example, the drying on asphalt surfaces takes advantage of the high temperatures of heated asphalt because it heats up faster. But it’s inappropriate because tars and other toxic substances from asphalt penetrate the beans and cause uneven drying and can pose a health hazard. Drying on the concrete area is much healthier because it heats up more slowly and raking beans is easier. On clay substrates, there is a risk of contamination during drying. The very good surface is sandy because it absorbs humidity well. For wet-processed coffee typically fruity, floral aroma and sour taste (Gebremariam Woldesenbet et al. 2014).

2.5. Coffee roasting

The required aroma and taste of coffee arise during the roasting and depend on temperature and time. During the roasting process, the coffee beans go through a series of reactions that lead to the formation of aromatic compounds. Also, there are several changes in chemical composition. Depending on the processing conditions, the composition of roast coffee varies and is characterized by the roasting grade reflected in the beans' external color. The color ranges from light to dark brown. In general, there are three types of roast coffee: light, medium, or dark, and depend on the degree of roasting (Franca et al. 2009). The temperatures are very high, so immediately after the beans must be quickly cooled to stop the process. Roasted beans are much lighter because the water has evaporated. It takes many years for experts to learn when perfectly roasted coffee is done, sometimes it can be ruined in seconds. It depends on a personal choice of which roast is perfect, which often has an impact on geographic location and national preferences (National Coffee Association of U.S.A. 2023).

In general, there are three stages of roasting: drying stage, browning stage, and development stage (roasting stage). The first stage is drying. The humidity of the coffee bean is 8-12 % so firstly it's necessary to dry it before the actual roasting begins. This phase takes about 4-8 minutes in a drum roaster with a temperature of 160 ºC. It's essential to be very careful with the beans which cannot burn at the beginning because of big heat. The second phase is the browning stage. When the temperature reaches 160 ºC the coffee begins to smell like toasted bread and hay at this moment aroma precursors start to transform into aroma compounds (PAULIG BARISTA INSTITUTE 2023). Finally, the beans are ground by using various types of grinders. Some of the roast beans are packed and supplied as whole unground beans. The final step is to vacuum pack the coffee and send it out into the world (Mussatto et al. 2011).

Figure 3. Coffee beans in each stage of the roast process from green to black

Author: Sweet Maria´s coffee library, 2023

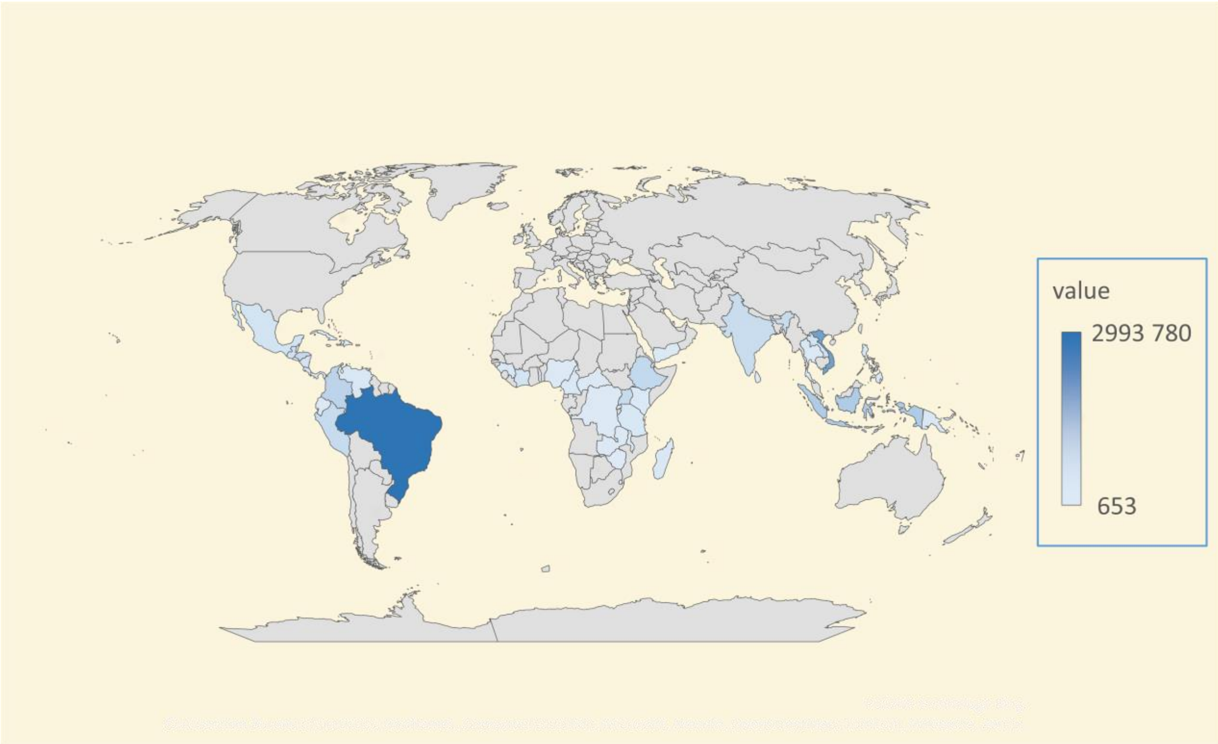
2.6. World production of coffee

As Tuomilehto 2013 coffee has become the second most-rated commodity in the world after oil. Coffee beverages belong to the most consumed pharmacologically active substances in the world. It is assumed that coffee drinking has become a regular part of life around the world. Some estimates claim that more than half of Americans drink coffee every day, but Europe is pretty much the same (Tuomilehto 2013).

The coffee year 2021/22 was a year of recovery after the global Covid-19 pandemic. The total production of coffee in the year 2021/22 was 167.2 million bags (1 bag is 60 kg) it´s a decrease of 2.1% compared to 170.83 million bags in the previous year. *C. arabica* had a production of approximately 93.97 million bags compared to *C. canephora* which had 73.2 million bags. Production in South America was about 77.5 million bags, in Asia and Oceania was 51.4 million bags, 19.0 million bags were produced in Mexico and Central America, and Africa´s production was 19.27 million bags in the year 2021/22 (International Coffee Organization 2023).

2.6.1. The biggest producers of coffee

Graph 1. The world´s biggest producers of coffee in 2022. Source: (FAO 2023)



The ranking of countries in the list of production over the years is changing. But for a long time, the biggest producer of coffee is Brazil, in second place is Vietnam, and the third biggest producer is Indonesia (FAO 2023).

2.7. Chemical composition of coffee

2.7.1. Caffeine

According to Santos et al. 2021 the chemical composition of coffee is strongly reflected in taste, aroma, and texture. One of the most well-known chemical substances is caffeine, which is part of the coffee beans and is also produced by a series of reactions during the roasting process. Nowadays, there is speculation that caffeine has become the most common psychoactive substance in the world (dos Santos & Boffo 2021). The value of caffeine which is in raw *C. arabica* coffee is between 0.8 % and 1.4 % (w/w) and for *C. canephora* the value is about 1.7 % and 4.0 % (w/w) (Mussatto et al. 2011). An ideal home-brewed coffee is between 30 mg and 175 mg of caffeine in a cup of 150 ml (Tuomilehto 2013). The coffee beverages are created of caffeine and chlorogenic acid in a molar ratio of 1:1 that is mean in the coffee sample is about 10 % of caffeine and 6 % of chlorogenic acid (Belitz et al. 2009).

Caffeine is a natural alkaloid, and it is also present in other commodities like tea, soft drinks, chocolate products, Yerba maté, energy drinks, and medicines. As for the plants, for example, caffeine is found in 23 different species of the genus *Camellia* (Mussatto et al. 2011). It is in the young leaves of the first flush shoots and has about 2-3 % caffeine depending on species. Another plant where caffeine is the genus *Theobroma* is in cotyledons of mature beans about 0.6 to 0.8 %, and a little less 0.5-0.6 % are in the shell of the seed. The next interesting plant in which we can find the substance of caffeine is *Ilex paraguariensis* from which the beverage produced is maté. The most caffeine is found in young maté leaves about 0.8 to 0.9 % therefore in fruits, bark, mature leaves, and wood is much less substance (Ashihara & Suzuki 2004).

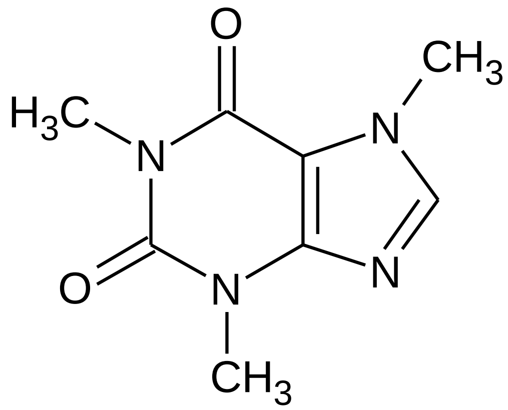


Figure 4. Chemical structures of caffeine

2.7.2. Effects of caffeine on health

After consuming coffee, caffeine is very quickly and essentially fully absorbed from the digestive tract to blood circulation. Also, is easily and quickly distributed throughout the body. Within an hour to an hour and a half after ingestion is the maximal concentration of caffeine in the blood (Nawrot et al. 2003). It is generally known that after cup of a coffee, the mood and behavior of humans change. Which is an increase in energy, motivation to work, more focus, self-confidence, mental well-being, and others (Depaula & Farah 2019). These effects of caffeine are perceived as positive ones. But there are a lot of discussions and opinions from experts if it is effects of caffeine are beneficial or harmful to health. The opinions of epidemiologists claim that regular coffee drinking has several health benefits as well as a lower risk of diseases such as Parkinson´s and Alzheimer´s. And not only this but it also has a beneficial effect on liver function and maybe even has an impact on weight loss (Mejia et al. 2014). From the information reviewed by Nawrot et al. 2003, it is a possible conclusion that with ordinary, healthy adults with a moderate income of caffeine (400 mg daily) it is not connected to unwanted effects. That means for example cardiovascular effects, general toxicity, increased risk of cancer, and influence on male fertility. On the other side, some studies show that coffee is linked with increased cholesterol levels and blood pressure (Nawrot et al. 2003).

The negative effects of caffeine on human health are considered to be depression, sleeping difficulties, rapid heart rate, and tremors. When someone suddenly drops the caffeine it may cause headaches, sleepiness, irritability, and sickness. In sensitive populations, such as pregnant women, children, the elderly, and people with heart diseases, the effects can be seen at lower doses of caffeine. So, they should limit their coffee consumption to avoid the side effects of caffeine (Wolde 2014).

Caffeine Intake Recommendations

Although there are many publications dealing with caffeine intake recommendations it is hard to describe an equal formula for everyone. It depends on many factors like age, body weight, sex, habituation, and nutritional status. According to European Food Safety Authority (2015) is the safe limit of caffeine is 400 mg per day for adults and 200 mg in single doses which is about two regular cups of coffee with 125 ml. The limit for a pregnant and lactating woman is 200 – 300 mg/day and for children is recommended max of 3 mg/kg per day (dePaula & Farah 2019). Once the daily dose is higher than 15 mg caffeine per 1 kg of body weight it can be toxic to gastrointestinal, nervous, and cardiovascular systems. If we convert it to a person weighing 70 kg, 1 g of caffeine can cause problems such as acute poisoning and intoxication (EFSA 2015).

2.7.3. Caffeine and students

It is common knowledge that students are the biggest consumers of coffee or caffeine. During the semester, they are required to do many seminar papers, presentations, and other stuff, and eventually comes the exam period. So, for several months most of the students live under pressure from deadlines and exams. To handle school and sometimes even work they need help from various supportive substances.

There are articles by (Ranjith Raj et al. 2018; Narayanan et al. 2021) that, through questionnaires target caffeine in college students. In each article, several hundred students were surveyed with similar questions. Mostly the questions were if the students drink coffee or other drinks containing caffeine. If the consumption helps with the school results, it extends focusing and working time, how often they used caffeine, and mainly why they consume the products with caffeine. The most common beverages were coffee, tea, energy drinks, and soft drinks. Most of the respondents in the questionnaire answered if they consume caffeine, it is for concentration, to handle stress, and just to be more productive. Even in Khan et al. 2015 article, some college students think that beverages with caffeine increase IQ and self-confidence.

A very important question is also if students have any negative side effects after ingestion of caffeine. According to Idris et al. 2022 over half of the responders answered that they have some untoward effects in connection with caffeine. Tachycardia, gastric pain, dizziness, and diarrhea were the most common issues. As R B Ranjith Raj et al. 2018, the students were asked the same question, but the answers are a little different. Less than half of respondents don´t know about any side effects, and the rest replied that they have insomnia, thirst, more frequent urination, et al. All these following symptoms are common in the general population.

2.7.4. Other substances than caffeine

Coffee is generally composed of many chemicals and the biggest representation has caffeine and chlorogenic acid. Of all the acids in coffee, chlorogenic acids are the most abundant. It is also one of the biologically active substances and slows the absorption of carbohydrates. Among other things, it also has some health benefits like anti-diabetic, anti-obesity, anti-carcinogenic, and more (Belitz et al. 2009). Another substance found in coffee is kahweol and cafestol they are classified as lipids. A major source of them can be found in unfiltered coffee which causes increased cholesterol. Interestingly, these two substances can be found only in Robusta coffee, and they are degraded in the roasting process. Anyway, chlorogenic acid, lipids, and caffeine are the most abundantly represented chemicals in coffee and some additional substances are carbohydrates, vitamins, minerals, alkaloids, and proteins. For an idea, a cup of black coffee consists of almost no carbohydrates, protein, or fat, and the energy value is about 1-2 kcal (Lire Wachamo 2017).

3. Aims of the Thesis

The first theoretical part of this thesis is focused on the history and botanical description of *Coffea spp.* It is also aimed at all the different processes involved in the processing of coffee beans, such as coffee harvest, coffee beans processing, and roasting. There is also a mention of world production and the biggest producers of coffee. An important part of this literature review is about caffeine, and it is properties, especially for students.

The practical part of this bachelor thesis is to compare caffeine in coffee beverages from seven different places on the campus of the Czech University of Life Sciences Prague.

4. Method

4.1. Materials and methods

4.1.1. Caffeine analysis using HPLC-DAD

Determination of caffeine was carried out based on high–performance liquid chromatography HPLC equipped with a diode array detector DAD. The principle of HPLC separation is about sample separation between a stationary phase (absorbent material on the column) and a mobile phase. Every component of the sample is expressed differently by the absorbent material, which causes a separation of the components and they flow out of the column. The detector records the volume of the substance and the time. In the experiment, the detector with an array diode is used for detecting particles that absorb ultraviolet or visible light. Dividing the remaining light spectrally, the separated light is detected by specific light–sensitive diodes (De Luca et al. 2018; da Silveira et al. 2020).

Chemicals used

- Demineralized water – purified using Milli-Q Plus (Millipore, Germany)
- Methanol for HPLC (Lach-Ner, Czech Republic)
- Caffeine (Sigma–Aldrich, Germany)

Apparatus

- Analytical scales (0.1 mg accuracy) Mettler AE 200 (Mettler Toledo, Switzerland)
- Infinity 1260 II. HPLC system (Agilent, USA):
  - Wide-range DAD detector 1260 Infinity II. (Agilent, USA)
  - Automatic Vialsampler 1290 Infinity II.
- Vortex SA 7 (Stuart, United Kingdom)
- A syringe with a PTFE membrane filter (0.45 µm)

Analysis conditions

- Column: Infinity Lab Poroshell 120, 2.7 µm C 18, size 150 x 3 mm (Agilent, USA)
- Mobile phase: methanol: demineralized water (ratio 40:60) – isocratic elution
- Detection: diode array detector at 264 nm
- Mobile phase flow rate: 0.25 ml/min
- Column temperature: 35 °C
- Length of analysis: 7 minutes
- Analyte (caffeine) retention time: 4.5 minutes

Collection of samples

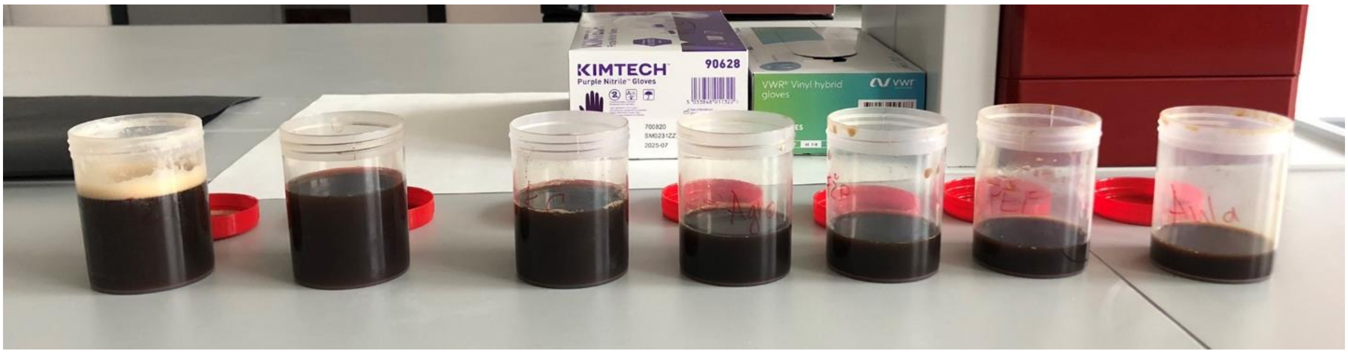


Figure 5. Collection of samples

The analysis of each sample was performed twice. In each of the locations where the research was carried out, the coffee was prepared differently. In the Faculty of Environmental Sciences is a bistro called Ledňáček. Coffee was prepared using a coffee machine and the brand of coffee is Costa coffee, which is only in this bistro. In the Faculty of Agrobiology, Food, and Natural Resources the samples were used from bistro Hodně dobré jídlo, and the coffee was prepared from the lever espresso machine. A coffee sample from the library was also prepared from the lever espresso machine. As well as coffee from AULA Café. In Club C, for the preparation of coffee, a coffee machine is used. In the Faculty of Economics and Management, samples were taken from two different places, the first and last samples were from bistro Hodně dobré jídlo and it was prepared from the lever espresso machine. And middle sample was from Fresh espresso prepared by the coffee machine. Only one coffee automat was used in this research and that was from the Faculty of Tropical AgriSciences and the type of coffee was black coffee.

Preparation of samples

Samples were prepared from espresso coffee. The diluted samples were prepared of 1 ml of coffee and 9 ml of distilled water. Then the samples were filtered through filter paper to get rid of the impurities. Via syringe membrane filter PTFE 13 mm 0.45µm were samples injected into vials and stored in a freezer.

A calibration set was prepared from the base solution with caffeine concentrations of 1; 5; 10; 50; 100 µg/ml. Volumes of base solution (0.25; 1.25; 2.5; 12.5 ml) were pipetted into 25 ml volumetric flasks and filled until the graduation marking with the mobile phase. Linear trend estimation was created using Microsoft Excel M365 (Graph 2).

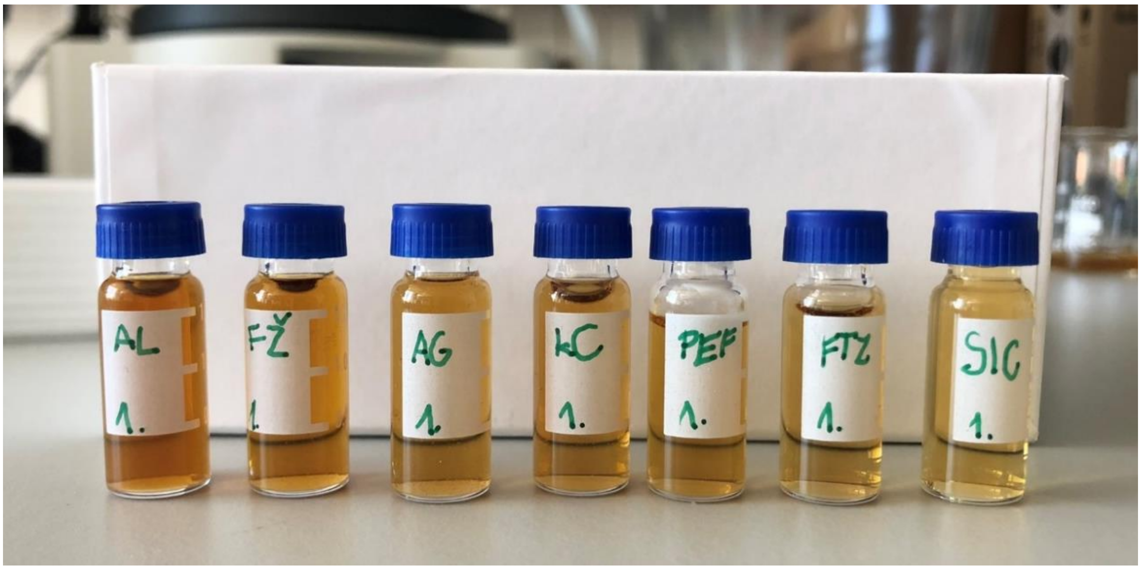
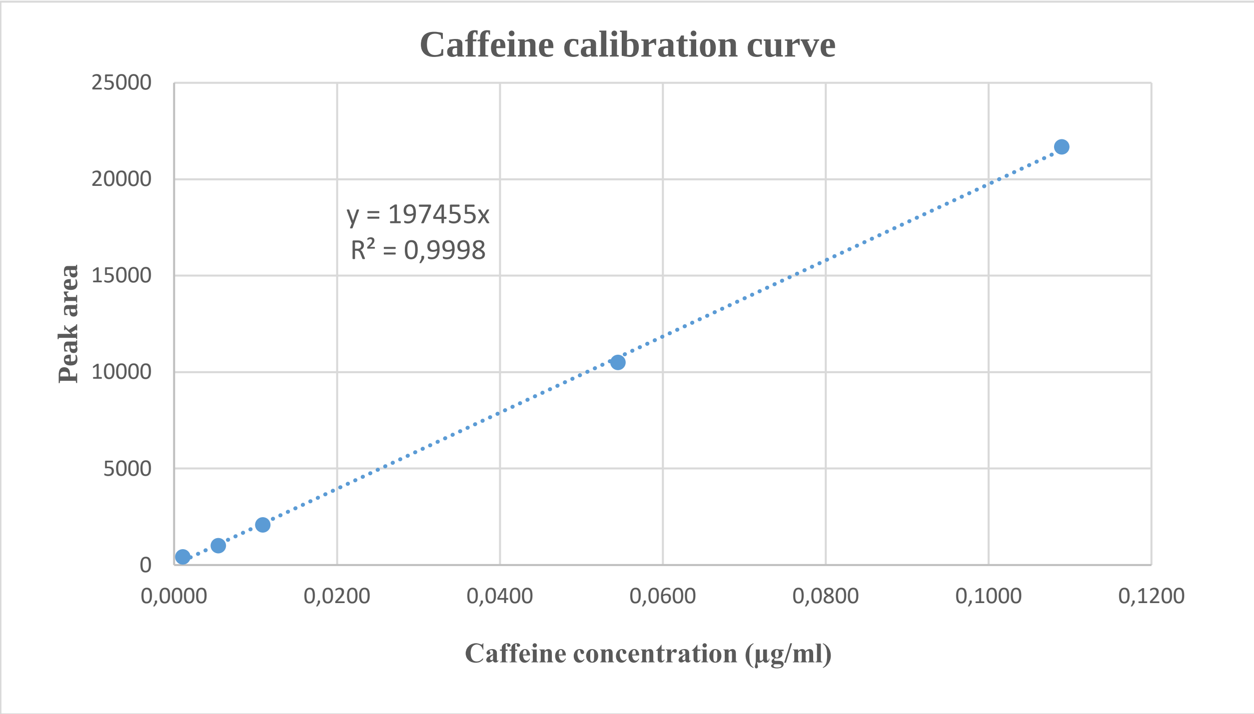


Figure 6. Prepared samples for measurement

Graph 2. Caffeine calibration curve



5. Results

The volume of coffee that varied the least over the course of the research is from FES and AULA Cafe, but the latter also has the lowest volume rate of all the studied samples. The highest and nearly consistent values in terms of volume are samples from Café + CO automat. In the Library buffet, the volume is the second largest. The biggest difference in volumetric values over the course of the research is in the samples from Club C. Otherwise, the volume remained more or less unchanged during the period under review.

Table 1. Samples of espresso coffee

Place of purchase	volume (ml)		
	02.06.2022	25.10.2022	11.01.2023
FES - Ledňáček	41	41	40
FAPPZ - Hodně dobré jídlo	50	50	58
SIC - buffet	115	108	114
AULA Cafe	30	25	26
Club C	60	58	90
FEM - Hodně dobré jídlo	36	*38	32
FTA - Café + CO automat	**120**	**120**	**118**

* FEM – Fresh espresso

The caffeine content mg/100 ml in the first two measurements of the highest values reaches espresso from AULA Café but in the last measurement, it is surpassed by coffee from FES. As for the caffeine content per serving (mg), the quality of the coffee did not change significantly over the period. In the first and last measurements, the highest caffeine content per serving have coffee from Club C, and in the second collection, it is FAPPZ.

Table 2. Caffeine content in samples

Place of purchase	caffeine content (mg/100 ml)		
	02.06.2022	25.10.2022	11.01.2023
FES - Ledňáček	261.23	282.60	**251.24**
FAPPZ - Hodně dobré jídlo	149.49	244.95	197.54
SIC - buffet	97.36	78.77	x
AULA Cafe	**298.99**	**296.56**	223.84
Club C	243.84	184.11	184.38
FEM – Hodně dobré jídlo	209.40	*161.67	210.72
FTA - Café + CO automat	51.65	63.59	53.71

* FEM – Fresh espresso x – invalid sample

Table 3. Caffeine content per serving

Place of purchase	caffeine content per serving (mg)		
	02.06.2022	25.10.2022	11.01.2023
FES - Ledňáček	107.10	115.87	100.50
FAPPZ - Hodně dobré jídlo	74.75	**122.48**	114.57
SIC - buffet	111.97	85.08	x
AULA Cafe	89.70	74.14	58.20
Club C	**146.30**	106.79	**165.94**
FEM - Hodně dobré jídlo	75.38	*61.43	67.43
FTA - Café + CO automat	61.98	76.30	63.38

* FEM – Fresh espresso x – invalid sample

6. Discussion

In this research, caffeine levels were measured from coffee samples obtained at the Czech University of Life Sciences Prague. The measurements were analyzed using high–performance liquid chromatography with a diode array detector. During the observation period, the caffeine content per serving of samples from the same place of purchase changed. A big difference is observed in the samples from FAPPZ, the increase in the second measurement from the first is 63.9 % higher. Samples that also experienced greater differences in caffeine content per serving are from Club C. In the first measurement they have the highest value of all, in the second is a decrease of 37 %, and in the last sample is an increase and again the highest value of all. The lowest average value of caffeine content per serving is 67.22 from FTA automat. As has already been written in this thesis recommended daily dose of caffeine is a max of 400 mg per day and 200 mg in single doses (EFSA 2015). Thus, all samples obtained fit within the limit of 200 mg of caffeine per dose. Coffee from Club C is possible to have only two coffees a day without being exceeded recommended daily dose. Very similarly, the coffee from FAPPZ uses coffee beans from the brand Costa coffee. It is possible to have 5 to 6 coffees a day from the FTA automat because the caffeine content is very low. Businesses in SIC and FEM are surprisingly in a very similar position as FTA automat but is necessary to note that there is a difference in the volumes of these individual samples. In general, the sample volume values are also very different, as is the caffeine content. The standard espresso size is around 30 ml which is a significant difference from the results in this thesis, which are very varied. This roughly corresponds to the samples from the AULA Café and FEM but the volume of coffee from SIC is almost four times bigger than it should be.

In this discussion is the intention to compare samples of this thesis with a student who did very similar research in the year 2020. The results from Sova (2020) generally reach much higher values compared to the results of this thesis. For example, the volume of samples from SIC in this thesis reaches on average 112.3 ml in Sova (2020) thesis is 31 ml, but the caffeine content per dose is similar. The average in this research from SIC is 98.52 compared to Sova (2020) which is 100.02. When comparing samples from FAPPZ the caffeine content per dose is more or less the same, versus caffeine content mg/100 ml in this thesis is about 78.7 % lower than the Sova (2020) thesis. According to the results of this research, coffee from Club C two times occupies a position in Table 3. with the highest values. And to compare it with the results of the Sova (2020) thesis the difference in caffeine content per serving is only about 10% lower than in this thesis. The average caffeine content per dose in the same places of purchase as Sova (2020) thesis is 123.16 mg in this thesis is 95.49 mg, which is about 22,5 % less. In summary, the results are different because there may now be different ways of preparing coffee in the places of purchase, different machines, or people who make the coffee.

The scientific article by Crozier et al. (2012) that inspired this thesis is primarily based on similar research. This article compared twenty different samples of espresso coffee from Glasgow. The range from the smallest volume to the largest is 23 to 100 ml in this bachelor thesis the range is 25 to 120 ml. The highest measured caffeine content per dose in the article is 322 mg, which is about 94 % higher than the values in this thesis. Otherwise, the lowest measured is 51 mg caffeine content per serving compared with this work is 58.20 mg. In Crozier et al. (2012) article, four samples have crossed the line of 200 mg of caffeine in one dose, which is according to EFSA (2015) the maximum recommended dose in one beverage. This was not proven in the research of this thesis. EFSA (2015) described that according to the recommended data, one cup of espresso (30 ml) has a range of 30 – 50 mg of caffeine. The recommended data have lower values and that makes a big difference between the results that came out in the article by Crozier et al. (2012) and the results in this thesis. Crozier et al. (2012) in the article write that values from EFSA (2015) do not show us a real image. Of course, there may be many other reasons why the results are different. It may be caused by the wrong preparation of coffee or improper use of the coffee machine. If the coffee machine is not cleaned regularly, various impurities can affect the coffee. It also depends on what kind of coffee you use in the preparation and their place of origin. May have used different blends of coffee *C. canephora* vs. *C. arabica*, different methods, and grade of bean roasting may have been used. How the beans were ground, very finely, medium, or coarse also makes a big difference in the final product.

Students, professors, and others should focus on how many of caffeine they daily consume. As uneducated people, we know that caffeine gives us energy, focus, and a clear head but we have to think about the flip side of caffeine. Especially vulnerable populations like pregnant women, children, and people with heart diseases should educate themselves about the effects caffeine has on their bodies and what can happen if they use more caffeine than the recommended daily dose for a long time. And students should also be careful not to become addicted to caffeine.

7. Conclusions

The first theoretical part of this bachelor thesis is aimed at the history of coffee. It deals with the coffee plant as a botanical description of *Coffea arabica* and *canephora*. Also, its focuses on techniques of coffee harvesting, methods of processing coffee beans, and the biggest producers of coffee in the world. Caffeine, the most abundant substance in coffee, is also mentioned in the theoretical part. It is the most consumed legal substance worldwide its effects on the psyche are scientifically proven. The really important part of this thesis concerns the effects of caffeine on students.

The second part is about research on the campus of CZU comparing caffeine in coffee beverages from different places. The research was conducted three times over a period of half a year. Results vary in both volume and caffeine content. The coffee volume is in the range of 25 ml to 120 ml. In caffeine content, in mg/100 the range is 51.65 to 298.99 and in caffeine content per serving is 58.20 to 165.94 mg. The results were also compared with each other over time, with some showing smaller or larger differences. It is important to focus on how the coffee is prepared and the employees in these specific companies should know the right way how to make excellent coffee. Students, professors, and the public should also watch how much caffeine they consume each day and educate themselves about the side effects of caffeine.

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