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The caffeine content in selected beverages and its health effect on vulnerable groups

BACHELOR'S THESIS

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Declaration

I hereby declare that I have done this thesis entitled The caffeine content in selected beverages and its health effect on vulnerable groups 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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Dušková Michaela

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Abstract

Caffeine consumption is a growing problem, especially among children and adolescents. Caffeine is a stimulant drug, widespread and used throughout the world. It is found naturally in nearly 100 species of plants, the most widely used being the genus *Coffea*. Caffeine is ingested daily in a variety of ways, most commonly from beverages. The most popular are coffee or tea, but it can also be found in soft drinks such as cola sodas or iced teas. These are the drinks most consumed by vulnerable groups. Caffeine is used to counteract fatigue or to stimulate. It can affect psychological and physical functions in the human body.

The aim of this research was to determine the content of caffeine in commonly available soft beverages and beverage powders using high performance liquid chromatography. The measured values ranged from 1.68 mg/100 ml to 24.18 mg/100 ml. Using the caffeine content and maximum daily caffeine intake, the amount of maximum daily consumption for the selected beverages was calculated, divided by age group, and then compared. The values were then compared with the stated caffeine levels from the labels and whether compliance with the stated amounts from major institutions occurred.

Key words: carbonated soft drinks, health, children, pregnant women, tea, cacao

Abstrakt

Konzumace kofeinu je rostoucím problémem, především mezi dětmi a adolescenty. Kofein je stimulační drogou, rozšířenou a užívanou po celém světě. Přírodně se nachází téměř ve 100 druzích rostlin, nejpoužívanější je rod *Coffea.* Kofein je každý den přijímán různými způsoby, nejčastěji z nápojů. Nejoblíbenější jsou káva nebo čaj, můžeme ho najít i v nealkoholických nápojích, jako jsou kolové limonády nebo ledové čaje. Právě tyto nápoje jsou nejčastěji konzumovány citlivými skupinami. Kofein se používá k potlačení únavy nebo povzbuzení. Může ovlivňovat psychologické i fyzické funkce v lidském těle.

Cílem tohoto výzkumu bylo stanovit obsah kofeinu v běžně dostupných nealkoholických nápojích a nápojových prášcích pomocí vysokoúčinné kapalinové chromatografie. Naměřené hodnoty sahaly od 1.68 mg/100 ml do 24.18 mg/100 ml. Pomocí obsahu kofeinu a maximální denní dávky kofeinu bylo vypočítáno množství maximální denní konzumace u vybraných nápojů a rozděleny podle věkových skupin a následně porovnány. Hodnoty byly následně srovnány s uváděným množstvím kofeinu z etiket a jestli došlo k jeho dodržení se stanoveným množství od významných institucí.

Klíčová slova: sycené nealkoholické nápoje, zdraví, děti, těhotné ženy, čaj, kakao

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List of the abbreviations used in the thesis

CDS	Chromatography data system
CNS	Central nervous system
CSD	Carbonated soft drinks
DAD	Diode Array Detector
DNA	Deoxyribonucleic acid
EC	European Commission
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
HPLC	High-Performance Liquid Chromatography
ICCO	International Cocoa Organization
USA	United State of America
WADA	World Anti-Doping Agency
WCF	World Cocoa Foundation
WHO	World Health Organization

1. Introduction

Caffeine is the most abundant methylxanthine in foods. It is present in nearly 100 species in 13 orders of the plant kingdom. Caffeine has been used in society for hundreds to thousands of years on every continent. In Asia it is consumed in the form of tea from *Camellia sinensis*, in Africa as coffee from *Coffea* spp., in South America, it is *Theobroma cacao*, *Paullinia cupana*, *Ilex paraquayensis*, *Cola nitida*. From these parts of the world, it later spread to Europe, Australia, and North America at the time of colonisation.

The effect of caffeine on the human body has been studied for several decades. Its consumption has an effect on all age groups. Consumption among children and teenagers is associated with changes in behaviour, mood, sleep duration, overweight or obesity, and even development itself. Its consumption is not recommended for pregnant and lactating women even though the effect on foetal development is not confirmed. In adults, it can have beneficial properties in effect on diabetes, exercise performance, or liver diseases. It is also used for its antioxidant, antimicrobial, and anti-inflammatory activities. Despite its many benefits, it also has many adverse effects on the human body, like poor bone structure, carcinogenicity, or the possible development of cardiovascular diseases. Among the elderly, has the potential to prevent the development of Alzheimer's and Parkinson's diseases.

Caffeine consumption among children and adolescents is on the rise. Mainly due to its easy availability in the form of beverages (carbonated soft drinks or energy drinks) but also in commonly sold sweets, like chocolate, chocolate bars, candies, or gums. The problem is low awareness of caffeine content in these products and a lack of information from producers. Caffeine concentration in commonly consumed beverages can be found further in the thesis.

2. Literature Review

2.1. Caffeine

Caffeine (1,3,7-trimethylxantine) is an organic compound with a purine base called xanthine, with theobromine and theophylline belonging in the methylxanthines. Methylxanthines are purine alkaloids, naturally found in plants, known for their stimulant effects. At room temperature, caffeine is a white crystalline substance, bitter in taste and odourless. It is well soluble in organic solvents, partially soluble in water but its solubility is enhanced by increasing temperature or by the formation of complexes, for example, with benzoate or citrate. Caffeine is completely absorbed by the gastrointestinal system within 45 minutes after consumption, with a half-time of approximately 4 – 6 hours. Its melting point is 234 – 239 °C (Bizzo et al. 2015; Willson 2018; dePaula & Farah 2019). The chemical formula of caffeine is shown in Figure 1.

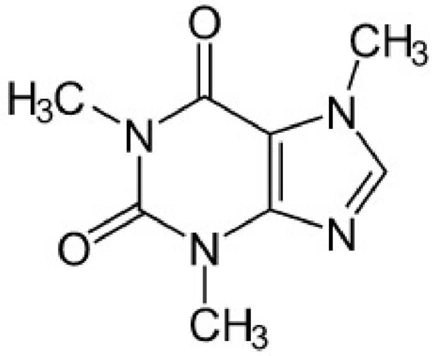

Figure 1. The chemical formula of caffeine
Source: Wikipedia (2022)

Caffeine is consumed by humans for thousands of years, the first known records come from China in 2737 BC consumed via tea (Willson 2018). *Coffea arabica* was used in Ethiopia and Arabia from 550 BC. In 1517, coffee was brought to Turkey, its consumption spread to Europe and was considered a luxury until the 18th and 19th centuries. Caffeine was first isolated in 1819 by German analytical chemist Friedlieb Ferdinand Runge (1794 – 1867). He also described the effect of caffeine on the human body. In 1875 the chemical structure was proposed by Ludwig Medicus (1847 – 1915). The first chemical synthesis was also performed by German chemist Hermann Emil

Fischer (1852 – 1919) in 1895 (Strunecká & Patočka 2011; Bizzo et al. 2015; Burdan 2015; dePaula & Farah 2019).

Caffeine is an antagonist to all types of adenosine receptors. There are four types of these receptors, A1, A2A, A2B, and A3. In caffeine's central effects, the A1 and A2A receptors are involved primarily and are widely spread throughout the brain. Adenosine is involved in the process of falling asleep and waking us up. Due to the similar chemical structure of caffeine and adenosine, these compounds compete for the receptors. Caffeine decreases the sensitivity of neurons to adenosine, which does not allow humans to feel tired. After the caffeine breaks down, the adenosine can again bind with these receptors and cause sleepiness. On the other hand, after habitual caffeine consumption, the neurons begin to synthesise more adenosine receptors which lead to the development of tolerance to caffeine (Roehrs & Roth 2008; Ribeiro & Sebastião 2010; Poltev et al. 2014; Ballesteros-Yáñez et al. 2018).

Caffeine affects the human body through these receptors that are not only in the brain but in almost all organs and tissue. Adenosine receptors in the heart are responsible for slowing down the heartbeat, and in the kidneys, they influence the volume of filtered blood and lead to less urine production. Caffeine blockages these receptors leads to increasing the heart rate and urine production (Ribeiro & Sebastião 2010; Poltev et al. 2014; Willson 2018; Ballesteros-Yáñez et al. 2018).

Caffeine stimulates the production of other chemical compounds, like adrenaline or dopamine. High adrenaline concentrations increase the heart rate or open up airways. High concentrations of dopamine increase the feeling of happiness, which is also one of the aspects that makes caffeine more addictive. There is also an association with coffee drinking along with eating something sweet, for example a cake. This fact leads to an increased risk of weight gain. It is also an example, of why artificial sweeteners are being added to beverages. These chemical reactions can trick our brain into enjoying these drinks and leads to buying them more (Temple 2009; Ribeiro & Sebastião 2010; Chu & Institute of Food Technologists 2012; Burdan 2015).

2.2. Sources of caffeine

Coffea species are the primary source of caffeine, it is also found in tea (*Camellia sinensis*), cocoa (*Theobroma cacao*), guarana (*Paullinia cupana*), maté (*Illex paraquayensis*), and in kola nuts (*Cola* spp.). It can also appear in several non-alcoholic beverages, sweets, dairy products, powders, capsules, medications, and cosmetics.

2.2.1. *Coffea* L.

Coffea is an evergreen shrub from the *Rubiaceae* family native to northeast Africa with over 120 species. Coffee beans are one of the most traded food products. In 2020, the world's production was 10.6 million tonnes, the biggest producer is Brazil with 3.7 million tonnes (FAO 2020). The majority of coffee plantations belong to the smallholders, it provides an important source of income and promotes employment and local development (Bichobr et al. 2013).

The most popular *Coffea* species are *C. arabica* (arabica) and *C. canephora* (robusta) because of their high caffeine concentrations. Lower levels of caffeine can be found, for example, in *C. liberica* (dewerei), *C. eugenioides*, or *C. excelsa*. Levels of caffeine widely differ in mentioned species. Robusta coffee has twice the amount of caffeine as arabica. There can be found even species with levels little as 0.6 – 1.2 % in dry seeds. Caffeine levels differ within the same plant species (*Coffea*), differs also within the state origin of the plant. Coffee that came from Brazil has a different concentration than coffee from Niger or Ethiopia. Caffeine concentrations in the coffee bean can change even during harvest, post-harvest manipulation, transportation, and even during the preparation of coffee drinks. In Cameroon in Central Africa can be found caffeine-free species *C. charrieriana*. This plant was found in 2008 and it is the first caffeine-free species found in Central Africa (Wintgens 2004; Stoffelen et al. 2008; Déchamp et al. 2015; Goldemberg et al. 2015; Ciaramelli et al. 2019).

Coffea arabica is a small evergreen shrub that grows up to 10 m. The leaves are simple, opposite, alternate, and dark glossy green, ovate, and 6-12 cm long. The buds often develop into lateral branches. The first flowering appears after 3 – 4 years of growth in late spring to early summer. The flowers are white, star-shaped, and grow in axillary clusters. The fruit is a drupe, immature berries are dark green and when ripe, they turn to bride red. Each fruit has 2 seeds, 8 – 12 mm long, and pressed together by a flattened side (Valíček 2002; Wintgens 2004; Déchamp et al. 2015).

Figure 2. *Coffea arabica*
Source: Iseli (2020)

Coffea canephora is the second favourite *Coffea* species. It is native to central sub-Saharan Africa. The leaves are large, pointed, and 15 – 30 cm long. The flowers are in clusters of 20 to 30, they are white to pinkish. The Robusta seeds are almost round and smaller than Arabica seeds. One of the many differences between *C. arabica* and *C. canephora* is that arabica is adapted to the tropical highlands 600 – 1500 m above sea level, and Robusta prefers tropical lowlands (0 – 800 m above sea level). *C. canephora* has a higher concentration also of chlorogenic acids and lower sucrose content than arabica. It is also considered to be more resistant to pests and diseases (Valíček 2002; Wintgens 2004; Bichobr et al. 2013; Goldemberg et al. 2015).

Coffea is primarily cultivated for the production of coffee from green beans. This favourite beverage has its characteristic flavour and aroma, which depend on the quality of the coffee bean. The quality can be affected by bean size, defects, moisture content, or chemical compounds (Bichobr et al. 2013). Caffeine and chlorogenic acids are responsible for bitterness and sucrose for their sweetness (Perrois et al. 2015). Coffee is also widely used as a flavouring agent, it can be found in dairy products like yogurts or ice creams, in pastries or sweets, also as an aroma agent in cosmetics. However, this plant has other uses, for example, the pulp is used to feed cattle in India.

Wood is used for the production of furniture. The coffelite is a type of plastic made from coffee beans. It can be also used as a biofuel or in the pharmaceutical industry.

Coffea harvest is an extremely critical part. Coffee seeds do not ripe at the same time and are manually or mechanically harvested when the majority is ripe. The seeds can be picked up one by one by hand, or the whole twigs are stripped. Mechanical harvest is done by shaking the tree or by stripping the branches. After harvest, the seeds need to be separated from the fruit. There are two types of processing; dry method and wet method. In the traditional dry method, the seeds are dried in the sun for up to 2 weeks. Dried seeds are then cleaned, and the husk is mechanically removed. The final product is the green coffee bean. The dry method is used in Brazil and Africa. During the wet processing are used only ripe berries. They are sorted, mechanically pulped, soaked in tanks, and fermented for 12 – 36 hours. After fermentation, the remaining pulp is removed, and the beans are then clean and dried on the tables or in the fields. This method is used in places where the fruit is harvested manually in Southern America or Asia. The wet method is used primally for *C. arabica* and produces better quality coffee. The dry method is preferred for *C. canephora* processing and produces more quantity. The most significant difference is that in wet processing the seeds are removed before drying (Wintgens 2004; Mussatto et al. 2011; Perrois et al. 2015)

2.2.2. Camelia sinensis

Camellia sinensis is a shrub from the family *Theaceae* native to the Yunan region in China and has been cultivated especially in Asian countries such as China, Thailand, and Japan for more than 5000 years (Chen et al. 2020). In Europe, tea is cultivated on Azores Islands in Portugal. According to FAO, the world's production of Camellia was 7 million tonnes in 2020 of which almost 6 million came from Asia and 3 million from China (FAO 2020).

Camellia sinensis is a small evergreen tree, trimmed to below 2 m and cultivated across the world in tropics and subtropics. In the wild tea tree can grow up to 15 m. The most important part of this plant is its leaves. They are 4 – 15 cm long and 2 – 5 cm wide. The leaves are light green, alternate, and coriaceous. Mature leaves are smooth, leathery, and bright green. In the leaves can be found a large number of polyphenols, like catechins, flavanols, or phenolic acid. The flowers are white to yellow-white, 2.5 –

4 cm in diameter, found in clusters of two or four with 7 – 8 petals. The fruit is a dark brown capsule containing 3 rounded-cuneate seeds (Valíček 2002; Tariq et al. 2010; Gupta 2012; Khurshid et al. 2016)

Figure 3. *Camellia sinensis*
Source: Bylinářství UH (2022)

Genus *Camellia* has 100 – 300 described species with around 1 500 cultivars. The two main varieties are *Camellia sinensis* var. *sinensis* and *Camellia sinensis* var. *assamica*. Var. *assamica* is used for black tea preparation, known as Assam tea, native to India. It prefers tropical areas and low elevation. The leaves are larger than in variety *sinensis*.

Camellia is primarily used for tea production, but it has other uses. Young leaves are used for the production of green tea, the older leaves for oolong and black tea, and the buds for white tea. The flower buds are used in Japan as a food garnish (Chen et al. 2020**).** The seeds, when pressed, are used as tea oil for cooking and sweetening (Namita et al. 2012). Tea extracts are also used in cosmetics, especially in skin care products, because of their antioxidant and anti-inflammatory properties. Green tea is used for the treatment of acne (Goméz 2019).

Namita et al. (2012) in a review suggest that Camellia may reduce risk of cardiovascular disease or some types of cancer. It also has other physiological functions such as body weight control, antioxidant, or antibacterial activities. Traditional tea is used as a blood sugar and body temperature regulator, or for improving mental health. Tea is recommended as a gastrointestinal remedy, like to stop vomiting or diarrhoea.

2.2.3. Theobroma cacao

Cocoa is a small tree from the *Sterculiaceae* family with origin in South America. The genus has 22 species of which *Theobroma cacao* L. is commercially the most important because of its seeds (Kongor et al. 2016). The International Cocoa Organization (ICCO 2022) reported that the worldwide cocoa bean production was almost 4 million tonnes for 2015–2016 and nearly 5 million tonnes in 2017. Cocoa is cultivated on over 70,000 km^2 worldwide between the Tropic of Capricorn and the Tropic of Cancer (23.3°) (Kongor et al. 2016). According to the World Cocoa Foundation (2012)**,** there are 5 – 6 million farmers in developing countries who produce around 90 % of cocoa worldwide.

Theobroma cacao is an evergreen tree (5 – 8 m) with a thick trunk and dense tree crown. The leaves are alternate, entire, dark green, and 30 cm long. The flowers are produced directly on the trunk and older branches. Cacao blooms in two main seasons but the flowers grow thought the entire year. One fruit is made from approximately 500 flowers. It is a 30 cm long berry with a smooth surface. Cocoa beans are about 2 cm long with white colour in the middle, they make about 25 % of the weight of the fruit. Freshly harvested seeds have on average 8.5 % protein, 30 % of fat, 10 % carbohydrates, 2.4 % theobromine, and 0.8 % caffeine (Valíček 2002).

Figure 4. *Theobroma cacao*

Source: Hill (2018)

We can divide this species into 3 variants, Forastero, Criollo, and Trinitario. The Forastero variety represents about 90 % of the world's cocoa production (Júnior et al. 2020). The Forastero beans have a higher pH after fermentation than Criollo beans. The chocolate produced from the Forastero beans is less bitter and less acidic than chocolate from either Criollo or Trinitario beans (Kongor et al. 2016). The Forastero variety is also more resistant to diseases and pests (Júnior et al. 2020). The Criollo beans can grow the fruits only on the trunk. It is cultivated especially in Central America, Madagascar, or Srí Lanka (Valíček 2002). The beans are white to ivory, their low yields and propensity to many diseases make them rare to cultivate. They represent about 5 – 10 % of the world's cocoa production (Júnior et al. 2020). The last variety is Trinitario which is a product of crossbreeding the two previous cocoa beans. The Trinitario trees are also susceptible to pests and diseases (Valíček 2002). This cultivar is known to have strong chocolate characters and a typical winery aroma (Kongor et al. 2016). Each variety provides beans with specific sensorial characteristics related to their origin, environmental conditions, and fermentation (Cinar et al. 2021).

Figure 5. Comparison of the cocoa variety
Author: Casa de Campo Living (2020)

Cocoa is mainly used in the food industry to produce chocolate, pastries, sweets, dairy products, and beverages. It can also be used in the non-food industry, in cosmetics, and in pharmaceuticals. The quality of the products depends on the cocoa variety, the climate, the soil, harvest, and post-harvest management (Júnior et al. 2020).

The cocoa tree becomes productive after four years from the planting and the yield increases annually until approximately 18 years old (Kongor et al. 2016). *Theobroma cacao* is harvested twice a year. The first harvest is at the end of the wet season in October and the second one is at the beginning of the wet season in March. It is crucial to harvest mature cocoa beans, they change their colour to red or yellow. They are chopped off with a special machete and then the seeds are removed from the husk. After separation, the cocoa seeds are placed in special containers, then fermented at 50 °C for a week. However, the length of the fermentation depends on the variety. Fermentation is one of the most important parts of cocoa processing because during the process the bean changes the colour to dark brown and the flavour is also changed to either bitter or slightly sweet (Valíček 2002). Another step is drying the seeds, usually in direct sunlight. During the drying process, the water content decreases. Roasting the cocoa beans can enhance the aroma, the bitterness alleviating. The roasting takes place at 80–130 °C. Roasted beans are separated into jute bags and transported to the manufacturer. The beans are then milled to produce chocolate. To produce cocoa powder, the beans are pressed. The pressed fat is known as cocoa butter (Valíček 2002).

Cacao may have beneficial and protective effects on human health because of more than 350 different components (Cinar et al. 2021). Bioactive compounds in cocoa beans are responsible for sweet, bitter, and acid taste. In cocoa, polyphenol compounds, like catechin, are around 12 – 18 % of total constituents (Ramos-Escudero et al. 2021). The content of cocoa phytochemicals can be affected by cocoa variety, bean maturity, the harvest time of post-harvest management. Cocoa beans are also rich in alkaloids, which are caffeine and theobromine, and minerals including calcium, magnesium, or potassium. Furthermore, cocoa has been shown to have higher antioxidant activity compared to tea (Cinar et al. 2021). According to various studies, cocoa polyphenols showed positive effects on reduction of the risk of cardiovascular diseases, colon and prostate cancer, Parkinson's and Alzheimer's diseases, or prevention of type 2 diabetes mellitus, and more (Cinar et al. 2021; Martín & Ramos 2017; Ramos-Escudero et al. 2021; Rusconi & Conti 2010). On the other hand, cocoa chocolate contains typically 15 – 35 % of added sugar, mostly sucrose. This can rapidly increase the glycaemic index with an impact on energy intake and health consequences (Cinar et al. 2021).

2.2.4. Paullinia cupana

Guarana is a ligneous liana in the family *Sapindaceae* with the origin in the Amazon basin. It was primarily found in the 109 southeast regions of Amazonas, in the Maués towns (Marques et al. 2019). The *Paullinia* genus has approximately 200 species, most of them are restricted to tropical and subtropical America (Schimpl et al. 2013). Nowadays, the guarana is mostly cultivated in Brazil, Venezuela, and Uruguay, almost 80 % of the world production comes from this region (FAO 2020). According to(Statista 2020), in 2019 approximately 2.76 thousand metric tons of guarana were produced in Brazil. Caffeine content can vary due to the preparation; however, the guarana provides about 50 mg of caffeine per gram. The stimulating effect of *Paullinia* can last longer than the effects of *Coffea* (Marques et al. 2019).

Paullinia cupana is a climbing plant from Amazon with ridged stem and alternate dark green leaves. It is a monoecious plant with small zygomorphic flowers. Guarana fruit is a green capsule when immature. When ripe, it has colours from yellow to red (Schimpl et al. 2013). The red fruit has one to four black seeds in a flashy red husk. The fruits ripen in October and November and are about the size of grapes. The seeds which did not fall from the liana, are then harvested (Valíček 2002). The seed's composition is estimated at 60 % starch, 15 % protein, 14 % phenolic constituents, and 2 – 6 % of caffeine (Santana & Macedo 2018). According to Marques et al. (2019), they found mean caffeine levels of 2.33 % in the kernel, 1.09 % in the tegument, and 2.15 % in the integral seed.

Figure 6. *Paullinia cupana*

Source: The tropical link (2022)

Guarana is cultivated only for its seeds which are consumed and commercialised. Around 70 % of guarana world production is used for beverages, while 30 % is used for the formulation of extracts, powder, and syrup (Santana & Macedo 2018). *Paullinia* seeds are ground and the pasta is made, which is used for making a drink similar to coffee (Valíček 2002). It is also included in energy drinks. As guarana powder, it is used in the cosmetics or pharmacology industry as an antidiarrheal and also helps against dysentery.

The processing of guarana seeds involves fermentation, roasting, and grinding. The seeds are manually picked up from the trees, and their shells are removed to facilitate fermentation. After fermentation, the seeds are roasted to decrease moisture which is important for beverage preparation. Then they are ground and separated into bags, and prepared for transportation to the manufacturer (Santana & Macedo 2018).

Guarana is mostly cultivated for its stimulating effects on the central nervous system; for example, it can improve alertness, reaction time, memory, mood, and physical performance which is why is the consumption of guarana associated with weight loss (Schimpl et al. 2013). The consumption of guarana is also associated with lipid metabolism and reduction of hunger. This is another reason why guarana is used in many weight loss products. Guarana is a promising option for the treatment of mental and physical fatigue related to cancer (Schimpl et al. 2013). Due to the content of tannins and amidons, guarana can be used as a treatment for diarrhoea and dysentery. According to case studies, guarana can be used as an adjuvant to diseases such as cancer, diabetes, or depression (Torres et al. 2022).

2.2.5. Ilex paraquayensis (Yerba maté)

Ilex paraguayensis, from the family *Aquifoliaceae*, is a native South American tree used for the production of Yerba Mate tea (Heck & de Mejia 2007). The family *Aquifoliaceae* is presented specially by the genus *Ilex*, with more than 600 species (Croge et al. 2021). *Ilex* is a tropical plant with about 80 % of the species native to Brazil. Most of the produced mate is consumed in South America. However, in the last few years maté beverages have become popular in the rest of the world (Croge et al. 2021). According to FAO (2020), the world's production of Maté was almost 1.5 million tonnes in 2020. All of this amount was produced in South America.

Ilex paraguayensis is a subtropical dioecious evergreen tree that can reach from 4 to 12 m in height. Its leaves are perennial, alternating, and ovate in shape with serrated edges. The flowers are greenish-white, unisexual, and in clusters. The fruits are 5 – 6 mm large, bright red drupes with four to five seeds (Valíček 2002). The Mate tree is flowering from October to November and the fruits are produced from March to June (Heck & de Mejia 2007). The leaves of *Ilex* contain 1 – 1.8 % of caffeine, 7 – 11 % of tannins and traces of vitamins (Valíček 2002).

Mate is mostly produced and also consumed in South America. The leaves are steeped in hot water to make a beverage called a mate or brewed in cold water to make tereré (Anesini et al. 2012). In the last decade, Yerba mate has gain a lot of popularity and it is used for the production of beers, soft drinks, sweets or cosmetics, and pharmaceutics (Croge et al. 2021). *Ilex* can be found in various energy drinks as an alternative to coffee, it is also added to ice tea (Anesini et al. 2012). Ilex species has the potential to be used as raw material in cosmetics or medicine due to its properties as a neurological stimulant, diuretic, antioxidant, or antibacterial (Heck & de Mejia 2007; Anesini et al. 2012; Gan et al. 2018; Croge et al. 2021).

Figure 7. *Ilex paraquayensis*
Source: Johnstone (2020)

The mate plant is cultivated for its leaves for tea preparation. Yerba mate is mostly cultivated in traditional or agroforestry systems; the products from these systems have a higher value (Croge et al. 2021). The harvest of mate is a combination of tradition and technology. The leaves are harvested when they are ripe. The leaves are directly exposed to a fire of 100°C to delay fermentation and to lost 25 % of water. The use of wood smoke during drying is important for flavour enhancement. After drying, the leaves are crushed and left in the bags to mature; this process is called aging and is strictly controlled by experts. Aging is important for developing flavour, colour, and aroma. The processing is completed by milling and packaging (Valíček 2002; Heck & de Mejia 2007; Croge et al. 2021).

Ilex paraguayensis has been proven to have several health benefits, including antioxidant, antibacterial, antimutagenic, and more. According to de Lima et al. (2019), the antioxidant and anti-inflammatory ingredients were proved to be effective against oxidative damage to DNA and have the potential to scavenge free radicals. Mate has been shown to have the potential for weight loss. It is used along with guarana in many dietary products (Heck & de Mejia 2007). Mate is also used for the treatment of fatigue, anxiety, and as a nervous system stimulant (Lutomski et al. 2020). These benefits are related to the phytochemical variability. In maté can be found several different chemical groups, such as alkaloids, saponins, polyphenols, or essential oils. The mate leaves also contain many vitamins or minerals (Croge et al. 2021).

2.2.6. Cola nitida

Cola nitida is a tropical tree from the family *Malvaceae* with origin in the West African rainforest. Even though the plant is indigenous to Angola, Togo, and Sierra Leone, it is extensively cultivated in Central and South America (Burdock et al. 2009). According to FAO (2020), the world's production in 2020 of kola nuts was 305 thousand tonnes. The caffeine content in cola nuts can vary between 1.5 % and 3.8 %, depending on the variety of the nut or harvest and post-harvest treatment.

Cola nitida is an evergreen, understorey tree of moderate height (9 – 12 m) with rigid, ovate leaves pointed at both ends (Burdock et al. 2009). The leaves are simple, usually sparse, and up to 33 cm long and 13 cm wide (Tachie-Obeng & Brown 2001)The flowers are yellow or white with dark red or purple stripes. The star-shaped fruit contain 8 – 12 seeds coated with slime. They need to be cleaned and dry to get a core that is known as a cola nut. The nuts are brown and have a bitter taste when fresh (Tachie-Obeng & Brown 2001; Burdock et al. 2009). The seeds contain 13.5 % water, 9.5 % protein, 1.4 % fat, and 45 % sugar and starch. The caffeine levels are about 2.8 % and theobromine 0.05 % (Brown 2001).

Figure 8. *Cola nitida*
Source: Hodge (2022)

Cola acuminata is another important *Cola* species. The main differences are that *C. acuminata* has curved and twisted leaves, fruits are rough to the touch, seeds have more cotyledons than *C. nitida*. Different is also the harvesting season, *C. nitida* is harvested from October to December and *C. acuminata* from April to June (Tachie-Obeng & Brown 2001).

Kola nut is mainly used as a flavouring agent in Coca-Cola, Pepsi Cola, and energy drinks. According to many institutions, like FDA or CoE, is Kola nut safe to use for consumption (Darfour et al. 2021). However, the nuts were primarily used as a medicine for the treatment of mental and physical fatigue. In the sub-Saharan countries chewing these nuts has a similar role and effects as drinking coffee or tea (Tachie-Obeng & Brown 2001). The kola nut pod husk is also used for feeding animals (Asogwa et al. 2011).

The kola nut is harvested by plucking the pod from the tree or manually picked from the ground. Traditionally they were consumed fresh or dried and cut into rounds. Nuts from *C. nitida* are sorted and dried on the ground, sometimes covered by jute bugs for up to 4 days. During this process, the immature nuts ripe and the testa wret which makes the nut easier to peel. The nuts can be also soaked in water for 24 hours, dehusked, and dried. They are stored in jute bags or baskets lined with sheets and inspection every week (Asogwa et al. 2011).

As stated above, cola nuts are used as herbal medicine. They have stimulating properties on the nervous system, antioxidant, anti-cancer, or antidiabetic properties (Darfour et al. 2021). Traditionally the leaves, flowers, or twigs are used as a remedy for fatigue, vomiting, diarrhoea, or chest complaints (Tachie-Obeng & Brown 2001). *C. nitida* have been associated with inhibitory activities against several pathogenic bacteria (Ebana et al. 1991).

2.3. Caffeine in beverages

Caffeine can be found in several other drinks than coffee, tea, and cacao. Caffeine is in carbonated soft drinks (CSDs), energy drinks, energy shots, and some fruit or fruit-flavoured and water beverages. CSDs are the primary source of caffeine among children, compared to coffee in adults (Mitchell et al. 2014). Typical soft drinks have moderated concentrations of caffeine; however, some beverages tend to have higher doses, such as energy drinks. Most soft drinks contain less than 25 mg per 250 ml (Allison & Chambers 2000). In 1980, the US Food and Drug Administration proposed the elimination of caffeine from soft drinks. This proposal was met with a response from soft drink manufacturers that caffeine is included in the drinks to impart the requisite flavour. This seems unlikely because most people cannot distinguish between caffeinated and non-caffeinated beverages at typical caffeine concentrations based on taste, and at higher doses, caffeine is extremely bitter (Temple et al. 2012). The flavour activity of caffeine in soft drinks is dependent on its concentration (Keast & Riddell 2007**).** According to Allison & Chambers (2000), the caffeine bitterness recognition in water is ranged from 58 to 602 ppm. Caffeine in higher concentrations may reduce the aromatic intensity of some flavours, for example, liquorice (Allison & Chambers 2000**).** In addition, caffeine may enhance the sweet taste of sweeteners (glucose), which can increase the density of dopamine D1 receptors in our brain. Caffeine can also activate the dopaminergic system and, paired with high doses of sugar may release more dopamine, which affects the preferences of soft drinks with added caffeine and sweeteners (Temple 2009).

In 2002, the European Commission released Commission directive 2002/67/EC of 18 July 2002 on the labelling of foodstuffs containing quinine, and of foodstuffs containing caffeine. In which the European Commission estimated the appearance of 'High caffeine content' label on beverages which is intended for consumption without modification with caffeine levels of 150 mg/l (European Commission 2002). This specified amount corresponds to 15 mg of caffeine in 100 ml of beverage. The FDA has also determined the maximum caffeine content in cola beverages. The amount allowed is 0.02 % of caffeine, or 71 mg/12 fl. oz, which is equivalent to 20 mg of caffeine in 100 ml (Reissig et al. 2009).

Caffeine used to be a naturally occurring component of cola and tea beverages because soft drink makers (such as Coca-Cola) used kola nut or black tea extract to make the drinks, and caffeine is naturally present in the kola nut and tea leaves (Kumar 2021). According to Juliano & Griffiths (2004), withdrawal symptoms can arise by staying away from a daily dose of only 100 mg of caffeine, which means that drinking 3 cans of Coke or Pepsi and 2 cans of Mountain Dew a day is enough to make you addicted. This can be one of the possible reasons why is caffeine added to soft drinks.

Coffee is also popular in its instant form. The production of instant coffee is the same as classic coffee. However, the roasted ground coffee is brewed, and the water is evaporated to produce the coffee extract. Instant coffee is made by using two methods: spray drying and freeze-drying. During the spray drying process, the coffee concentrate is atomised in the drying chamber. Stifling air with temperatures between 200 and 300 °C is blown into the chamber, which separates the water from the concentrate. The dry coffee extract (powder) then falls to the bottom of the chamber. This method is more popular because it allows large-scale production, reduces the cost, and the final products have a better appearance and solubility. During the freeze-drying methods, the coffee concentrate freezes to temperatures around - 40 °C, the water particles crystallise, and the ice is then extracted from the granules by sublimation. This method is more expensive because of the higher energy demand (Mussatto et al. 2011).

There is also an alternative for people sensitive to caffeine, decaffeinated coffee. However, during the chemical process of removing the caffeine, the coffee loses also compounds responsible for its signature taste and aroma. There are many ways of removing caffeine from coffee. They can be divided into three main groups: solvent decaffeination (methylene chloride and ethyl acetate), decaffeination using carbon dioxide, and water decaffeination (Swiss water and French water decaffeination). The solvent decaffeination process starts before the coffee beans are roasted. Green coffee beans are soaked in methylene chloride or ethyl acetate for several hours. Methylene chloride was very favourite until 1970 when was discovered in rats experiments that high doses of this chemical are carcinogenic. In response, the FDA has estimated its levels in decaffeinated coffee to be below 10 ppm. This solvent is removed by drying the beans at 100 °C and roasting. Ethyl acetate is a chemical compound that naturally occurs in coffee aromas and many fruits, like bananas or apples. In 1982, it was officially approved by FDA for decaffeination. After 3 to 5 hours of wetting, the beans are stream stripped, which helps to remove the residual solvent. Decaffeination with carbon dioxide is highly effective because of its ability to remove only the caffeine. Carbon dioxide is a natural coffee compound that leaves the roasted beans in gas form over several days. The green coffee beans are soaked with water to increase moisture content to 50 %. During the soaking, the caffeine transforms into the mobile form, which helps the caffeine to penetrate the bean. The swollen beans are put into the metal extractor, which is filled with liquid carbon dioxide, which dissolves the caffeine. The caffeine is later removed from carbon dioxide using water, which carries it away. This process takes place from 8 to 12 hours, until the caffeine content in the beans is 0,08 %. This method has several advantages, like non-toxicity of the carbon dioxide or low loss of other coffee compounds. Another way how to remove caffeine is water decaffeination. Caffeine has the ability to solubilise in water that varies with temperature. During the Swiss water decaffeination coffee beans are soaked in water, which absorbs caffeine along with flavour. Caffeine is removed using a carbon filter and the flavoured caffeine-free water is then used in another batch of beans. The caffeine is dissolved but the flavour components stay in the beans because they cannot pass into the flavour-saturated water. This process removes 100 % of caffeine. The French water decaffeination is similar; however, the water with flavour compounds and caffeine is purified through filters. The already dried beans are then resoaked in this caffeine-free water and the flavours return to the beans. This method removes 99.9 % of caffeine (Ramalakshmi & Raghavan 1999).

There are different types of tea, varying by colour, smell, and taste. All types come from the same plant and depended on changes during harvest and processing. Tea leaves are harvested manually to provide the highest quality. After plucking the leaves are withered to reduce the moisture. Green tea is a non-fermented tea, prepared by withering for up to 10 hours, then steaming to prevent fermentation. During the steaming process, colour pigments are broken down, which gives the tea its natural green colour. Rolling and drying follow (Chacko et al. 2010). Black tea is completely oxidised. After the withering process, the leaves are bruised or rolled to disrupt cell structures and release enzymes that activate oxidation. This process takes between 60 minutes to 3 hours in 20 to 30 °C. During fermentation, the leaves change colour to dark red, and a specific aroma is created. Fermented leaves are dried and stored, during this time the tea production is completed. Oolong tea is on the borderline between green tea and black tea. Fresh leaves are dried in sunlight, and then lightly bruised. The main difference between black and oolong tea is the length of oxidation which ranges 5 – 40 % for light oolong teas and between 60 to 70 % for dark oolong teas. After fermentation, the leaves are rolled and dried. White tea is known for its minimal processing. Young buds are harvested, withered for several hours or days, and then dried. It is produced predominantly in China and not well known in non-Asia countries because of its lower quantity production and high prices (Hilal & Engelhardt 2007).

There are many types of teas that differs not only in the way they are processed, but also in the origin, cultivation, harvesting and preparation. For Vietnam is characteristic green tea with jasmine or lotus flower, for Japan it is matcha, in England is preferred Earl grey served with milk.

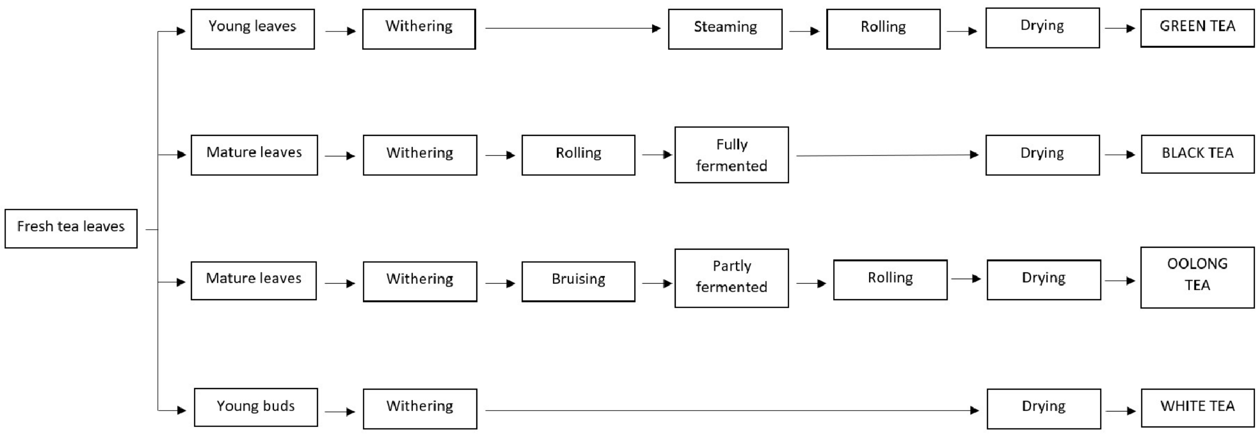

Figure 9. Comparison of tea processing
Author: Author's graph

Caffeinated beverages are also made from other plants than mentioned above where is caffeine naturally occurs. One of them is beverages made from Yerba maté, which is usually prepared as herbal tea. It is consumed in Brazil, Argentina, Uruguay, and Paraguay. The beverage is known as mate or chimarrão in Brazil, Argentina, and Uruguay, and it is drunk with hot water. In Paraguay, it is drunk with cold water and is known as Tererê. The leaves and twigs are dried, typically over a fire, and steeped in hot water (Colpo et al. 2016). The second plant is guarana, plants are commercially cultivated in Brazil of which 70 % are consumed by soft-drink industries to produce non-alcoholic carbonated drinks (Brazilian Amazon Consortium for Genomic Research (REALGENE) et al. 2008).

Caffeine has also been added to other beverage products such as caffeine-containing water Water Joe™ or Coffizz by Tchibo. It can also be found in non-beverage products, for example, caffeinated gum (Stay Alert™) and mints (Viter Energy™ or Rally). The Mars™ Corporation released a caffeinated version of the Snickers™ bar, called Snickers Charged (Temple 2009). Caffeine is also added to some non-food products. For example, shampoos that claim to promote hair growth, cosmetics, or weight loss pills.

2.4. Effect of caffeine on vulnerable groups

Caffeine is a worldwide consumed stimulant drug via several beverages, like tea or coffee. Effect on the human organism is a widely discussed topic. Caffeine can have a positive and negative influence on behaviour, mood, cardiovascular system, central nervous system, diabetes, obesity, fertility of men and women, miscarriage, cancer, and even brain development or taste preferences. Several of these effects are discussed below.

The government of Canada has determined the recommended maximum daily intake of caffeine for children under 12 years old to be under 2.5 mg/kg of body weight. Based on the average body weight of children, Health Canada sets the maximum daily intake (Tab. 1.).

Table 1. Maximum daily caffeine intake

Age	mg/day	References
4 to 6	45.0	Health Canada (2012)
7 to 9	62.5	Health Canada (2012)
10 to 12	85.0	Health Canada (2012)
13 to 17	100.0	dePaula & Farah (2019)
Pregnant and lactating women	200.0	EFSA (2015)
	300.0	Nawrot et al. (2003)
Adult	400.0	FDA (2018)

In addition, FDA has also determined the lethal dose of caffeine to be 1,200 mg which is equivalent to 0.15 tablespoons of pure caffeine.

2.4.1. Withdrawal symptoms

Firstly, it is necessary to specify the differences between tolerance, withdrawal symptoms, and dependence on caffeine. Tolerance is a reduced reaction to an agent that is often administrated for some time. Tolerance to caffeine usually develops within a couple of days, followed by a reduced release of several chemical compounds like adrenaline or renin (dePaula & Farah 2019). Withdrawal symptoms can occur after the disconnection of a regular administration or after the development of tolerance. Dependence is a state associated with withdrawal symptoms upon cessation of repeated exposure to the agent (Dews et al. 2002).

Withdrawal symptoms of caffeine generally occur about 12 to 24 h after cessation of caffeine consumption, reach a peak after 20 to 48 h and duration of about 1 week (Nawrot et al. 2003). For the body to desensitise to the acute effects of caffeine, it is required at least 4 days of withdrawal (Irwin et al. 2011). According to dePaula & Farah 2019, in some individuals, symptoms can appear 3 to 6 hours after consumption and can last for a week. More common is a shorter period of deprivation; for example after overnight caffeine abstinence, when someone did not have their morning coffee (Rogers et al. 2013). Caffeine withdrawal symptoms can be avoided if the caffeine doses slowly decrease.

One of the best-known symptoms is headache, which was described in 1952. According to Phillips-Bute (1997), withdrawal symptoms are not associated with high levels of caffeine. People who consume about 100 mg of caffeine per day; may experience a headache, sleepiness, yawning, fatigue, also a decreased alertness and activity. In moderate caffeine consumers, there can be found some severe symptoms, it was reported an increase of depression and anxiety after 2 days of abstinence (Phillips-Bute 1997). The effects of caffeine on humans are extremely variable, some people may never develop a tolerance or withdrawal symptoms (Temple 2009).

2.4.2. Mood and behaviour

Caffeine also has the ability to affect human mood and behaviour. According to scientific studies, caffeine improves basic cognitive functions such as alertness, attention, or reaction time. It has also influenced exacerbated jitteriness and anxiety, especially in individuals with pre-existing psychiatric disorders or those with a genetic predisposition to anxiety or depression. However, there are also inconsistent results of caffeine's impact on memory or vigilance. In adults, there are also reports of an enhanced feeling of self-confidence, sociability, motivation to work, or increased talkativeness (Richardson et al. 1995; Herz 1999; Castellanos & Rapoport 2002; Smith 2002; Aepli et al. 2015).

Caffeine consumed by children leads to an increase in fidgetiness, nervousness, and decreased sluggishness. These outcomes are associated only with high doses of caffeine or its habitual consumption. High doses also increased physical activity, task performance, and speech rate. Self-reported anxiety was not affected (Castellanos & Rapoport 2002; Temple 2009; Aepli et al. 2015; Watson et al. 2017).

2.4.3. Sleep

Sleep deprivation is a growing problem among teenagers. According to the United States National Sleep Foundation (Sleep Health 2015), as much as 80 % of adolescents do not receive enough sleep. There are many reasons why they go to bed late, for example, for educating purposes, reading, watching movies, or even playing video games, or for spending time on social media. Caffeine is the most widely used stimulant for delaying sleep (James et al. 2011; Lodato et al. 2013).

According to many studies, caffeine consumed before bedtime increases sleep latency and decreases sleep duration but do not affect REM sleep (Nawrot et al. 2003; Roehrs & Roth 2008). Sleep duration is also not affected by doses lower than 100 mg/day. Caffeine may affect sleep patterns and daily activities in children. According to Lodato et al. (2013), some teenagers even experienced withdrawal symptoms during the day, like headaches.

Higher caffeine consumption is associated with attending public schools, a lower education level of parents, or more sedentary activities. Caffeine intake increases on weekends, probably because of higher social interactions. Its intake also increases before or right after school (Clark & Landolt 2017).

2.4.4. Obesity and weight loss

Caffeine consumption is associated with both obesity and weight loss. As stated above, caffeine can be found in several soft drinks and sweets. The problem in these products is not the caffeine itself but the added sugars. This is concerning especially among children because adults prefer black coffee or diet soda (Temple 2009). Several studies have shown that children who consume sugar-sweetened carbonated beverages are more likely to be overweight. Consumption of soft drinks is linked with a poor lifestyle. According to Harnack et al. (1999), children who consume more soda per week also eat less milk, fruits, and vegetable.

On the other hand, caffeine increases metabolic rate, exercise performance, coordination, lipid oxidation, and lipolytic activities. It is used for weight management, especially weight loss. The influence on weight loss also has another coffee compound, chlorogenic acids. The regular consumption of this coffee polyphenol can reduce body fat, especially the abdominal fat, including visceral fat (Gökcen & Şanlier 2019). For these reasons, caffeine can be found in many weights' loss pills and metabolism accelerators. In 2003, the World Anti-Doping Agency (WADA) included caffeine in the list of stimulants banned from sports competitions. However, it was removed in 2004, because of the difficulty to differentiate if the caffeine was ingested to improve the athletic performance or as a habitual consumption (dePaula & Farah 2019).

2.4.5. Pregnancy

Caffeine consumption during pregnancy is a greatly discussed topic, and many studies have been done. However, there is a nonfinal verdict that indicates caffeine's influence on fertility or miscarriage. Several studies associated coffee drinking during pregnancy with low birth weight, subfertility, and spontaneous abortion (Fenster et al. 1991; Weng et al. 2008; Lyngsø et al. 2017).

Caffeine use has been linked to changes in hormone levels, tubal illness or endometriosis, a change in tubal transport time, and a reduction in fertilised ovum viability (Nawrot et al. 2003). Consumed caffeine is quickly absorbed from the gastrointestinal tract and easily crosses the placenta, where it is distributed to foetal tissues, including the CNS (dePaula & Farah 2019). That may affect the development of the foetus and generate adult-onset diseases (Lyngsø et al. 2017). Caffeine clearance is prolonged in pregnant women and its metabolic rate in the foetus is low due to low enzyme levels. It may also affect cell growth by raising cellular cyclic adenosine monophosphate levels and decreasing intervillous placental blood flow by raising circulating catecholamine levels (Weng et al. 2008). After the first trimester, the caffeine's metabolic rate decreases and the half-life increases from 2.5–4.5 hours to 15 hours during the pregnancy (Qian et al. 2020).

It is important to note that caffeine ingestion may also affect men fertility. That said, caffeine is capable of easily crossing human tissues, like blood-testis barriers (Nawrot et al. 2003). Men who consumed one to two cups of coffee per day had higher sperm motility and density than men who did not drink any. Men who consumed more than two cups per day, on the other hand, had lower sperm motility and density (Nawrot et al. 2003). According to Dlugosz & Bracken (1992), doses of caffeine greater than 400 mg/day may reduce sperm motility and/or increase the proportion of dead spermatozoa.

2.4.6. Diabetes

Diabetes mellitus is a chronic, metabolic disease characterised by hyperglycaemia, which is elevated levels of blood glucose. Type 2 diabetes usually occurs in adults resistant to insulin (Emami et al. 2019; WHO 2022). In 2019 was an estimated 4.2 million deaths among adults (20 – 79 years old) which contribute to 11.3 % globally (Saeedi et al. 2020). Diabetes is also associated with several other diseases and hazards, like a higher risk of renal disease, blindness, or amputation. Myocardial infarction or stroke are also experienced in patients with type 2 diabetes more than in healthy people (Emami et al. 2019).

Type 2 diabetes mellitus is a global health concern that has affected 2.2 % of the world population in 2000 and coffee consumption is associated with its reduction and better glucose tolerance (Heckman et al. 2010). Caffeine also reduces insulin sensitivity and has adverse effects on glucose metabolism. Insulin sensitivity is linked with caffeine's role as an adenosine receptor antagonist, which leads to difficulties in the transportation of glucose from the blood into the muscles (Emami et al. 2019).

According to the Strong Heart Study, a high level (12 cups/day) of coffee reduced the risk of developing diabetes by 67 % (Zhang et al. 2011).

In addition, studies comparing decaffeinated and regular coffee suggested beneficial effects of both drinks on glucose homeostasis if the dose is higher than 250 mg of caffeine. This indicates that other coffee compounds, like chlorogenic acids or magnesium, also have positive effects on glucose metabolism (Gökcen & Şanlier 2019).

2.4.7. Cardiovascular

Caffeine also has an influence on the heart. It is negatively and positively associated with cardiovascular effects, affecting heart rate, blood pressure, arrhythmia, and cardiovascular diseases, like coronary heart disease and acute myocardial infarction. Heart rate and blood pressure are affected by the activation of adenosine receptors and the activity of the sympathetic nervous system. After 1 hour of caffeine ingestion, the blood pressure slightly increases by 10 to 20 mm Hg (Cano-Marquina et al. 2013). Hypertensive individuals can experience a slight increase in both systolic and diastolic pressure for up to 3 hours after consumption of caffeine. Interestingly, decaffeinated coffee has also the ability to increase blood pressure (Jee et al. 1999; Nurminen et al. 1999).

Coffee compounds, cafestol, and kahweol are responsible for increasing blood lipids. These diterpenoid alkaloids are also known as coffee fats (Gökcen & Şanlier 2019). Elevated levels of blood lipids can cause fat build-up in artery walls which increases the risk for heart disease. Levels of these chemicals in coffee can be partially removed by preparing coffee using a paper filter.

Coffee drinking has been linked with the potential of developing coronary heart disease (CHD). This topic is widely discussed and yet there is no definitive answer if caffeine is responsible for the increase or decrease of developing this disease (Turnbull et al. 2017).

Caffeine consumption is also associated with increased occurrence of arrhythmias mostly because of experimental studies on animals. The reduced risk of arrhythmias is associated only with the consumption of high doses of caffeine. One of the less investigated heart diseases is stroke. There may exist an association between caffeine ingestion and a 10 – 20 % reduction of the risk of stroke (Cano-Marquina et al. 2013).

The inconclusive results of many studies may be due to a failure to consider using other substances like alcohol or nicotine. According to Nawrot et al. (2003), a single dose of caffeine less than 450 mg does not increase the risk of cardiac arrhythmia in healthy adults. It is important to note that currently available studies indicate that moderate caffeine consumption (up to 400 mg/day) does not negatively affect cardiovascular health.

3. Aims of the Thesis

The aims of this bachelor thesis were divided into two parts. Firstly, it is the literature review focused on caffeine, its chemical features, and natural sources in six different plants, their morphology, harvest, processing, and health properties. Basic information about caffeine in beverages, and its effects on human health, focused primarily on vulnerable groups, children, adolescents, and pregnant or lactating women.

The second part of this bachelor thesis was a determination of caffeine content in commonly available selected beverages and beverage powders. A comparison of measured caffeine and maximum daily consumption of these drinks is also included.

4. Methods

4.1. Materials and methods

Caffeine content was measured using 17 samples of non-alcoholic beverages and 2 samples of beverage powder (Figure 10). Analysis was performed using high-performance liquid chromatography (HPLC) with a diode array detector (DAD).

Figure 10. Store bought iced tea beverages

Author: Author's photography

Figure 11. Store bought cola beverages

Author: Author's photography

4.2. Determination of caffeine content using HPLC

Chemicals used:

- Caffeine (C0750, Sigma-Aldrich)
- Methanol (LiChrosolv, 1.06018, Merck)
- Demineralised water – purified using Neptune Analytical, Purity

Apparatuses used:

- Automatic pipets (Eppendorf)
- Ultrasonic bath (DT 103 H, Bandelin)
- Filtration apparatus
- Microfiltration (MicroSpin, PVDF, 0.2um, Chromservis)
- Centrifuges (EBA 200, Hettich)
- High-Performance Liquid Chromatography (1260 Series, Aglient)
- Computer (OpenLab CDS)

Conditions of analysis:

- Column: Eclipse XDB C18, size 150 x 4,6 mm (Agilent, USA)
- Mobile phase: methanol: demineralised water (40:60)
- Detection: DAD at 265 nm
- Mobile phase flow rate: 1,0 ml/min
- Column temperature: 35 °C
- Injection volume: 20 µl
- Analysis length: 10 min

Preparation of caffeine standard and calibration curve

The stock solution was prepared by dissolving 10 mg of caffeine in the mobile phase (100 ml) inside a 100 ml volumetric flask at a concentration of 100 µg/ml. A calibration series was prepared from stock solution with caffeine concentrations of 1; 2; 5; 10; 20 µg/ml. Volumes of stock solution (Table 2.) were pipetted into 1 ml HPLC transparent glass vials and a calibration curve (Figure 14.) was constructed.

Table 2. Caffeine standard preparation

	Stock solution 100 µg/ml [µl]	Mobile phase [µl]	Caffeine concentration [µg/ml]
K0	0	1000	0.0
K1	10	990	1.0
K2	20	980	2.0
K3	50	950	5.0
K4	100	900	10.0
K5	200	800	20.0

Sample preparation

Samples with cocoa had to be prepared differently than the rest of the samples. Recommended preparation of Granko by Nestlé is a 13.5 g (4 – 6 spoons) of cocoa powder mixed with 250 ml of milk. This is equivalent to 0.54 g in 10 ml. The Granko sample was prepared by dissolving 0.54g with 10 ml of demineralised water. Recommended preparation of Holandské kakao by internet recipes is 2 spoons mixed with 250 ml of milk. According to the internet page Rady kutilům (2015), 1 spoon of cacao powder measure 8 g. This is equivalent to 0.64 g in 10 ml. The cacao sample was prepared by dissolving 0.64 g with 10 ml of demineralised water. Both cocoa samples were dissolved in the ultrasonic bath (Figure 12.) for 30 min at 60°C. Firstly the samples were filtrated in a filter kit; secondly, by microfiltration using a filter with a pore size of 0.45 µm in a centrifuge EBA 200 with 4000 revolutions per minute for 5 minutes.

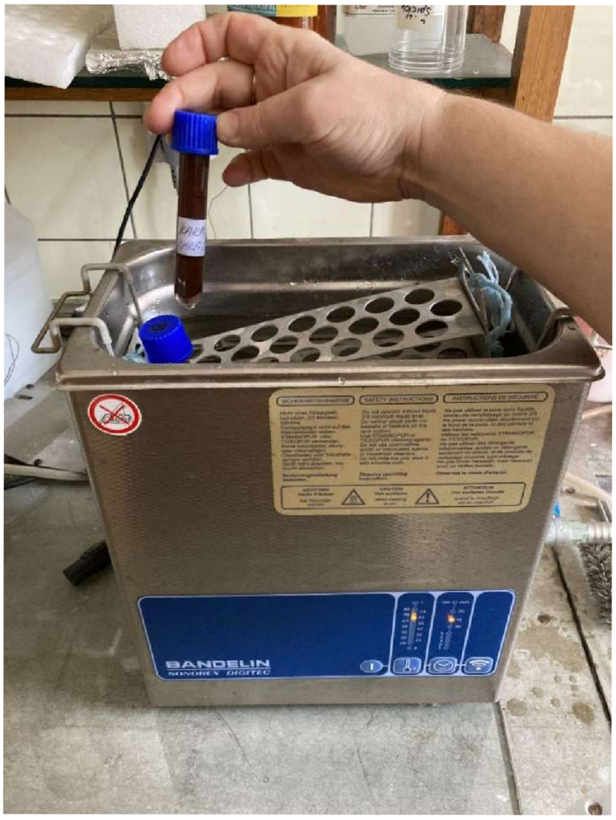

Figure 12. Cocoa samples in an ultrasonic bath
Author: Author's photography

Each sample was diluted 10 times with mobile phase into 1 ml HPLC vials (Figure x.) and analysed.

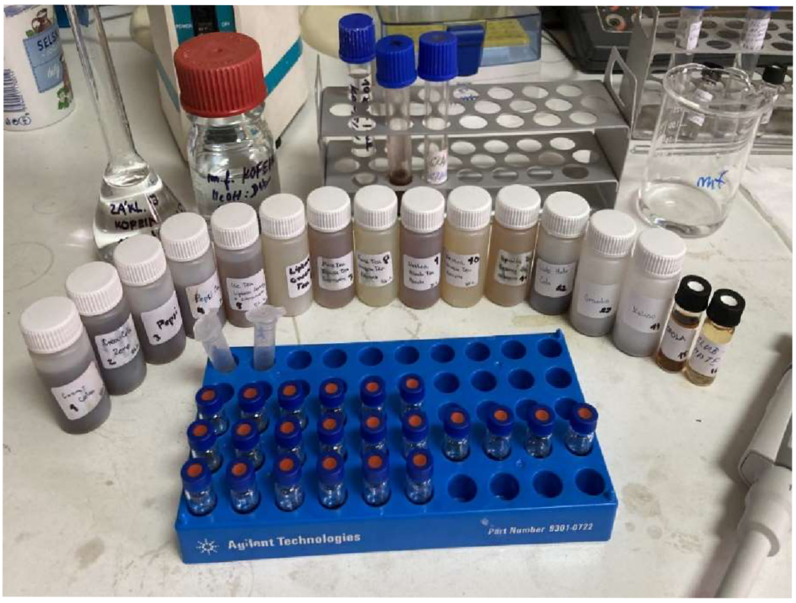

Figure 13. Caffeine standard and samples
Author: Author's photography

5. Results and Discussion

The calibration curve was created with peak areas and caffeine concentration obtained from HPLC determination. The creation of these curves is important for the determination of the concentration of an unknown sample solution.

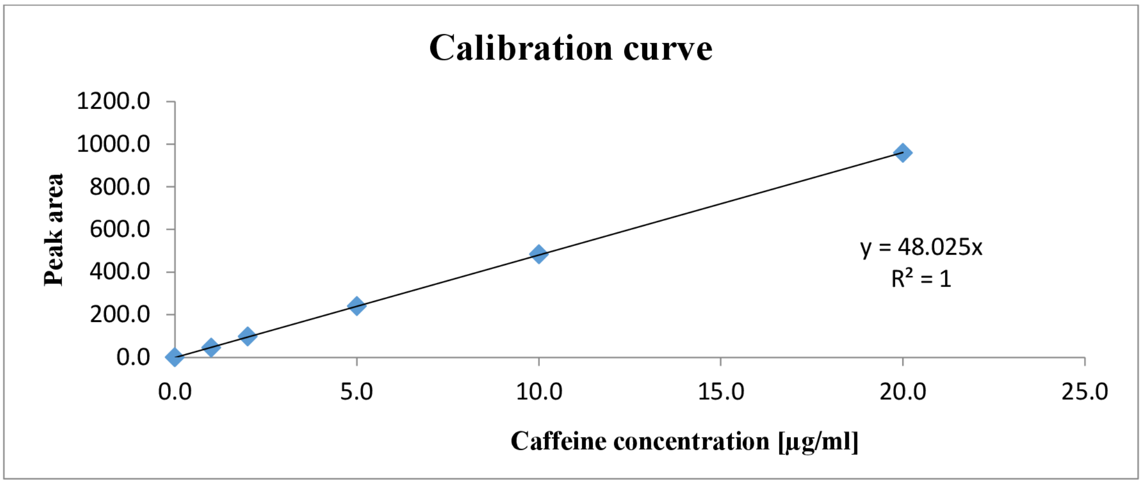

Figure 14. The calibration curve
Author: Author of this thesis

In figures 14 and 15. we can see chromatograms. It is a chromatograph record showing the individual analytes in the form of chromatographic peaks. The retention time under these chromatographic conditions is 2.53 minutes. In the chromatogram of the caffeine standard (Figure 14.) we can see only the pure caffeine without impurities. In the chromatogram of Aquila Black Tea Citron (Figure 15.), there can be found also other ingredients of this drink along with caffeine.

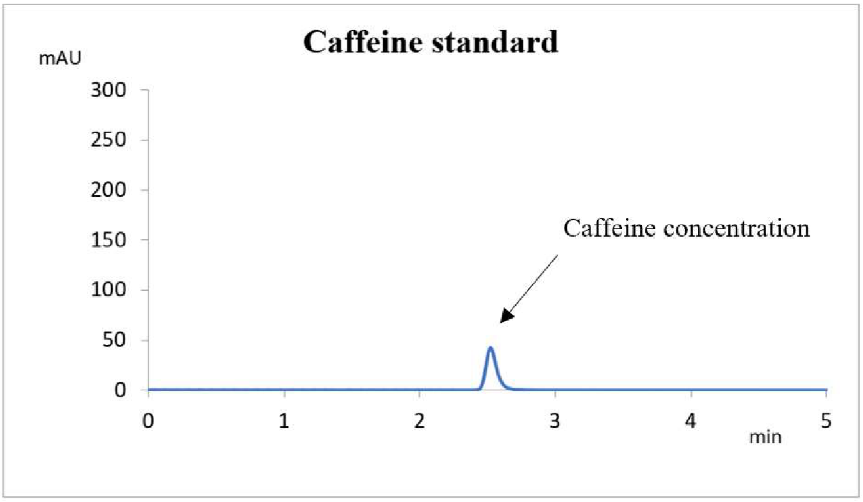

Figure 15. Chromatogram of caffeine standard
Author: Author of this thesis

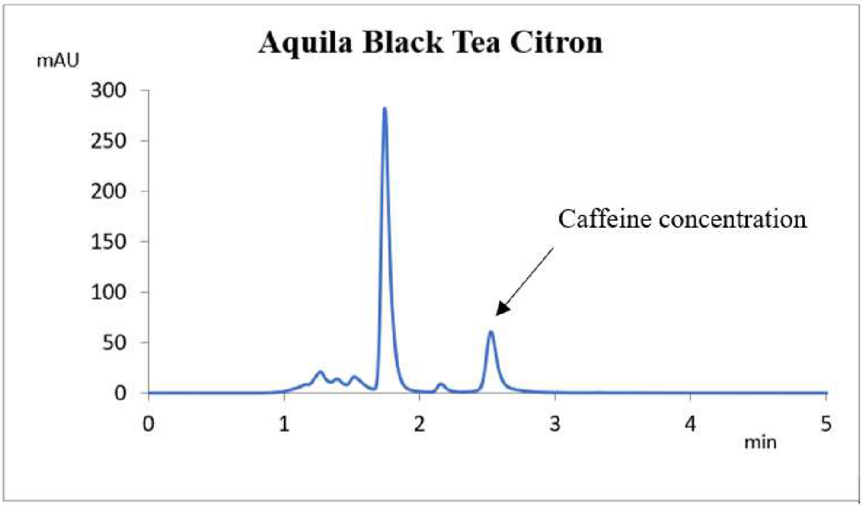

Figure 16. Chromatogram of Aquila Black Tea Citron
Author: Author of this thesis

The peak area of each sample was divided by the y-axis (y = 48,025x). The obtained caffeine concentration in every sample can be found in Table 3. below.

From the results in Table 3 is possible to determine the amount of these beverages that children should consume in one day (Tab. 4.). The amount was calculated using recommended daily caffeine intake (Chapter 2.4.) and caffeine concentration in selected beverages measured using HPLC, then multiplied by 100.

Table 3. Caffeine concentration in beverages

Beverage	The concentration of caffeine in beverage mg/100 ml
Coca-Cola	9.47
Coca-Cola Zero	9.23
Pepsi Cola	10.38
Pepsi Cola Max	11.69
Kofola	7.64
Black Tea Ahmad	24.18
Black Tea	18.36
Green Tea	10.97
Lipton Black Tea Lemon	3.71
Lipton Green Tea	5.38
FuzeTea Black Tea Citron	5.30
FuzeTea Green Tea Citron	7.10
Nestea Black Tea Peach	3.68
Nestea Green Tea Citrus	3.69
Aquila Black Tea Citron	4.59
Club Maté	20.92
Club Maté Cola	9.49
Nestlé Granko	1.68
Holandské kakao	6.45

The caffeine content of selected beverages was measured using high-performance liquid chromatography (HPLC) with DAD detection. As stated in Table 3, the lowest caffeine content was measured in Nestlé Granko compared to the second cocoa drink Holandské kakao which has a caffeine concentration of 6.45 mg/100 ml. The highest caffeine content was measured in black tea from Ahmad (24.18 mg/100 ml) and in Club Maté (20.92 mg/100 ml). Even though classic green tea has lower caffeine content than classic black tea, iced tea beverages with green tea have higher caffeine than black ice teas from the same brand. From the ice teas, the lowest concentration was in Nestea Black Tea Peach (3.68 mg/100ml) and the highest caffeine content was in FuzeTea Green Tea Citron (7.1 mg/100 ml). The rest of the ice teas had caffeine concentrations between 3 and 5 mg/100 ml. On the other hand, the caffeine content in cola beverages was between 7 and 12 mg/100 ml. The highest concentrations were measured in Pepsi Colas, from which Pepsi Cola Max has 11.69 mg/100 ml. The lowest caffeine content was in Kofola (7.64 mg/ 100 ml).

Table 4. Maximum daily consumption of selected beverages by age

	4 – 6 years (ml)	7 – 9 years (ml)	10 – 12 years (ml)	13 – 17 years (ml)	Pregnant women (ml)	Adult (ml)
Coca-Cola	475	660	898	1056	2112	4224
Coca-Cola Zero	488	677	921	1083	2167	4334
Pepsi Cola	434	602	819	963	1927	3854
Pepsi Cola Max	385	535	727	855	1711	3422
Kofola	589	818	1113	1309	2618	5236
Black Tea Ahmad	186	258	352	414	827	1654
Black Tea	245	340	463	545	1089	2179
Green Tea	410	570	775	912	1823	3646
Lipton Black Tea Lemon	1213	1685	2291	2695	5391	10782
Lipton Green Tea	836	1162	1580	1859	3717	7435
FuzeTea Green Tea Citron	634	880	1197	1408	2817	5634
Nestea Black Tea Peach	1223	1698	2310	2717	5435	10870
Nestea Green Tea Citrus	1220	1694	2304	2710	5420	10840
Aquila Black Tea Citron	980	1362	1852	2179	4357	8715
Club Maté	215	299	406	478	956	1912
Club Maté Cola	474	659	896	1054	2107	4215
Nestlé Granko	2679	3720	5060	5952	11905	23810
Holandské kakao	698	969	1318	1550	3101	6202

The results in Table 4. indicate that children from 4 to 6 years old should consume less than 0,5 litres of cola beverages. The exception is Kofola, which they can drink at 589 ml. On the other hand, it is safe to consume more than one litre of several iced teas, like Lipton Black Tea or both Nesteas. They can also consume more than 2 litres of Nestlé Granko or can put more spoons of power into one cup, the healthy amount of consumed milk is not considered. For children from 7 to 9 years old is safe to daily consume only 258.48 ml of Black Tea Ahmad, which is equivalent to one cup. Except for FuzeTea Green Tea Citron, they can drink in one day more 1 l of iced teas. They can consume a standard 0,5l bottle of cola drinks a day. Unlike children (7 – 9), 10 to 12 years old kids can consume 1 l of Holandské kakao and more than 2 l of both Nestea iced teas and Lipton Black Tea Lemon. Adolescents are the first ones who can drink more than 0,5 l of Black Tea and almost 1 l of Green Tea. Both Coca-Cola beverages can be consumed at one litre a day.

Pregnant and lactating women can drink slightly above 1 l of Black Tea and almost 2 l of Green Tea. They should not consume more than 2 l of both Pepsi colas. The results for adult consumption show that they should not consume beverages in excessive amounts. The largest packages commonly found in supermarkets do not exceed a volume of 2.5 litres which is a daily intake of fluids for the average person. It is important to consider that it is assumed the consumption of other types of beverages during the day, like water, fruit flavoured water, fruit juice, and others.

The measured caffeine contents were compared with values from the (Caffeine Informer 2022). On this website, the caffeine content is given in mg per fl. oz. with different volumes per beverage. In Table 5. there is the volume in fl. oz. of every drink with their amount of caffeine and caffeine content calculated in mg per 100 ml. There is also included measured caffeine in beverages from Table 3. for better comparison.

Table 5. Comparison of caffeine content

Beverage	Volume in fl. oz.	Caffeine content in fl. oz. [mg]	Caffeine content in 100 ml [mg]	Measured caffeine content in 100 ml [mg]
Coca-Cola	12.0	34.0	9.6	9.47
Coca-Cola Zero	12.0	34.0	9.6	9.23
Pepsi Cola	12.0	38.0	10.7	10.38
Pepsi Cola Max (UK)	11.2	43.0	13.0	11.69
Black Tea	8.0	42.0	17.8	18.36
Green Tea	8.0	18.0	7.6	10.97
Lipton Black Tea	20.0	25.0	4.2	3.71
Lipton Green Tea	20.0	25.0	4.4	5.38
FuzeTea	24.0	24.0	3.4	5.30
Nestea	17.0	23.0	4.6	3.68
Club Maté	17.0	100.0	20.0	20.92

As stated in Table 5., the caffeine content in all cola-type soft drinks is lower in samples from this thesis. On the other hand, the dose in both Black and Green tea is significantly higher than from the Caffeine informer (2022). In iced tea beverages, there are two drinks with higher and two with lower caffeine content. The higher levels have the Lipton Green Tea and FuzeTea, and the lower levels were found in Lipton Black Tea and Nestea. The sample of Club Maté also has a higher caffeine amount. The largest difference between the caffeine contents is found in the Green tea, where the change is 3.37 mg, while the Coca Cola has the smallest difference, at 0.13 mg. The caffeine content in selected beverages is in accordance with the determined levels by FDA and EC and none has been found to be in breach of them.

The caffeine volumes in every beverage are different and it is a great challenge to assign specific levels because of their variability. It is influenced by the plant, the origin, and the treatment during its growth, harvest, and processing. Even the preparation of the final drink affects caffeine content, including temperature, and time of steeping or brewing. The effects of caffeine consumption are very variable and individual. The same dose has different effects on a person of the same gender, age, or health status. Therefore, it is not possible to accurately determine the probable outcome of caffeine ingestion and prevent the onset of the specific disease. It is also important to consider the usage of other substances, like sugar, alcohol, or nicotine.

6. Conclusions

The first part of this bachelor thesis was focused on the literature review of the chemical compound caffeine, its chemical properties, history, and pharmacokinetics. Natural sources of caffeine, their morphology, varieties, cultivation, harvest, processing, and health effects were described. Furthermore, caffeinated beverages were discussed, the recommended levels of caffeine in several drinks, processing of tea and special types of coffee, and also non-beverage caffeinated products. Finally, the effects of caffeine on human health, both physical and psychical.

In the practical part, where 19 samples were analysed, the experiment revealed differences in caffeine content. The selected beverages were divided into groups according to the origin of caffeine. Caffeine levels varied from 1.68 to 24.2 mg per 100 ml. The average caffeine content in cola drinks is 9.7 mg per 100 mg, in tea beverages 8.7 mg/100 ml, in mate drinks 15.2 mg and in cocoa beverages, the average is 4.1 mg in 100 ml. The maximum daily consumption of selected beverages was calculated, divided into age groups focused on children and adolescents and analysed. Children from 4 to 6 years old should not consume more than 500 ml of cola-type drinks and not more than 1 litre of iced tea beverages. The consumption of cola beverages in children to 9 years old should not exceed 700 ml and more than 1.5 litres of iced teas. The children between 10 and 12 years old can drink 1 litre of cola beverages and 2 litres of tea drinks. Adolescents to 17 years old can not consume more than 1 litre of cola-type drinks per day. Pregnant and lactating women should not drink more than 2.5 litres of cola beverages and 5 litres of iced tea drinks. All selected beverages meet the mandated caffeine levels.

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Figure 2:

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Figure 9:

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