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Differences in caffeine content in various coffee beverages

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Declaration

I hereby declare that I have done this thesis entitled Differences in caffeine content in various coffee beverages 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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Sova Peter

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Abstract

Coffee is the most frequently used caffeinated drink worldwide. Caffeine, as a main psychoactive substance, has various effects on its consumers and affects physiological and psychological processes in human and animal organisms, therefore research is still very important.

Aims of theoretical part of this thesis was to describe and summarize the most economically important species of genus *Coffea* spp., the most common types of coffee preparation, caffeine as a chemical substance synthesised by plants, its metabolism and sources of caffeine, effects of caffeine ingestion on human physical and psychical health and possible consequences resulting from excessive ingestion of substance.

In practical part, research of the coffee, that is sold all around the campus of the Czech University of Life Sciences (CZU) in Prague, took place. Research was inspired by similar analysis, that took place in Scotland, United Kingdom. For analyses of espresso samples obtained at CZU, high performance liquid chromatography was used. Caffeine content varied from 55.36 to 150.61 mg of substance per serving, 61.09 to 931.56 mg of caffeine per 100 ml respectively. A lot of students and professors drink coffee on a daily basis and therefore, over-ingestion of caffeine is possible. With plausible side effects, whether negative or positive, it might be easy to reach daily maximum advised amounts of caffeine in the bloodstream, ergo knowing the levels of substance was essential.

Key words: *Coffea* spp., caffeine, HPLC-DAD, human health, espresso, coffee

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List of the abbreviations used in the thesis

AD - Anno Domini

AMP - Adenosine monophospfate

APA - American Psychiatric Association

C.- Coffea

cAMP - Cyclic adenosine monophospfahte

CZU - Czech University of Life Sciences

CYP1A2 - Cytochrome P 450 1A2

DAD - Diode Array Detector

FEM - Faculty of Economics and Management

FES - Faculty of Environmental Sciences

FTA - Faculty of Tropic Agrisciences

GMP - Guanosine monophosphate

HPLC - High Performance Liquid Chromatography

IFIC - International Food Information Council Foundation

PTFE - Polytetrafluorethylene

SAM - S-Adenosyl methionine

SIC - Study and Information Centre

1. Introduction

Coffee culture is ubiquitous. We can buy and enjoy this excellent beverage almost everywhere; in cinemas, coffee shops around the city, in shopping malls, all around the University campus, on farmers markets, in hospitals, libraries and many more places. Even during those hard times marked by a global epidemic, cafes survive thanks to their loyal customers, who cannot imagine their day without a cup of strong espresso or smooth cappuccino. This observation raises a question...Why is coffee so popular? How is it possible, that even if you open a new cafe next to an older one, you will still be profitable? What lies behind the worldwide success of coffee? We must look for answers in the chemical composition of coffee beans, their processing, preparation and especially their effect on the human body and the psychological side. One of the acceptable answers might be hidden behind the main active compound, caffeine. Thanks to this substance, many of us could survive hard times during studies and endless work. Its energizing effects are the first reason why everybody consumes caffeinated beverages. Secondly, the pleasing feelings during consumption can easily boost actual mood. And, let's be honest, we all need that time to time.

Green coffee beans that we pick, roast, grind, and brew to produce the most popular caffeinated beverage known all over the world are actually the seeds contained in drupe fruits from trees and shrubs naturally grown in the shade of African forests, including the islands of Mauritius and Madagascar (the latter formerly known as one of the Mascarene Islands). They are also cultivated in tropical areas such as equatorial Africa, Java, Sumatra, and other islands of the Dutch East Indies, West Indies, India, Arabia, the islands of the Pacific, Mexico, and Central and South America (Davis et al. 2007).

Coffee is, inter alia, a beverage, mostly served hot, prepared from pre-dried, then roasted coffee beans. Those are sold in supermarkets as a whole or ground beans or a water-soluble powder. Many possibilities of coffee preparation are known from all around the world. Some of them are discussed later in this work. Also views on human health vary from possible toxicity to positive effects on cells and physiology of human and animal body. Nevertheless, it is still one of the most consumed beverages all around

the globe. It is mainly thanks to the effects of caffeine on cognitive enhancement and lack of tiredness, which is known to every culture from East to West.

Caffeine is the most widely consumed psycho stimulant in every culture around the world. This alkaloid is naturally present in variable amounts in the leaves, seeds, beans and fruits of over 60 plants species. Caffeine (1,3,7-trimethylxanthine) is the main active ingredient of coffee. It is a natural alkaloid that, together with theophylline (1,3-dimethylxanthine), theobromine (3,7-dimethylxanthine), and paraxanthine (1,7-dimethylxanthine), belongs to methylxanthines. But are there any differences in its effects on each individual, or in the types of preparation? And how much is too much?

Roasted and ground coffee beans and dried tea leaves are the primary sources of caffeine in human diet. It is also found in kola nuts, cocoa beans, yerba mate, and guarana berries. Heckman et al. (2010) stated, that caffeine is ingested most frequently in drinks such as coffee (71%), soft drinks (16%), and tea (12%). Over the last two decades or so, functional beverages, such as energy drinks, caffeinated sport drinks, juices, and waters have been introduced. In addition, caffeine is also found in cocoa, chocolate, and medications such as analgesic formulations and dietary supplements. Let's have a closer look at the plant and then focus on the main topic of this thesis - caffeine. What do we actually know about it? And how it affects our everyday life as consumers?

But first, let me tell you little story about how did coffee found its way to the human diet. It all begin in the shades of bushes in Ethiopian Highlands. According to the Ethiopian legend, coffee waited for its discovery till the 6th century AD. One day shepherd named Kaldi was on the pasture with his goats. It was day like every other, until the evening came. His goats were abnormally active, seemed a bit nervous and were running all around the hills. Even on their way back home, whole herd acted really strange. During that night, goats could not fell asleep, neither Kaldi did. The next morning, herd was behaving totally normal, but Kaldi was tired because of lack of sleep. Once they came back to the hill, Kaldi saw goats eating cherry-like fruits and leaves from shrubs. After a while, goats were again full of energy. He decided to try few drupes and leaves. Although, fruits did not taste like cherries, after a short while, he felt full of life and missing energy from the sleepless night came out of nowhere. Knowing this new discovery, Kaldi picked few branches with fruits and brought them to the local monastery. After he shared the story of last night and morning to the abbot and monks,

abbot decided to boil branches with water. Once boiled, abbot had few sips of the liquid. Thus, taste was horrible, so he threw whole pot with branches to the fire. After a while, seeds inside the fruits began to roast and the smell attracted everybody to have a closer look. Abbot felt influx of energy from sips he took before, so he decided to cook fruits again. Surprisingly, taste of the potion was absolutely different and pleasant. Everybody, who tried newly discovered infusion, felt energised almost instantly. Legend has it, that monks stayed awake whole night praying until the sun came up, and that was only thanks to the newly discovered beverage, coffee.

Even if this legend was not true, the most important fact is, that coffee is one of the most traded tropical crops worldwide and provides many people with work and livelihood.

2. Literature review

2.1. History of coffee

Firstly, origin of this popular beverage was unclear to the Western world. Well known botanist Carl von Linné classified coffee as *Coffea arabica,* as he thought it origins in Arabic world. The reason Linné thought coffee originates in Arabian Peninsula was the fact, that Arabic nations were known for growing and processing coffee beans, until the end of 18th century. Later then, coffee found its way to the Indian Peninsula and Indonesia, followed by South America, mainly Colombia, Venezuela and Brazil - countries that are considered to be the biggest producers of coffee nowadays. Thanks to the merchants and traders, coffee finally entered Europe. Unfortunately, it was very pricy and therefore its popularity rose slowly. Firstly, it was considered as a medication and was sold by pharmacies, later by liquor stores and after many years first real coffee selling stores and coffee-shops, as we know them today, were opened for business (Mangal 2007).

2.1.1. Origins of coffee

Coffee plants can be found all over the tropical areas around the world. Although, African continent and islands in Indian Ocean close to Africa are two main regions of origin. The species *C. liberica* originates from lowland habitats in West Africa, often coastal. The centre of origin of *C. arabica* is in Ethiopia (Abyssinia) in high plateaux areas at altitudes between 1300 and 2000 meters above sea level. On the other hand, the origins of *C. canephora* are more widely dispersed in tropical Africa at altitudes below 1000 meters above sea level. A further species, *C. excelsa,* closely related to *C. liberica,* is also a native of lowland forest habitats in West and Central Africa. The original identification of this species was made in Liberia, hence its name.

2.2. The coffee plant and beans

The coffee shrub or tree is a part of the subkingdom of plants known scientifically as the Angiosperm, or Angiospermae, which means that the plant reproduces by seeds that are enclosed in a box-like compartment, known as the ovary, at the base of the flower (Ukers 1922). It belongs to the botanical family Rubiaceae, which has some 500 genera and over 6000 species (Ukers 1922). This family comprises many genera including *Gardenia, lxora, Cinchona* (quinine) and *Rubia.* The latter includes *Rubia tinctoria* (the Turkey Red), from which the name of the family Rubiaceae was derived (Wintgens 2004). Coffee belongs to genus *Coffea*, which is by far the most economically important member of the Rubiaceae family(Murthy & Naidu 2012). The genus *Coffea* covers approximately 100 species. One of them, to which coffee belongs, is the subgenus *Eucoffea*. Another important genus in the Rubiaceae family is *Psylanthus. Psylanthus* species often have been confounded with *Coffea* genus species (Ukers 1922).

Coffee is a short-day plant, and hence the floral initiation takes place in conditions of 8 - 11 h of daylight. As Wintgens (2004) stated, the plant takes approximately 3 years to develop from seed germination to first flowering and fruit production. A well-managed coffee tree can be productive for up to 80 years or more, but the economic lifespan of a coffee plantation is rarely more than 30 years.

Flowers form only on 1-year-old branches that is only slightly hardened. Pollination takes place within 6 h after flowering. The process of fertilization is completed within 24 - 48 h after pollination. After pollination, a fruit develops into a 10 to 15 mm long cherry-like drupes containing two seeds. *Coffea arabica* with inflorescences and fruits, is displayed on the Figures 1 & 2. Size of the fruit and its seeds depends on the variety. The fruit comprises of the skin (epicarp or exocarp), which is a monocellular layer covered with a waxy substance that protects the fruit; it is usually yellow, dark pink, or red; the mesocarp, which comprises a fleshy pulp and, in ripe fruits, a slimy pectinaceous layer of mucilage adhering to parchment; the parchment or parch (endocarp), which is a crumbly, thin, paper-like polysaccharide covering the silverskin, which is the seed coat composed of mainly polysaccharides, especially cellulose and hemicelluloses, in addition to monosaccharides, proteins, polyphenols, and other minor compounds; and two egg-shaped or elliptical seeds

containing endosperm and embryos displayed on Figure 3 (FAO 2005; CAC 2008; Murthy & Naidu 2012).

Figure 1. ***Coffea arabica***

Author: Koehler H A 1887

Figure 2. Drupes of *Coffea arabica*

Author: Author's photography

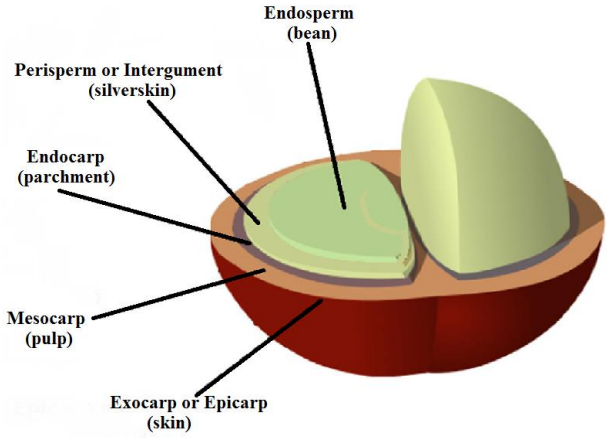


Figure 3. Parts of the coffee drupe fruit

Author: Geromel et al. 2006

The weight of a parchment seed at 18% moisture content is 0.45-0.50 g for Arabica and 0.37-0.40 g for Robusta. The average weight of hulled beans of both species at 12-13 % moisture content varies from 0.17 to 0.40 g. The shape of a coffee tree varies depending on the species and variety. In general, the coffee tree consists of an upright main trunk with primary, secondary, and tertiary lateral branches. Primary branches grow off the main stem. They develop secondary branches that, in turn, give rise to tertiary and quaternary ramifications. These branches are called suckers at the

developing stage and stems at the final stage. The leaves are opposite decussate on suckers. Each leaf pair is cross-positioned to the next leaf pair. Leaves appear shiny, wavy, and dark green in colour with conspicuous veins. In the axil of each leaf are four to six serial buds, and directly above them is one slightly bigger bud called the extra-axillary bud, because of its relatively distant position. This extra-axillary bud develops into a plagiotropic or lateral, horizontal branch. Plagiotropic or lateral branches grow at almost right angles from the main stems (Figure 4). No other bud in the same axil can grow into a lateral branch, which means that if such a branch is cut off, no lateral regeneration can occur on the node of a main vertical stem. Laterals are commonly called primaries. Each serial bud on a primary can develop into an inflorescence (flowers) or into a secondary branch, which has a structure similar to that of the primary branch, with serial buds that develop either into small bunches of condensed cymose flowers or into tertiary branches. Serial buds can remain dormant for a long time. If a secondary branch is cut or removed, another secondary on the same axil can replace it, so regeneration of secondaries on primaries is possible. Depending on the species and environmental conditions, a 1-year-old coffee plant develops approximately six to ten levels of plagiotropic branches. After 2 years, the coffee plant can reach a height 1.5-2 m and the first flowers appear. After approximately 3years, the coffee tree reaches full maturity and begins to yield a normal crop.

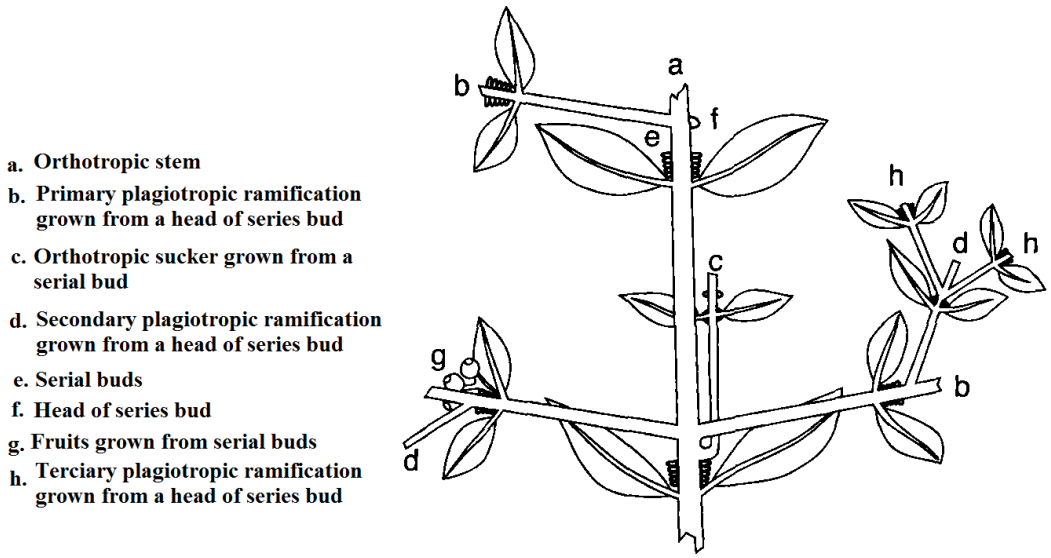


Figure 4. Buds and shoots of the coffee plant

Author: Wintgens 2004

A shallow root system comprises a main taproot and lateral and small feeder roots. The root hairs (feeders) are the main providers of mineral nutrition to the coffee plant. The feeder roots of *C. arabica* trees penetrate relatively deep into the soil, whereas *C. canephora* trees have feeder roots concentrated very close to the soil's surface. Therefore *C. arabica* is more resistant to drought. In favourable soils coffee tree roots can expand up to 15 m^3 of ground. In humid, heavy soils, the superficial roots concentrate mostly in the upper layers. In dry, sun-exposed soils, the root system is less superficial. Coffee trees can root up to 3 m deep in a normal soil although about 90% of their roots develop in the upper 30 cm layer. This part of the root system is sensitive to weather changes (moisture, temperature, drought). Protection can be provided by mulch that maintains the humidity of the topsoil and provides added mineral nutrition to the coffee plant. Under mulch protection, the density of the superficial root system can triplicate. Optimum temperatures of the soil for root development and effective functioning of the rooting system are around 26 °C during the day and no less than 20 °C during the night (Wintgens 2004; FAO 2005;Murthy & Naidu 2012).

Coffee seeds do not require a period of dormancy. It is essential for seeds to be sown as soon as possible after ripening, when their moisture content is over 50%. At this stage their germination rate is over 90%. Viability decreases rapidly after 6 months (for Arabica) and 2 months (for Robusta) when stored at ambient temperature. If seeds have to be stored, they should be stored as parchment seeds which have to be dried slowly at a low temperature (not above 40 °C) down to 12 - 13% moisture content. The dried parchment seed stored at 10 °C will maintain a high germination capacity for several months. It has been demonstrated that Arabica seeds with 10-11% moisture content stored at 15 °C can be maintained for approximately 2 years. Attempts at chilling seeds to temperatures below 10 °C and drying below 9% moisture content have both resulted in a rapid loss of viability. Temperatures of below -15 °C are lethal for coffee seeds. When storing coffee seeds, the parchment should not be removed. The germination of coffee seeds is slow, taking 30 - 60 days under the most favourable conditions, which are: air temperature of 30 - 35 °C, soil temperature of 28 - 30 °C and high rate of ambient humidity. Germination is slowed down by lower air temperature. This means that during the dry season, germination may be delayed by up to 90 days. If temperatures remain below 10 °C for longer periods, germination might not occur at all. The rate of germination can be accelerated by 6-10 days by removing the parchment

before sowing, preferably by hand. Germination can also be increased by soaking the seeds in water for about 24 hours before sowing. As the hypocotyl axis grows, it lifts the seed out of the ground, this is known as an epigeous germination. Ten weeks after sowing, the parchment splits and the two cotyledons unfurl into two circular leaf-like structures. At this stage the root system consists only of a tap root with laterals (Figure5). Three months after sowing, the apical bud lodged between the two cotyledons develops the first true leaves (Wintgens 2004).

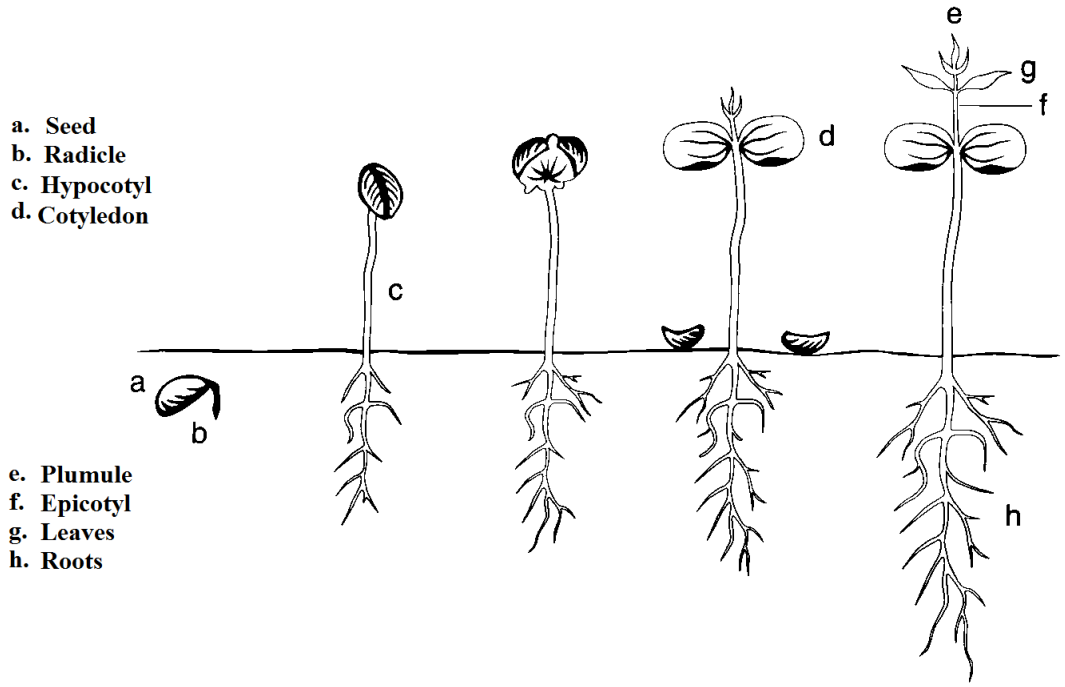


Figure 5. Epigeous germination of a coffee plant

Author: Wintgens 2004

2.2.1. Plantation cultivation

Coffee plants start to yield approximately 3 years after germination. Economically advantageous is to grow and cultivate plantation for maximum 30 - 40 years, after that, the yield starts to decrease significantly. Although, bearing fruits was observed even on the trees nearly 80 years old.

Climatic conditions are the biggest issue in cultivating new plantation. As for *Coffea arabica,* it is especially altitude and yearly rain falls that are restrictive. In subtropical areas, altitude must be in-between 1800 - 3600 metres above sea level with typical dry/rainy seasons. Those might be in Zimbabwe, Peru, Honduras, Nicaragua, Costa Rica, Mexico, Jamaica, Sau Paulo and Minas Gerais in Brazil. Growing is also

possible in equatorial areas with higher altitude from 3600 to 6300 metres above sea level, namely in countries like Ethiopia, Kenya and Colombia. Although, because of the high precipitation throughout whole year, it is necessary to dry coffee beans only mechanically with suitable technique. On the other hand, *Coffea canephora* grows naturally in lower altitudes and is less prone to higher temperatures and its fluctuations, especially in countries like Vietnam, Indonesia, India, Malaysia, Thailand. (Mangal 2007).

2.2.2. Economically substantial species

***Coffea arabica* L.**(syn. *C. vulgaris* Moench) - known also as "arabica", is the most important species of the genome *Coffea*, mainly due to its high quality of produced seeds. Furthermore, around 70 % of the world's coffee production is maintained by this exact species. There are many varieties, namely bourbon, mocca, murta, amarella or Blue Mountain from Jamaica, which is considered as the one with the highest quality yield. Nowadays, arabica is cultivated dominantly in Central America and Brazil.

Second most important coffee species is ***Coffea canephora*** Pierre ex Froehn (syn. *C. robusta* Lind) also known as "robusta". Robusta contributes almost 30% to the worlds production. Nevertheless, its production is rising every year at the expense of "arabica". The main reasons are; easier adaptability to climate changes, higher resistance to pests and diseases and faster growing in comparison to "arabica", *Coffea canephora* begets smaller and rounder seeds, which contains twice more caffeine. However, those are considered as lower quality material for coffee preparation, therefore its price is significantly lower. Areas, where "robusta" is cultivated are mainly in Africa (Congo river valley) and in Southeast Asia. New hybrid called "arabusta" was created by crossing *C. arabica* and *C. canephora* and shows perspective in a growing coffee worldwide (Kadlec 2012).

Only less than 1% of the coffee production is maintained by ***Coffea liberica*** Bull ex Hiern, also known as Liberian coffee. Beverage prepared from those seeds tastes bitter-sour and it is considered as low quality. Nonetheless, its hybrid with arabica is known as "maragogipe" and brings higher quality yield.

Coffea Dewevrei Chev. var. *excelsa*, coffee plant called "chari" or "excelsa" has the lowest quality seeds with the sharp and distasteful aroma, although with the highest concentration of caffeine. It is usually grown in Congo and Vietnam (Kadlec 2012).

2.2.3. Caffeine-free species in the genus *Coffea*

The popularity of coffee is also considerably high among people for whom its active compound, caffeine, might have troubling effects on their health. Moreover, consumption of even small doses of this substance can seriously endanger them and cause serious damage to well-being. These include mainly pregnant women, breast-feeding mothers, people with heart dysfunctions as hypertension, arrhythmias and others, abnormalities in central nervous system functions and mental disorders, people with calcium deficit, sleeping problems and few more. Especially for those groups of consumers, scientists found ways to make decaffeinated coffee and tea. Although, chemical processes used to make coffee decaf are in conflict with healthy approach to life. Therefore, researchers are finding ways how to grow coffee that will be caffeine free in natural conditions.

Two main groups of coffee plants were recognized based on their caffeine content; caffeine-free species originating from the Indian Ocean islands and caffeinated coffees natively growing on the African continent. The first African wild caffeine free coffee (namely *Coffea pseudozanguebariae*) was from Kenya, East Africa. Moreover, a new species from Cameroon called *Coffea charrieriana* was also tested and described as caffeine free (Campa et al. 2005; Stoffelen et al. 2008). Within the genus, caffeine content varies from total absence to more than 3% dry matter basis in *Coffea canephora.* As predicted, African coffee beans contain caffeine in different raging amounts (higher than 0.4% in dry matter), except in four species. Besides the two totally caffeine-free species (the Central African *C. charrieriana* and the East African *C. pseudozanguebariae*), there are two others, *Coffea salvatrix* from East Africa and *Coffea rhamnifolia* from Northeast Africa, present very low amounts of caffeine (less than 0.04% content in dry matter). While both economically cultivated species *Coffea arabica* and *C. canephora* only produce caffeine as trimethylxanthine, two other purine alkaloids (theophylline, up to 0.20% in dry matter, and theobromine) were also found in addition to caffeine in the mature seeds of the East African species *Coffea racemosa* and *Coffea sessiliflora*. On the contrary, *Coffea* species found on Indian Ocean islands were believed to be caffeine free, except for a very low content (0.07%) reported in seed of *Coffea mauritiana*. The detection of a remarkable amount of caffeine in seeds (0.55% and 0.81%) of two species found on Madagascar (*Coffea kianjavatensis* and *Coffea*

lancifolia var. auriculata) led to investigations on a wider sample of coffees native to the Indian Ocean islands (Clifford et al. 1991; Rakotomalala et al. 1992). Results with zero to only a trace of caffeine content was found in 30 out of the 47 species. Very low amounts (0.01 - 0.20%) were discovered in 17 species. These studies, implemented on four populations, supported results previously obtained for both species *C. kianjavatensis* and *C. lancifolia* var auriculata. Their caffeine content (0.20 - 0.80%) was as high as some wild East African species, such as *Coffea eugenioides* and *C. sessiliflora*. Especially *Coffea tetragona*, *Coffea heimii* and *Coffea dubardii*, naturally growing on Indian Ocean islands do not produce purine alkaloids at all (Rakotomalala et al. 1992).

Interestingly, caffeine-free species were observed as well as species containing very various amounts of caffeine. For example, samples of *Coffea millotii* were mostly caffeine free (15 out of 18 samples). The two remaining species (*Coffea ambodirianensis* and *Coffea farafanganensis*) contain very low caffeine amounts (0.03 - 0.05%). A similar situation is observed for the *Coffea boiviniana*, *Coffea jumellei*, *Coffea sakarahae,* and *Coffea pervilleana*, where caffeine content ranged from 0.01% to 0.21% in dry matter. Within this series, Rakotomalala et al. (1992) detected theobromine only in *Coffea tsirananae* (0.03%). Moreover, five non-Madagascar species, of which two were analyzed, revealed low caffeine content. Also *C. mauritiana* was reported with absence of caffeine and theobromine, accordingly for Comorian *C. humblotiana,* which has also revealed as caffeine free (Rakotomalala et al. 2004; Campa et al. 2005).
Rakotomalala et al. (1992) also confirmed the complete absence of caffeine and theobromine in the populations of *Coffea homollei* contrasted with the presence of caffeine and theobromine in seeds from *C. lancifolia* var auriculata and *C. kianjavatensis* (0.55% and 0.81%) found in the same area. Interestingly, the two *C. kianjavatensis* populations, growing only 200 km from each other, were tested with results showing high genetic divergence. Moreover, differences were found also in their composition of purine alkaloids (Krishnan et al. 2012; Razafinarivo et al. 2013; Andrianasolo et al. 2013). In contrary, Nagai et al. (2008) found that African species showed caffeine content in the leaves as very low in *C. eugenioides* (0.01%).However, leaves of *C. pseudozanguebariae* and *C. arabica* are caffeine-rich while the compound is lower or absent in the seeds. Caffeine was lacking in all adult leaves of ten species, including *C. kianjavatensis* and *C. lancifolia* var auriculata. Although, only

theobromine was detected in *C. lancifolia* var auriculata. No methylxanthines were present in *Coffea kianjavatensis, Coffea ankaranensis, Coffea perrieri, Coffea leroyi,* and *Coffea augagneuri* (Charrier & Berthaud 1975).

Geographical distribution of *Coffea* species collide with their caffeine content. Interestingly, some exceptions exist in both African and Madagascar species. Ultimately, caffeine content ranges from 0 to less than 1% in dry matter in seeds of Indian Ocean islands species, while in the African beans it varies from 0 to more than 3% in dry matter. In Africa, species native to the western coast and central parts are medium to rich in caffeine, whereas species from the eastern region are low to moderate-rich in alkaloids content.

Barre et al. (1998) and Akaffou et al. (2012), independently of each other, studied the inheritance of genes responsible for the synthesis of caffeine. Both teams were focused on inter-specific progenies. The caffeine content is under polygenic control, although its production is regulated by a single main gene with two alleles. The recessive allele is responsible for the absence of the compound. This main gene constitutes 57% of the overall variation among interspecific back-cross hybrids (*C. pseudozanguebariae* × *C. liberica* var dewevrei) × *C. seudozanguebariae* (Akaffou 1999). When the dominant allele is present, caffeine is produced. It seems that other genes are responsible for the difference in the amount of alkaloid. The caffeine content for the interspecific cross between *C. pseudozanguebariae* × *C. dewevrei* is not additive. Results showed that the mean value of substance for F1 hybrids is 60% lower than expected under the additive principle (0.18% vs. 0.43%). Charrier & Berthaud (1975) studied impact on the caffeine level of many environmental influences. They found out that traits like the harvest date of mature fruits, length of the flowering and fructification period, year, field locality or growth development alter the caffeine levels. For instance, the amount of caffeine declined between the beginning and the end of the harvest season, with a variation estimated between 0.15% and 0.22%. Nevertheless, the impact of these external influences compared to the genetic effects appears small (Cailleux et al. 2004). It seems that longer flowering–fructification period of African species results in the higher caffeine levels. The lack of caffeine in mentioned species is, however, not due to a short flowering and fruit bearing time. In comparison, the observation of few groups exhibiting lack of caffeine as well as a medium term flowering-fructification indicates a potential recombination of the two attributes. The association in not that

clear for the Indian Ocean islands species. Those can be easily divided into three groups, based on the time span of their flowering and fruit bearing length. Short duration from 2 to 5 months, medium duration from 6 to 8 months and long duration, lasting up to 1 year and longer. The two species containing high levels of caffeine come from Eastern Madagascar and are part of a short flowering group (Akaffou 1999). Thanks to all those researchers, we can assume, that caffeine free species will bear seeds soon and producers of decaffeinated coffee would not have to use chemical pathways or to wash caffeine out of seeds. There is a potential for growers to make caffeine free products even in bio quality.

2.3. World trade with coffee

On the report of FAO, coffee is grown on 10 million hectares of land with an average global yield of almost 1 tonne per hectare (973 kg). Therefore, worldwide coffee production is over 10 million tons (FAO 2018). Due to the rising demand on coffee by consumers, growing and processing is widely spread from regions where it was cultivated naturally to the all suitable tropic areas. Even though *Coffea* spp. has specific demands on the climate, it is possible to produce high quality yields in many countries around the globe. In Figure 6 & Figure 7 are listed countries that are the biggest producers of green coffee seeds of *Coffea arabica* and *Coffea canephora* respectively with estimation on yields in year 2020.

Green *Coffea arabica* production by country in 1000 x 60 kg bags

Rank	Country	Arabica Production (1000 x 60 kg bags)
1	Brazil	39,900
2	Colombia	14,300
3	Ethiopia	7,350
4	Honduras	6,500
5	Peru	4,500
6	Mexico	4,350
7	Guatemala	3,500
8	China	2,300
9	Nicaragua	2,300
10	Costa Rica	1,375

Figure 6. Top 10 world producers of green *Coffea arabica* seeds (year 2020 estimation)

Data from: USDA FAS

Green *Coffea canephora* production by country in 1000 x 60 kg bags

Rank	Country	Robusta Production (1000 x 60 kg bags)
1	Viet Nam	31,105
2	Brazil	18,100
3	Indonesia	9,450
4	India	3,910
5	Uganda	3,500
6	Malaysia	2,000
7	Côte D'Ivoire	1,800
8	Thailand	700
9	Tanzania, United Republic Of	600
10	Lao People's Democratic Republic	475

Figure 7. Top 10 world producers of green *Coffea canephora* seeds (year 2020 estimation)

Data from: USDA FAS

Green coffee is marketed on the world market under the name of the country that produced it, or the port from which it was exported. The labelling according to the botanical species of the coffee is not usual, only when it was grown on the varieties that

are considered as the ones with highest or lowest quality, namely bourbon, Blue Mountain, liberica, robusta and chari. (Kadlec 2012). Consumers can choose from wide range of varieties of coffee products, from classic roasted beans (whole, ground), soluble powder, via coffee extracts as in a form of liquid or powder, to paste. All of the stated products are also available as a decaffeinated products. Moreover, there are plenty aromatised and flavoured options.

2.4. Coffee preparation

Important step in coffee processing is roasting, because of the significant structural, physical, chemical and sensorial changes. During this procedure, coffee beans are exposed to high temperature for a time-length depending on the type of desired properties, variety (coffee bean characteristics), roaster and geographical origin (Severini et al. 2017). Roasting leads to overall reduction of caffeine levels by 30% from 0.89% ± 0.02 in green beans to 0.6% ± 0.03 in roasted arabica beans (Franca et al. 2005). The increase of the caffeine solubility in water (water vapour) with rising temperature causes decrease of caffeine content by evaporation as it is dragged out of beans by water vapour (Dutra et al. 2001).

Effects of grinding on caffeine extraction

The grinding is a key step for preparation of the coffee brew. In whole roasted beans, chemical compounds are incorporated in cells and they cannot dissolve freely in hot water. To obtain better dissolution, seeds are reduced to small particles by grinding. Size of particles may vary from few μm to 1 mm, allowing volatiles and other substances to extract in hot water (Wang & Lim 2014). Ground coffee may be divided in four groups, such as very fine, fine, medium and coarse. Moroney et al. (2015) stated that "particle size of coffee ground is vitally important in coffee extraction in that it affects both the fluid flow through the grind and the grind's extraction kinetics". The degree of wetting of each coffee particle, water flow through capillaries in coffee cake and the diffusion of substances from ground coffee to hot water are the main characteristics controlling levels of chemicals released into the coffee beverage (Baggenstoss et al. 2008). When fine or very fine grounds are used, the percolation rate is low, due to the lower porosity of coffee cake. This situation contributes to a

significant increase in overall extraction of chemical compounds. In contrary, coarse ground has bigger particles, therefore contact with hot water is lower, resulting in less extraction ability of chemical substances. Therefore, the size of particles must be always adjusted by grinding for each specific type of desired coffee preparation and final product. Coarse particles are used in French press coffee, as the leaching of coffee ground in hot water takes several minutes. If used ground was too fine, final product may result in over-extracted, bitter liquid. In contrary, espresso preparation takes only 25 - 30 seconds, therefore fine ground is required. From 1 coffee bean, 100 - 300 particles are obtained for French press, 3,500 for espresso and 25,000 (± 10,000) particles for Turkish coffee. For all types of coffee preparation, solid to liquid extraction is the main principle of dissolving all chemical compounds from roasted-ground coffee to hot water (Barbanti &Nicoli 1996; Vignoli et al. 2011).

Several variables may modify the quality of final product. For differences in caffeine content are important mainly; time of extraction, water temperature, vapour pressure (for espresso and moka), filtration, coffee:water ratio, type of contact between coffee ground and water, the extract volume and boiling process (Petracco 2001; Gloess et al. 2013; Caporaso et al. 2014). Required final volume of the beverage widely varies simultaneously with caffeine content. In espresso (25 ml) levels of caffeine were measured ranging from 2.4 to 4.5 mg/ml, in filtered coffee (200 ml) from 0.4 to 1.4 mg/ml, in moka (30 ml) from 0.7 to 5.4 mg/ml, in French press (100 ml) from 0.2 to 0.5 mg/ml and in Turkish coffee (50 ml) 1.94 mg/ml. This confirms that volume of drink has a significant impact on the caffeine content. Another variable is ground:water ratio, which affects final properties of beverage and depends on the selected type of preparation. As reported, 7 g is usual amount used to prepare 25 ml espresso coffee, 12 g of ground for 200 ml of filtered coffee, 8 g for 100 ml of French press and 5 g for 50 ml of Turkish coffee. Moreover, time for extraction depends on the size of grounds and varies widely, from 25 seconds for espresso to 5 - 7 minutes for French press and filtered coffee brews (Pérez et al. 2010; Ludwig et al. 2012; Gloess et al. 2013; Caporaso et al. 2014).

2.4.1. Boiled coffee/Turkish coffee

Turkish coffee is made by boiling grounded coffee in water. The fine powder is inserted in a kettle (e.g., cezves), water is poured and brought up to simmer. The

infusion is primarily based on the extraction of soluble substances at higher temperatures. At such high temperatures, also fewer water-soluble compounds are derived which give the usual strong, dark-chocolate and bitter flavours. Usually the heating is halted after reaching the boiling point, however the coffee powder stays in contact with boiling water and the extraction process proceeds. Boiling can occurs several times, based on the technique of the person preparing beverage. Sediment ought to be settled while pouring. The outcome is a very good and strong coffee with a little bit of residue in a mug (Mestdagh et al. 2017).

2.4.2. Pour over brew/Coffee percolation

For the drip or pour over brew, the coffee beans are ground at greater particle sizes allowing the water to flow by gravity itself. Ground coffee shall be inserted in a container comprising of a filtering unit. Numerous filter types, styles, and components are widely available, enabling barista to adjust the form of filter head and level of filtration. Regulation of the particle size by changing the grinder, enables for adjustments in percolation speed and time of contact between water and coffee. Percolation is achieved by adding boiling water, which is often measured very accurately and at an ideal temperature for a different coffee beans. Water is poured manually or by an automated dripping filter system, which can also be designed to produce various amount of water at particular temperatures over time. The water flows through the coffee in filtering part, primarily by the influence of gravity. The intensity of pressure is also very restricted relying on the water column above the coffee. The water flows into the serving reservoir and particles are held by the filter membrane. This process is often referred to as a drip filter method. There is a lot of debate about the form of the filter and thickness of the coffee layer, which defines consistency of the extraction. A very large, flat filter unit extracts coffee differently from a broad, little one. The explanation for this is the time of interaction between the liquid and coffee. Many drip coffee devices use conical shaped filters that create a conic-like remains These differences will also have impact on optimizing the absorption of certain aromas and flavours and will have an effect on the outcome product(Mestdagh et al. 2017).

2.4.3. French Press/Immersion brew

This method is also called cafetière and is well known around the world, especially in francophone nations. For the French press method, coffee should be coarsely ground. Boiling water is then poured over the beans, stirred and left for about 4 to 7 minutes to infuse. Time length depends mainly on the desired intensity of beverage. Shorter extractions underline sweetness and acidity, on the other side, longer extractions bring intensity and bitterness. Filtering equipment is incorporated in a plunger, which is used for dividing the liquid beverage from grounds. This final product is ready to be poured into the mugs. Although only inefficient metallic filters are used in French press pots, higher amount of dusty-like remains and sediments may be present in comparison to the pour over method or espresso. The pressure made on the coffee also squeezes out oils of the coffee ground, which remarkably increase the concentration of oily particles in final beverage (Zhang et al., 2012). An alternative method called Aero Press combines drip filter methods and immersion. Firstly, it starts with absorption, pouring hot water over the ground beans. After a while, piston made pressure pushes mixture towards paper filter and final product is poured straight to the cups without visible remains in the liquid part (Mestdagh et al. 2017).

2.4.4. Espresso

The first espresso machines were manufactured at the end of 19th century in Italy after the demand on coffee grew rapidly throughout middle class. Everyone wanted to have a cup of freshly prepared coffee anytime during the day, without the need to go home. Initially, machines were using hot steam for extraction. However, really high temperature of steam caused the burnt taste of beverage without pleasing flavours. Steaming mechanism was exchanged by hot water and this method is used until today. Nowadays, the machines are set up for the traditional Italian espresso coffee. As Istituto Nazionale Espresso Italiano states, the best quality product is obtained with water temperature of 88 °C (± 2 °C), pressure of the water 0.9 MPa (± 0.1) and short time for extraction between 20 - 30 seconds. Standardised amount of coffee used is 7 g (± 0.5 g). Ideally, final product has 25 - 40 ml créma (special name for coffee foam) rich beverage with a various pleasing flavours and aromas (Petracco, 2001). However, trends are

changing and sometimes, espresso is prepared from as much as 20 g of ground beans for 40 ml cup or the higher water pressure is used, still obtaining high quality espresso (Rao, 2013). Thus, pressure and water:coffee ratio have the biggest impact on caffeine content, flavours and richness of aromas extracted from coffee cake. The character of an espresso is connected to this pressure and a low water-to-coffee ratio. Flavour profile is affected mainly by the extraction pressure. Moreover, characteristics of the pump and its type are decisive forces for espresso making. The pump operating specifications give exact characteristics, given by the manufacturers in factories. Normally, the pump delivers higher pressure when the water flow decreases and vice versa. Properties of coffee cake, like permeability, actual operating pressure and corresponding water flow through coffee cake, brewing time and finally water residence time, have the significant impact on profile of flavours, aromas, caffeine content and overall quality of beverage in cup. Operating time is set up to pour the exact amount of hot water over the coffee cake to produce certain amount of beverage. Freshly ground coffee is put into small basket placed in portafilter and pressed automatically or by barista. If the pressure was too high, cake permeability is low, overall flow rate decreases and extraction takes longer. This results with over-extracted, burnt to spicy taste caused by, among other things, higher caffeine extraction in final espresso (Corrochano et al., 2015). Nowadays, available technologies implemented into the espresso machines allow baristas to change different parameters of extraction such as variable water flow, pressure and temperature of water or exact time of extraction. Options may vary machine to machine and are defined by manufacturer of each model. Thanks to those possibilities, skilled baristas can easily change and deliver specific flavours particular to all coffee variants from around the globe to each prepared cup. High importance of ground size is essential for the results in the mug. For instance, coffee grounds with bigger particles have smaller aromatic profile and lower caffeine content (caused by smaller extraction area) than finely ground beans (Severini et al., 2015). On the other hand, beans that are ground too finely can easily extend the time of extraction, resulting in different flavours, aromas and caffeine volume released to the beverage. To gain required profile of aromas and flavours, barista always needs to check and adjust technical issues of machine and size of the coffee powder particles. Additionally, new espresso machines, made mainly for use at home, work with pods or capsules technologies.

Level of class of beverages prepared on those new cafeterias is considered as high, depending on the quality of coffee used inside the capsules and technical properties of each machine. Thanks to the system of packing of pods and capsules, shelf-life of coffee grounds remain longer than freshly ground beans without vacuum handling. Also coffee:water ratio and pressure are equivalent to the machines used in the coffee shops, allowing even inexperienced users to prepare high quality coffee at home. Coffee made on those cafeterias is usually strong, with pleasing aromas and rich créma on top of it.

2.4.5. Moka pot/Stove-top coffee maker

Moka pot was invented by Italian constructer Alfonso Bialetti in 1933 and became big hit throughout whole Italy and later whole Europe and world. Unsurprisingly, it is still the most popular coffee brewing method for household use in Italy. The construction of moka pot is comprised of three chambers, folded together, using pressure of hot water for extraction (Figure 6).

Figure 8. Moka pot - parts, coffee extraction

Author: Author's collage of photographs

Chamber on the base works as water reservoir, the middle one comprises basket for coffee grounds and filter and top chamber works for collecting of final beverage. As the water heats on the bottom, air and vapour pressure increases, steam and hot liquid pass through middle part with coffee, extracting espresso like beverage flowing inside the upper part. Swelling of coffee particles decrease permeability of the coffee cake. That and rising of water flow rate are the main drivers of the increased pressure inside the bottom chamber. Although créma is usually missing. In comparison to espresso machine, moka pot provides considerably lower pressure. Regardless of relatively simple functioning and manufacture of moka pots, research showed complex thermodynamic behaviour in equation to the other methods of coffee preparation (Navarini et al. 2009). Nevertheless, rapid over extraction is easily obtained as several variables are not facile to control and cause almost burnt taste of the coffee. To avoid this issue, timing is essential. The most important is to stop the extraction exactly when espresso like liquid pours into the top chamber. Well known rattling sound of the last stage of preparation in moka pots resembles the eruption of volcano. This sound is caused by the increased pressure and low water content inside of the bottom chamber. Fluid should be dark, almost creamy, with syrupy consistency and a no to very little créma on top. When the extraction takes too long, water temperature and pressure rise, causing solution of less desired chemical compounds from coffee ground, which worsen overall quality of beverage. This may lead to the bitterness and astringency of moka coffee.

2.4.6. Cold Brew and Iced Coffee

Cold water, usually room temperature or lower, is used for cold brew preparation. French press, pour over or immersion might be used as technique for extraction. Obtained beverage can be diluted with hot or cold water, depending on the required temperature, although dilution is not obligatory at all. In contrary, iced coffee is generally prepared by the drip-brewing method using hot water for extraction. Beverage is then cooled down usually by ice cubes or crushed ice and served on more ice inside the glass. If the ice-cream is used for cooling down the beverage, the final product is called "affogato" and is very popular during summer in Mediterranean countries. Interestingly, if cold water is used for extraction of coffee, low polar

compounds, such as oils from coffee beans, could not get leached by water. Although, substances with higher polarity do not have problems with extraction by water with lower temperature. As stated before, temperature affects solubility of flavours and aromas from coffee powder. Thus, each compound has different chemical properties and its extraction depends on the temperature of water. This results in various final products even from the same initial beans. When cold water is used, extraction should take longer and might last up to 24 hours. Unsurprisingly, cold brew coffee is significantly different from the one that is prepared by hot water techniques. This beverage has usually lighter colour, emphasized body and sweet and chocolate notes in flavour. Extraction that takes too long may lead to oxidization of soluble compounds, which results in less pleasing taste and overall quality. To prevent oxidization, the regulation of air exposure throughout the preparation is essential. This led to the invention of vacuum powered cold brew mechanisms.

2.5. Caffeine

Caffeine is a tri-methylated xanthine (Figure 9), whereas theobromine and theophylline are only a di-methylated form. Xanthosine is initial substance in purine alkaloid biosynthesis and leads to the caffeine through one hydrolysis and three *N*-methylations via theobromine and theophylline.

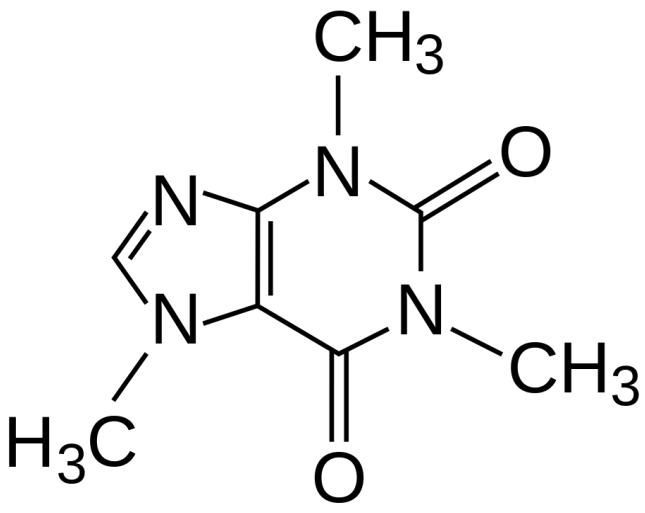


Figure 9. Structural formula of caffeine

Source: wikipedia.sk

Various *N*-methyltransferases function as catalysers in methylation steps (Ashihara & Crozier 2001). Not only the main caffeine biosynthesis pathway exists, also various minor routes with paraxanthine as a intermediate product may operate. However, these are principally relying on the wide specificities of the *N*-methyltransferases (Ashihara et al. 2008). Caffeine is disrupted predominantly to xanthine via theophylline and 3-methylxanthine (Romero & Waller 1988). However, Ashihara et al. (2011) found that via minor route of caffeine degradation, theobromine might come out as a by-product.

In contrary, other purine alkaloids such as liberine, theacrine and methylliberine are also produced from the caffeine in the mature leaves of coffee (*Coffea liberica* and *Coffea dewevrei*). These methyluric compounds are the products of the third pathway emerging from caffeine (Petermann & Baumann 1983).

Properties of caffeine are numerous. Besides its most popular role as a stimulant to the central nervous system, allelopathic properties of caffeine have been postulated. It

could reduce the predatory activities of animals. It might also effectively inhibit the production of the toxin *Aspergillus ochraceus*. Caffeine seems to act as a herbicide and could help regulate agricultural and landscape development. In cosmetics, caffeine is becoming widely used to reduce cellulite, as it accelerates lipids degradation (Frischknecht & Baumann 1985).

Recently, based on a literature review concerning the effects of caffeine on the central nervous system and on the cardiovascular system, the European Food Safety Authority (2015) has established safe levels of caffeine consumption in the population. Adults are advised to limit their caffeine consumption in one sitting to 200 mg (about two regular 125 ml cups of coffee) and to 400 mg over a daytime period. Concentrations higher than 15 mg of caffeine per 1 kg of bodyweight may be toxic for the cardiovascular, nervous, and gastrointestinal systems. Calculated for 70 kg weighing person, 1 g of caffeine might cause acute intoxication and poisoning. Pregnant women should not exceed 200 mg caffeine per day, and for children and adolescents, the safe limit is set at 3 mg/kg per day. There is a large inter-individual variability in caffeine consumption, metabolism and in its effects on the human body. The daily consumption of coffee/caffeine in various individuals ranges from zero to very high levels (that can sometimes be well above the European food safety authority advised levels of consumption) and most reasons underlying this inter-individual variability are not clearly understood. Various factors that could contribute have been identified over the years, including age, sex, hormones, type of activity, co-ingestion with food, drugs or alcohol. In the last two decades or so, numerous genetic studies aimed at clarifying how the genetic variability in the enzymes metabolizing caffeine and in the expression of adenosine receptors, mainly the A_{2A} receptor underlying most of the physiological effects of caffeine in the body and brain, might underlie the pharmacodynamics and pharmacokinetics of caffeine and hence the sensitivity of different population subsets to the effects of caffeine ingestion. Caffeine exerts a wide range of effects on the body, both positive and negative, that might partly influence the variability of caffeine intake between theinter-individual differences (Nehlig 2018).

2.5.1. Biosynthesis pathways

There are several lines of evidence, using coffee and tea (*Camelia sinensis*, Kuntze) plants in experiments, that indicate the purine alkaloids, theobromine and caffeine, are synthesized via xanthosine from purine nucleotides (Suzuki &Waller 1988; Waller et al. 1991; Suzuki et al. 1992). However, only a few investigations, mostly with tea plants, have been carried out to examine the relationship between biosynthesis of caffeine and theobromine and purine metabolism **(**Ashihara & Kubota 1986; Fujimori et al. 1991). Xanthosine is the primary substance for synthesis of purine alkaloid (Figure 10). As discovered, substrate can be supplied and created by at least five various pathways (Figure 11): the degradation pathways guanine nucleotides (GMP route) and adenine nucleotides (AMP route), de novo purine biosynthesis (de novo route), salvage pathways and the S-adenosyl-L-methionine (SAM) cycle also called SAM route(Ashihara & Crozier, 1999a; Stasolla et al., 2003; Zrenner et al., 2006).

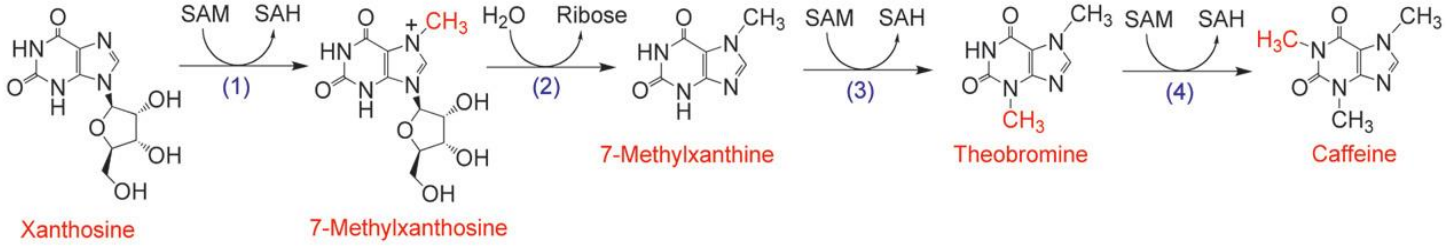


Figure 10.The major biosynthetic pathway of caffeine from xanthosine

Author: Ashihara et al. 2008

Suzuki and Waller (1984) reported that the integration of exogenously supplied guanine and adenine into purine alkaloids occurred in unripe fruits of coffee plants. Moreover, Negishi et al. (1985) demonstrated the incorporation of xanthosine into purine alkaloids in young shoots, and they suggested the first step of caffeine biosynthesis is methylation of xanthosine to 7-methylxanthosine, followed by series of

methylation reactions.

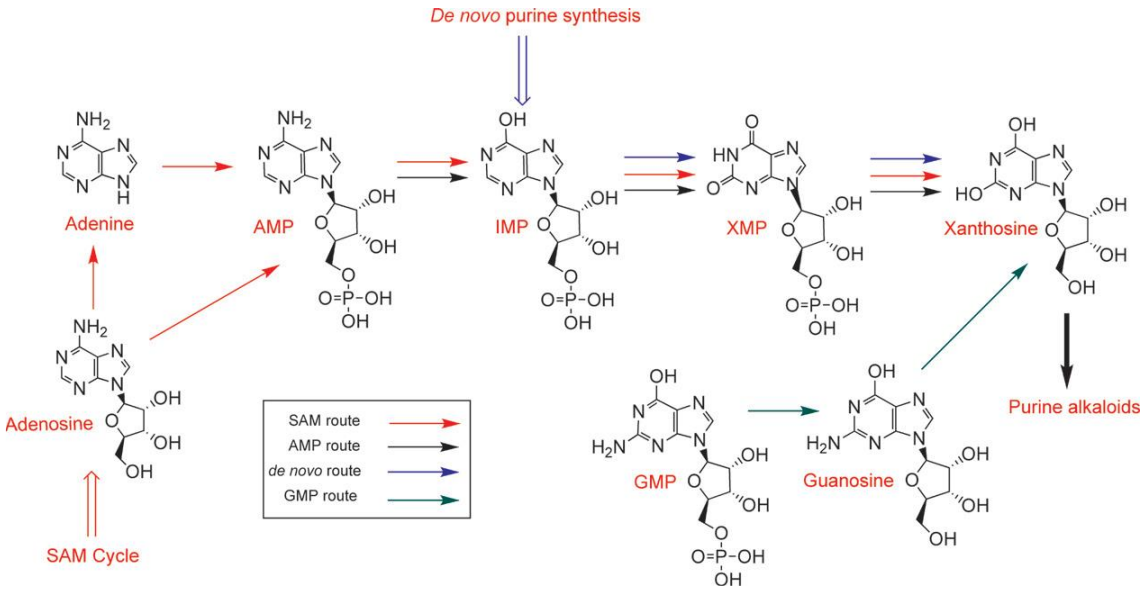


Figure 11. Four of five routes of purine alkaloids synthesis.

Author: Ashihara et al. 2008

In higher plants, almost all exogenously supplied adenine is restored by adenine phosphoribosyltransferase, and AMP is formed (Ross 1981; Hirose & Ashihara 1984). Furthermore, most plants have little or no detectable adenine deaminase activity (Yabuki & Ashihara 1991).

Fujimori et al. (1991) suggested the conversion of adenosine monophosphate to caffeine via theobromine. It has been proposed that in tea leaves the major pathway to caffeine from adenine nucleotide proceeds as follows: adenosine monophosphate → inosine monophosphate → xanthosine monophosphate → xanthosine → 7-methylxanthosine → 7-methylxanthine → theobromine →caffeine.

The biosynthesis of caffeine from adenine nucleotides occurs only at the very early stages of leaf development. Frischknecht et al. (1986) found out that levels of caffeine increase at the very early phases of development of coffee leaves, reaching a maximum when the leaves were fully opened. Similar age-dependent biosynthesis of caffeine has been reported in other parts of coffee plants, especially fruits, leaves flowers and fruits of tea plants (Suzuki &Waller 1984; Ashihara & Kubota 1986).

The activity of related enzymes is one of the limiting factors in the biosynthesis of caffeine. Some of the enzymes are; S-adenosylmethionine xanthosine N-methyltransferase, 7-methylxanthosine nucleosidase, S-adenosylmethionine 7-

methylxanthine N-methyltransferase. Those may be blocked by naturally occurring enzyme inhibitors. Seasonal variations in the activities of some of these enzymes have been observed in tea leaves (Fujimori et al. 1991). The physiological importance of the biosynthesis of caffeine in plants is unclear. However, Frischknecht et al. (1986) stated that the synthesis of caffeine in young leaves and buds of coffee plants is due to the prevention of pests. Moreover, caffeine and other purine alkaloids contained in the drupes, also function as allelochemical, suppressing other plants from growing in the same area (Waller 1987).

2.5.2. Pharmacokinetics

Pharmacokinetics of caffeine and its metabolites differs significantly throughout animal kingdom and even between Animalia and human kind. Therefore, it is difficult to estimate impact on different genus based on observations of another. Although, clinical tests revealed, that in healthy human, elimination of caffeine is via first-order kinetics in a one compartment open model system. This model assumes that the drug can enter or leave the body (that is why the model is "open"), and the entire body acts like a uniform, single compartment. The intakes ranged from 2 to 10 mg/kg (Bonati et al. 1982; Blanchard & Sawers 1983). The first-pass metabolism for caffeine is minimal (Arnaud, 1993). In the case of intoxicated infant, Jarboe et al. (1986) revealed a dose dependent kinetics with levels of caffeine over 30 mg/ml in blood plasma.

Arnaud & Enslen (1992) discovered, that demethylation of caffeine to paraxanthine in adults saturates at low levels (1 – 4 mg/kg).

Nonlinear clearance was confirmed in various *in vivo* tests, suggesting that metabolism of caffeine is dose-dependent (Denaro et al. 1990). In addition to the dose, delayed gastric emptying might be caused by the dietary residues inside the stomach or gastric diseases and it may affect the plasma kinetics of caffeine (Arnaud 1993). Ingestion of liquids also alter renal clearance and affect overall pharmacokinetics of caffeine and its total clearance from body (Trang et al., 1985). Environmental and genetics factors have the main influence on the variations in caffeine kinetics. After administration of 4 mg/kg dosage of caffeine by humans, half-lives varied from 2.5 to 5 hours and its plasma concentrations reached highest rate from 1 to 2 hours (Arnaud, 1993). Significant differences in half-lives, that ranged from 2.3 to 9.9 hours, reported

by Blanchard &Sawers (1983) signalise obvious inter-individual variations in caffeine clearance. After oral ingestion, the total plasma clearance of paraxanthine and caffeine is identical (2.07 - 2.20 ml/min per kg; Lelo et al., 1986). The essential metabolites of caffeine, theophylline, theobromine and paraxanthine, have biological activity. Theophylline acts on inflammatory pathways and is used in asthma as a bronchodilator. Theophylline, same as caffeine and paraxanthine, also antagonizes and blockades A_1, A_{2A}, and A_{2B} adenosine receptors (Fredholm et al. 1999; Haskó & Cronstein, 2011). Besides that, histone acetylation pathways, that are affected by theophylline, promote deacetylation, consequently reduce the inflammatory expression of proteins and genes (Ito et al. 2002). In comparison, theobromine has lower biologic effects on human body. Although, it is precursor of pentoxyphylline, which functions as inhibitor of cytokines (Whitfield et al. 2009). Nowadays, researchers are concentrated on biological effects of caffeine only, without its cooperation with metabolites and xanthine substances. Therefore, there are still unknown pharmacokinetic pathways of trimethylxanthine dissociation and more research is needed.

2.5.3. Metabolism

After orally ingested, caffeine is absorbed really fast by gastrointestinal system straight to the blood circulation. Average intake 5 - 8 mg/kg is followed by concentration in blood serum 8 - 10 mg/l. Placental, nor hemato-encephalic barrier do not stop caffeine from transfer. Absorption in gastrointestinal system is affected by many factors, as the overall intake of caffeine, food content in stomach, sex, age, smoking habit, pH of the food or beverage ingested, dysfunctions in gastrointestinal system and many more. After caffeine is absorbed, it is present not just in blood, but even in all body fluids like urine, saliva, sweat, cerebrospinal fluid, bile, breast milk, semen and others. Caffeine is metabolised in the liver thanks to microsomal oxidative complex P-450, especially CYP1A2 where N-demethylation takes place. By-products of metabolism of caffeine are paraxanthine (1,7-dimethylxanthin), theophylline (1,3-dimethylxanthin) and theobromine (3,7-dimethylxanthin). Average half-life of caffeine is 3 to 5 hours after consumption, but differences may occur as in inter-individual aspects takes place (Arnaud 2005).

As the main impact of caffeine is antagonism to adenosine receptors, therefore it suppresses the effects of adenosine, which is nucleotide that affects many physiological

processes in human and animal body. For example, regime of sleep, excitement and many more. We distinguish four types of adenosine receptors - A_1, A_{2A}, A_{2B} and A_3, naturally occurring in vascular endothelium, central nervous system, liver, fat layer, heart, muscles etc. All of the receptors affect levels of cyclic adenosinemonophosphate (cAMP), A_{2A} and A_{2B} rises those levels, whereas A_1 and A_3 lowers its amounts. Function of cyclic adenosine monophosphate is also called "second messenger" in cells signalisation. This, and the placement of receptors in a whole body, are the main reasons why caffeine has so many variable effects on organisms (Zapletalová 2011).

Lethal dosage for adult person is set to 10 g of pure caffeine, which may be reached by drinking more than 1.6 litre of coffee when caffeine content in 100 ml of beverage is around 60 mg. Although, this depends on preparation and type of coffee seeds used. However, caffeine poisoning occurs after lower intake than lethal dosage. This highly depends on inter-individual differences and is influenced by routine consumption (Hrubá 1997; Velíšek 2002).

Accumulation of caffeine and its metabolites was not proven by any research, everything is excluded from body and around 2% of caffeine leaves by urine in a non-changed form. Physical activity nor heat expenditure does not have impact on caffeine metabolism. On the other hand, sex, age, smoking, alcohol consumption and other chemical substances do have effects, whether positive or negative. For instants smoking of tobacco products accelerate metabolism process and exclusion from body. Cigarettes contain polycyclic aromatic hydrocarbons that promote a greater liver enzyme activity, thereby increase caffeine metabolism. Smoking may accelerate the pre-systemic (i.e., first-pass) and systemic (i.e., second-pass) metabolism of caffeine, with the hepatic microsomal oxidative enzymes causing a faster demethylation. Vice versa, after interruption of smoking, speed of metabolism slows significantly (levels of caffeine may reach up to 200 % in comparison to previous rate). Therefore, smokers who decided to quit, should be aware of their caffeine intake, as it may reach very high levels in blood-serum and lead to possible poisoning and intoxication. Reduced ability to eliminate caffeine is also observed in females with long-term contraceptive intake, people with liver dysfunctions as CYP1A2 microsomal oxidative complex may be less active and women in late stadium of gravidity when by 35^{th} week of pregnancy it may reach up to 18 hours (Hrubá & Kachlík 1997; Hrubá 1997; Tsutsumi 2001).

Acute poisoning might occur after higher consumption of caffeine, which does not have to be strictly from coffee. The symptoms include tachycardia, anxiety, restlessness, heart arrhythmia, severe headaches, sleeping deprivation, urgent or constant need to urinate and dizziness. Chronic intoxication develops after longer period of constant intake of caffeine in high doses. In that case, symptoms are more serious and dangerous, for instance nausea, vomitus, chronic tiredness, insomnia, dysfunctions of gastrointestinal system, chronic anxiety, jitters, mood swings, depression, loss of focus and heart dysfunctions. Continual high intake is characterized as dependence, since after skipping in intake, light withdrawal symptoms occur. Those are mainly restlessness, anxiety, severe sleep disorders and deprivation and attenuation. It is highly recommended to keep intoxicated person in a well-ventilated room, put him/her in a stabilized position and to induce vomiting. Although pharmaceuticals might be used, namely propanolol 10 - 20 mg/kg or esmolol 50 mg/kg (Arnaud 2005).

2.5.4. Sources of caffeine

Naturally synthesised caffeine has various functions in plants. Degradation of caffeine, even in matured and aged leaves of many species, is relatively slow. Therefore, because of remaining levels even after abscission, it cannot be considered as nitrogen reserve. There are two theories concerning the imposition of caffeine in plants, the "allelopathic function" and "chemical defence" hypotheses. For instance, its allelopathic effect may be used by farmers on coffee and tea plantations, when there is less weeds growing around. Interestingly, caffeine has also a positive impact on biological suppression of pests and vermin. No wonder there is a lot of plant species producing even small amounts of caffeine. Although, just some of them synthesise levels high enough for commercial processing. Purine alkaloids, in comparison to other plant alkaloids, such as nicotine and morphine, are widely distributed throughout the plant kingdom. Although, the accumulation of significant concentrations in beans and/or leaves is limited to a small number of plants, namely *Camellia* spp. (tea), *Coffea* spp. (coffee), *Cola* spp., *Ilex guayusa* (wayusa), *Ilex paraguariensis* (maté), *Paulina cupana* (guarana) and *Theobroma* spp. (cocoa). The most popular purine alkaloids are theophylline, theobromine and caffeine, which are present mainly in coffee, tea and cocoa beverages along with other non-alcoholic drinks. Synthesised in the young leaves and unripe fruits of those plants, caffeine accumulates during their maturation.

Therefore, alkaloids in various amounts can be found in leaves and in the pericarp of fruits and seeds (Ashihara 2006).

Leaves and buds of *Camellia sinensis* produce significant amounts of caffeine (also called "theine") and theophylline. The highest amount of caffeine was found in black Assam tea from India, reaching up to 6.2% in dry matter. Moreover, infusion time plays very important role for extraction of caffeine, when after two minutes, almost 70% of substance is steeped into the beverage (Pössl, 2010). After coffee and tea consumption, beverage called "maté" plays noteworthy part in caffeine intake, mainly in Latin America. Maté is prepared from leaves of *Ilex paraguariensis*, which may be dried and grinded. Similarly to tea, caffeine from maté takes longer to start affecting adenosine receptors and overall effect lasts longer. This prolongation in metabolism is caused by different preparation and chemical composition of leaves. Another species of genus *Ilex*, specifically *Ilex guayusa* contains significant amount of caffeine and is branded under the name "wayusa". A shrub or small tree, producing theobromine as the derivate of caffeine is called *Theobroma cacao* originating in Ecuador. Its seeds are well known and very important trade article, not only for production of cocoa powder and chocolate, but also for its high composition of oils and use in native medicine. Although, another beverage gained popularity, especially among young generations around the world. Soft drinks sold under the name "cola" originally contained extracts of nuts from *Cola* spp. plants, which are from the same botanical family (Malvaceae) as cocoa. Another species that produce high amounts of caffeine, *Paullinia cupana*, also called guaraná-da-amazônia or simply guarana, originates in the Amazon region. Indigenous tribes and communities used guarana for centuries, mainly for its medicinal and stimulant properties. Effects on health include diuretic properties and beneficial effects against fever, cramps and headache (Henman, 1986). Caffeine is prepared also synthetically in the laboratories and then used in various medications. Many pills are easily obtained in pharmacies like over the counter drugs.

2.6. Effects of caffeine on human health

The side effects of higher intake of caffeine varies from person to person and are based on genetics, age, liver cytochrome P450-CYP1A2 function, sensitivity, hormones levels, pregnancy, smoking of tobacco and interaction with other substances (drugs). Moreover, it is unknown whether addition of other stimulating substances decrease or increase the possible side effects (Campbell et al. 2013).

Withdrawal syndrome

It has been observed that the acute interruption of regular caffeine intake might generate particular symptoms, which are named caffeine withdrawal syndrome as stated by the Diagnostic and Statistical Manual of Mental Disorders of the American Psychiatric Association (Silverman et al. 1992; APA 1994; Strain et al. 1994). Symptoms, characteristic for caffeine-withdrawal include fatigue, headache, difficulties at work (missing motivation for work and loss of focus and concentration), lethargy, decreased wellbeing (including increased irritability and decreased self-confidence), lowered blood pressure, drowsiness and rise in cerebral blood flow. These are the exact opposite effects to those caused by caffeine consumption (Silverman et al. 1992; Strain et al. 1994; Evans et al. 1999). Symptoms usually start around 12 to 24 h after caffeine withdrawal and their peak is being reached after 20 to 48 h. Although, depending on inter-individual differences, some subjects confirmed first symptoms only after 3 - 6 hours with a week-long lasting (Lima & Farah 2019). Even a short-term withholding from caffeine, such as skipping morning coffee, can cause severe adverse effects in regular consumers. Symptoms and syndrome itself as well, can be averted by ingestion of caffeine The truth is that frequent caffeine intake will lead to physical dependency, and avoiding caffeine triggers signs of withdrawal. Caffeine withdrawal syndrome may be prevented if the intake of caffeine is reduced slowly (Griffiths et al. 1986; Höfer & Bättig 1994; Phillips-Bute & Lane 1998).

2.6.1. Cardiovascular system

Caffeine is a non-selective competitive antagonist of adenosine receptors, mainly A_1 and A_{2A} subtypes. The antagonistic effect occurs after moderate coffee intake due to the plasmatic concentration reached. On the other hand, other caffeine effects, such as calcium release from intracellular stores or inhibition of phosphodiesterase, occur at higher concentrations (Matioli 2007).

All tissues with adenosine receptors may be influenced by caffeine consumption. McCusker et al. (2003) examined the amount of caffeine in different espresso and brewed coffees and found a wide range of caffeine due to variable preparations. The amount of caffeine in 240 ml of brewed coffee ranged from 72 to 130 mg while caffeine in espresso coffee ranged from 58 to 76 mg in a single shot (28 ml). The variability in caffeine content in coffee beverages might be caused by different factors: quality of coffee beans, roasting method, length of brewing time or variations in preparation.

There is little information about the effects of caffeine on the development of cardiac arrhythmias, even though the effects of coffee on the cardiovascular system have been mainly related to caffeine. Although physiologic studies present insights into the effects of caffeine on the cardiac system. High caffeine intake increases levels of adenylate cyclase, cyclic adenosine monophosphate and intracellular calcium levels. Moreover, the increase of renin activity and the increase of plasma catecholamines are also well-known effects. Several observations of animal models took place and results confirmed that adenosine receptors are involved in arrhythmias events. The selective blockade of A_1 receptors induces the prolongation of the atrial refractory period and can prevent atrial fibrillation (Brandts et al. 2003). From these data, caffeine seems to provide a protective effect. A recent study on human atrial myocytes from patients with atrial fibrillation suggests that an adenosine-mediated signalling pathways can possibly contribute to the formation of atrial fibrillation (Llach et al. 2011).

Moderate intake of caffeine seems to act positively, especially when the source is coffee, which contains antioxidants and anti-inflammatory substances (Rauh et al. 2006). It is thought-provoking, that the first reported physiological actions of adenosine are its calmative effects on heart rate and atrioventricular conduction. This expertise has continuously supported considerable interest in implementing adenosine receptor agonists as medicine for cardiac arrhythmias, including atrial flutter, supraventricular arrhythmias, supraventricular tachycardia, and atrial fibrillation (Gao & Jacobson 2011).

Another possible link between caffeine and arrhythmias has been related to heart rate variability (Matioli 2007).

Consummation of caffeine increases sympathetic nervous system activity and therefore have a possible action on heart rate variability. There have been few studies evaluating the effects of caffeine on the cardiac parasympathetic nervous system activity. Sondermeijer et al. (2002) rated the effect of acute caffeine intake on parasympathetic nervous system activity in infrequent caffeine consumers. Authors stated that acute ingestion of caffeine (100 or 200 mg of caffeine) in healthy subjects resulted in decreased heart rate variability measures and increased blood pressure, without obvious changes in heart rate. These findings suggest direct reduction in parasympathetic nervous system activity after sudden ingestion of caffeine.

Effects of enormous caffeine intake on heart rate variability have been studied, leading to controversial conclusions. The clinical impact of these researches need to be confirmed with further experiments (Yeragani et al 2005; Bonnett et al. 2005).

Levels of cholesterol and lipid activity

Double-sided impact of coffee on blood lipid levels is caused by various preparation methods. Unfiltered coffee is rich in diterpenes (kafestol, kahweol), which boost levels of absolute concentration of cholesterol, mainly low-density lipoproteins and triacylglycerols. Therefore, malfunctions and damage on cardiovascular system may occur with higher probability. On the other hand, coffee beverage prepared with some of filtration methods, demonstrate the opposite effect. Consumption of diterpene-free coffee reveals higher levels of high-density lipoprotein in bloodstream, which has reductive effects on overall concentration of cholesterol (Kempf et al 2010; Cai et al. 2012). High amount of polyphenols in coffee, which have limitation effects on the lipid oxidation process, may have positive impact on lowering levels of cholesterol (Yukawa et al. 2004).

2.6.2. Central nervous system

Unsurprisingly, of substances contained in coffee, caffeine has serious impact on central nervous system of human body. Its ability to permeate blood brain barrier allows caffeine to affects the functions of brain, especially adenosine receptors. Moreover,

effects on levels of some endocrine hormones were confirmed by animal testing. Specifically, decrease of levels of serum growth hormone and thyreotropine and increase in concentrations of β-endorphine and serum corticosterone were observed. Although, very high doses of caffeine were used during the experiments, thus moderate ingestion has negligible effects on levels of hormones in human body (Arnaud 2005). Caffeine acts as a stimulant on central nervous system. After ingestion, increase of levels of some neurotransmitters is demonstrated, namely serotonin, catecholamine, γ-aminobutiric acid and acetylcholine. As a result of these changes, behavioural effects appear, for instance improvement in cognitive enhancement may occurs after ingestion of 1 to 5 mg/kg of caffeine. With doses higher than 15 mg/kg, the risk of anxiety, shivers, jitters and sweating significantly increases (Caballero 2009).

Alzheimer disease

Nowadays, around 25 - 30 million of people worldwide suffer from Alzheimer disease, it is the most frequently diagnosed kind of dementia linked to older age. Many scientific studies are concerned about mutual interaction between coffee consumption and outbreak of the disease. Assumptions based on animal experiments suggest, that caffeine and chlorogenic acids from coffee, may act against symptoms of Alzheimer disease and cognitive recession in central nervous system. Although, clear conclusion has not been reached yet. Consumption of coffee might lower the risk of the onset of the disease and another types of dementia, but the mechanism of action is not absolutely clear, therefore coffee could not be considered a preventive factor (Eskelinen & Kivipelto 2010; Gelber et al. 2011).

Parkinson disease

Parkinson disease is, after Alzheimer disease, second most frequent neurodegenerative condition, with characteristic shivers, muscle twitching, reduced ability to move and muscle rigidity. In last few years, coffee consumption was connected to prevention of Parkinson disease. The vast majority of scientific studies, undertaken since 2000, confirmed lowered risk (up to 30%) of outbreak of this condition, thanks to the coffee consumption. Interestingly, the higher the consumption, the lower the risk of outbreak. At this place, it is important to take a look even on different effects of hormones of both sexes. The presumed neuroprotective effect of

estrogens results in a lower incidence of the disease in women. On the other hand, estrogens seems to suppress neuroprotective effects of caffeine and therefore, those actions are dependent on the actual levels of estrogens in bloodstream. This results in observation, that men and women (without exogenous treatment by hormones) who consume coffee have lower risk of outbreak of Parkinson disease. Contrariwise, women under hormone treatment might be in group with higher risk of outburst of this condition (Ascherio et al. 2004; Palacios et al. 2010).

In treatment and prevention of Parkinson disease, adenosine receptors antagonists are used, therefore caffeine with its antagonism on A_{2A} receptors seems like protecting agent (Zapletalová 2011). Sadly, the results cannot be applied for prevention on whole population, as many different factors might have a significant impact on functions of caffeine. It is possible that patients diagnosed with Parkinson disease may have reduced appetite for coffee because of neurodegenerative dysfunctions, which leads to lower consumption and therefore effects of caffeine are not substantial (Ascherio et al. 2004; Palacios et al. 2010). Nevertheless, caffeine appears as neuroprotective factor, which may help reduce the prevalence of this serious condition, but still more detailed research is needed.

2.6.3. Respiratory system

In individuals, who are not used to consume caffeine regularly, even small doses (4 mg/kg) can increase the activity of respiratory system. Although, regular intake eliminates this effect. Historically, theophylline and caffeine were used as bronchodilators and even today, doctors use those substances in small infants with respiratory dysfunctions to improve airflow (Arnaud 2005).

2.6.4. Gastrointestinal system

Moderate intake of caffeine causes relaxation of smooth muscle tissue in gastrointestinal system and slightly increases intestinal peristalsis. Although, higher doses firstly cause contractions of the smooth muscle tissue and after a while the relaxation phase begins.

Many epidemiological and clinical studies did not prove or refuted the negative effect of caffeine on gastric ulcers, further research is still needed. However, the greatest

importance in scientific studies is nowadays put on the effects of caffeine and coffee consumption on diabetes mellitus type II. and liver dysfunctions (Arnaud 2005).

Liver

A lot of studies, that took place during last two decades, brought evidence of beneficial effects of coffee consumption on liver, especially regarding hepatocellular carcinoma and cirrhotic liver disease (Vecchia 2005). Liver tests, involving five different parameters, are commonly used as a laboratory method to assess liver status. Tests include the determination of plasma concentrations of four hepatic enzymes (alanine aminotransferase, gamma-glutamyl transpeptidase, aspartate aminotransferase and alkaline phosphatase) and bilirubin, whose increased concentrations indicate the degree of damage. Many scientific studies have shown a beneficial effect of coffee consumption on liver enzymes. Regular coffee drinkers, compared to non-drinkers, who digest 5 or more cups of coffee daily, show lower serum levels of alanine aminotransferase and aspartate aminotransferase. Alcohol users, on the other hand, have higher levels of hepatic enzymes, leading to the cirrhotic damage. Although, after coffee intake, the concentration of gamma-glutamyl transpeptidase was significantly lower, causing liver cell renewal (Nakanishi et al. 2000; Honjo et al. 2001).

Variability between genders depends mainly on higher alcohol intake by men, no hormonal activity was proven yet (Gallus et al. 2002). The beneficial effect of coffee consumption on the risk of developing primary sclerosing cholangitis (chronic progressive disease affecting the bile ducts) has also been proven by research (Andersen et al. 2013). Although, most of the researches took coffee as a whole substance and therefore, no significant effect of caffeine was studied. Nevertheless, coffee has been proved to have protective functions on liver by many studies.

Type II. diabetes mellitus

There are presumptions, that drinking coffee is associated with a reduced risk of developing type II. diabetes mellitus (Hu et al. 2006). Although, scientific opinions are not uniform and the mechanism of action is not fully understood yet. Also, it is not clear, whether only caffeine, or the interaction of coffee compounds together play a major role. In addition, the consumption of decaffeinated coffee is also (but on a smaller

scale) associated with a reduced risk of type II. diabetes mellitus. In this case, particularly important components of coffee are potassium, magnesium and antioxidants. One of the possible action of protection is the influence on C-peptide levels, which is monitored marker of insulin secretion (Salazar-Martinez et al. 2004; Lee et al. 2005; Wu et al. 2005).

2.6.5. Renal system

Caffeine intake from 4 mg/kg has positive impact on renal excretion of calcium, potassium, magnesium and sodium cations. At the same time, excretion of chlorides and overall volume of urine is increased. This effect is caused by increased blood flow through kidneys, filtration rate in glomerulus and decreased tubular re-absorption of sodium, calcium and other ions.

Even though caffeine might function as diuretic substance, it was reported that diuretic effect of coffee consumption is caused even by higher intake of water. Therefore, there is no need to worry about dehydration caused by coffee consumption. Although, it is necessary to be careful, especially when subject suffers from deficiency of minerals (Arnaud 2005; Killer et al. 2014).

2.6.6. Muscular & skeletal system

In general public exist assumptions, that coffee consumption increases calcium loss via urine as a result of acidification of the body, therefore its drinking might promote decalcification of bones and the development of osteoporosis (Rapuri et al. 2001; Welch & Bingham 2007). A study conducted in 2006 confirmed, that a daily intake of caffeine more than 330 mg may increase risk of fractures, especially in older women with insufficient calcium intake. Also genetic predispositions play significant role. Although, people with balanced diet rich in minerals and vitamins, containing at least 1000 mg of calcium per day, do not have to worry about moderate coffee consumption due to the risk of development of osteoporosis (Arnaud 2005). Nevertheless, research should continue, as we still do not perfectly know the functions of coffee on osteoclasts and other compartments of skeletal system.

Energetic metabolism

Due to energy metabolism, caffeine intake has a significant impact. After consumption, there is an increase in basal energy metabolism by approximately 5 to 25%. However, with habitual regular consumption this percentage decreases. Changes in metabolism are connected to decrease of insulin sensitivity, impaired glucose tolerance and with increased lipolysis, followed by an increase in serum concentration of lactate, glycerol and free fatty acids. Anyhow, recent studies show a higher effect of caffeine directly on the central nervous system and inside the muscles than on lipolysis itself. Diet high in carbohydrates, together with caffeine intake, provide dietary combination used by top athletes before exercise. Thus, given effect of caffeine potential is not significant in short-term nor intense physical activity (Manore 2012).

Precisely because of the positive impact of caffeine on promotion of physical performance and activity, the International Olympic Committee has set an upper limit for the caffeine content in the urine of athletes at 12 µg/ml (Arnaud 2005).

2.6.7. Carcinoma diseases

Various substances, that were found in coffee, have the potential to act against cancer. Several mechanisms affect and determine the anti-carcinogenic effects of coffee. For instance, the acceleration of intestinal peristalsis and motility, results in shorter contact of carcinogens, contained in the diet, with the mucosa of the colon. At the enzymatic level, diterpenes (kafestol and kahweol) promote the detoxification of carcinogens and inhibit their activation. Polyphenols in coffee, with their high antioxidant activity, play significant role in protection of cells (Lee et al. 2007; Cárdenas et al. 2011; Farah 2012).

Colorectal carcinoma

Colorectal carcinoma is the most common type of cancer in the world. Precisely because of this fact, the protective effects of coffee on this type of carcinoma was investigated. Studies shown, that increased motility of the intestines and increased excretion of bile caused by coffee consumption, may have anti-carcinogenic impact on gastrointestinal system and lower the risk development of colon cancer (Li et al. 2013).

Carcinoma of bladder and kidneys

Until today, there were not enough scientific experiments focused on the effect of coffee on development of kidney carcinoma, although some previous studies confirmed the possible protective effect of caffeine on kidneys. Thus, further research is still needed (Lee et al. 2007). Some studies on development of bladder cancer suggest, that excessive coffee consumption may increase the risk of its outbreak, but the research is still highly insufficient and need more experiments (Pelucchi & Vecchia 2009).

Breast cancer

The anti-carcinogenic effect on this type of cancer after the coffee consumption has been demonstrated by several studies, mainly in premenopausal women. It may be deduced, that regular coffee ingestion (at least 4 cups a day) is associated with slightly lowered risk of prevalence of breast tumour (Baker et al. 2006; Nkondjock et al. 2006).

Endometrial carcinoma

Consistent results of epidemiological researches that studied the development of endometrial cancer in women and their coffee consumption showed, that regular intake significantly lowers the risk of outbreak of carcinoma. Also the fat content in the body, which acts as a risk factor for this cancer, has considerable impact. Especially in a women with a higher body fat content, coffee operates as even stronger protective agent (Friberg et al. 2009; Je 2011).

Prostate cancer

Some experts have studied the correlation between development of prostate carcinoma in men and coffee consumption. Results, however, showed minimal to no risk factors and did not confirm the onset of the disease caused by coffee ingestion (Ganmaa et al. 2002).

Cancer of oral cavity and throat

It has been scientifically confirmed, that coffee consumption reduces the risk of pharyngeal and oral cavity cancer (Galeone et al. 2010).

Stomach carcinoma

Consumption of black coffee on an empty stomach is often associated with a risk of a gastric dysfunction and carcinoma development. Despite few studies confirming this hypothesis, there is more evidence of no significant effect of coffee on gastric carcinogenesis (Botelho et al. 2006).

Liver cancer

Liver cirrhosis is a major risk factor for the development of hepatic cancer. As indicated above, the protective effect of coffee has an inhibitory impact on liver enzymes and thus reduces the risk of cirrhosis and hepatocellular carcinoma (Bravi et al. 2013).

2.6.8. Coffee and psychic health

Since coffee contains, among other substances, caffeine, which is considered as psycho-stimulant, we must also look at its effects on the human psyche. Precisely due to the wide and extensive influence of coffee on human physical body, we can also assume a broad effects on cognitive functions and mood. Caffeine is the most frequently consumed legal substance worldwide, mainly due to its positive effect on the human psyche. We live in a time, when it is very difficult to avoid stress and this factor is the main trigger of depressive behaviour, that can sadly result in suicide. Every year, more than one million suicides happen worldwide (Wasserman et al. 2007).

Depression

Two long-term scientific studies, dealing with the development of depression in connection with drinking coffee have shown a positive effect of consuming this beverage on human psyche, therefore the risk of development was significantly decreased (Klatsky et al. 1993; Kawachi et al. 1996). Alleviation of depressive states is probably based on the protective effects of coffee on the human body, as well as the antidepressant impact of drinking coffee, which is associated and conditioned with socialization and induction of pleasant feelings (Smith 2009; Ruusunen et al. 2010). A very important factor, that played a crucial role in these studies was moderate daily intake of caffeine. Excessive coffee consumption and caffeine overdose, which occurs at doses higher than 400 mg/day, is often accompanied by alcohol and tobacco abuse. This behaviour has the exact opposite effect and increases the risk of suicide (Tanskanen et al. 2000; Tanskanen et al. 2000).

Cognitive enhancement

Among human cognitive functions we include collection, evaluation and selection of information from the environment and their subsequent processing. Whether the certain activity takes place or not, is also determined by cognitive component of human mind and thinking. The cognitive side of the personality is also strongly conditioned by the current mood. Mood is characterized as a certain actual state of mind. It has been scientifically proven that coffee consumption can increase some implications of cognitive enhancement, such as alertness, vigour, concentration, mood, memory skills and overall performance. Regular moderate coffee intake prolongs and improves long-term and short-term memory and also helps to obtain information quickly from the depths of human mind. Shortened reaction time and refinement of the reactions performed were also observed (Haskell et al. 2005; Hewlett & Smith 2007; Glade 2010).

2.6.9. Caffeine metabolism during pregnancy

Metabolism of caffeine is various among human population and is conditioned by health status and many other factors. During pregnancy, a woman's body produces various levels of hormones. This fact and presence of the foetus in the uterus have impact on differences in caffeine metabolism and its action on central nervous system. As stated earlier in this text, no blood barrier can stop caffeine from its transfer throughout the body fluids. Placenta is not an exception, therefore caffeine has a significant impact on the developing foetus. For the correct and healthy degradation pathways, liver cytochrome P450 is necessary, especially its subunit CYP1A2. Unfortunately, unborn foetus nor placenta do not comprise any of those cytochromes. These begin to develop from a certain age of the newborn, hence elimination of caffeine takes less safe pathway via kidneys. Eliminated caffeine can still accumulate in placental water and cause very serious health damage (Fontana 1998). A significant decrease in the rate of caffeine metabolism and degradation was monitored mainly during the second and third trimesters of pregnancy. Because of this reduced ability to eliminate substance from the body, its prolonged duration of action takes place. The release of catecholamines into the bloodstream of pregnant women stimulated by caffeine consumption substantially increases the level of cyclic adenosine monophosphate, which may interfere with the cell division of the foetus. Abnormally high levels of catecholamines can also cause premature uterine contractions (Brazier 1983; Kirkinen et al. 1983). From the above information on the effects of caffeine during pregnancy on the foetus and pregnant women, it may appear that the intake of this substance is highly undesirable. However, many studies have confirmed that moderate caffeine intake during pregnancy, which does not exceed 200 mg per day, is not a health hazard (EFSA 2015). Problems might occur in different content of this psychoactive substance in various beverages, depending on many preparation factors. A low risk of miscarriage occurred mainly after higher intake of caffeine, exceeding 300 mg/day (Giannelli et al. 2003; Tolstrup et al. 2003; Morgan et al. 2013).

3. Aims of the Thesis

Aims of this bachelor thesis were divided into two parts. Firstly, it was the literature review focused on the genus *Coffea* spp., caffeine-free species and its cultivation. Also coffee preparation with caffeine extraction was discussed and the most used techniques were described. As the main part of literature review was to classify caffeine as the main psychoactive compound of coffee and to summarize its impact after ingestion on human health.

In the practical part the experiment took place. As the major aim of experiment was to measure volumes of samples of espresso coffee obtained around the campus of Czech University of Life Sciences (CZU) in Prague, analyse their caffeine content, compare findings with each other and to draw possible consequences from excessive caffeine ingestion caused by its unequal levels in samples.

4. Methods

4.1. Materials and methods

Caffeine content was measured in 15 different samples (Table 1; Figure 12) purchased around the campus of Czech University of Life Sciences in Prague. Analysis was performed using high performance liquid chromatography (HPLC) with diode array detector(DAD).

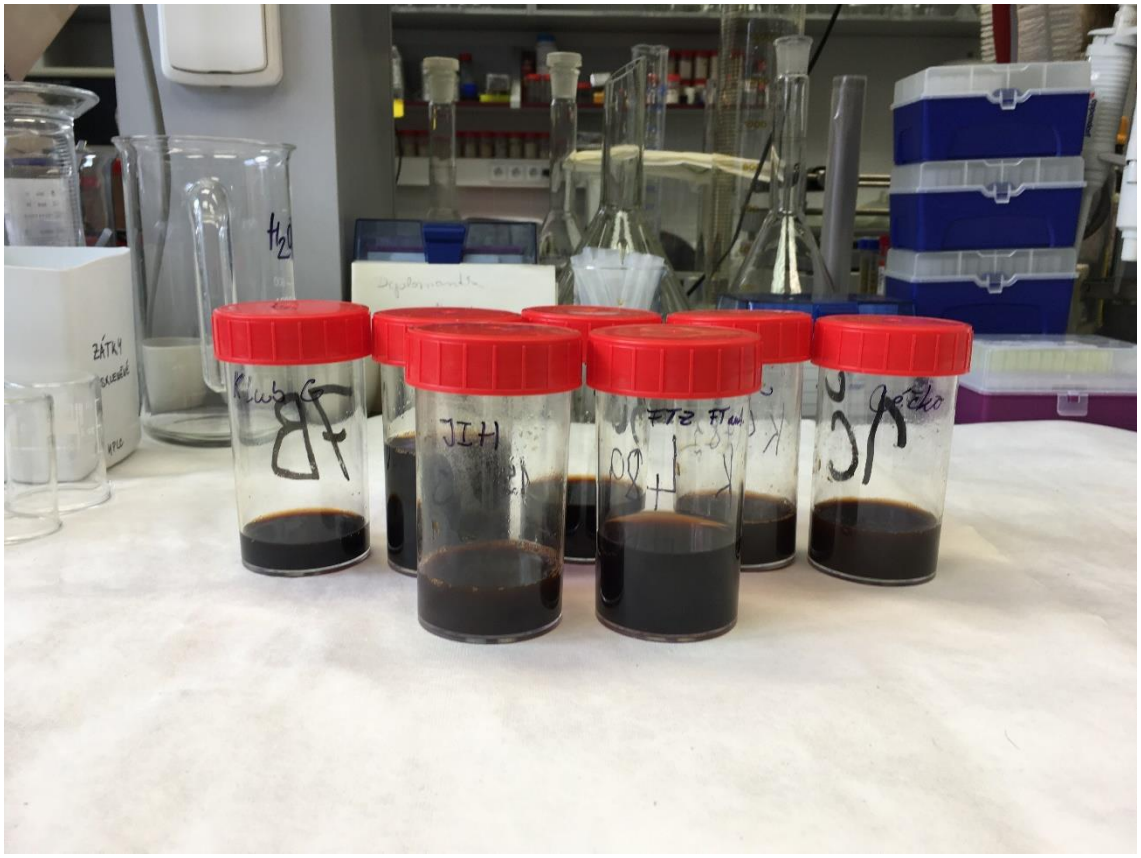


Figure 12. Second batch of samples

Author: Author's photography

Table 1. Samples of espresso coffee

Shop / place of purchase	Volume (ml)
Agrofaculty - Hodně dobré jídlo	26
Café + CO automat	72
Club C	69
Club G	40
Farma	29
FEM - Fresh espresso	45
FEM - Hodně dobré jídlo	**15.5**
FES - buffet	40
FTA - FairTrade automat	55
JIH - buffet	17
Secretary of FTA	105
SIC - buffet	31
Academic Administration Office	**115**
Technical faculty - buffet	92
University canteen - Deli buffet	45

4.1.1. Determination of caffeine content using HPLC - DAD

Chemicals used:

Methanol for HPLC (Lach-Ner, Czech republic)

Caffeine (Sigma - Aldrich®, Germany)

Demineralised water – purified using Milli-Q Plus (Millipore, Germany)

Apparatuses used:

Milli Q Plus (Millipore,Germany)

Ultrasonic bath (Tesla, Czech republic)

Analytical scales (accuracy 0.1 mg) Mettler AE200 (Mettler - Toledo, Switzerland)

Chromatographic system :

- thermostatted column compartment TCC - 100 (Dionex, USA)
- HPLC pump P680 (Dionex, USA)

- detector UVD340U (Dionex, USA)
- manual injection valve Rheodyne 7725i (Rheodyne, USA)

Syringe with PTFE membrane filter

Desktop computer LYNX with Chromeleon software

Conditions of analysis

Column: Luna ® 5 µm C18 (2), size 250 x 4.6 mm (Phenomenex, Germany)

Mobile phase: methanol : demineralised water (40:60) - isocratic elution

Detection: DAD at 264 mm

Mobile phase flow rate: 0.8 ml/min

Column temperature: 35 °C

Injection volume: 20 µl

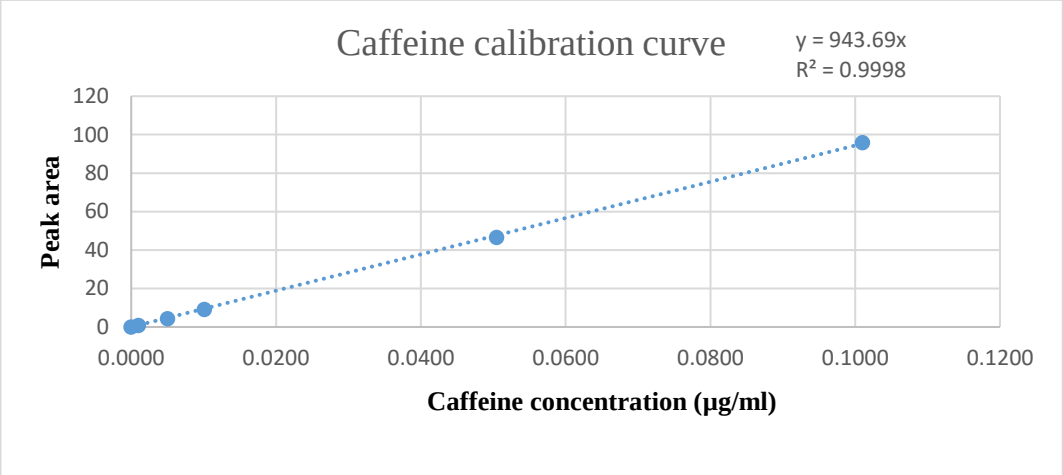
Analysis length: 8 min

Analyte retention time: 6.028 min

Preparation of caffeine standard

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 the stock solution with caffeine concentrations of 1; 5; 10; 50; 100 µg/ml. Volumes of stock solution (0.25; 1.25; 2.5; 12.5 ml) were pipetted into 25 ml volumetric flasks and replenished to the mark with mobile phase. A calibration curve was constructed in Excel using HPLC and Chromeleon software (Table 2).

Table 2. Caffeine calibration curve



Sample preparation

Each sample was filtered through a syringe microfilter with a PTFE (Polytetrafluoroethylene) membrane (0.45 µm). Solutions were diluted 20 times with mobile phase into the volumetric flasks. Each sample was analysed in three parallel tests (Figure 13).

Figure 13. Samples ready for parallel analysis

Author: Authors' photography

5. Results

Each sample was analysed three times for caffeine content. Thanks to parallel analyses, it was able to eliminate errors in measurement and thus satisfactory results were achieved. Median values were used to recalculate levels of caffeine with respect to the sample size and per 100 ml. As stated in Table 3, lowest volume of liquid was measured in the sample from buffet "Hodně dobré jídlo" at Faculty of Economics and Management (FEM). On the other hand, the largest volume was measured in sample from Academic Administration Office of Faculty of Tropical Agrisciences (FTA). However, samples obtained from Academic Administration Office of FTA and Secretariat of FTA were prepared by automatic espresso machines, with automatic coffee beans grinder and a present volume size, therefore their volumes were significantly higher. The highest levels of caffeine (931.56 mg/100 ml) were observed in the smallest sample, obtained in "Hodně dobré jídlo" buffet at FEM, which may significantly affect biological processes after only 2 shots of espresso. The highest volume of caffeine per serving was measured in sample obtained at Faculty of Environmental Sciences' buffet (FES), with the same conditions as the sample from FEM - "Hodně dobré jídlo". On the contrary, the lowest amount of caffeine was measured in sample obtained from the buffet at the JIH dormitories with only 55.36 mg of caffeine per serving and the lowest volume of substance in terms of 100 ml was observed in sample from Secretariat of FTA. The results in the table were highlighted in bold and arranged alphabetically (A-Z).

Table 3. Caffeine content in examined samples

Shop/place of Purchase	Volume (ml)	Caffeine content per serving (mg)	Caffeine content (mg/100ml)
Agrofaculty - Hodně dobré jídlo	26	128.35	488.22
Café + CO automat	72	116.27	161.49
Club C	69	126.71	183.63
Club G	40	80.86	202.15
Farma	29	75.97	261.99
FEM- Fresh espresso	45	95.15	211.45
FEM - Hodně dobré Jídlo	**15.5**	144.39	**931.56**
FES - buffet	40	**150.61**	376.52
FTA - FairTrade Automat	55	96.12	174.76
JIH - buffet	17	**55.36**	325.66
Secretariat of FTA	105	64.14	**61.09**
SIC - buffet	31	100.02	322.65
Academic Administration Office	**115**	75.25	65.43
Technical faculty - Buffet	92	113.66	123.54
University canteen - Deli buffet	45	75.83	168.50

6. Discussion

Caffeine content was measured by high performance liquid chromatography (HPLC) with DAD detection in various samples of espresso coffee. As mentioned earlier in this thesis, espresso volume should vary from 25 - 40 ml (Petracco 2001). Only 5 out of 15 samples were in this range, just 2 were smaller than minimum limit and 8 were larger than upper limit. This was most likely caused by the different techniques of each barista, their coffee, ground too fine (smaller volume) or too coarse (higher volume), their compression force, different machines with various technical settings and other variables. Caffeine, as a measured substance, also varied in each sample. Difference between the lowest and highest content was 95 mg of caffeine per serving, meaning that the espresso sample with 150.61 mg per serving (the highest level) was close to the upper safe level stated by the European Food Safety Authority (2015), which is 200 mg in one sitting. Although, after drinking more than 2 shots of this espresso, the maximum daily limit is obtained and slightly exceeded by 50 mg of caffeine. Seven samples contained more than 100 mg of caffeine per serving, resulting in advised maximum intake of 2 espresso shots in one sitting and no more than 3 during one day. Interestingly, the smallest sample (only 15.5 ml) contained the highest levels of caffeine per 100 ml. Reason for this small volume of coffee with high levels of caffeine was mainly preparation technique by barista. Coffee was ground too fine and coffee cake was pressed too much, resulting in small strong espresso. The alarming number was as high as 931.56 mg of caffeine, meaning that after ingestion of 6 or more shots of this espresso (to fulfil 100 ml condition), upper daily limit is extremely exceeded by more than 500 mg. To compare obtained results with the article that inspired this research, Crozier et al. (2012) tested 20 samples of espresso with serving sizes from 23 - 70 ml and range of caffeine content from 51 - 322 mg/serving. McCusker et al. (2003) examined espresso samples with 28 ml volume and described range from 58 to 76 mg of caffeine (207.14 - 271.42 mg/100 ml) In contrary, data shared by International Food Information Council Foundation (IFIC) in 2008 state, that caffeine volumes in one espresso serving (30 ml) varies only from 30 - 50 mg. Interestingly, none of three researches showed such low values. This may lead to disinformation and possible intoxication by enormous caffeine intake. As results by

Crozier et al. (2012) and those obtained by this research are similar and both show significant differences from IFIC, there is a point of reflection, what were main reasons of such high levels of caffeine? Most likely it was caused by different blends of coffee ground (arabica × robusta), grade of roasting, size of ground particles, different practices of baristas and various coffee machines used for preparation. Even Caporaso et al. (2014) reported caffeine content in espresso from 60 - 112.5 mg per 25 ml serving (2.4 - 4.5 mg/ml). From all of these data we can conclude, that the results obtained in this research do not deviate from the average values as much as from the data shared by IFIC. Such low values shared by IFIC might be caused by a measurement error, use of coarse ground, special or stale coffee beans or inadequate technological settings of the espresso machines. Although, new testing with specified conditions should take place.

As discussed, those alarming amounts of caffeine discovered in samples, might have severe damaging effects on the consumers' health, moreover if any health condition already exists. People with dysfunctions in central nervous system, psychical issues such as anxiety disorder, mental dysfunctions, liver abnormalities, pregnant or breastfeeding women, women with osteoporosis and non-habitual caffeine consumers might experience intoxication and poisoning. However, this scenario is not likely to happen, as after the digestion of fourth shot of espresso, first symptoms would appear, such as jitters, shivers, overall nervousness, increased need to urinate etc., and those might signal cessation of ingesting more caffeine. Two samples with the highest volumes were prepared on automatic espresso machines, therefore human interaction could not affect a preset values and functions of these cafeterias as radically as on classic espresso machines operated by baristas. On the other hand, coffee sample that contained only 55.36 mg of caffeine per serving was also the second smallest sample obtained (17 ml), pointing to the too coarse ground and low pressure used. This amount is considered as safe for all adult users regardless of health status. It is important to note, that there are large inter-individual disparities in caffeine metabolism, thus every person reacts to caffeine intake differently. Differences are affected mainly by age, hormone levels, tobacco smoking habit, liver function and/or drug abuse. Therefore, it is difficult to determine and evaluate the overall impact and effects on individuals, mainly after moderate intake. Although, caffeine withdrawal syndrome is recognised by American Psychiatric Association (1994) in regular caffeine users. With mild to serious symptoms that affects subjects based on inter-individual differences (Phillips-Bute &

Lane 1998), including headaches, fatigue, loss of focus and concentration etc., caffeine withdrawal syndrome may be easily avoided by decreasing daily intake of substance leisurely. Effects of caffeine (and its withdrawal) on human physical health are based on inter-individual differences and that is why it is difficult to draw one-way conclusions that would apply to the entire population. The risk group consist mainly of pregnant and breastfeeding women (Fontana 1998), because the foetus does not have developed liver cytochromes CYP1A2 for the breakdown of caffeine, which can results in serious damaging defects. According to EFSA (2015), pregnant women should not exceed daily caffeine intake 200 mg. As this research revealed high amounts of caffeine, it is important to be a careful user and prioritize decaffeinated coffee and tea.

Although observed differences in caffeine content were high, it might be caused by various places of origin of each coffee, its variety (arabica × robusta) or their blends, freshness and size of ground particles, level of roast and mainly by baristas' preparation techniques.

7. Conclusions

First part of this bachelor thesis was focused on literature review of coffee origin, history, its cultivation, various (potentially) economically important species, different beverage preparation methods and caffeine as a main psychoactive substance. Furthermore, biosynthesis, pharmacokinetics, metabolism and interpersonal disparities in metabolism were described. The most important was the impact of caffeine on human physical health and psychical performance, as it is the most consumed legal substance with effects on psyche worldwide.

In practical part experiment revealed differences in 15 various samples of espresso coffee obtained around the campus of CZU in Prague. Differences were observed not only in liquid volume, but also in caffeine content. Volumes of samples varied from 15.5 ml to 115 ml, making almost 100 ml difference. Also caffeine levels varied considerably, from 55.36 to 150.61 mg per serving or from 61.09 to 931.56 mg per 100 ml. These differences proved the importance of right preparation techniques and emphasised the need to raise awareness in general public, as acute intoxication by high levels of caffeine could be possible. For all University employees and students, caution in their caffeine intake is highly advised and further research with more measurements should take place to confirm or refute obtained data.

8. References

Akaffou DS.1999. Recherche des possibilités d'amélioration des caféiers cultivés par transfert de gènes des caféiers sauvages: étude des hybrides interspécifiques entre Coffea pseudozanguebariae Bridson et C. liberica var. dewevrei De Wild et Th. Dur [Ph.D. thesis]. Université of Cocody; Abidjan, Côte d'Ivoire.

Akaffou S, et al. 2012. Inheritance and relationship between key agronomic and quality traits in an interspecific cross between *Coffea pseudozanguebariae* Bridson and *C. canephora* Pierre. Tree Genetics & Genomes**8**: 1149–1162.

American Psychiatric Association. 1994. Diagnostic and Statistical Manual of Mental Disorders; American Psychiatric Association: Washington, DC, USA.

Andersen I M, et al.2014. Effects of Coffee Consumption, Smoking, and Hormones on Risk for Primary Sclerosing Cholangitis. Clinical Gastroenterology and Hepatology**12**: 1019–1028

Andrianasolo DN, Davis AP, Razafinarivo NJ, Hamon S, Rakotomalala JJ, Sabatier SA, Hamon P. 2013. High genetic diversity of in situ and ex situ populations of Madagascan coffee species: further implications for the management of coffee genetic resources. Tree Genetics & Genomes**9**: 1295–1312.

Arnaud M. 1993. Metabolism of caffeine and other components of coffee. Caffeine, Coffee and Health **1** 43–95,

Arnaud M, Enslen M. 1991. The role of paraxanthine in mediating physiological effects of caffeine. Pages 71–79 in 14th International Colloquium on Coffee Science; ASIC, Paris, France.

Arnaud M. 2011. Pharmacokinetics and metabolism of natural methylxanthines in animal and man. Handbook of Experimental Pharmacology **200**: 33–91

Arnaud M. 2005. Caffeine. Pages 247–252. Allen L., Caballero B., Prentice A., editors. Encyclopedia of Human Nutrition. Elsevier. Amsterdam, Netherlands

Ascherio A, et al. 2004. Coffee Consumption, Gender, and Parkinson's Disease Mortality in the Cancer Prevention Study II Cohort: The Modifying Effects of Estrogen. American Journal of Epidemiology**160**: 977–984

Ashihara H, Crozier A. 2001. Caffeine: a well known but little mentioned compound in plant science. Trends in Plant Science **6**: 407–413.

Ashihara H, Kato M, Crozier A. 2011. Distribution, biosynthesis and catabolism of methylxanthines in plants. Pages 11–33. Fredholm BB, editor. Methylxanthines, handbook of experimental pharmacology. Springer - Verlag. Berlin Heidelberg, Germany.
Ashihara H, Sano H, Crozier A. 2008. Caffeine and related purine alkaloids: biosynthesis, catabolism. Phytochemistry **69**: 841–856.

Ashihara H. 2006. Metabolism of alkaloids in coffee plants. Brazilian Journal of Plant Physiology**18**: 1–8.

Ashihara H, Kubota H. 1986.Patterns of adenine metabolism and caffeine biosynthesis in different parts of tea seedlings. Physiologia Plantarum **68**:275-281.

Ashihara H, Crozier A, 1999. Biosynthesis and metabolism of caffeine and related purine alkaloids in plants. Advances in Botanical Research **30**: 118–205.

Baggenstoss J, Poisson L, Kaegi R, Perren R, Escher F. 2008. Roasting and aroma formation:Effect of initial moisture content and steam treatment. Journal of Agricultural and FoodChemistry**56**: 5847–5851.

Baker J A, et al. 2006. Consumption of coffee, but not black tea, is associated with decreased risk of premenopausal breast cancer. The Journal of Nutrition**136**: 166–171.

Barbanti D, Nicoli MC. 1996. Estrazione e stabilità della bevanda di caffè: Aspetti chimici etecnologici. Tecnologie Alimentari**1**: 62–67.

Barre P, Akaffou S, Louarn J, Charrier A, Hamon S, Noirot M. 1998. Inheritance of caffeine and heteroside contents in an interspecific cross between a cultivated coffee species *Coffea liberica* var *dewevrei* and a wild species caffeine-free *C. pseudozanguebariae*. Theoretical and Applied Genetics **96**: 306–311.

Blanchard J, Sawers SJ. 1983. The absolute bioavailability of caffeine in man. European Journal of Clinical Pharmacology**24**: 93–98.

Bonati M, Latini R, Galletti F, Young JF, Tognoni G, Garattini S. 1982. Caffeine disposition after oral doses. Clinical Pharmacology & Therrapeutics **32**: 98–106.

Bonnett M, Tancer M, Uhde T, Yeragani VK. 2005. Effects of caffeine on heart rate and QT variability during sleep. Depression and Anxiety **22**: 150–155.

Botelho F, et al. 2006. Coffee and gastric cancer: systematic review and meta-analysis. Cadernos de saúde pública**22**: 889–900.

Brandts B, et al. 2003. Diadenosine-5-phosphate exerts A1-receptor–mediated proarrhythmic effects in rabbit atrial myocardium. British Journal of Pharmacology **139**: 1265–72.

Bravi F. et al. 2013. Coffee reduces risk for hepatocellular carcinoma: an updated meta-analysis. Clinical gastroenterology and hepatology: the official clinical practice journal of the American Gastroenterological Association**11**: 1413–1421.

Brazier J L, et al. 1983. Pharmacokinetics of caffeine during and after pregnancy. Developmental pharmacology and therapeutics**6**: 315–322.

Caballero B. 2009. Guide to nutritional supplements. Academic Press, Cambridge, Massachusetts, USA

CAC, FAO,WHO. 2008. Report of the 2nd session of the codex committee on contaminants in foods. Codex Alimentarium Commission, The Hague, The Netherlands.

Cai L, et al.2012. The effect of coffee consumption on serum lipids: a meta-analysis of randomized controlled trials. European journal of clinical nutrition **66**: 872–877.

Cailleux E, Bullier E, Moreau C, Campa C, Noirot M, Hamon S, De Kochko A,. 2004. Some genes encoding enzymes involved in the caffeine biosynthesis of *Coffea pseudozanguebariae* are regulated by an incomplete splicing of their pre-messenger RNA. Pages 696–698 in Proceedings of the 20th congress of the international scientific association of coffee Bangalore, India. ASIC, Paris, France.

Campa C, Doulbeau S, Dussert S, Hamon S, Noirot M. 2005. Diversity in bean caffeine content among wild *Coffea* species: evidence of a discontinuous distribution. Food Chemistry**9**: 633–637.

Campbell B, et al. 2013. International Society of Sports Nutrition position stand: energy drinks. The International Society of Sports Nutrition. ISSN (1550-2783) DOI: https://doi.org/10.1186/1550-2783-10-1

Caporaso N, Genovese A, Canela MD, Civitella A, Sacchi R. 2014. Neapolitan coffee brewchemical analysis in comparison to espresso, moka and American brews. Food ResearchInternational**61**: 152–160.

Cárdenas C, et al. 2011. Anti-angiogenic and anti-inflammatory properties of kahweol, a coffee diterpene. PLoS ONE *(*e23407) DOI: https://doi.org/10.1371/annotation/38262cc6-07cc-4074-8ce7-2181d4d0fbdc

Charrier A, Berthaud J. 1975. Variation de la teneur en caféine dans le genre *Coffea*. *Café Cacao Thé* **19**: 251–264.

Chevalier A. 1938. Essai d'un groupement systématique des caféiers de Madagascar et des Iles Mascareignes. Revue International de Botanique Appliquee et d'Agriculture Tropicale **18**: 825–843.

Clifford M N, Gibson C L, Rakotomalala J J R, Cros E, Charrier A. 1991. Caffeine from green beans of Mascarocoffea. Phytochemistry**30**: 4039–4040.

Corrochano B R, Melrose J R, Bentley A C, Fryer P J, Bakalis S. 2015. A new methodology to estimate the steady-state permeability of roast and ground coffee in packed beds. Journal of Food Engineering **150**, 106 - 116.

Crozier T W M, Stalmach A, Lean M E J, Crozier A. 2012. Espresso coffees, caffeine and chlorogenic acid intake: potential health implications. Food & Function **3**: 30–33.

Davis A P, Chester M, Maurin O, Fay M F. 2007. Searching for the relatives of *Coffea* (Rubiaceae, Ixoroideae): the circumscription and phylogeny of Coffeeae based on plastid sequence data and morphology. American Journal of Botany **94**: 313–329.

Denaro C P, Brown C R, Wilson M, Jacob P, Benowitz N L. 1990. Dose-dependency of caffeine metabolism with repeated dosing. Clinical Pharmacology & Therapeutics **48**: 277–285.

Dutra ER, Oliveira LS, Franca AS, Ferraz VP, Afonso RJCF.2001.A preliminary study on thefeasibility of using the composition of coffee roasting exhaust gas for the determinationof the degree of roast. Journal of Food Engineering **47**: 241–246.

EFSA. European Food Safety Authority. 2015. Scientific opinion on safety of caffeine. EFSA. Parma, Italy. Available from : https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2015.4102 (accessed March 2020).

Eskelinen M H, Kivipelto M. 2010. Caffeine as a Protective Factor in Dementia and Alzheimer's Disease. Journal of Alzheimer's Disease 20: 167–174.

Evans S M; Griffiths R R. 1999. Caffeine withdrawal: A parametric analysis of caffeine dosing conditions. Journal of Pharmacology and Experimental Therapeutics **289**: 285–294.

FAO. 2005.FAOSTAT: Arabica coffee manual for Lao PDR. FAO Regional Office for Asia and the Pacific, Bangkok. Available from: http://www.fao.org/docrep/008/ae939e/ae939e00.htm (accessed March 2020)

FAO. 2018. FAOSTAT: Production - Coffee,green. FAO, Rome. Available from http://faostat.fao.org/ (accessed May 2020).

Farah A. 2012. Coffee Constituents. Pages 21–58 in Chu YF editor. *Coffee :* Emerging Health Effects and Disease Prevention. Wiley-Blackwell, Hoboken, New Jersey, USA.

Fontana R J,et al. 1998. Caffeine based measures of CYP1A2 activity correlate with oral clearance of tacrine in patients with Alzheimer's disease. British Journal of Clinical Pharmacology **46**: 221–228.

Fox GP, Wu A, Yiran L, Force L. 2013. Variation in caffeine concentration in single coffee beans. Journal of Agricultural and Food Chemistry**61**: 10772-10778.

Franca AS, Mendonca CFJ , Oliveira SD. 2005.Composition of green and roasted coffees of different cup qualities. Lebensmittel-Wissenschaft & Technologie**38**: 709–715.

Fredholm BB, Bättig K, Holmén J, Nehlig A, Zvartau EE. 1999. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacological Reviews **51**: 83–133.

Friberg E, et al. 2009. Coffee drinking and risk of endometrial cancer - a population-based cohort study. International journal of cancer. Journal international du cancer**125**: 2413–2417.

Frischknecht PM, Baumann TW. 1985. Stress induced formation of purine alkaloids in plant tissue culture. Phytochemistry**24**: 2255–2257.

Frischknecht P M, Ulmer-Dufek J, Baumann T W. 1986.Purine alkaloid formation in buds and developing leaflets of *Coffea arabica*: expression of an optimal defence strategy?Phytochemistry **25**:613–616.

Fujimori N, Suzuki T, Ashihara H. 1991. Seasonal variations in biosynthetic capacity for the synthesis of caffeine in tea leaves. Phytochemistry**30**: 2245–2248.

Galeone C, et al. 2010. Coffee and Tea Intake and Risk of Head and Neck Cancer: Pooled Analysis in the International Head and Neck Cancer Epidemiology Consortium. Cancer Epidemiology Biomarkers & Prevention **19**: 1723–1736.

Gallus S, et al. 2002. Does Coffee Protect Against Liver Cirrhosis? Annals of Epidemiology**12**: 202–205.

Ganmaa D, et al. 2002. Incidence and mortality of testicular and prostatic cancers in relation to world dietary practices. International Journal of Cancer **98**: 262–267.

Gao ZG, Jacobson KA. 2011. Emerging adenosine receptor agonists—an update. Expert Opinion on Emerging Drugs**16**: 597–602.

Gelber R P, et al. 2011. Coffee Intake in Midlife and Risk of Dementia and its Neuropathologic Correlates. Journal of Alzheimer's Disease**23**: 607–615.

Geromel C, et al. 2006. Biochemical and genomic analysis of sucrose metabolism during coffee (*Coffea arabica*) fruit development. Journal of Experimental Botany **57**: 3243–3258.

Giannelli M, et al. 2003. The effect of caffeine consumption and nausea on the risk of miscarriage. Paediatric and perinatal epidemiology **17**: 316–323.

Glade M J. 2010. Caffeine-Not just a stimulant. Nutrition**26**: 932–938.

Gloess AN, Schonbachler B, Klopprogge B, D'Ambrosio L, Chatelain K, Bongartz A,Strittmatter A, Rast M, Yeretzian C. 2013. Comparison of nine common coffee

extractionmethods: Instrumental and sensory analysis. European Food Research and Technology**236**: 607–627.

Griffiths R R; Bigelow G E; Liebson I A. 1986. Human coffee drinking: Reinforcing and physical dependence producing effects of caffeine. Journal of Pharmacology and Experimental Therapeutics **239**: 416–425.

Haskell C F, et al. 2005. Cognitive and mood improvements of caffeine in habitual consumers and habitual non-consumers of caffeine. Psychopharmacology**179**: 813–825.

Haskó G, Cronstein B. 2011. Methylxanthines and inflammatory cells. Handbook of Experimental Pharmacology **200**: 457–468.

Henman A R. 1986. Vida Natural. O Guaraná: Sua cultura, propriedades, formas de preparação e uso. Global/Ground, São Paulo, Brazil.

Hewlett P, Smith A. 2007. Effects of repeated doses of caffeine on performance and alertness: new data and secondary analyses. Human Psychopharmacology: Clinical and Experimental**22**: 339–350.

Hirose F, Ashihara H. 1984. Changes in activity of enzymes involved in purine "salvage" and nucleic acid degradation during growth of *Catharanthus roseus* cells in suspension culture. Physiologia Plantarum**60**:532–538

Höfer I F; Bättig K. 1994. Psychophysiological effects of switching to caffeine tablets or decaffeinated coffee under field conditions. Pharmacopsychoecologia **7**:169–177.

Honjo S, et al. 2001. Coffee consumption and serum aminotransferases in middle-aged Japanese men. Journal of Clinical Epidemiology**54**: 823–829.

Hrubá D. 1997. Konzumace kávy ve vztahu k poruchám reprodukce. Hygiena **42**: 37–43.
Hrubá D, Kachlík P. 1997. Konzumace kávy a frekvence poruch reprodukce. Praktický Lékař**77**: 491–493.

Hu G, et al. 2006. Joint association of coffee consumption and other factors to the risk of type 2 diabetes: a prospective study in Finland. International Journal of Obesity **30**: 1742–1749.

International Food Information Council Foundation. 2008. IFIC Review: Caffeine & Health: Clarifying The Controversies. IFIC, Washington DC. Available from https://foodinsight.org/ific-review-caffeine-and-health-clarifying-the-controversies/(Accessed Ma 2020).

Ito K, Lim S, Caramori G, Cosio B, Chung KF, Adcock I M, Barnes PJ. 2002. A molecular mechanism of action of theophylline: induction of histone deacetylase activity to decrease inflammatory gene expression. Proceedings of the National Academy of Sciences**99**: 8921–8926.

Jarboe CH, Hurst HE, Rodgers G C Jr, Metaxas JM. 1986. Toxicokinetics of caffeine elimination in an infant. Journal of Toxicology: Clinical Toxicology **24**: 415–428.

Je Y A. 2011. Prospective Cohort Study of Coffee Consumption and Risk of Endometrial Cancer over a 26-year of Follow-Up. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology**20**: 2487–2495.

Kadlec P, et al. 2012. Přehled tradičních potravinářských výrob : technologie potravin. Key Publishing, Ostrava.

Kawachi I, et al. 1996. A prospective study of coffee drinking and suicide in women. JAMA Internal Medicine**156**: 521–525.

Kempf K, et al. 2010. Effects of coffee consumption on subclinical inflammation and other risk factors for type 2 diabetes: a clinical trial. The American Journal of Clinical Nutrition**91**: 950–957.

Killer S C, Blannin A K, JeukendrupAE. 2014. No Evidence of Dehydration with Moderate Daily Coffee Intake: A Counterbalanced Cross-Over Study in a Free-Living Population. PLoS ONE (e84154) DOI: 10.1371/journal.pone.0084154.

Kirkinen P, et al. 1983. The effect of caffeine on placental and fetal blood flow in human pregnancy. American journal of obstetrics and gynecology **147**: 939–942.

Klatsky A L, et al. 1993. Coffee, tea, and mortality. Annals of epidemiology**3**: 375–381.

Krishnan S, Ranker TA, Davis AP, Rakotomalala J-J. 2012. An assessment of the genetic integrity of *ex situ* germplasm collections of three endangered species of *Coffea* from Madagascar: implications for the management of field germplasm. Genetic Resources and and Crop Evolution **60**: 1021–1036.

Lee J E, et al. 2007. Intakes of coffee, tea, milk, soda and juice and renal cell cancer in a pooled analysis of 13 prospective studies. International Journal of Cancer**121**: 2246–2253.

Lee K J, et al. 2007. Hepatoprotective and antioxidant effects of the coffee diterpenes kahweol and cafestol on carbon tetrachloride-induced liver damage in mice. Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association**45**: 2118–2125.

Lee K J, Jeong H G. 2007. Protective effects of kahweol and cafestol against hydrogen peroxide-induced oxidative stress and DNA damage. Toxicology Letters **173**: 80–87.

Lee S, et al. 2005. Caffeine ingestion is associated with reductions in glucose uptake independent of obesity and type 2 diabetes before and after exercise training. Diabetes care **28**: 566–572.

Lelo A, Birkett DJ, Robson R A, Miners JO. 1986. Comparative pharmacokinetics of caffeine and its primary demethylated metabolites paraxanthine, theobromine and theophylline in man. British Journal of Clinical Pharmacology **22**: 177–182.

Li G, et al. 2013. Coffee consumption and risk of colorectal cancer: a meta-analysis of observational studies. Public health nutrition**16**: 346–357.

Lima J P; Farah A. 2019. Potential negative effects of caffeine consumption on health. Pages 489–508 in Farah A. editor. Coffee: Consumption and Health Implications. Royal Society of Chemistry: London, UK, 2019;

Llach A, et al. 2011. Abnormal calcium handling in atrial fibrillation is linked to up-regulation of adenosine A2A receptors. European Heart Journal **32**:721–729.

Ludwig IA, Sanchez L, Cammerer B, Kroh LW, de Peña MP, Cid C. 2012. Extraction of coffee antioxidants: Impact of brewing time and method. Food Research International**48**:57–64.

Mangal S K. 2007. *Coffee : Planting, Production and Processing*. Global Media, Delhi, India.

Manore M M. 2012. Dietary supplements for improving body composition and reducing body weight: where is the evidence? International journal of sport nutrition and exercise metabolism**22**: 139–154.

Mattioli A V. 2007. Coffee and caffeine effects on hypertension. Current Hypertension Reviews**3**: 250–254.

Mattioli AV. Effects of caffeine and coffee consumption on cardiovascular disease and risk factors. Future Cardiology **3**: 203–212

McCusker R R, Goldberger B A, Cone E J. 2003. Caffeine content of specialty coffees. Journal of Analytical Toxicology **27**: 520–522.

Mestdagh F, Glabasnia A, Giuliano P. 2017. The Brew - Extracting for Excellence. Pages 355–378 in Folmer B, Blank, I, Farah A, Giuliano P, Sanders D, Wille Ch, editors. The Craft and Science of Coffee. Academic Press, London, United Kingdom.

Morgan S, et al. 2013. Is caffeine consumption safe during pregnancy? Canadian family physician Médecin de famille canadien **59**: 361–362.

Moroney KM, Lee WT, O'Brien SBG, Suijver F, Marra J. 2015. Modelling of coffee extractionduring brewing using multiscale methods: An experimentally validated model.Chemical Engineering Science**137**: 216–234.

Murthy PS, Naidu MM. 2012. Sustainable management of coffee industry by-products and value addition- a review. Resources, Conservation & Recycling **66**: 45–58.

Nagai C, Rakotomalala J-J, Katahira R, Li Y, Yamagata K, Ashihara H. 2008. Production of new low-caffeine hybrid coffee and biochemical mechanism of low caffeine accumulation. Euphytica **164**: 133–142.

Nakanishi N, et al. 2000. Lifestyle and serum gamma-glutamyltransferase: a study of middle-aged Japanese men. Journal of Hepatology**50**: 115–120.

Navarini L., Nobile E., Pinto F., Scheri A., Suggi-Liverani F. 2009. Experimental investigation of steam pressure coffee extraction in a stove-top coffee maker. Applied Thermal Engineering **29**: 998–1004.

Negishi O, Ozawa T, Imagawa H. 1985. The role of xanthosine in the biosynthesis of caffeine in coffee plants. Agricultural and Biological Chemistry **49**: 2221–2222.

Nehlig A. 2018. Interindividual Differences in Caffeine Metabolism and Factors Driving Caffeine Consumption. Pages 384–411in Alexander S.P.H. editor. Pharmacological reviews. The American Society for Pharmacology and Experimental Therapeutics. Pediatric Neurology, Necker-Enfants Malades Hospital, University of Paris Descartes, Paris, France

Nkondjock A, et al. 2006. Coffee consumption and breast cancer risk among BRCA1 and BRCA2 mutation carriers. International journal of cancer. Journal international du cancer **118**: 103–107.

Palacios N, et al. 2010. Polymorphisms of caffeine metabolism and estrogen receptor genes and risk of Parkinson's disease in men and women. Parkinsonism & Related Disorders**16**: 370–375.

Pelucchi C, Vecchia C L A. 2009. Alcohol, coffee, and bladder cancer risk: a review of epidemiological studies. European journal of cancer prevention: the official journal of the European Cancer Prevention Organisation (ECP) **18**: 62–68.

Pérez-Martínez M, Caemmerer B, De Peña MP, Cid C, Kroh LW. 2010. Influence of brewingmethod and acidity regulators on the antioxidant capacity of coffee brews. Journal ofAgricultural and Food Chemistry**58**: 2958–2965.

Petermann J, Baumann TW. 1983. Metabolic relations between methylxanthines and methyluric acids in *Coffea*. *Plant Physiology* **73**: 961–964.

Petracco M., 2001. Beverage preparation: brewing trends for the new millennium. Pages 140–164; 230 in Clarke, R, Vitzthum, O, editors., Coffee: Recent Developments. Blackwell Science, Oxford.

Phillips-Bute B G.; Lane J D. 1998. Caffeine withdrawal symptoms following brief caffeine deprivation. Physiology & Behaviour **63**: 35–39.

Pössl, M. 2010. *Čaj jako životní styl*. Grada, Praha

Rakotomalala J-J, Cros E, Clifford MN, Charrier A. 1992. Caffeine and theobromine in green beans from *Mascarocoffea*. Phytochemistry **31**: 1271–1272.

Rakotomalala J-J, Kumamoto T, Aburatani T, Rabemiafara A, Nagai C, Sanbongi K, et al. 2004. Caffeine content distribution among *Mascarocoffea* species in Madagascar. Pages 154–160 in Proceedings, 20th ASIC international conference on coffee science, ASIC, Bangalore, India.

Rao S. 2013. Espresso Extraction: Measurement and Mastery. Self-publication, USA

Rapuri P B, et al. 2001. Caffeine intake increases the rate of bone loss in elderly women and interacts with vitamin D receptor genotypes. The American journal of clinical nutrition**74**: 694–700.

Rauh R, Burkert M, Siepmann M, Mueck-Weymann M. 2006. Acute effects of caffeine on heart rate variability in habitual caffeine consumers. Clinical Physiology and Functional Imaging**26**: 163–166.

Razafinarivo NJ, et al. 2013. Genetic structure and diversity of coffee (*Coffea*) across Africa and the Indian Ocean Islands revealed using microsatellites. Annals of Botany**111**: 229–248.

Richardson T, Rozkovec A, Thomas P, Ryder J, Meckes C, Kerr D. 2004. Influence of caffeine on heart rate variability in patients with longstanding type 1 diabetes. Diabetes Care**27**: 1127–1131.

Romero JCL, Waller GR. 1988. Production of a new compound by metabolism of theophylline in *Coffea arabica* L. Revista Latinoamericana de Química **19**: 7–12.

Ross C W. 1981. Biosynthesis of Nucleotides. Pages 169–205in Stumpf P K, Conn E E, editors. The Biochemistry of Plants Vol. 6. Academic Press, New York.

Ruusunen A, et al. 2010. Coffee, tea and caffeine intake and the risk of severe depression in middle-aged Finnish men: the Kuopio Ischaemic Heart Disease Risk Factor Study. Public health nutrition**13**: 1215–1220.

Salazar-Martinez E, et al. 2004. Coffee consumption and risk for type 2 diabetes mellitus. Annals of internal medicine**140**: 1–8.

Severini C, Ricci I, Marone M, Derossi A, De Pilli T. 2015. Changes in the aromatic profile of espresso coffee as a function of the grinding grade and extraction time: a study by the electronic nose system. Journal of Agriculture and Food Chemistry **63**: 2321–2327.

Severini C, Derossi A, Ricci I, Fiore A G, Caporizzi R. 2017. How Much Caffeine in Coffee Cup? Effects of Processing Operations, Extraction Methods and Variables. Pages 45–85 in Latosińska M, Latosińska J L ,editors. The question of caffeine. Intech, Rijeka, Croatia.

Silverman K; Evans S M; Strain E C; Griffiths R R. 1992. Withdrawal syndrome after the double-blind cessation of caffeine consumption. The New England Journal of Medicine **327**:1109–1114.

Smith A P. 2009. Caffeine, cognitive failures and health in a non-working community sample. Human psychopharmacology**24**: 29–34.

Sondermeijer H P, van MarleAGJ, Kamen P, Krum H. 2002. Acute effects of caffeine on heart rate variability. American Journal of Cardiology **90**: 906–909.

Stalmach A, et al. 2011. Phytochemicals on Coffee and the Bioavailability of Chlorogenic Acids. Teas, Cocoa and Coffee : Plant Secondary Metabolites and Health. Wiley- Blackwell, Hoboken, New Jersey, USA.

Stasolla C, Katahira R, Thorpe T A, Ashihara H, 2003. Purine and pyrimidine nucleotide metabolism in higher plants. Journal of Plant Physiology **160**: 1271–1295.

Stoffelen P, Noirot M, Couturon E, Anthony F. 2008. A new caffeine-free coffee from Cameroon. Botanical Journal of the Linnean Society **158**: 67–72.

Strain E; Mumford G; Silverman K; Griffiths R R. 1994. Caffeine dependence syndrome. Evidence from case histories and experimental evaluations. JAMA **272**: 1043–1048.

Suzuki T, Waller G R. 1984. Biosynthesis and biodegradation of caffeine, theobromine, and theophylline in *Coffea arabica* L. fruits. Journal of Agricultural and Food Chemistry **32**: 845–848.

Suzuki T, Waller G R. 1988. Metabolism and analyses of caffeine and other methylxanthines in coffee, tea, cola, guarana and cacao. Pages 184–220 inLinskens, H F, Jackson, J F, editors. Modern methods of Plant Analysis, New Series Volume 8, Analysis of Non-alcoholic Beverages. Springer, Berlin.

SuzukiT, AshiharaH, WallerG R. 1992. *Purine and* purine alkaloid metabolism in *Camellia* and *Coffea* plants. Phytochemistry**31**: *2575*–2584.

Tanskanen A, et al. 2000. Heavy coffee drinking and the risk of suicide. European Journal of Epidemiology**16**: 789–791.

Tanskanen A, et al. 2000. Joint heavy use of alcohol, cigarettes and coffee and the risk of suicide. Addiction**95**: 1699–1704.

Tolstrup J S, et al. 2003. Does caffeine and alcohol intake before pregnancy predict the occurrence of spontaneous abortion? Human reproduction**18**: 2704–2710.

Trang J M, Blanchard J, Conrad KA, Harrison G G. 1985. Relationship between total body clearance of caffeine and urine flow rate in elderly men. Biopharmaceutics & Drug Disposition **6**:51–56.

Tsutsumi K, et al. 2001. The effect of pregnancy on cytochrome P4501A2, xanthine oxidase, and N-acetyltransferase activities in humans. Clinical pharmacology and therapeutics**70**: 121–125

Ukers W H. 1922. All about coffee. The Tea and Coffee Trade Journal Company; New York, USA.

United States Department of Agriculture, Foreign Agricultural Service. 2019. Coffee: World Markets and Trade. USDA/FAS. Available from https://apps.fas.usda.gov/psdonline/circulars/coffee.pdf (accessed May 2020).

Vecchia C L A.2005. Coffee, liver enzymes, cirrhosis and liver cancer. Journal of Hepatology**42**: 444–446.

Velíšek J. 2002. *Chemie potravin II.* OSSIS, Tábor.

Velíšek J. 2002. *Chemie potravin III.* OSSIS,Tábor.

Vignoli JA, Bassoli DG, Benassi MT. 2011. Antioxidant activity, polyphenols, caffeine andmelanoidins in soluble coffee: The influence of processing conditions and raw material. Food Chemistry**124**: 863–868.

Waller GR. 1987. Allelochemicals: Role in agriculture and forestry. American Chemical Society, Washington DC

Waller G R, Ashihara H, Suzuki T. 1991. Comparison of caffeine, theobromine and theophylline metabolism and distribution between coffee and tea plants. 14th International Scientific Colloquium on Coffee **14**: 258–268

Waller G R, Ashihara H, Suzuki T. 1993. .Updated review of purine and purine alkaloid metabolism in Coffea and Camellia plants. 15th International Scientific Colloquium on Coffee **1**: 141–154.

Wang X, Lim LT. 2014. Effect of roasting conditions on carbon dioxide degassing behaviour incoffee. Food Research International**61**: 144–151.

Wasserman D, et al. 2007. Nature and nurture in suicidal behaviour, the role of genetics: some novel findings concerning personality traits and neural conduction. Physiology & behaviour. **92**: 245–249.

Welch A A, Bingham S A. 2007. More acidic dietary acid-base load is associated with reduced calcaneal broadband ultrasound attenuation in women but not in men: results from the EPIC-Norfolk cohort study. The American journal of clinical nutrition **85**: 1134–1141.

Whitfield K, Rambaldi A, Wetterslev J, Gluud C. 2009. Pentoxifylline for alcoholic hepatitis. The Cochrane Database of Systematic Reviews **4**: 1469–1493.

Wintgens J N. 2004.Coffee: Growing, Processing, Sustainable Production A Guidebook for Growers, Processors, Traders, and Researchers. Wiley-VCH, Weinheim, Germany.

Wu T, et al. 2005. Caffeinated Coffee, Decaffeinated Coffee, and Caffeine in Relation to Plasma C-Peptide Levels, a Marker of Insulin Secretion, in U.S. Women. Diabetes Care**28**: 1390–1396.

Yabuki N, Ashihara H. 1991. Catabolism of adenine nucleotides in suspension-cultured plant cells. Biochimica et Biophysica Acta **1073**: 474–480.

Yeragani VK, Krishnan S, Engels HJ, Gretebeck R. 2005. Effects of caffeine on linear and nonlinear measures of heart rate variability before and after exercise. Depression and Anxiety**21**: 130–134.

Yukawa G S, et al. 2004.Effects of coffee consumption on oxidative susceptibility of low-density lipoproteins and serum lipid levels in humans. Biochemistry. Biokhimiya **69**: 70–74.

Zapletalová M. 2011. Adenosinové receptory a jejich agonisté v hematologii a onkologii. Masarykova univerzita, Přírodovědecká fakulta, Brno.

Zhang C, Linforth R, Fisk I. 2012. Cafestol extraction yield from different coffee brew mechanisms. Food Research International **49**:27–31.

Zrenner R, Stitt M, Sonnewald U, Boldt R. 2006. Pyrimidine and purine biosynthesis and degradation in plants. Annual Review of Plant Biology **57**: 805–836.