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Effect of coffee preparation on caffeine content

BACHELOR'S THESIS

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Declaration

I hereby declare that I have done this thesis entitled Effect of coffee preparation on caffeine content 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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Marek Gawlik

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Abstract

Caffeine is the most widespread psychoactive stimulant in the world, with coffee accounting for the highest amount of caffeine consumed worldwide. Theoretical part of the thesis summarized coffee history, botany and different brewing methods. Most importantly it reviewed caffeine's chemistry, synthesis and its effects on human health, positive and adverse. The practical research focused on evaluating impact of beverage preparation method on caffeine content, eliminating other variables influencing caffeine extraction. Twelve different brews of *Coffea arabica* from northern Tanzania were analyzed using high-performance liquid chromatography. Recorded values ranged from 35.216 ± 0.212 to 66.715 ± 8.588 mg of caffeine per 100 ml. Showing significantly higher caffeine extraction in immersion methods than percolation techniques.

Key words: *Coffea* spp.; *Coffea arabica*; coffee; caffeine; HPLC-DAD; human health; brewing methods

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List of the abbreviations used in the thesis

AMP – Adenosine monophosphate

ANOVA – Analysis of variance

CZU – Czech University of Life Sciences

DAD – diode–array detection

EFSA – European Food Safety Authority

GMP – Guanosine monophosphate

HPLC – High performance liquid chromatography

IARC – International Agency for Research on Cancer World Health Organization

IMP – Inosine monophosphate

SAH – *S*-Adenosyl-L-homocysteine

SAM – *S*-adenosyl-L-methionine

Spp. – several species

XMP – Xanthosine monosphosphate

1. Introduction

Caffeine is the world's most popular and widely used psychoactive drug. It is available, in many forms, almost anywhere in the world, being sold without any restrictions. It has also overcome the negative stigma other intoxicating substances face. With that in mind, coffee is the second most enjoyed caffeine-containing drink after tea. It even surpasses tea in the amount of caffeine consumed worldwide (Harland 2000).

Concerns about the amount of coffee consumed date back to the seventeenth century, when the recommendation was "moderate use." In the nineteenth century, moderate intake was supposed to relieve from fatigue and promote organ function. Excessive use could classify coffee as a poison (Alcott 1998; Bizzo et al. 2015). Since the nineteenth century, a toxic dose has been acknowledged as several times human consumption. In the past big emphasis was, by the medical and broad public, put on caffeinism – addiction to coffee, which led to waves of demonization. It is nowadays well known that determination of recommended intake is very difficult due the variability of its metabolization by individuals and the large scale of its bioactive compounds (Bizzo et al. 2015); nevertheless, the content of caffeine in coffee is an essential information for the proper assessment of its health effect.

2. Literature Review

2.1. Etymology and History of Coffee

The European name coffee comes from the original Arabic word ‘quahweh’, a poetic term for wine, which was later transferred to coffee, meaning the drink. Through the Turkish form *kahwheh* became *café* (French), *caffè* (Italian), *Kaffee* (German) and *coffee* (English), later becoming the basis for the botanical genus *Coffea* (Ukers 1935; Smith 1985). In Abyssinia coffee is called *bunn* and the beverage *bunchum*. Under this term, we observe the first written mention by Rhazes (850-922), a Hippocratic Arabian physician. Although that we only know through the writings of Philippe Sylvestre Dufour (1622-1697), who was a French coffee merchant and writer (Dufour 1693). The oldest surviving document referring to bunchum is Avicenna’s (980-1037) The Canon of Medicine. In this respected work he dedicates an entry to bunchum: “*As to the choice thereof, that of a lemon color, light, and of a good smell, is the best; the white and the heavy is naught. It is hot and dry in the first degree, and, according to others, cold in the first degree. It fortifies the members, it cleans the skin, and dries up the humidities that are under it, and gives an excellent smell to all the body“* (Ukers 1935). The Colour of unroasted coffee beans ranges from white to yellow and green, which concurs with Avicenna’s description.

With Canon of Medicine, being translated into Latin and becoming a respected work on theory and practice of medicine, we can observe many mentions of coffee, similar and even referring to Avicenna’s. The first European mention of coffee, in this fashion, is Leonhart Rauwolff (1535-1596), a German botanist and physician who travelled through the Middle East and wrote accounts of the beverage being enjoyed in the region (Ukers 1935).

According to Arab historians, coffee drinking as we know it began in the region of Yemen in the middle of the fifteenth century. First, being used by a Sufi orders for ceremonial uses. Through natural transition of the first coffeehouses, called *kahwe khaneh*, were born. By 1510 coffee had spread from had spread to Islamic capitals such as Cairo and Mecca and became widely used by every layer of society (Weinberg et al. 2001).

2.2. Botanical classification and description

While hundreds of species have been described, the taxonomic classification of *Coffea* genus is confusing and everchanging (Charrier & Berthaud 1985). *Coffea arabica* and *C. canephora* (known as Robusta variant) are the two most widely known species of coffee. Coffee tree is a plant of the *Rubiaceae* family, which produce fruit called cherry, inside which develops a seed, called bean. This bean is used for production of roast coffee, ground coffee and other products. Coffee is a short-day plant, meaning it starts blooming in its relevant photoperiod of < 12 hours of daylight.

A. de Jussieu was the first to make a botanical description of the coffee tree in 1713, although it was under the name *Jasminum arabicanum* (Charrier & Berthaud 1985). However, Carolus Linnaeus (1707-1778) was the first botanist to categorize and name the species *Coffea arabica* (Fischer et al. 2019). First herbarium specimen can be seen in Figure 1.

The natural habitat of *C. arabica* is the understory of tropical forests in Abyssinia and Ethiopia Although it grows well in equatorial Africa, Arabia, Central and South America, Mexico, the islands of Pacific, India and Vietnam. (Ukers 1935). The second most economically important *Coffea canephora* var. robusta comes from the tropical forests of central Africa (Wintgens 2004).

C. arabica is the only known tetraploid species (2n = 4x = 44) of the *Coffea* genus and self-pollinating. All other species are diploid and mostly self-incompatible (Charrier & Berthaud 1985). *C. arabica* is the result of hybridization between diploid *C. canephora* and *C. eugenioides*, this event occurred between 1.08 million and 543 thousand years ago (Bawin et al. 2021).

Figure 1 – First herbarium specimen Linnaeus (Natural History Museum London 2006)

Aerial parts of the coffee tree consist of an upright main shoot with primary, secondary, and tertiary lateral branches. Orthotropic (vertical) branches have regularly distanced nodes with opposite leaves. In the leaves axil, there are four to six serial buds. Directly above it is extra-axillary bud, which develops into a plagiotropic (lateral) branch. No other bud in the axil can generate a lateral branch; thus, no regeneration can occur. Lateral branches grow out at right angles from the vertical stems. Serial buds on primary branches can develop into inflorescence or a secondary branch, which resembles the primary. Regeneration of secondary branches is possible, as they can grow out of any bud in the axil. At each leaf node, there are 5 buds, each one with 4 flowers, thus being able to create 20 fruits at each node (Ukers 1935; FAO 2005; Winston et al. 2005). A one-year-old plant has six to ten levels of plagiotropic branches, depending on the species and conditions. At 2 years it reaches heigh of 1.5—2 m and starts flowering. It reaches full maturity at 3 years and begins to bear fruit (Wintgens 2004).

The mature leaves are dark green on the upper surface and lighter underneath. They are shiny and waxed. Their shape is elliptical with conspicuous veins that can be netted. *C. arabica* leaves are thinner and more delicate, than those of *C. canephora* or *C. liberica* species. Young leaves of *Coffea arabica* are light green or bronze (Ukers 1935; Wintgens 2004).

The root system consists of a central taproot (0.45—1 m in length), axial roots which exceed the length of the taproot and run in various directions, and lateral roots that run parallel to soil surface. In good conditions, the root system can spread to a volume of 15 m^3. In heavy and humid soils, roots concentrate in upper layers, whereas in dry soils they are less superficial. With 90% of roots developing in the upper layer, mulching is a good practice by providing needed humidity and nutrition. Bent or deformed tap root leads to a decrease of nutritional uptake and a shorter lifespan. *C. arabica* has a deeper root system than *C. robusta* and therefore has a higher resistance to drought (Thurber 1889; Wintgens 2004; FAO 2005).

Figure 2 – *Coffea arabica* (Author: Köhler HA, 1887)

Seed and germination

The seed consists of a hard endosperm (bean), which is encased in two husks, the inner integument (silverskin) and outer endocarp (parchment). The embryo, being 3–4 mm long, consists of the embryo axis (hypocotyl) and two cotyledons (Figure 3). The size of the coffee seed (bean) differs in shape and size, on average, it is approximately 10 mm long and 6 mm wide. According to Wintgens (2004) the average weight of parchment seed at the moisture content of 18% is 0.45–0.5 g for arabica and 0.37–0.4 g for robusta.

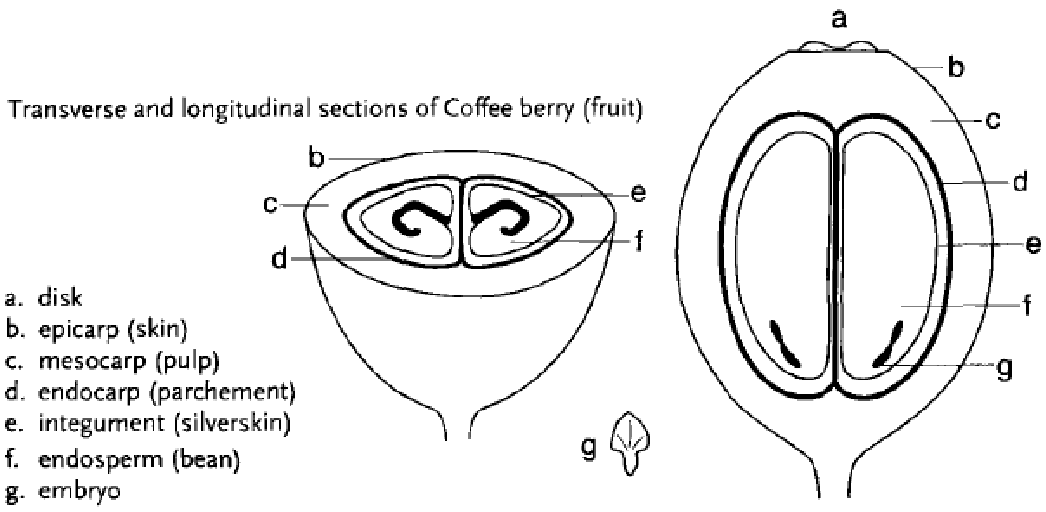


Figure 3 – Coffee berry (Author: Wintgens 2004)

Coffee seeds are not dormant and therefore it is best to propagate them right after their ripening, with moisture content over 50% (Wintgens 2004). Germination begins in a sufficiently moist environment, ideal soil temperature is 28–30 °C. Lower temperatures slow down germination, if air temperature is below 10 °C may not begin at all. Removing the parchment speeds up the germination by 6-10 days. The germination of Coffea is epigeous, the growing hypocotyl raises the seed out of the ground (Figure 4) (Wintgens 2004). The first cotyledon leaves develop after four to six weeks (FAO 2005; Winston et al. 2005). In this stage, the plant has only the taproot with laterals (Figure 4).

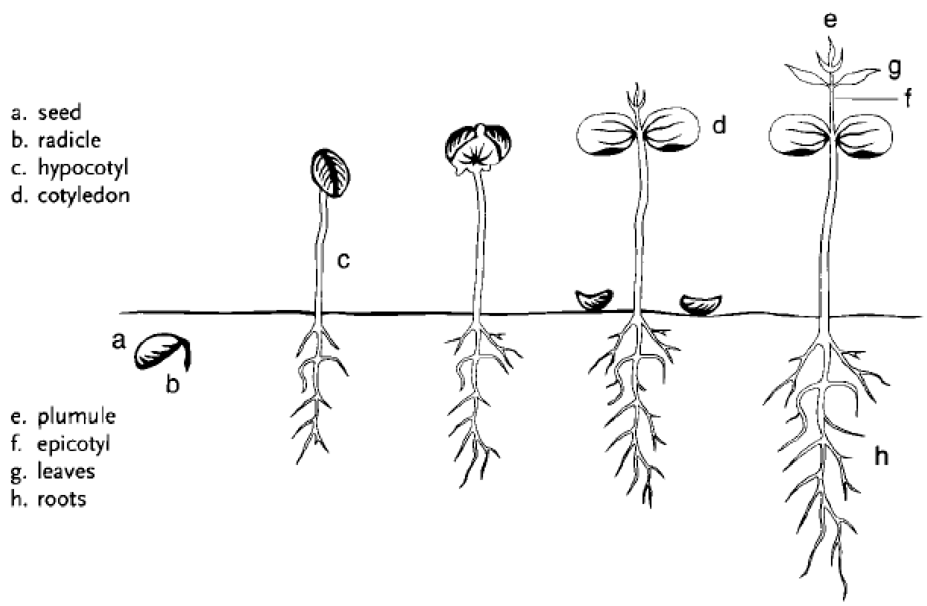


Figure 4 – Seed germination (Author: Wintgens 2004)

Flower and pollination

The flower is white in appearance, it is formed by a corolla, consisting of five lobes, calyx, five stamens and a pistil. Its ovary contains two ovules, able of producing two coffee beans. Generally, an inflorescence consists of four floral buds, known as cyme, the cyme in most cases generates four flowers. Differences appear with variety and conditions. *C. arabica* generates 16–48 flowers per node, while *C. robusta* 30–100. The buds remain dormant in dry season for 2–3 months, which is broken by rehydration of the plant. In equatorial climates, trees bloom during the whole year and the dormancy can be broken by irrigation. Flowers open roughly 12–15 days after the stimuli, they do so early in the morning and can remain receptive for some days. The pollen is very light in weight and most pollination is done by the wind. While *C. arabica* is mostly self-pollinating, in *C. canephora* fertilization will only occur by cross-pollination (Wintgens 2004).

2.3. Coffee Growing

Plants start to produce fruit approximately 3 years after germinating. The average yield declines after 30–40 years of cultivation and the plantation needs to be renewed. Although in most cases, this is done continuously.

Factors affecting yield and quality include: genotype species and varieties of plant, environment and the plants management. Prior to setting-up or renewing a plantation location and suitable stock should be considered. The variety of coffee should be selected based on several factors, such as productivity, quality of fruit and production costs. Productivity depends on the variety of clone selected, however adaptability to local conditions and right cultivation methods are crucial for desired results. Coffee quality is based on the variety of coffee, with harvesting and post-harvesting methods having considerable impact. *C. arabica* is regarded as the species with highest quality of fruit. Production costs are directly related to chosen cultivation system – intensive, semi-intensive or extensive (Wintgens & Zamarripa C. 2004; FAO 2005).

2.4. Economically important species

The *Coffea* genus includes a total of 124 species, which origin is distributed throughout Africa, on some Indian Ocean Islands and in the Australasian block. Natural habitats include temporarily flooded riverbanks and all types of tropical forests: dry, semideciduous and riverine. Coffee's natural elevation ranges from sea level to 2000 m and can grow in various types of soil. The most economically substantial species is Arabica (*Coffea arabica*), which accounts for 60% of all coffee traded. Arabica has been harvested for millennia and farmed for several hundred years. Mainly due to its quality of seed. There are many varieties, the most important are Typica and Bourbon, with its descendants such as Blue Mountain, which is resistant to coffee berry disease. Arabica is grown mainly in Central and South America. The second most important species is Robusta (*Coffea canephora*). Robusta has been recognized by science only in 1897. Due to its resistance to coffee leaf rust, higher productivity, and caffeine content, it has grown from an unknown and underutilized African crop to a major commodity in just 150 years. Today it makes up for 40% of globally traded coffee and is mainly used in instant coffees. Although due to negative organoleptic properties of robusta, arabica still has a higher market share (Davis et al. 2019). Liberica coffee (*Coffea liberica*) is the third most grown species, mainly as a rootstock for Arabica and Robusta. Its insignificance in terms of economic importance is due to its bad cup qualities (Ukers 1935; Davis et al. 2019; "International Coffee Organization - Trade Statistics Tables" 2020).

2.5. Decaffeinated coffee

The recent availability of decaffeinated coffee and raise of awareness of coffees health benefits makes it a viable option for people with health disorders, caffeine intolerance or those in search of a healthy lifestyle. Nowadays, it comprises of 10% of total coffee consumption. Decaffeination is done before roasting. The least costly and historically most used method is extraction by an organic solvent (dichloromethane or ethyl acetate), vapor is used to open pores and wash the seeds. After removal of caffeine the seeds are dried to reach moisture content similar to before extraction. There is a general concern about remaining dichloromethane in the beverage and key flavor components can be lost using this method. Nowadays extraction by using water and supercritical carbon dioxide is the only method used in Europe and United States. As it poses no health concerns and original chemical composition is better preserved, thus maintaining its flavor (Farah 2012; Ludwig et al. 2014).

Caffeine-free species are natural alternative to artificially decaffeinated coffee. Within the *Coffea* genus there are more caffeine-free or low-caffeine species than caffeine-rich ones. Most notable is *Coffea charrieriana*, endemic to Cameroon, as it was the first caffeine-free coffee available on the market. Caffeine-free species may be used for biotechnology and hybridization of coffee species (Preedy 2014).

2.6. Caffeine

Caffeine is the main active constituent of coffee and one of the most popular and widely used drugs and psychoactive substances in the world. It is naturally occurring in over 60 plants (Harland 2000). Other primary sources are tea, maté, kola and cacao. Biological effects of caffeine are numerous, the most common are shown in Table 1. It has both positive and beneficial effects on health, while having negative impacts on well-being. Caffeine consumption creates an immediate and generally pleasant effect of alertness. Though, excessive consumption can lead to unpleasant sensation of anxiety and excitement. Caffeine doses impact each individual differently, which can be attributed to genetic susceptibility and habituation to the effects of caffeine. This well-observed mechanism is attributed to upregulation of adenosine receptors (Depaula & Farah 2019). For some people even a small dose of caffeine (50–60 mg) can be unpleasant and be a cause of insomnia and racing mind, while other individuals are not susceptible to much higher doses.

Table 1 – Biological properties of Caffeine (Crozier et al. 2011)

Biological properties of caffeine
CNS and sympathetic nervous system stimulant
Alertness, heightened awareness
Agitation, anxiety
Tremor
Sleep disturbances
Addiction
Lowered seizure threshold
Diuretic
Polyuria, nocturia
Relative dehydration
Cardiac stimulant
Sinus tachycardia, (palpitations)
Increased cardian muscle contractility (treatment of heart failure)
Arrgythmias: ventricular extrasystole ('missed beats', palpitations)
Smooth muscle relaxant
Gastro-oesophagaeal (reflux, hearthburn)
Bronchodilatation (asthma treatment, illegal sports performance, enhancement
Uterine muscle relaxation (possibly miscarriage)
Vasodilator
Headechaes on caffeine withdrawal
Synergism with nitrites
Synergism with analgesics

2.6.1. History of caffeine

Caffeine was discovered in 1819 by Friedrich Runge (1795-1867), an influential pioneer of chromatography. Runge analyzed coffee beans on the initiative of Wolfgang Goethe (1749-1832), who was at the time interested in plant chemistry and coffee as a beverage itself. (Runge 1821; Bizzo et al. 2015).

With general concern of adverse effects of caffeine emerge publications on caffeine-free coffee, the earliest dates back to 1898. In the early twentieth century such coffee could be purchased in Europe and North America, with industrialization making it a widespread commodity (Bizzo et al. 2015).

The physiological activity of substances other than caffeine starts to be of significance on the edge of twenty-first century. Mainly because of their antioxidant properties, which could help prevent degenerative diseases. Before then, these known substances were believed to have no biological effects. First studies on the bioavailability of chlorogenic and caffeic acids started in the 1950s (Bizzo et al. 2015).

Other uses of caffeine stem from its ability to act as a herbicide (M. Frischknecht & W. Baumann 1985). *Coffea* varieties with higher caffeine content inhibit production of ochratoxin A produced by *Aspergillus* section *Nigri* and section *Circumdati* groups. This could be of great potential of inhibiting growth and production of mycotoxin by mycotoxigenic fungi even beyond coffee cultivation (Akbar et al. 2016). It is also used in cellulite creams, because of caffeine’s lipolytic properties proven in clinical trials (Byun et al. 2015).

2.6.2. Chemistry

Caffeine is a purine based heterocyclic organic compound called 1,3,7-trimethylxanthine. Although naturally it is accompanied by theophylline (1,3-dimethylcanthine) and theobromine (3,7-dimethylxanthine), which in coffee and tea are in lower concentrations, that do not create notable physiological response (Harland 2000). It is known as a stimulating alkaloid, that is heat stable and water soluble (Farah 2012; Depaula & Farah 2019).

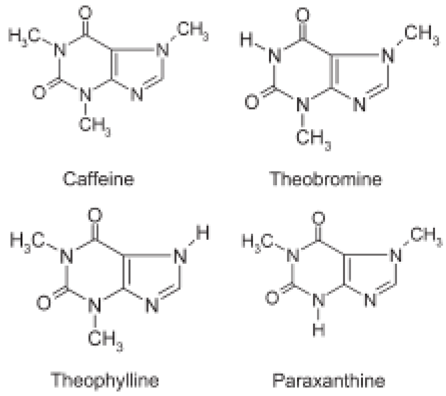


Figure 5 – Chemical structure of natural methylxanthines (Author: Burdan 2015)

Caffeine's mode of action involves its attachment to adenosine receptors, which are located on cell membranes in central and peripheral nervous system. Thus it is called a competitive antagonist of adenosine, which is caused by their very similar structure (Harland 2000). This causes elevation of the neurotransmitter dopamine, rise in its concentration is cause to caffeine's stimulating and addictive properties. Dopamine also enhances serotonin mood-raising effects (Crozier et al. 2011).

2.6.3. Biosynthesis

The role of caffeine in plants is based on two hypotheses. The chemical defense theory suggests that high concentrations of caffeine in young leaves, flowers, and fruit of *Coffea arabica* and *Camelia sinensis* protect them from pests and predators. The other described as allelopathic or autotoxic theory suggests that caffeine in seeds is released into the soil and inhibits germination of other seeds (Ashihara & Crozier 1999).

Caffeine synthesis occurs in young tissues and caffeine synthase is in chloroplasts of young leaves. The primary pathway to biosynthesis of caffeine is xanthosine → 7-methylxanthosine → 7-methyxanthine → theobromine → caffeine (Figure 6). Some alternative pathways also exist, due to the substrate specificity of *N*-methyltransferases. Xanthosine as a primary substrate is a purine nucleoside that is produced by the degradation of purine nucleotides. The known pathways to its synthesis are shown in Figure 7: de novo purine synthesis, the degradation of adenine (AMP) and guanine (GMP) nucleotide pools and salvage of adenosine from the S-adenosyl-L-methionine (SAM) cycle (Suzuki et al. 1992; Ashihara & Crozier 1999; Ashihara 2004; Ashihara et al. 2008).

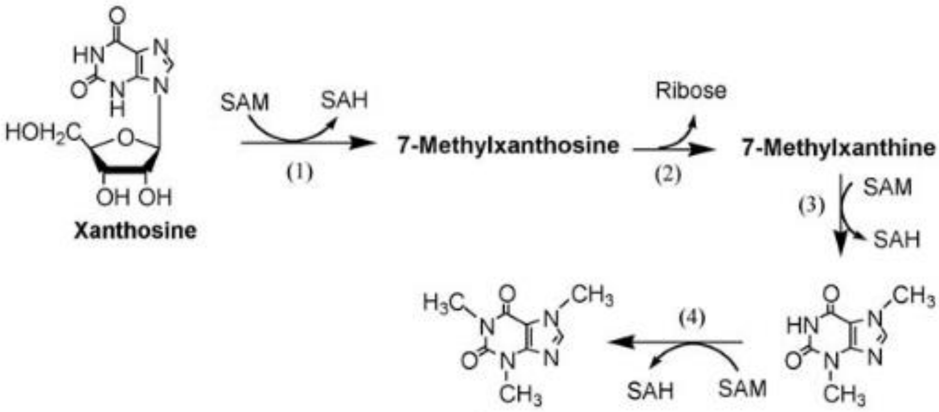


Figure 6 - Core pathway of caffeine biosynthesis in plants (Suzuki et al., 2004)

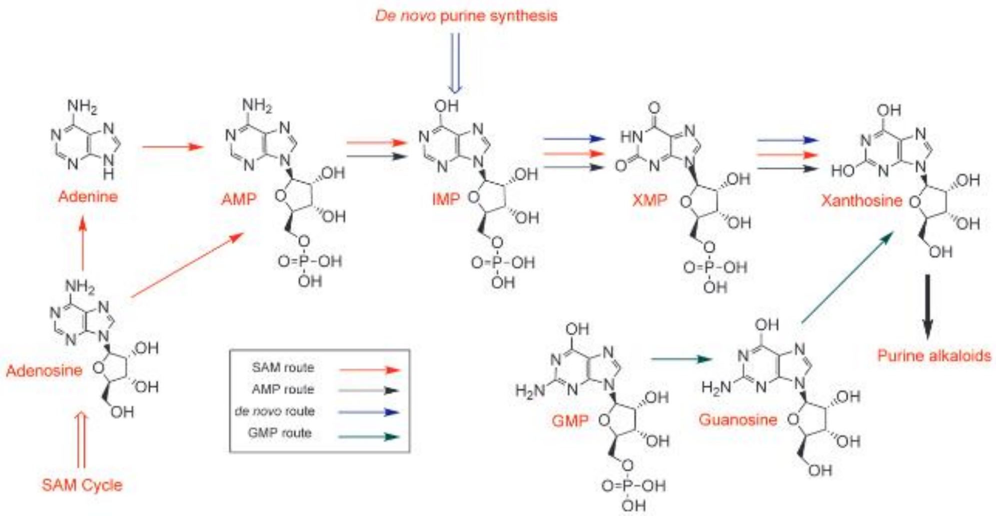


Figure 7 – Different pathways to Xanthosine synthesis (Ashihara et al. 2008)

With the rising popularity of decaffeinated coffee understanding these mechanisms could be beneficial for suppressing or enhancing production through genetic engineering. They can also serve as a natural pesticide by creating caffeine-producing transgenic plants, which did not originally synthesize it. This has been described, with satisfactory results, on trans-genic caffeine producing tobacco (Kim & Sano 2008). Although further research is needed to determine to what extent these effects occur in other species, especially those of agricultural importance (Ashihara et al. 2008).

2.6.4. Caffeine tolerance

Long-term ingestion of caffeine stimulates creation of new adenosine receptors; thus, the individual tolerance is increased over time. Chronic usage may induce withdrawal symptoms, which are described as headache, drowsiness, fatigue, and negative mood (Harland 2000).

Even with this knowledge it is very difficult to study long-term effects of caffeine. Because consumption habits change over time and even types of caffeine products. Caffeine intake might not be exclusive only to the consumption of coffee but might come from several sources.

Table 2 – Effects of caffeine based on dose

Caffeine amount	Consequences
100 or 200 mg	Increased mental alertness, faster flow of thought, wakefulness, restlessness, fatigue is reduced, sleep need is delayed
1 g	Caffeinism, anxiety, insomnia, mood changes, cardiac arrhythmias, gastrointestinal disturbances
1.5 g	Agitation, anxiety, tremor
2–5 g	Spinal cord stimulated
10 g	Lethal dose

2.6.5. Effects of caffeine on health

Fertility and Pregnancy

It is suggested that consumption of caffeine doses greater than 300 mg/day may reduce fecundability in fertile women, as for men doses higher than 400 mg/day might decrease sperm motility and increase dead spermatozoa, although not sufficiently enough to affect male fertility altogether (Depaula & Farah 2019).

Caffeine easily travels through placenta into the fetus; however, the fetal liver cannot metabolize it, thus prolonging caffeine’s half-life. Regarding the effects of caffeine on pregnancy, the evidence is mixed. Recent meta-analysis found no significant adverse effects on fetal growth and pre-term birth, but suggest it may still contribute to miscarriage (Maslova et al. 2010). Other substances contained in coffee, such as theobromine and theophylline, might also have an influence. Habits generally linked with coffee use, such as alcohol and tobacco consumption may also have their effect (Hinds et al. 1996; Harland 2000).

According to EFSA caffeine intake lower than 200 mg/day by pregnant women in the general population does not raise any concerns in the development of the fetus (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) 2015).

Osteoporosis and bone health

There are several described ways caffeine could influence bone health. Its antagonistic property to adenosine is one of them. Adenosine stimulates bone metabolisms in several ways, *in vitro* studies indicate that stimulation of adenosine A_{2A} and A_{2B} receptors stimulate bone formation by activating osteoblast. It also negatively affects calcium metabolism, f.e. an increase in calcium excretion. This is prevalent especially in older adults and premenopausal middle-aged women. However, rather indirect association exists between caffein consumption and osteoporosis and bone health. According to some sources these issues could be offset by ingesting small amounts of milk with coffee (Depaula & Farah 2019). Further studies, especially long-term, need to done in order clarify these issues (Cooper et al. 2009; Berman et al. 2022).

Caffeine and Cardiovascular System

Effects of coffee on the cardiovascular system have been historically a key topic of research; often with controversial findings (Preedy 2014). High doses of pure caffeine (200–250 mg) administered to healthy individuals have been observed to raise blood pressure and induce cardiac arrythmias (Depaula & Farah 2019). However, these short-term increases later normalized and proved to be transient and reversible in majority of the cases. Existing literature shows that moderate caffeine intake (< 600 mg/day) is not associated with increased risk of cardiovascular disease; arrythmia; heart failure; blood pressure changes among regular caffeine consumers and hypertension in general healthy population (Turnbull et al. 2017). Recent evidence suggests that caffeine consumption does not increase the risk of coronary heart disease (Lopez-Garcia et al. 2006). No association was found between coffee and risk of stroke (Grobbee et al. 1990).

Hypertensive individuals might be more sensitive to some caffeine effects. For example, pre/hypertensive populations experienced an acute increase in blood pressure with caffeine consumption of 100–400 mg/day. To conclude, epidemiological studies show an increased risk in cardiovascular disease only when five or more cups of coffee were consumed representing ≥ 500 mg of caffeine daily (Depaula & Farah 2019).

Caffeine and Neurodegenerative Diseases

Caffeine, as a known psychoactive stimulant, that is permeable across the blood-brain barrier and is antagonist to adenosine receptors, has a tremendous impact on the central nervous system. Recent investigations of caffeine's impact on neurodegenerative diseases suggest, that it is associated with prevention of development of Alzheimer's and Parkinson's and is associated with better cognitive performance (Preedy 2014; Depaula & Farah 2019). Caffeine creates new connective pathways in the brain through changing morphology of neural synapses, supporting formation of larger dendritic spines and altering neural networks (Preedy 2014).

Alzheimer's disease leads to cognitive decline and is the lead cause of dementia. In affected individuals develop neurofibrillary tangles and senile plaques, which are caused by accumulation of toxic beta-amyloid peptide, or Tau protein in the brain (Checler 1995; Ittner et al. 2010). Caffeine's protection is related to anti-inflammatory effects on A1 and A2 receptors and the reduction of deposits of the beta-amyloid peptide. A mouse model study reported that heavy caffeine intake (the human equivalent of 500 mg caffeine) was able to protect against and could even treat Alzheimer's disease (Arendash et al. 2009).

Parkinson's disease affects motor and non-motor skills through degeneration of dopaminergic neurons in the midbrain (Dauer & Przedborski 2003). Meta-analysis of 26 studies suggests that coffee drinkers were at a 25% lower risk of developing Parkinson's disease than non-coffee drinkers. With total risk reduction of 24–32% per 300 mg increase in caffeine intake (Depaula & Farah 2019).

Caffeine and liver disease

Caffeine consumption is associated with decreased risk of cirrhosis and hepatocellular carcinoma. Furthermore, it could be helpful in treating chronic hepatitis C and reduction of fibrosis in alcoholic liver disease. Recent meta-analysis suggests that a regular intake of three cups a day reduces these risks by 40% (Preedy 2014).

Caffeine and Glucose Metabolism

Habitual coffee consumption is associated with reduced risk of diabetes. However, studies indicate that caffeine itself promotes adverse effects on glucose metabolism and reduces insulin sensitivity. Which points out that other coffee constituents, especially chlorogenic acids, show positive effects on glucose homeostasis and balance effects of caffeine. In conclusion regular coffee consumption of both decaffeinated and regular coffees is proven to reduce risk of diabetes, with decaffeinated seemingly more beneficial for glycemic control. Studies of different coffee constituents and their effects on glucose metabolism would be beneficial, as they may lead to developments of coffees that can maximize health benefits (van Dam & Hu 2005; Depaula & Farah 2019).

Carcinogenicity of Caffeine

In the 1980s reviews on coffee consumption created by national governments raised concerning questions whether caffeine may be carcinogenic, especially regarding bladder and colon cancer. However, most of these conclusions have been made due to inadequate control for tobacco smoking, which has a strong association with heavy coffee ingestion (IARC 1990; Depaula & Farah 2019). Most evidence finds no substantial relation between caffeine intake and various types of cancer. Furthermore, meta-analysis of this topic show that coffee consumption may reduce the total cancer incidence and has inverse association with some type of cancers (Yu et al. 2011).

Caffeine Intake Recommendations

Even though there is extensive research regarding the potential health effects and safety aspects of caffeine consumption, no consensus on accepted daily intake exists. Safe limit for its consumption is hard to determine, because of different effects on individuals, based on caffeine sensitivity and habituation. The exact amount of caffeine which creates adverse and unpleasant effects varies from person to person. Unexperienced individuals should ingest it with caution until a person understands how it interacts with their body. General standards set by government authorities range from 200–400 mg/day in adults.

Caffeine ingestion in children and adolescents interferes with their sleep and possibly hinders brain development. EFSA recommends no caffeine consumption for children under 12 months. In older children and teenagers, it is recommended that intake is lower 3 mg/kg bw/day (<120 mg/day in 40 kg bodyweight). Due to the reasons abovementioned pregnant and lactating women should also limit their caffeine intake; recommended amounts are 200–300 mg/day (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) 2015; Depaula & Farah 2019).

2.7. Coffee preparations

Although caffeine is nonvolatile and stable upon roasting small fraction may be lost to sublimation during the roasting process (Farah 2012). Studies reported (Dutra et al. 2001) that caffeine was detected in the exhaust gas released during roasting, implicating that some caffeine loss may be attributed to water vapour released during seed fracturing caused by pressure. Throughout roasting beans are exposed to a temperature of 100–245 °C for a different time depending on the variety, geographical origin and desired properties. Roasting leads to overall reduction of caffeine by 30% from 0.89% ± 0.02 in green beans to 0.6% ± 0.03 in roasted arabica beans (Franca et al. 2005); thus, darker roasts can lower caffeine content.

Grinding is a vital step in coffee preparation. As chemical compounds in whole beans are incorporated into cells and cannot be dissolved. Grinding reduces beans to small particles ranging from a few µm to 1–2 mm; this releases aromatic volatiles and allows chemical compounds to dissolve easily. Ground coffee is classified into four groups: coarse, medium, fine, and very fine. Generally, finer ground coffee allows higher extraction of caffeine and other chemical compounds due to higher surface are in contact with water.

There are several other variables affecting the caffeine content in the final beverage. Coffee brewing is a solid-liquid extraction of chemical compounds in coffee (soluble solids) into hot water (solvent). The main parameters are; type of contact between ground coffee and water, coffee/water ratio, extraction time, volume of the extract and water temperature. Vapour pressure in Espresso making and boiling also play a great role in the final caffeine extraction (Petracco 2008; Severini et al. 2017).

Ancient preparationsUnfortunately, there is no evidence that people on African continent made use of coffee. We might suggest, based on traditions and accounts of European travelers from the seventeen century, that coffee was ingested before recorded history. The prevalence of the genus *Coffea* as wild and cultivated plant in this region is another supportive argument of this conclusion. With regard to African tradition, it is assumed, that before tenth century, wine was made from the pulp of ripe berries (Ukers 1935; Weinberg et al. 2001). It is also believed that in the eleventh century, in Ethiopia, began the practice of boiling unripe coffee beans in their husks to create a beverage

(Weinberg et al. 2001). With Arab traders bringing back coffee from Africa they began to prepare two distinctly different beverages. The first drink was called *kisher*, beverage similar to tea, steeped from dried husk, which, according to testimonies, tastes nothing like modern coffee, but similar to an aromatic or spiced tea (Davids 1996; Weinberg et al. 2001). The second *bounya*, called after *bunn*, is a thick brew made from ground or crushed beans, it was drunk unfiltered and ingested with the sediment, as was common practice for several hundred years (Weinberg et al. 2001). Early preparations of *bounya* were made from raw, boiled beans. In the Levantine arose the practice of roasting of beans before boiling the several times in clean water, while reserving the extracted liquid for later serving. Islamic coffee drinkers in sixteenth century invented the *ibrik*, a small metal vessel used to boil coffee easier, with the addition of a lid the prototype of modern coffee pot was created. The newest method of preparing coffee, dating back to eighteenth century, was infusion. Ground coffee was put in a cloth bag, which was steeped in hot water (Ukers 1935; Weinberg et al. 2001).

2.7.1. Decoction and immersion methods

Decoction or immersion regard to a process whenever a soluble solid is kept in contact with a given amount of water, at a certain temperature for an appropriate amount of time. Decoction in coffee brewing regards to a process when water is boiled with the grounds, whereas immersion suggests already boiled water was put in contact with ground coffee. When concentration increases the extraction rate decreases, making high-ratio decoctions ineffective and possibly with and unpleasant aroma. Time of contact between solvent and solid also increases extraction. Higher temperature of water is another plausible method of obtaining higher extraction of caffeine and other compounds, as they are easier soluble at higher temperatures (Petracco 2008; Mestdagh et al. 2017).

Boiled and Turkish Coffee

Boiled coffee is the most basic brewing method, that is popular in northern and central Europe. Medium to coarsely ground coffee is put in a pot and heated on a stove until boiling. If the beverage is kept at a boiling point for a prolonged time it allows for further extraction.

Turkish coffee is a type of boiled coffee where beans are ground to a very fine powder. This allows for a high extraction because of the increased surface area and makes the ground coffee unable to float, forcing it to settle down. It is traditionally prepared in a long-handled pot, called *cesve*. It produces a strong beverage with some sediment, that is generally served in a small cup.

French press and other immersion brews.

The use of electric kettles led to a simplification of boiled coffee, whereas ground coffee is simply put into a mug and poured over with freshly boiled water, letting it steep in a decreasing temperature. The beverage is consumed when it has cooled down sufficiently. This method is popular in central and eastern Europe particularly for its ease of production and no specialty equipment needed.

French press is a type for container with a fine wire mesh plunger used to separate the grounds and liquid. Boiled water it is poured over medium to coarse ground coffee, it is generally stirred and let to soak for 2–8 minutes. Time length depends on the desired intensity of extraction. Longer extraction generates higher caffeine content and bring intensity and bitterness, whereas shorter extractions bring out acidity and floral notes of coffee. Before serving the plunger with mesh strainer is pressed down, allowing the liquid to pass through, thus separating the grounds. Although due to inefficient mesh filtration some fine particles and sediment occur in the final beverage (Mestdagh et al. 2017). By pressing the coffee oils are squeezed out from the coffee bed, increasing their content in the final brew (Zhang et al. 2012).

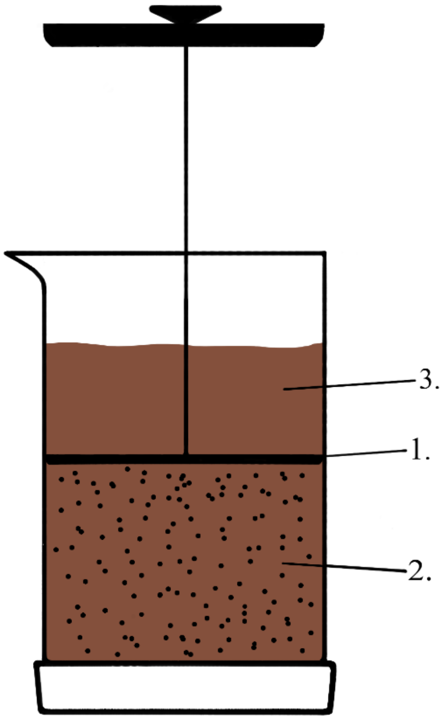


Figure 8 – French press diagram: 1. Plunger, 2. Separated ground coffee, 3. Filtered beverage (Source: Author)

Vacuum coffee

This method utilizes a thermodynamic difference in temperature. The device consists of a funnel-shaped glass flask, with a filter screen, that is put on top a second flask. Water is placed in the bottom flask, while ground coffee upon the filter in the upper one. It is then heated above a heat source, typically a spirit burner, allowing the water to vapor into the upper flask and mix with the coffee. After the heat source is put out and the assembly is allowed to cool down, until the pressure in bottom flask has lowered allowing the liquid to flow down through the funnel (Petracco 2008).

2.7.2. Percolation methods

Percolation, sometimes called pour over or filtered coffee, is a method where hot water is freely allowed to flow through a bed of coffee, by gravity alone, allowing for a short contact time, which creates a beverage milder in flavor, but able to extract certain nuanced aromas. The ground coffee is generally put into a conical holder with a filter device. Various filter shapes, sizes and materials are commercially available each producing a specific result. Particle size, coffee bed shape, water amount and temperature greatly influence the final beverage. Water can be applied manually or by an automatic drip filter machine (Mestdagh et al. 2017).

2.7.3. Pressure methods

Moka pot

Moka pot is a type of a stove-top coffee maker, which is "the most popular household coffee brewing in Italy (Mestdagh et al. 2017; Severini et al. 2017)." It was invented in 1993 by Alfonso Bialetti. It is comprised of three chambers. The bottom chamber provides pressurized water/steam to pass through the middle chamber, containing the ground coffee and finally collect in the upper chamber. The final drink is compared with Espresso, although pressures in preparation are much lower. Although its simple construction, moka thermodynamic and extraction behavior is quite complex. The extraction process is not easy to control and can lead to overextraction, solubilizing undesired compound, resulting in a harsh and bitter drink.

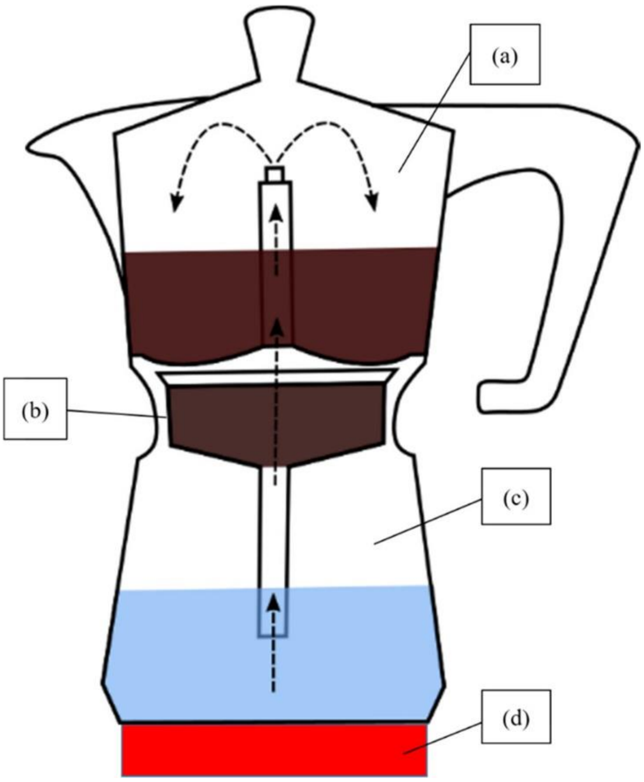


Figure 9 – Diagram of a Moka pot: (a) collection chamber, (b) basket chamber (containing ground coffee), (c) bottom chamber, and (d) heat source (Windisch et al. 2020).

Espresso

The Italian Espresso gained worldwide popularity for its intense aromatic drink of small volume made for immediate consumption. “In general, an Espresso coffee (~25 mL) is prepared by ground roasted coffee beans (6.5 ± 1.5 g), by means of hot water (90 ± 5°C) under pressure (9 ± 2 bar) applied for a short extraction time (30 ± 5 s) to a compact roast and ground coffee cake by a percolation machine, to obtain a small cup of a concentrated foamy elixir (Severini et al. 2017).“ It gives different sensory satisfaction to other brewing methods, based on the high pressure and low water/coffee ratio. It is very difficult to obtain quality espresso cup, as the high extraction can show defects of the raw material and other variables (Petracco 2008). Espresso is used mainly commercially due to the quickness of preparation and high price of Espresso machines. Thus, it plays a miniscule role in coffee homebrewing.

AeroPress

AeroPress is a cross between an immersion and pressurized brewing methods. In a cylindrical chamber, coffee grounds are placed on a paper filter and let to steep. After which pressure is manually added by a plunger, to extract more from the coffee bed and allowing the liquid, to exit through the filter (Mestdagh et al. 2017; de Figueiredo Tavares & Mourad 2020).

3. Aims of the Thesis

First aim was to create a literature review that summarizes botany of *Coffea* spp., and different types of coffee preparation, with their historical context. The most important part of the theoretical review summarizes importance of caffeine and its impact on human health and cognition.

In the practical part, caffeine content was determined in beverages prepared by different kinds of coffee brewing methods. Its aim was to evaluate potential of caffeine extraction in different coffee preparations.

4. Methods

4.1. General

The same coffee was used for all beverages. Bourbon variety of *C. arabica*, from the region of northern Tanzania, grown in elevation of 1000–1400 m asl, harvested during October – December of 2020. Beans were washed and roasted at temperature ranging from 220 to 240 °C for 15 minutes. Coffee was ground using a Comandante C40 hand grinder, using the medium to fine setting for all extracts. Ratio of 6 g of ground coffee and 100 ml of water was used for all preparations. In order to preserve homebrewing conditions, communal tap water was used (water source: Želivka, Prague).

Standardized test was created to determine extraction potential of caffeine in different coffee preparations. As most research to this day dealt with several variables that influenced caffeine content, such as different coffee samples, roast, grind setting and water : ground coffee ratio. This experiment tried to eliminate these unwanted variables and focused solely on the potential of brewing methods.

4.2. Coffee extraction

Twelve different brewing methods were used, which are described in the literature review above. They were performed in duplicate. Four of the samples were previously made batches, which were microwaved. For this experiment, percolation methods were chosen, in order to stop further extraction of caffeine.

	Extraction methods used
1	Infusion (time: 5 minutes; temperature: 100 °C)
2	Infusion (time: 5 minutes; temperature: 90 °C)
3	Infusion (time: 10 minutes; temperature: 100 °C)
4	Infusion (time: 10 minutes; temperature: 90 °C)
5	Moka pot (removed from heat source 5 minutes after first boiling)
6	Manual pour-over (percolation)
7	Sample 6 microwaved (2 minutes; 700 W)
8	Aeropress
9	Sample 8 microwaved (2 minutes; 700 W)
10	Cezves (removed from heat after first boiling)
11	Vacuum pot
12	Home espresso machine (Ariete Café Retro 1385)

4.3. Caffeine analysis using HPLC-DAD

High-performance liquid chromatography is an analytical method used to separate and quantify each constituent of a mixture. The separation principle of HPLC is based on the distribution of sample between a mobile phase (eluent) and stationary phase (adsorbent material on the column). Each sample component reacts differently to the adsorbent material, causing separation of constituents as they flow out of the column. Time and volume of substance is registered by a detector. Diode array detector used in the experiment, records particles that absorb ultraviolet or visible light, by dividing remaining light spectrally and the separated light is detected by specific light-sensitive diodes (Meyer 2010).

Chemicals used

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

Methanol for HPLC (Lach-Ner, Czech republic)

Caffeine (Sigma–Aldrich, Germany)

Apparatus

Analytical scales (0.1 mg accuracy) Mettler AE 200 (Mettler Toledo, Swirzerland)

Ultrasonic bath (Elma, Germany)

Infinity 1260 II. HPLC system (Agilent, USA):

- Wide-range DAD detector 1260 Infinity II. (Agilent, USA)
- Automatic Vialsampler 1290 Infinity II.

Vortex SA 7 (Stuart, United Kingdom)

Syringe with PTFE membrane filter (0.45 µm)

DELL computer with an OpenLab software

Analysis conditions

Column: Infinity Lab Poroshell 120, 2.7 µm C 18, size 150 x 3 mm (Agilent, USA)

Mobile phase: methanol : demineralized water (ratio 40:60) – isocratic elution

Detection: DAD at 264 nm

Mobile phase flow rate: 0.25 ml/min

Column temperature: 35 °C

Length of analysis: 7 minutes

Analyte retention time: 4.5 minutes

Caffeine standard preparation

Base solution was prepared dissolving 10 mg of caffeine in the mobile phase (100 ml) creating a concentration of 100 µg/ml. A calibration set was prepared from the base solution with caffeine concentrations of 1; 5; 10; 50; 100 µg/ml. Volumes of base solution (0.25; 1.25; 2.5; 12.5 ml) were pipetted into 25 ml volumetric flasks and filled until the graduation marking with mobile phase. Linear trend estimation was created using Microsoft Excel 2016 (Table 3; Table 4).

Table 3 – Caffeine calibration curve for first batch

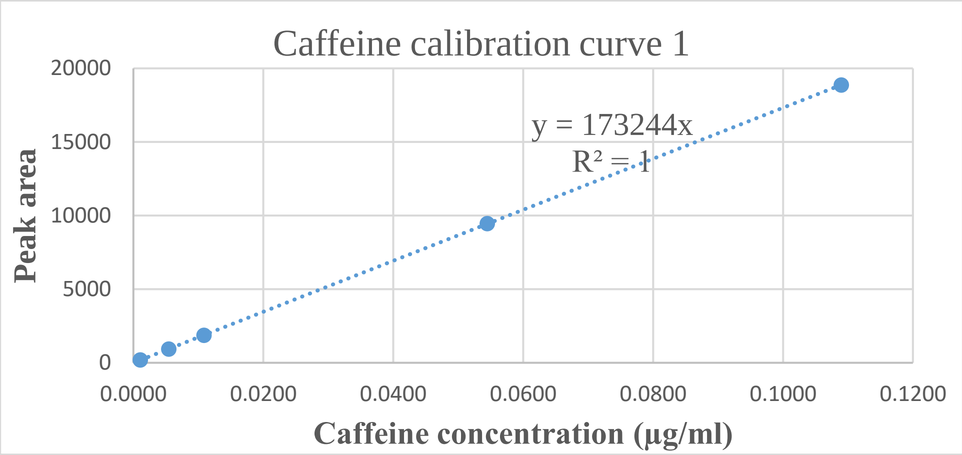
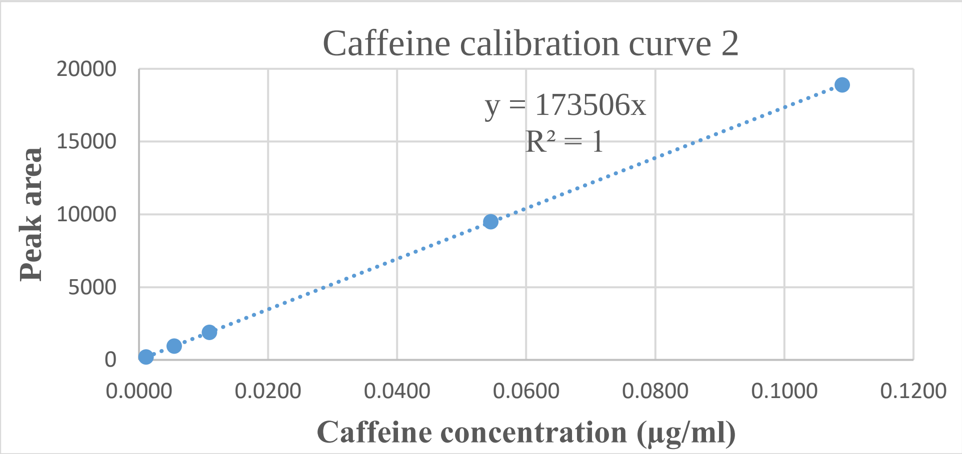


Table 4 – Caffeine calibration curve for second batch



4.4. Statistical analysis

Descriptive statistic was used to summarize and describe obtained data. Content of caffeine was measured in duplicate for each sample. Values such as minimum, maximum and arithmetic means were determined to interpret results. In order to establish significant differences ANOVA single factor was used. This statistical analysis was performed using StatSoft 12.

5. Results

Sufficient amount of samples was obtained, by performing each examined brewing method in duplicate. Although, values show some variance, it was important, to replicate homebrewing conditions, where coffee preparations can never be repated exactly. Errors in HPLC analysis were eliminated by analysing each obtained sample twice. Peak area data was recalculated into concentrations of milligram per 100 ml, using linear trend estimation, obtained by caffeine standard preparation. Arithmetic mean values were calculated, as seen in Table 5. The highest concentration of caffeine was observed in Moka pot preparation (66.715 ± 8.588 mg/100 ml**)**, which also showed highest variance, which could be caused by preheated heatsource, or vapour channeling in the basket chamber. Interestingly, Aeropress preparation yielded the lowest extraction (35.216 ± 0.212 mg/100 ml), which can be attributed to a short time of infusion before extracting. Indexed brewing methods in Table 5 are of signifact difference with the corresponding value. Contrary to predictions home espresso machine did not show the highest caffeine extraction. It is not suitable for such extraction ratio and grind setting. Moreover, infusion temperature and microwaving showed no significant difference in caffeine concentration. Detailed results of statistical analysis performed using ANOVA can be seen in Table 6, Table 7 and Table 8.

Table 5 – Caffeine content in examined samples. Indexed brewing methods are of significant difference to each other. (Example: If 6. And 7. are indexed on brewing method 1., they were statistically proven to be of significant difference to 1.)

	Extraction methods used	Caffeine concentration: mg/100 ml (Mean values ± SD)
1.	Infusion (time: 5 minutes; temperature: 100 °C)[6; 7; 8; 9; 11]	56.80 ± 0.30
2.	Infusion (time: 5 minutes; temperature: 90 °C)[6; 7; 8; 9; 11]	56.63 ± 2.23
3.	Infusion (time: 10 minutes; temperature: 100 °C)[6; 7; 8; 9; 11; 12]	65.37 ± 2.73
4.	Infusion (time: 10 minutes; temperature: 90 °C)[6; 7; 8; 9; 11; 12]	63.37 ± 0.20
5.	Moka pot (removed from heat source 5 minutes after first boiling)[6; 7; 8; 9; 11; 12]	**66.72 ± 8.59**
6.	Manual pour-over (percolation)[1; 2; 3; 4; 5; 10; 12]	39.72 ± 0.86
7.	Sample 6 microwaved (2 minutes; 700 W)[1; 2; 3; 4; 5; 10; 12]	39.68 ± 2.51
8.	Aeropress[1; 2; 3; 4; 5; 10; 12]	**35.22 ± 0.21**
9.	Sample 8 microwaved (2 minutes; 700 W)[1; 2; 3; 4; 5; 10; 11; 12]	37.09 ± 1.03
10.	Cezves (removed from heat after first boiling)[6; 7; 8; 9; 12]	62.39 ± 0.46
11.	Vacuum pot[1; 2; 3; 4; 5; 10]	41.50 ± 0.35
12.	Home espresso machine (Ariete Café Retro 1385)[3; 4; 5; 6; 7; 8; 9; 10]	51.04 ± 4.28

Table 6 – ANOVA results; One-way significance test

Efect	One-way significance test for Caffeine content (mg/100 ml) Sigma-limited parameterization Decomposition of efective hypothesis				
	Sum of squares	Degrees of freedom	Average sum of	F	p
Abs. member	126287,2	1	126287,2	13402,50	0,00
Brewing methods	6354,7	11	577,7	61,31	0,00
Error	339,2	36	9,4		

Table 7 – ANOVA Graph

X-axis (brewing methods): 1. Infusion (time: 5 minutes; temperature: 100 °C); **2**. Infusion (time: 5 minutes; temperature: 90 °C); **3**. Infusion (time: 10 minutes; temperature: 100 °C); **4**. Infusion (time: 10 minutes; temperature: 90 °C); **5**. Moka pot (removed from heat source 5 minutes after first boiling); **6**. Manual pour-over (percolation); **7**. Sample 6 microwaved (2 minutes; 700 W); **8**. Aeropress; **9**. Sample 8 microwaved (2 minutes; 700 W); **10**. Cezves (removed from heat after first boiling); **11**. Vacuum pot; **12**. Home espresso machine

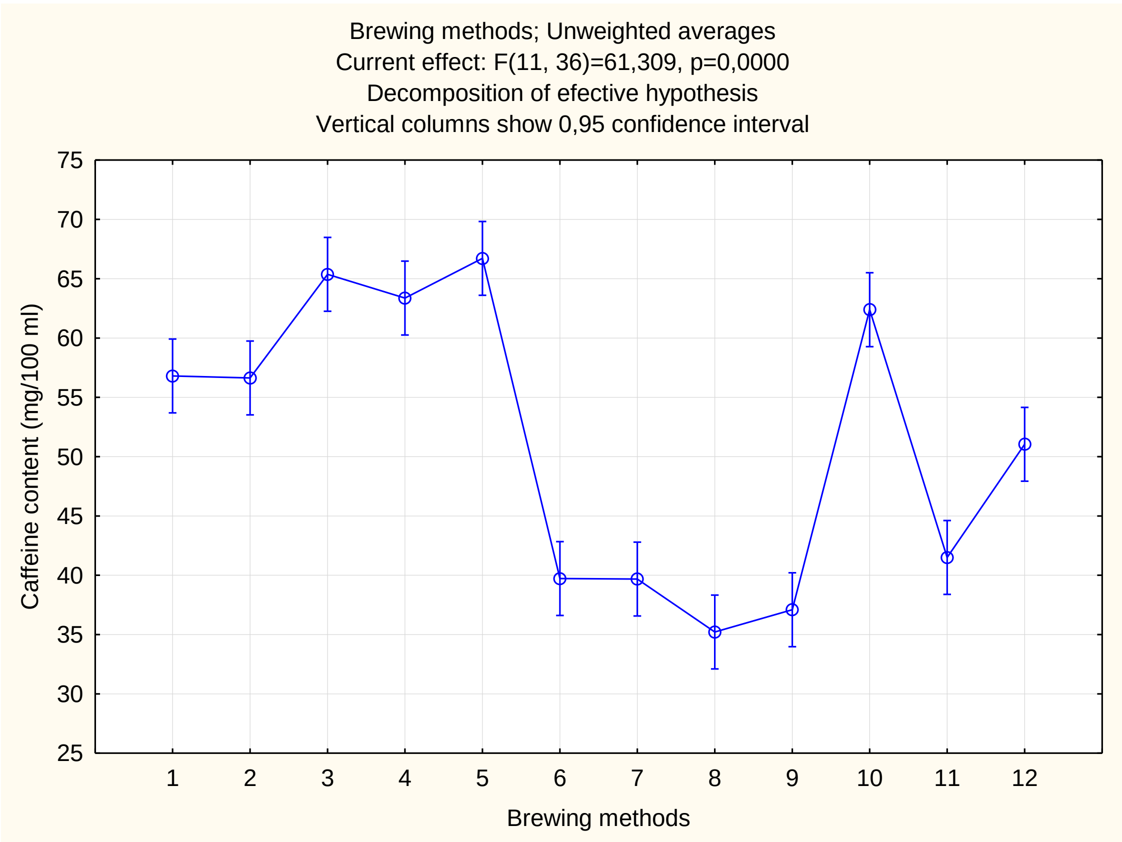


Table 8 – ANOVA Scheffe test; $p < 0.05$ indicate significant difference between brewing methods (red values). Brewing methods refer to Table 9.

Scheffe test; variable caffeine content (mg/100 ml)
Probability for post-hoc test
Error: intergroup PČ = 9,4227, sv = 36,000

Cell number	brewing methods	{1} 56,803	{2} 56,631	{3} 65,368	{4} 63,365	{5} 66,715	{6} 39,721
1	1		1,000000	0,208310	0,611285	0,073314	0,000035
2	2	1,000000		0,184538	0,571550	0,063166	0,000043
3	3	0,208310	0,184538		0,999962	0,999999	0,000000
4	4	0,611285	0,571550	0,999962		0,995143	0,000000
5	5	0,073314	0,063166	0,999999	0,995143		0,000000
6	6	0,000035	0,000043	0,000000	0,000000	0,000000	
7	7	0,000034	0,000041	0,000000	0,000000	0,000000	1,000000
8	8	0,000000	0,000000	0,000000	0,000000	0,000000	0,950657
9	9	0,000002	0,000002	0,000000	0,000000	0,000000	0,999458
10	10	0,814255	0,782587	0,998284	1,000000	0,963011	0,000000
11	11	0,000272	0,000331	0,000000	0,000000	0,000000	0,999989
12	12	0,782125	0,813827	0,000824	0,007272	0,000179	0,020072

Scheffe test; variable caffeine content (mg/100 ml)
Probability for post-hoc test
Error: intergroup PČ = 9,4227, sv = 36,000

Cell number	brewing methods	{7} 39,679	{8} 35,216	{9} 37,091	{10} 62,390	{11} 41,497	{12} 51,041
1	1	0,000034	0,000000	0,000002	0,814255	0,000272	0,782125
2	2	0,000041	0,000000	0,000002	0,782587	0,000331	0,813827
3	3	0,000000	0,000000	0,000000	0,998284	0,000000	0,000824
4	4	0,000000	0,000000	0,000000	1,000000	0,000000	0,007272
5	5	0,000000	0,000000	0,000000	0,963011	0,000000	0,000179
6	6	1,000000	0,950657	0,999458	0,000000	0,999989	0,020072
7	7		0,953812	0,999535	0,000000	0,999986	0,019255
8	8	0,953812		0,999981	0,000000	0,674604	0,000150
9	9	0,999535	0,999981		0,000000	0,957746	0,001254
10	10	0,000000	0,000000	0,000000		0,000000	0,019524
11	11	0,999986	0,674604	0,957746	0,000000		0,099601
12	12	0,019255	0,000150	0,001254	0,019524	0,099601	

Table 9 – Explanation for brewing methods in Table 8

	Extraction methods used
1	Infusion (time: 5 minutes; temperature: 100 °C)
2	Infusion (time: 5 minutes; temperature: 90 °C)
3	Infusion (time: 10 minutes; temperature: 100 °C)
4	Infusion (time: 10 minutes; temperature: 90 °C)
5	Moka pot (removed from heat source 5 minutes after first boiling)
6	Manual pour-over (percolation)
7	Sample 6 microwaved (2 minutes; 700 W)
8	Aeropress
9	Sample 8 microwaved (2 minutes; 700 W)
10	Cezves (removed from heat after first boiling)
11	Vacuum pot
12	Home espresso machine (Ariete Café Retro 1385)

6. Discussion

It was statistically proven, that immersion brewing methods generally show higher caffeine extraction, than percolation, which corresponds with Gloess et al. (2013), whose team tested different brewing methods and their extraction. In their research espresso had the highest caffeine content (210 ± 4x mg/100ml). Caffeine concentrations than decreased from Nespresso pods to Moka pot, French press, and filtered coffee. With the last showing lowest concertation of 47 ± 1 mg/100ml.

In an early scientific study on caffeine content influence by grinding and brewing techniques Bell et al. (1996). concludes that finely ground and larger volumes of coffee yielded higher caffeine content. Boiled coffee also showed higher caffeine contents than filtered coffee, although depending on length of boiling time. This is consistent with the measured data, where longer infusion (10 minutes; 65.368 ± 2.730 mg/100 ml) time showed higher caffeine extraction, than shorter brew (5 minutes; 56.803 ± 0.299 mg/100 ml).

Moka pot showed highest caffeine extraction, as was the case in a recent study by Bobková et al. (2021), where the effect of Moka pot and filtered coffee preparation was tested. Several types of *C. arabica* were prepared by a ratio of 7 g ground coffee to 120 ml of water. Caffeine content obtained from these methods ranged from 1.37% to 1.78%, which are similar to values in this research.

Measured values in the range of 35.216 ± 0.212 – 66.715 ± 8.588 mg/100 ml are consistent. These values also do not excede recommended caffeine intake in normal daily consumption. They were even below the EFSA guideline for pregnant women of < 200 mg per day (EFSA Panel on Dietetic Products, Nutrition and Allergies 2015). Showing, that choice of a homebrewing technique should not be of a worry for caffeine sensitive individuals. Whereas commercial preparations show much higher concern as was documented by Crozier et al. (2012). In the recent study 20 espresso beverages were obtained in local café shops, with caffeine content ranging from 51 mg to 322 mg, corresponding to caffeine extraction of 1.6–6.5 mg/ml. This difference in caffeine content is concerning, as some espressos exceeded recommended intake of caffeine for pregnant and lactating women. It also poses difficulty of caffeine dosing for general population, as the actual caffeine content in a certain beverage might not correlate with government guidelines.

With comparison to other research, in can be concluded that other variables affect caffeine extraction more than brewing techniques. Therefore should be of greater interest in terms of establishing safe limits for consumption. As Budran (2014) reported, that final caffeine content is highly dependent on coffee processing and final preparation, suggesting that lighter roast contain more caffeine than darker, although espresso preparations are able to extract more of this methylxanthine from darker roasts. Regarding the concentration of caffeine in different *C. arabica* samples Fujioka & Shibamoto (2008) tested various commercial ground-roasted samples. Same brewing method was applied. The caffeine content ranged from 10.9 ± 0.04 mg/g of ground coffee to 16.5 ± 0.24 mg/g (1.09–1.65% caffeine content). According to several researchers *C. arabica* should have 40–50% less caffeine than *C. canephora*. This fact might make it more suitable for pregnant, caffeine intolerant or health problematic consumers (Preedy 2014). Obtained values also showed, that consumption of filtered coffee should be less risky for sensitive indivduals, as it yields lower caffeine extractions.

7. Conclusions

Twelve brewing methods were examined for their caffeine content. Significant difference was found between coffee preparations, concluding that daily caffeine intake is to some extent influenced by a type of coffee ingested. Although obtained caffeine content results, ranging from 35.216 ± 0.212 to 66.715 ± 8.588 mg/100 ml, are in accordance with recommended daily intake, in terms of normal coffee consumption. These values are even with compliance with recommendations for pregnant and lactating women. Concluding the obtained data, choice of homebrewing technique proposes no significant risk to human health. With comparison to other research other variables such as coffee variety, roast and grind setting influence caffeine extraction substantially more. Especially commercial espresso preparations showed alarming values, some greatly exceeding recommended limits. Further research investigating caffeine extraction should be done, especially focusing on other variables. As high caffeine intake could have potentially adverse effects, albeit moderate coffee consumption has many positive health benefits. Furthering knowledge in terms of other coffee constituents and their extraction should lead to better comprehension of coffees health impacts.

To conclude, each cup of coffee is different in size and composition, thus raising awareness in general public is of great importance. Understanding individual consumption habits is crucial for future choice making in respect to our health.

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