CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

Effect of coffee preparation on the contents of caffeine and chlorogenic acid

MASTER'S THESIS

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Declaration

I hereby declare that I have done this thesis entitled Effect of coffee preparation on the contents of caffeine and chlorogenic acid 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 25.4.2024

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Marek Gawlik

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Abstract

Coffee is a widely enjoyed beverage across the world and accounts for the highest amounts of caffeine & chlorogenic acids consumed. The potential health effects of coffee are well known and are largely attributed to these two compounds. Caffeine is a psychoactive stimulant, with positive and adverse effects on human health, especially in sensitive individuals. While chlorogenic acids are linked to antioxidant, anti-inflammatory, therapeutic and cardiovascular effects. Different coffee preparations have a potential effect on extraction kinetics of these compounds in the final beverage. This research evaluated the impact of coffee preparation methods on pH values, caffeine and chlorogenic acid content, and the impact of these compounds on human health. Twelve different brewing methods of *Coffee arabica* from two sources were analyzed using high-performance liquid chromatography. The measured caffeine content ranged from 31.4 mg to 75.3 mg per 100 ml, falling below established safety guidelines for pregnant women. Chlorogenic acid content ranged from 118 mg to 274 mg per 100 ml. The Moka pot showed significantly higher extraction of both compounds. Based on these findings, it can be concluded that the choice of homebrewing method does not have a significant effect on extraction of these compounds. Further research is necessary to explore other factors that may affect caffeine and chlorogenic acid extraction into the final beverage.

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

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List of the abbreviations used in the thesis

5-CQA – 5-caffeoylquinic acid

AMP – Adenosine monophosphate

ANOVA – Analysis of variance

CGA – chlorogenic acid

CGAs – chlorogenic acids

CZU – Czech University of Life Sciences

DAD – diode–array detection

DM – diabetes mellitus

Dx – particle size distribution

EFSA – European Food Safety Authority

FAO – Food and Agriculture Organization

GABA – γ-Aminobutyric acid

GMP – Guanosine monophosphate

HPLC – High performance liquid chromatography

SAM – S-adenosyl-L-methionine

SD rat – Sprague-Dawley rat

TXA2 – thromboxane A2

1. Introduction and Literature Review

1.1. Economically important species

There are 124 species in the *Coffea* genus, with their origin distributed throughout Africa, on some Indian Ocean Islands and in the Australasian block. Natural habitats include seasonally flooded riverbanks and several types of tropical forests: dry, semideciduous and riverine. Coffee can grow in a variety of soil types and can naturally grow in elevations up to 2000 MASL. Arabica (*Coffea arabica*) is the most economically important species and accounts for 60 % of all coffee traded. Arabica has been harvested for millennia and farmed for several centuries, mainly due to its seed quality. While there are many varieties, the most important are Typica and Bourbon, and their descendants, such as Blue Mountain, which is resistant to coffee berry disease. Arabica is primarily grown in Central and South America. The second most important species, Robusta (*Coffea canephora*), was recognized by science only in 1897. In just 150 years, it has gone from being an unknown and underutilized African crop to a significant commodity due to its resistance to coffee leaf rust, better productivity, and higher caffeine content. Currently, it accounts for 40 % of coffee traded internationally and is mostly used in instant coffee. Although due to the less favourable organoleptic properties of robusta, arabica still has a higher market share (Davis et al. 2019). The third most widely grown species is Liberica coffee (*Coffea liberica*), used mainly as a rootstock for Arabica and Robusta. Its insignificance in terms of economic importance is due to its poor cup qualities (Ukers 1935; Davis et al. 2019; “International Coffee Organization - Trade Statistics Tables” 2020).

1.2. Botanical classification and description

Although hundreds of species of *Coffea* have been identified, the taxonomic classification of the genus is unclear and everchanging (Charrier & Berthaud 1985; Davis et al. 2006; Davis & Rakotonasolo 2021). *Coffea arabica* and *C. canephora* (known as Robusta) are the two most well-known species of coffee. The coffee tree is a plant from the Rubiaceae family; it produces a fruit called cherry, which develops a seed called bean. This bean is utilized to create coffee products for consumption. As a short-day plant, coffee starts blooming in its relevant photoperiod of < 12 hours of daylight.

A. de Jussieu was the first to describe the coffee tree in 1713 botanically; however, it was under the name *Jasminum arabicanum* (Charrier & Berthaud 1985). Carolus Linnaeus (1707-1778) was the first botanist to classify and name the species as *Coffea arabica* (Fischer et al. 2019). Figure 1 displays the first herbarium specimen.

C. arabica's natural habitats are the understory layers of tropical forests in Ethiopia. Nevertheless, it thrives in areas of equatorial Africa, Arabia, Central and South America, Mexico, the islands of the Pacific, India and Vietnam. (Ukers 1935). As he second most economically important species, *Coffea canephora* var. robusta originates from the tropical forests of central Africa (Wintgens 2004a).

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

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

The coffee tree's aerial parts consist of an upright main shoot with primary, secondary, and tertiary lateral branches. Nodes on orthotropic (vertical) branches are regularly distributed and carry opposite leaves. There are four to six serial buds in the leaf axil. The extra-axillary bud, which develops into a plagiotropic (lateral) branch, is located directly above it. Only the extra-axillary bud can generate a lateral branch; therefore, no regeneration can occur. Lateral branches grow at right angles with the vertical stems. The serial buds on primary branches can develop into an inflorescence or a secondary branch resembling the primary branch. Secondary branches have the ability to regenerate, as they can grow out of any axillary bud. Each leaf node contains

five buds, each one with four flowers. This means a node is able to produce 20 fruits (Ukers 1935; FAO 2005; Winston et al. 2005). Depending on the species and environment, a one-year-old plant may have six to ten levels of plagiotropic branches. After two years, it reaches a height of 1.5–2 m and begins to bloom. After three years, it reaches maturity and begins to produce fruit (Wintgens 2004a).

The mature leaves have a dark green upper surface and a lighter appearance underneath. They are noticeably shiny and feel waxy. They have a prominent elliptical shape with netted veins. *C. arabica* leaves are thinner and more delicate, compared with leaves of *C. canephora* or *C. liberica* species. Young leaves of *Coffea arabica* have a light green or bronze colour (Ukers 1935; Wintgens 2004a).

The three main components of the root system are a central taproot (0.45–1 m in length), axial roots that extend beyond the length of the taproot and run in various directions, and lateral roots which run parallel to the soil's surface. In ideal conditions, the root system can reach a volume of 15 m^3. In heavy and humid soils, roots concentrate in the upper layers. In contrast, the roots in dry soil are less superficial and run deeper. Since 90 % of roots develop in the top layer, mulching is a good practice that provides needed humidity and nutrition. A tap root that is bent or deformed leads to a decrease in nutritional uptake and, therefore, a shorter lifespan. As *C. arabica* has a deeper root system than *C. robusta*, it is more drought-resistant (Thurber 1889; Wintgens 2004a; FAO 2005).

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

Seed and germination

The seed consists of a hard endosperm (bean), which is encased by two layers: the inner integument (silverskin) and outer endocarp (parchment). The embryo is 3–4 mm long and comprises the embryo axis (hypocotyl) and two cotyledons (Figure 3). Although the shape and size of the coffee seed (bean) varies, generally, it is about 10 mm long and 6 mm wide. Wintgens states that the average weight of parchment seed at a moisture content of 18 % ranges from 0.45–0.5 g for arabica coffee and 0.37–0.4 g for robusta coffee (Wintgens 2004).

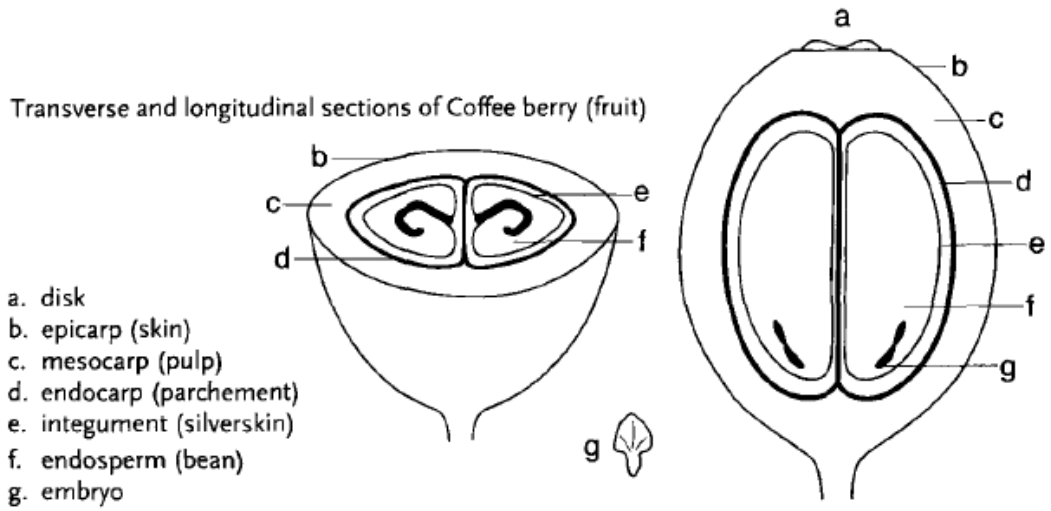

Figure 3 – Coffee fruit (berry) (Author: Wintgens 2004)

Coffee seeds should be used for propagation right after ripening, as they are not dormant; the optimal moisture content of the seed should be over 50 % (Wintgens 2004a). A sufficiently moist environment is vital for germination, and the ideal soil temperature should be 28–30°C. As lower temperatures slow down germination and with air temperatures below 10 °C, it may not begin at all. Removing the parchment speeds up the germination by 6-10 days. Plants in the genus *Coffea* germinate epigeously, meaning the growing hypocotyl raises the seed off the soil's surface (Figure 4) (Wintgens 2004a). After four to six weeks, the first cotyledon leaves develop (FAO 2005; Winston et al. 2005). At this stage, the plant only has a taproot and lateral roots (Figure 4).

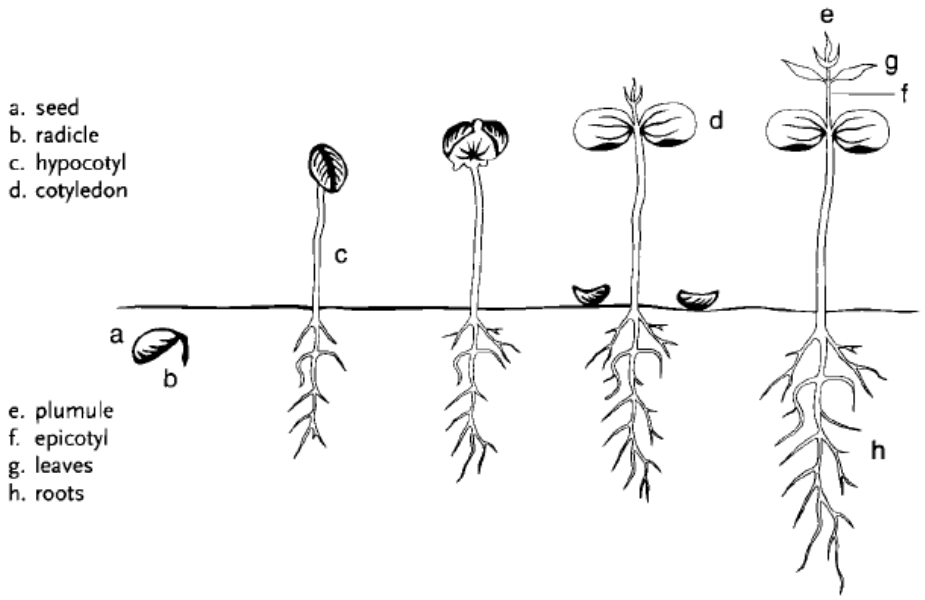

Figure 4 – Seed germination of *Coffea* spp. (Author: Wintgens 2004)

Inflorescence and pollination

The inflorescence has a white appearance; it is formed by a five-lobed corolla, calyx, five stamens and a pistil. The ovary has two ovules each able to produce a seed. Its inflorescence typically consists of four floral buds, known as cyme, each can generate four flowers. Differences may appear based on variety and conditions. *C. arabica* generates 16–48 flowers per node, whereas *C. robusta* 30–100. During the dry season, the buds remain dormant for 2–3 months. The rehydration breaks the dormancy of the plant. Trees in equatorial climates bloom throughout the year, and the dormancy can be broken by irrigation. Coffee flowers open roughly 12–15 days after the break of dormancy; they do so early in the morning and remain receptive for some days. The pollen is very light, so most of the pollination is done by the wind. Although *C. arabica* is predominantly self-pollinating, fertilization *C. canephora* is only possible by cross-pollination (Wintgens 2004a).

1.3. Coffee Propagation

Grafted plants start to produce fruit around 3 years after germination. After 30–40 years of cultivation, the average yield declines, and the plantation needs to be renewed. However, this is often done continuously.

Plant genotype, species, and varieties, as well as the environment and plant management practices, all impact yield and quality. A suitable location for the plantation and suitable stock should be considered before establishing or renewing it. Several criteria, including fruit quality, production costs, and productivity, should be taken into consideration when choosing a coffee variety. The type of clone chosen will determine productivity, but appropriate cultivation techniques and adaptation to local conditions are essential for the intended outcomes. The coffee variety determines its quality. However, harvesting and post-harvesting methods have a considerable impact. *C. arabica* is generally regarded as the species with the highest quality fruit. Production costs are directly linked to chosen cultivation systems – intensive, semi-intensive or extensive (Wintgens & Zamarripa 2004; FAO 2005).

1.4. Coffee processing

The quality and chemical composition of the final beverage is influenced not only by the coffee variety but also by the processing method. In which green coffee beans are isolated and subsequently roasted (Duarte et al. 2010; Bastian et al. 2021).

1.4.1. Post-harvest processing

After harvesting, three coffee processing methods are used: dry (natural), semi-dry (pulped-natural) and wet method (washed) (Brando 2004; Bastian et al. 2021).

Dry processing

Most coffee varieties marketed as natural are processed by the dry method, in which the whole cherry (including exocarp, mesocarp and endosperm) is dried to the moisture content of 10–12 % without any prior treatment (Cwiková et al. 2022). The whole hull (dried pulp and parchment) is then removed mechanically (Brando 2004). This method is used by 60 % of farmers in Brazil and Ethiopia and is generally used for

robusta coffee (Poltronieri & Rossi 2016). During the dry process the metabolism of coffee beans continues, as germination is inhibited. In the dry process, accumulation of γ-aminobutyric (GABA) acid occurs significantly more compared to the wet process (Bytof et al. 2005). GABA is a stress metabolite that indicates drought stress. GABA has a role in neurotransmission, neurodegenerative diseases, sleep and insomnia. It is associated with many health advantages, such as antidiabetic, hypotensive, anti-anxiety and antidepression properties (Shelp et al. 1999; Sahab et al. 2020).

Semi-dry processing

During semi-dry processing, the pulp (exocarp and part of the mesocarp) is removed mechanically. The leftover mucilage and parchment are dried with the beans, after which the dry parchment and adhering mucilage are hulled to obtain green coffee (Brando 2004; Cwiková et al. 2022). This method is sometimes known as the honey process as the sucrose content in the final product is higher than in the dry or wet method. However, the semi-dry process shows lower concentrations of chlorogenic acid (CGA) and trigonelline (Schwan et al. 2012).

Wet processing

In the wet process, the pulp is removed similarly to the semi-dry method, after which the coffee is left to ferment naturally, which degrades the mucilaginous residue. The beans are then washed and dried (Brando 2004; Cwiková et al. 2022). The de-pulping is a crucial step in the germination process, as the pulp contains germination inhibiting abscisic acid. The germination process, occuring during wet processing, produces several amino acids that become flavour precursors (Valio 1980). The wet processing creates more flavourful coffee with a pleasant acidity, but having less body than the dry processing method (Kulapichitr et al. 2019). The increase of amino acids in comparison to dry-processed coffee ranges from 3.3–20.9 % (Selmar et al. 2005).

1.4.2. Roasting methods

It is well known that the roasting process is an essential stage in coffee manufacturing, affecting the taste, colour, and aroma of the coffee. This process is divided into three main types: light-roast, medium-roast and dark-roast. Heat can be transmitted directly by conduction, by free or forced convection by streaming hot air, or by radiation (Bastian et al. 2021).

Effect of roasting on caffeine

Caffeine is moderately heat stable. However, due to sublimation, caffeine concentration in darker roasts is generally lower (Hečimović et al. 2011). Bastian et al. reviewed current literature and found caffeine content loses of 40–60 % in dark roasts (Bastian et al. 2021).

Effect of roasting on chlorogenic acid

Chlorogenic acid content decreases continuously with roasting. Mild roasting temperature demonstrates a 30–55 % drop in chlorogenic acid compared to green coffee. Dark roast shows an average decrease of chlorogenic acid by 90 % (Bolka & Emire 2020). Roasting breaks down chlorogenic acid feruloyl quinic acid lactones, caffeoylquinic acid lactones, and p-coumarylquinic acid lactones, which are some of the important taste components in coffee, to get the most desired effect light-medium roast should be used (Bastian et al. 2021).

Effect of Roasting on Acrylamide

While roasting of coffee beans creates acrylamide, there are currently few alternatives to mitigating its accumulation. Roasting is subsequently connected with the development of the flavour and colour of the final product. Therefore, reducing coffee's acrylamide levels is practically impossible, as the roasting process cannot be altered (Soares et al. 2015). However, fluidized bed-roaster shows lower acrylamide accumulation and might be useful in decreasing the risk of acrylamide poisoning in humans (Bolka & Emire 2020). Interestingly, higher concentrations of acrylamide are present in lighter roasts, as acrylamide levels rapidly elevate in the initial stage and are subsequently degraded during roasting (Clifford et al. 2003; Soares et al. 2015).

1.4.3. Decaffeinated coffee

The recent rise in the availability of decaffeinated coffee and general awareness of coffee's health benefits makes it a viable option for people with health disorders, caffeine intolerance or those searching for a healthier alternative. Currently, it accounts for about 10 % of total coffee consumption. Decaffeination is done before roasting. Historically, the most important and least costly method is extraction by an organic solvent (dichloromethane or ethyl acetate); vapour is used to open the seed's pores and wash them. After removing caffeine, the seeds are dried to a moisture content comparable to before extraction. There is a general health concern about remaining dichloromethane after this processing, simultaneously key flavour components can be lost using this method. Today, extraction using water and supercritical carbon dioxide is the only method used in Europe and the United States. Since it poses no health risks and it better preserves its original chemical composition, which maintains its flavour (Farah 2012; Ludwig et al. 2014).

Natural substitutes for artificially decaffeinated coffee include species that lack caffeine. There are more low- or no-caffeine species in the *Coffea* genus than high-caffeine species. The most famous is *Coffea charrieriana*, which is native to Cameroon and was the first coffee on the market to be caffeine-free. Caffeine-free species may be used for biotechnology and hybridization of new coffee species (Preedy 2014a).

With the general concern of adverse effects of caffeine, publications on caffeine-free coffee emerged, the earliest dates being in 1898. Such coffee has become widely available due to industrialization (Bizzo et al. 2015).

1.5. Caffeine

Caffeine is the primary active constituent of coffee and is also one of the most popular and widely used drugs and psychoactive substances in the world. It occurs naturally in over 60 plants (Harland 2000). Other plant sources are *Camelia sinensis* (tea), *Ilex paraguariensis* (maté), *Cola acuminata* (kola) and *Theobroma cacao* (cacao). Caffeine has many biological effects, the most common of which are shown in Table 1. It has both positive and beneficial effects on health, while it can negatively impact well-being. Consumption of caffeine creates an immediate and typically pleasant effect of alertness. However, excessive consumption may lead to unpleasant sensations of anxiety and over-excitement. Individuals are impacted differently by caffeine doses, which are attributed to genetic susceptibility and habituation to the effects of caffeine. Caffeine's mechanism is attributed to the upregulation of adenosine receptors (Depaula & Farah 2019a). For some individuals, even smaller doses of caffeine (50–60 mg) can be unpleasant and cause insomnia and a racing mind, while other individuals will not be affected by much higher doses.

Caffeine's other uses stem from its ability to function as a herbicide (Frischknecht & Baumann 1985). *Coffea* varieties with higher caffeine content inhibit the production of ochratoxin A produced by *Aspergillus* genus, sections *nigri* and *circumdati*. This could have a significant impact on inhibiting the growth and production of mycotoxin by mycotoxigenic fungi, even outside the coffee industry (Akbar et al. 2016). Because of caffeine's lipolytic properties, it is used in cellulite creams, which was proven in clinical trials (Byun et al. 2015).

The physiological activity of other compounds in coffee starts to be explored on the edge of the twenty-first century, primarily because of their antioxidant properties and the associated ability to prevent degenerative diseases. Previously, these known substances were believed to have no biological effects. The first studies on the bioavailability of chlorogenic and caffeic acids started in the 1950s (Bizzo et al. 2015).

Table 1 – Biological properties of Caffeine in humans (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 cardiac muscle contractility (treatment of heart failure)
Arrhythmias: ventricular extrasystole (“missed beats“, palpitations)
Smooth muscle relaxant
Gastro-oesophageal (reflux, heartburn)
Bronchodilatation (asthma treatment, illegal sports performance, enhancement)
Uterine muscle relaxation (possibly miscarriage)
Vasodilator
Headaches on caffeine withdrawal
Synergism with nitrites
Synergism with analgesics

1.5.1. Chemistry of caffeine

Caffeine was discovered in 1819 by Friedrich Runge (1795-1867), an influential pioneer of chromatography. (Runge 1821; Bizzo et al. 2015). It is a purine-based heterocyclic organic compound called 1,3,7-trimethylxanthine. Naturally, it is accompanied by theophylline (1,3-dimethylcanthine) and theobromine (3,7-dimethylxanthine), which are present in lower concentrations in coffee and tea and do not create notable physiological responses (Harland 2000). Caffeine is heat-stable and water-soluble and is regarded to as a stimulating alkaloid (Farah 2012; Depaula & Farah 2019).

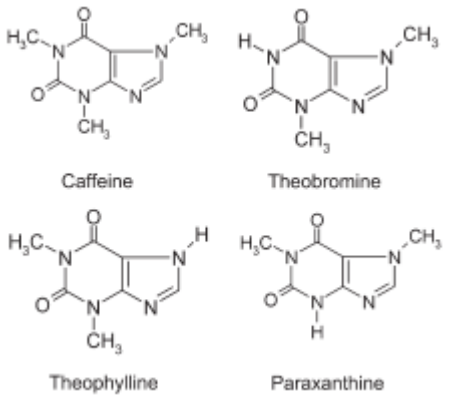

Figure 5 – Chemical structure of natural methylxanthines (Author: Burdan 2015)

Caffeine metabolism involves attachment to adenosine receptors located on cell membranes in the central and peripheral nervous system. It is referred to as a competitive antagonist of adenosine because of their structural similarity (Harland 2000). As a result, levels of dopamine are elevated. A rise in the concentration of this neurotransmitter is causing caffeine's stimulating and addictive properties. Additionally, dopamine enhances the mood-raising effects of serotonin (Crozier et al. 2011).

1.5.2. Biosynthesis

There are two hypotheses on the role of caffeine in plants. The theory of chemical defence 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 second theory, described as allelopathic or auto-toxic, suggests that caffeine in seeds is leached into the soil and inhibits the germination of other plants (Ashihara & Crozier 1999).

Synthesis of caffeine occurs in young tissues and chloroplasts of young leaves containing caffeine synthase enzyme. Caffeine's primary pathway to biosynthesis is xanthosine → 7-methylxanthosine → 7-methyxanthine → theobromine → caffeine (Figure 6). Due to the substrate specificity of *N*-methyltransferases alternative pathways also exist. Xanthosine is a purine nucleoside, which is a primary substrate, is produced by the degradation of purine nucleotides. Figure 7 shows the known pathways to its synthesis: 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).

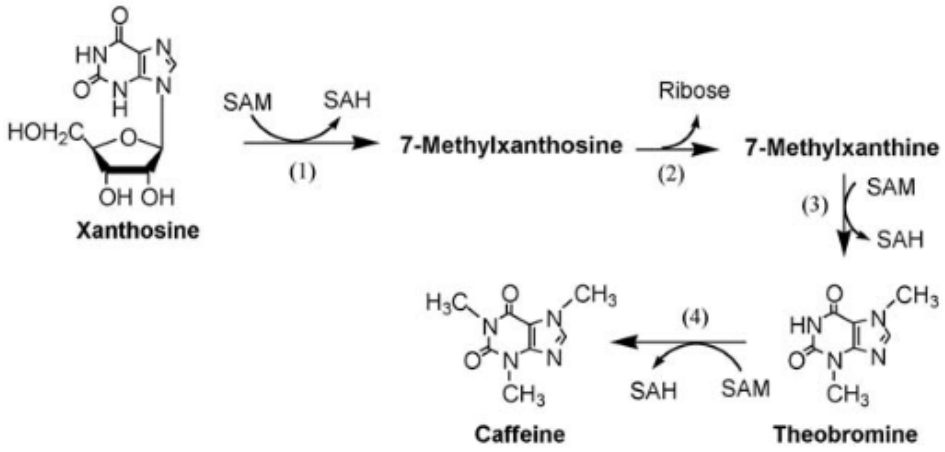

Figure 6 - Core pathway of caffeine biosynthesis in plants (Suzuki et al., 2004)

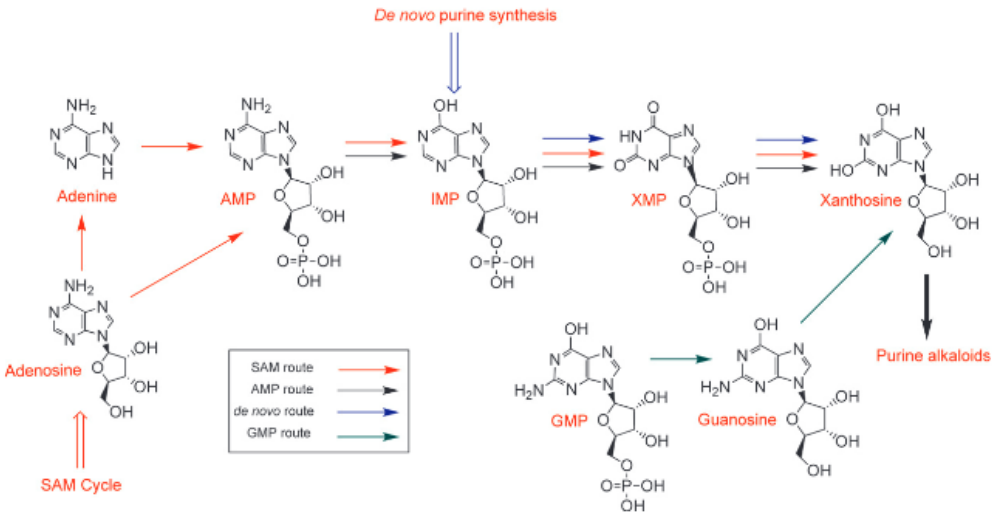

Figure 7 – Different pathways to Xanthosine synthesis (Ashihara et al. 2008)

Given the growing popularity of decaffeinated coffee, understanding these mechanisms could be advantageous for suppressing or enhancing production through genetic engineering. The creation of caffeine-producing transgenic plants may serve as a natural pesticide. This has been described in trans-genic caffeine-producing tobacco, with satisfactory results (Kim & Sano 2008). More investigations are needed to determine to what extent these effects are accomplished in other species, especially those of agricultural importance (Ashihara et al. 2008).

1.5.3. Caffeine tolerance

Caffeine consumption over time stimulates the creation of new adenosine receptors; henceforth, individual tolerance increases over time. Chronic usage may cause withdrawal symptoms, some of which are headache, drowsiness, fatigue, and negative mood (Harland 2000).

Studying the long-term effects of caffeine is difficult because consumption habits change over time, and 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 in healthy adult individuals

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

1.5.4. Effects of caffeine on health

Fertility and Pregnancy

Current research suggests that consumption of caffeine doses greater than 300 mg/day may reduce fecundability in fertile women, while for men doses higher than 400 mg/day could decrease sperm motility and increase dead spermatozoa, though not sufficiently enough to affect male fertility altogether (Depaula & Farah 2019a).

Caffeine easily crosses through the placenta into the fetus; however, the fetal liver cannot metabolize it, thus prolonging the half-life of caffeine. The evidence of caffeine's effects on pregnancy is mixed. A meta-analysis on caffeine consumption during pregnancy did not find any significant adverse effects on fetal growth and pre-term birth but suggested it could nevertheless contribute to miscarriage (Maslova et al. 2010). Other compounds present in coffee, such as theobromine and theophylline, might also be of influence. Habits that are generally linked with coffee use, such as alcohol and tobacco consumption, may also have an impact (Hinds et al. 1996; Harland 2000).

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

Caffeine and bone health

Caffeine has been shown to have several effects on bone health. Among them is its antagonistic nature toward adenosine. Adenosine stimulation of bone metabolisms is one of them. According to *in vitro* studies, the stimulation of adenosine A_{2A} and A_{2B} receptors encourages bone formation by activating osteoblast, this effect can be prevented by the binding of caffeine to adenosine receptors. Caffeine also negatively affects calcium metabolism, f.e. an increase in calcium excretion. This is particularly common, especially in older adults and premenopausal middle-aged women. Nonetheless, the association between caffeine consumption and osteoporosis and bone health is rather indirect. Some sources suggest that these issues could be offset by ingesting small amounts of milk with coffee (Depaula & Farah 2019a). Further long-term studies need to be done to clarify these issues (Cooper et al. 2009; Berman et al. 2022b).

Caffeine and Cardiovascular System

Research on coffee's effects on the cardiovascular system has been historically a key topic; often with controversial findings (Preedy 2014a). Administering high doses of pure caffeine (200–250 mg) to healthy individuals has been observed to elevate blood pressure and induce cardiac arrhythmias (Depaula & Farah 2019a). However, these short-term raises later normalized and proved to be temporary and reversible in the majority of the cases. Existing research indicates that moderate caffeine intake (< 600 mg/day) is not linked with increased risk of cardiovascular disease, arrhythmias, heart failure, blood pressure changes and hypertension among regular caffeine consumers in healthy population (Turnbull et al. 2017). Recent evidence also suggests that caffeine consumption does not increase the risk of coronary heart disease (Lopez-Garcia et al. 2006b). Finally, no association was found between coffee ingestion and a risk of stroke (Grobbee et al. 1990).

Hypertensive individuals are more sensitive to some effects of caffeine. For instance, pre/hypertensive populations experienced an acute rise in blood pressure with consumption of caffeine around 100–400 mg/day. In summary, epidemiological studies indicate an increased risk of cardiovascular disease only when five or more cups of coffee were consumed, representing ≥ 500 mg of caffeine daily (Depaula & Farah 2019a).

Caffeine and Neurodegenerative Diseases

The central nervous system is greatly affected by caffeine. It is a known psychoactive stimulant that permeates the blood-brain barrier and is antagonistic to adenosine receptors. Recent studies of caffeine's impact on neurodegenerative diseases suggest that it can be associated with preventing the development of Alzheimer's and Parkinson's and may be linked with better cognitive performance (Preedy 2014a; Depaula & Farah 2019a). Caffeine does this by creating new connective pathways in the brain, altering the morphology of neural synapses, supporting the formation of larger dendritic spines, and changing neural networks (Preedy 2014a).

Alzheimer's disease causes cognitive decline and is the primary cause of dementia. Affected individuals develop neurofibrillary tangles and senile plaques, which are triggered by the accumulation of toxic beta-amyloid peptide or Tau protein in

the brain (Checler 1995; Ittner et al. 2010). Caffeine's protection is linked with anti-inflammatory effects on A1 and A2 receptors and the decrease of deposits of the beta-amyloid peptide. A model study on mice reported that high caffeine intake (human equivalent of 500 mg) can both prevent and even treat Alzheimer's disease (Arendash et al. 2009b).

Parkinson's disease worsens motor and non-motor skills by degenerating dopaminergic neurons in the midbrain (Dauer & Przedborski 2003). According to a meta-analysis of 26 studies, coffee drinkers were at a 25 % lower risk of developing Parkinson's disease compared to non-drinkers. For every 300 mg increase in caffeine intake, there was a total risk reduction of 24–32 % (Depaula & Farah 2019a).

Caffeine and liver disease

Consumption of caffeine is associated with a lower risk of cirrhosis and hepatocellular carcinoma. Moreover, it might be beneficial in treating chronic hepatitis C and reducing fibrosis in alcoholic liver disease. According to a recent meta-analysis, a regular intake of three cups a day reduces these risks by 40 % (Preedy 2014).

Caffeine and Glucose Metabolism

Regular coffee consumption is related to a reduced risk of diabetes. However, research suggests that caffeine itself encourages adverse effects on glucose metabolism and reduces insulin sensitivity. This emphasizes the positive effects of other coffee constituents, especially chlorogenic acids, on glucose homeostasis and their ability to balance the effects of caffeine. In conclusion, it has been proven that consistent coffee consumption of both decaffeinated and regular coffees reduces the risk of diabetes, with decaffeinated seemingly better for glycemic control. Research on various coffee constituents and their effects on glucose metabolism would be helpful, as it may lead tothe development of coffee beverages that can maximize positive effects on health (van Dam & Hu 2005; Depaula & Farah 2019a).

Carcinogenicity of Caffeine

In the 1980s the reviews of national governments on coffee consumption raised alarming concerns whether caffeine may be carcinogenic, particularly regarding bladder and colon cancer. However, most of these assumptions have been made due to inadequate control for tobacco smoking, which is strongly linked to heavy coffee

ingestion (IARC 1990; Depaula & Farah 2019a). The majority of research indicates no conclusive link between coffee consumption and any kind of cancer. Furthermore, a meta-analysis on this subject revealed that coffee consumption has an inverse relationship with some types of cancer and may lower the incidence of cancer overall (Yu et al. 2011).

Caffeine Intake Recommendations

Despite extensive research focusing on the potential health effects and safety aspects of caffeine consumption, no universally accepted daily intake guideline exists. Safe limit for its consumption is difficult to establish because of different effects on individuals, which are based on sensitivity and habituation to caffeine. The dose of caffeine, which creates adverse and unpleasant effects, varies from individual to individual. Inexperienced users should consume it with caution until a one learns how it interacts with their body. The general standards by government authorities range from 200–400 mg/day in adults.

Children and adolescents who consume caffeine experience sleep disturbances and could hinder their brain development. The EFSA recommends no caffeine intake for children under 12 months. For older children and teenagers, it is recommended that intake is below 3 mg/kg bw/day (<120 mg/day in 40 kg bodyweight). Pregnant and lactating women should limit their caffeine intake for the reasons mentioned above; the maximum recommended daily dose is 200–300 mg/day (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) 2015a; Depaula & Farah 2019a).

1.6. Chlorogenic acid

Most studies up to date have focused on the potential health benefits of caffeine (Haskell et al. 2008; Arendash et al. 2009a; Depaula & Farah 2019b; Berman et al. 2022a). Nonetheless, information on the potential health benefits of other coffee compounds is scarce (Zang et al. 2003).

Chlorogenic acids (CGAs) are biologically active polyphenols which have alleged antioxidant properties (Nardini et al. 2002), are produced by certain plant species and are a major component of coffee. The term CGAs stands for a group of hydroxycinnamic esters with quinic acid, including caffeoyl-, feruoyl-, dicaffeoyl- and coumaroylquinic acids. Furthermore, there are several isomeric forms of these subgroups. The major polyphenol in coffee is caffeoylquinic acid, which is an ester of caffeic acid with quinic acid and is often referred to as chlorogenic acid. Its most common isomer in coffee is 5-caffeoylquinic acid (5-CQA) and accounts for 76-84 % in green coffee beans or 10 g/100 g in roasted coffee beans (Clifford & Ramirez-Martinez 1991; Perrone et al. 2008, 2010; Tajik et al. 2017).

CGAs are present in raw coffee and in other seeds and fruits such as sunflower seeds and blueberries. Lower content has also been observed in potatoes, tomatoes, apples, pears and eggplants, but consumption of CGAs from these sources only accounts for 5–10 % compared to coffee (Clifford 2000; Manach et al. 2004). While the method of processing might effect CGAs content in green coffee. The change is miniscule with the effect of roasting on CGAs content, which decomposes during the roasting process, especially in darker roasts (Várady et al. 2022a). 5-CQA is still a major isomer in roasted coffee and is considered to be the main source of CGA in the human diet (Clifford 1999; Renouf et al. 2010).

Although a limited number of studies focused on the metabolism and bioavailability of CGAs in humans. Lafay et al. reported that CGA is absorbed unchanged in the stomach of rats (Lafay et al. 2006). The majority of CGA is then hydrolysed to caffeic acid and quinic acid before their absorption by esterases in small and large intestine (Konishi & Kobayashi 2004). Subsequently, it is metabolized to glucuronide and sulphite metabolites, which circulate in human plasma (Nardini et al.

2002). However, Monteiro et al. reported a significant variability in the absorption and metabolism of CGAs between individuals (Monteiro et al. 2007).

1.6.1. Effects of chlorogenic acid on health

CGA and Cardiovascular health

According to meta-analysis conducted by Tajik et al. 23 studies examined the association between consumption of CGAs and cardiovascular health. Fifteen employed animal models, while eight were performed on humans. Based on statistical analysis, they found an association between dietary consumption of CGAs and a significant reduction is systolic and diastolic blood pressure. Although the mechanisms of CGA on endothelial dysfunction, which is a major factor in developing atherosclerosis, remain unclear. There have been suggested protective properties associated with antioxidant and anti-inflammatory properties of CGA. As well as the protective effects of CGA by the release of vasoactive molecules such as nitric oxide (NO) and thromboxane A2 (TXA2) (Taguchi et al. 2014; Tajik et al. 2017). Though there is only one human study up do date (Tajik et al. 2017) there are claims that CGA can modulate lipid and glucose metabolism to prevent dyslipidaemia, which is linked with fatty liver disease and cardiovascular disease (Rodriguez de Sotillo & Hadley 2002). Lecoultre et al. has shown positive effects of caffeinated coffee with 9 % concentration and decaffeinated coffee with 3 % concentration of CGA on glucose and lipid metabolism in healthy men (Lecoultre et al. 2014). Current evidence in SD rats also suggests cholesterol-lowering effects of CGA, that are most likely mediated by increasing utilization of fatty acids in the liver via upregulation of PPAR-α mRNA (Wan et al. 2013).

CGA and Diabetes mellitus

Type-2 diabetes mellitus (T2DM) is a metabolic disease that involves impaired glucose and fat metabolism (Kamtchouing et al. 2006). Tajik et al. summarized 17 studies (12 animal models and 5 human) examining the association between consumption of CGAs and Diabetes mellitus (DM). Huxley et al. reported that the risk of Type-2 DM is reduced by 30 % in those who drink 3–4 cups of decaffeinated coffee containing high concentrations of CGA (2009). Van Dijk et al. examined the immediate effects on glucose tolerance in overweight men; they found improved insulin responses and early fasting plasma glucose when compared to placebo (Van Dijk et al. 2009). Ahrens et al. suggest that regular ingestion of CGA containing supplements is able to lower the glycaemic impact of food and continually lower background blood glucose levels of Type-2 DM (Ahrens & Thompson 2013). Moreover, In vitro and human study evidence suggests that CGA increases cell insulin secretion (Johnston et al. 2003; Tousch et al. 2008).

CGA and obesity

Obesity is a worldwide severe health problem that is the leading risk factor for cardiovascular disease (Ogden et al. 2007). The results from two prospective cohort studies showed that caffeinated and decaffeinated coffee were both associated with weight loss, which suggests that non-caffeine compounds, such as CGAs, have a positive effect on weight reduction (Greenberg et al. 2005; Lopez-Garcia et al. 2006a). In a meta-analysis of randomised clinical trials, Onakpoya et al. observed an average weight reduction of 2.5 kg in individuals ingesting green coffee extracts with high CGA concentrations (Onakpoya et al. 2011). However, another randomized study performed on 30 healthy individuals did not observe body weight change while ingesting CGA-rich coffee (CGA 4.5 mmol/L); it should be noted that the study time was only 4 weeks (Kotyczka et al. 2011). Although there is positive evidence that long-term consumption of decaffeinated coffee has positive impact on weight-loss. The results are incomplete and further investigations into the influence of CGAs on appetite and satiety, thermogenesis, thermic effect of food should be conducted.

CGA and cancer

Early in-vitro studies show that cellular damage caused by reactive oxygen species (ROS) is responsible for a number of diseases, including coronary heart disease, diabetes and cancers (Hussain et al. 2003; Valko et al. 2007). CGA should, therefore, have beneficial affects to such diseases due to its ability to prevent oxidative stress and oxidative damage (Valko et al. 2007). Tajik et al. examined 11 studies, which focused on the relationship of CGAs consumption and cancer. They reported that "the current animal data on CGAs is promising, beneficial effects of CGAs on human cancer have not been studied extensively" (Tajik et al. 2017). Nonetheless, a randomized controlled trial by Bakuradze et al. reported the beneficial effects of coffee rich in CGA on DNA integrity (Bakuradze et al. 2015).

CGA and brain health

While numerous studies explore the effects of coffee on the human nervous system, most of them focus solely on caffeine. Tajik et al. report only 13 studies examining the association of CGAs consumption and brain health. Three of those were performed on humans. Although they improved cognition and neuroprotective effects, further research should be conducted to evaluate these properties over extended periods of exposure and in different age groups (Tajik et al. 2017).

CGA and gastrointestinal health

Some animal studies have explored the benefits of CGAs anti-inflammatory properties on gastrointestinal health. In an experimental model of colitis on rats, CGA supplementation reduced the appearance of diarrhoea. These effects were attributed to the reduction of pro-inflammatory cytokines and activation of NF-KappaB (Di Paola et al. 2010). Gut-health is also greatly influenced by an intact intestinal barrier. Ruan et al. reported that CGA supplementation in rats mitigated mucosal inflammation by decreasing the intestinal permeability and by the increased expression of tight junction proteins (Ruan et al. 2014). However, these mechanisms have not been yet determined in humans (Tajik et al. 2017).

CGA and hepatic health

In a pharmacological review, Xue et al. explored recent research and reported that “CGAs have an excellent protective effect against various liver diseases,” such as alcoholic liver disease, drug-induced liver injury, metabolic fatty liver disease, cholestatic liver disease and liver cancer. CGAs beneficial effects were attributed to their antioxidant and anti-inflammatory properties (Xue et al. 2023).

CGA and its effects on inflammation and pain

Tajik et al. describe several animal studies that show positive effects of CGAs on inflammation and pain. CGA can supposedly reduce inflammation by decreasing the production of certain markers involved in the inflammatory process (Krakauer 2002). It has also been observed to inhibit the formation of oedema and alleviate pain (Dos Santos et al. 2006). Furthermore, CGA has shown the ability to treat neuropathic pain, with studies suggesting that it can reduce hyperalgesia and modulate certain ion channels involved in pain perception (Bagdas et al. 2013; Hara et al. 2014; Qu et al. 2014).

1.7. Coffee preparation methods

Although caffeine is non-volatile and stable after roasting, a small amount may be lost to sublimation during roasting (Farah 2012). Dutra et al. reported that caffeine was detected in the exhaust gases released during roasting, assuming that some caffeine loss may happen because of water vapour being released during seed fracturing caused by pressure (Dutra et al. 2001). During roasting, the beans are exposed to a temperature of 100–245 °C for varying periods of time depending on the variety, geographical origin and desired characteristics. Darker roasts can have lower caffeine content as roasting leads to a 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).

Grinding is a crucial step in coffee preparation. Chemical compounds in whole beans are inside cells and cannot be dissolved. Grinding breaks beans into small particles ranging from a few μm to 1–2 mm. This allows easy dissolvement of chemical compounds and releases aromatic volatiles. There are four categories of ground coffee: coarse, medium, fine, and very fine. Typically, finer ground coffee has a higher extraction of caffeine and other compounds due to higher surface area being in contact with solvent.

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

Unfortunately, there is no evidence that people on the African continent consumed coffee before the common era. Based on traditions and accounts of European travellers from the seventeenth century, we might suggest that coffee was ingested before recorded history. The prevalence of the genus *Coffea* in this region is another supportive argument for this hypothesis. According to African tradition, we can assume that wine was made from the pulp of ripe coffee berries, prior to the tenth century (Ukers 1935; Weinberg et al. 2001). It is believed, that during the eleventh century in Ethiopia, began the practice creating a beverage by boiling unripe coffee beans in their

husks (Weinberg et al. 2001). After Arab traders brought back coffee, they started to prepare two different beverages. The first drink was called *kisher*. Nowadays similar to *cascara*, it is steeped from dried husk, and according to testimonies, has a taste similar to an aromatic or spiced tea (Davids 1996; Weinberg et al. 2001). The second drink was called *bounya*, or *bunn*, is a thick brew made from ground or crushed beans. It was drunk unfiltered and ingested with the sediment, as was usual practice for several hundred years (Weinberg et al. 2001). Early preparations of *bounya* were made by boiling raw beans. The practice of roasting beans arose in the Levantine. Islamic coffee drinkers in the sixteenth century invented the *ibrik*, a small metal vessel used to boil coffee, which was the prototype of the coffee pot. Younger method of preparing coffee is infusion. During the eighteenth century, ground coffee was put in a cloth bag and steeped in hot water (Ukers 1935; Weinberg et al. 2001).

1.7.1. Decoction and immersion methods

Decoction or immersion describe a process where a soluble solid is kept in contact with a specific volume of water at a certain temperature for a specific period of time. Decoction in coffee brewing describes a process when water is boiled together with the ground coffee, while immersion indicates that already boiled water was put in contact with the grounds. With increasing concentration, the extraction rate decreases, making high-ratio decoctions ineffective and possibly having an unpleasant aroma. The time of contact between solvent and solid increases extraction. The temperature of water is another parameter, as caffeine and other compounds are more readily soluble at higher temperatures (Petracco 2008; Mestdagh et al. 2017).

Boiled and Turkish Coffee

Boiling of coffee is the most rudimentary brewing method, it has been historically famous in northern and central Europe. Coffee that has been medium or coarsely ground is heated in a pot until boiling. Keeping the beverage at a boiling point increases the extraction.

Turkish coffee is a type of boiled coffee where the beans are ground very fine. This makes the coffee to settle on the bottom, as it is unable to float. It also increases the extraction due to increased surface area. Traditionally, it is prepared in a long-

handled pot, called *cesve*. The product is a small, strong beverage which contains some sediment.

French press and other immersion brews

Boiling coffee has become easier with the invention of electric kettles; ground coffee is simply added to a mug, then freshly boiled water is poured over it, allowing the coffee to steep at a lower temperature. Once the drink has cooled down enough, it is consumed. The ease of production and lack of specialized equipment required for this method make it popular in central and eastern Europe.

French press is a type of vessel with a fine wire mesh plunger that is used to separate the grounds and liquid. Boiling water is poured over medium to coarse ground coffee, mixed, and left to sit for 2–8 minutes. The desired intensity of extraction determines the duration. While shorter extraction times highlight the coffee's acidity and floral notes, longer extraction times result in higher caffeine content as well as intensity and bitterness. The mesh strainer plunger is pressed down before serving, allowing the liquid to flow through and separate the grounds. Some fine particles and sediment enter the finished beverage as a result of ineffective mesh filtration (Mestdagh et al. 2017). By pressing the plunger, coffee oils are squeezed out from the coffee bed, increasing their content in the final beverage (Zhang et al. 2012).

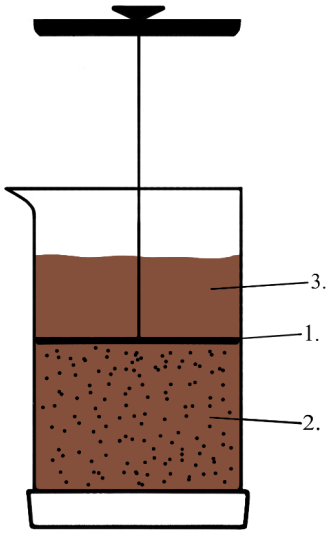

Figure 8 – French press diagram: 1. Plunger, 2. Separated ground coffee, 3. Filtered beverage (Source: Author 2022)

1.7.2. Percolation methods

Percolation, also known as pour-over or filtered coffee, is a technique in which hot water is allowed to run freely through a bed of coffee using gravity alone. This allows for a brief period of contact time, resulting in a beverage that has a milder flavour but still maintains some subtle aromas. Typically, the ground coffee is placed in a conical holder fitted with a filter. Commercially available filters come in a variety of sizes, shapes, and materials, each of which yields a particular outcome. The final beverage is significantly influenced by temperature, water volume, coffee bed shape, and particle size. Water can be applied manually or through a drip filter machine that operates automatically (Mestdagh et al. 2017).

1.7.3. Pressure methods

Moka pot

According to literature, the moka pot is a type of stove-top coffee maker that is "the most popular household coffee brewing in Italy (Mestdagh et al. 2017; Severini et al. 2017b)." It was invented by Alfonso Bialetti in 1993. It comprises of three chambers. Pressurized water or steam is supplied by the bottom chamber and travels through the middle chamber, which holds the ground coffee, before collecting in the upper chamber. Despite significantly lower preparation pressures, the finished beverage is compared to espresso. Although it has a straightforward construction, the thermodynamic and extraction behaviour of moka is highly complicated. The extraction process is difficult to control and can lead to overextraction and dissolution of undesired compounds, which results in a harsh and bitter drink.

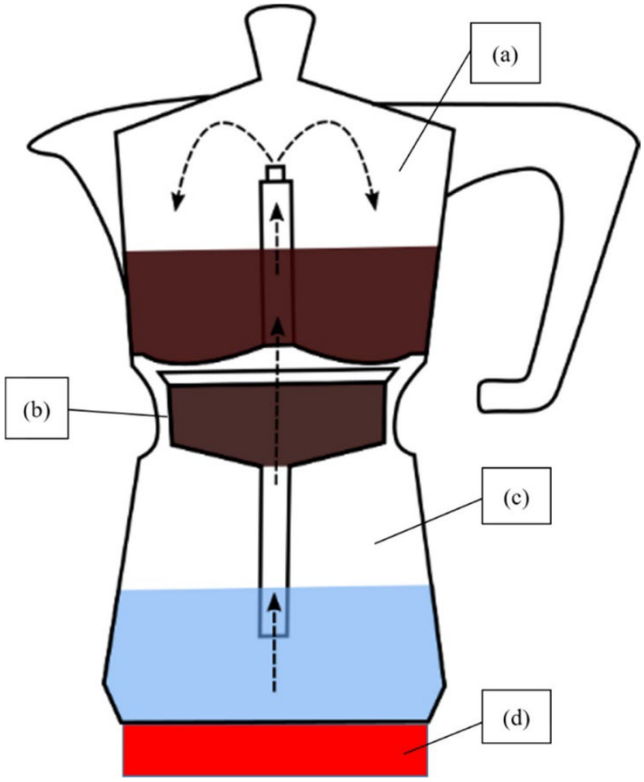

Figure 9 – Diagram of a Moka pot: (a) collection chamber, (b) basket chamber (containing ground coffee), (c) bottom chamber, and (d) heat source (Windisch et al. 2020)

Espresso

The Italian espresso became well-known throughout the world for its potent, highly aromatic, small-volume beverage that is meant to be consumed right away. “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. 2017b). Because of its high pressure and low water/coffee ratio, it provides a different kind of sensory satisfaction than other brewing methods. Acquiring high-quality espresso cups is quite challenging, as higher extraction may indicate flaws in the source material and other factors (Petracco 2008a). Espresso is used mainly commercially due to the quickness of preparation and the high price of Espresso machines. Thus, it plays a miniscule role in coffee homebrewing.

Single-serve coffee makers

The fastest-growing preparation method in the coffee sector is single-serve coffee makers. The coffee pod market is expected to hit $ 25 billion by 2025, in USA alone (Eiermann et al. 2020). The beverage is prepared by forcing an exact volume of water through pre-filled coffee pods or capsules. The water is compressed at a pressure ranging from 3–19 bar and is heated to temperatures of 93–95 °C, the power input of these machines ranges from 400–1500 W (Cibelli et al. 2021). Different systems vary in the shapes and sizes of capsules, but the most common machine is the Espresso system (Eiermann et al. 2020). Because they are generally hermetically sealed capsule and pod systems are supposed to keep the ground coffee as fresh as possible. They are also supposed to provide consistent brew, as the human factor is eliminated from the brewing process (Severini et al. 2017b; Eiermann et al. 2020). However, they pose ecological concerns as they pose increased production of packaging material and post-consumer waste disposal. The carbon footprint of a single coffee made by a pod or capsule machine has been established as 12.6 to 27.6 g CO_2 higher than an induction Moka pot (Cibelli et al. 2021).

AeroPress

AeroPress is a combination of immersion and pressurized brewing methods. The coffee grounds are placed in a cylindrical chamber and let to steep. After which the beverage is extracted through a paper filter by manual pressure added by a plunger to extract more from the coffee (Mestdagh et al. 2017; de Figueiredo Tavares & Mourad 2020).

2. Aims of the Thesis

The main objective of this thesis was to investigate the impact of coffee preparation techniques on the content of caffeine and chlorogenic acid. The goal was to establish a safe guidelines for sensitive groups of individuals. To achieve this, the experiment tried to eliminate all other variables (grind size, water/coffee ratio, etc.) to focus on the impact of brewing method.

3. Materials & methods

3.1. Coffee samples

Two types of *C. arabica* sourced from roastery Českáva s.r.o. were used. The first sample ("Ethiopia") origin is Sidamo in southwestern Ethiopia, it was grown in elevations 1600–1900 msl. The coffee was processed using washed fermentation. The second sample ("Nicaragua") was grown in Nicaragua on plantation Finca El Cipres by Isac Javier in elevations around 1200 msl. It was processed using anaerobic natural fermentation. Samples were roasted in Českáva roastery on a commercial grade fluid-bed hot air roaster Novoroaster. The roasting recipe for Nicaragua sample was based on time, for 60 s hot air of 180 °C was supplied, after which it was raised to 220 °C for 165 s and finished at 223 °C for 230 s. Resulting in 455 s roasting time for 750 g of green coffee beans. While the recipe for Ethiopia sample was based on heat of the beans. They were heated in increments while the highest obtained temperature was 216 °C. The time of roasting is dependent on the batch, but is is generally between 15 to 17 minutes for 1500 g of green coffee.

3.2. Brewing methods

Following preparation methods were used: AeroPress™, Cezves, French press, Filtered – V60, Moka pot, Nespresso pods and De'Longhi Espresso Maker. These should reflect the general homebrewing methods available to most of the population. Specialty and commercial methods were omitted, as they do not represent the conditions of a home setting and result in a very different beverage.

Moka-pot (Moka). A Bialetti moka pot of 220 ml volume, up to the pressure valve, was used. The diameter of the basket chamber was 53 mm. It is necessary to never fill up a moka pot fully, as the pressure might get too high and be potentially dangerous. This method was prepared in two ways:

- First by adding cold water and heating it up for 5 minutes on the full power of the gas stove. At this time the moka pot sputters and most of the water has

passed through the chambers and extracted. The extracted beverages ranged from 62.1 g to 72 g. It is generally not recommended to let it boil past the distinctive sound as the beverage might become over-extracted and not palatable (Mestdagh et al. 2017).

- In the second method already boiling water was added to the chamber and let on the stove for 2 minutes. The obtained yields ranged from 58.4 g to 67.2 g. These losses of liquid are contributed to by the evaporation of hot steam, absorption of moisture by coffee grounds, and some water left in the bottom chamber.

French press (French). An Orion brand glass home French press was used with a metal mesh plunger, it had a diameter of 80 mm. The coffee grounds were poured over with boiling water and left to steep for 3 min, 5 min and 10 min. After which the plunger was gently pressed to the water surface and the liquid was strained.

Filtered coffee (V60). For the filtered coffee a Hario V60-01 plastic dripper was used. Its height was 83 mm and width at the top 95 mm. Hario VCF-01-100W paper filters were used alongside with it. The different temperatures of hot water (80 °C, 90 °C and 96–98 °C) were poured over the coffee slowly in two to three pours.

AeroPress (Aero). The AeroPress GO used has a diameter of 95 mm. The paper filters used with it were AeroPress Micro-Filters. Coffee grounds were poured over with boiling water and the beverage was let to steep in the plunger turned upside down for 30, 120 and 180 s. After which the filter in its case was screwed on and the beverage plunged out into a vessel.

Cezves (Cezv). A traditional brass Cezves of a two-walled construction, which was acquired from a Turkish market was used. Its top diameter was 60 mm and bottom diameter 85 mm. The grounds with cold water were brought to a boil on the gas stove. As soon as it was staring to boil over from the cezves it was taken of heat and strained through a paper filter. The boiling time was somewhat consistent with the minimum of 175 s and maximum of 216 s.

Nespresso – pods (Nesp). For the pod coffee a Krups Nespresso XN110110 was used together with reusable aluminium capsules from SEALPOD. The Nespresso machine has a power input of 1200W and a stated pressure rating of 19 bar. A big

coffee setting on the machine dispensed 100 ml of hot water, creating the desired beverage.

Espresso machine (Esp). A lever coffee machine De'Longhi Dedica EC 685.M was used to make espresso. Its power input is 1300 W and it can generate pressure of 15 bar. A plastic lever with a porta filter, which was provided by the manufacturer, was used with it. The double coffee button dispensed 100 ml of water without the coffee grounds.

3.3. Sample preparation and filtration

The acquired samples were filtered immediately through a paper filter and afterwards through a microfilter of 0.45 μm. After which they were diluted with MiliQ water in a ratio 1:11 and put into vials, these were stored at – 20 °C until analysis.

Samples of each method were prepeared in **four replications** per type of coffee. The ration of coffee grounds to water was 6 g to 100 ml in all preparations. Twice with double distilled water and twice with local tap water from water source Želivka. For preparations needing a heat source a home gas stove MORA P4251AW was used. The smallest burner outputting 1 kW was always put on full power. For methods needing boiled water an electric kettle with temperature setting was used (Sencor SWK 1796SS). Most preparations, except the Filtered coffee, used 96-98 °C water.

3.4. Experiment design

A total of seven main brewing methods, including their respected variations, were tested. These were then prepared for both types of coffee (Ethiopia & Nicaragua) and concurrently for both types of water (distilled & tap). Detailed diagram of the experiment design is explained in Figure 10 below. As a result, 56 distinct samples obtained. To ensure accuracy, the entire experiment was conducted in duplicate, resulting in a total of 112 samples for analysis.

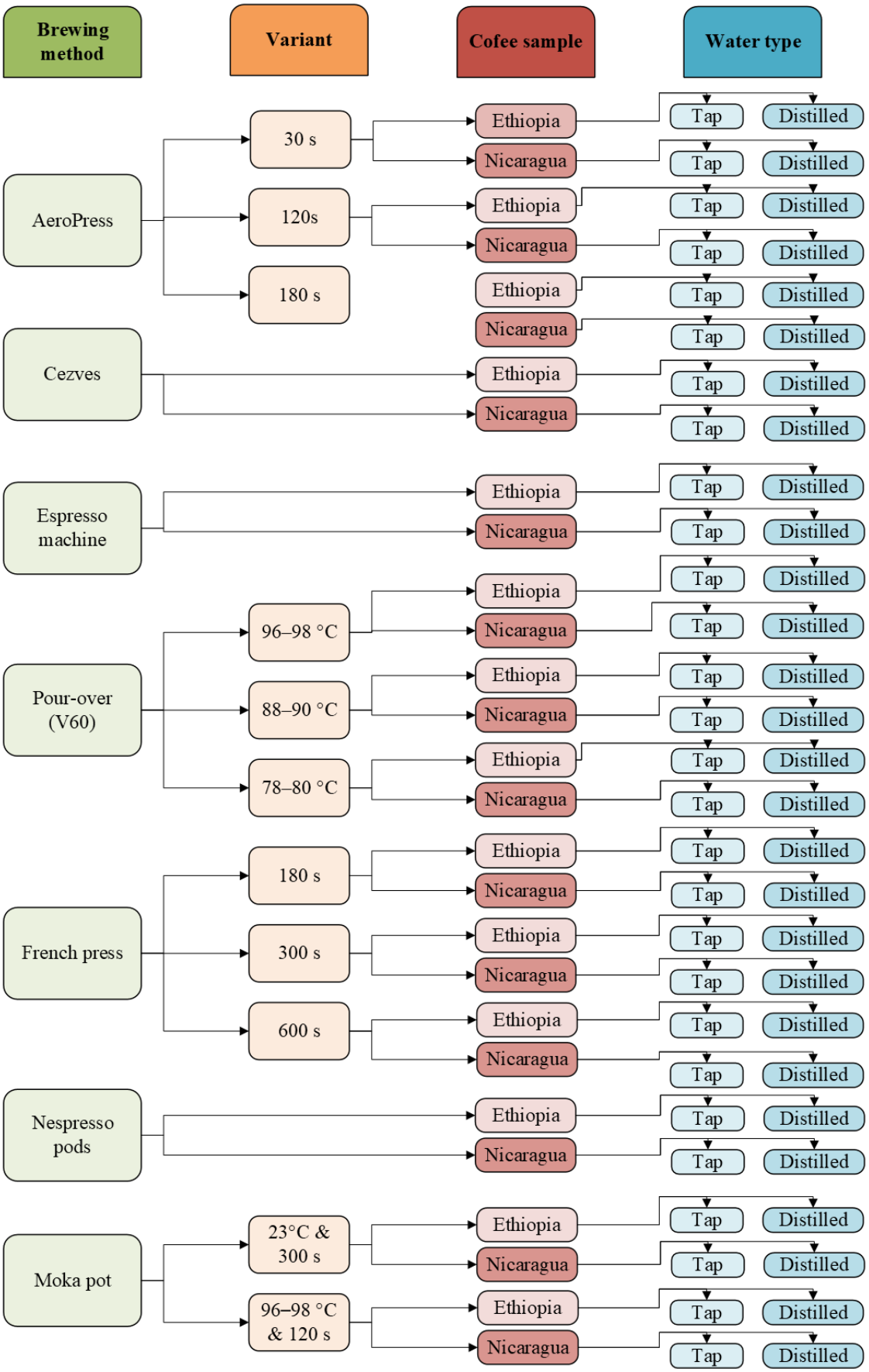

Figure 10 - Experiment design diagram (Source: Author)

3.5. Secondary analyses

The pH analysis was performed after the first filtration immediately when the beverage has cooled to temperature of 23 °C. An electrode pH meter WTW from InoLab was used.

Particle size analysis was performed using laser diffraction. Mastersizer 3000 (Malvern Instruments, UK) was used with a Aero S unit (Malvern Instruments, UK). The refraction index was set to 1.46 and absorption index to 0.01. Evaluation of the measurements was performed using the instrument software (Malvern Instruments, UK) and the particle size percentage was expressed as Dx (10), Dx (50), Dx (90).

3.6. High Performance Liquid Chromatography using Diode Array Detection (HPLC-DAD)

The caffeine and chlorogenic acid (CGA) content was analyzed using HPLC-DAD.

Chemicals used

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

Methanol for HPLC (Lach-Ner, Czech republic)

Caffeine (Sigma–Aldrich, Germany)

Chlorogenic acid – EP Reference Standard (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 for caffeine, and 320 nm for CGA

Mobile phase flow rate: 0.3 ml/min

Injection: 20 µl of sample

Column temperature: 35 °C

Length of analysis: 5 minutes

Analyte retention time: 4.5 minutes

Caffeine standard. 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, where measured peak areas were compared to known concentrations (Figure 10).

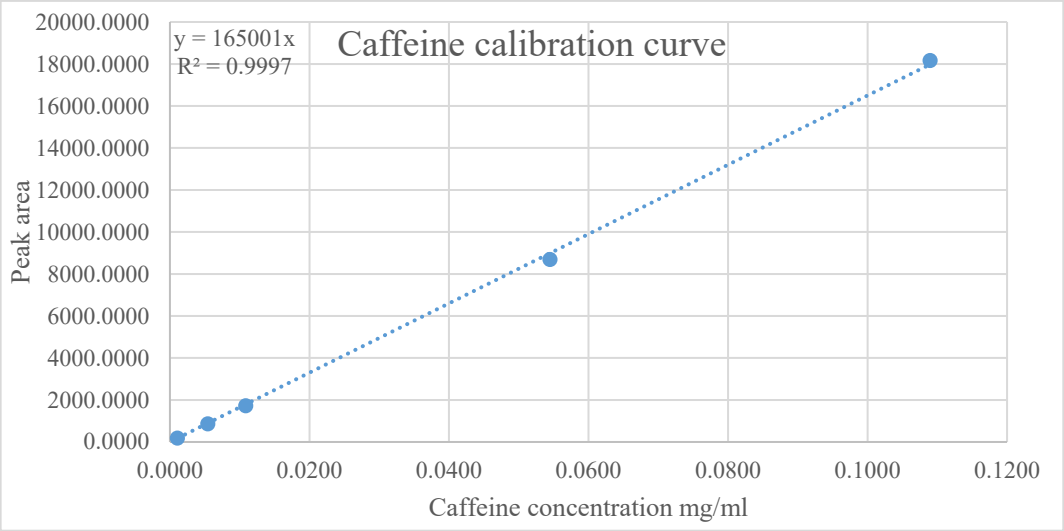

Figure 11 - Caffeine calibration curve.

Chlorogenic acid standard was prepared in concentrations of 150; 200; 250 and 500 mg/l. Similarly to caffeine calibration, the curve for CGA is shown in Figure 11.

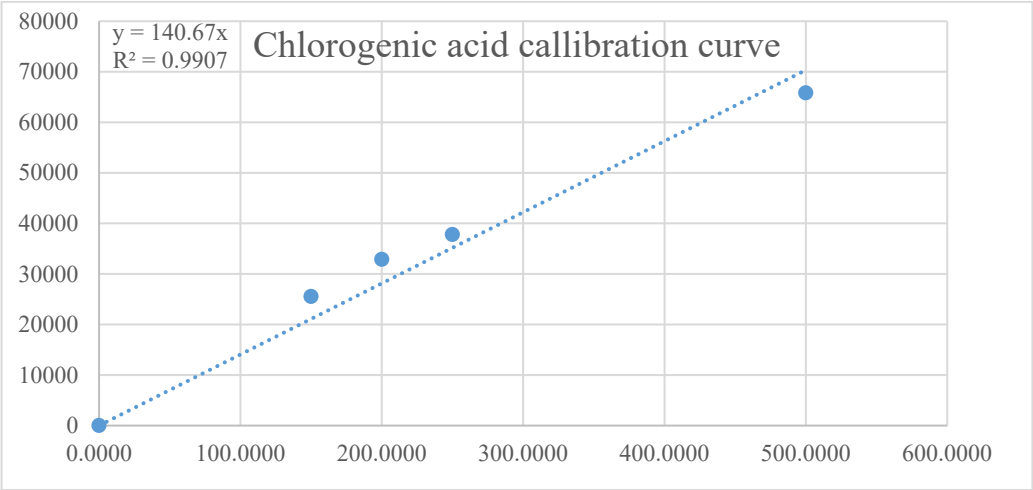

Figure 12 - Chlorogenic acid calibration curve.

3.7. Statistical analysis

The statistical analysis was performed using R language system Jamovi. Independent samples T-test was used for comparison of the difference in coffee's origin. One-way ANOVA was utilized to explore effect of method on measured variables. And MANCOVA was performed to showcase interactions of these variables.

4. Results

In total, 111 samples were tested for pH, caffeine and CGA content. Table 3 below displays all measured values. These values were further statistically analyzed.

Table 3 - Raw results (Source: Author)

Brewing method	Variant	Coffee sample	Water type	Sample 1			Sample 2		
				pH	caffeine mg/100 ml	CGA mg/100 ml	pH	caffeine mg/100ml	CGA mg/100 ml
AeroPress	30 s	Ethiopia	Distilled	5.01	35.43	164.96	4.96	36.05	167.22
			Tap	4.99	36.28	160.85	4.94	36.64	172.65
		Nicaragua	Distilled	4.94	43.91	174.49	4.92	44.11	176.45
			Tap	4.79	44.90	166.12	4.79	44.72	164.22
	120 s	Ethiopia	Distilled	4.96	40.68	190.85	5.02	36.46	177.67
			Tap	4.91	37.03	173.37			
		Nicaragua	Distilled	4.94	46.24	174.30	4.91	45.39	171.08
			Tap	4.83	48.22	178.86	4.78	47.09	178.03
	180 s	Ethiopia	Distilled	5.10	39.34	181.88	5.02	39.62	181.40
		Nicaragua	Distilled	4.96	47.09	178.00	4.91	49.43	184.28
Cezves		Ethiopia	Distilled	5.00	41.93	197.92	5.04	40.99	191.42
			Tap	4.99	39.57	163.04	5.03	38.49	183.32
		Nicaragua	Distilled	4.92	53.21	194.76	4.93	50.28	171.97
			Tap	4.83	49.52	182.30	4.83	47.97	182.14
Espresso machine		Ethiopia	Distilled	4.97	35.93	165.59	5.03	37.85	167.64
			Tap	5.08	34.01	155.73	5.02	34.72	158.39
		Nicaragua	Distilled	4.89	47.64	166.94	4.87	47.59	161.03
			Tap	5.05	37.70	143.42	4.92	47.46	179.37
Pour-over (V60)	96–98 °C	Ethiopia	Distilled	4.95	42.07	192.18	4.93	45.52	204.92
			Tap	4.91	42.06	194.22	5.01	37.11	180.38
		Nicaragua	Distilled	4.94	34.49	132.68	4.92	51.55	191.12
			Tap	4.86	41.37	125.21	4.81	44.62	164.01
	88–90 °C	Ethiopia	Distilled	5.05	43.80	200.25	5.02	43.25	199.40
			Tap	5.11	40.64	189.17	5.09	41.48	185.89
		Nicaragua	Distilled	4.90	57.32	193.65	4.90	56.90	202.00
			Tap	4.97	54.03	190.43	4.90	51.48	184.14
	78–80 °C	Ethiopia	Distilled	5.02	39.21	185.19	5.01	40.87	188.68
			Tap	5.16	51.12	223.58	5.11	39.73	185.28
		Nicaragua	Distilled	4.93	54.51	203.92	4.93	46.72	173.53
			Tap	4.97	45.83	164.60	5.07	35.22	170.77
French press	180 s	Ethiopia	Distilled	5.02	38.35	190.95	4.99	40.95	191.18
			Tap	5.05	35.84	179.94	4.90	42.51	199.76
		Nicaragua	Distilled	5.01	47.66	167.12	4.99	50.07	190.27
			Tap	4.88	45.79	170.07	4.87	44.91	166.66
	300 s	Ethiopia	Distilled	5.02	37.43	191.72	4.98	52.65	198.20
			Tap	5.03	35.84	179.94	5.01	35.98	180.97
		Nicaragua	Distilled	5.01	51.49	190.26	5.02	48.85	182.21
			Tap	4.85	41.90	141.16	4.82	41.41	140.34
	600 s	Ethiopia	Distilled	5.04	37.27	176.31	5.06	37.56	177.17
			Tap	5.02	35.15	182.57	4.99	35.44	178.72
		Nicaragua	Distilled	4.97	51.80	203.31	4.96	51.30	177.71
			Tap	4.85	46.74	167.47	4.83	46.22	190.12
Nespresso pods		Ethiopia	Distilled	5.03	39.35	173.14	5.00	31.39	150.73
			Tap	5.07	39.92	183.95	5.07	38.99	186.19
		Nicaragua	Distilled	4.93	34.63	127.73	4.92	40.95	153.08
			Tap	4.97	36.75	118.41	4.98	64.35	217.84
Moka pot		Ethiopia	Distilled	4.91	45.07	204.81	4.99	45.40	210.04
			Tap	4.99	66.84	258.67	5.00	53.14	227.27
		Nicaragua	Distilled	4.88	59.48	201.36	4.87	46.15	170.86
			Tap	4.82	60.09	209.38	4.72	61.99	202.26
		Ethiopia	Distilled	4.99	51.35	219.97	5.00	53.17	221.26
			Tap	4.82	70.40	273.96	4.84	48.67	217.81
		Nicaragua	Distilled	4.86	71.42	228.94	4.90	63.17	216.86
			Tap	4.73	75.32	230.69	4.83	65.87	211.32

4.1. Particle analysis

Table 3 shows the mean particle size distribution between the coffee samples. With Dx (50) describing the median value. Whereas Dx (10) means that 10 % of particles were smaller than the measured value and Dx (90) stating that 10 % of particles were larger than the measured value. Figure 10 shows the graph comparison of these distributions.

Table 4 - Particle size distribution (µm)

Coffee sample	Dx (10)	Dx (50)	Dx (90)
Ethiopia	311.83	658.53	1154.33
Nicaragua	292.23	642.87	1129.70

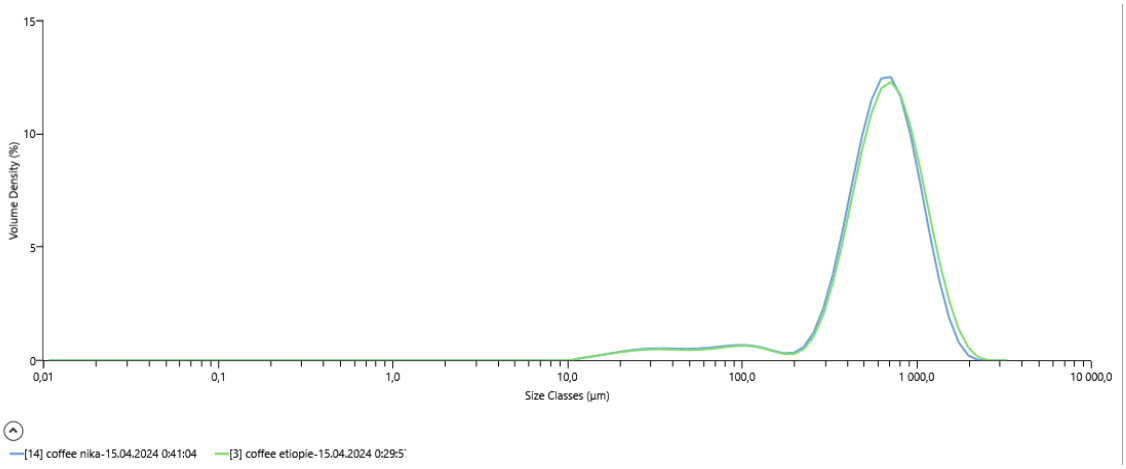

Figure 13 - Comparison of Particle sizes between coffee samples ("[14] coffee nika" - Nicaragua sample; "[3] coffee etiopie" - Ethiopia sample).

4.2. Effect of preparation method

The brewing method had significant impact on the pH, caffeine and chlorogenic acid content. The mean values for pH ranged from 4.92 (Cezves) to 5.0 (Nespresso). Caffeine content showed mean values from 40.36 mg/100 ml (Espresso) to 55.34 mg/100 ml (Moka). CGA content had a range of mean values of 162.26 mg/100 ml (Espresso) to 212.23 mg/100 ml (Moka).

Table 5 - Group descriptives of brewing methods

Group Descriptives

	method	N	Mean	SD	SE
pH	Moka	20	4.89	0.0943	0.0211
	Aero	21	4.93	0.0813	0.0177
	Cezv	7	4.92	0.0749	0.0283
	V60	24	4.98	0.0878	0.0179
	Nesp	8	5.00	0.0576	0.0203
	French	23	4.97	0.0763	0.0159
	Esp	8	4.98	0.0783	0.0277
caffeine (mg/100 ml)	Moka	20	55.34	11.0315	2.4667
	Aero	21	41.59	4.7330	1.0328
	Cezv	7	46.91	5.6216	2.1247
	V60	24	45.04	6.6095	1.3492
	Nesp	8	40.79	10.0271	3.5451
	French	23	43.07	6.2039	1.2936
	Esp	8	40.36	6.1040	2.1581
CGA (mg/100 ml)	Moka	20	212.23	26.8157	5.9962
	Aero	21	174.69	7.2458	1.5812
	Cezv	7	180.08	10.0011	3.7800
	V60	24	184.38	21.6677	4.4229
	Nesp	8	163.88	32.8674	11.6204
	French	23	178.89	15.4674	3.2252
	Esp	8	162.26	10.4797	3.7051

Effect of brewing method on pH. The only statistical evidence observed was between the Moka pot and Nespresso coffee (Table 7).

Table 6 - Effect of brewing method on pH

		Moka	Aero	Cezv	V60	Nesp	French	Esp
Moka	Mean difference	—	-0.0341	-0.02407	-0.0834	-0.1018 *	-0.07376	-0.0842
	p-value	—	0.875	0.992	0.062	0.031	0.105	0.253
Aero	Mean difference		—	0.01000	-0.0493	-0.0677	-0.03969	-0.0502
	p-value		—	1.000	0.456	0.214	0.642	0.726
Cezv	Mean difference			—	-0.0593	-0.0777	-0.04969	-0.0602
	p-value			—	0.589	0.353	0.724	0.730
V60	Mean difference				—	-0.0183	0.00966	-8.33e−4
	p-value				—	0.9t92	1.000	1.000
Nesp	Mean difference					—	0.02799	0.0175
	p-value					—	0.924	0.998
French	Mean difference						—	-0.0105
	p-value						—	1.000
Esp	Mean difference							—
	p-value							—

Note. * p < .05, ** p < .01, *** p < .001

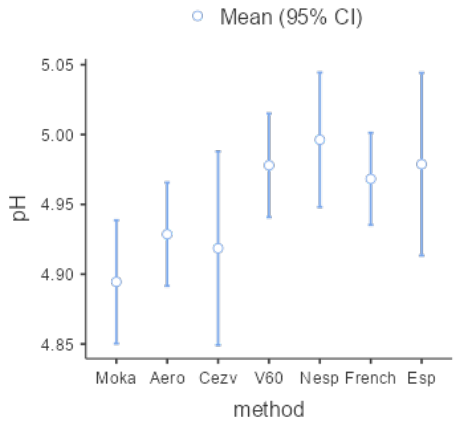

Figure 14 - Boxplot of brewing method on pH.

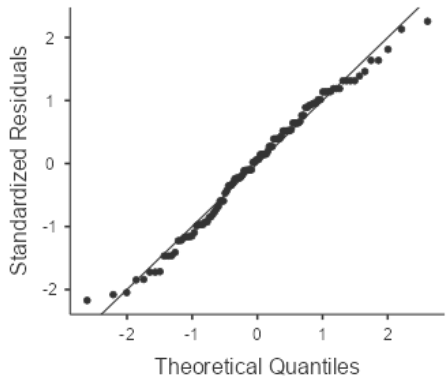

Figure 15 - Quantiles of pH.

Effect of brewing method on caffeine content. The lowest recorded caffeine content was present in Nespresso 31.39 mg/100 ml and the highest content was present in Moka 75.32 mg/100 ml. The Moka pot had statistically higher extraction of caffeine than AeroPress, Filtered – V60, French Press and Espresso (values in Table 8, which are highlighted by *).

Table 7 - Effect of brewing method on caffeine

Games-Howell Post-Hoc Test – caffeine (mg/100 ml)

		Moka	Aero	Cezv	V60	Nesp	French	Esp
Moka	Mean difference	—	13.8 ***	8.43	10.31 *	14.553	12.27 **	14.982 **
	p-value	—	< .001	0.179	0.015	0.054	0.002	0.002
Aero	Mean difference		—	-5.32	-3.45	0.800	-1.48	1.228
	p-value		—	0.356	0.413	1.000	0.972	0.998
Cezv	Mean difference			—	1.87	6.119	3.84	6.548
	p-value			—	0.986	0.751	0.716	0.375
V60	Mean difference				—	4.246	1.97	4.675
	p-value				—	0.907	0.938	0.549
Nesp	Mean difference					—	-2.28	0.429
	p-value					—	0.995	1.000
French	Mean difference						—	2.707
	p-value						—	0.924
Esp	Mean difference							—
	p-value							—

Note. * p < .05, ** p < .01, *** p < .001

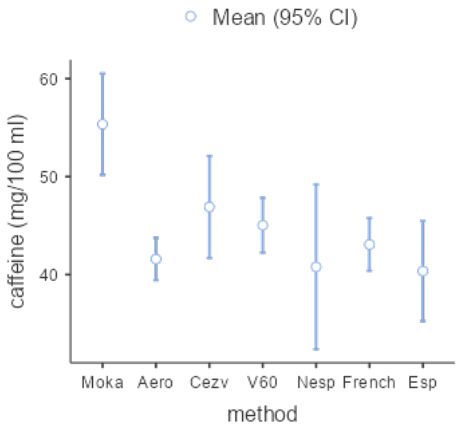

Figure 16 - Boxplots of caffeine/method.

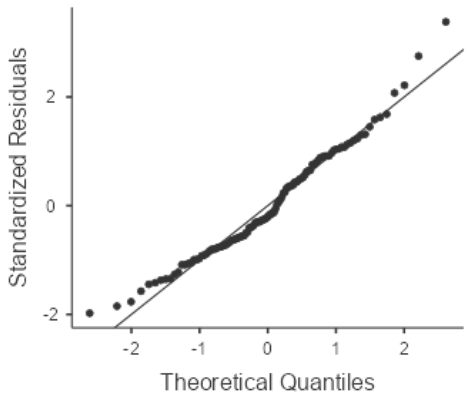

Figure 17 - Quantiles of caffeine.

Effect of brewing method on CGA content. The lowest recorded CGA content was present in again in Nespresso 118.42 mg/100 ml, while the highest content also in Moka 273.96 mg/100 ml. The Moka pot showed statistically significant higher CGA content compared to all the other methods. Moreover, Espresso showed significantly lower CGA content compared to French press and Filtered coffee – V60 (Table 9).

Table 8 - Effect of brewing method on CGA content

Games-Howell Post-Hoc Test – CGA (mg/100 ml)

		Moka	Aero	Cezv	V60	Nesp	French	Esp
Moka	Mean difference	—	37.5 ***	32.15 **	27.85 *	48.3 *	33.35 ***	49.97 ** *
	p-value	—	< .001	0.002	0.010	0.040	< .001	< .001
Aero	Mean difference		—	-5.39	-9.69	10.8	-4.19	12.43
	p-value		—	0.829	0.400	0.957	0.901	0.114
Cezv	Mean difference			—	-4.30	16.2	1.20	17.82
	p-value			—	0.988	0.824	1.000	0.059
V60	Mean difference				—	20.5	5.50	22.12 *
	p-value				—	0.660	0.950	0.011
Nesp	Mean difference					—	-15.00	1.62
	p-value					—	0.859	1.000
French	Mean difference						—	16.62 *
	p-value						—	0.042
Esp	Mean difference							—
	p-value							—

Note. * p < .05, ** p < .01, *** p < .001

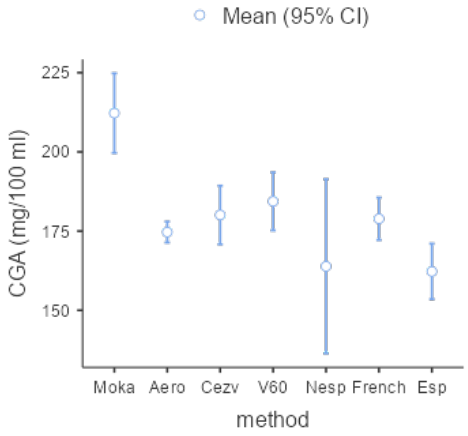

Figure 18 - Boxplot of brewing method/CGA content.

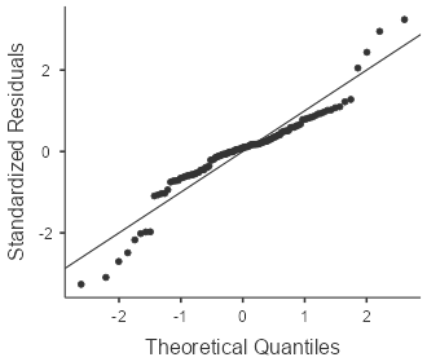

Figure 19 - Quantiles of CGA content.

Interactions of parameters. The interactions between non-dependent parameters were assessed using MANCOVA. Singular analysis confirmed that the type of coffee and preparation method influence caffeine and CGA content. It has also shown that the type of water has significant effect only on pH, which was to be expected. No interactions of these variables had effect on caffeine or CGA content, which were the observed attributes.

Univariate Tests

	Dependent Variable	Sum of Squares	df	Mean Square	F	p
water	caffeine (mg/100 ml)	0.87772	1	0.87772	0.0255	0.873
	CGA (mg/100 ml)	175.44369	1	175.44369	0.5224	0.472
	pH	0.03571	1	0.03571	15.5253	< .001
coffee	caffeine (mg/100 ml)	1875.17057	1	1875.17057	54.5073	< .001
	CGA (mg/100 ml)	3375.11809	1	3375.11809	10.0502	0.002
	pH	0.29346	1	0.29346	127.5877	< .001
method	caffeine (mg/100 ml)	2988.86328	6	498.14388	14.4800	< .001
	CGA (mg/100 ml)	24779.10942	6	4129.85157	12.2976	< .001
	pH	0.14462	6	0.02410	10.4796	< .001
water ✻ coffee	caffeine (mg/100 ml)	64.92290	1	64.92290	1.8872	0.173
	CGA (mg/100 ml)	1020.88997	1	1020.88997	3.0400	0.085
	pH	0.02058	1	0.02058	8.9470	0.004
water ✻ method	caffeine (mg/100 ml)	518.40892	6	86.40149	2.5115	0.028
	CGA (mg/100 ml)	3940.15866	6	656.69311	1.9555	0.081
	pH	0.12637	6	0.02106	9.1569	< .001
coffee ✻ method	caffeine (mg/100 ml)	94.77115	6	15.79519	0.4591	0.837
	CGA (mg/100 ml)	2513.48848	6	418.91475	1.2474	0.291
	pH	0.00249	6	4.16e-4	0.1808	0.981
water ✻ coffee ✻ method	caffeine (mg/100 ml)	131.73212	5	26.34642	0.7658	0.577
	CGA (mg/100 ml)	1087.94840	5	217.58968	0.6479	0.664
	pH	0.01800	5	0.00360	1.5653	0.179
Residuals	caffeine (mg/100 ml)	2889.78164	84	34.40216		
	CGA (mg/100 ml)	28209.25924	84	335.82451		
	pH	0.19320	84	0.00230		

4.3. Coffee sample differences

Independent Samples T-Test (Table 5) was performed to establish the difference between the Ethiopia and Nicaragua coffee samples. There have been found significant differences in the pH, caffeine and CGA content in these groups. Table 4 showcases main descriptives.

Table 9 - Group descriptives of coffee samples

	Group	N	Mean	Median	SD	SE
pH	Ethiopia	54	5.00	5.01	0.0655	0.00891
	Nicaragua	57	4.90	4.90	0.0750	0.00994
caffeine (mg/100 ml)	Ethiopia	54	41.09	39.34	7.2375	0.98490
	Nicaragua	57	49.31	47.64	8.3587	1.10713
CGA (mg/100 ml)	Ethiopia	54	188.74	184.57	23.0778	3.14049
	Nicaragua	57	177.73	178.00	24.4632	3.24023

Table 10 - Independent Samples T-Test

		Statistic	df	p
pH	Student's t	7.65	109	< .001
caffeine (mg/100 ml)	Student's t	-5.53	109	< .001
CGA (mg/100 ml)	Student's t	2.44	109	0.016

Note. H_a $\mu_{Ethiopia} \neq \mu_{Nicaragua}$

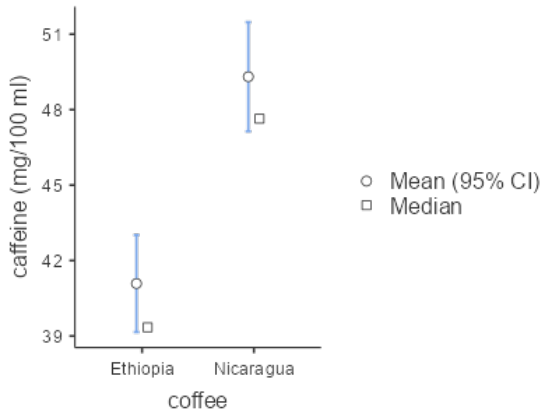

Figure 21 - Boxplot of caffeine content based on coffee type.

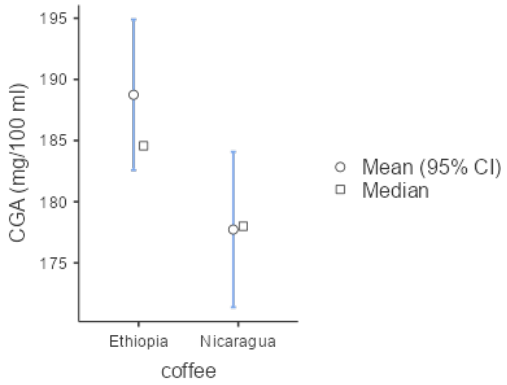

Figure 22 - Boxplot of CGA content based on coffee type.

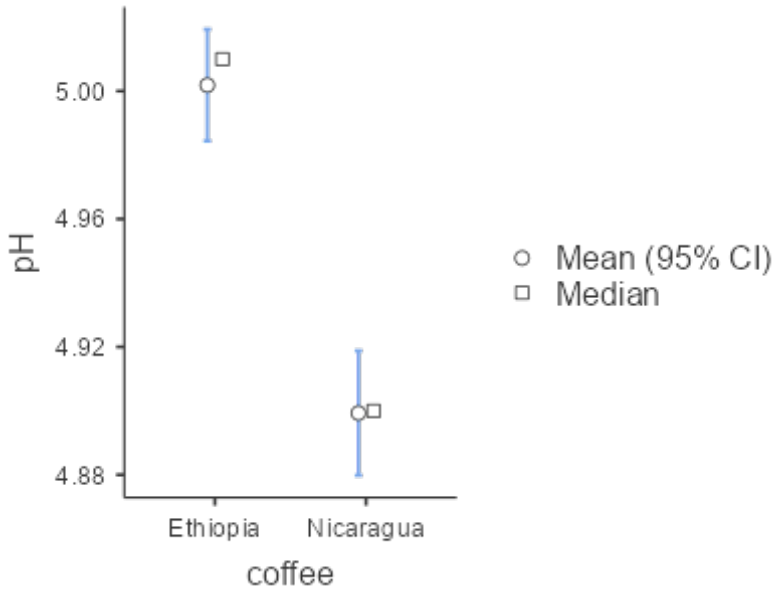

Figure 20 - Boxplot of pH based on coffee type.

5. Discussion

Although, Fuller & Rao suggest that the grind setting does not have an influence on equilibrium concentrations of CGA and caffeine in long-term extractions. They highlight that with the increase of grind size the extraction time increases (Fuller & Rao 2017). As none of the brewing methods used in this research are of a long-term characteristic (f.e. cold brew) a particle analysis was necessary to show that the data of different coffee samples is comparable. The median value of particle size for Ethiopia sample was 658.53 μm and 642.87 μm for Nicaragua sample. Despite this slight variance, both samples exhibited very similar grind and distribution profile. It should be noted that under real life circumstances the same grind setting would not be used for different brewing methods, as it would have negative effect on organoleptic properties (VOILLEY et al. 1981). This might show the results of the thesis as not reflective of everyday life. Nevertheless, it was necessary to remove all unwanted variables, that might influence the CGA and caffeine extraction, other than the brewing method. As these effects show to have detrimental effect on the extraction yield (Wang & Lim 2023).

The only statistically proven difference in pH was between Moka and Nespresso samples. As the Nespresso pods were reported to show water channelling and therefore bad extraction (Schmieder et al. 2023). As is supported by the non-normality of the Nespresso values. Therefore, it can be assumed, that the brewing method had no effect on the pH of the final beverage. When evaluating the pH, Ethiopian coffee sample had values ranging from 4.82 to 5.16. While the Nicaraguan coffee was more acidic with pH values of 4.72 to 5.07. These values are comparable to other research. Bobková et al. reported values of pH in *C. arabica* beverages ranging from 4.60 to 5.35. Being consistent with other studies, which reported values of 4.95 to 5.99 (Fujioka & Shibamoto 2008; Moon et al. 2009). Obviously, the type of water used (distilled or tap) showed to have an effect on the final pH values, as water is the main component of coffee beverage and its original pH will affect the final values.

The measured caffeine content ranged from 31.4 mg/100 ml in Nespresso to 75.3 mg/100 ml in Moka. This range is similar with the previous experiments performed (Gawlik et al. 2022) and other research (Gloess et al. 2013; Bobková et al. 2021). In my

previous bachelor's thesis, I recorded values of caffeine ranging from 35.22 to 66.72 mg/100 ml. Bobková et al. measured caffeine content for several types of *C. arabica*, using a similar ratio to this thesis (7 g of coffee to 120 ml of water). Their recorded values ranged from 1.37 to 1.78 %, these values are similar compared to this research. Values measured in this research show no health concern, as they are below the guideline set by EFSA, which suggests that pregnant women should consume < 200 mg of caffeine per day, keeping in mind they would consume a 250 ml beverage daily (EFSA Panel on Dietetic Products, Nutrition and Allergies 2015b). However, it should be noted that commercial preparations, such as espresso, pose a higher risk. Crozier et al. measured 20 espresso beverages obtained from different café shops and reported caffeine concentrations ranging from 51 to 322 mg in a cup. Although, this research utilized a lever espresso machine, the coffee to water ratio, pressure and extraction kinetics were much different (6 g to 100 ml) compared to a commercial setup (Crozier et al. 2012; Schmieder et al. 2023). The values for Espresso (34–47.6 mg/100 ml) and Nespresso (31.4–64.3 mg/100 ml) were unexpectedly lower than anticipated, considering these brewing methods are commonly associated with having high extraction rates (Severini et al. 2017). This is due to the choice of the experiment, as these methods should use smaller grind size and higher coffee to water ratio to be effective. The large variability in recorded concentrations is due to water channelling. This phenomenon happens in the coffee bed when the grind setting, amount of coffee and its distribution are not ideal and reduces the amount of coffee being exposed to the pressurized water (Schmieder et al. 2023). Under these parameters, the machines do not reflect the professional setting, as it is very difficult to obtain a quality espresso cup (Petracco 2008). As mentioned, Moka pot showed the highest caffeine extraction and was proven to be statistically significant compared to all other methods. This corresponds to previous experiments (Gawlik et al. 2022) and other research (Bobková et al. 2021). No other methods showed a statistically significant difference in caffeine extraction compared to each other, which is contrary to a previous experiment (Gawlik et al. 2022). That is due to robustness of this research (111 samples), whereas the 2022 experiment measured only 24 different samples and one type of coffee. Moreover, the coffee in the previous experiment was ground using a hand grinder, possibly yielding different results, as particle analysis was not performed.

CGA content ranged from 118 mg/100 ml in Nespresso to 274 mg/100 ml in Moka. These values are in accordance with other authors. For example, Bobková et al. stated that CGA content in a cup of coffee ranges from 15–325 mg, although she does not specify the volume of such cup. While Vollmanová et al. suggest concentrations per cup of 70–350 mg, not stating the mass of coffee used (Bobková et al. 2021; Vollmanová et al. 2022). It is hard to determine what is a cup of coffee, as not all beverages are consumed in the same volume. Although, the higher extraction rates in this research could be attributed to the roasting degree of coffee samples, as light-medium roast beans were used. Blumberg et al. reports that increased roasting temperatures and time lead to degradation of CGA precursors and lower their final extraction into the beverage (Blumberg et al. 2010; Król et al. 2020). The statistical analysis showed notably higher CGA content in Moka compared to all the other methods. Filtered coffee (V60) and French Press had also significantly greater CGA extraction compared to Espresso machine. This finding contradicts conclusions made by Bobková et al. (2021), which states that the content of CGA is not statistically dependent on beverage preparation method, albeit only Moka and French press were tested in their research. Nevertheless, they observed that "generally, samples prepared by moka method showed both higher content of caffeine and chlorogenic acid" (Bobková et al. 2021).

While the health benefits of coffee have been long known, it has a been a part of scientific discourse to which compounds we can attribute these effects (Ludwig et al. 2014; Preedy 2014b; Poole et al. 2017). New studies explore the possibility that chlorogenic acids could be responsible for some of the beneficial health effects, such as their antioxidant and anti-inflammatory properties; therapeutic effect on the treatment of hyperlipidemia and diabetes; neuroprotective, immunoprotective and anti-mutagenic effects; ability to promote cardiovascular health and treat hypertension (Li & Chang 2005; Tajik et al. 2017; Farah & de Paula Lima 2019). Moreover, it has been shown that the variety, growing conditions and processing of coffee has a detrimental effect on the final CGA content (Perrone et al. 2008; Sherge et al. 2016; Fuller & Rao 2017). With coffee being the biggest source of CGA in human diet (Clifford 2000; Manach et al. 2004), it is important to better understand the mechanisms of CGA extraction, in order to fully leverage these health benefits.

Interestingly, there was no statistically significant difference of caffeine and CGA extraction based on time in French press (3, 5 & 10 min) and temperature of water in Filtered coffee – V60 (80, 90 & 100 °C). Fuller & Rao also reported, that temperature of brew (hot & cold) had no effect on CGA and caffeine content. Moreover, Várady et al. observed little effects of brew temperature in drip coffee on total dissolved solids (Várady et al. 2022b). As CGA is freely soluble in water at room temperature (Budavari et al. 1996). While there are no measured kinetics of CGA extraction in hot water. The extraction of CGA in cold water is known to increase rapidly in the first 180 minutes and reach an equilibrium at approximately 400 minutes (Fuller & Rao 2017). Possibly suggesting that CGA solulability in hot water, at this grind setting, is fairly quick from the perspective coffee brewing. Moreover, the use of brewing water (tap & distilled) had no effect on CGA and caffeine extraction. Fibrianto et al. also reported that "taste and flavour attributes were not affected by the pH of brewing water within the range of 5.5 to 9.1" (Fibrianto et al. 2018).

6. Conclusions

Coffee is considered as one of the most popular beverages in the world and it is prepared using many brewing methods. It is important to highlight that these methods are crucial in the properties of the final product and may affect it in multitude of effects. This research focused on quantitative analysis of CGA content, caffeine content and pH in twelve brewing methods and their variances. Based on obtained results it can be said that pH values did not show significant differences between brewing methods. Using ANOVA single factor, we found out that CGA and caffeine content were both significantly different in analyzed samples. Especially Moka pot showing higher exactions of both compounds comparing to other methods. It has also been found that under these test conditions Espresso and Nespresso pod coffee showed great variance in results, due to abovementioned water channelling, displaying results of these brewing methods as unreliable. Although there were no statistical differences in CGA and caffeine contents in methods except Moka pot. Some certain variances were observed and show potential for further examinations on coffee brewing methods.

Reviewing results and other research, it can be concluded that other variables affect CGA and caffeine extraction more than brewing techniques. As all caffeine levels measured in this work are in accordance with safety guidelines for pregnant and lactating women. Thus, the choice of homebrewing technique poses no significant health risk. Nevertheless, further research on caffeine extraction should be concluded, especially focusing on other variables. As excessive caffeine consumption could have potentially adverse health effects. Although moderate coffee consumption has many positive health benefits, some of which are linked to chlorogenic acids. Gaining a better understanding of these and other constituents in coffee, as well as their extraction into the beverage, should enhance comprehension of the health benefits associated with coffee.

To conclude, it is important to understand that each cup of coffee is different in composition and size. Furthermore, individual sensitivity plays a great role in metabolization of active compounds in coffee. It is therefore necessary to raise awareness in general public that coffee consumption and its impact on health is highly individual.

7. References

Ahrens MJ, Thompson DL. 2013. Effect of emulin on blood glucose in type 2 diabetics. Journal of Medicinal Food **16**:211–215.

Akbar A, Medina A, Magan N. 2016. Efficacy of different caffeine concentrations on growth and ochratoxin A production by *Aspergillus* species. Letters in Applied Microbiology **63**:25–29.

Arendash GW et al. 2009a. Caffeine Reverses Cognitive Impairment and Decreases Brain Amyloid-β Levels in Aged Alzheimer's Disease Mice. Journal of Alzheimer's Disease **17**:661–680.

Arendash GW et al. 2009b. Caffeine Reverses Cognitive Impairment and Decreases Brain Amyloid-β Levels in Aged Alzheimer's Disease Mice. Journal of Alzheimer's Disease **17**:661–680. IOS Press.

Ashihara H. 2004. Distribution and biosynthesis of caffeine in plants. Frontiers in Bioscience **9**:1864.

Ashihara H, Crozier A. 1999. Biosynthesis and Metabolism of Caffeine and Related Purine Alkaloids in Plants. Pages 117–205 Advances in Botanical Research. Elsevier. Available from https://linkinghub.elsevier.com/retrieve/pii/S0065229608602281 (accessed March 5, 2022).

Ashihara H, Sano H, Crozier A. 2008. Caffeine and related purine alkaloids: Biosynthesis, catabolism, function and genetic engineering. Phytochemistry **69**:841–856.

Bagdas D, Cinkilic N, Ozboluk HY, Ozyigit MO, Gurun MS. 2013. Antihyperalgesic activity of chlorogenic acid in experimental neuropathic pain. Journal of Natural Medicines **67**:698–704.

Bakuradze T, Lang R, Hofmann T, Eisenbrand G, Schipp D, Galan J, Richling E. 2015. Consumption of a dark roast coffee decreases the level of spontaneous DNA strand breaks: a randomized controlled trial. European Journal of Nutrition **54**:149–156.

Bastian F, Hutabarat OS, Dirpan A, Nainu F, Harapan H, Emran TB, Simal-Gandara J. 2021. From Plantation to Cup: Changes in Bioactive Compounds during Coffee Processing. Foods **10**:2827. Multidisciplinary Digital Publishing Institute.

Bawin Y, Ruttink T, Staelens A, Haegeman A, Stoffelen P, Mwanga Mwanga JI, Roldán-Ruiz I, Honnay O, Janssens SB. 2021. Phylogenomic analysis clarifies the evolutionary origin of *Coffea arabica*. Journal of Systematics and Evolution **59**:953–963.

Berman NK, Honig S, Cronstein BN, Pillinger MH. 2022a. The effects of caffeine on bone mineral density and fracture risk. Osteoporosis InternationalDOI: 10.1007/s00198-021-05972-w. Available from https://doi.org/10.1007/s00198-021-05972-w (accessed March 7, 2022).

Berman NK, Honig S, Cronstein BN, Pillinger MH. 2022b. The effects of caffeine on bone mineral density and fracture risk. Osteoporosis InternationalDOI: 10.1007/s00198-021-05972-w. Available from https://doi.org/10.1007/s00198-021-05972-w (accessed March 7, 2022).

Bizzo MLG, Farah A, Kemp JA, Scancetti LB. 2015. Highlights in the History of Coffee Science Related to Health. Pages 11–17 Coffee in Health and Disease Prevention.

Blumberg S, Frank O, Hofmann T. 2010. Quantitative Studies on the Influence of the Bean Roasting Parameters and Hot Water Percolation on the Concentrations of Bitter Compounds in Coffee Brew. Journal of Agricultural and Food Chemistry **58**:3720–3728. American Chemical Society.

Bobková A, Jakabová S, Belej A, Jurčaga L, Aapla J, Bobko M, Demianová A. 2021. Analysis of caffeine and chlorogenic acids content regarding the preparation method of coffee beverage. International Journal of Food Engineering **17**:403–410.

Bolka M, Emire S. 2020. Effects of coffee roasting technologies on cup quality and bioactive compounds of specialty coffee beans. Food Science & Nutrition **8**:6120–6130.

Brando CHJ. 2004. Harvesting and Green Coffee Processing. Pages 604–715 Coffee: Growing, Processing, Sustainable Production. John Wiley & Sons, Ltd. Available from http://onlinelibrary.wiley.com/doi/abs/10.1002/9783527619627.ch24 (accessed February 22, 2022).

Budavari S, O'Neil M, Smith A, Heckelman P, Obenchain J. 1996. The Merck Index, Print Version, Twelfth Edition12th edition. CRC Press, Whitehouse Station, NJ.

Bytof G, Knopp S-E, Schieberle P, Teutsch I, Selmar D. 2005. Influence of processing on the generation of γ-aminobutyric acid in green coffee beans. European Food Research and Technology **220**:245–250.

Byun S-Y, Kwon S-H, Heo S-H, Shim J-S, Du M-H, Na J-I. 2015. Efficacy of Slimming Cream Containing 3.5% Water-Soluble Caffeine and Xanthenes for the Treatment of Cellulite: Clinical Study and Literature Review. Annals of Dermatology **27**:243.

Charrier A, Berthaud J. 1985. Botanical Classification of Coffee. Coffee: botany, biochemistry, and production of beans and beverage. New YorkDOI: 10.1007/978-1-4615-6657-1_2.

Checler F. 1995. Processing of the β-Amyloid Precursor Protein and Its Regulation in Alzheimer's Disease. Journal of Neurochemistry **65**:1431–1444.

Cibelli M, Cimini A, Cerchiara G, Moresi M. 2021. Carbon footprint of different methods of coffee preparation. Sustainable Production and Consumption **27**:1614–1625.

Clifford MN. 1999. Chlorogenic acids and other cinnamates - Nature, occurrence and dietary burden. Journal of the Science of Food and Agriculture **79**:362–372.

Clifford MN. 2000. Chlorogenic acids and other cinnamates – nature, occurrence, dietary burden, absorption and metabolism. Journal of the Science of Food and Agriculture **80**:1033–1043.

Clifford MN, Johnston KL, Knight S, Kuhnert N. 2003. Hierarchical Scheme for LC-MSn Identification of Chlorogenic Acids. Journal of Agricultural and Food Chemistry **51**:2900–2911. American Chemical Society.

Clifford MN, Ramirez-Martinez JR. 1991. Phenols and caffeine in wet-processed coffee beans and coffee pulp. Food Chemistry **40**:35–42.

Cooper C, Atkinson EJ, Wahner HW, O'Fallon WM, Riggs BL, Judd HL, Melton LJ. 2009. Is caffeine consumption a risk factor for osteoporosis? Journal of Bone and Mineral Research **7**:465–471.

Crozier A, Ashihara H, Toms-Barbran F, Tomás-Barbéran F. 2011. Teas, Cocoa and Coffee: Plant Secondary Metabolites and Health. John Wiley & Sons, Incorporated, Hoboken, UNITED KINGDOM. Available from

http://ebookcentral.proquest.com/lib/czup/detail.action?docID=819329 (accessed March 7, 2022).
Crozier TWM, Stalmach A, Lean MEJ, Crozier A. 2012. Espresso coffees, caffeine and chlorogenic acid intake: potential health implications. Food Funct. **3**:30–33.
Cwiková O, Komprda T, Šottníková V, Svoboda Z, Simonová J, Slováček J, Jůzl M. 2022. Effects of Different Processing Methods of Coffee Arabica on Colour, Acrylamide, Caffeine, Chlorogenic Acid, and Polyphenol Content. Foods **11**.
Dauer W, Przedborski S. 2003. Parkinson's Disease: Mechanisms and Models. Neuron **39**:889–909. Elsevier.
Davids K. 1996. Home Coffee Roasting, Revised, Updated Edition: Romance and Revival. Available from https://www.scribd.com/book/190494280/Home-Coffee-Roasting-Revised-Updated-Edition-Romance-and-Revival (accessed April 6, 2022).
Davis AP, Chadburn H, Moat J, O'Sullivan R, Hargreaves S, Lughadha EN. 2019. High extinction risk for wild coffee species and implications for coffee sector sustainability. Science Advances **5**.
Davis AP, Govaerts R, Bridson DM, Stoffelen P. 2006. An annotated taxonomic conspectus of the genus Coffea (Rubiaceae). Botanical Journal of the Linnean Society **152**:465–512.
Davis AP, Rakotonasolo F. 2021. Six new species of coffee (Coffea) from northern Madagascar. Kew Bulletin **76**:497–511.
de Figueiredo Tavares MP, Mourad AL. 2020. Coffee beverage preparation by different methods from an environmental perspective. The International Journal of Life Cycle Assessment **25**:1356–1367.
Depaula J, Farah A. 2019a. Caffeine consumption through coffee: Content in the beverage, metabolism, health benefits and risks. Beverages **5**.
Depaula J, Farah A. 2019b. Caffeine consumption through coffee: Content in the beverage, metabolism, health benefits and risks. Beverages **5**.
Di Paola R, Esposito E, Mazzon E, Caminiti R, Toso RD, Pressi G, Cozzocrea S. 2010. 3,5-Dicaffeoyl-4-malonylquinic acid reduced oxidative stress and inflammation in a experimental model of inflammatory bowel disease. Free Radical Research **44**:74–89.
Dos Santos MD, Almeida MC, Lopes NP, De Souza GEP. 2006. Evaluation of the anti-inflammatory, analgesic and antipyretic activities of the natural polyphenol chlorogenic acid. Biological and Pharmaceutical Bulletin **29**:2236–2240.
Duarte GS, Pereira AA, Farah A. 2010. Chlorogenic acids and other relevant compounds in Brazilian coffees processed by semi-dry and wet post-harvesting methods. Food Chemistry **118**:851–855.
Dutra ER, Oliveira L, Franca A, Ferraz V, Afonso R. 2001. A preliminary study on the feasibility of using composition of coffee roasting exhaust gas for the determination of the degree of roast. Journal of Food Engineering **47**:241–246.
EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). 2015a. Scientific Opinion on the safety of caffeine. EFSA Journal **13**:4102.
EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). 2015b. Scientific Opinion on the safety of caffeine. EFSA Journal **13**:4102.
Eiermann A, Smrke S, Guélat L-M, Wellinger M, Rahn A, Yeretzian C. 2020. Extraction of single serve coffee capsules: linking properties of ground coffee to extraction dynamics and cup quality. Scientific Reports **10**:17079. Nature Publishing Group.

FAO RO for A and the P. 2005. Arabica coffee manual for Lao PDR. Available from https://www.fao.org/3/ae939e/ae939e04.htm#bm04.6 (accessed February 23, 2022).
Farah A. 2012. Coffee Constituents. Pages 21–58 Coffee. John Wiley & Sons, Ltd. Available from http://onlinelibrary.wiley.com/doi/abs/10.1002/9781119949893.ch2 (accessed February 25, 2022).
Farah A, de Paula Lima J. 2019. Consumption of Chlorogenic Acids through Coffee and Health Implications. Beverages **5**:11.
Fibrianto K, Ardianti AD, Pradipta K, Sunarharum WB. 2018. The influence of brewing water characteristic on sensory perception of pour-over local coffee.
Fischer E, Victor B, Robinson D, Farah A, Martin P. 2019. CHAPTER 1. Coffee Consumption and Health Impacts: A Brief History of Changing Conceptions: Consumption and Health Implications. Pages 1–19.
Franca AS, Mendonça JCF, Oliveira SD. 2005. Composition of green and roasted coffees of different cup qualities. LWT - Food Science and Technology **38**:709–715.
Fujioka K, Shibamoto T. 2008. Chlorogenic acid and caffeine contents in various commercial brewed coffees. Food Chemistry:5.
Fuller M, Rao NZ. 2017. The Effect of Time, Roasting Temperature, and Grind Size on Caffeine and Chlorogenic Acid Concentrations in Cold Brew Coffee. Scientific Reports **7**.
Gawlik M, et al. 2022. Effect of coffee preparation on caffeine content. Czech University of Life Sciences.
Gloess AN, Schönbächler B, Klopprogge B, D'Ambrosio L, Chatelain K, Bongartz A, Strittmatter A, Rast M, Yeretzian C. 2013. Comparison of nine common coffee extraction methods: Instrumental and sensory analysis. European Food Research and Technology **236**:607–627.
Greenberg JA, Axen KV, Schnoll R, Boozer CN. 2005. Coffee, tea and diabetes: The role of weight loss and caffeine. International Journal of Obesity **29**:1121–1129.
Grobbee DE, Rimm EB, Giovannucci E, Colditz G, Stampfer M, Willett W. 1990. Coffee, caffeine, and cardiovascular disease in men. The New England Journal of Medicine **323**:1026–1032.
Hara K, Haranishi Y, Kataoka K, Takahashi Y, Terada T, Nakamura M, Sata T. 2014. Chlorogenic acid administered intrathecally alleviates mechanical and cold hyperalgesia in a rat neuropathic pain model. European Journal of Pharmacology **723**:459–464.
Harland BF. 2000. Caffeine and nutrition. Nutrition **16**:522–526.
Haskell CF, Kennedy DO, Milne AL, Wesnes KA, Scholey AB. 2008. The effects of l-theanine, caffeine and their combination on cognition and mood. Biological Psychology **77**:113–122.
Hečimović I, Belščak-Cvitanović A, Horžić D, Komes D. 2011. Comparative study of polyphenols and caffeine in different coffee varieties affected by the degree of roasting. Food Chemistry **129**:991–1000.
Hinds TS, West WL, Knight EM, Harland BF. 1996. The effect of caffeine on pregnancy outcome variables. Nutrition Reviews **54**:203–207.
Hussain SP, Hofseth LJ, Harris CC. 2003. Radical causes of cancer. Nature Reviews Cancer **3**:276–285.

Huxley R, Lee CMY, Barzi F, Timmermeister L, Czernichow S, Perkovic V, Grobbee DE, Batty D, Woodward M. 2009. Coffee, decaffeinated coffee, and tea consumption in relation to incident type 2 diabetes mellitus: A systematic review with meta-analysis. Archives of Internal Medicine **169**:2053–2063.

IARC. 1990. Coffee, Tea, Mate, Methylxanthines and Methylglyoxal. Available from https://publications.iarc.fr/Book-And-Report-Series/Iarc-Monographs-On-The-Identification-Of-Carcinogenic-Hazards-To-Humans/Coffee-Tea-Mate-Methylxanthines-And-Methylglyoxal-1991 (accessed March 11, 2022).

International Coffee Organization - Trade Statistics Tables. 2020. Available from https://www.ico.org/trade_statistics.asp (accessed March 17, 2022).

Ittner LM et al. 2010. Dendritic Function of Tau Mediates Amyloid-β Toxicity in Alzheimer's Disease Mouse Models. Cell **142**:387–397. Elsevier.

Johnston KL, Clifford MN, Morgan LM. 2003. Coffee acutely modifies gastrointestinal hormone secretion and glucose tolerance in humans: Glycemic effects of chlorogenic acid and caffeine. American Journal of Clinical Nutrition **78**:728–733.

Kamtchouing P, Kahpui SM, Dzeufiet P-DD, Tédong L, Asongalem EA, Dimo T. 2006. Anti-diabetic activity of methanol/methylene chloride stem bark extracts of Terminalia superba and Canarium schweinfurthii on streptozotocin-induced diabetic rats. Journal of Ethnopharmacology **104**:306–309.

Kim Y-S, Sano H. 2008. Pathogen resistance of transgenic tobacco plants producing caffeine. Phytochemistry **69**:882–888.

Konishi Y, Kobayashi S. 2004. Transepithelial Transport of Chlorogenic Acid, Caffeic Acid, and Their Colonic Metabolites in Intestinal Caco-2 Cell Monolayers. Journal of Agricultural and Food Chemistry **52**:2518–2526.

Kotyczka C, Boettler U, Lang R, Stiebitz H, Bytof G, Lantz I, Hofmann T, Marko D, Somoza V. 2011. Dark roast coffee is more effective than light roast coffee in reducing body weight, and in restoring red blood cell vitamin E and glutathione concentrations in healthy volunteers. Molecular Nutrition and Food Research **55**:1582–1586.

Krakauer T. 2002. The polyphenol chlorogenic acid inhibits staphylococcal exotoxin-induced inflammatory cytokines and chemokines. Immunopharmacology and Immunotoxicology **24**:113–119.

Król K, Gantner M, Tatarak A, Hallmann E. 2020. The content of polyphenols in coffee beans as roasting, origin and storage effect. European Food Research and Technology **246**:33–39.

Kulapichitr F, Borompichaichartkul C, Suppavorasatit I, Cadwallader KR. 2019. Impact of drying process on chemical composition and key aroma components of Arabica coffee. Food Chemistry **291**:49–58.

Lafay S, Gil-Izquierdo A, Manach C, Morand C, Besson C, Scalbert A. 2006. Chlorogenic acid is absorbed in its intact form in the stomach of rats. Journal of Nutrition **136**:1192–1197.

Lecoultre V, Carrel G, Egli L, Binnert C, Boss A, MacMillan EL, Kreis R, Boesch C, Darimont C, Tappy L. 2014. Coffee consumption attenuates short-term fructose-induced liver insulin resistance in healthy men1-4. American Journal of Clinical Nutrition **99**:268–275.

Li SY, Chang CQ. 2005. Biological effects of chlorogenic acid and body health. Wei sheng yan jiu = Journal of hygiene research **34**:762–764.

Linné Carl. 2006. Coffea arabica - Linnaean Typification Project. Available from https://www.nhm.ac.uk/our-science/data/linnaean-typification/search/detail.dsml?ID=246200&listPageURL=list%2Edsml%3FSpecies%3Darabica%26Genus%3DCoffea (accessed March 20, 2022).

Lopez-Garcia E, Van Dam RM, Rajpathak S, Willett WC, Manson JE, Hu FB. 2006a. Changes in caffeine intake and long-term weight change in men and women. American Journal of Clinical Nutrition **83**:674–680.

Lopez-Garcia E, van Dam RM, Willett WC, Rimm EB, Manson JE, Stampfer MJ, Rexrode KM, Hu FB. 2006b. Coffee consumption and coronary heart disease in men and women: a prospective cohort study. Circulation **113**:2045–2053.

Ludwig IA, Clifford MN, Lean MEJ, Ashihara H, Crozier A. 2014. Coffee: biochemistry and potential impact on health. Food Funct. **5**:1695–1717.

M. Frischknecht P, W. Baumann T. 1985. Stress induced formation of purine alkaloids in plant tissue culture of Coffea arabica. Phytochemistry **24**:2255–2257.

Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. 2004. Polyphenols: Food sources and bioavailability. American Journal of Clinical Nutrition **79**:727–747.

Maslova E, Bhattacharya S, Lin S-W, Michels KB. 2010. Caffeine consumption during pregnancy and risk of preterm birth: A meta-analysis. American Journal of Clinical Nutrition **92**:1120–1132.

Mestdagh F, Glabasnia A, Giuliano P. 2017. The Brew—Extracting for Excellence. Pages 355–380 The Craft and Science of Coffee. Elsevier. Available from https://linkinghub.elsevier.com/retrieve/pii/B9780128035207000153 (accessed March 18, 2022).

Monteiro M, Farah A, Perrone D, Trugo LC, Donangelo C. 2007. Chlorogenic acid compounds from coffee are differentially absorbed and metabolized in humans. Journal of Nutrition **137**:2196–2201.

Moon J-K, Yoo HS, Shibamoto T. 2009. Role of roasting conditions in the level of chlorogenic acid content in coffee beans: correlation with coffee acidity. Journal of Agricultural and Food Chemistry **57**:5365–5369.

Nardini M, Cirillo E, Natella F, Scaccini C. 2002. Absorption of phenolic acids in humans after coffee consumption. Journal of Agricultural and Food Chemistry **50**:5735–5741.

Ogden CL, Yanovski SZ, Carroll MD, Flegal KM. 2007. The Epidemiology of Obesity. Gastroenterology **132**:2087–2102.

Onakpoya I, Terry R, Ernst E. 2011. The use of green coffee extract as a weight loss supplement: A systematic review and meta-analysis of randomised clinical trials. Gastroenterology Research and PracticeDOI: 10.1155/2011/382852.

Perrone D, Donangelo R, Donangelo CM, Farah A. 2010. Modeling weight loss and chlorogenic acids content in coffee during roasting. Journal of Agricultural and Food Chemistry **58**:12238–12243.

Perrone D, Farah A, Donangelo CM, de Paulis T, Martin PR. 2008. Comprehensive analysis of major and minor chlorogenic acids and lactones in economically relevant Brazilian coffee cultivars. Food Chemistry **106**:859–867.

Petracco M. 2008a. Technology IV: Beverage Preparation: Brewing Trends for the New Millennium. Pages 140–164 Coffee: Recent Developments.

Petracco M. 2008b. Technology IV: Beverage Preparation: Brewing Trends for the New Millennium. Pages 140–164 Coffee: Recent Developments.

Poltronieri P, Rossi F. 2016. Challenges in Specialty Coffee Processing and Quality Assurance. Challenges **7**:19. Multidisciplinary Digital Publishing Institute.

Poole R, Kennedy OJ, Roderick P, Fallowfield JA, Hayes PC, Parkes J. 2017. Coffee consumption and health: umbrella review of meta-analyses of multiple health outcomes. BMJ:j5024.

Preedy VR. 2014a. Coffee in Health and Disease Prevention. Elsevier Science & Technology, San Diego, UNITED STATES. Available from http://ebookcentral.proquest.com/lib/czup/detail.action?docID=1826804 (accessed March 9, 2022).

Preedy VR. 2014b. Coffee in Health and Disease Prevention. Elsevier Science & Technology, San Diego, UNITED STATES. Available from http://ebookcentral.proquest.com/lib/czup/detail.action?docID=1826804 (accessed March 13, 2022).

Qu Z-W, Liu T-T, Qiu C-Y, Li J-D, Hu W-P. 2014. Inhibition of acid-sensing ion channels by chlorogenic acid in rat dorsal root ganglion neurons. Neuroscience Letters **567**:35–39.

Renouf M et al. 2010. Measurement of caffeic and ferulic acid equivalents in plasma after coffee consumption: Small intestine and colon are key sites for coffee metabolism. Molecular Nutrition and Food Research **54**:760–766.

Rodriguez de Sotillo DV, Hadley M. 2002. Chlorogenic acid modifies plasma and liver concentrations of: Cholesterol, triacylglycerol, and minerals in (fa/fa) Zucker rats. Journal of Nutritional Biochemistry **13**:717–726.

Ruan Z et al. 2014. Chlorogenic acid decreases intestinal permeability and increases expression of intestinal tight junction proteins in weaned rats challenged with LPS. PLoS ONE **9**.

Runge F. 1821. Neueste phytochemische Entdeckungen zur Begründung einer wissenschaftlichen Phytochemie. Reimer.

Sahab NRM, Subroto E, Balia RL, Utama GL. 2020. γ-Aminobutyric acid found in fermented foods and beverages: current trends. Heliyon **6**:e05526.

Schmieder BKL, Pannusch VB, Vannieuwenhuyse L, Briesen H, Minceva M. 2023. Influence of Flow Rate, Particle Size, and Temperature on Espresso Extraction Kinetics. Foods **12**.

Schwan R, Silva C, Batista L. 2012. Coffee Fermentation. Pages 677–690.

Selmar D, Bytof G, Knopp S, Bradbury A, Wilkens J, Becker R. 2005. Biochemical insights into coffee processing: quality and nature of green coffees are interconnected with an active seed metabolism. Available from https://www.semanticscholar.org/paper/Biochemical-insights-into-coffee-processing%3A-and-of-Selmar-Bytof/4b4703cd09c50c31cdf68b9e03e9e8906265f472 (accessed April 14, 2024).

Severini C, Derossi A, Ricci I, Fiore A, Caporizzi R. 2017a. How Much Caffeine in Coffee Cup? Effects of Processing Operations, Extraction Methods and Variables.

Severini C, Derossi A, Ricci I, Fiore A, Caporizzi R. 2017b. How Much Caffeine in Coffee Cup? Effects of Processing Operations, Extraction Methods and Variables.

Shelp BJ, Bown AW, McLean MD. 1999. Metabolism and functions of gamma-aminobutyric acid. Trends in Plant Science **4**:446–452.

Sherge K, D'heer J, Duchateau L, Boeckx P. 2016. Influence of growing altitude, shade and harvest period on quality and biochemical composition of Ethiopian specialty coffee. Journal of the science of food and agriculture **97**.

Soares C, Alves R, Oliveira M. 2015. Chapter 24. Factors Affecting Acrylamide Levels in Coffee Beverages. Pages 217–224 Coffee in Health and Disease Prevention.

Suzuki T, Ashihara H, Waller GR. 1992. Purine and purine alkaloid metabolism in Camellia and Coffea plants. Phytochemistry **31**:2575–2584.

Taguchi K, Hida M, Matsumoto T, Ikeuchi-Takahashi Y, Onishi H, Kobayashi T. 2014. Effect of short-term polyphenol treatment on endothelial dysfunction and thromboxane A2 levels in streptozotocin-induced diabetic mice. Biological and Pharmaceutical Bulletin **37**:1056–1061.

Tajik N, Tajik M, Mack I, Enck P. 2017. The potential effects of chlorogenic acid, the main phenolic components in coffee, on health: a comprehensive review of the literature. European Journal of Nutrition **56**:2215–2244.

Thurber FB. 1889. Coffee: from Plantation to Cup: A Brief History of Coffee Production and Consumption. American Grocer Publishing Association.

Tousch D, Lajoix A-D, Hosy E, Azay-Milhau J, Ferrare K, Jahannault C, Cros G, Petit P. 2008. Chicoric acid, a new compound able to enhance insulin release and glucose uptake. Biochemical and Biophysical Research Communications **377**:131–135.

Turnbull D, Rodricks JV, Mariano GF, Chowdhury F. 2017. Caffeine and cardiovascular health. Regulatory Toxicology and Pharmacology **89**:165–185.

Ukers WH. 1935. All about Coffee. Library of Alexandria.

Valio IFM. 1980. INHIBITION OF GERMINATION OF COFFEE SEEDS (COFFEA ARABICA L. cv. MUNDO NOVO) BY THE ENDOCARP. Journal of Seed Technology **5**:32–39. Association of Official Seed Analysts.

Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. International Journal of Biochemistry and Cell Biology **39**:44–84.

van Dam RM, Hu FB. 2005. Coffee Consumption and Risk of Type 2 Diabetes: A Systematic Review. JAMA **294**:97.

Van Dijk AE, Olthof MR, Meeuse JC, Seebus E, Heine RJ, Van Dam RM. 2009. Acute effects of decaffeinated coffee and the major coffee components chlorogenic acid and trigonelline on glucose tolerance. Diabetes Care **32**:1023–1025.

Várady M, Tauchen J, Frankova A, Kloucek P, Popelka P. 2022a. Effect of method of processing specialty coffee beans (natural, washed, honey, fermentation, maceration) on bioactive and volatile compounds. LWT **172**:114245.

Várady M, Tauchen J, Klouček P, Popelka P. 2022b. Effects of Total Dissolved Solids, Extraction Yield, Grinding, and Method of Preparation on Antioxidant Activity in Fermented Specialty Coffee. Fermentation **8**:375. Multidisciplinary Digital Publishing Institute.

VOILLEY A, SAUVAGEOT F, SIMATOS D, WOJCIK G. 1981. INFLUENCE OF SOME PROCESSING CONDITIONS ON THE QUALITY OF COFFEE BREW. Journal of Food Processing and Preservation **5**:135–143.

Vollmanová J, Urminská D, Bajčan D, Bobková A, et al. 2022. Chémia potravin (Food Chemistry), 2022nd edition. Slovenská pol'nohospodárska univerzita v Nitre, Nitra.

Wan C-W, Wong CN-Y, Pin W-K, Wong MH-Y, Kwok C-Y, Chan RY-K, Yu PH-F, Chan S-W. 2013. Chlorogenic acid exhibits cholesterol lowering and fatty liver attenuating properties by up-regulating the gene expression of PPAR-α in hypercholesterolemic rats induced with a high-cholesterol diet. Phytotherapy Research **27**:545–551.

Wang X, Lim L-T. 2023. Effects of grind size, temperature, and brewing ratio on immersion cold brewed and French press hot brewed coffees. Applied Food Research **3**.

Weinberg BA, PH.D PDBAW, Bealer BK. 2001. The World of Caffeine: The Science and Culture of the World's Most Popular Drug. Psychology Press.

Windisch J, Keppler BK, Jirsa F. 2020. Aluminum in Coffee. ACS Omega **5**:15335–15343. American Chemical Society.

Winston E, Op de Laak J, Marsh T, Lempke H, Aung O, Nyunt T, Chapman K, Agriculture Group. 2005. Arabica coffee manual for Myanmar. FAO, Bangkok, Thailand. Available from https://www.fao.org/publications/card/en/c/5edb5e0f-2cec-5e49-8fd5-1e83dd91b944 (accessed February 22, 2022).

Wintgens JN. 2004a. The Coffee Plant. Pages 1–24 Coffee: Growing, Processing, Sustainable Production. John Wiley & Sons, Ltd. Available from http://onlinelibrary.wiley.com/doi/abs/10.1002/9783527619627.ch1 (accessed February 22, 2022).

Wintgens JN. 2004b. The Coffee Plant. Pages 1–24 Coffee: Growing, Processing, Sustainable Production. John Wiley & Sons, Ltd. Available from http://onlinelibrary.wiley.com/doi/abs/10.1002/9783527619627.ch1 (accessed February 22, 2022).

Wintgens JN, Zamarripa C. A. 2004. Coffee Propagation. Pages 87–136 Coffee: Growing, Processing, Sustainable Production. John Wiley & Sons, Ltd. Available from http://onlinelibrary.wiley.com/doi/abs/10.1002/9783527619627.ch4 (accessed February 22, 2022).

Xue H, Wei M, Ji L. 2023. Chlorogenic acids: A pharmacological systematic review on their hepatoprotective effects. Phytomedicine **118**.

Yu X, Bao Z, Zou J, Dong J. 2011. Coffee consumption and risk of cancers: a meta-analysis of cohort studies. BMC Cancer **11**:96.

Zang L-Y, Cosma G, Gardner H, Castranova V, Vallyathan V. 2003. Effect of chlorogenic acid on hydroxyl radical. Molecular and Cellular Biochemistry **247**:205–210.

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