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How Much Caffeine in Coffee Cup? Effects of Processing Operations, Extraction Methods and Variables

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Abstract

About 80–90% of the adults are regular consumers of coffee brews. Its consumption has positive effect on energy expenditure, power of muscle, while over consumption has negative effects widely debated. Across geographical areas, coffee brews may notably change when preparing Espresso, American, French, Turkish, etc. This chapter reviewed the phases able to affect the amount of caffeine in cup. Three most important areas will be addressed: (1) coffee varieties and environment; (2) coffee processing operations; (3) brewing methods extraction variables. What arises from the state of art is that, although there is a significant agreement on the effect of each critical variable on caffeine extraction, there is also a great difficulty to precisely know how much caffeine is in a coffee cup, although this is the most important information for the consumers. The number of affecting variables is very high, and some of them are inversely related with caffeine content (brewing time and brew volume), while others exhibit a direct relationship (grinding level, dose, and tamping). Finally, some variables under the control of barista rarely are accurately reproduced during brewing. For instance, it was found that the caffeine content in a Starbuck's coffee cup during different days varied significantly.

Keywords: caffeine, coffee, extraction, processing conditions, effect of variables

1. Introduction

The most studied component of the coffee is certainly caffeine (1,3,7-trimethylxanthine). It is present in the form of salt of chlorogenic acid and, in the roasted coffee, in free form. The caffeine

amount present in raw coffee can significantly vary, depending on many factors, among which the most important are the origin and cultivar, Arabica or Canephora (var. Robusta).

On average, the raw Arabica shows a caffeine content ranging from 0.9 to 1.5% (dry weight), while the Robusta contains about twice as much between 1.2 and 2.4% [1–5].

But it is far from being considered a bad news. The World Health Organization (WHO) considers the coffee a “non-nutritive dietary component” because of its 2 calories per cup of bitter coffee. In fact, the numerous compounds formed during the roasting process come primarily from Maillard Reaction, and are considered as fiber. Like caffeine, they are hydrosoluble and can be easily disposed by the kidneys. From this point of view, the coffee and caffeine seem to be “neutral” component of human diet. Nevertheless, this is not true.

The positive effects of caffeine on the human organism are now widely known, with particular reference to the improvement of cognitive skills, as a stimulant of attention and concentration. From this point of view, coffee can therefore be considered a “functional” product according to the European Parliament and Council Regulation No. 1924/2006 of 2006 December 20 on “Nutrition and health claims made on foods” [6], it responds to the claims of type A which is related to the “improvement of a biological function related to specific physiological, psychological, and biological activities, beyond their established role in growth, development, and other normal functions”.

However, in the past, caffeine was often demonized as responsible for diseases. Today, in the lights of the numerous studies conducted worldwide, it can be stated that caffeine is neither responsible for any disease related to cancer, development of cardiovascular diseases [7], nor related with problems that may arise during pregnancy, such as a shorter gestation or reduced birth weight (from a study of 12,208 women) [8]. Also in breastfeeding, the nurse can continue to drink coffee. It has been observed, in fact, that in the milk of women who drink coffee, caffeine reaches its maximum rate after about 1 h. Its concentration depends on the fat content of milk, and the infant absorbs only 0.06 - 1.5% of caffeine. So, there is no justification to prohibit nurses from a moderate coffee consumption [8].

On the contrary, a study carried out in Bhabha Atomic Research Centre in Bombay demonstrates that caffeine is able to contrast and prevent oxidative damage of human organism cell membranes, caused by free radicals, and shows an antioxidant capacity similar to the glutathione (antioxidant naturally present in the human intracellular fluid) and greater than the vitamin C [9].

From this point of view, the coffee, simply by the presence of caffeine, can also be considered a “functional” product that responds to the claims of type B related to the “reduction of disease risk that relate to consumption of a food or a food component that might help to reduce the risk of a given disease or medical condition because of specific nutrients or non-nutrients contained in it”.

These topics about coffee, caffeine, and human health will be deeply discussed in Section 2 of this chapter.

Concerning the features of coffee beverage, it is known that its quality depends on a number of variables, so that starting from the same raw material we could obtain a coffee with completely different characteristics, in terms of pH, flavor, “body”, cream, caffeine, phenols, Maillard Reaction Products (MRPs), etc.

For this reason, the chapter will consider all the principal aspects, which affect the quality of coffee “in cup”.

Section 3 deals with the properties of raw material, the green coffee, and how its variability could affect the final quality of coffee brew.

Section 4 is aimed to deepen the central technological process that coffee undergoes during roasting. In this case, the different process conditions, applied in different countries and cultures, may lead to a range of possible chemical compositions (i.e. phenol content and MRP content) and sensory characteristics of coffee “in cup” (acidity).

In Section 5, the author investigates a particular aspect, which has been neglected so far, however being one of the most important for the quality of coffee beverage. The grinding process, which could dramatically affect all the features of beverage, such as volume, total solid content, caffeine content, pH, and flavor in general.

Sections 6 and 7 are totally dedicated to the different types of extraction and their fallout on caffeine content and other characteristics of coffee “in cup”. Obviously, the single-service size systems are also described, even considering the more recent results of our research.

2. Coffee consumption and its debate on health

Coffee is an extremely popular beverage, which has become the second most valuable commodity after oil [10]. Annually, 120 million of coffee bags are consumed in the world, corresponding to over 7 million of tons [11]. Coffee consumption is a regular part of daily life worldwide [12], in fact in the European Community, as well as in the United States, the average consumption of coffee per capita is of 5.1 kg/year [13]. Americans consume more than 400 million of coffee cups daily, making this beverage the major source of caffeine in the adult diet [14].

The coffee is a complex mixture of thousands of chemicals. It contains, besides caffeine, more than 1,000 chemical compounds responsible of its flavor and aroma, carbohydrates, lipids, nitrogenous compounds, vitamins, minerals, alkaloids, and phenolic compounds [15, 16]. Anyway, among these, the caffeine is that on which is focused the majority of debates regarding the coffee consumption and its effects on health. Caffeine content in coffee is highly variable depending on a huge number of factors, such as variations in environmental and climatic conditions, features of raw materials, agricultural practices, post-harvest techniques, duration and conditions of storage, roasting degree, roasting process, type of commercial coffee, grinding, and brewing methods [16]. Caffeine is an alkaloid that is found in more than 60 plants

which has a protective effect against insects [17]. The world's primary sources of dietary caffeine are roasted coffee beans and tea leaves. Caffeine is the most widely consumed psychoactive substance throughout the world, and it has been used for thousands of years [18].

Other common sources of caffeine are the kola nut, cacao bean, yerba mate, and guarana berries [19]. It has been estimated that 80–90% of adults are regular consumers of caffeine-containing brews, such as tea, coffee, cocoa, cola, and energy drinks [20]. A study on the caffeine intakes of the US population (considering a total of 37,602 consumers) showed that 85% of people consumed at least one caffeinated beverage per day. Caffeine intake in adults increases by age with the highest consumption for people of 50–64 years old (226 mg/day). Adult men consumed more total caffeine from beverages than adult women, as confirmed by Frary et al. [21]. In particular, the most frequently consumed beverages containing caffeine are coffee (71%), soft drinks (16%), and tea (12%) [19]. Although each of these has strong economic, social, and cultural impact, coffee brew remains the most important both economically and socially. In fact, coffee brew significantly contributes to the overall caffeine consumption of the adult populations [22].

In 2012, FDA [23] stated that for healthy adults, a caffeine intake up to 400 mg/day is not associated with adverse effects. Obviously for children, different and specific recommendations exist. Health Canada issued recommendations in 2009 specified the caffeine intake at 45–85 mg/day as healthy levels for children aged 6–12 years and 100–175 mg/day for adolescents of ages >12 years [24]. Health Canada recommended, for pregnant women, a daily dose of caffeine lower than 300 mg, while UK Food Standard Agency restricted this amount below 200 mg/day [12]. Brent et al. [25] and Peck et al. [26] do not support adverse effects for this caffeine consumption on reproductive health or pregnancy outcomes. Furthermore, another study [8] found that the caffeine consumption is not related with problems that may arise during pregnancy, such as a shorter gestation or reduced birth weight (from a study of 12,208 women).

Caffeine is rapidly absorbed in the stomach and small intestine, and it is distributed to all tissues, including the brain. Once caffeine is absorbed, it exhibits numerous and well-studied physiological effects.

However, apart from caffeine, coffee brews are also rich in other bioactive substances with a wide range physiological effects [27]. The list comprises of many phytochemicals, such as phenols, lactones, niacin, trigonelline, melanoidins, choline, etc.

An understanding of the physiological effects of coffee beverage is limited by the wide array of components included in the extracted product and by the numerous effects of each of these compounds.

However, it can be stated that the majority of the research carried out on the physiological properties of coffee has concerned the caffeine, which principally has stimulatory effects, including enhanced perception, reduced fatigue, enhanced memory consolidation, improved mental alertness, and reduced sleep duration [28]. A moderate consumption of caffeine has shown to increase strength and power of muscle, as well as energy expenditure. In fact, the consumption of 300 mg caffeine per day increases energy expenditure by approximately 79 kcal/day [29]. Moreover, it enhanced lipid oxidation and lipolytic and thermogenic activities [19].

On the other hand, an over consumption of caffeine might have negative effects, such as ringing in the ears, mood diarrhea, delirium, muscle tension, gastric acid secretion, etc. [30]. Excess caffeine intake is also involved in a state of excitement, anxiety, tachycardia, headache, palpitations, insomnia, nervousness, and tremor [31].

Wide differences in the dose-response of caffeine among individuals were observed as a result of genetic variation of susceptibility [32].

Furthermore, experimental and clinical evidences confirm tolerance from caffeine, which produce a reduction in the response as a consequence of previous exposure; consequently, the observed effects after a series of repetitive caffeine dosage may be very different from those highlighted after the first intake.

Apart from the well-known physiological properties of caffeine, more recent investigations indicated potential healthy effects of coffee, which are to a certain extent correlated with caffeine [31].

Some epidemiological studies suggested that coffee beverage is inversely associated with risk of various diseases [16, 33, 34].

Most of the more recent studies reported a relationship between a significant risk reduction of 30–60% in the development of type 2 diabetes and coffee consumption [34]. In particular, some studies reported a significant dose-dependent reduction in the risk of developing type 2 diabetes with a long-term coffee consumption [35, 36]. Moreover, this positive effect was observed both for caffeinated and decaffeinated coffee [37]; thus, it is possible to ascribe these effects to other phytochemicals.

Some studies reported controversial effects on the post-prandial glucose peak [38] as affected by coffee consumption. As reported from Greenberg et al. [39], part of these effects might be attributable to caffeine.

Furthermore, coffee intake has shown to reduce the liver damage in people at risk for liver diseases, such as hepatic injury, cirrhosis, and hepatocellular carcinoma [40, 41]. It was suggested that the coffee may preserve hepatocytes from damage, regardless of whether the aggressive agent is a virus, alcohol, drugs, or others [42]; however, the mechanisms associated with the protective effect of coffee on the liver are still unclear.

Coffee consumption is also inversely associated with the risk of Parkinson's disease in men and women, who have never used postmenopausal estrogen [43]. A meta-analysis found a risk reduction of 49% by consuming three additional coffee cups per day, whereas no effects were found for the cohort study that included only women [44, 45]. The well-reported protective effect of coffee on Parkinson's disease could be ascribed to its caffeine content, which acts to the dopaminergic system [31]. However, the mechanisms involved were not fully understood. Still about the risk of neuro-degenerative diseases, coffee drinkers have a lower risk of Alzheimer's disease respect to people who do not drink coffee [46], even if this outcome is under debate.

Some experimental studies asserted that cognitive deterioration of Alzheimer's disease in the central nervous system may be prevented by caffeine and/or chlorogenic acid [47, 48]. In addition, Gelber et al. [49] pointed out neither coffee and caffeine intake could be associated with any form of cognitive deterioration.

Regarding cancer, coffee consumption is inversely correlated with the risk of liver and colon-rectum cancers, even if the mechanisms involved are not clear yet [27]. Moreover, two meta-analysis concluded that there is a clear dose-dependent inverse association between hepatocellular cancer and the increase in coffee consumption, suggesting that by raising the intake of coffee, the possibility of developing hepatocellular carcinoma may be reduced [50, 51]. Also, a strong protective association has been found between coffee consumption and the reduction of endometrial cancer [52], while coffee intake might be weakly associated with breast cancer risk [53]. To highlight the protective effect of coffee extracts, it is worth noting that any association between these diseases and decaffeinated coffee was not observed. Under these considerations, it is possible that caffeine might be responsible for the protective role [31].

Only few studies have linked coffee consumption with an increased risk in developing cardiovascular (CV) disease. However, this risk is related to the ingestion of the diterpenes cafestol and kahweol, which have been shown to increase serum total and LDL cholesterol [54]. These compounds are mainly found in high amounts in boiled and unfiltered coffee. Besides these diterpenes, caffeine might exert negative effects on CV health too, by increasing heart rate and blood pressure [31, 55]. In a paper just published by a group of researchers from California [56] the effect of a diet rich in caffeine (coffee, tea, and cocoa) on the electrocardiographic profile of 1,388 study participants was tested. The subjects were followed up with clinical analysis and annual or semi-annual visits for 10 years and contacted every 6 months after this period. From results, there is no evidence (95% confidence) that frequent consumption of products containing caffeine is associated with heart problems. Patients with a history of heart problems showed no induction or cardiac arrhythmia aggravation within 1 h of taking 2 or 3 cups of coffee (275 mg caffeine). Moreover, one study involving about 3,000 patients hospitalized for cardiac arrhythmia showed an inverse relationship between consumption of coffee and caffeine and frequency of hospitalizations for arrhythmia, suggesting that it is highly unlikely that caffeine intake increases the risk of arrhythmia [57].

Coughlin and Nehlig [7] conducted a large study, which collects all the data made available by the worldwide research over the last 30 years, considering the balance of risks and benefits of coffee consumption as a whole. There is a plethora of potentially carcinogenic compounds (tested at high doses in animals) in coffee, but considered within the whole food ("whole food approach"), they produce a protective effect against many forms of cancer (lung, bladder, colon-rectal, endometrial, liver, prostate, leukemia, mouth, and throat). It is what the authors called "coffee paradox".

Therefore, the coffee beverage is now an important item in the lives of billions of people which is traditionally used to complement meals, as well as for hedonistic and psychostimulant purposes. Epidemiological data support the view that habitual coffee consumption has several health benefits because of its content of bioactive compounds and caffeine, which can exert physiological and healthy effects. Caffeine intakes up to 400 mg/day do not give rise to safety concerns for healthy adults in the general populations.

3. Coffee species, origin and blending

Coffee's most studied component, caffeine, varies substantially as a function of coffee plant species [58]. Green coffee beans are used by the International Standard (ISO 3509-1989) to define "a commercial term designating the dried seeds of the coffee plant" [2]. Coffee beans are produced from the cotyledons of seeds belonging to the genus *Coffea*, which includes approximately 70 species. Some of these are of small-scale, they are cultivated in some African countries, but the resultant beverages are generally of low quality and most of the beans are not exported [59]. Three coffee species are mostly commercialized: *Coffea arabica*, *Coffea canephora* Pierre, and *Coffea liberica* Bull worldwide known as Arabica, Robusta, and Liberica or Liberian coffee, respectively. However, only the first two have a commercial importance; in particular, *C. arabica* provides for 60% of world production, while the remaining 40% are from *C. canephora* var. Robusta (**Figure 1**) [60]. These two species display differences, deriving from optimal climate of growing, physical aspects, chemical composition, and quality of the beverages. Generally, coffee extract prepared by *C. arabica* is more appreciated than Robusta because of its superior quality in terms of aroma and, therefore, it reaches higher prices in the international market [61]. On the other hand, the Robusta coffee, characterized by a more bitter and persistent taste, shows a high amount of antioxidants and soluble solids [3]. However, green beans are especially featured by their content in caffeine, trigonelline, and chlorogenic acids. The two main species exhibit differences in caffeine percentages ranged between 1.2–2.4% for Robusta and 0.9–1.5% for Arabica [1–5]. Caffeine is formed in immature coffee fruits, and it gradually accumulates all along seed development [62]. The lower content in caffeine for *C. Arabica* is explained by a lower expression of some genes (CaXMT1, CaMXMT1, and CaDXMT2) respect to *C. canephora* [63]; these genes were positively correlated with the caffeine accumulation in coffee beans. Likewise, geographical origins may have influence on caffeine accumulation and its final concentration. Babova et al. [5] reported that Arabica coffee from Brazil contains more caffeine than the same species growth in Ethiopia and Kenya; similarly, the Robusta coffee from Uganda shows more caffeine than the same species coming from Vietnam. Furthermore, Cheng et al. [63] deeply reviewed the metabolism of the most important components in coffee as affected by genotype (G) and environment (E), showing as both affect seed development and the final concentration of metabolites in coffee beans,



Figure 1. Green beans of *C. arabica* and *C. canephora* var. Robusta.

especially caffeine content. Specifically, the authors highlighted as G and E, as well as their interaction (G×E), may affect the overall quality of coffee; similar results were found in a recent study, in which 20 samples of *C. arabica* and *C. canephora* were investigated [5]. This study highlighted a clear separation among *C. arabica* accessions based on their geographical origins, with Ethiopia and Mexico's accessions which exhibit the lowest content of caffeine [5]. Despite the high differences between the two most important species of coffee beans, in terms of caffeine content and geographical origins, there are variations within the same species and across the different cultivars [5].

Other than genotype and geographical origins, other environmental factors may affect caffeine accumulation. For example, light exposure is required for caffeine synthesis, although its optimal level is very low [63]. Indeed, some researchers demonstrated as increased level of shade improves caffeine content in *C. arabica* [64, 65], while seedlings of Robusta coffee completely grown in darkness showed a remarkable decrease in caffeine content [66]. Furthermore, among environmental conditions, also the high altitude was positively related to caffeine content [67].

Fox et al. [68] studied the variations in caffeine concentration for 25 single beans from 5 selected coffees. They found a positive relationship between the weight of beans and caffeine content, but a very low determination coefficient, r^2 , of 0.31 was calculated. This proved that selecting the beans for weight would not ensure an increase in caffeine concentration.

However, apart from geographic origins, rarely the coffee used to prepare a beverage consists in a unique species; the blending is a technique used to improve the overall aroma, body, and flavor of coffee, with the main aim to obtain a coffee having excellent sensorial properties on the final roasted product. Blending may be done before or after roasting, even though, traditionally, the retailer and the roasters perform it before the roasting, by combining green beans with similar characteristics, to obtain same physicochemical changes during the thermal process. To date, many popular blends are available on the market which may show notable changes in caffeine content based on origin, species, cultivar, and ratios used in blending. Generally, commercial blends available in the market present a great variability, mainly depending on the species used, even if other factors may influence caffeine content; for example, roasting degree, grinding level of coffee powder, etc. which will be well presented in the next section.

4. Changes in caffeine content as affected by roasting

Roasting is one of most important step in coffee processing because of the marked chemical, physical, structural, and sensorial changes that confer the worldwide appreciated properties. During this process, coffee beans are exposed to high temperature for a time length that can greatly vary according to the type of roaster, geographical origin, variety, coffee bean characteristics, and the desired sensorial properties. Coffee roasting is a process carried out in different ways throughout the world (**Figure 2**) [69].



Figure 2. Coffee roast levels (adapted from Ref. [69]).

In terms of structure, the beans increase their volume becoming up to almost a double of the original. Moreover, the beans lose weight in a range of the 15–25% as well as a continuous change in color is commonly observed (**Figure 3**) [70]. The modifications involved during the roasting are the result of hundreds of chemical reactions and thermal decompositions occurring on thousands of chemical compounds. Four regions of decomposition of the green coffee beans have been reported: (1) dehydration; (2) hydrolysis; (3) desmolysis; (4) catalysis [71]. The decomposition begins at 100°C by a significant endothermic reaction that is followed by a drop in temperature (**Figure 3**) [72, 73]. Among the reactions occurring during this endothermic step, the major contribution seems to be given by the phenols. Considering caffeine, in spite of its high sublimation point (178°C), a reduction is observed by evaporation because it is dragged by the water vapor [73]. This phenomenon is also allowed by the increase of the caffeine solubility in water as a function of temperature.

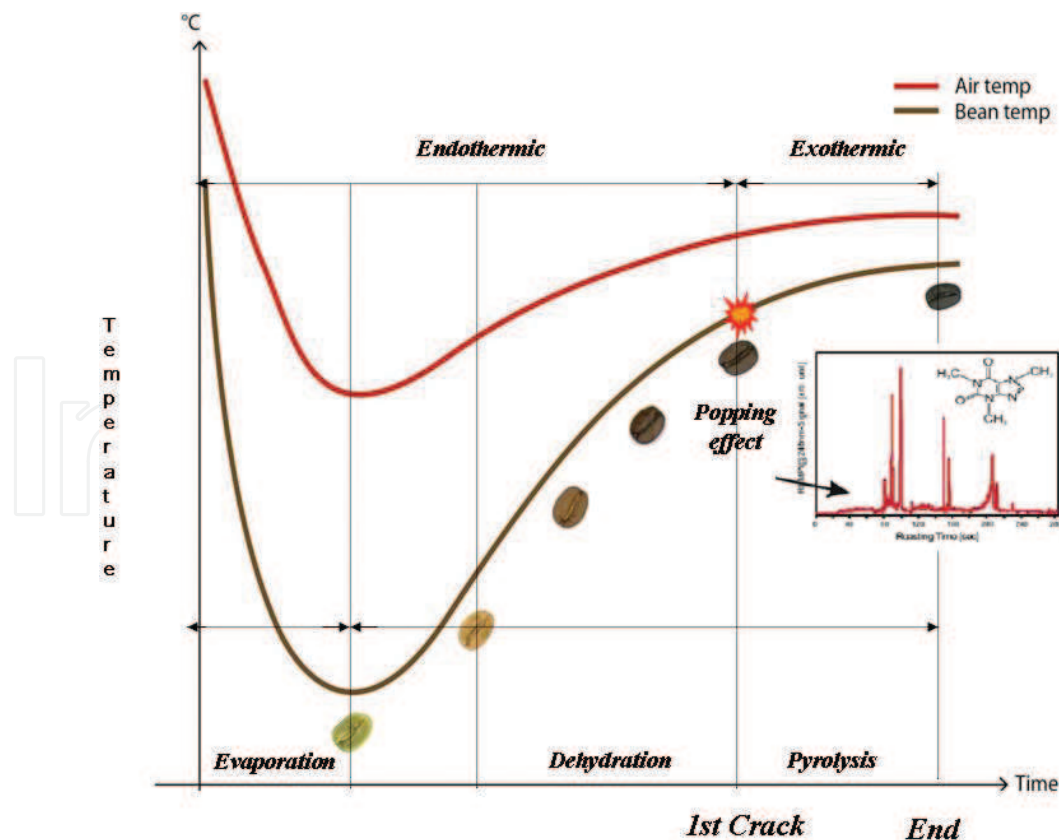


Figure 3. Roast profile analysis (adapted from Refs. [70, 80]).

In general, the roasting causes a reduction in caffeine content of 30% (from $0.89\% \pm 0.02$ of green beans to $0.6\% \pm 0.03$ for roasted Arabica beans) [74]. Farah [3] confirmed that even though caffeine is not involved in chemical reactions, being stable upon roasting, a small fraction may be lost by sublimation. Analyzing the evolution of gas composition during roasting, it was observed that in the same temperature region ($100\text{--}245^\circ\text{C}$), an increase of nitrogen-containing heterocyclic compounds, such as indole and caffeine occurs.

The stability of caffeine, during roasting process, was also reported for the roasted coffee oil (an important by-product with aromatic properties of the Brazilian soluble coffee industries) obtained by mechanical pressing of beans before the extraction of soluble coffee. During mechanical *via* expelling extraction (high pressure and high temperature), a large amount of caffeine is incorporated into the roasted coffee oil because it is not thermally degraded [75].

Due to the temperature of sublimation (178°C) [76, 77], it would be expected that the loss of caffeine would occur to a higher extent when this temperature is reached. Macrae [78] reported that these phenomena could be related with porosity and the internal pressure created into the beans that may cause some difficulties for the sublimation of caffeine. Nevertheless, in a model system, where caffeine is probably free of chemical and physical linkages, a similar gradual decrease of its content occurs [79].

Moreover, important microstructural changes occurring during roasting can drive an additional loss in caffeine. The high temperature reached during roasting causes bursts accompanied by popping sounds [80]. During popping phenomena, caffeine is easily detectable in the roasting gas, because it is emitted during seed fracturing (**Figure 3**). Popping is a consequence of the accumulation of inorganic gases formed into the closed pores of beans, during the pyrolysis of several compounds. When the pressure reaches a critical limit, the seeds crack and the entrapped gases are abruptly released. Under these conditions, darker roasting degrees could present less caffeine amount.

However, the roasting variables may be classified as intrinsic and extrinsic process. The first class includes all that can be controlled and changed depending on the desired degree of roasting (methods of roasting, time and temperature profiles, and coffee's load), while the latter depends on the features of the green beans (variety, species, origin, and quality) and its pre-processing (batch-to-batch differences in the coffee beans, semi-dry or wet post-harvesting method, and humidity).

Among the extrinsic variables, Crozier et al. [12] reported that caffeine content depends on preliminary processing to which beans are subjected. For example, both the washed and unwashed Arabica beans submitted to different time/temperature profiles, such as high temperature for short time (H-S) and low temperature for a longer time (L-L) led to a reduction in caffeine of 80% (in comparison with green beans) in the corresponding coffee brews prepared by adding 5 g of ground beans in 100 mL of boiling water for 5 min than unroasted samples. However, the brew obtained from washed Arabica roasted beans retained the 20.6 and 19.6% for H-S and L-L, respectively, while a better retention of caffeine was observed when using unwashed Arabica beans with values of 19.2 and 18.6% for H-S and L-L, respectively. Coffee bean's humidity markedly affects the time of roasting; as well as the temperature of the beans at the end of roasting.

Taking into account roasting techniques, coffee beans are traditionally roasted in batch, working hundreds of kilograms, or in continuous systems. The heat can be transferred to the beans by conduction at direct contact with hot metal surfaces, by free or forced convection due to a streaming media (hot air), or by radiation [81]. Moreover, non-conventional microwave roasting or combined techniques were also studied [82, 83]. The authors reported that the application of microwave roasting determined a lower loss in caffeine (10.38%, from 2.12 to 1.90 g/100 g) rather than conventional roasting (14.15%, from 2.12 to 1.90 g/100 g). However, combined methods (convective and microwave) enabled to obtain a further preservation in caffeine content exhibiting a total loss of 8%.

The microwaves operate directly in the core of the beans, so that the process of roasting is intensified throughout the whole interior of the bean. This leads to a very intensive heating from the core to the surface of beans. The application of combined methods resulted in the increasing of heating and chemical reactions, a reduction of roasting time, while the ultimate temperature of coffee is lower than the values measured by traditional convective heating. The same changes in caffeine content were observed by headspace analysis of corresponding ground of green and roasted coffee beans (**Table 1**) [82, 83]. Because of these reasons, the microwave roasting method was found to be the most advantageous for caffeine retention.

Another key factor for the process is, of course, roasting time. The quantity of heat transferred to the beans is the result of temperature and roasting time [84]. According to the widespread opinion, the degree of roast in the product is correlated to the final roasting temperature [84, 85]. In general, temperature must exceed 190°C to provide a sufficiently reactive roast environment; therefore, the residence time and the process temperature should be precisely measured to describe the overall thermal behavior. For example, **Table 2** reports the changes in caffeine for Arabica and Robusta coffee beans during two roasting experiments [79]. The first trial was performed at constant roasting time of 15 min by increasing temperature, while the second one was performed by increasing roasting time at fixed temperature of 240°C. At constant roasting time, caffeine content decreases of 11.3% (from 1.24 to 1.10 g/100 g d.w.) and 7.7% (2.08 and 1.92 g/100 g d.w.) in Arabica and Robusta coffee beans, respectively. Roasting temperatures until 220°C did not caused any loss in caffeine content in Arabica coffee beans, while a slight decrease of 4.3% (from 2.08 to 1.99 g/100 g d.w.) in Robusta coffee occurred

Roasting method (medium roast degree 9.5% of solids substances)	Roasting time (min)	Beans temperature (°C)	Caffeine in beans (g/100 g d.w.)	Caffeine in headspace surface area of GC peak (%)
Unroasted bean	–	–	2.12 ± 0.03	2.01
Convective	9.75 ± 0.21	238 ± 3	1.82 ± 0.02	0.23
Microwave	11.08 ± 0.17	207 ± 2	1.90 ± 0.05	0.30
Convective- microwave	5.33 ± 0.12	195 ± 0.08	1.95 ± 0.08	0.37

Table 1. Caffeine content in green and roasted beans (g/100 g dry weight) and in corresponding headspace after roasting process with different methods (adapted from Refs. [82, 83]).

Experiment 1			Experiment 2		
Roasting temperature (°C)	Caffeine (g/100 g d.w.)		Roasting time (min)	Caffeine (g/100 g d.w.)	
	Arabica	Robusta		Arabica	Robusta
Green	1.24	2.08	Green	1.24	2.08
140	1.44	2.21	5	1.14	2.04
160	1.52	2.17	8	1.14	2.04
180	1.36	1.98	12	1.05	1.98
200	1.39	1.99	15	0.99	1.93
220	1.29	1.99	20	0.93	1.91
240	1.10	1.92			

Table 2. Changes in caffeine content during roasting at fixed time of 15 min (experiment 1) and at fixed temperature of 240°C (experiment 2) (adapted from Ref. [79]).

probably due to its higher water content. At constant temperature of roasting (240°C), caffeine content decreases of 20% (from 1.24 to 0.93 g/100 g d.w.) and 7.21% (2.08 to 1.91 g/100 g d.w.) in Arabica and Robusta coffee, respectively, after 15 min of process. These data state that the temperature, rather than time, is the main factor affecting caffeine loss during roasting. Therefore, the evolution of caffeine content from raw or green to the roasted beans depends to the chemical and physical changes that occur during process. Although it not degrades, caffeine content could be reduced in two phase of roasting, during dehydration in which caffeine is dragged by water vapor and during the first crack of the beans, with other volatile compounds, as well as when its sublimation temperature is reached. However, this slight reduction is observed in the final step of roasting, determining a less caffeine concentration in the dark roasted beans.

5. Effects of grinding on caffeine extraction

The grinding is a crucial step for coffee brew preparation. In roasted whole beans, the volatiles and the chemical compounds are entrapped in cells and they barely can dissolve in hot water. After grinding, the beans are reduced to small particles having micro- and meso-scale dimension (from few micrometers to ~1,000 µm) from which volatiles may be released and chemical compounds are easily dissolved in hot water, giving the worldwide appreciated aroma [86]. Consistently, from coffee powder about the 60% of aroma is lost during the first 15 min after grinding. For this reason, coffee brews should be rapidly prepared, with the aim to keep its aroma as much as possible. Moreover, in terms of chemical compounds dissolved in coffee brew, the grinding process is one of the most important critical control points for extraction phenomena. Moroney et al. [87] stated that “*particle size of coffee ground is vitally important in coffee extraction in that it affects both the fluid flow through the grind and the grind’s extraction kinetics*”. Commonly, ground coffee is classified in four groups, such as coarse, medium, fine, or very fine. However, across different countries various particle

size distributions may be indicated with the same name, as in the case of Europe and USA where the coarse coffee ground has an average size of 850 and 1,130 μm , respectively, likewise the fine ground coffee, which shows an average size of 430 and 800 μm , respectively [2]. The percolation of water inside the voids (capillaries) in coffee cake, the wettability of each coffee particle, and the diffusion of chemicals from coffee particles to hot water are the main phenomena controlling the amount of chemical compounds released in coffee beverage [88]. When coarse particles are used, the percolation rate is high, due to the greater porosity fraction of coffee cakes and the dimension of its capillaries. This condition leads to an overall decrease in extraction of chemicals. Moreover, diffusion process is reduced due to the decrease in surface contact area between particles and hot water. On the other hand, fine or very fine coffee ground may create a coffee cake very close to its percolation threshold. In this case, the extraction time significantly increase, and a different extraction may occur. A proper equilibrium between percolation, diffusion, and wettability of coffee particles drives the type and the amount of chemicals in coffee hence its quality in cup. Therefore, as a rule, the grinding must be adjusted on the basis of the sensorial and chemical properties desired in coffee brew (i.e. the type of coffee brew). French press coffee, for which the infusion of coffee ground in hot water takes several minutes, needs coarse particles with the aim to get slower diffusion avoiding the extraction of bitter compounds. When preparing espresso coffee with automatic machines, working under pressure, extraction time is reduced to 25–30 s, and finer particles are needed to increase extraction rate of chemicals and volatiles. For French press coffee, about 100–300 particles are usually obtained from each coffee bean, while 3,500 and 15,000–35,000 particles are obtained for preparing Espresso and Turkish coffee, respectively [89]. **Figure 4** schematically depicts the overall particle size distribution for the most common coffee preparation. However, a bimodal particle size distribution is generally preferred being

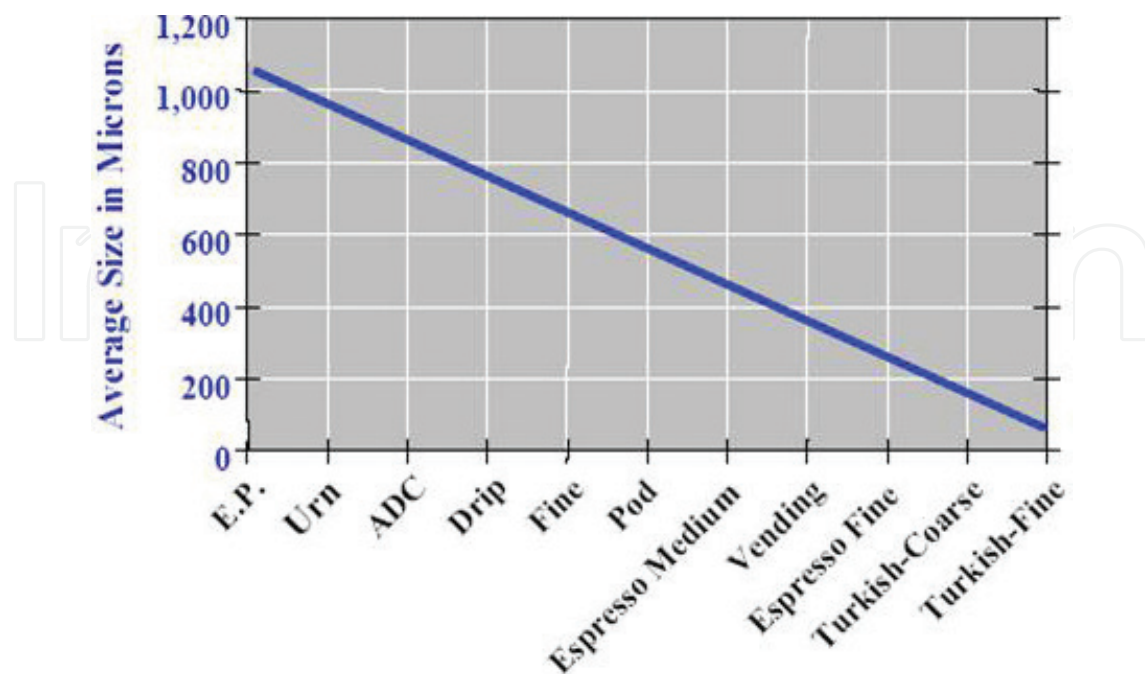


Figure 4. Average particle size of coffee ground for different preparation methods [89].

it able to keep a good equilibrium between wettability, percolation, and diffusion phenomena [88, 90]. Petracco [91] reported that a bimodal distribution of coffee particles is the starting point to obtain a good espresso coffee. The result of grinding operation is affected by several variables, such as the mechanical properties of coffee beans, the moisture content of roasted beans, the type of grinders (blade grinder, conical, or flat burrs grinder). Also, the grinding affects the stability of coffee powder during storage being strictly related with the agglomeration phenomena and aroma retention [88, 92].

The grinding uniformity, that is, how large is the particle size distribution, of crucial importance. If it is poor, an extraction time ideal for the smallest particles will be incorrect for the larger ones, thus leading to a tea-like taste [89]. The impact of particles size of ground coffee on the quality of the brew was widely studied by several authors [90, 93–96]. However, the effect of grinding on the caffeine extraction and its amount in cup have not been studied yet in details.

Spiro and Selwood [97] explored the effects of particle size on the kinetic of caffeine infusion. By separating the coffee ground in sub-groups of particles having different size, the authors estimated the rate constants for extraction caffeine by infusion in water. They reported an increase from 0.207×10^{-3} to $22 \times 10^{-3} \text{ s}^{-1}$ for particles size of 1,700–2,400 and 152–211 μm , respectively. Of course, this is in accordance with the general decrease of coffee particle-water contact area. Bell et al. [93] studied the effect of grinding level on the caffeine content of coffee brew. Although the authors did not analyze the particle size distribution of coffee ground, they showed that by using 8 g of coffee ground for 355 mL of filtered brew, the finest powder yielded the higher caffeine content of 70 mg/177 mL, while when the coarse coffee ground was used, the caffeine content was of 50 mg/177 mL. Again, this was an effect of the greater surface contact area between the fine coffee ground and hot water, which favored the caffeine extraction. On the other hand, when the authors used 32 g of coffee powder for 1,420 mL of water any difference in caffeine content was not observed by using coarse-medium or coarse ground. In spite of the same ratio coffee ground/water, when the authors used more coffee ground, the grinding levels did not have effect. This was due to the longer extraction time of 10 min during which the caffeine was completely extracted, independently from the particles size. Instead, when 8 g of coffee ground was used, for a brewing time of 3 min, the effect of the grinding levels was statistically significant.

An interesting result was obtained for people who prefer to perform the grinding at home with commercial grinders. By home-grinding, no influence of grinding time on caffeine content of the brews was observed. As reported from the authors, the low efficacy of the home-grinder produced very large particle size distribution function being overlapped for 8 or 18 s of grinding time. The authors used the term “*less distinctive grinding patterns*” to explain that no statistical differences were observed increasing the grinding time of 10 s. Similar results were reported by Buchmann et al. [98], who studied the impact of grind size, water temperature, and coffee/water amount on trigonelline and caffeine in Espresso and American brew coffee. In accordance with above discussion, the authors showed the increase of caffeine content from coarse to fine

particles. For Espresso coffee, values of ~25 mg/65 mL, ~62 mg/65 mL, and ~75 mg/65 mL were measured by using 7.5 g of coarse, medium, and fine coffee ground, respectively. Similarly, for Fresh Brew (American filtered coffee), values of ~45 mg/125 mL, ~65 mg/125 mL, and ~62 mg/125 mL were determined when 9 g of coarse, medium, and fine coffee ground were used. However, the authors did not report the particle size distribution of coffee grounds.

These observations enable to introduce the importance of the relationships among particle size, extraction time, and volume of the brew. Severini et al. [90, 95, 96] deeply studied how these variables affect the quality of espresso coffee. The authors analyzing the effect of using coarse, fine-coarse, and fine coffee ground (Table 3) [95] on the caffeine concentration collecting three brew fractions: the first 8 s (Ft1), from 9 to 16 s (Ft2), and from 17 to 24 s (Ft3). Without regard to the fraction time, the caffeine concentration exhibited the following order: fine > fine-coarse > coarse. For instance, values of 4.98, 4.35, and 2.41 mg/mL were measured for Ft1 samples [95]. It was highlighted that this increase was not only the result of the reduction of particles size, but also the consequence of a reduced brew volume for a less percolation rate that, in turn, was due to the lower porosity in coffee cakes.

Under this consideration, the authors modeled the caffeine extraction through coarse, fine-coarse, and fine coffee ground [90]. First, the authors proved that among grinding, doses, and tamping, the former was statistically the most important to explain the caffeine behavior during extraction. Nonetheless, when considering the total amount of caffeine in cup an opposite order was observed. For instance, the authors reported caffeine content of 75.60, 98.97, and 128.79 mg/cup for fine, coarse-fine, and coarse coffee ground after 14 s of extraction. The volumes of coffee brew, after 14 s, were 10, 22, and 50 mL for fine, fine-coarse, and coarse powder, respectively. By using coarse coffee ground, a greater percolation rate (i.e. the amount of water that flowed through coffee cake in the unit of time), due to the large pores available, increased the extraction of caffeine. In spite of the use of fine coffee powder gives a greater particles-water contact area, the lower percolation rate reduced the total amount of caffeine in cup. The authors stated that these results proved that the major contribution to the total caffeine content of espresso coffee in cup was given by the percolation rate, rather than the grinding level.

Particle size (μm)	Grinding grade		
	Fine	Fine-coarse	Coarse
>600	0.21 ± 0.11	0.68 ± 0.22	2.93 ± 0.53
400 < X < 600	5.69 ± 1.26	13.80 ± 0.82	33.87 ± 1.63
250 < X < 400	32.00 ± 4.89	47.15 ± 14.12	35.64 ± 1.88
180 < X < 250	52.60 ± 6.12	37.18 ± 14.10	26.42 ± 1.07
<180	9.52 ± 3.21	1.19 ± 1.02	1.15 ± 0.85

Table 3. Distribution (%) of particle size in each grinding grade of coffee powder (mean values ± standard deviation) (adapted from Ref. [95]).

6. Coffee preparation: methods

The consumer preferences in terms of the sensorial properties of coffee are affected by different factors, such as culture, lifestyle, social behaviors, habits, and economic aspects. Moreover, more recently, the attention of consumers is focused on the outcomes of coffee intake on health and well-being of specific components, such as caffeine and bioactive compounds. In this contest, brewing methods and the extraction conditions are essential to obtain the desired chemical, sensorial, and healthy properties of coffee in cup. A wide literature, across the last 20 years, is available but often the published data are difficult to compare due to the difference in coffee preparation conditions. On the other hand, all authors revealed that there is not *“the best coffee preparation method”*, but every extraction has its own peculiar characteristics [20, 99–104]. In the following sections, we present the most relevant data and discussion on the different brewing methods and their effect of coffee beverage quality.

6.1. Brewing methods: geographical and cultural aspects

Depending on geographical origins and cultural traditions, different brewing techniques are commonly used to make a coffee cup in the world. Among the most important and popular, a coffee cup may be prepared as Espresso, Turkish, American, Moka, Neapolitan, and French press coffee. However, as reported by Petracco [105] under a physical point of view, the coffee preparations may be classified in three main methodologies: (1) the “original Italian method” under high pressure (i.e. Espresso and Moka); (2) infusion by pouring hot water on ground coffee followed by a filtration (i.e. Drip filter, French press or Plunger, and Neapolitan); (3) decoction methods (i.e. Turkish, boiled, percolator, and vacuum). All these methods noticeably affect the type and the amount of chemical compounds extracted, including the caffeine content. Drip filter coffee is the most popular brewing method in the world. It is largely diffused in USA, while in north Europe, France, and Scandinavian region, the plunger or French press coffees are the most consumed. When considering the southern European countries, a greater variability in the coffee brew methods is observed. The Turkish coffee is consumed in the Middle East, North Africa, Balkans, Greece, Turkey, and various locations within Eastern Europe [106]. In Italy, Spain, and Portugal, coffee cups are generally prepared by using the Espresso method and Moka [100, 102, 103, 107, 108]. The instant coffee, also known as soluble coffee, initially consumed mainly in Great Britain and Japan, later has been spread all over the world [20, 109, 110]. Finally, in the last 10 years, the single-dose pod or capsule system has gained interest for the preparation of coffee at home or at work [104, 111–113].

6.2. Variables affecting caffeine content

Once the blending of coffee varieties, the roasting level likewise the grinding degree has been chosen, obtaining the desired roasted-ground coffee, several brewing methods may be used to prepare our coffee cup. However, for all of these, the theoretical principle consists in a solid-liquid extraction of all chemical compounds from roasted-ground coffee (soluble solid) to hot water (solvent) [114]. Considering the brew preparation at coffee shops, bar, or at home, several variables may modify the coffee quality in cup. The type of contact between water and

coffee ground, the extraction time, the roasted-ground coffee/water mass ratio, the extract volume as well as water temperature, the vapor pressure in the case of Espresso coffee, filtration, and boiling process play important roles on the caffeine content of the beverage, as well as on functional and sensorial compounds [20, 102, 104, 105, 109, 115, 116]. First of all, the volume of the brew in cup is the variable exhibiting the wider variance mostly due to the personal appreciation. For instance, the coffee cup may vary from the “*Ristretto espresso coffee*” [117] of about 15–20 mL to the “*American filtered coffee*” of 125–400 mL [100]. This, of course, greatly affects the caffeine intake every day. Several studies indicated that the caffeine contents ranging from 2.4 to 4.5 mg/mL for Espresso (25 mL), from 0.4 to 1.4 mg/mL for American or filtered (200 mL), from 0.2 to 0.5 for French or Plunger (100 mL), from 0.7 to 5.4 mg/mL for Moka (30 mL), 1.6 mg/mL for Neapolitan (30 mL), and 1.94 mg/mL for Turkish (50 mL) coffee brews. This highlights that the volume of beverage per cup has a profound effect on the assumption of caffeine. A second variable, which significantly changes among the brewing method, is the powder/water (p/w) ratio. It was reported that 7 g/25 mL are commonly used to prepare an Italian espresso coffee, 12 g/200 mL are adopted for American or filtered, while 8 g/100 mL and 5 g/50 mL are used for French and Turkish coffee brew, respectively. Moreover, extraction time is subject to a huge variability. Taking into account the difference in coffee powder/water ratio, several authors highlighted that about 25 s are necessary to prepare an Espresso coffee and 5–7 min would be needed for American and French coffee brews [100–103, 107].

In the following sections, the most important brewing methods and the extraction conditions will be analyzed in detail, paying attention on the quality of the beverage, particularly concerning the caffeine content.

6.3. Espresso coffee

The Italian Espresso coffee (EC) is one of the most appreciated coffee brews, an intense aromatic beverage made for immediate consumption. EC may be defined as “*a brew obtained by percolation of hot water under pressure through compacted cake of roasted-ground coffee, where the energy of the water pressure is spent within the cake*” [84]. In general, an Espresso coffee (~25 mL) is prepared by ground roasted coffee beans (6.5 ± 1.5 g), by means of hot water ($90 \pm 5^\circ\text{C}$) 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 [105]. However, important differences are commonly observed, such as the so-called *ristretto* with a volume of brew < of 20 mL and the *lungo* espresso coffee > of 30 mL, which are often consumed in Italy and in other countries [117]. Apart from the above overall definition, the in-cup quality of espresso coffee and, particularly caffeine content, is affected by several variables under control of the *barista* (espresso coffee professional bartender) as shown in **Figure 5**.

After the choice of coffee blend and roasting degree, the first step to produce the EC brew is the grinding of the roasted beans at the optimal level. As previously reported, grinding level of roasted coffee powder greatly affects the caffeine content in coffee brew. In general, until a certain level of finesse, a decrease of the particle size of the coffee ground implies an increase of the caffeine content due to the larger surface area. For an espresso cup prepared by fine ground

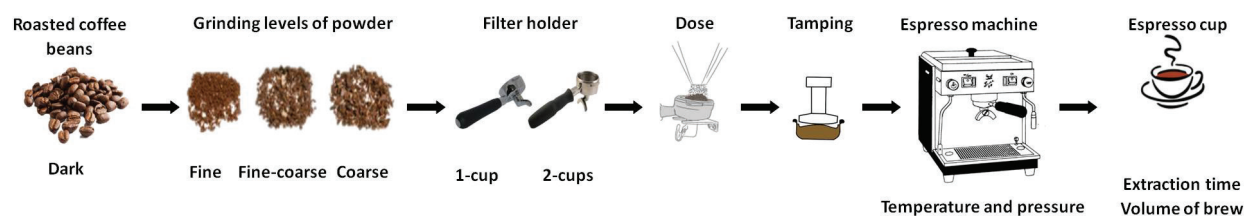


Figure 5. Espresso coffee: variables under control of the *barista*.

coffee, the caffeine content varies from 2.1 to 4.2 mg/mL, while using the coarse coffee powder, caffeine concentration was ranged from 0.5 to 3.2 mg/mL [90, 94, 95, 102]. Andueza et al. [94] reported that the caffeine in EC, from roasted coffee blend (20% Arabica – 80% Robusta), was of 3.80, 3.19, and 3.05 mg/mL for very fine, fine and coarse, ground coffee respectively. Severini et al. [90, 95, 96] confirmed that, maintaining constant the dose of coffee ground, the pressure on the upper surface of cake and the extraction time, the caffeine content in espresso was strictly correlated with the grinding level. In general, therefore, all authors agree with an increased caffeine extraction as finer is the ground coffee used for brewing. However, as reported below, several other variables may affect the caffeine concentration with some of these having a direct relationship, while others are inversely related. So, as explained in the following section, in some extraction conditions, the effect of grinding level could be also completely invalidated.

Among these, one of the most important is the dose of ground coffee, that is the amount of coffee powder used to prepare an espresso coffee cup. Romani et al. [118] reported that the dose of roasted coffee powder used to prepare a cup of espresso is found to be between 6 and 8 g, until a limit of 9 g. This variability has an important effect on the caffeine content of espresso coffee. Andueza et al. [116] highlighted that the caffeine content in EC cup is greatly affected by the quantity of coffee powder used. By preparing an EC of 40 mL, using 6.5, 7.5 and 8.5 g of ground coffee, the authors showed several differences in caffeine with values of 1.80, 1.88, and 2.21 mg/mL, respectively, when using doses of 100% Arabica coffee. Similarly, by using a blend of 20% Arabica – 80% Robusta, higher caffeine values of 3.01, 3.17, and 3.31 mg/mL were, respectively, obtained for the same doses.

However, the effect of ground coffee dose on caffeine concentration could be roughly analyzed without taking into account the corresponding amount of water used to prepare the brew (i.e. coffee powder/water mass ratio).

The analysis of this variable is correctly interpreted for brewing methods in which the amount of water is defined before coffee extraction, such as American coffee, Turkish, etc., but it would be wrongly analyzed for espresso coffee methods for which the variable ground coffee/water ratio is rather a ground coffee/brew volume ratio.

However, depending on the traditions, a cup of espresso coffee in Italy is of 20–25 mL, in Spain of 40–60 mL, and in Scotland about 30–50 mL. From these data, roasted coffee powder/water ratios were 7 g/20 mL, 9 g/60 mL, and 11 g/30 mL, and the caffeine contents resulted, in mean, of 5.4, 1.8, and 3.9 mg/mL, respectively [110].

Several studies highlighted that the increase in dose and/or grinding level, keeping constant the total volume of EC, determines an increase of caffeine concentration. Moreover, when the dose

of ground coffee is higher, being the powder/water surface greater, the percolation pathway for hot water through the compact cake is more tortuous, increasing the brewing time and more aromatic and chemical compounds in coffee beverage [90, 116].

Furthermore, the pressure on the upper surface of the coffee cake (tamping) is a step of crucial importance for both the microstructural properties of the coffee cake and, therefore, on the pathway of water during the percolation [119]. Severini et al. [90] highlighted that differences may be in the chemical composition of espresso cup, including the caffeine content, applying, for 5 s, different pressures (0.75, 1.5 and 2.25 kg) on the coffee cake. On these basis, the tamping step which could be underestimated at bar could have a significant effect on the caffeine content of espresso coffee independently from the grinding level and the coffee powder/water ratio. Also, several studies on the espresso machine conditions (pressure and temperature) are available. Masella et al. [120] studied the effect of temperature and pressure of water on the quality of espresso coffee. They found that the combination between three temperatures (75, 80, and 85°C) and two machine pressures (15 and 20 atm) not influenced the caffeine content of the coffee samples, showing an average value of 2.25 mg/mL. These data well agree with Andueza et al. [121] who tested three water pressures (7, 9, and 11 atm) on caffeine content of espresso coffee showing a mean value of 2.04 mg/mL. On the other hand, Salamanca et al. [122] proved that by applying a gradient of temperature to prepare an espresso cup across different varieties of roasted coffee, an increase or decrease of some chemical compounds was highlighted, among these the caffeine.

The extraction time is also a crucial variable in terms of chemical extraction. Nicoli et al. [1] divided the volume of beverage in five fractions of 10 mL each during espresso coffee preparation. In the first fraction, the highest caffeine concentration with a value of 6.5 mg/mL was observed, while a value of 0.2 mg/mL was found in the last fraction. According to these data, Mora and Rodriguez [123] reported that are necessary only 10 s to extract the 60% of caffeine from roasted coffee powder (100% Arabica, 100% Robusta, and blend) when preparing an EC cup of 30 mL. Ludwig et al. [101], monitoring some chemical compounds during the extraction time, measured a caffeine concentration of 4.36 and 0.57 mg/mL in the first 0–8 s and 16–24 s, respectively. Severini et al. [90, 95, 96] proved that the extraction time highly affected the aromatic and chemical compounds of EC brew. Taking into account the caffeine content, it was shown as during the first 8 s of extraction, the caffeine concentration was comparable with the value measured in a cup of 25 mL. Therefore, all authors agree with progressive reduction of extracted caffeine as a function of brewing time, obviously caused by the reduction of remaining caffeine in ground coffee. In general, all papers confirmed that the majority of caffeine is extracted in the first phase of brewing. For example, considering a fine roasted coffee powder, the caffeine concentration was of 4.98 and 4.18 mg/mL after 8 s of extraction (Volume ~16 mL) and in the final EC cup (25 mL), respectively. Of course, the reduction of 0.8 mg/mL of caffeine in the final cup is due to the dilution effect since in the last seconds of extraction, only water falls in the cup, in practice. Using a coffee powder fine, fine-coarse (or medium), and coarse, it was reported that 22, 15, and 10 s were necessary to produce a volume of 20–25 mL for espresso cup, and their caffeine content were of 4.2, 4, and 3.2 mg/mL, respectively [90].

Finally, when people consume espresso coffee at coffee shop, the *barista* can use two types of filter holder at 1 cup or 2 cups. Severini et al. [96], who studied the potential effect of some

variables under the control of *barista*, reported that the espresso coffees from 2-cups filter holder presented a higher amount of caffeine. This was explained by the higher extraction of water-soluble compounds as a consequence of the greater amount of coffee ground (~ 14 g) in the 2-cups filter holder respect to 1 cup (~ 7 g) [124]. As proved by the authors, during the first 8 s of percolation, the caffeine content in each coffee cup, prepared with fine-coarse (or medium) powder, resulted of 4.51 and 3.46 mg/mL for the beverage prepared with 2-cups and 1-cup filter holder, respectively [96].

On the basis of above discussion, we must state that although the initial choice of coffee blend and the roasting level are important factors affecting the chemical composition of coffee beverage, they are not able to definitively control the amount of caffeine in espresso coffee cup. Many other factors may also counterbalance their effects; likewise, some of these may exhibit an opposite effect. For instance, by increasing the dose of ground coffee, an increase of caffeine content would be expected, but a slight pressure on the surface of coffee cake and a negligible increase of brewing time could increase the amount of water falling in the cup reducing the concentration of caffeine. Therefore, several EC preparation factors should be taken into account contemporaneously, such as the grinding level of coffee powder, the dose, the tamping, the extraction rate, and the volume of extract. Each of them should be precisely defined to obtain the desired chemical compounds and caffeine concentration in EC.

6.4. American coffee

Drip filter or American coffee brew is prepared using an automatic machine (**Figure 6**) [125] equipped of a tank in which the water is heated ($92\text{--}96^\circ\text{C}$), a container in which, using a single-use paper filter, is placed the roasted coffee powder. At the bottom of the device, a glass flask collects the coffee beverage. Being the most diffused preparation coffee method in the world, a wide literature on American coffee brew is available. As for other preparation methods, several factors affect the caffeine content in filtered coffee, such as roasting degree, grinding level of ground coffee, dose of coffee, powder/water ratio, brewing time, and final volume of beverage. Tfouni et al. [126] evaluated that the caffeine concentration in filtered coffee brew, obtained by Brazilian coffee beans, roasted at two different levels (medium and dark), varies from 0.92 to 0.99 mg/mL and 1.23–1.65 mg/mL for Arabica and Robusta, respectively. However, another study on the two varieties reported that the amounts of caffeine in American coffee ranged from 0.35 to 1.07 mg/mL and 0.65 to 1.58 mg/mL for Guatemala (Arabica) and Vietnam (Robusta) coffee, respectively. Considering the same extraction time (375 s), the average content of caffeine in filtered coffee brew is about 0.57 and 1.15 mg/mL [101] for Arabica and Robusta, respectively. Bell et al. [93] reported that the finely ground coffee powder yielded a significantly higher caffeine content due to the larger surface area, they highlighted that by using a powder/water ratio of 0.023 g/mL, the caffeine concentration of 0.2, 0.35, and 0.40 mg/mL for coarse, medium, and fine ground coffee were observed, respectively. Also, a longer brewing time (from 3 to 10 min) implies a longer contact time between the water and coffee powder, leading a more complete caffeine extraction.

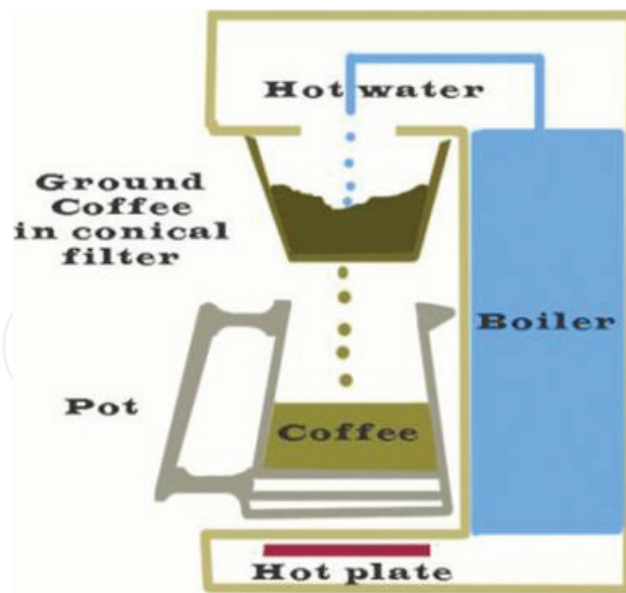


Figure 6. Schematic representation of an American coffee device [125].

6.5. Other coffee preparation methods

As previously reported, apart the preparation of espresso coffee, several other brewing methods may be used to prepare coffee beverages. Among these, some are widely used, popular, and very appreciated in the world, such as filtered coffee, while others are only linked to some cultural tradition and used exclusively in restricted geographical areas. In this section, we summarized the most important brewing method and their specific effects on caffeine content.

The most popular household coffee-brewing method in Italy is the Moka that uses a stove-top coffee maker invented in 1933 by the aluminum technologist, Alfonso Bialetti. Due to its low cost and easy-to-handle characteristics, Moka is used in other countries where it is called stove-top espresso or often misnamed mocha or mocca. In **Figure 7** [127, 128], the moka apparatus is shown, that consists of a metallic tank base, used as a water boiler, a metallic filter to contain the coffee powder, and the cylindrical tank on the upper part in which the coffee brew is collected. The extraction steps are also reported [127]. Boiling water is forced through the filter, containing the coffee ground, up to the tank in which is collected the coffee beverage. Nicoli et al. [1] highlighted that, using a roasted coffee blend and coffee powder/water ratio of 8 g/80 mL, the caffeine content in beverage was 2.56 mg/mL. López-Galilea et al. [100], by using a lower ratio of 40 g/500 mL, reported a caffeine content of 0.28 mg/mL. By using 100% Arabica roasted coffee, with a coffee powder/water ratio of 10 g/50 mL, a high value of caffeine content was found of 5.40 mg/mL [108], while a lower value of 1.68 mg/mL was found employing a ratio of 11.3 g/80 mL [103]. Another study showed that in 100% Arabica coffee brew from moka, with a p/w ratio of 7 g/110 mL, the caffeine content resulted to 0.75 mg/mL [102]. Briefly, exclusively considering the brewing method, the core of moka system is the coffee powder/water ratio used during brew preparation. Of course, when this is reduced, a less

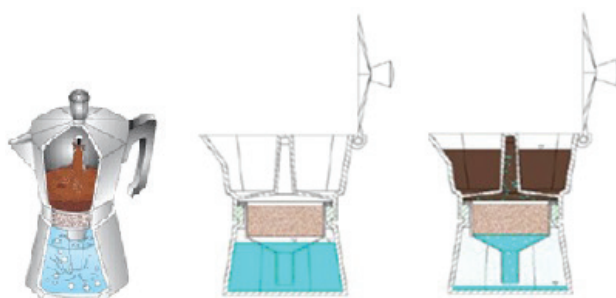


Figure 7. Schematic representation of the use of moka for coffee preparation (adapted from Refs. [127, 128]).

caffeine concentration in the brew is obtained. In these conditions, the total amount of caffeine intake should be linearly related with the volume of brew. Finally, it can be taken into account that not all water is used for coffee brew since that a small part of it remains in the metallic tank base, and another fraction remains in the wet spent coffee.

Another typical Italian method of coffee preparation consists in the use of the Neapolitan pot, also called *cuccumella* that in, Southern Italy, has been very popular. This method is based on the percolation of hot water under gravity through a bed of medium-coarse ground coffee. The *cuccumella* consists of a special coffee pot in aluminum, in which there is a tank filled with water at the bottom, a filter containing the unpressed ground coffee in the middle, and a tank which sealed the upper side of coffee pot on the top. The process consists of heating water in the boiler tank of the coffee pot. When the water reaches the boiling temperature quickly, the Neapolitan machine is overturned, enabling the hot water to percolate across coffee powder and to collect the brew in the upper tank, now down (**Figure 8**) [128, 129]. As reported from Santini et al. [108], 10 g of ground coffee and 50 mL of water are typically used to prepare Neapolitan coffee. According to the limited use of this method, which is restricted in some regional area of Italy, very few studies reported scientific data on the quality of coffee prepared with Neapolitan pot. By using a 100% Arabica roasted coffee, some researches highlighted that a caffeine content of 1.89 mg/mL was measured when using a roasted coffee powder/water ratio of 10 g/50 mL [108], while a value of 1.3 mg/mL was found using a ratio of 15.4 g/145 mL [103]. A coarse coffee powder is necessary to prepare a coffee cup, and after the filtration, the light brown beverage obtained resulted to be very similar to the American coffee.

French coffee, also known as European coffee, is prepared using the French press or plunger pot schematically depicted in **Figure 9** [128]. In this apparatus, the coarse-roasted coffee powder is soaked with hot water for 2 or 5 min, then a separation of ground spent coffee is made pushed down the wire-mesh filter (or plunger) toward the bottom of the tank. Finally, the infused coffee brew may be easily spilled in cup. Also in this case, very few experiments explored the caffeine content of French coffee. López-Galilea et al. [100], who prepared a plunger coffee brew using 40 g of roasted coffee powder and 500 mL of hot water, measured caffeine content of 0.20 mg/mL. Also, Gloess et al. [102] investigated the caffeine concentration of different obtained coffee brews, according to different extraction methods. Among these brewing techniques, the French coffee samples, prepared by 27.5 g of ground coffee in 500 mL of hot water (90°C) for an extraction time of 4 min, exhibited an average caffeine content of 0.49 mg/mL.

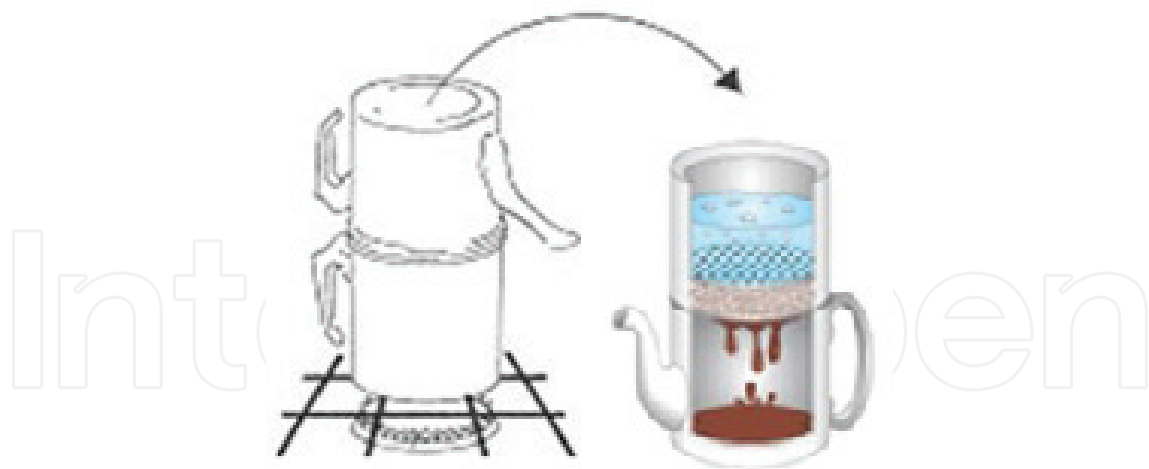


Figure 8. Schematic representation of Neapolitan coffee preparation with Neapolitan pot “Cuccumella” (adapted from Refs. [128, 129]).



Figure 9. Schematic representation of French coffee preparation (adapted from Ref. [128]).

Turkish coffee is the most ancient preparation method of coffee brew. Usually, roasted beans of *C. arabica*, after milling to obtain the finest powder, are boiled in a pot called “cezve” (**Figure 10**) [128] previously added with sugar. The coffee is served in a cup where the grounds are allowed to settle. The amount of water necessary for brewing is measured by using the coffee cups but, usually, is the range of 60–90 mL. For each cup, between 5 and 10 g of finest coffee powder are used [106]. A slow heating until the boiling is performed allowing the development of the foam on the beverage surface. Then, the process is interrupted for few seconds before repeating the boiling with the aim to facilitate the precipitation of insoluble compounds. Niseteo et al. [20] reported that the caffeine content of Turkish coffees prepared by using coffee powder/water ratio of 7 g/50 mL, resulted between 2 and 2.8 mg/mL. Similar results were found by Santini et al. [108], who found caffeine content of 1.9 mg/mL, by using 100% Arabica roasted coffee, with a coffee powder/water ratio of 10 g/100 mL.

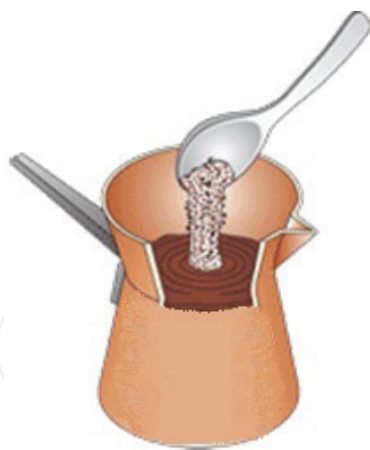


Figure 10. Cezve (adapted from Ref. [128]).

6.6. The use of soluble coffee

Instant, soluble or dried coffee is referred to the soluble portion of roasted-ground coffee, in either powder or granule form, which produces, in a very short time, a coffee beverage adding only hot water to the powder in cup [130]. The production of instant coffee involves the treatment of roasted-ground coffee with hot water and high pressure to extract the water-soluble compounds. Then, the obtained product is subjected to cooling, centrifugation, and concentration by heat and freeze drying at low temperatures [3]. Depending on the coffee species (Arabica or Robusta), roasted degree, and the extraction methods (using hot water or double-extraction, modulating temperature and pressure), different caffeine content may be observed. Vignoli et al. [109] reported that the caffeine content of dark soluble coffee resulted, for both extraction methods, as an average of 3.49 g/100 g and 4.82 g/100 g for Arabica and Robusta soluble coffee, respectively. Niseteo et al. [20] reported that, using a coffee powder/water ratio of 7 g/50 mL, the average caffeine content in two blends of instant coffee was of 4.5 mg/mL. Moreover, by studying eight different brands of instant coffee, Ludwig et al. [110] highlighted that the amount of caffeine content in coffee brews, prepared by 2 g of instant coffee dissolved in 125 mL of boiling water, ranged from 0.38 to 0.70 mg/mL, with an average value of 0.46 mg/mL.

6.7. Single service size systems: pods and capsule

In the last decade, a new coffee preparation method was developed to fulfill the increasing needs of consumers, such as convenience, high quality, quickness, and ease of use. The roasted coffee powder is dosed, tamped, and hermetically packaged following two methods: (1) pods, obtained by sealing the ground coffee between two layers of filter paper; (2) capsules of different size and shape but essentially in plastic or aluminum. The key factor of their success is to make possible for anyone to prepare a like-espresso coffee anytime and everywhere. However, the use of pods or capsules shows great differences, and each coffee brand has developed brewer machines with specific features, such as pressure, percolation time, water temperature, flow rate of water, etc. to obtain a quality of coffee as best as possible.

Several studies reported on the use of capsule or pods to make an espresso coffee cup. Albanese et al. [111] studied five blends of roasted coffee (100% Arabica (A), 100% Robusta (R), 80% A–20% R, 40% A–60% R, and 20% A–80% R) packaged in pods and extracted by three water temperature (90, 100, and 110°C) and their effects on chemical properties of espresso coffee brews (coffee powder/water ratio: 7 g/25 mL). As expected, the caffeine content was strictly depended from coffee blend; in fact, increasing the percentage of Robusta coffee, the caffeine content in the extracts resulted higher. In addition, the high temperature of water promoted the extraction of chemical compounds among which the caffeine. As reported, the caffeine contents in ECs were of 2.59 mg/mL (100% A) and 3.55 mg/mL (100% R) when extracted at water temperature of 90°C and 3.31 mg/mL (100% A) and 4.65 mg/mL (100% R), when extracted at water temperature of 110°C.

Bartel et al. [131] studied several single-service systems (pods and capsules) to prepare espresso coffee samples. The caffeine content for an espresso “lungo” (100% A) prepared by pods (coffee powder/water ratio: 6.9 g/115 mL) was 0.79 mg/mL, while similar values of 0.80 and 0.77 mg/mL were measured using plastic or aluminum capsules, respectively. However, it must be considered that the use of the above three systems implies the use of different extraction conditions with coffee powder/water ratios of 6.9 g/115 mL, 7.9 g/115 mL, and 5.2 g/85 mL for pods, plastic capsule, and aluminum capsules, respectively. Obviously, differences were also found in caffeine content for EC from coffee blend (35% A–62% R; coffee powder/water ratio: 5.2 g/85 mL) in aluminum capsule with a mean value of 1.08 mg/mL, while, Gloess et al. [102] reported that the caffeine content in a regular EC, from aluminum capsule (100% A; coffee powder/water ratio: 5.5 g/30 mL), resulted of 1.4 mg/mL.

A recent research reported that, using the same roasted coffee powder, comparing two single-dose capsule systems to the classic bar machine, the caffeine content in ECs resulted equivalent, having an average value of 2.22 mg/mL [112].

Another study observed that, keeping constant the particle size distribution, the pressure on the upper surface of coffee cake (i.e. the tamping) in different brands of single-dose capsule, may have an important effect on the extraction of caffeine in ECs due to the changes in microstructure of coffee cake [113].

A complex research on single-serve capsule brewer to prepare the American coffee highlighted that several parameters, such as the origin of raw material, the roasting degree, the particle size distribution of coffee powder, the dose in capsule and the cup volume, significantly affected the chemical and sensorial attributes of coffee brews [104]. Considering constant some variables, as the dose of coffee (8.9 g) and the grinding level (volume mean diameter = 734 μ m), the caffeine content in American coffee brews increased when the roasting level was high (dark > medium) and resulted lower when the volume of beverage increased from 113 to 226 mL.

Of course, independently on the extraction system used, classic coffee machine (i.e. Espresso or American) or single-dose systems, the same variables affect the chemical and sensorial properties of coffee brew, such as the grinding level, the dose of powder, the tamping (Espresso), the extraction time, and the volume of beverage.

7. How much caffeine in a single cup? Differences through brewing method and conditions

How much caffeine is actually assumed for coffee cup? Even though it is recognized that 50 mg of caffeine for cup and 4 cups/day (total amount of 200 mg/day) is acceptable for people, a real assessment of caffeine intake for consumers is very difficult. It is a non-trivial question in consideration of that it depends on the brew volume (i.e. how big is the cup), the grinding grade, the dose, the tamping, the brewing method used, how much coffee ground is used to prepare the brew, the coffee varieties, and blending. Of course, this could become a problem when considering that each people cannot know the total content of caffeine inside a cup consumed at home, at coffee shops, by self-service coffee machine, etc. Crozier et al. [12] did a snapshot of the variability of caffeine content of espresso coffees sold in several coffee shops. They reported that caffeine may vary of 6-fold from 51 mg/cup in Starbucks to 322 mg/cup in Patisserie Françoise. This impressive variability is certainly the result of different extraction conditions, mainly the dose but also grinding level, roasting conditions, volume of coffee cup, etc.

The web site caffeine informer [132] enables to examine the content in caffeine of hundreds of coffee brews sold by different brands. By sorting in ascending order, the first one is the Nescafé Ice Java having a caffeine content of 100 mg in 25 mL (4 mg/mL), while the last one is, as expected, the decaffeinated instant coffee with 2.5 mg in 236 mL (0.0106 mg/mL). In **Table 4** [132], the amount of caffeine for the most popular coffee brews and the related volume are resumed.

McCusker et al. [18] analyzing the caffeine content of “speciality” reported a great variability among coffee types as well as among coffees sold in different days but in the same coffee bar. As example, they reported caffeine doses of 75.8 mg and 140.4 mg for 1-shot (42 mL) and for 2 short shots (40 mL) of espresso coffee respectively, while, when a 1-shot of coffee (42 mL) was sold by Starbucks, a significantly lower content in caffeine of 58.1 mg was measured. Similarly, Crozier et al. [12], by considering espresso coffees sold by Starbucks, showed caffeine content of 51 mg for a serving size of 27 mL. This first data clearly indicate a great variability in caffeine content in cup among the brewing methods, total brew volume in cup as well as inside the same coffee shop.

McCusker et al. [18], analyzing some brands specialty coffees in a 16-oz cup (473 mL), reported caffeine content between 143.4 and 259.3 mg respectively for Dunkin’ Donuts and Starbucks. A very interesting finding was the high variability observed by analyzing Starbucks’ coffees, although it was expected a high standardization of preparation conditions. Particularly, analyzing six consecutive days, the authors reported caffeine contents between 259.2 and 564.4 mg for a 473 mL cup. Furthermore, by consulting the website of Starbucks [133] caffeine content of 155, 235, 319, and 410 mg are reported for a short 8-oz cup (236 mL), a “tall” coffee of 12-oz (354.72 mL), a “grande” coffee of 16-oz (472.96 mL), and a “venti” coffee of 20-oz (591.2 mL), respectively. By plotting the caffeine contents vs volume, we evaluate a direct linear relationship between volume and caffeine content but this was not confirmed by scientific literature. This was because in the coffee shop like Starbucks, coffee brew is continuously prepared and stored in big urn until where, of course, caffeine content is an average of several

Coffee brew	mL	Caffeine (mg)	mg/mL
Nescafe Ice Java	25.14	100	3.98
Black Insomnia Coffee	354.88	702	1.98
Coffee (Espresso)	44.36	77	1.74
Nespresso Coffee Capsules	39.92	60	1.50
Robusta Coffee	236.59	265	1.12
Turkish Coffee	59.15	50	0.85
Illy Issimo Cafe	201.10	155	0.77
Starbucks Grande Coffee	473.18	330	0.70
High Brew Coffee	236.59	163	0.69
Starbucks Doubleshot	192.23	125	0.65
Dunkin' Donuts Brewed Coffee	414.03	210	0.51
Starbucks Grande Caffè Americano	473.18	225	0.48
Americano Coffee	354.88	154	0.43
Caffè Mocha	354.88	152	0.43
Starbucks Grande Caffè Mocha	473.18	175	0.37
McDonalds (McCafé) Mocha	473.18	167	0.35
McDonalds Coffee	473.18	145	0.31
Starbucks Verismo Coffee Pods	236.59	60	0.25
Coffee (Instant)	236.59	57	0.24
Caffè Nero Coffee	354.88	80	0.23
Starbucks Decaf Coffee	473.18	25	0.05
Nescafé Ricoffy	236.59	6	0.03
Coffee (Decaf, Brewed)	236.59	6	0.03
Coffee (Decaf, Instant)	236.59	3	0.01

Table 4. Caffeine content in cup for coffee brews sold by several brands [132].

extractions. In these conditions, the average caffeine concentration will be exactly the same for each coffee cup, while the only significant variable becomes the total volume of beverage.

Ludwig et al. [101] studied the effect of brewing time and two different methods, such as espresso and filtered coffee brews, on caffeine content. Filtered coffee brews were prepared by using 36 g of ground coffee, 600 mL of water at 90°C, and a brewing time of 6

min. Espresso coffee samples were obtained employing a conventional coffee machine from 7 g of ground coffee for a brew volume of 45 mL. The authors separated coffee samples in 5 and 3 fraction for espresso and filtered coffee, respectively, and they analyzed the changes in caffeine concentration (mg/10 mL) and volumes (mL) of each brew fraction. For instance, caffeine content reduced from 85.44 mg (106.8 mg/100 mL) in the first fraction (75 s, 80 mL) to 23.14 mg (89 mg/100 mL) in the fifth fraction (75 s, 26 mL) in the case of filtered coffee. On the other hand, values from 47.50 mg (in the first fraction of 8 s and 16 mL) to 5.03 mg (for the third fraction of 8 s and 17 mL) were reported for espresso coffee. By using these data, it was possible to calculate that, for people consuming a 473 mL of coffee cup (i.e. a part of the total volume of 600 mL), a caffeine intake of 304.02 and 545.36 mg should be considered for filtered brew prepared by Guatemala and Vietnam coffee, respectively. Similarly, values of 63.63 and 131.98 mg were found assuming 45 mL of espresso, when using Guatemala and Vietnam coffee, respectively. These data are in accordance to that reported by McCusker et al. [18] for 1-shot of espresso coffee, while they were significantly higher for filtered coffee.

Parenti et al. [112] reported the differences in caffeine content of espresso coffee comparing different brewing techniques. The authors compared the espresso coffees from conventional bar machine, the hyper espresso method (HIP) and the I-Espresso (IE) capsule systems, reporting a total volume of 25–30 mL (with a flow rate of 1 mL/s) for conventional bar machine, while, for HIP and IE, the volume of EC brews was weighed until 25 g. However, if let us consider that the authors prepared a regular coffee of 25 mL for each type of brewing method, we estimated values of 55.5, 57.75, and 53.5 mg of caffeine for conventional bar machine, hyper espresso and IE systems, respectively. These data are lower than those previously discussed from Ludwig et al. [101], who used a lower total volume (25 vs. 45 mL) and a less amount of ground coffee (6.7 vs. 7 g).

Caporaso et al. [103] analyzed the caffeine content of Neapolitan, Moka, Espresso, and American (filtered) coffee. For espresso coffee (25 mL), a caffeine content of 60.95 mg for cup was obtained, being in good agreement with the findings of Parenti et al. [112]. On the other hand, no accordance there was for the American coffee samples for which the authors measured a dose of caffeine of 173.25 mg that is significantly lower than the data reported from both McCusker et al. [18] and Ludwig et al. [101]. However, this was mainly due to the changes in total volume of the brew that in the case of the paper of Caporaso and coauthors. was considered of 125 mL. By considering the caffeine concentration of 1.39 mg/mL as reported from the authors, it is possible to estimate a total content of caffeine for a 16-oz cup (473 mL) of 657.47 mg. This value is greater than those reported from McCusker et al. [18] and Ludwig et al. [101] as above reported for the same volume of coffee cup.

Relationship between caffeine content and four brewing procedures (filter, plunger, mocha, and espresso coffee method) were also studied by López-Galilea et al. [100]. Considering a commercial blend of Arabica and Robusta, caffeine concentrations of 0.22, 0.20, 0.28, and 0.63 mg/mL were measured for filter, plunger, mocha, and espresso coffee, respectively. From these data, total caffeine of 25, 140, 100, and 88 mg may be estimated for espresso (40 mL), mocha (500 mL), plunger (500 mL), and filtered coffee (400 mL), respectively.

All above data indicates two main aspects: (1) a very high variability of caffeine content in cup, also when we consider the same brewing conditions and (2) the difficulty to critically compare the literature data since they are obtained in different operative conditions. Particularly, for the latter consideration, the kinetic of caffeine extraction should be always taken into account by the researchers, who wish give information on caffeine intake as well as for each other chemical compounds. This is because the caffeine kinetic extraction is not linearly related with time. For instance, if we consider the data published by López-Galilea et al. [100], an average caffeine concentration of 0.22 mg/mL was measured for filtered coffee, leading to a total content of 88 mg in a 400 mL of total volume. However, the comparison of these results and those reported by McCusker et al. [18] and Ludwig et al. [101] who analyzed a 16-oz cup (473 mL) is not possible. This is because, in both papers any information on the kinetic of caffeine extraction were not reported. Severini et al. [90] studied how the variance of some extraction variable may affect the quality of espresso coffees served every day. The authors modeled the kinetic of caffeine extraction by changing the grinding (coarse, fine-coarse, and fine ground coffee), the dose (6, 7, and 8 g), and the tamping on the upper surface of coffee cake (0.75, 1.5, and 2.25 kg). **Figure 11** [90] reports the kinetic extraction of caffeine and its cumulative dose as a function of extraction time for sample prepared by coarse (grinding level, 7), fine-coarse (grinding level, 6.5), and fine ground coffee (grinding level, 6). The authors estimated total caffeine contents of a 25 mL cup as 77.4, 105.83, and 98.97 mg for brew prepared by coarse, fine-coarse, and fine ground coffee, respectively.

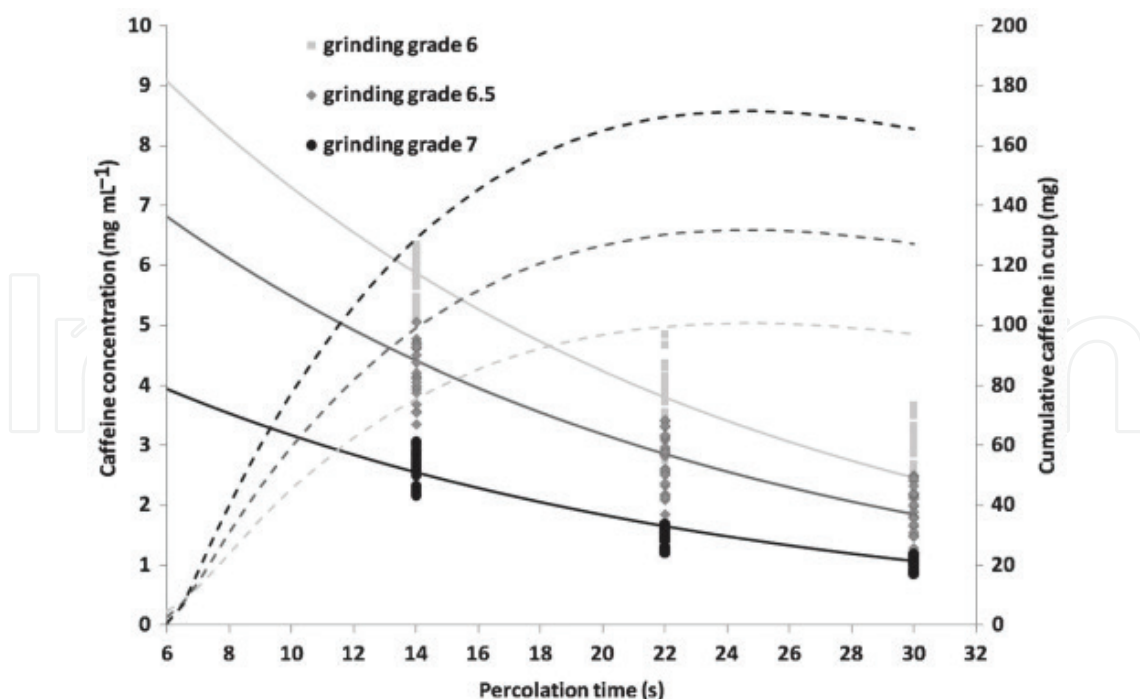


Figure 11. Changes in caffeine concentration of espresso coffee as a function of grinding level and extraction time [90].

8. Conclusion

From the huge number of researches and results, which we can find in literature, it becomes quite impossible to answer a simple question: how much caffeine we take with a cup of coffee?

Different cultivar, origins, agronomic conditions, post-harvest treatments, transport and conservation, as well as the blending before the roasting could affect the caffeine content in green coffee seeds.

The roasting process seems to be the only step almost irrelevant, because the caffeine remains more or less unaltered by the roasting temperature.

Each different operative condition, such as grinding level, dose of ground coffee, tamping, water temperature, water pressure, water/coffee ratio, extraction rate, volume of beverage, etc. could produce differences in the extraction kinetic of caffeine which should be considered when comparing the caffeine content in cups. Unfortunately, despite the wide bibliography concerning the caffeine content in coffee brew, few papers reported the differences in extraction kinetic of caffeine by changing type of coffee brewing.

Among all process parameters, doubtless the grinding level plays an important role for the caffeine content “in cup”, due to its effect on extraction kinetic. However, the considerable variability in the composition of the coffee beverage, as well as the significant differences in volume of a single coffee cup, makes it very hard to accurately define the average of daily intake of caffeine and of other bioactive constituents of coffee.

From the point of view of caffeine effects on human health, its content in coffee cup and its intake should be far to be a trouble. In the same way, it seems unjustified the choice of a pure variety of green coffee, based on the less content in caffeine.

Despite 20 years of reassuring researches, many people still avoid caffeinated coffee because they worry for the biological effects of caffeine [10].

A difficulty in interpreting epidemiological data is that some surveys were not specifically designed to quantify coffee consumption; thus, the debate about the coffee consumption, its beneficial or detrimental effect for human health, still persists. Pending that these encouraging observations could be confirmed and be widely spread, further experiments are needed particularly on the bioavailability of coffee components in order to elucidate their responsibility as well as the mechanisms involved in the observed positive effects. It may be concluded, therefore, that labeling the coffee as a harmful beverage and caffeine as a dangerous compound for human health lacks of support in the light of present knowledge.

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