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What kind of coffee do you drink? An investigation on effects of eight different extraction methods

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ABSTRACT

The chemical composition of brewed coffee depends on numerous factors: the beans, post-harvest processing and, finally, the extraction method. In recent decades, numerous coffee-based beverages, obtained using different extraction techniques have entered the market. This study characterizes and compares eight extraction coffee methods from a chemical-physical point of view, starting from the same raw material. Specifically, three types of Espresso, Moka, French Press, and 3 filter coffee that for the first time are reported in the scientific literature Cold Brew, V60, and Aeropress are compared.

Physical measurements included the quantification of total dissolved solids, density, pH, conductivity, and viscosity. Chemical analyses identified 15 chlorogenic acids (CGAs): six caffeoylquinic acids, one p-Coumaroylquinic acid, one Feruloylquinic Acid, four Caffeoylquinic lactones, and three Dicaffeoylquinic acids. Maximum caffeine and CGA concentrations were found in Espresso coffees, while Moka and filtered coffees were three to six times less concentrated. The classic Espresso method was most efficient for caffeine and CGA recovery, with a yield almost double that of other methods. Per-cup caffeine and CGAs were higher in Cold Brew than Espresso coffees, as a function of the volume of beverage, which ranged from 30 mL (for espresso) to 120 mL (for filtered coffees). In light of these results, it is not possible to establish how many cups of coffee can be consumed per day without exceeding the recommended doses, since according to the applied brewing method, the content of the bioactive substances varies considerably.

1. Introduction

Coffee is one of the most widely-consumed beverages worldwide (ICO, 2016), and numerous brewing and extraction methods are used depending on the geographic, cultural and social context, not to mention personal preferences. Typically, its preparation involves three main stages. First, the green beans are roasted. Following this, the roasted beans are ground to facilitate extraction during the final, brewing, stage. In beverage form, quality characteristics such as smell, taste, color, and body are relevant, and highly appreciated attributes (Nunes, Coimbra, Duarte, & Delgadillo, 1997). The flavor of a freshly-prepared cup of coffee is the final expression, and perceptible result of a long chain of transformations (Yeretzian, Jordan, Badoud, & Lindinger, 2002).

This complex beverage contains over 1000 compounds that are responsible for its pleasant flavor and aroma (Nijssen, Visscher, Maarse, Willemsense, & Boelens, 1996). Of these, caffeine (1,3,7-trimethylxanthine) is the most widely studied. Caffeine exerts most of its biological effects through the antagonism of the adenosine receptor inducing generally stimulatory effect in the central nervous system (Bae, Park, Im, & Song, 2014; Cano-Marquinaa, Tarínb, & Canoc, 2013). Infact, its positive effects are well-known; in particular, improvements related to cognitive abilities such as better perception, reduced tiredness, and shorter duration of sleep (Borota et al., 2014). Recently, it was demonstrated that the risk of Alzheimer disease was lower in those who regularly consume caffeine-containing coffee than those who did not drink it. In addition, the physiolocal effects of caffeine intake include acute elevation of blood pressure, increasing metabolic rate and diuresis (Bae et al., 2014).

The alkaloid is heat stable, and the amount present in raw coffee can vary significantly depending on many factors, among which the most important are origin and cultivar. Its concentration and biological

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activity depend on a blend of factors, such as raw materials (Arabica or Canephora) (Severini, Derossi, Ricci, Fiore, & Caporizzi, 2017), agricultural practices (traditional or organic), post-harvest techniques (wet or dry), duration and conditions of storage, roasting degree (light, medium, or dark), roasting process (standard or torrefacto), type of commercial coffee (ground roasted or instant), and grinding and brewing method (boiled, filtered, or expresso). Altogether, this means that we never drink two cups of coffee with the same chemical composition, even when they come from the same outlet (De Mejia & Ramirez-Mares, 2014).

Many studies have demonstrated that coffee is one of the most important sources of polyphenols and caffeoylquinic acids (CQAs) (Kamiyama, Moon, Jang, & Shibamoto, 2015).

The major polyphenol in coffee in chlorogenic acid and it is one of the major strong antioxidant compounds in coffee (Bae et al., 2014).

It is known that, on average, about one third of the ingested amount of chlorogenic acids through coffee can be absorbed in the human gastrointestinal tract, metabolized in the stomach, intestine, liver, and kidney and can probably exert a series of beneficial biological properties in the body, explaining at least partially why coffee consumption has been associated with higher longevity and lower incidence of various degenerative and nondegenerative diseases in epidemiological studies (Farah & Duarte, 2015).

These water-soluble acids are abundant in coffee, and they are formed by the coffee plant through esterification of trans-cinnamic acids (most notably caffeic, ferulic, and p-coumaric) with quinic acid (Higdon & Frei, 2006). CGAs and their derivatives are known to contribute to the acidity, astringency, and bitterness of the final coffee beverage (Scholz & Maier, 1990; Trugo & Macrae, 1984). The main CGAs are 5-O-caffeoylquinic acid (5-CQA), and its isomers 3-O-caffeoylquinic acid (3-CQA) and 4-O-caffeoylquinic acid (4-CQA), which together account for 80% of total CGAs (Farah & Donangelo, 2006; Moeenfard, Rocha, & Alves, 2014).

Coffee preparation is a solid–liquid extraction process, involving: (1) water absorption by ground coffee; (2) mass transfer of soluble solids from ground coffee into hot water; and (3) separation of the resulting extract from spent solids. Several variables can modify in-cup coffee quality, including the contact time between the water and ground coffee, extraction time, the ground coffee/water ratio, water temperature and pressure (for espresso coffee), type of filter, and the boiling process. All of these factors play important roles in modifying caffeine content and other compounds (Andueza et al., 2003; Andueza, Vila, De Peña, & Cid, 2007; Gloess et al., 2013; Niseteo, Komes, Belščak-Cvitanović, Horžić, & Budeč, 2012).

There are many ways to prepare coffee and consumer preferences for a particular mode are influenced by various factors such as lifestyle, culture, and flavor preferences (Illy & Viani, 2005). Of the various brewing methods that use pressure, the most famous is the espresso machine. Espresso coffee (EC) is one of the most appreciated brews; the term *espresso* is derived from the Italian word for 'express' since expresso is made for, and served immediately to, the customer. EC is prepared on request from roasted and ground coffee beans. A limited amount of pressurized hot water quickly percolates through a ground coffee cake to yield a small cup of concentrated foamy beverage (Petracco, 2001). The original EC formulation used 7 g of coffee powder to obtain around 30 g of expresso beverage. Nowadays, there are many different recipes, of which the most popular is specialty coffee. This preparation uses 7 g of coffee powder to produce 14 g of expresso beverage. As every gram of ground coffee turns into 2 g of liquid the final beverage is a strong expresso with an extraction formula of 50% (SCAA 2016). Recently, a new expresso brewing method, namely *Caffè Firenze* (EU Patent 06023798.9; US 2010/0034942 A1) has been developed, which uses a sealed chamber and pressurized air (Masella et al., 2015). Another pressurized method is the Moka pot. Traditionally, this is the most popular method in Italian homes as the machine is cheap, and it is quick to brew. However, quality is often compromised as the risk of over or under extraction is high (depending on the grind).

Lungo is an alternative to EC. This less-intense beverage is characterized by a different water/ ground coffee ratio and a larger cup size (100–250 mL), depending on cultural habits. Numerous brewing methods may be used to prepare lungo coffee: steeping using a French press, filtration or dripping in the V60, Aeropress and cold drip technique, and boiling.

Standard preparation methods have been developed for different types of extraction. These methods differ in terms of the process, grams of coffee, amount of water, and grain size of ground coffee. Several studies have compared these different techniques, and described the physicochemical attributes and sensory profile of the coffees that are produced (Andueza et al., 2003; Caporaso, Genovese, Canela, Civitella, & Sacchi, 2014; Gloess et al., 2013; Masella et al., 2015; Parenti et al., 2014). These studies reveal that there is no 'best' extraction method, but that each technique has its own characteristics. This study extends the literature and examines several new brewing techniques that are already well-known by baristas and consumers, but for which there are, as yet, no data.

The aim was to describe and compare eight extraction methods: three espresso systems, classic (EC), specialty espresso (ECS), and *Caffè Firenze* (ECF); one cold brew system (Cold Brew); and four filter methods (V60, Aeropress, French Press, and Moka) that use different pressures and filter techniques. These methods were characterized by the analysis of physicochemical parameters. This was supplemented by an in-depth investigation of caffeine and CGA content based on high-performance liquid chromatography with diode-array detector (HPLC-DAD) analyses. Quantitative data related to bioactive substances were expressed as concentration (mg/mL of beverage), extractive capacity (mg/g of ground coffee) and per-cup dosage (mg/cup).

This study provides a comprehensive scientific overview of the most common coffee extraction methods currently used worldwide. It compares eight different extraction methods in term of it provides the concentration (mg/mL), extraction capacity (mg/g), and per-cup content of caffeine and CGA. To the best of our knowledge, this is the first time that data for Cold Brew, V60, and Aeropress techniques are reported in the literature.

2. Materials and methods

2.1. Experimental design

The experiment was designed to highlight differences between extraction methods in terms of the physicochemical characteristics of brewed coffee, and its sensory aspects. A specific recipe was followed for each of the eight methods. Standardized procedures were developed that differed in terms of the grind, the amount of coffee used, water temperature and, last but not least, the equipment. The extraction parameters were summarized in Table 1. Six replicates were performed for each brewing method. The order of beverage preparation was completely randomized.

2.2. Coffee samples and extraction methods

The same batch of 100% Arabica coffee (Ethiopian, Gera Estate) was used for all extractions. Each pack of beans (250 g) was opened immediately before brewing to avoid oxidative damage. Beans were ground using a professional grinder (EK43 Mahlkönig AG, Switzerland). Coarse-ground coffee was used for all lungo and filter methods (Clarke & Vitzthum, 2008), while a fine grind was used for expresso and Moka methods. Size distribution was analyzed using laser diffractometry, which is suitable for ground coffee particles ranging from 5 to 2000 µm. As water quality plays an important role in coffee beverage quality (Navarini & Rivetti, 2010) all samples were prepared using the same commercial brand of mineral water.

Table 1
Extraction parameters: extraction method [1], grind, amount of ground coffee in grams, volume of water per cup or jug in milliliters, temperature in degrees centigrade, pressure in bar, time in seconds, total amount of beverage in milliliters, and extraction %.

Extraction method	Grinding level	Powder (g)	Water (mL)	Temperature (°C)	Pressure (bar)	Time	Beverage (g)	Extraction%
EC	Fine	14	-	93	9	27 ± 1.7(s)	29.6 ± 1.7	22.8 ± 1.3
ECF	Fine	15	-	92	20	70 ± 10(s)	30 ± 5	13.1 ± 1.6
ECS	Fine	18	-	93	9	26.50 ± 1.8(s)	17.4 ± 1.6	17.5 ± 0.9
Moka	Fine	15	150	100	1.5	2.13 ± 0.13 (min)	134 ± 1.8	28.4 ± 1.1
V60	Coarse	15	250	93	1	2.3 ± 0.1(min)	206 ± 5	22.1 ± 0.7
Cold Brew	Coarse	25	250	20	1	4.7 ± 0.1(h)	199 ± 10	23.3 ± 0.9
Aeropress	Coarse	16.5	250	93	1	1.35 ± 0.08(min)	212 ± 4	20.4 ± 1.2
French Press	Coarse	15	250	93	1	5(min)	199 ± 4	18.7 ± 1.1

[1] EC, espresso coffee; ECS, specialty espresso, ECF, Caffè Firenze;

2.3. EC Espresso classical method

A conventional bar machine (GS3, LaMarzocco, Italy) was used. Two cups of EC were prepared (14.5 ± 0.2 g). Physicochemical analyses were only performed on one of the two ECs. Extraction parameters were: water temperature 92 °C, water pressure 9 bar, and 30 s of percolation time, assuming an optimal flow rate of about 1 mL s^{-1} (Illy & Viani, 2005).

2.4. ECS Espresso Specialty method

ECS was produced with the bar machine described above. This preparation follows the Specialty Coffee Association of America (SCAA) standard procedure (SCAA, 2016), and differs from the classic method in two respects: more coffee powder (18 g), and slower percolation (25 s).

2.5. ECF Espresso Caffè Firenze

Caffè Firenze (ECF) samples (Patent 06023798.9; US 2010/0034942 A1) were produced following the procedure given in Masella et al., 2015. The method uses a sealed extraction chamber in which water and air are at higher pressures than other extraction methods, resulting in a pronounced difference in foam characteristics.

2.6. Cold Brew

Samples were prepared using cold drip equipment with 25 g coffee powder and 250 mL mineral water at room temperature (22 °C). Equipment comprised three parts. An upper (glass) section, containing water, was equipped with a tap. The tap was used to control the flow rate and extraction time. The coffee/water mixture was placed in a central container. Water entering from above passed through a filter and into a lower carafe, where the final brew was collected. Spent coffee grounds were retained in the filter. The average extraction time was approximately 5.5–6 h.

2.7. Moka

A three-cup expresso maker was used (Bialetti Industrie SpA, Italy). Moka is the most popular technique in Italian households. Samples were produced following the procedure given in Navarini, Nobile, Pinto, Scheri, & Suggi-Liverani, 2009.

2.8. French press

Coarse-ground coffee (25 g) and hot water (250 g at 95 °C) were mixed in a brewer fitted with a mesh plunger. The mixture was brewed for 5 min, then the plunger was pressed to trap coffee grounds at the bottom of the container, following the SCAA standard procedure *(SCAA 2016)*.

2.9. V60

This coffee maker consists of three parts: a cone-shaped upper dripper with ridges along the inner edges and a single, large hole at the bottom, a paper filter, and a glass vessel (Hario server, 300 mL). Water was poured into the V60 to create a small crater in the middle of the ground coffee. Next, 70 mL of water at 98 °C, w*as* poured over the coffee, which was left to pre-infuse for 30 s. Finally, 180 mL of water was added in concentric circles and left to drawdown for three minutes. The brew ratio was 60 g/L.

2.10. Aeropress

The Aeropress was invented in 2005 by Aerobie; the device consists of two nested cylinders. One has a flexible airtight seal, and fits inside the larger cylinder, similar to a syringe. The procedure was as follows: first, 16.5 g of ground coffee was put into the cylinder, and then 250 mL of water at 93 °C was added. Coffee was steeped for one minute and then forced through a filter by pressing the plunger through the tube. Paper filters were used. The average quantity of beverage obtained was 215 mL.

2.11. Physicochemical analyses

2.11.1. Physical analyses

All samples were brought to 20 °C before selected parameters were analyzed and evaluated. A digital pH meter (GLP 21, Crison Instruments, Spain) was used to determine pH. Viscosity was measured with a capillary viscometer (Ostwald-type) fitted with an automatic optical reader (ViscoClock, Schott Instruments, Germany) and expressed as mN s m^{-2}. Relative density was measured with a 25 mL pycnometer. Total dissolved solids (TDS) was measured using a refractometer (VST LAB Coffee III Refractometer, USA) to calculate extraction yields. TDS was converted into the total percentage of ground coffee dissolved in the brewed coffee: Total Coffee Brewed (g) * TDS % / powder used (g).

2.11.2. Analyses of caffeine and CGAs

Coffee samples were centrifuged at 12000 rpm for 5 min and diluted 1:10 with water before HPLC-DAD analysis.

HPLC was carried out using an Agilent HP 1100 system equipped with an autosampler, column heater module and quaternary pump, coupled to a diode array detector (DAD) all from Agilent Technologies (Palo Alto, CA, USA). A 150 mm × 3 mm i.d., 2.7 μm Poroshell 120, EC-C18 column (Agilent Technologies) was used, equipped with a pre-column of the same phase, and maintained at room temperature. Injection volume was 5 μL. The elution method was performed at a flow rate of 0.4 mL/min using water at pH 3.2 by formic acid (solvent A) and acetonitrile (solvent B). All solvents used were Chromasolv for HPLC grade (Sigma Aldrich S.R.L). The multistep linear solvent gradient technique is described in detail in Angeloni et al. (2018). Starting from

95% A, up to 10% A, over 24 min (the total analysis time) UV–vis spectra were recorded in the range 220–600 nm. Chromatograms were registered at 330 nm for CGAs, and 278 nm for caffeine. Caffeine and CGAs were identified by comparing their retention times, UV–vis spectra to those of the respective standard, when it was possible, or with published data (Angeloni et al. 2018). CGAs were evaluated by HPLC- DAD using a five-point calibration curve of chlorogenic acid (purity 99%) (Extrasynthèse, Genay, France) at 330 nm (0–1.776 μg; $r^2 = 0.9991$) and caffeine content was determined by HPLC-DAD using a six-point calibration curve from Extrasynthèse (purity 95%) at 278 nm (0–0.632 μg; $r^2 = 0.9994$).

Quantitative data related to bioactive substances were expressed as concentrations (mg/mL of beverage), extractive capacity (mg/g of coffee powder) and per-cup dosage (mg/cup).

2.12. Cluster analysis

Cluster analysis is an exploratory, multivariate technique used to explore the data structure and overall characteristics when little (or even no) information about group structure is available (Ares, 2014). It is a convenient method for identifying homogenous groups of objects. Objects (in our case, brewing methods) in a specific cluster share many characteristics and are dissimilar to objects not belonging to that cluster (Sarstedt & Mooi, 2014). It is a hierarchical approach, based on the determination of the distance between objects (degree of similarity/ dissimilarity), and the application of an agglomerative (amalgamation) method to establish clusters of n-objects. Variables included in the analysis were physical measurements, and concentrations (mg/mL) of caffeine and CGAs for each brewing method.

2.13. Statistical analyses

Conventional analysis of variance (ANOVA) was used to compare means and standard deviation determined for the different extraction methods. The tested factors were considered significantly different at $p < .05$. All statistical analyses were performed using R software (version 3.4.0 for Windows).

3. Results and discussion

Extraction parameters were optimized for each brewing method in order to follow, as closely as possible, the settings used by baristas, while guaranteeing the best possible comparability.

3.1. Cluster analysis

Homogenous groups of brewing techniques were identified by a cluster analysis. As shown in Fig. 1, cluster analysis made it possible to divide the eight methods into two main groups, with four subclasses in each group: the first group comprised Cold Brew, Aeropress, French Press, and V60 and a second included Moka, ECF, ECS, and EC.

Similar concentrations were frequently found for these two groups of extraction methods. Within the filter group the French Press method could be distinguished from the other methods, probably due to a different time of extraction and temperature, as reported in Table 1.

Within the expresso group, another differentiation was found between ECS–EC and ECF–Moka, confirmed by the results of physico-chemical analyses.

As expected, EC and ECS resulted similar because the extraction method was the same and the only difference it was in the ratio of powder/water.

3.2. Physical analyses

The physical characterization of the coffee beverage produced using the different preparation methods is shown in Table 2. This analysis highlighted significant differences between the eight brewing methods for TDS %, extraction %, and viscosity. Concerning TDS %, the highest values were found for ECS followed by EC, Moka and ECF methods. No difference was found among the remaining extractive methods, where values were lower. TDS % directly correlates with coffee strength: high TDS % is consistent with a strong brew. It reflects the level of extraction of the coffee. High temperature and pressure increase extraction yield and rate, seen in the difference between expresso and Moka coffees, and filtered brews (López-Galilea, de Peña, & Cid, 2007). It is well-known that TDS % affects the sensory property described as 'body' (Gloess et al., 2013), and seems to be related to the coffee/water ratio (Andueza et al., 2007), and the brewing procedure (López-Galilea et al., 2007). Although the literature contains no data related to TDS, this factor is employed by baristas, and is recommended by SCAA to assess the correct degree of extraction.

Concerning extraction %, the highest value was found for Moka (28.6 ± 1%) and the lowest value for ECF. Intermediate values were recorded for the other two expresso preparations, EC and ECS. Percentages were similar for Cold Brew and Aeropress, although different quantities of ground coffee were used. The value for the V60 method was similar to the EC method, and the value for the French Press method was similar to the ECS method. SCAA guidelines state that extraction % should be in the range 18–23%. Our data is generally consistent with this range, except for ECF (which appears to be under-extracted), and Moka (which appears to be over-extracted).

Relating viscosity, Moka and ECF were similar to each other but different from other expresso coffees. No significant differences were found among the remaining methods (V60, Aeropress, Cold Brew, and French Press).

No significant differences were found for densities, which were around 1.05 g/mL, and for pH values, which were around 5.16.

3.3. Chemical analyses

The qualitative profile of bioactive substances detected by HPLC-DAD was almost the same for all samples. A total of 15 CGAs were detected. Fig. 2 presents chromatographic profiles at 278 and 330 nm. Peaks were identified based on UV spectra and elution/retention sequences reported in the literature, and confirmed by their mass spectrometric behavior, as reported in our earlier work (Angeloni et al., 2018).

Fujioka and Shibamoto (2008) report that the most abundant CGAs in coffee are caffeoylquinic acids (CQAs), notably 5-O-caffeoylquinic (5-CQA) followed by its isomers 3-and 4-CQA. Dicaffeoylquinic acid (3,4-, 3,5- and 4,5-diCQA), feruloylquinic acid (3-, 4-and 5-FQA), diferuloylquinic acid (dFQA) and p-coumaroylquinic acid (3-, 4- and 5-p-CoQA) isomers were also found in our samples, although less abundant.

Any comparison of caffeine and CGAs must take into consideration the fact that every operational condition (e.g. particle size and dose of ground coffee, tamping, water temperature and pressure, coffee/ water ratio, and the final volume of the drink) create considerable differences in bioactive compound extraction kinetics. Of these, one of the most important factors is the ratio of ground coffee to the final volume of water (Andueza et al., 2007). For this reason, the results of chemical analyses are presented in three ways: concentration (mg/mL), extraction efficiency (mg/g of ground coffee), and total bioactive content per cup (mg/cup), (Tables 3, 4, and 5 respectively). Furthermore, Fig. 3 (a, b, and c) reports mean values for caffeine and total CGAs.

3.3.1. Concentration of bioactive compounds (mg/mL)

Table 3 shows that there was a significant difference in caffeine concentration for the methods tested ($p \leq .05$). Values were highest for ECS and EC, on the contrary lowest concentrations were observed for Aeropress, V60 and French Press methods. Significant differences were found between these groups and other extraction methods (Cold Brew, ECF, and Moka).

Fig. 1. Cluster analysis of extraction methods. List of acronyms: EC, espresso coffee; ECS, specialty espresso, ECF, Caffè Firenze;

These data agree with Severini (2017), who assessed the main variables that affect caffeine concentrations in coffee-based beverages. Several studies have indicated that caffeine content ranges from 2.4 to 4.5 mg/mL for expresso (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), and from 0.7 to 5.4 mg/mL for Moka (30 mL) (Caporaso et al., 2014; López-Galilea et al., 2007). Caffeine is moderately soluble in water at room temperature 20 °C (1.46 mg/mL), it increases at 80 °C (180 mg/mL), but becomes very soluble at 100 °C (670 mg/mL) (Prankerd, 2007). Despite the lower solubility of caffeine in water at room temperature, data for the Cold Brew method shows that concentrations are similar to Moka and ECF. This fact could be explained by the extensive contact time between water and the ground coffee (around six hours). Regarding ECF, the lower caffeine concentration could be due to the fact that the chamber in which the coffee panel was placed in direct contact with water at 75 °C (Masella et al., 2015). Consequently, water that is in contact with the coffee panel is at a lower temperature than classic espresso.

Concerning CGAs, CQAs dominated for all preparations ranging about 75% of the total, followed by CQLs (about 12%) then di-CQAs (about 7%), 5-FQAs (about 4.5%) and finally 5-pCoQAs (about 1.5%) according to previous literature data (Ludwig et al., 2012). Moreover, 5-CQA was always the most abundant compound, ranging from 35 to 39% of total CGAs (for ECF and Moka, respectively), followed by 4-CQA and 3-CQA. CGA concentrations followed the trend observed for caffeine. For all 15 CGAs, values were highest for EC and ECS preparations. An interesting finding is that ECF, Cold Brew, and Moka methods have a mean total CGA concentration that is significantly different from the other two expresso methods, and from Aeropress, French Press and V60 preparations ($p \leq .05$). Intermediate values were found for the latter (Table 3 and Fig. 3a). Several studies have assessed the influence of contact time and brew ratio on bioactive compound extraction (Andueza et al., 2007; Caprioli, Cortese, Sagratini, & Vittori, 2015; Crozier, Jaganath, & Clifford, 2009). The results show that most extractable compounds are brought into solution in the first few seconds of the extraction process under higher pressure, as previously reported by Ludwig et al., 2012, that evidenced the technological differences between espresso and filter coffeemaker. This could explain the highest CGA concentrations in EC and ECS coffees compared to the other preparation methods.

These trends agree with the results reported by Gloess et al. (2013), in which the highest concentration of CGAs was reported for espresso,

Table 2
Physical characterization of coffee beverages[1,2].

	pH	TDS %	Extraction %	Density 20°(g/mL)	Viscosity (mN s m $^{-2}$)
ECF	5.16 ± 0.10 a	3.32 ± 0.40 a	13.46 ± 1.56 a	1.02 ± 0.03 a	115.15 ± 3.29a
ECS	5.30 ± 0.25 a	8.44 ± 0.38 b	17.54 ± 0.86 b	1.01 ± 0.01 a	151.59 ± 7.01b
EC	5.17 ± 0.07 a	5.20 ± 0.35 c	22.59 ± 1.51 c	1.04 ± 0.03 a	123.13 ± 2.70c
V60	5.15 ± 0.12 a	1.55 ± 0.04 d	22.14 ± 0.65 c	1.07 ± 0.09 a	99.76 ± 3.44d
Cold Brew	5.12 ± 0.10 a	1.54 ± 0.06 d	20.89 ± 0.82 d	1.05 ± 0.05 a	100.83 ± 2.40d
Aeropress	5.16 ± 0.11 a	1.52 ± 0.06 d	20.56 ± 0.67 d	1.06 ± 0.05 a	101.74 ± 2.62d
French Press	5.16 ± 0.13 a	1.35 ± 0.03 d	18.61 ± 1.20 b	1.07 ± 0.07 a	98.25 ± 3.97d
Moka	5.10 ± 0.24 a	3.40 ± 0.15 a	28.60 ± 1.03 e	1.06 ± 0.02 a	111.61 ± 2.56a

[1] Data are expressed as mean ± standard deviation. Letters (a,b,c,d,e) indicate statistically significant differences between extraction methods.
[2] EC, espresso coffee; ECS, specialty espresso, ECF, Caffè Firenze.

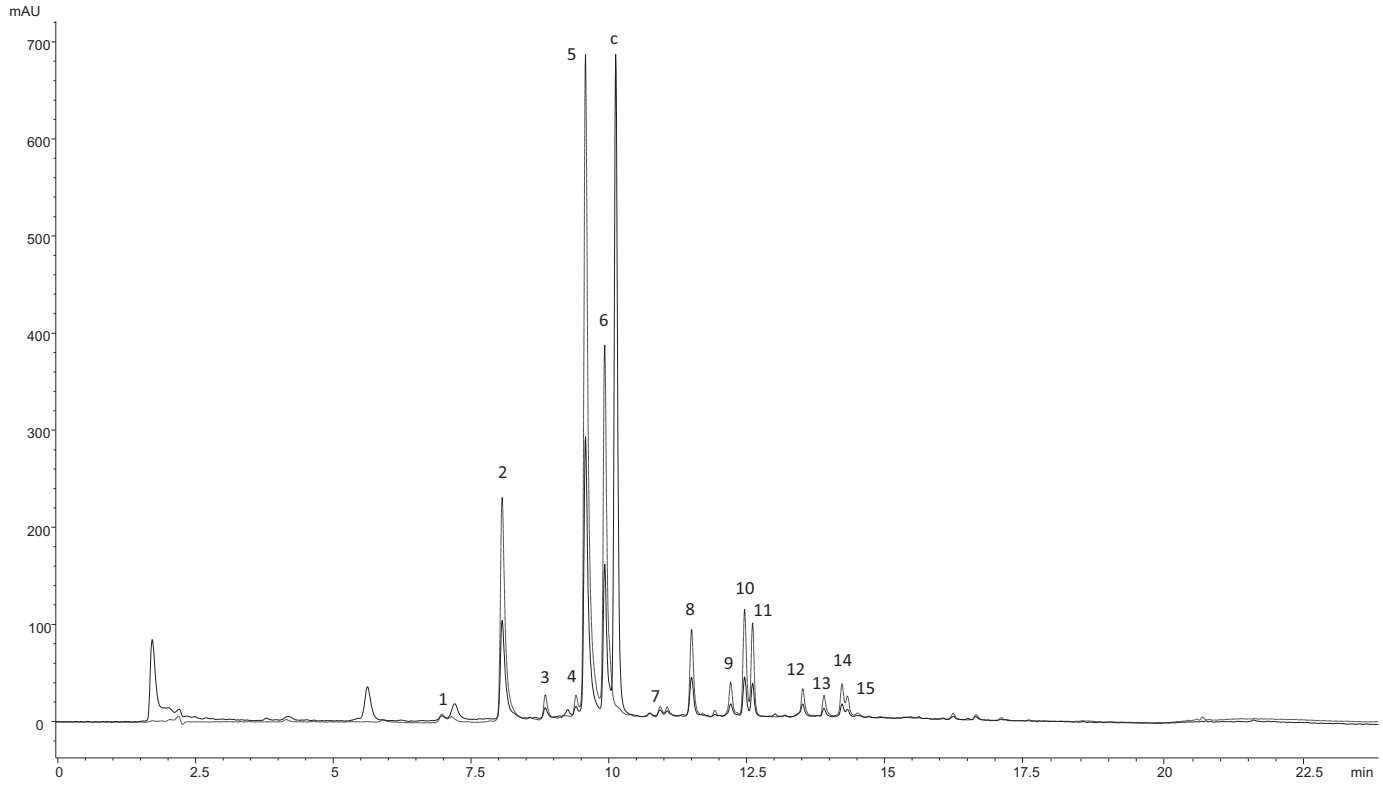

Fig. 2. Overlapping of HPLC/DAD chromatograms at 278 nm (whole line) and 330 nm (dotted line) for CGAs and caffeine monitoring of a representative coffee sample.
1: CQA*; 2: 3-CQA; 3: CeQA*; 4: CeQA*; 5: 5-CQA (chlorogenic acid); 6: 4-CQA; 7: 5-p-CoQA; 8: 5-FQA; 9:CQL*; 10:4-CQL*; 11: CQL*; 12:CQL*; 13:1,4-diCQA; 14: 3,5-diCQA;15: 4,5-diCQA. *acylation position in uncertain. List of acronyms: CQA: Caffeoyl Quinic Acid; CeQA: caffeoyl epi-quinic acid; p-CoQA:p-Coumaroyl Quinic Acid; FQA: Feruloyl Quinic Acid; CQL: Caffeoyl Quinic Lactone Acid; diCQA: di-Caffeoyl Quinic Acid.

followed by Moka and, finally, filter coffee. In this earlier work, concentrations ranged from 17.0 mg/mL for expresso, to 2.43 mg/mL for French Press. The present study evaluated five other methods that are not widely known in the scientific literature; of these, concentrations in at least three methods (Aeropress, French Press, and V60), were comparable to those of the filter coffees reported by Gloess et al. (2013).

3.3.2. Extraction efficiency (mg/g ground coffee)

Extraction efficiency can be defined as the ratio of the mass of ground coffee powder that passes into the cup, and the total amount of ground coffee used (Clarke & Vitzthum, 2008). Table 4 shows that there was a significant difference in extraction efficiency among all 15 CGAs, for the tested methods ($p \leq .05$, letters indicate statistically significant differences between groups). The analysis showed that extraction

Table 3
Chemical characterization beverages. Concentrations (mg/mL) of Caffeine, CQAs, CeQAs, 5-FQA, 5-pCoQA, CQLs and diCQAs are reported [1,2].

	ECF	ECS	EC	V60	COLD BREW	AEROPRESS	FRENCH PRESS	MOKA
Caffeine	1.43 ± 0.07b	4.20 ± 0.09a	4.10 ± 0.16 a	0.74 ± 0.09 c	1.25 ± 0.12 b	0.78 ± 0.09 c	0.52 ± 0.06 c	1.28 ± 0.04 b
CQA†	0.07 ± 0.02b	0.20 ± 0.02a	0.18 ± 0.03 a	0.03 ± 0.00 c	0.04 ± 0.00 c	0.02 ± 0.01 a	0.02 ± 0.00 c	0.04 ± 0.01 c
3-CQA†	0.60 ± 0.06b	1.86 ± 0.01a	1.80 ± 0.30 a	0.31 ± 0.05 b	0.50 ± 0.06 b	0.27 ± 0.04 b	0.21 ± 0.03 b	0.45 ± 0.07 b
CeQA†	0.08 ± 0.01b	0.23 ± 0.02a	0.24 ± 0.04 a	0.03 ± 0.00 c	0.06 ± 0.01 b	0.03 ± 0.01 bc	0.02 ± 0.00 c	0.05 ± 0.01 bc
CeQA†	0.08 ± 0.02b	0.17 ± 0.02a	0.17 ± 0.02 a	0.03 ± 0.00 c	0.05 ± 0.01 b	0.03 ± 0.01 c	0.02 ± 0.00 c	0.04 ± 0.01 bc
5-CQA	1.56 ± 0.17b	4.80 ± 0.30a	4.46 ± 0.10 a	0.80 ± 0.08 c	1.39 ± 0.15 b	0.72 ± 0.11 c	0.53 ± 0.07 c	1.22 ± 0.18 b
4-CQA	0.85 ± 0.11b	2.50 ± 0.30a	2.59 ± 0.14 a	0.44 ± 0.04 c	0.76 ± 0.08 b	0.31 ± 0.16 c	0.31 ± 0.04 c	0.50 ± 0.20 bc
5-pCoQA	0.09 ± 0.02b	0.27 ± 0.07a	0.23 ± 0.05 a	0.03 ± 0.00 b	0.06 ± 0.02 b	0.04 ± 0.02 b	0.02 ± 0.00 b	0.05 ± 0.01 b
5-FQA	0.22 ± 0.04b	0.71 ± 0.08a	0.50 ± 0.20 a	0.09 ± 0.01 cb	0.18 ± 0.03 b	0.09 ± 0.01 c	0.07 ± 0.01 c	0.15 ± 0.03 b
CQL†	0.04 ± 0.01b	0.12 ± 0.04a	0.17 ± 0.01 a	0.01 ± 0.00 c	0.02 ± 0.01 b	0.02 ± 0.00 b	0.01 ± 0.00 c	0.01 ± 0.00 bc
4-CQL	0.11 ± 0.02b	0.31 ± 0.07a	0.31 ± 0.06 a	0.04 ± 0.01 c	0.07 ± 0.02 bc	0.05 ± 0.02 c	0.03 ± c 0.00	0.06 ± 0.02 bc
CQL†	0.21 ± 0.04b	0.61 ± 0.07a	0.43 ± 0.19 a	0.09 ± 0.02 bc	0.16 ± 0.02 c	0.11 ± 0.02 bc	0.07 ± 0.01 c	0.16 ± 0.03 b
CQL†	0.19 ± 0.03b	0.52 ± 0.09a	0.41 ± 0.09 a	0.08 ± 0.02 c	0.12 ± 0.02 bc	0.07 ± 0.02 c	0.05 ± 0.00 c	0.13 ± 0.02 bc
1,4-diCQA	0.10 ± 0.03b	0.28 ± 0.08a	0.33 ± 0.09 a	0.03 ± 0.00 b	0.06 ± 0.02 b	0.05 ± 0.02 b	0.02 ± 0.00 b	0.05 ± 0.02 b
3,5-diCQA	0.08 ± 0.02b	0.21 ± 0.07a	0.26 ± 0.11 a	0.02 ± 0.00 b	0.04 ± 0.01 b	0.03 ± 0.00 b	0.02 ± 0.00 b	0.04 ± 0.01 b
4,5-diCQA	0.15 ± 0.03b	0.41 ± 0.11a	0.38 ± 0.04a	0.05 ± 0.01b	0.09 ± 0.02b	0.07 ± 0.03b	0.03 ± 0.01 b	0.09 ± 0.02 b

p-CoQA, p-Coumaroyl Quinic Acid; FQA, Feruloyl Quinic Acid; CQL, Caffeoyl Quinic Lactone Acid; diCQA:, di-Caffeoyl Quinic Acid. EC, espresso coffee. ECS, specialty espresso, ECF, Caffè Firenze.

[1] Data are expressed as mean ± standard deviation. Letters indicate statistically significant differences between extraction methods.

† Indicates that the acylation position was uncertain.

[2] CGA, chlorogenic acid; 5-CQA, 5-O-caffeoylquinic acid; 3-CQA, isomers 3-O-caffeoylquinic acid; 4-CQA, 4-O-caffeoylquinic acid; CeQA. caffeoyl epi-quinicacid.

Table 4
Chemical characterization of beverages. Extraction efficiency (mg/g) of caffeine, CQAs, CeQAs, 5-FQA, 5-pCoQA, CQLs and diCQAs are reported[1,2].

	ECF	ECS	EC	V60	COLD BREW	AEROPRESS	FRENCH PRESS	MOKA
Caffeine	5.76 ± 0.33 d	8.50 ± 0.12 c	17.40 ± 0.62 a	10.19 ± 0.97 b	9.67 ± 0.64 b	10.14 ± 1.21 b	6.89 ± 1.00 c	10.17 ± 0.33 b
CQA[†]	0.30 ± 0.08 b	0.42 ± 0.05 b	0.77 ± 0.12 a	0.35 ± 0.05 b	0.33 ± 0.06 b	0.29 ± 0.08 b	0.26 ± 0.03 b	0.29 ± 0.15 b
3-CQA[†]	2.42 ± 0.27 b	3.79 ± 0.21 b	6.82 ± 0.32 a	4.29 ± 0.57 b	3.90 ± 0.63 b	3.63 ± 0.56 b	2.76 ± 0.41 b	3.06 ± 1.55 b
CeQA[†]	0.34 ± 0.06 b	0.48 ± 0.05 b	1.00 ± 0.17 a	0.43 ± 0.03 b	0.50 ± 0.13 b	0.42 ± 0.06 b	0.30 ± 0.03 b	0.35 ± 0.19 b
CeQA[†]	0.31 ± 0.07 b	0.34 ± 0.05 b	0.72 ± 0.11 a	0.37 ± 0.04 b	0.39 ± 0.10 b	0.34 ± 0.06 b	0.23 ± 0.04 b	0.30 ± 0.16 b
5-CQA	6.32 ± 0.70 c	9.75 ± 0.66 b	18.91 ± 0.18 a	11.02 ± 0.95 b	10.39 ± 1.73 b	9.52 ± 1.49 b	7.06 ± 1.10 c	8.17 ± 4.12 b
4-CQA	3.44 ± 0.45 c	5.20 ± 0.53 b	11.00 ± 0.47 a	6.04 ± 0.47 b	5.70 ± 0.95 b	4.16 ± 1.21 b	3.99 ± 0.54 c	3.22 ± 2.48 bc
5-pCoQA	0.37 ± 0.10 b	0.55 ± 0.16 b	0.98 ± 0.21 a	0.35 ± 0.06 b	0.44 ± 0.14 b	0.54 ± 0.33 b	0.28 ± 0.03 b	0.32 ± 0.18 b
5-FQA	0.91 ± 0.14 b	1.44 ± 0.17 b	2.11 ± 0.93 a	1.27 ± 0.11 b	1.38 ± 0.26 b	1.22 ± 0.19 b	0.91 ± 0.14 b	0.99 ± 0.53 b
CQL[†]	0.15 ± 0.03 b	0.24 ± 0.08 b	0.71 ± 0.49 a	0.09 ± 0.01 b	0.14 ± 0.07 b	0.30 ± 0.43 b	0.09 ± 0.01 b	0.09 ± 0.06 b
4-CQL	0.45 ± 0.07 b	0.64 ± 0.15 b	1.33 ± 0.28 a	0.51 ± 0.09 b	0.55 ± 0.16 b	0.68 ± 0.28 b	0.35 ± 0.04 b	0.43 ± 0.23 b
CQL[†]	0.84 ± 0.18 b	1.23 ± 0.15 b	1.82 ± 0.14 a	1.31 ± 0.25 b	1.17 ± 0.22 b	1.39 ± 0.20 b	0.87 ± 0.16 b	1.10 ± 0.56 b
CQL[†]	0.79 ± 0.13 b	1.06 ± 0.19 b	1.73 ± 0.32 a	1.04 ± 0.25 b	0.95 ± 0.19 b	0.92 ± 0.32 b	0.70 ± 0.08 b	0.88 ± 0.45 b
1,4-diCQA	0.41 ± 0.10 b	0.58 ± 0.17 b	1.40 ± 0.41 a	0.45 ± 0.03 b	0.46 ± 0.13 b	0.69 ± 0.38 b	0.30 ± 0.03 b	0.36 ± 0.21 b
3,5-diCQA	0.32 ± 0.11 b	0.45 ± 0.14 b	1.20 ± 0.48 a	0.32 ± 0.04 b	0.28 ± 0.07 b	0.43 ± 0.03 b	0.22 ± 0.03 b	0.26 ± 0.14 b
4,5-diCQA	0.60 ± 0.13 bc	0.84 ± 0.21 bc	1.63 ± 0.18 a	0.71 ± 0.13 bc	0.59 ± 0.13 bc	1.03 ± 0.44 b	0.46 ± 0.06 c	0.62 ± 0.32 bc

p-CoQA, p-Coumaroyl Quinic Acid; FQA, Feruloyl Quinic Acid; CQL, Caffeoyl Quinic Lactone Acid; diCQA:, di-Caffeoyl Quinic Acid. EC, espresso coffee; ECS, specialty espresso, ECF, Caffè Firenze.

[1] Data are expressed as mean ± standard deviation. Letters indicate statistically significant differences between extraction methods

[†] indicates that the acylation position was uncertain.

[2] CGA, chlorogenic acid; 5-CQA, 5-O-caffeoylquinic acid; 3-CQA, isomers 3-O-caffeoylquinic acid; 4-CQA, 4-O-caffeoylquinic acid; CeQA. Caffeoyl epi-quinic acid

efficiency was highest for the EC method, both for caffeine and all CGAs.

Specifically, for EC caffeine extraction efficiency was about double that of the ECS method (17.4 ± 0.62 mg/g compared to 8.5 ± 0.12 mg/g for ECS). Given that the extraction time was similar (25 ± 5 s), this observation could be explained by the different ground coffee/mL beverage ratio (7 g/30 mL for EC and 9 g/18 mL for ECS). For Moka, although the concentration was similar to that of ECF, extraction efficiency was similar to V60, Cold Brew, and Aeropress. This could be explained by the contact time, which was much longer than that used for expresso preparation (25 ± 5 s). Finally, extraction efficiency was lowest for ECF (5.76 ± 0.33 mg/g).

Concerning CGA concentrations, trends were similar to those for caffeine for all 15 detected compounds. Fig. 3b show that EC was able to extract 52.09 ± 4.81 mg/g of total CGAs, with an extraction capacity about twice that of ECS, Moka and ECF. French Press and ECF they were been least efficient and significantly different to V60, Cold Brew, and Aeropress methods. These trends agree with earlier data (Gloess 2013), which found highest concentrations of the most abundant CGAs for expresso, followed by Moka and filter coffee.

3.3.3. Bioactive content per cup

In the context of caffeine and CGA content in a coffee brew, some factors must be taken into consideration. First, the usual amount of coffee in a cup varies enormously in different cultures and traditions, ranging from 18 to 30 mL for expresso, to over 200 mL for filtered coffee. Therefore, we adopted a 'typical' volume for each type of beverage: 30 mL for expresso; 18 mL for ECS; 40 mL for Moka; and 120 mL for the other types. Romani, Severini, Fiore, and Pinnavaia (2004) argues that the ratio between the dose of ground coffee, and volume of coffee is a variable that strongly affects the final caffeine content in the Espresso cup. Similarly, it is reasonable to affirmative that this could explain the high caffeine content in a cup of Cold Brew coffee (149.52 ± 13.80 mg/cup).

As reported in Table 5, EC contained much more caffeine than ECS. However, these two expresso were prepared with different cup volumes

Table 5
Chemical characterization of beverages. Bioactive content per cup (mg/cup) of caffeine, CQAs, CeQAs, 5-FQA, 5-pCoQA, CQLs and diCQAs are reported[1,2].

	ECF	ECS	EC	V60	COLD BREW	AEROPRESS	FRENCH PRESS	MOKA
Caffeine	42.78 ± 2.15 e	75.51 ± 1.54 d	122.40 ± 4.95 b	89.04 ± 11.25 c	149.52 ± 13.80 a	93.36 ± 10.32 c	62.16 ± 6.92 d	51.14 ± 1.43 e
CQA[†]	2.12 ± 0.54 c	3.67 ± 0.37 b	5.46 ± 0.88 a	3.05 ± 0.47 b	5.28 ± 0.55 a	2.64 ± 0.76 b	2.28 ± 0.17 c	1.69 ± 0.29 c
3-CQA[†]	17.97 ± 1.89 c	33.52 ± 1.86 b	54.02 ± 10.08 a	37.50 ± 6.37 b	61.20 ± 6.69 a	32.42 ± 5.05 b	24.96 ± 3.17 b	18.12 ± 2.85 c
CeQA[†]	2.52 ± 0.42 c	4.20 ± 0.41 b	7.08 ± 1.18 a	3.78 ± 0.39 b	7.68 ± 1.65 a	3.80 ± 0.60 b	2.69 ± c 0.34	2.05 ± 0.47 c
CeQA[†]	2.27 ± 0.49 c	3.00 ± 0.39 b	5.05 ± 0.69 a	3.24 ± 0.43 b	6.17 ± 1.26 a	3.05 ± 0.60 b	2.08 ± 0.36 c	1.73 ± 0.48 c
5-CQA	46.92 ± 4.91 d	86.03 ± 5.97 c	133.86 ± 2.91 b	96.04 ± 9.21 c	167.29 ± 13.26 a	86.08 ± 13.26 c	63.81 ± 8.72 d	48.63 ± 7.23 d
4-CQA	25.54 ± 3.22 c	45.73 ± 4.51 b	77.76 ± 4.08 a	52.66 ± 5.30 b	90.96 ± 8.58 a	37.71 ± 19.17 b	36.78 ± 4.30 bc	19.78 ± 9.75 c
5-pCoQA	2.70 ± 0.75 b	4.81 ± 1.29 a	6.98 ± 1.55 a	3.04 ± 0.56 b	6.61 ± 2.15 a	4.91 ± 2.95 a	2.54 ± 0.40 b	1.80 ± 0.57 b
5-FQA	6.73 ± 1.08 c	12.73 ± 1.73 b	15.14 ± 6.81 a	11.02 ± 0.87 b	21.77 ± 3.28 a	10.93 ± 1.73 b	8.24 ± 3.53 bc	5.85 ± 1.23 c
CQL[†]	1.12 ± 0.22 bc	2.09 ± 0.63 b	4.99 ± 3.36 a	0.81 ± 0.09 c	2.44 ± 1.25 a	2.80 ± 3.92 a	0.79 ± 0.12 c	0.57 ± 0.16 c
4-CQL	3.41 ± 0.56 dc	5.63 ± 1.33 bc	9.39 ± 1.83 a	4.42 ± 0.69 c	8.48 ± 2.62 ab	6.21 ± 2.55 b	3.18 ± 0.21 d	2.49 ± 0.59 d
CQL[†]	6.28 ± 1.33 c	10.99 ± 1.33 b	13.03 ± 3.78 ab	11.30 ± 1.84 b	18.78 ± 2.73 a	12.59 ± 1.88 b	7.83 ± 1.31 c	6.53 ± 1.10 c
CQL[†]	5.73 ± 0.92 c	9.34 ± 1.74 b	12.33 ± 2.52 ab	9.01 ± 1.88 b	14.91 ± 2.87 a	8.30 ± 2.81 b	6.35 ± 0.39 bc	5.26 ± 0.90 c
1.4-diCQA	3.06 ± 0.79 c	5.05 ± 1.57 b	9.95 ± 2.75 a	3.92 ± 0.35 cb	7.01 ± 2.32 a	6.34 ± 3.63 ab	2.68 ± 0.14 c	2.15 ± 0.67 c
3.5-diCQA	2.41 ± 0.77 c	3.79 ± 1.24 b	7.83 ± 3.21 a	2.77 ± 0.35 bc	4.44 ± 1.04 a	3.97 ± 0.32 b	1.90 ± 0.23 c	1.56 ± 0.36 c
4.5-diCQA	4.41 ± 1.10 c	7.45 ± 1.91 b	10.53 ± 1.32 a	6.17 ± 0.96 bc	10.22 ± 2.18 ab	8.88 ± 3.96 ab	3.92 ± 0.45 c	3.68 ± 0.70 c

p-CoQA, p-Coumaroyl Quinic Acid; FQA, Feruloyl Quinic Acid; CQL, Caffeoyl Quinic Lactone Acid; diCQA:, di-Caffeoyl Quinic Acid. EC, espresso coffee; ECS, specialty espresso, ECF, Caffè Firenze;

[1] Data are expressed as mean ± standard deviation. Letters indicate statistically significant differences between extraction methods

[†] indicates that the acylation position was uncertain

[2] CGA, chlorogenic acid; 5-CQA, 5-O-caffeoylquinic acid; 3-CQA, isomers 3-O-caffeoylquinic acid; 4-CQA, 4-O-caffeoylquinic acid; CeQA. Caffeoyl epi-quinic acid

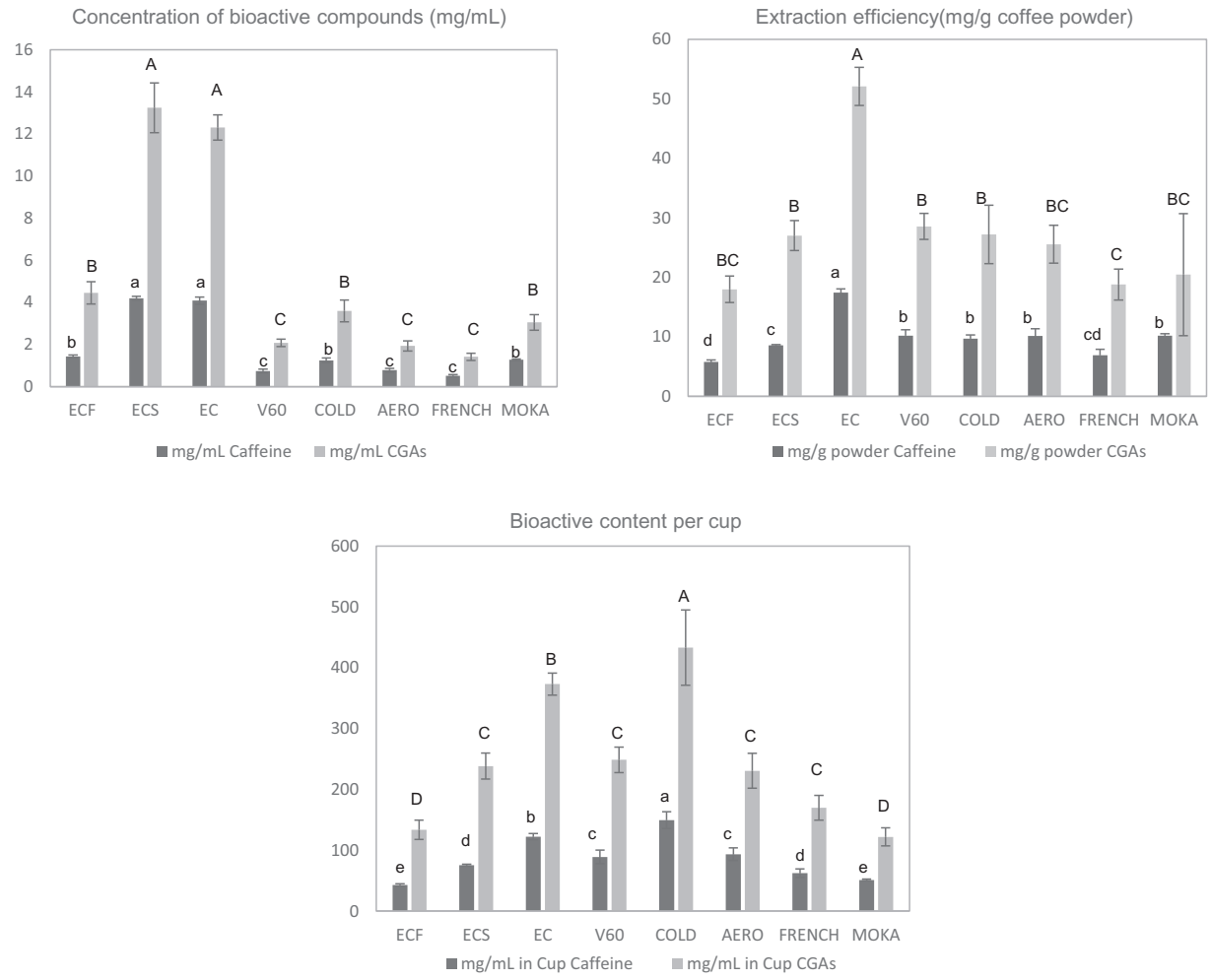

Fig. 3. Content per mL of extract (a), per gram of coffee powder (b), and per cup of coffee brew (c) of caffeine and of sum of CGAs. Letters indicate statistically significant differences between extraction methods. Capital letters indicate difference in CGAs while lowercase letters indicate differences in caffeine. Error bars correspond to the standard deviation (95%).

the ECS cup being almost half the size of the EC cup. Caffeine content for a cup of Moka and ECF was lower than for the other expresso methods, although the ANOVA analysis found that these two methods were not significantly different from each other, they showed different to other extraction methods. High per-cup levels of caffeine were found for V60 and Aeropress methods, these values were lower than the Cold Brew method, and different to the other methods.

Concerning per-cup CGA content, the same trend was observed for all individual compounds. The highest level was observed for Cold Brew followed by EC. As reported in Table 5 and Fig. 3c, highest concentrations of all 15 compounds were detected for the Cold Brew method (sum of CGAs 433.25 ± 52.50 mg/cup). This result was expected as extraction is cold, limiting the degradation of compounds.

This information is relevant in the context of the maximum recommended daily dose of caffeine. In 2012, the FDA (2012) stated that, for healthy adults, a dose of caffeine up to 400 mg/day was not associated with adverse effects. This work highlights that the intake of bioactive components is highest for lungo coffee, although the consumer often considers that a long coffee is more diluted and therefore contains less bioactive substances.

4. Conclusions

This study provides important information on concentrations (mg/mL), extraction capacity (mg/g), and per-cup caffeine and CGA content for eight types of beverage preparation. Some of these methods, which are very popular among consumers and industry experts, have not previously been investigated in the scientific literature. Here, they are assessed and compared for the first time.

Technical differences in these extraction methods led to quantitative differences in extraction efficiencies, and produce coffees with different profiles. In general, the concentration of bioactive compounds was higher for the expresso group than the filter group. However, when content per cup was compared, filter coffees were found to have a higher content. The cluster analysis identified clear differences between and among these two groups. Clusters can be distinguished based on caffeine and CGA concentrations.

This study reviewed extraction methods for coffee production. The aim was not to establish “the best method” but to highlights that different extraction methods produce coffee beverages with different qualitative and quantitative characteristics, starting from the same raw material.

In light of these results it is not possible to establish how many cups of coffee can be consumed per day without exceeding the recommended doses, since according to the applied brewing method, the content of the bioactive substances varies considerably.

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