DOTTORATO DI RICERCA IN
GESTIONE SOSTENIBILE DELLE RISORSE AGRARIE, FORESTALI E ALIMENTARI

CICLO XXXI

COORDINATORE Prof.ssa Susanna Nocentini

PHYSICAL AND MECHANICAL METHODS OF EXTRACTION FROM GROUND COFFEE AND SPENT COFFEE GROUND

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ABSTRACT

Coffee, one of the most popular beverages in the world, is consumed by millions of people every day. The result in the cup is strongly affected by the extraction method, and many papers have focused on this subject (Niseteo 2012), (Andueza. S. 2007), (Gloess 2013). In recent decades, various coffee-based beverages, obtained using different extraction techniques have entered the market, but there aren't reported in the scientific literature.

During the preparation of a coffee beverage, a solid residue known as spent coffee grounds (SCG) is produced. In recent years, however, the growing awareness of the necessity for waste reduction and environmental protection has stimulated the search for possible methods of using this waste (Kondamudi 2008), (Adi 2009), (Fenoll 2011), (Janissen and Huynh 2018).

The aim of the work was at first, to characterize and compare eight different coffee extraction methods from a physical and chemical point of view, starting from the same raw material. The study describes, three types of Espresso, Moka, French Press, and three filter coffee that for the first time are reported in the scientific literature Cold Brew, V60, and Aeropress.

After, the attention was focused on cold extraction: cold brew and cold drip. The effects of the primary process variables (temperature and contact time) were assessed in a full factorial experiment.

Finally, the focus of the third study concern to test which variables could influence the amounts of phytochemicals extracted, to optimize a green extraction method for water-soluble compounds that do not require the use of organic solvents and that maximizes the recovery of phytochemicals from spent coffee grounds (SCG). We used a Plackett-Burman design to estimate which factors have more influence on the amount of phytochemicals to be recovered. In the second part of the experiment, we have tested only the

significant factors with a fully factorial scheme. For each study has been performed physical measurements included the quantification of TDS, density, pH. Furthermore, the phytochemicals have been quantified using HPLC-DAD.

Technical differences in these 8 extraction methods led to quantitative differences in extraction and produced coffees with different profiles. Maximum caffeine and CGA concentrations were found in Espresso coffees, while Moka and filtered coffees were three to six times less concentrated. The Espresso method was most efficient for caffeine and CGA recovery. Per-cup caffeine and CGAs were higher in Cold Brew than Espresso coffees, as a function of the volume of beverage.

Concerning the cold extraction techniques, significant differences were found in the chemical and physical parameters, both between and within the two methods. The temperature was found to increase the concentrations of several compounds. Conversely, the contact time between the coffee powder and water has a limited effect on brew characteristics.

Regarding the study of the recovery of phytochemicals, the results obtained from the fractional design showed that the significant factors to recover phytochemicals were Temperature and type of SCG. Afterwards, four temperature and two type of SCG have been tested. At 110°C has been observed the higher concentration values of caffeine and CGAs. Moreover, a significant effect was revelated for the different type of SCG. The amounts of phytochemicals recovered from SCG was significantly higher in French Press than the Espresso. These conditions of temperatures, as reported in Conde and Mussatto study (2016), could be considerate mild conditions, combined with the use of water as a solvent. The system of recovery it was demonstrated to be an efficient method with the added value of being a green and low-cost system.

LIST OF ORIGINAL PUBLICATIONS

This study is based on the following original publications:

- G Angeloni, L Guerrini, P Masella, M Bellumori, S Daluiso, A Parenti, M Innocenti

What kind of coffee do you drink? An investigation on effects of eight different extraction methods.
Food Research International, *in press,* 2018.

- G Angeloni, L Guerrini, P Masella, M Innocenti, M Bellumori, A Parenti

Characterization and comparison of cold brew and cold drip coffee extraction methods.
Journal of the Science of Food and Agriculture, 99 (1), 391-399, 2018.

- G Angeloni, P Masella, L Guerrini, M Innocenti, M Bellumori, A Parenti

A green method to recover phytochemicals from spent coffee grounds
In preparation for submission to Food and Bioprocess Technology.

Poster-presentation:

- G Angeloni, L Guerrini, P Masella, M Innocenti, M Bellumori, A Parenti

Comparison of cold brew and cold drip coffee extraction methods.
CoCoTea- Cocoa Coffee and Tea 25 - 28 Giugno 2017, Centro Congressi Torino, Torino.

- G Angeloni, L Guerrini, P Masella, A Parenti

Effects of pressure and air volume on Espresso foam.
CoCoTea- Cocoa Coffee and Tea 25 - 28 Giugno 2017, Centro Congressi Torino, Torino.

- G Angeloni, L Guerrini, M Bellumori, M Innocenti

Characterization and comparison of chlorogenic acids content in eight coffee brewing methods.

Food Bioactive and health, Universiade NOVA de Lisboa (26-28 Settembre 2018).

-   M. Bellumori, G. Angeloni, L. Guerrini, P. Masella, A. Parenti, N. Mulinacci, M. Innocenti.

Recovery of bioactive compounds from spent coffee grounds.
Chimali 2018 - XII Italian Food Chemistry Congress, Camerino, September 24-27 2018.

-   G. Angeloni, P. Masella, L. Guerrini, M. Innocenti, M. Bellumori, A. Parenti.

Fractional design to estimate the significant factors to recovery phytochemicals from spent coffee grounds.
GENP 2018 III Edition "Green Extraction of Natural Products", University Aldo Moro Bari-Italy, November 12-13, 2018.

1.INTRODUCTION

1.1 Coffee: general aspects, cultivation and species

Coffee is one of the important world trade commodities. According to the ICO, International Coffee Organization, (ICO 2018), in 2016 around 151.3 million 60-kg bags of coffee were consumed worldwide. The USA is the largest coffee consumer as a country (25 million bags). Brazil is the second largest consumer (20 million bags) and the largest coffee producer (55 million bags) in the world. The European Union have a consumption of 42 million bags, Scandinavians have the highest per capita coffee consumption (Finland 12,2 kg). Italy, a country known for its strong coffee culture, has a per capita coffee consumption of 5,6kg (Wissen 2017).

Coffee is produced in tropical and equatorial countries; the major producers are Brazil, Vietnam, Colombia, India, Indonesia, Mexico, Guatemala, Ethiopia, and Uganda (ICO- International Coffee Organization 2015).

The coffee tree is a shrub with a straight trunk which can survive for about 70 years. The first flowers appear during the third year, but production is only profitable from the fifth year onwards. Botanists classify Coffee as a member of the Rubiaceous family. Of around sixty different species of coffee tree, two alone dominate world trade - the Coffee Arabica (*Coffea Arabica L.*), or, more simply, Arabica, which represents 75% of production; and the *Coffea Canephora*, which is commonly known by the name of the most widespread variety, Robusta (Mutua 2000).

The cherry is the name usually given to the fruit of the coffee tree. These cherries ripen over several months, becoming yellow, then red, and finally almost black. The ideal time for harvesting is when the berries are red. The red skin is called the exocarp. Beneath the pulp (the mesocarp), each surrounded by a parchment-like covering (the endocarp), lie two beans, flat sides together. When the fruit is ripe a thin, slimy layer of mucilage surrounds

the parchment. Underneath the parchment the beans are covered in another thinner membrane, the silver skin (the seed coat), (Caballero 2015).

The processing of coffee initiates with the conversion of coffee cherries into green coffee beans and starts with the removal of both the pulp and hull using either a wet or dry method. Depending on the method of coffee cherries processing, i.e., wet or dry process, the solid residues obtained have different terminologies: pulp or husk, respectively (Pandey 2000).

The dry method (also called the natural method) is the simplest and involves drying the whole cherry.

In this process, the newly harvested coffee cherries are sorted, and sun dried. In some cases, depending upon the plantation production, the cherries are machine dried after being in the sun for a few days. To ensure even drying, the cherries are spread evenly and raked regularly throughout the day. It could take up to four weeks in the sun before the cherries are free of excess moisture. The optimum moisture content is around11% (Adams 1987).

It is important that the cherries are dried to the correct degree because over drying will result in brittle coffee beans that will not produce a good roast. On the other hand, cherries with too much water content cannot be easily stored, because they will be prone to attack from bacteria and fungi.

Almost all Robusta are processed by this method. It is not practical in very rainy regions, where the humidity of the atmosphere is too high or where it rains frequently during harvesting.

The wet process involves several stages that comprise considerable amounts of water and includes a microbial fermentation step in order to remove any mucilage still attached to the beans.

After sorting and cleaning, the pulp is removed from the cherry. This operation is the difference between the dry and the wet methods, since in

the wet method the pulp of the fruit is separated from the beans before the drying stage (S. M. Mussatto 2011).

After the berries have had the pulp removed, they must go through a process of washing and sieving to remove any remaining pulp and skin.

Next the coffee beans are placed into a fermentation tank to remove the mucilage.

Mucilage, composed of natural sugars and alcohols, plays a crucial role in developing the sweetness, acidity and overall flavor profile in the coffee beans. It is important in the washed process that all mucilage is removed from the bean.

The relationship between fermentation and the corresponding coffee aroma profile can be described as intricate and delicate. Under controlled condition, the fermentation can impart desirable attributes to the corresponding coffee aroma while uncontrolled fermentation inevitably leads to off-flavors (Lee 2015).

The length of time that the beans are left in the fermentation tank – usually from 8 to 36 hours, depends on various factors including the temperature (Vincent 1987).

Once the mucilage has dissolved, it is removed completely through repeated washings. The beans are ready now for the next stage, which is the drying phase.

The wet method is generally used for all the Arabica coffees. It is rarely used for Robusta. Figure 1, illustrates all the steps applied in both methods of processing.
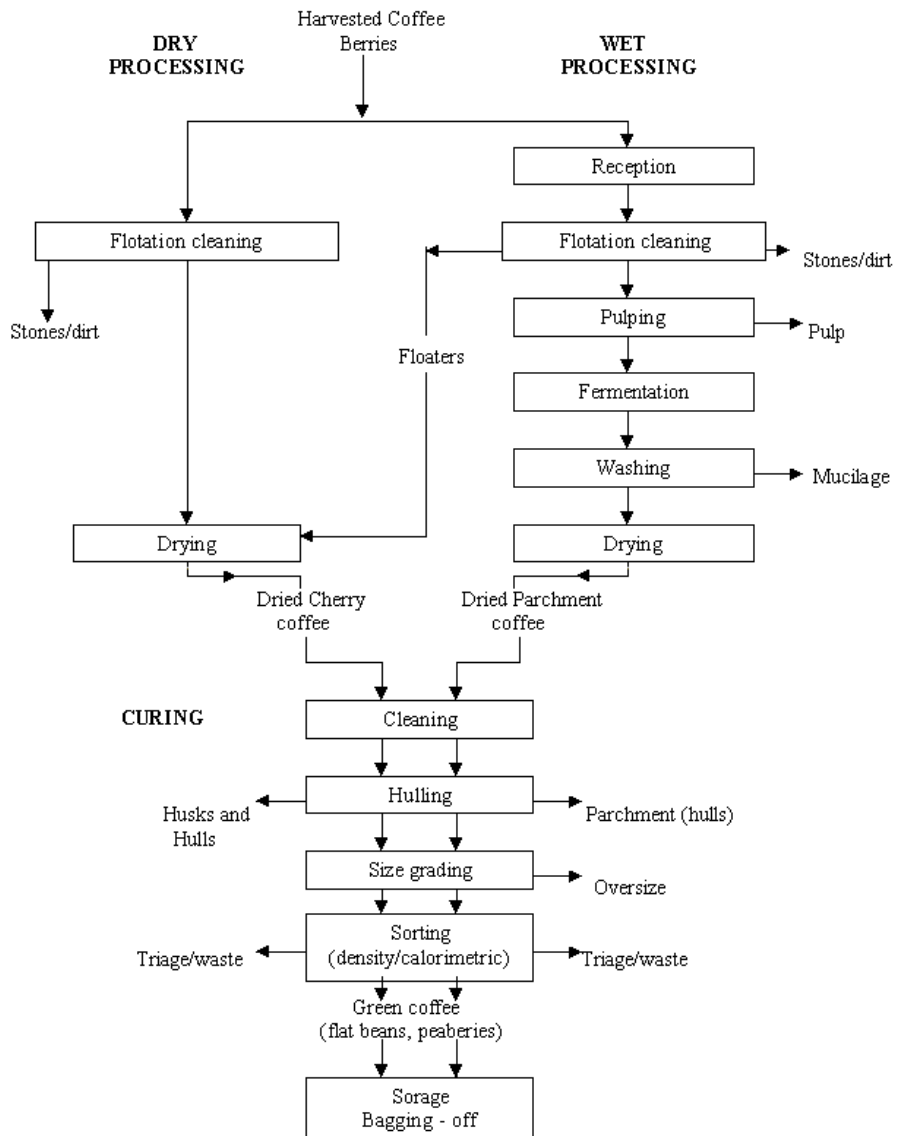
Figure 1: Flow sheet illustrating the stages of wet and dry processing of coffee.

Original habitats and climatic tolerance distinguish Arabica and Robusta. In general, Arabica dominates the coffee-growing area in most North, Central, and South American countries, e. g. Costa Rica, Brazil, and Columbia, while Robusta takes up most of coffee plantations in African and Asian countries (Smith 1985) (Schoenholt 1992). Arabica coffee grows well at medium to high

altitudes (1000 to 2100 m) with daily average temperatures of around 18 to 22 °C, typical of equatorial regions. Also, arabica grows best in partial shade whereas Robusta in the sun. In general, cooler climatic conditions improve the quality of arabica coffee. In contrast, Robusta coffee requires a hot and humid climate and grows best at lower altitudes (100 to 1000 m) with average temperatures of 22 to 26 °C, as found in tropical regions (Bertrand B 2012), (Illy A 2005).

These differences can also be found for the content of caffeine, chlorine-based acids (CGAs) and total oils. The Arabica species contains lower levels of caffeine, amino acids, and CGAs, but a higher total oil content.

The different concentration of these compounds besides influences the sensory and aromatic aspects of the beans and, at last, on the drinks.

In general, Robusta has a strong and overpowering taste but also possesses "earthy" and "musty" flavor notes which are undesirable in many consuming countries. Arabica is considered of better quality due to its more delicate flavor (Briandet 1996) and therefore is more expensive (Martín 1998).

1.2 Roasting process

Green coffee has no desirable taste or aroma of its own; the desired flavor is developed in the roasting of the beans.

The coffee beans need to be introduced to the roasting process before they can be used to produce the beverage.

Roasting stage produces significant chemical-physical changes in green coffee. From an engineering point of view, roasting is a complex process that involves heat and mass transfer coupled to chemical reactions and structural mechanics. Roasting is a process during which coffee beans are brought for a

given time to a temperature in the range 170–230 °C for 10-15 minutes (Illy 1995).

Mainly, the beans are affected by two different heat sources during the roasting process:

- Conductive heat from the metal of the roasting drum.
- Convection heat from hot air flow moving through the bean mass.

The roaster modulates the amount of heat and amount of airflow to change how quickly or slowly the beans go through that process.

The roasting methods are mainly two: a fluidized bed, in which the raw coffee beans are hit by hot air remaining in suspension in the toasting chamber, and with a rotating drum, in which a metal drum is used, inside which there are augers or fins to continuously invert the product and make it roast; a gas burner conveys the hot air needed for the process, for a variable amount of time.

Briefly, as temperature increases to about 100°C, green coffee beans undergo moisture loss from 8-12% in green coffee beans to about 5% in the roasted coffee beans (Hernández 2007). The smell of the bean's changes from herb-like green bean aroma to bread-like, the color turns from green to yellowish, and the structure changes from strength and toughness to crumblier and brittle. When the internal temperature of beans reaches 100°C, the color darkened slightly for about 20-60 s due to the vaporization of water. At 160-170°C, the beans become lighter in color for about 60-100 s. As roasting continues at this temperature, Maillard and pyrolytic reactions start to take place, resulting in gradually darkening of the beans (Hernández 2007). The buildup of water pressure, along with a large amount of gases generated causes the cellulose cell wall to crack, giving rise to the so-called "first crack." As heating continues at the roasting temperature (160-170 °C), the coffee becomes darker and more rapid popping of coffee bean occurs ("second

crack") as the carbon dioxide (CO_2) buildup exceeds the strength of the cellulosic walls of the bean.

As a consequence of the increase in temperature, the beans become darker and lose 18-20% of its weight, mainly due to the evaporation of water. This change in the structure induces significant increases in fragility and friability, which are essential for ensuring an efficient grinding.

Finally, after roasting, the freshly roasted coffee beans are quickly cooled to stop roasting (Yeretzian 2002).

As shown in Figure 2, when the green coffee at room temp is added to roaster machine the temperature drops quickly, but after some time it is possible to relate a turning point where the temperature starts rising.
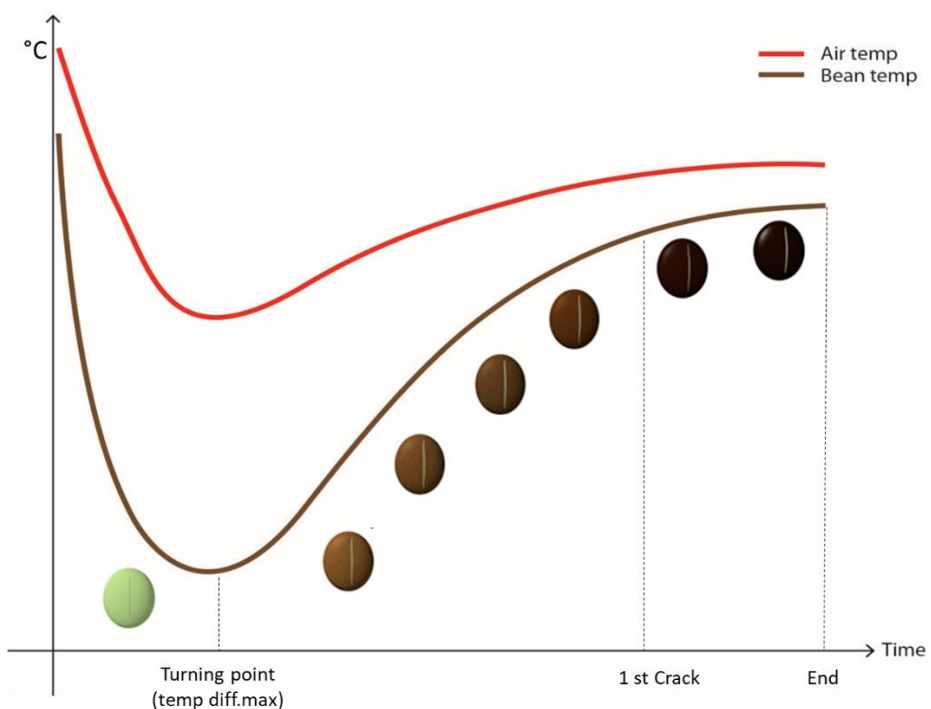
Figure 2: An example of roasting profile, adapted from Dorfner et al., 2004 in process control of coffee roasting analytical chemistry.

After the turning point, there is a period of the roast with maximum temperature increment speed. After this quick rising of temperature is possible to register the 1st crack. While is called " development" the phase that elapses from the first crack and the end of roasting.

The temperature difference between air and beans is an interesting measure since it gives in indication how much convection drives the roasting process.

Finally, a cooling phase is required to avoid burning the coffee. Thus, the freshly roasted coffee is quickly cooled to halt roasting (Sivetz 1979).

During the drying phase, the beans are brought to around 100 °C, most of the free water is driven out.

The 10–12% moisture content of green beans is reduced to just a few percent. At the end of this phase, toasted beans are obtained whose appearance, chemical, physical and aromatic characteristics depend directly on the type of roasting to which they have been subjected.

The increase in temperature, causes the pressure of the gas and steam inside the grains, as the temperature rises, the structure of the grains becomes more porous and friable.

The grains undergo a weight loss between 12% and 24% (due to the evaporation of water and volatile substances), the density decreases between the 11% and 2%. Their volume increases due to the leakage of anhydride carbon dioxide (a phenomenon that continues even in the following days).

In Table 1, were summarized the effects of temperature increase during the roasting phase.

Table 1: Effect of roasting temperature of coffee beans

T (C°)	Effects on beans
20-130	Colour changes, weight loss
130-140	Intense yellow colouring; beginning of non-enzymatic browning; increase in volume; evaporation of gases formed with roasting.
140-190	Colouring light brown; bean volumes increase and increase of the fragility and friability; crack formation on the external wall; beginning of the formation of the aromas;
190-220	Colouring brown for the cellulose carbonization and also due by the sugar caramelization; internal crack formation; bean volumes increase due to the elimination of the dioxide of carbon; develop of toasted flavour.

Adapted from "Textural Changes of Coffee Beans as Affected by Roasting Conditions" (P. M. Pittia 2001).

Roasting, in addition to inducing physical changes, such as increasing the porosity and friability of the grains that make grinding possible, thus improving the extraction capacity (given by the increase in the contact surface between water and coffee powder), leads to optimizing the flavor, making the soluble chemical compounds of coffee, are dragged into the cup. With this process, the taste is optimized so that the solids are dissolved in the drinks extracted from the coffee, also reporting in the solution the volatile aromatic compounds and oils, optimizing the aroma.

During roasting, the content of volatiles changes and 650 new volatile compounds build-up: about 850 volatiles have been identified in roasted coffee (Flament 2001). During roasting, the content of volatiles changes and 650 new volatile compounds build-up: about 850 volatiles have been identified in roasted coffee (Czerny 2000), (Flament 2001).

Several compounds are responsible for the sensory profile of roasted coffee (Czerny 2000), (Mayer 2001), and in particular, some peculiar sensory attributes are positively related to a roasting degree (Bhumiratana 2011).

1.2.1 The evolution of coffee aroma during roasting condition

The typical coffee aroma develops during the roasting process, in which the beans undergo some transformations, and through reactions, they modify their chemical composition.

The green beans have 300 volatile compounds; the roasting process has degraded some of these while other remain steady.

On the complex, the roasting condition increases the concentration of volatile compounds that have been originated from non-volatile compounds (Bonnlaender 2005).

Usually, green coffee beans are mainly non-aromatic and lack the characteristic aroma of roasted coffee but contain many chemical precursors such as chlorogenic acids (CGAs) and trigonelline that contribute to the flavor of coffee (Ludwig 2014).

Several compounds from the volatile composition of the roast coffee, nowadays there are 900 compounds revelated, but only a minor part of them contribute to characteristic odor and flavor (Buffo 2004), (Yeretzian C. 2002). During the temperature increases phase, and in high-pressure condition, there are made caramelization reaction and carbohydrate degradation, mainly responsible for the formation of aldehyde, furans and other volatile compounds (Yeretzian C. 2002).

The protein fraction is affected by denaturation and degradation reactions. The free amino acids with the reducing sugar are involved in Maillard reaction. This reaction contributes to the formation of several volatile compounds, like furans, pyridines, pyrazines, pyrroles, aldehydes and brown

polymers called melanoidins, responsible to coffee color, and in part of its antioxidant activity (Farah 2011).

The content of thermolabile compounds, like chlorogenic acids and trigonelline, changes in function of the roasting degree. In fact, in roasted coffee, there are lower level of these compounds respect to green coffee beans (Farah 2009).

The chlorogenic acids are almost complete degraded in high roasting condition, for their thermal instability. They are also responsible for producing phenolic compounds and aromatic compounds.

The sugars pyrolysis creates several furan compounds. They are one of the groups of compounds more abundant, they are identified through the headspace analysis. They represent only the 2% of all the odorant present in green coffee beans, but they increase in roasted coffee and confer the typical caramel and burnt aroma (Nebesny E. 2006).

The pyrazines represented roughly the 12% of the total aromatic compounds of green coffee. There are present also in the volatile fraction of roasted coffee, and it was confirmed their contributions in the coffee beverage aroma.

Green coffee and roasted coffee contain several carbonyl compounds; the roasted coffee presents these compounds derived from the auto-oxidation of fatty acids. The carbonyl compounds there are important for coffee aroma. Some of them are volatiles and conferred different sensory attributes; for example, low molecular weight aldehydes, such as acetaldehyde, propanal, 2-, and butanal, are important carbonyl constituents. They are very volatile and transmit the typical malt note to the coffee (Czerny 1999). Sulfuric compounds are only 1% of the volatile fraction of roasted coffee (Clarke et al., 1990). Methyl-thiazoles are responsible for unpleasant aromas, similar to that of burnt rubber (Nebesny 2006). While, some terpenic compounds in

particular monoterpenes (linalool, limonene, geraniol, α-terpineol) are responsible for the main positive notes of coffee aroma, in particular of floral, citrus and fruity smell.
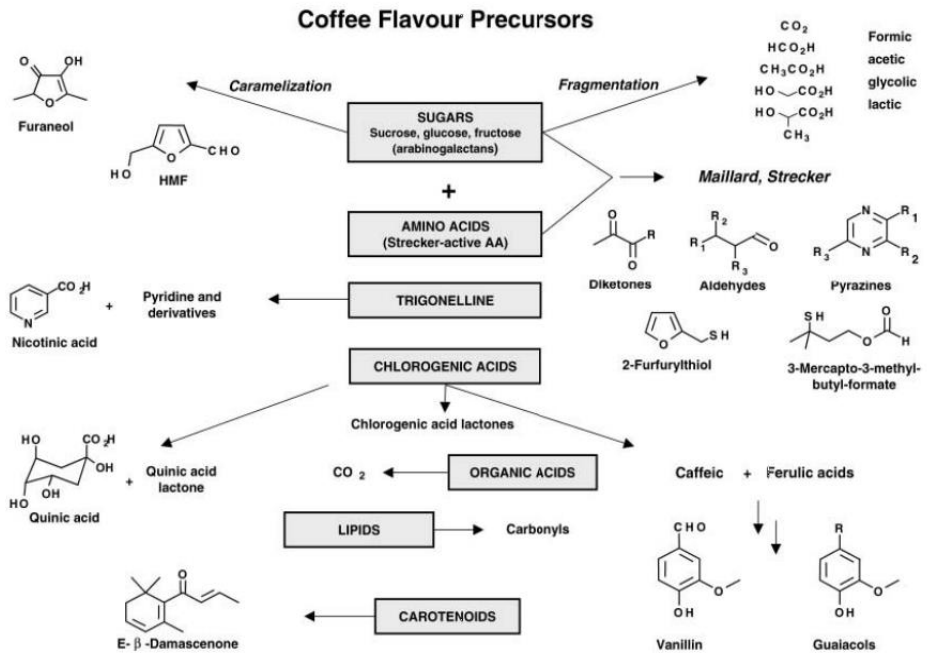
Figure 3: Simplified scheme showing the main classes of volatile compounds formed from non-volatile precursors in the green beans during roasting (Yeretzian 2002)

1.3 Grinding

The grinding step is a size reduction process in order to obtain a homogeneous product. The primary objective of this process is to increase the specific extraction surface, or rather to increase the extent of the interface between water and coffee, to facilitate the transfer of soluble and emulsifiable substances into the brew (Andueza 2003).

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. Also, the grinding affects the stability of coffee powder during storage is strictly related to the agglomeration phenomena and aroma retention (Baggenstoss 2008).

During grinding, beans are reduced to particles in size from a few micrometers to ~1000 μm, from which volatiles may be released, and chemical compounds are quickly dissolved in hot water, giving the worldwide appreciated aroma (Wang 2014).

Moroney and co-authors (Moroney 2015) stated that the particle size of the 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.

Ground coffee is commonly classified into different groups: coarse, medium, fine and very fine. Average particle size needs to be adequately adjusted for each type of coffee brew (Severini 2017).

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 (Clarke 2003).

Recent studies have reported that the grinding level of coffee powder greatly affects the chemical and aromatic compounds of the beverage (Salamanca 2017), (Derossi 2018).

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 water are the main phenomena controlling the amount of chemical compounds released in coffee beverage. 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 water (Baggenstoss 2008).

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 correct equilibrium between percolation, diffusion, and wettability of coffee particles drives the type and the amount of chemicals in coffee hence its quality in cup (Severini C 2016).

Therefore, the grinding must be regulated both based on the sensory and chemical properties that the beverage should possess and related to the type of extraction technology that we use.

For 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, working under pressure, extraction time is reduced to 25–30 s, and finer particles are needed to increase extraction rate of chemicals and volatiles (Illy A 2005).

In the FIGURE 4 as reported an example of a bi-modal curve representing the particles sizes of an espresso coffee.
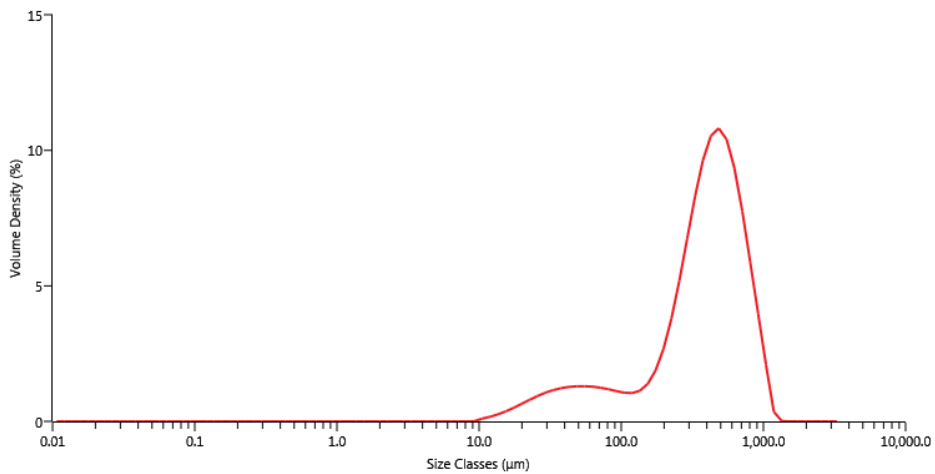
Figure 4: Particle size distribution of an espresso coffee

Is reported a description of the three main types of coffee grinders which can give a better understanding regarding different technologies used in the grinding process of coffee as well as assert the importance of such machines in the process of brewing coffee.

The most commonly used grinding devices in coffee processing are disc grinders and roller grinders. Disc grinders are typically used at smaller scales (households and coffee shops). Particle size reduction is achieved by the impact of blades at high speed on the beans. Roller grinders are preferably used at the industrial level. In this case, particle size reduction is achieved by the forces exerted by two rotating rollers when the beans pass through the gap left between them. The resulting particle size distribution depends on the configuration of the grinder, e.g., the distance between the discs or separation between the rollers, type of rollers, number of rollers and the mechanical properties of the beans. The mechanical properties of the beans have been shown to depend on moisture content mainly, and to a lesser extent on a roasting degree and beans type (P. D. Pittia 2001).

1.4 Extraction Methods

Typically, the coffee beverages 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 1997). The flavor of a freshly-prepared cup of coffee is the final expression, and perceptible result of a long chain of transformations (Yeretzian 2002).

This complex beverage contains over 1000 compounds that are responsible for its pleasant flavor and aroma (Nijssen 1996).

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.

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. Accounting the difference in the 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 (López-Galilea I 2007), (Caporaso 2014), (Pérez-Martínez M 2010).

All of these factors play important roles in modifying caffeine content and other compounds (Gloess 2013), (Niseteo 2012), (Andueza 2003), (Andueza. S. 2007).

There are many ways to prepare coffee and consumer preferences for a mode are influenced by various factors such as lifestyle, culture, and flavor preferences (Illy 2005). They can be brewed in several different ways, but these methods generally classified under into four main groups depending on how the water is introduced to the coffee grounds:

• Decoction (Boiling):

Is the technical name for extraction via water boiling. Coffee grounds are placed in boiling water to extract their flavor.

In decoction, ground coffee is in contact with high-temperature water for a period (generally a few minutes), causing a more intense extraction. The simplest method is to put the ground coffee in a cup, pour hot water over it and let cool while the grounds founder to the bottom. If the coffee beans are not ground finely enough, the grounds do not go under the bottom. *Turkish coffee* is an example of boiled coffee.

• Gravitational feed (Filtering):

Coffee is usually placed in a filter of some kind, and hot water is poured into it. The hot water extracts the flavor and seeps through the filter. Percolators and drip brewers use this technique.

In filters category are includes a variety of devices that use gravity to push water through a filter that holds coffee grounds. Some of the devices include:

- *Electric percolator*

Electric percolator is a popular device in western countries. Coffee is placed in a paper filter and placed into the machine. A container is placed beneath the filter to catch the coffee. The coffee machine drips hot water onto the

coffee filter, soaking the coffee. The filter allows the brewed coffee to flow through, but not the coffee grounds.

- *Cold brew coffee*

Coarsely ground coffee is placed in a large container with cold water. The coffee is allowed to brew in this cold water for a very long period

Coffee is 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.

- Infusion (Steeping):

it is the process of gradually extracting flavors from the coffee grounds by placing them in a solvent like hot or cold water.

An infusion involves steeping coffee in water before filtration and creates a milder brew with more acidity. (Sunarharum 2014). Steeping involves letting the coffee sit in hot or cold water for a set period to extract flavor from the coffee grounds.

The most famous device for the infusion brewing of coffee is the *French press*. It is a tall and narrow cylinder that comes with a plunger that has a fine mesh filter. The coffee grounds are placed into the container with the hot water; then after a few minutes of brewing, the plunger is used to push the coffee grounds to the bottom of the device. The coffee can be poured out while the plunger holds the coffee grounds at the bottom of the container.

- Pressurized Percolation:

Pressure is used to push hot water through tightly compacted coffee grounds. It is the technique used by espresso machines.

This technique involves hot water being pushed through coffee grounds under pressure.

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 thick foamy beverage (Petracco 2001).

The original EC formulation used 7 gr of coffee powder to obtain around 30 gr of espresso beverage. Nowadays, there are many different recipes, of which the most popular is specialty coffee. This preparation uses 7 gr of coffee powder to produce 14 gr of espresso beverage. As every gram of ground coffee turns into 2 grams 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 06 023 798.9; US 2010/0034942 A1) has been developed, which uses a sealed chamber and pressurized air (Masella 2015).

Other methods that use pressurized percolation include *Aeropress.*

It is a handheld, non-electrical device that uses pressure to push medium-temperature water through coffee grounds. 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.

The Moka Pot is another pressured method to obtained coffee. Moka is the most popular technique in Italian households. It is a three-chamber pot with water in the lower section and coffee in the middle. As it boils, the pressure created by the steam forces the water upwards, through the coffee grounds and into the third section.

The Figure **5** illustrates some of the extraction machine mentioned.

Figure 5: Different coffee brewing methods. A- French Press, B- Espresso, C- Filter, D- Cold Drip, E- Turkish, F- Boiling, G- Aeropress, H- Moka Pot.

The one reported is one of the classifications that is used to discriminate the various extraction methods; but is a common practice to sort the coffee beverage in long, filter and espresso-short coffee, thus classify respected to the type of beverage.

1.5 Bioactive compounds in coffee

The World Health Organization (WTO) considering coffee beverage a "non-nutritive dietary component", because of its two calories per cup of bitter coffee.

Among the many bioactive compounds present in coffee are methylxanthines (caffeine, theobromine, theophylline) diterpene alcohols (cafestol, kahweol), chlorogenic acids (caffeoylquinic acids, feruloylquinic acids, p-coumaroylquinic acids), flavonoids (catechins, anthocyanins), hydroxycinnamic acids (ferulic acid, caffeic acid, p-coumaric acid), tocopherols, and melanoidins. Caffeine is the most studied due to its numerous effects (de Mejia 2014).

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" (European Parliament and of the Council of Nutrition and Health Claims Made on Foods, 2006). It responds to the claims 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."

Several studies, have demonstrated that the consumption of coffee for a long time reduces both 30-50% the risk to develop the type 2 diabetes, this effect

it was also found in the decaffeinated coffee, and for this reason it attributed this benefit action to other compounds presents in the coffee as the chlorogenic acids and their derivates (Johnston K.L. 2003).

Other studies have demonstrated that coffee is one of the most important sources of polyphenols and caffeoylquinic acids (CQAs) (Kamiyama 2015).

Trigonelline is present in green coffee at 1% dry weight, with slightly higher values found in Arabica coffees than in Robusta. During the roasting process, trigonelline is partially degraded to nicotinic acid and several pyridine derivatives (Muriel 2010).

Trigonelline has been shown to possess hypoglycemic, neuroprotective, anti-invasive, estrogenic, and antibacterial activities.

Many investigations pointed out that also the melanoidins may have benefited in vivo effects, such as antioxidant activity, and phase I and phase II enzyme-modulating activity, including to be beneficial to human health, displaying in vivo antioxidant, antimicrobial, and prebiotic activity in the intestine (Borrelli 2004); (Daglia 2000); (Morales 2012).

1.5.1 Caffeine

Caffeine is a natural alkaloid present in coffee plants, cocoa, tea, cola, guarana and mate and in the drinks obtained from there. The Figure 6 shows the Caffeine chemical structure.

The alkaloid is heat stable, and the amount present in a raw coffee can vary significantly depending on many factors, among which the most important are origin and cultivar. Its concentration and biological activity depend on a blend of factors, such as raw materials. 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% (Severini 2017). Also the 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) are responsible to the different concentration of caffeine in beverage. 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 2014).
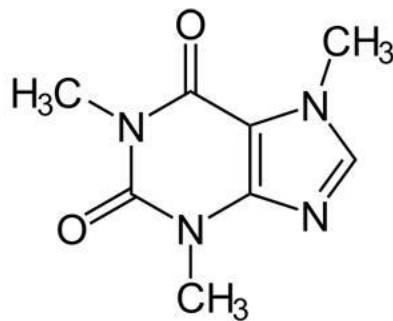
Figure 6: Chemical structure of the caffeine molecule

Caffeine exerts most of its biological effects through the antagonism of the adenosine receptor generally inducing the stimulatory effect in the central nervous system (Cano-Marquinaa A. 2013), (Bae J.H. 2014).

The positive effects of caffeine on the human body are widely known today, with particular reference to the improvement of cognitive abilities, including improved perception, reduction of tiredness, strengthening of memory performance, improvement of vigilance and a reduction in the duration of sleep (Capek 2009) (Borota 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 (Butt 2011). Besides, the physiological effects of caffeine

intake include acute elevation of blood pressure, increasing metabolic rate, and diuresis. (Bae J.H. 2014).

1.5.2 Chlorogenic Acids

The major polyphenol in coffee in chlorogenic acid and it is one of the significant strong antioxidant compounds in coffee (Bae 2014), (Ayseli 2016). CGA are esters of transcinnamic acids, such as caffeic, ferulic and p-coumaric acids, with (-)-quinic acid (QA), (Figure 7) (Clifford 2000).

These water-soluble acids are abundant in coffee, and the coffee plant forms them through esterification of trans-cinnamic acids (Higdon 2006). CGAs and their derivatives are known to contribute to the acidity, astringency, and bitterness of the final coffee beverage (Trugo 1984).

Quantitatively the main CGA subclasses in coffee are:

• caffeoylquinic acids (CQAs including the three isomers: 3-, 4- and 5-CQA)

• dicaffeoylquinic acids (diCQAs including the three isomers: 3.4-, 3.5 and 4.5-diCQA)

• feruloylquinic acids (FQAs: including the three isomers: 3-, 4- and 5-FQA)

• ρ-coumaroylquinic acid (pCoQA)

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 and Donangelo, 2006).
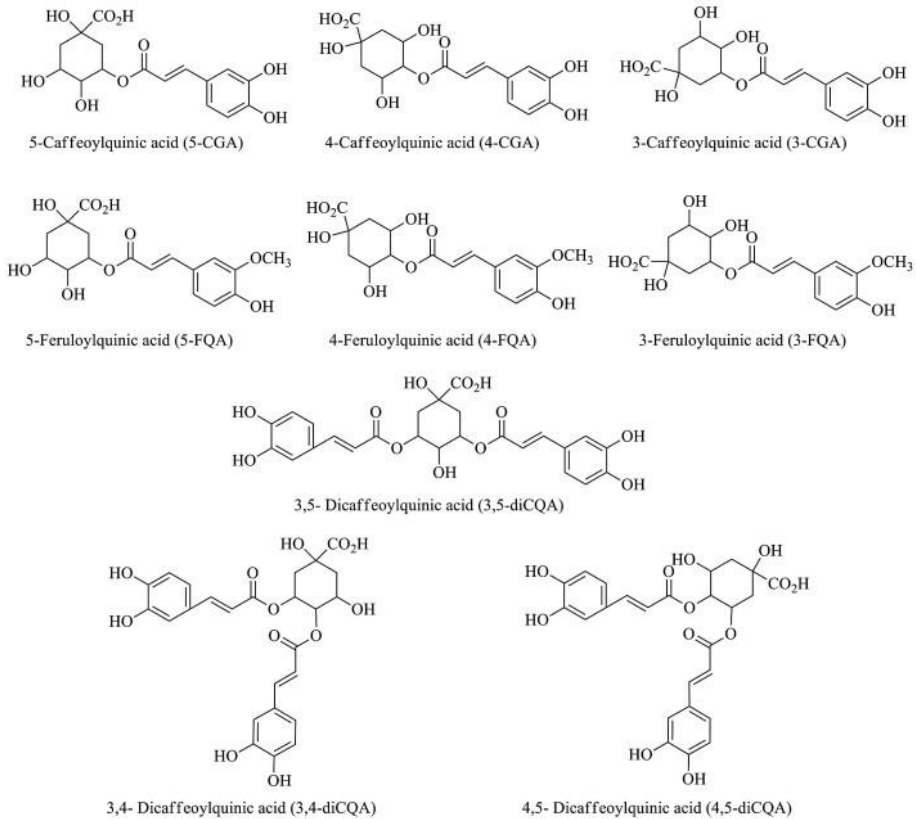
Figure 7: Structural representation of the major chlorogenic acids found in coffee. (Mills 2013)

Due to the abundance of CGA and their effect on sensory character in coffee, several attempts have been made to correlate the levels of CGA with beverage quality particularly trying to find correlations between specific sensory attributes, such as astringency, and the presence of specific CGA isomers.

It is well-known that CGAs contribute to the sweet and oily odor of green coffee beans odor (Gonzalez-Rios 2007). Besides, CGAs also generate volatile compounds such as phenol, γ-Butyrolactone and 2-methoxyphenol (guaiacol)

which give antimicrobial and, antioxidant properties to roasted coffee (Arumugam 2012).

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 (A. &. Farah 2015).

Nowadays, many studies have linked CGAs consumption to a wide range of health benefits, including antidiabetics, anti-obesity and anti-inflammatory effects (Ayseli 2016), (Shin 2015).

1.6 Properties of coffee by-products

The food industry produces large quantities of waste. The massive consumption of coffee creates large volumes of waste, due to the high production of this beverage. The coffee industry produces a large number of by-products during the transformation from bean to the coffee beverage (Nabais 2008).

Bioactive compounds are essential and non-essential compounds (e.g. vitamins or polyphenols) that occur in nature, are part of the food chain, and can be shown to affect human health (Biesalski 2009). These properties justify their isolation from the industrial wastes. These recovered compounds could be an alternative source for obtaining natural antioxidants.

Coffee is subjected to several stages of processing, such as pulping, washing, drying, curing, roasting, and brewing, and during the process, various by-

products such as coffee pulp (CP), cherry husk (CH), parchment husk (PH), silver skin (SS), and spent coffee ground (SCG) are obtained (Pushpa 2011).

Spent coffee ground (SCG) is the residue obtained during the brewing process (R. Cruz 2012). SCGs are the insoluble residue that remains after coffee beans are dehydrated, milled and brewed. There are two main sources of SCGs: those generated by the soluble coffee industry, which accounts for ~50% of the global coffee harvest each year, and those produced by cafés and by private consumption, accounting for the remaining 50% (Scully 2016).

From each cup of coffee made with an averaged 7 g of coffee powder, is obtained 13 g of spent coffee, where the increment of weight is due to the residual brewing water. It has been estimated a yearly production of 6 million tons of spent coffee (Janissen 2018).

SCG contain large amounts of organic compounds (i.e. fatty acids, lignin, cellulose, hemicellulose, and other polysaccharides) that can be exploited as a source of value-added products.

The different brewing systems commonly used (espresso, Moka, filter) present different extraction yield, generally lower than 50%. This has been reported in several studies. Gleoss et al. (Gloess 2013), reported that the extraction yield of Moka system was 31.2 %, while the filter coffee showed an extraction yield of 19 %. Merritt and Proctor (Merritt MC 1959) reported an extraction yield of 13.8–20.4 % on a dry basis at an extraction temperature of 93 °C and an extraction time of 0.5 min to 10 min for filtered coffee. Angeloni and co-authors (Angeloni G. 2018 b), reported several extractions yields for different coffee preparation systems. For the espresso preparations it was revelated 22.6±1.5 % and for French Press 18.61±1.20 %.

In the last decade the increase of knowledge and the necessity to reduce the waste environmental impact have stimulated the research of different methods to use the by-products of the coffee industry. Furthermore, the

presence in the spent coffee powder of bioactive compounds such as tannin, caffeine, and polyphenolic compounds limits the use of this waste in animal feedstuffs due to their antinutritional effects. The storage of this waste is a serious environmental problem and requires sustainable ways for its management, as well as new utilization solutions.

Recently, the coffee residue has been investigated for biodiesel production (Kondamudi 2008) (Kwon 2013), as a possible fertilizer after composting (Adi 2009), a versatile barrier to reduce pesticide leaching through the soil (Fenoll 2011).

However, the presence of chlorogenic acids limits its use as a fertilizer due to its phytotoxic action, and it had an adverse effect on germination of seeds and plant growth (Janissen and Huynh 2018).

The possibility to use SCG as animal feed for ruminants, pigs, chickens, and rabbits (Givens 1986) have already been also verified, but the high lignin content (≈25%) in this material was considered a limiting factor for its application. More recently, it has been studied the positive effect of the addition of spent coffee to the substrate for the edible fungi cultivation (Murthy P.S. 2012).

Several studies have been focused on using the spent coffee without treatments, in particular, many researches evidences the possible use of this waste as an adsorbent for the removal of cationic dyes (Franca 2009), or as a heavy metal chelator in wastewater treatment (Tokimoto 2005), (Utomo 2006), (Zuorro 2012).

An overview of the potential usages of SCG is presented in figure 8 .
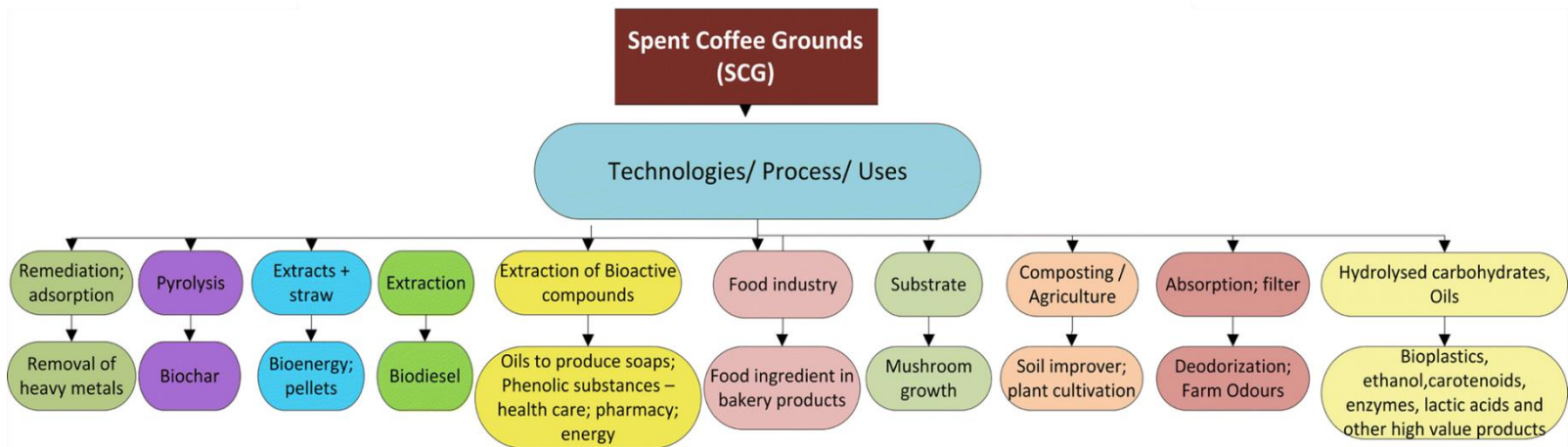
Figure 8: Overview of potential usages of SCG (Stylianou 2018).

In the last few years, the research was focused on the recovery of by-products from coffee as a source of bioactive compounds. Caffeine is undoubtedly the most studied compound given its widely known psychoactive effects and its exciting action of energy metabolism.

Chlorogenic acids (CGAs) are the main components of the phenolic fraction of green coffee seeds; several studies report their strong antioxidant activity in vitro (Yen W.J. 2005), (Brezová 2009) and numerous health benefits (Rawel 2007). The main CGAs present in coffee are highly bioavailable, easily absorbed or metabolized throughout the gastrointestinal tract.

These compounds are only partially extracted during the preparation of the beverage, which is why exhausted coffee is still a vital source of potentially useful bioactive compounds.

Recent studies highlight the high potential of coffee grounds for the presence of natural phenolic antioxidants, including CGAs and derivatives, as well as caffeine (Panusa 2013) (J. J. Bravo 2012).

Among the chlorogenic acids, the exhausted coffee contains caffeoylquinic acids, feruloylquinic acids, p-cumaroylquinic acids, and mixed diesters of caffeic acid and ferulic acid with quinic acid (Farah 2006).

Due to these essential biofunctionalities, phenolic compounds have found numerous applications in food and pharmaceutical areas. Thus, extracting antioxidant phenolic compounds from SCG can be thus considered a stimulant alternative to obtaining these important industrial ingredients from low-cost raw material, while thus add value to coffee waste.

2. Aim

The aim of the work was at first, to characterize and compare eight different coffee extraction methods from a physical and chemical point of view, starting from the same raw material. The aim was not to establish the "best" practice. Instead, it highlights that different extraction methods produce coffee with different qualitative and quantitative characteristics, starting from the same raw material.

After, the attention was focused on cold extraction: cold brew and cold drip. The effects of the primary process variables (temperature and contact time between coffee powder and water) were assessed in a full factorial experiment.

Finally, the focus of the third study concern to develop a green extraction method for water-soluble compounds from spent coffee ground (SCG), and to test which physical and chemical variables influence the amounts of phytochemicals extracted.

Spent coffee grounds (SCG) are the most abundant coffee by-product (45%) generated in coffee beverage preparation and instant coffee manufacturing. One approach to reducing the environmental impact of these residues is to transform them into value-added products using SCG as a raw material for the recovery of phytochemicals.

3. INVESTIGATION AND COMPARISON OF DIFFERENT COFFEE EXTRACTION METHODS

3.1 Introduction

As previously mentioned, there are several methods to extract the beverage, and for each method, there is various type of coffee.

Standard preparation methods have been developed for different types of extraction. These methods differ concerning 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 2003), (Gloess 2013), (Caporaso 2014), (Parenti 2014), (Masella , 2015).

These studies reveal that there is no 'best' extraction method, but that each technique has its 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. The analysis of physicochemical parameters characterized these methods. 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).

The positive effects of the caffeine are well-known; in particular, improvements related to cognitive abilities such as better perception, reduced tiredness, and shorter duration of sleep (Borota 2014). Besides, the physiological effects of caffeine intake include acute elevation of blood pressure, increasing metabolic rate, and diuresis (Bae 2014). Its

concentration and biological activity depend on a blend of factors, such as raw materials (Arabica or Canephora) (Severini 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).

Many studies have demonstrated that coffee is one of the most important sources of polyphenols and caffeoylquinic acids (CQAs). Chlorogenic acid it is one of the significant strong antioxidant compounds in coffee (Bae 2014).

These water-soluble acids are abundant in coffee, and the coffee plant forms them through esterification of trans-cinnamic acids (most notably caffeic, ferulic, and p-coumaric) with quinic acid (Higdon, 2006). CGAs and their derivatives are known to contribute to the acidity, astringency, and bitterness of the final coffee beverage (Scholz and Maier 1990), (Trugo and 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 and Donangelo 2006), (Moeenfard 2014).

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.

3.2 Materials and methods

3.2.1 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, finally, the equipment. The extraction parameters were summarized in Table 2.

Six replicates were performed for each brewing method.

Table 2: Extraction parameters

Extraction method	Grinding	Powder (g)	Water (mL)	T (°C)	Pressure (bar)	T ime	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

3.2.2 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, 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 5–2000 μm. In Figure 9 has shown the bi-modal curve for three coffee powder grinders, using Laser diffraction technique, with the Mastersizer laser granulometer.

	Record Number	Sample Name	Dx (10) (μm)	Dx (50) (μm)	Dx (90) (μm)
	17	Average of 'ESPRESSO	27,4	232	445
	8	COLD BREW	129	553	956
	6	V60	68,8	440	767
Mean			75,0	408	723
1xStd Dev			50,9	163	258
1xRSD (%)			67,9	39,9	35,7
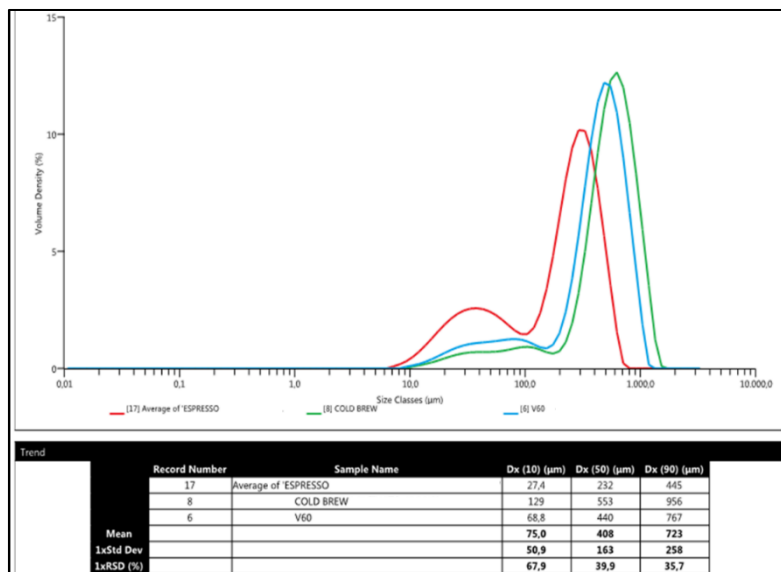
Figure 9: Bi-modal curve for the espresso, cold brew and v60 powders particle sizes.

As water quality plays an important role in coffee beverage quality (Navarini and Rivetti, 2010) all samples were prepared using the same commercial brand of mineral water (physicochemical characteristics are shown in Table 3).

Table 3: Physicochemical characteristics of mineral water used in trials

Analytical parameter	Values
pH	8.1
Electrical conductivity (20 °C)	249 µS/cm
Total dissolved solids	148 mg/L
Hardness	14 °F
Kubel oxydability	0.6 mg/L
Free carbon dioxide	3.3 mg/L
Calcium (Ca^{2+})	30.1 mg/L
Magnesium (Mg2+)	15.0 mg/L
Sodium (Na^+)	1.4 mg/L
Potassium (K^+)	0.5 mg/L
Hydrogen carbonate (View the MathML source)	157 mg/L
Sulfate (View the MathML source)	10.7 mg/L
Nitrate (View the MathML source)	5.0 mg/L
Chloride (Cl^-)	1.5 mg/L
Fluoride (F^-)	0.06 mg/L
Silicon dioxide (SiO_2)	6.6 mg/L

EC Espresso classical method

A conventional bar machine (GS3, La Marzocco, 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 and Viani, 2005).

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).

ECF Espresso Caffè Firenze

Caffè Firenze (ECF) samples (Patent 06 023 798.9; 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.

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.

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 (L. N.-L. Navarini 2009).

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).

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, was 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.

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, like 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.

3.2.3   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).

3.2.4   Analysis 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. (Angeloni 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; r2=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; r2=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).

3.2.5 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 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.

3.2.6 Statistical analyses

Conventional analysis of variance (ANOVA) was used to compare means determined for the different extraction methods. The tested factors were considered significantly different at $p < 0.05$. All statistical analyses were performed using R software (version 3.4.0 for Windows).

3.3   Results and discussion

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

Caffeine and CGAs were compared and expressed in term of concentration (mg/ml), extraction efficiency (mg/g), and bioactive compounds per cup. Physical measurements included TDS, density, pH, conductivity and viscosity. 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.

3.3.1 Cluster analysis

Homogenous groups of brewing techniques were identified by a cluster analysis. As shown in Figure 10, 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 2.
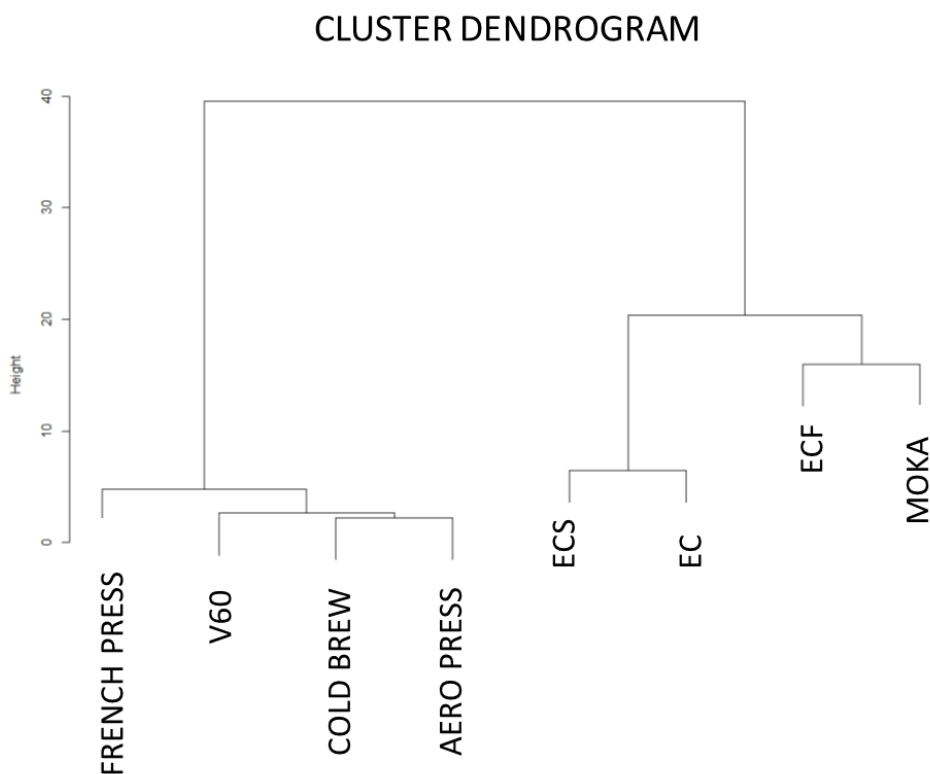
Figure 10: Cluster analysis of extraction methods. List of acronyms: EC, espresso coffee; ECS, specialty espresso, ECF, caffè Firenze.

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

3.3.2   Physical analyses
The physical characterization of the coffee beverage produced using the different preparation methods is shown inTable 4.
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 I 2007) . It is well-known that TDS % affects the sensory property described as 'body' (Gloess 2013) and seems to be related to the coffee/water ratio (Andueza 2007), and the brewing procedure (López-Galilea 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.

Table 4: Physical characterization of coffee beverages [1, 2].

	ECF	ECS	EC	V60	Cold Brew	Aero-press	French Press	Moka
pH	5.16 ± 0.1 a	5.3 ± 0.25 a	5.17 ± 0.07 a	5.15 ± 0.12 a	5.12 ± 0.1 a	5.16 ± 0.11 a	5.16 ± 0.13 a	5.1 ± 0.24 a
TDS %	3.32 ± 0.4 a	8.44 ± 0.38 b	5.2 ± 0.35 c	1.55 ± 0.04 d	1.54 ± 0.06 d	1.52 ± 0.06 d	1.35 ± 0.03 d	3.4 ± 0.15 a
Extraction %	13.46 ± 1.56 a	17.54 ± 0.86 b	22.59 ± 1.51 c	22.14 ± 0.65 c	20.89 ± 0.82 d	20.56 ± 0.67 d	18.61 ± 1.2 b	28.6 ± 1.03 e
Density 20°(g/mL)	1.02 ± 0.03 a	1.01 ± 0.01 a	1.04 ± 0.03 a	1.07 ± 0.09 a	1.05 ± 0.05 a	1.06 ± 0.05 a	1.07 ± 0.07 a	1.06 ± 0.02 a
Viscosity (mN s m-2)	115.15 ± 3.29 a	151.59 ± 7.01 b	123.13 ± 2.7 c	99.76 ± 3.44 d	100.83 ± 2.4 d	101.74 ± 2.62 d	98.25 ± 3.97 d	111.61 ± 2.56 a

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;

3.3.3   Analysis of caffeine and CGAs

The analyzed samples showed almost the same qualitative profile of bioactive substances found in HPLC/DAD profiles at 278 nm for monitoring caffeine, and at 330 nm for CGA detection.

A total of 15 CGAs were detected. Figure **11** presents chromatographic profiles at 278 and 330 nm.
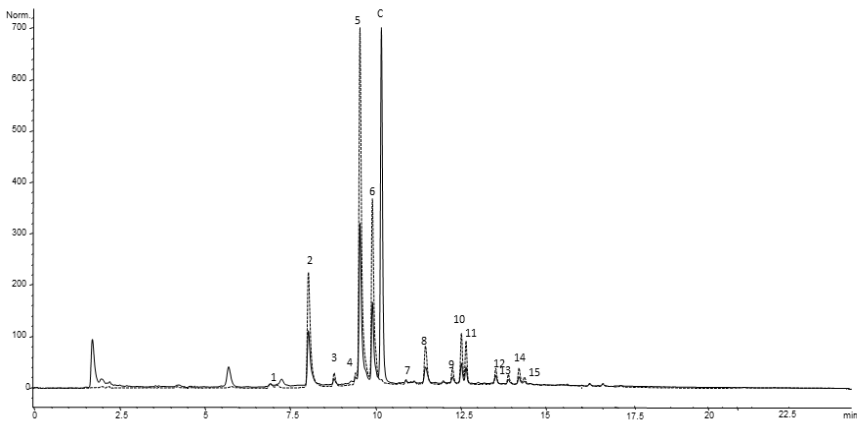
Figure 11: Overlapping of HPLC/DAD chromatograms at 278 nm (whole line) and 330 nm (dotted line) for CGAs and caffeine monitoring of a representative coffee sample.

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.

Fujioka and Shibamoto (Fujioka 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 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 5, 6, and 7 respectively). Furthermore, figure 11, 12, 13 reports mean values for caffeine and total CGAs.

Concentration of bioactive compounds (mg/mL)

Table 5 shows that there was a significant difference in caffeine concentration for the methods tested ($p \leq 0.05$). 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).

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) (López-Galilea 2007), (Caporaso 2014). 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 because the chamber in which the coffee panel was placed in direct contact with water at 75 °C (Masella 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 35–39% 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 ($p \leq 0.05$). Intermediate values were found for the latter (Figure 12 and table 5).
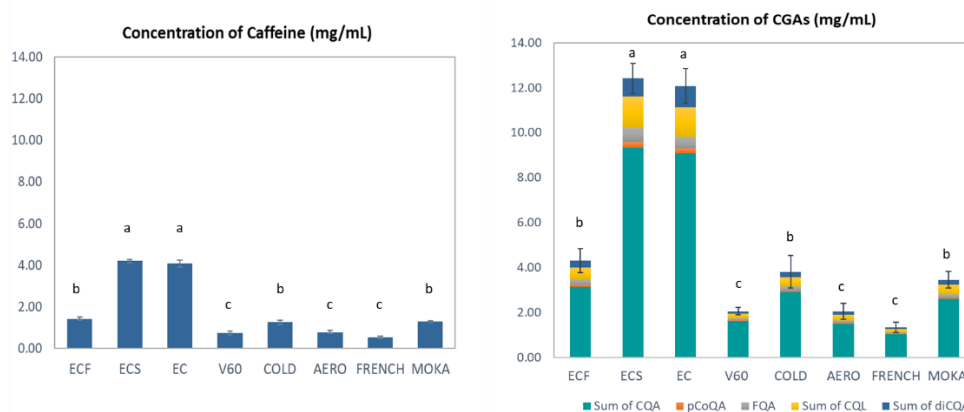

Figure 12 : Content per ml of extract of caffeine and of sum of CGAs. Letters indicate statistically significant differences between extraction methods. Error bars correspond to the standard deviation (95 %).

Table 5: Chemical characterization beverages. Concentrations (mg/ml) of caffeine, CQAs, CEQAs, 5-FQA, 5-pCoQA, CQLs and diCQAs are reported[1].

	ECF	ECS	EC	V60	COLD BREW	AEROPRESS	FRENCH PRESS	MOKA
Caffeine	1.43 ± 0.07 b	4.20 ± 0.09 a	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 ± 0.02 b	0.20 ± 0.02 a	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 ± 0.06 b	1.86 ± 0.01 a	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 ± 0.01 b	0.23 ± 0.02 a	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 ± 0.02 b	0.17 ± 0.02 a	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 ± 0.17 b	4.80 ± 0.30 a	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 ± 0.11 b	2.50 ± 0.30 a	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 ± 0.02 b	0.27 ± 0.07 a	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 ± 0.04 b	0.71 ± 0.08 a	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 ± 0.01 b	0.12 ± 0.04 a	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 ± 0.02 b	0.31 ± 0.07 a	0.31 ± 0.06 a	0.04 ± 0.01 c	0.07 ± 0.02 bc	0.05 ± 0.02 c	0.03 ± 0.00 c	0.06 ± 0.02 bc
CQL†	0.21 ± 0.04 b	0.61 ± 0.07 a	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 ± 0.03 b	0.52 ± 0.09 a	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 ± 0.03 b	0.28 ± 0.08 a	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 ± 0.02 b	0.21 ± 0.07 a	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 ± 0.03 b	0.41 ± 0.11 a	0.38 ± 0.04 a	0.05 ± 0.01 b	0.09 ± 0.02 b	0.07 ± 0.03 b	0.03 ± 0.01 b	0.09 ± 0.02 b

1. Data are expressed as mean ± standard deviation. Letters indicate statistically significant differences between extraction methods. † indicates that the acylation position was uncertain

Several studies have assessed the influence of contact time and brew ratio on bioactive compound extraction (Andueza 2007), (Crozier 2009), (Caprioli 2015). 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 at 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, 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).

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, 2008). Table 6 shows that there was a significant difference in extraction efficiency among all 15 CGAs, for the tested methods ($p \leq 0.05$, letters indicate statistically significant differences between groups). The analysis showed that extraction 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 (7g/30mL for EC and 9g/18mL 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).

Table 6: Chemical characterization of beverages. Extraction efficiency (mg/g) of caffeine, CQAs, CEQAs, 5-FQA, 5-pCoQA, CQLs and diCQAs are reported[1].

	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

1. Data are expressed as mean ± standard deviation. Letters indicate statistically significant differences between extraction methods. † indicates that the acylation position was uncertain.

Concerning CGA concentrations, trends were similar to those for caffeine for all 15 detected compounds. Figure 13 shows 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 have been least efficient and significantly different to V60, Cold Brew, and Aeropress methods. These trends agree with earlier data (Gloess 2013), which found the highest concentrations of the most abundant CGAs for expresso, followed by Moka and filter coffee.

Figure 13: Content per gram of coffee powder of caffeine and of sum of CGAs. Letters indicate statistically significant differences between extraction methods. Error bars correspond to the standard deviation (95 %).

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 18-30 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 and co-authors (Romani S. 2004) argue 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 7, EC contained much more caffeine than ECS. However, these two expressos were prepared with different cup volumes 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 **Errore. L'origine r iferimento non è stata trovata.** and table 7, 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.
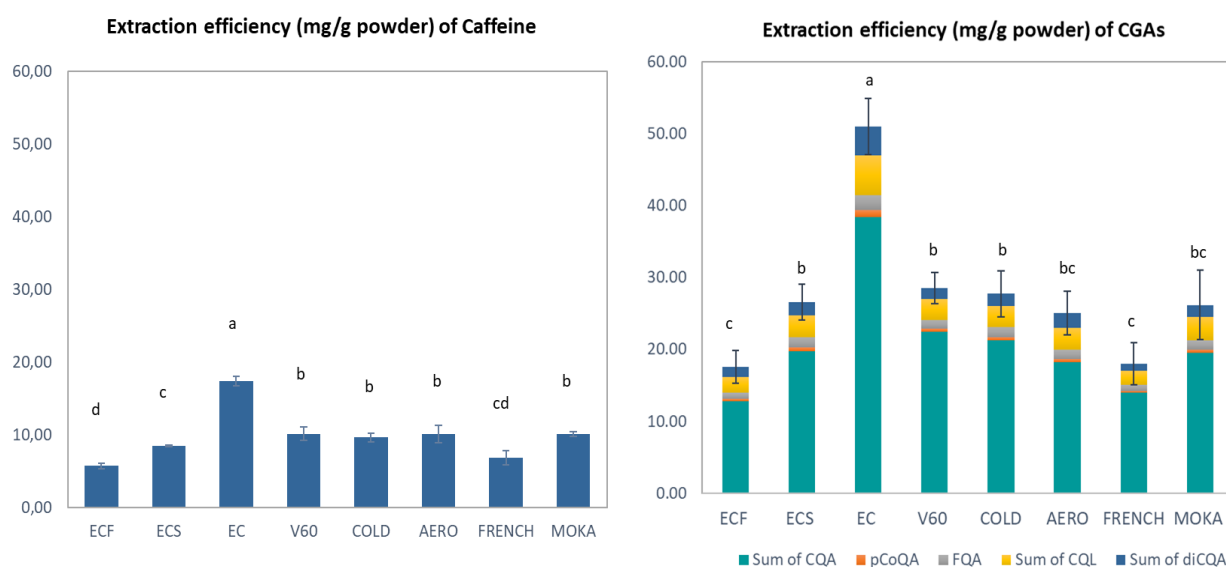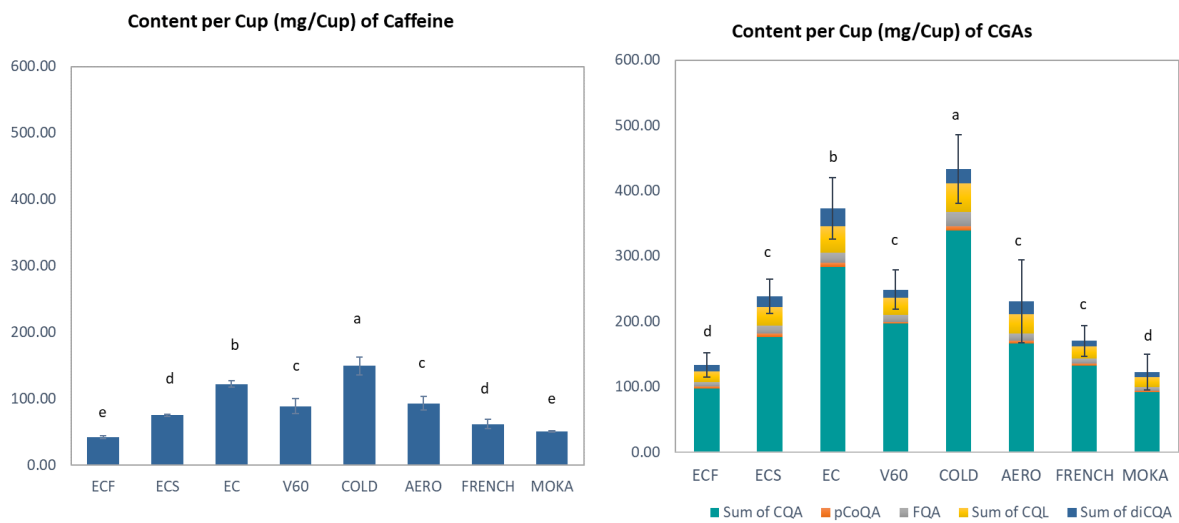

Figure 14: Content per cup of caffeine and of sum of CGAs. Letters indicate statistically significant differences between extraction methods. Error bars correspond to the standard deviation (95 %).

Table 7: Chemical characterization of beverages. Bioactive content per cup (mg/cup) of caffeine, CQAs, CEQAs, 5-FQA, 5-pCoQA, CQLs and diCQAs are reported[1].

	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 ± 0.34 c	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

1. Data are expressed as mean ± standard deviation. Letters indicate statistically significant differences between extraction methods. † indicates that the acylation position was uncertain

3.4. 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. Instead, it highlights that different extraction methods produce coffee with different qualitative and quantitative characteristics, starting from the same raw material.

4. Characterization and comparison of Cold Brew and Cold Drip Coffee Extraction Methods

4.1 Introduction

The brewing method affects the composition of the final coffee beverage: notably, considerable differences are found in polyphenol extraction, caffeine content, total solids, antioxidant activity and volatile profile (López-Galilea I 2007).

During extraction, soluble compounds are dissolved and, depending on the extraction methods, non-soluble compounds are washed with the extraction water, ending up in the extract as dissolved or suspended solids (López-Galilea I 2007), (Caprioli 2015).

However, the temperature of the brewing water is usually consistent. Hot water is used in order to increase the extraction yield, whereas several chemical extraction studies have shown that different aromatic compounds are extracted at different temperatures (Salamanca 2017), (Andueza. S. 2007). Although consumers traditionally drink hot coffee, in recent times the consumption of cold coffee has increased in northern European countries, the United States and Japan (FiorMarket 2017), due to new preparation methods that involve longer extraction times at colder temperatures (i.e. room temperature or less), rather than rapid exposure to high temperatures. This, cold brew method, indicates a coffee produced by cold extraction, and should not be confused with cold coffee, which is usually produced by a hot system and left to cool down. Several recipes for the extraction of coffee powder with cold water have been developed.

They differ concerning apparatus configuration, the contact time between powder and water, and water temperature, but can be categorized into two broad methods: cold brew and cold drip.

In the cold brew method, coffee powder is steeped in a volume of water at room temperature (or colder) for a long time (six hours or more), then separated by filtering.

In the cold drip method, water at room temperature (or colder) is slowly dripped onto a coffee panel supported by a filter, and the beverage is recovered.

For these new methods, there are not specific and unequivocal recipes, regarding times and extraction temperatures, baristas rely on their perception and experience to set extraction parameters. However, there have been few empirical investigations of these slow, cold extraction methods that are designed to produce a lungo coffee.

The aim of this study was to characterize and compare cold brew and cold drip extraction methods in terms of the chemical composition, physical properties, and sensory evaluation of the coffee that is produced. The effects of the main process variables (temperature and contact time between coffee powder and water) were assessed in a full factorial experiment. In order to introduce a

benchmark for beverage characterization, a third extraction method, the French press, was included in the experimental design.

4.2 Materials and Methods

4.2.1 Experimental design

The experiment was designed to highlight differences between two cold extraction methods: cold brew and cold drip. Two temperatures (room temperature, 22°C; refrigerator temperature, 5°C) and two powder–water contact times were tested. Coffee preparation methods and operative conditions are shown in Table 8.

Three replicates were performed for each sample. The order of beverage preparation was completely randomized.

For the cold drip method, contact time between the powder and water was tested at two flow rates: one drops every 5 s and one drop every 10 s.

For the cold brew method, extraction time was calculated from the two overall extraction times for the respective cold drip method.

In addition, French Press extraction method has been chosen as benchmark.

Table 8: Coffee preparation methods and operative conditions.

Extraction Procedure	Temperature	Time - Flow Rate
Cold Drip	22 °C	1 drop /5 s
Cold Drip	5 °C	1 drop /5 s
Cold Drip	22 °C	1 drop /10 s
Cold Drip	5 °C	1 drop /10 s
Cold Brew	22 °C	3 h
Cold Brew	5 °C	6 h
Cold Brew	22 °C	6 h
Cold Brew	5 °C	3 h
French Press	95 °C	5 min

4.2.2 Coffee samples and Extraction methods

The same batch of coffee was used for all extractions (Illy Rosso 100% Arabica). Each pack of coffee beans (250 g) was opened immediately before brewing to avoid oxidative damage. Beans were coarse-ground using a professional coffee grinder (KE640, Ditting Maschinen AG, Switzerland).

The coffee was grinded 'coarse' as well as for all the other lungo and filter methods (Clarke R 2008). Water quality plays an important role in coffee beverage quality (L. R. Navarini 2010) , so all samples were prepared using the same commercial brand of mineral water, described in previous study.

For cold drip method, samples were prepared using a cold drip coffee equipment with 25 g coffee powder and 250 mL mineral water at different temperatures of extraction and times/flow rates. The Equipment comprised three parts, as shown in Figure 14.

An upper (glass) part (a), 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 (b). Water entered from above, passed through a filter, and into a lower carafe (c), where the final brew was collected. Used coffee grounds were retained in the filter.

The average extraction time was 6.5 h for the slower times of extraction/flow rate, and 3.3 h for the faster times of extraction/flow rate. Extraction was performed at room temperature (22°C) and at refrigerator temperature (5°C).
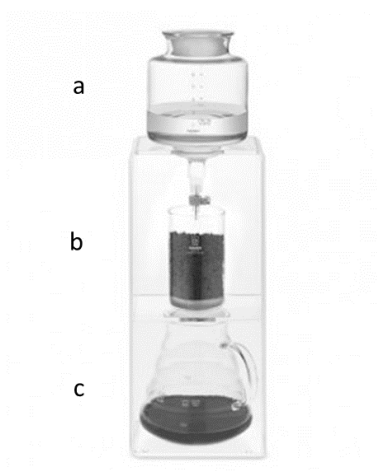
Figure 14: Cold drip equipment.

Cold brew coffee was prepared using 25 g of coffee powder and 250 mL of water. Cold brew extraction was performed under static conditions.

Powder and water were in contact for the same amount of time as the cold drip method (6.5 h for the slower, and 3.3 h for the faster), as shown in Figure 15.

When extraction ended, the beverage was filtered through a paper filter. Extraction temperatures were the same as for the cold drip method.

Figure 15: Cold brew equipment.

French press was prepared with 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.

4.2.3 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. Refractive index was measured with a portable digital refractometer (Refracto 30PX, Mettler Toledo, Italy) using the total internal reflection method. Total solids, expressed as mg/mL, were measured gravimetrically by drying about 10 mL (less than ± 0.5 mL) of coffee at 100°C for 24 h, until a constant weight was reached (Caporaso 2014).

4.2.4 Analysis of caffeine and chlorogenic acids

Coffee samples were centrifuged at 12000 rpm for 5 min and diluted 1:10 with water before high performance liquid chromatography (HPLC) analysis. HPLC was carried out using an Agilent HP 1100 system equipped with an autosampler, column heater module and quaternary pump, and coupled to a diode array detector (DAD) and a time-of-flight (TOF) mass spectrometer equipped with an electrospray interface (ESI), all from Agilent Technologies (Palo Alto, CA, USA).

HPLC was performed under the following conditions: gas temperature 300°C, nitrogen flow rate 12 L/min, nebulizer pressure 20 psi, capillary voltage 3800 V, and fragmentors in the range 120–300 V, operating in negative ion mode for CGAs and in positive ion mode for caffeine.

An InfinityLab 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. Elution 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 HPLC grade. Starting from our previous work the applied multistep linear gradient was modified as follow start from 95% A followed by a plateau for 5 min, 15 min to 56% A, 2 min to 10% A, and a final plateau of 5 min at 10% A.

The total analysis time was 24 min. UV–vis spectra were recorded in the range 220–600 nm, and the detector was set at 330 nm for CGAs and 278 nm for caffeine.

CGAs and caffeine were identified by comparing their retention times, UV–vis and MS spectra to those of the respective standards. Identification of other CGAs was performed by ESI-MS/MS using an API 4000 triple quadrupole mass spectrometer equipped with a TurboIonSpray® source (Applied Biosystems/Sciex, Toronto, Canada). The source was operated in negative ionization mode with a needle potential of −4500 V and turbo gas flow rate of 10 L min^{-1} of air heated to 150°C (nominal heating-gun temperature).

Mass calibration and resolution adjustments on the resolving quadrupoles were performed automatically using a 10^7 mol L^{-1} polypropylene glycol (PPG) solution introduced via a built-in infusion pump. The peak width was set on both resolving quadrupoles at 0.7 Th (measured at half height) for all MS and MS/MS experiments.

Collision-activated dissociation (CAD) MS/MS was performed in the LINAC Q2 collision cell, operating with nitrogen at 10 mTorr as the collision gas. The declustering potential and collision energy were automatically optimized for all species studied using Analyst 1.4 software. The acquired data were processed using Analyst 1.5.2 proprietary software, with the 'Explore' option for spectral interpretation.

CGAs were evaluated by HPLC/DAD using a five-point calibration curve of chlorogenic acid (purity 99%) (Extrasynthèse, Genay, France) at 330 nm (r^2 = 0.999), and caffeine content was determined by HPLC/DAD using a six-point calibration curve from Extrasynthèse (purity 95%) at 278 nm (r^2 = 0.999).

4.2.5 Sensory evaluation

Sensory evaluation was performed by a panel of eight trained sensory experts. Each brew was tested in duplicate in an air-conditioned room at 22°C in independent sessions. The classification system developed by SCAA was used (S. C. SCAA 2009). This includes a standard list of attributes, divided into broad and specific categories.

For each sample, trained panelists were first asked to rate the intensity of odor descriptors perceived by the nose (aroma). Then, they were asked to sip the sample and rate the intensity of odors perceived retronasally. Finally, they took a second sip and rated taste and mouthfeel attributes. The odor attribute was Overall Intensity. Flavor attributes were Overall Intensity, Acidity, Sweetness, Bitterness, Enzymatic (flowery, fruity, herby), Sugar Browning (nutty, caramelly, chocolatey), Distillation (carbon, spicy, resinous), and Astringency. The perceived intensity of each sensation was rated on a nine-point scale ranging from 1 (extremely weak) to 9 (extremely strong).

4.2.6 Statistical analysis

Differences between means were assessed using a conventional analysis of variance (3 way-ANOVA) in a full factorial experiment. Three factors, and all their interactions were tested at two levels: extraction methods (Cold Drip and Cold Brew), temperature (5°C and 22°C) and times/flow rates (fast/slow). In cases where the F-test was significant at the $p < 0.05$ level, multiple paired-means tests checked for significance using the post hoc Tukey Honest Significance Difference test ($p < 0.05$). All statistical analyses were performed using R software (version 3.4.0 for Windows).

4.3 Results and discussion

4.3.1 Physical analyses

The physical characterization of beverages is shown in ᴛᴀʙʟᴇ 9, which compares preparation methods, temperature and flow rate conditions. For each parameter, the mean, standard deviation, and p-value of the three variables and the significant interactions are reported. This analysis highlights differences between beverages prepared with different methods and under different conditions.

Significant differences were found for refractive index, pH and total solids. With respect to the refractive index, for the cold drip method, the higher temperature resulted in a higher refractive index, whereas no difference was found for the cold brew method (interaction extraction method * temperature p = 0.025). Furthermore, refractive index consistently increased as the time of contact between powder and water increased.

Similarly, temperature was found to influence pH (p = 0.0051). Infusion temperature is recognized as an important factor in coffee beverage preparation, and lower temperatures usually reduce the quantity of extracted beverage (Andueza 2003).

Here, temperature proved to be a decisive influence on the measured physical parameters. Coffee prepared at lower temperature (5°C) had a higher pH, regardless of the extraction method (drip or brew). pH varied from 5.5 ± 0.1 (at 22°C) to 5.7 ± 0.1 (at 5°C) (Table 8). These values were higher than the French press method (5.2 ± 0.1).

Nicoli and co-authors (Nicoli 1991) showed that total solids are regulated by the brewing formula, coffee/water ratio, roast and percolation temperature. Similarly, in the present study, temperature was found to have a significant effect (p = 0.033), with more total solids in coffee prepared at 22°C (20.115 ± 1.992 mg/mL) than that prepared at 5°C (17.56 ± 2.38 mg/mL). Total solids were lower compared to the benchmark French press method (27.358 ± 3.71 mg/mL), probably due to the lower extraction temperature.

However, a high variance in the total solids from different preparation methods has been reported in literature (Gloess 2013), (López-Galilea I 2007).

No significant difference was found for viscosity and density for either method, or for temperature or contact time. Furthermore, the values of these parameters were similar to measurements using the French press method. These parameters changed in different Espresso brewing techniques (Parenti 2014) .

Table 9: Physical characterization of coffee beverages, comparing extraction method, temperature and flow rate.

Extraction Temperature FlowRate	Drip 22°C Fast	Brew 22°C Fast	Drip 5°C Slow	Brew 5°C Slow	Drip 22°C Slow	Brew 22°C Slow	Drip 5°C Fast	Brew 5°C Fast	T	F	E:T	French Press
Refractive Index	1.37 ±0.50	0.77 ±0.06	0.9 ±0.62	1.2 ±0.2	1.5 ±0.1	1.13 ±0.06	0.63 ±0.35	0.67 ±0.06	0.019 *	0.0248 *	0.024 *	0.98 ± 0.19
pH	5.5 ±0.02	5.63 ±0.3	5.79 ±0.1	5.67 ±0.0	5.44 ±0.1	5.5 ±0.1	5.67 ±0.1	5.64 ±0.1	0.005 **	ns	ns	5.24 ± 0.02
Density 20° (g mL^{-1})	1.05 ±0.005	1.05 ±0.01	1.02 ±0.01	1.08 ±0.01	1.07 ±0.03	1.06 ±0.03	1.05 ±0.01	1.05 ±0.03	ns	ns	ns	1.06 ± 0.01
Viscosity (mN s m^{-2})	1.08 ±0.06	1.07 ±0.03	1.03 ±0.11	1.14 ±0.07	1.11 ±0.02	1.12 ±0.11	1.06 ±0.05	1.04 ±0.07	ns	ns	ns	1.13 ± 0.08
Total solid mg/mL	19.41 ±2.9	20.3 ±2.13	14.02 ±3.43	18.42 ±0.69	18 ±4.76	22.75 ±0.68	18.89 ±0.74	18.94 ±0.10	0.033*	ns	ns	27.35 ± 3.7

Mean, standard deviation, and p-value for significant variables, and their significant interactions are reported. In a separate column are reported the mean and standard deviation for the French press extraction used as the benchmark. (T= Temperature, E= Extraction, F= FlowRate).

4.3.2 Analysis of caffeine and chlorogenic acids

Qualitative results

CGAs and their derivatives are known to contribute to the acidity, astringency and bitterness of the final coffee beverage (Trugo 1984). Chlorogenic acid lactones (CGLs) are formed from CGAs during roasting, through a process that involves the loss of a water molecule from the quinic acid moiety and the formation of an intramolecular ester bond. Along with CGAs, CGLs contribute to coffee flavor and, despite their low concentrations, their impact on the final cup quality may be significant. CGLs have also been studied for their potential hypoglycemic effects, and their action on opioid and adenosine brain receptors (Farah 2009), (Jaiswal R 2010). The analyzed samples showed almost the same qualitative profile of bioactive substances found in HPLC/DAD profiles at 278 nm for monitoring caffeine, and at 330 nm for CGA detection (as shown previously in Figure 11).

A total of 14 CGA compounds were detected in coffee samples. Their peaks were identified based on UV spectra and elution/retention sequences reported in the literature and confirmed by their mass spectrometric behavior.

In the first set of experiments, coffee samples were analyzed by ESI-TOF mass spectrometry in negative ionization mode, to define the molecular weights of the different compounds. Five Caffeoylquinic acids (CQAs), one feruloylquinic acids (FQA), one *p*-coumaroylquinic acids (*p*-CoQA), four caffeoylquinic acid lactones (CQLs), and three dicaffeoylquinic acids (diCQAs) were identified,

according to Clifford (Clifford 2000) and Jaiswal (Jaiswal R 2010). In a second set of experiments, samples were subject to product ion scan measurement (MS2) in negative ionization mode using an ESI triple quadrupole mass spectrometer. Table 10 summarizes MS2 data for monoacyl and diacyl CGAs and CGLs.

Table 10: Negative ion MS^2 fragmentation data for CGAs.

MS^2 ion

Identity	Rt	Parent ion (m/z)	MS^2 base peak m/z	m/z	int	m/z	int	Ref
3-CQA	8.1	353.2	190.9	179.0	61.6	173.0	2.9	Jaiswal et al. 2010; Scutz et al. 2004Clifford et al. 2005;
C*e*QA [†]	8.9	353.2	191.1	179.0	4.4	173.0	6.7	Jaiswal et al. 2010
C*e*QA[†]	9.45	353.2	179.0	190.7	85.7	173.2	28.6	Jaiswal et al. 2010
5-CQA	9.69	353.2	190.8	178.8	2.9	160.6	2.9	Clifford et al. 2005; Jaiswal et al. 2010; Schutz et al. 2004
4-CQA	10.07	353.2	173.0	178.9	81.2	191	37.3	Clifford et al. 2005; Jaiswal et al. 2010; Schutz et al. 2004
5-p-CoQA	11.06	337.2	190.9	172.9	47	162.9	18	Jaiswal et al. 2010
5-FQA	11.63	367.2	191.2	173.0	72.1	193.0	18.8	Jaiswal et al. 2010
CQL[†]	11.94	335.3	160.6	173.1	94.7	178.8	30.7	Jaiswal et al. 2014
4-CQL	12.23	335.3	160.6	178.9	34.7	172.8	27.1	Jaiswal et al. 2014
CQL[†]	12.46	335.3	161.0	178.8	9.3	172.9	7.9	Jaiswal et al. 2014
CQL[†]	12.64	335.3	161.0	179.1	8.8	172.8	3.4	Jaiswal et al. 2014
1.4-diCQA	13.79	515.3	353.1	335.0	6.4	317.0	2.7	Clifford et al. 2005
3.5-diCQA	14.34	515.3	352.9					Clifford et al. 2005
4.5-diCQA	14.57	515.3	353.1	335.0	3.5	317.0	4.4	Clifford et al. 2005

Quantitative results

Caffeine content has been shown to vary substantially as a function of the variety and geographical origin of the coffee bean, and the extraction method (Severini 2017). Caffeine and CGA content (mg/mL) for this experiment are shown in Table 11.

Caffeine concentration was found to differ significantly as a function of both extraction method and temperature. Concentrations were higher in cold drip than cold brew beverages, and in beverages extracted at 22°C (1.03 ± 0.19 mg/mL and 0.853 ± 0.15 mg/mL, respectively).

This result was unsurprising as dynamic methods (drip) involve the continuous renewal of the extraction solvent. Since the matter transfer from the solid to the liquid phase are driven by the concentration gradient, this is a more efficient way to extract relevant molecules than a static system. In the latter case, coffee powder is in contact with the total volume of extractive solvent in a unique solution, leading to saturation. The highest caffeine concentration was measured using the drip extraction method at room temperature.

Caffeine content in French press coffees (1.09 ± 0.11 mg/mL) was similar to levels obtained with drip extraction at 22°C. It may be that the longer brewing time used in the cold method (6 h compared to 5 min) compensates for the difference in temperature (roughly 90°C compared to 22°C). No significant differences in caffeine content were found between the two contact times for the cold brew method.

CGAs are abundant phenolic compounds in coffee, while the literature reports that caffeoylquinic (CQAs) are the major subclass. (Fujioka 2008). These compounds are known to influence flavor, contributing to acidity and conferring astringency and bitterness (Clifford 2000).

TABLE 11: Concentrations (mg/ml) of caffeine, CQAs, CGAs, FQA, 5-pCoQA, CQLs and diCQAs as a function of extraction method, temperature and flow rate.

Mean, standard deviation, and p-value for each of the three variables, and their interactions are reported. In a separate column are reported the mean and standard deviation for the French press extraction used as the benchmark T= Temperature, E= Extraction, F= FlowRate).

Extraction Temperature FlowRate	Drip 22°C Fast	Brew 22°C Fast	Drip 5°C Slow	Brew 5°C Slow	Drip 22°C Slow	Brew 22°C Slow	Drip 5°C Fast	Brew 5°C Fast	E	T	F	F:E	F: T	E:T	*French* *Press*
Caffeine	1.14 ± 0.12	0.78 ± 0.11	0.79 ± 0.32	0.89 ± 0.21	1.27 ± 0.15	0.97 ± 0.12	0.94 ± 0.16	0.76 ± 0.17	0.026*	0.018*	ns	ns	ns	ns	1.09 ± 0.11
3-CQA	0.19 ± 0.02	0.14 ± 0.02	0.16 ± 0.05	0.14 ± 0.04	0.22 ± 0.04	0.18 ± 0.03	0.14 ± 0.02	0.16 ± 0.03	ns	0.0241*	ns	ns	ns	ns	0.20 ± 0.02
CeQA[†]	0.05 ± 0.01	0.04 ± 0.00	0.04 ± 0.02	0.03 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	ns	0.0249*	ns	ns	ns	ns	0.05 ± 0.00
CeQA[†]	0.03 ± 0.01	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.11 ± 0.14	ns	ns	ns	ns	ns	ns	0.03 ± 0.00
5-CQA	0.40 ± 0.05	0.28 ± 0.04	0.33 ± 0.12	0.27 ± 0.07	0.45 ± 0.04	0.35 ± 0.05	0.29 ± 0.06	0.24 ± 0.02	ns	0.037*	ns	ns	ns	ns	0.40 ± 0.03
4-CQA	0.27 ± 0.03	0.19 ± 0.03	0.22 ± 0.07	0.20 ± 0.05	0.31 ± 0.03	0.25 ± 0.04	0.19 ± 0.04	0.22 ± 0.05	ns	0.0195*	ns	ns	ns	ns	0.23 ± 0.10
5-pCoQA	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.0413*	0.0397*	ns	ns	0.0478*	0.0423*	0.03 ± 0.01
5-FQA	0.08 ± 0.01	0.06 ± 0.01	0.07 ± 0.02	0.06 ± 0.02	0.10 ± 0.01	0.08 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.0495*	0.0117*	ns	ns	ns	0.0398*	0.08 ± 0.01
CQL[†]	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.01	0.06 ± 0.02	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.03 ± 0.03	0.0315*	0.0255*	ns	ns	ns	0.0007 ***	0.02 ± 0.01
4-CQL	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.03 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.01	0.00017 ***	0.00013 ***	ns	ns	0.0298*	0.033*	0.03 ± 0.00
CQL[†]	0.07 ± 0.01	0.04 ± 0.01	0.05 ± 0.03	0.01 ± 0.01	0.09 ± 0.01	0.06 ± 0.00	0.05 ± 0.01	0.04 ± 0.02	0.00017 ***	0.0001 ***	ns	ns	ns	ns	0.09 ± 0.01
CQL[†]	0.06 ± 0.01	0.03 ± 0.01	0.04 ± 0.02	0.04 ± 0.01	0.07 ± 0.00	0.04 ± 0.00	0.03 ± 0.01	0.04 ± 0.01	0.0108*	0.005**	ns	ns	ns	0.0036 **	0.07 ± 0.01
1.4-diCQA	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	ns	ns	ns	ns	ns	0.0068 **	0.02 ± 0.00
3.5-diCQA	0.03 ± 0.01	0.02 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.04 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.01 ± 0.01	0.00018 **	0.00006 ***	0.044*	ns	ns	ns	0.04 ± 0.01
4.5-diCQA	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.01	ns	ns	ns	ns	ns	ns	0.02 ± 0.01

Data are expressed as mean ± standard deviation. Letters indicate statistically significant differences between extraction methods. † indicates that the acylation position was uncertain

Figure 16 shows that in this study, concentrations of 5-CQA (chlorogenic acid) and CQAs were significantly different at the two temperatures (p = 0.037 and 0.023, respectively).

The values of 5-CQA ranged from 0.37 ± 0.07 mg/mL for the extraction at 22°C, to 0.28 ± 0.03 mg/mL for the extraction at 5°C, from 0.51 ± 0.08 mg/mL (extraction at 22°C) to 0.41 ± 0.03 mg/mL (extraction at 5°C) for the sum of CQA.

The French press values revealed were 0.39 ± 0.03 mg/mL for 5-CQA and, for the sum of other CQA 0.51± 0.12mg/mL. These values were consistent with values reported in the literature for the filter coffees (Andueza. S. 2007), (Gloess 2013).

Concentrations increase with temperature, regardless of the extraction method, flow rate, or contact time.

Significant differences are found for several compounds; notably there are significant interactions between extraction method and temperature for 5-pCoQA (p = 0.0423), 5-FQA, (p = 0.0398) and other classes of CQL compounds.

More specifically, the higher temperature increases concentrations of these compounds using the cold drip method, while this is not the case for the cold brew method. Concentrations were significantly higher in drip extraction at ambient temperature.
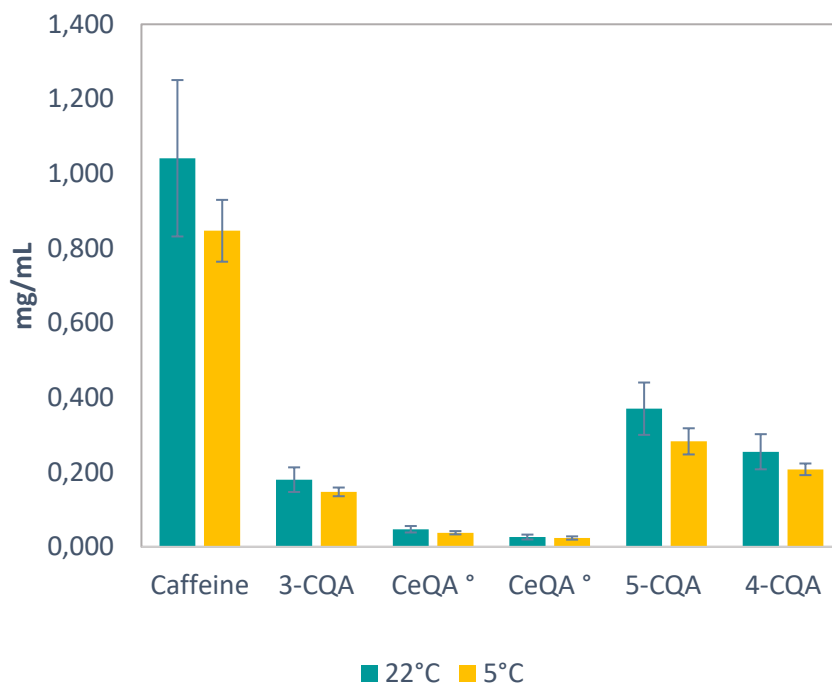

Figure 16: Concentrations (mg/mL) of Caffeine, 3-CQA, CeQAS, 5-CQA and 4-CQA at different extraction temperatures.

A significant interaction between time and temperature was found for only two compounds (5-pCoQA and 4-CQLs). In this case, concentrations were highest for low flow rates/contact times, at ambient temperature.

Finally, significant differences related to extraction and temperature were found for di-CQA compounds.

4.3.3 Sensory evaluation

Aromatic components are particularly important in coffee beverages as they are the main constituents of the sensory experience of coffee drinkers. Overall, the sensory evaluation found that *lungo* coffee is a little less intense that the typical Italian coffee. Significant differences ($p < 0.05$) were found for the Extraction method respect to overall intensity of odor, bitterness, sugar caramelization and sweet taste (Table 12).

The cold brew method was characterized by a higher intensity of sugar caramelization attribute and sweet taste, while the cold drip method by a higher overall intensity of odor and bitterness.

In the drip method, the high intensities of the bitter attribute were also confirmed by the caffeine content, greater than the cold brew. It's known in the scientific literature, that the concentration of caffeine influences the perceived strength, body and bitterness of a brewed coffee (Gloess 2013), (Clarke 2003). Similar values were also found for the French press, which showed the value of bitter intensity slightly lower than the cold drip but higher to the cold brew system.

No significant differences were found for enzymatic and distillation attributes.

Temperature had a particularly dominant effect on the sour taste ($p = 0.000$, $F = 27.01$). Coffee extracted at temperatures of 22 ° C were evaluated in terms of intensity sourer than those obtained at 5 ° C. On the other hand, coffee obtained with french press have shown much lower value.

Temperature increased intensity, and a significant interaction was found between this and flow rate/contact time ($p = 0.024$).

Coffees extracted slowly at 22°C were more intense than those extracted at 5°C.

Extraction method has influenced the intensity of sweet taste. Indeed, the coffee extracted by cold brew method it was been sweetest than drip method, and also regard the French press.

Table 12: Sensory evaluation n of coffee beverages, comparing extraction method, temperature and flow rate.

Mean, standard deviation, and p-value for each of the three variables, and their interactions are reported. In a separate column are reported the mean and standard deviation for the French press extraction used as the benchmark.

Extraction / Temperature °C / FlowRate	Drip 22°C Fast	Brew 22°C Fast	Drip 5°C Slow	Brew 5°C Slow	Drip 22°C Slow	Brew 22°C Slow	Drip 5°C Fast	Brew 5°C Fast	E	T	F	F:E	F:T	E: T	French Press
				Average values ± sd								p			
O- Global Intensity	6.47 ± 1.21	4.94 ± 0.42	6.78 ± 0.19	4.64 ± 0.53	7.11 ± 0.84	5.31 ± 0.80	6.96 ± 0.40	5.11 ± 1.05	0.0002 ***	ns	ns	ns	ns	ns	5.45 ± 0.63
F- Global Intensity	5.57 ± 0.49	5.69 ± 0.05	4.97 ± 0.55	5.17 ± 0.29	6.04 ± 0.63	4.89 ± 0.98	5.93 ± 0.61	5.17 ± 0.29	ns	ns	ns	ns	ns	ns	3.50 ± 0.55
Enzymatic	3.58 ± 0.62	3.54 ± 1.70	4.42 ± 1.21	3.83 ± 1.66	3.81 ± 0.17	3.53 ± 1.32	4.58 ± 0.98	4.03 ± 0.59	ns	ns	ns	ns	ns	ns	3.08 ± 0.58
Sugar caramelization	4.33 ± 0.58	6.17 ± 1.04	4.67 ± 0.58	5.06 ± 0.48	4.94 ± 0.59	4.90 ± 1.04	5.25 ± 0.25	5.38 ± 0.67	0.05	ns	ns	ns	ns	ns	5.66 ± 0.61
Distillation	3.44 ± 1.39	4.03 ± 0.63	3.42 ± 0.72	4.03 ± 0.63	4.78 ± 0.38	3.74 ± 0.65	4.20 ± 0.35	3.80 ± 0.52	ns	ns	ns	ns	ns	ns	3.25 ± 0.42
Bitter	7.44 ± 0.47	5.92 ± 0.14	6.93 ± 0.32	5.83 ± 0.29	7.89 ± 0.67	5.89 ± 1.01	7.43 ± 0.40	5.83 ± 0.29	0.0001 ***	ns	ns	ns	ns	ns	6.33 ± 0.26
Sweet	2.58 ± 1.01	3.83 ± 0.29	2.67 ± 0.58	3.75 ± 0.25	2.89 ± 0.51	3.11 ± 0.19	3.19 ± 0.17	3.58 ± 0.52	0.002 **	ns	ns	ns	ns	ns	2.16 ± 0.41
Sour	6.06 ± 0.48	5.78 ± 0.69	4.83 ± 0.29	5.75 ± 0.43	6.94 ± 0.53	6.90 ± 0.74	5.64 ± 0.27	5.05 ± 0.52	ns	0.00081 ***	0.039 *	ns	0.027 *	ns	1.75 ± 0.42
Astringency	4.89 ± 1.51	4.53 ± 1.14	3.89 ± 1.39	3.42 ± 0.72	4.78 ± 0.38	3.63 ± 0.55	4.15 ± 0.25	3.39 ± 0.67	ns	ns	ns	ns	ns	ns	1.25 ± 0.42

Intensities for cold brew and cold drip and French press methods are shown in the spider plot in Figure 17 which reveals clear differences in the flavor profile of the respective extraction methods in terms of bitterness. sourness. astringency and global intensity.
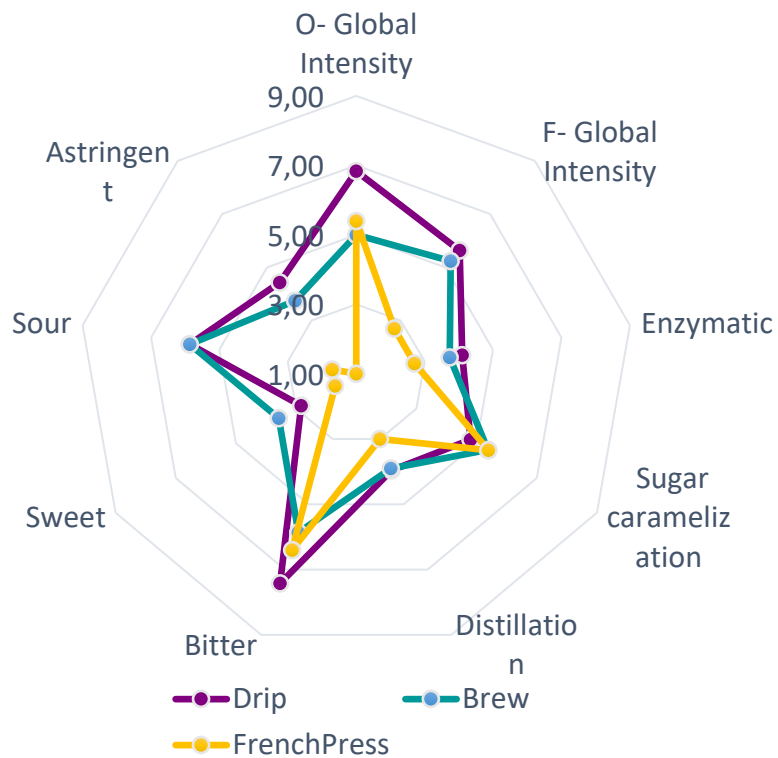
Figure 17: Spider plot of sensory attributes for drip, brew and French press extraction methods.

Particularly, the increase in bitterness and astringency is consistent with the higher concentration of caffeine and chlorogenic acids as previously found by Gloess et al. 2013 for hot extractions.

4.4 Conclusions

Two extraction methods for preparing a cold coffee were characterized: cold drip and cold brew.

The results show that the differences during cold coffee preparation lead to differences in physical parameters, the concentration of chemical compounds, and the sensory profiles of coffees.

Cold drip coffees were recognized as more bitter with more content in caffeine and chlorogenic compounds than cold brews.

The temperature was found to increase the concentrations of several compounds. Particularly, higher temperature increases the total solid, concentration of caffeine, CQAs and 5 CQA. However, refractive index and the remining CGAs, are increased by temperature only in cold drip, while no difference was found for cold brew.

Conversely, the contact time between the coffee powder and water has a limited effect on brew characteristics.

5. Optimization of a green method to recover phytochemicals from spent coffee grounds

5.1 Introduction

As mentioned in the introduction section, the spent coffee ground is generated in large quantities around the world (approx. 6.000.000 tons/year) (Mussatto 2011). Even though some researches have revealed functional potential of different compounds found in SCG such as polysaccharides, proteins, phenolic compounds, minerals, among others, this residue still has not largely been used as raw material in industrial processes. Nevertheless, interest in reusing these residues has increased in the last years.

It was estimated that only from the province of Rome over 10000 t of SCG are available per year to produce polyphenols rich extracts and bioenergy. This means that about 1400t/year of polyphenol extract could be produced (Zuorro and Lavecchia, 2012).

Such interest has been motivated by environmental concerns and also because these residues contain several components that can be valuable for application in food, cosmetic, and pharmaceutical areas (S. Mussatto 2015).

It has to be mentioned that food commodities are usually fortified with additional quantities of antioxidants, either natural or synthetic due to depletion of antioxidants present in raw food when subjected to processing, preservation, and storage.

Besides, the presence of caffeine in spent coffee grounds has been proposed as one of the main problems for use in agriculture because of its toxicity.

Several studies show the possibility of using different extraction techniques and solvents (Mussatto 2011), (Ludwig 2012), (Bravo 2013), (Panusa 2013), (Ranic M. 2014), in order to obtain extracts that exhibit high antiradical power.

Different studies reported the influence of various factors on the extraction capability of the phenolic compounds from spent coffee, to establish the more effective procedure (J. M. Bravo 2013) (Zuorro A. 2012). The methods proposed exploit the use of different solvent, generally a mix of ethanol or methanol and

isopropanol in a different ratio, or even with a pure solvent with a solid\liquid ratio variable (Bravo et al., 2102; Panusa et al., 2013; Zuorro et al., 2102; Murthy and Naidu, 2012). Among the solvents used, water has been shown convenient and efficient for obtaining extracts with a high content of bioactive compounds, also with a single extraction (Bravo 2013).

However, there is a necessity of evaluating and identifying more eco-friendly methodologies that do not require the use of organic solvents and may enhance the extracts compatibility for the food industry and enable their use as an added-value constituent for different applications.

A Recent study showed the capability of the autohydrolysis technique as an alternative application for recovery the recovery of antioxidant compounds since it does not require organic solvents for the reaction, but only water (Ballesteros 2017). In a previous study, autohydrolysis under mild reaction conditions was demonstrated to be a technology with great potential to recover phenolic compounds from SCG (Conde 2016).

Nevertheless, the efficiency of the extraction process is affected by the type of solvent used and its concentration, the solvent/solid ratio, time of contact, temperature, and particle size of the solid matrix (S. I. Mussatto 2011 b). Therefore, it is necessary to select the conditions that maximize the recovery of the desired compound for each raw material.

Temperature is one of the most critical factors contributing to the compound's recovery. Generally, the higher the temperature applied, the higher the recovery yield. However, high temperatures may cause degradation of some compounds. Furthermore, the solid/solvent ratio is also an important parameter yielding higher recoveries when using more diluted conditions (S. M. Mussatto 2011). The use of diluted conditions may be, however, economically disadvantageous, increasing the costs of the process for the recovery of the compounds from the liquid phase.

The aim of this research has been to test which physical and chemical variables could influence the amounts of bioactive compounds extracted from SCGs, identifying optimal range of extraction conditions, and to develop an effective green extraction method to maximize the recovery of these molecules.

The knowledge of the effect of these factors on the recovery of bioactive compounds is very important to optimize the extraction system conditions. To develop an extraction system as possible green and eco-friendly that allows using a waste of coffee and as a solvent the water. This makes the recovery system less expensive respect to others that use organic solvents.

5.2 Materials and Methods

The experiments presented in the literature typically assess the relationship between various chemical and physical factors and the recovery performance, but these experiments usually test only one, or a few, variables at a time.

Instead, simultaneously testing the effect of more factors at different levels with replicates could be more informative, but this poses a problem for researchers, as many factors affect recovery performance, and it is complicated to include all the elements in a single experimental plan. Furthermore, it is often difficult to understand the importance of each factor concerning the others and therefore, which to select to optimize the system. The method uses in the present work was based on an experimental design that simultaneously tests a large number of variables. Accordingly, we have used a Plackett-Burman screening design for the estimate which factors have more influence on the amount of caffeine and phytochemicals to be recovered, without interest in studying the interactions. Subsequently, we have tested only the factors that have shown a significant effect on the recovery of bioactive compounds and phytochemicals, adopting a full factorial scheme.

5.2.1 Samples preparation and selected factors for screening design

A brand of coffee beans, and two different extraction techniques for the production of SCG, were selected. We have selected the spent coffee ground from Espresso and French Press. We had chosen these methods because they are the most used techniques for coffee extraction. The coffee brews were prepared as described by Angeloni 2018 (Angeloni G. 2018 b).

A Plackett-Burman screening design (or 2(7-4) fractional design) was adopted. In our configuration, this design made it possible to test seven factors at two levels using eight experiments. The chosen variables and their settings for the two levels are shown in Table 13, while the combinations used in the eight experiments are shown in Table 14.

Table 13: Tested factors with maximum (+) and minimum (-) values

Factors	+	-
Temperature	115°C	100°C
Time of extraction	10 min	3 min
SCG/Water	1/6	1/3
Source of SCG	French Press	Espresso
SCG storage time	48h	0
Water	Light mineralization	Medium mineralization
SCG treatment	Blast Chiller	No treatment

Three replicates were performed, making a total of 24 trials. Trials were presented in a completely randomized order.

Table 14: Tested factors in fractional design ('+' represents the upper bound, while '–' is the lower bound).

Trials	Temperature	Time	SCG/ Water	Source of SCG	Storage time	Water	SCG treatment
T1	-	-	-	+	+	+	-
T2	+	-	-	-	-	+	+
T3	-	+	-	-	+	-	+
T4	+	+	-	+	-	-	-
T5	-	-	+	+	-	-	+
T6	+	-	+	-	+	-	-
T7	-	+	+	-	-	+	-
T8	+	+	+	+	+	+	+

Among the selected variables, blast chilling was applied to SCG. The blast chilling is a method of cooling foods quickly to a temperature level that is relatively safe from bacterial growth. For the experimental trial, it has been established the temperature of -18°C in 20 minutes.

Moreover, it has been established, for every SCG produced, that the solvent for the extraction of the bioactive compounds and the phytochemicals it was the water, to propose a green method of compounds recovery. We have chosen two different commercial water with different level of mineralization (Light and Medium) light mineral content water (fixed residue < 50 mg/L) and medium mineral content water (fixed residue between 500 and 1500 mg/L) (Rizzo R 2011). Other important factors that we have tested in this first screening part, have been the ratio of SCG to water (two powder/water ratio of 1:3 and 1:6), two different extraction times (3 or 10 minutes), and two different extraction methods (Espresso or French Press).

Part of the samples (water + SCG) were extracted in a pressure cooker for different time (3 or 10 minutes). Other samples, instead, were extracted in a conventional open pot for different time (3 or 10 minutes). The critical difference

between these two extraction systems it was the temperature of extraction. In fact, in the pressure cooker the temperature was around 110°C at a pressure of 1.4 bar, whereas in the conventional open pot the extraction was conducted at 100°C.

The last factor that has been considered is the short-term storage of the SCG before treatment. Specifically, the coffee grounds were extracted as above-mentioned, both immediately after being produced, and after 48 hours of storage at room temperature (21 °C). We have selected this factor because we thought the storage of the SCG it could influence the extraction efficiency, and because this factor it could be interesting to export the extraction system from a laboratory (our) scale to an industrial scale.

At the end of extraction, each extract has been weighed, filtered and centrifugated at 5500rpm for 5 minutes (Hermle centrifuge Z 206 A) to separate the solid part and liquid extract.

5.2.2 Samples preparation and selected factors for fully factorial scheme

After the analysis of screening design results, a full factorial experimental design was performed with the factors that showed significance for the recovery of caffeine and phytochemicals, as shown in Table 15.

It has been considerate the SCGs produced from espresso and French press and tested at 4 different temperatures of water. The higher temperature levels, 110°C and 115° were regulated with the pressure pot, the other temperatures were 100°C (i.e. the boiling temperature of water without over-pressure) and 80°C obtained by means of thermostatic laboratory water bath.

At the end of extraction, each extract has been weighed, filtered and centrifugated at 5500rpm for 5 minutes (Hermle centrifuge Z 206 A) to separate the solid part and liquid extract. In the Figure 18 samples preparation scheme is shown.
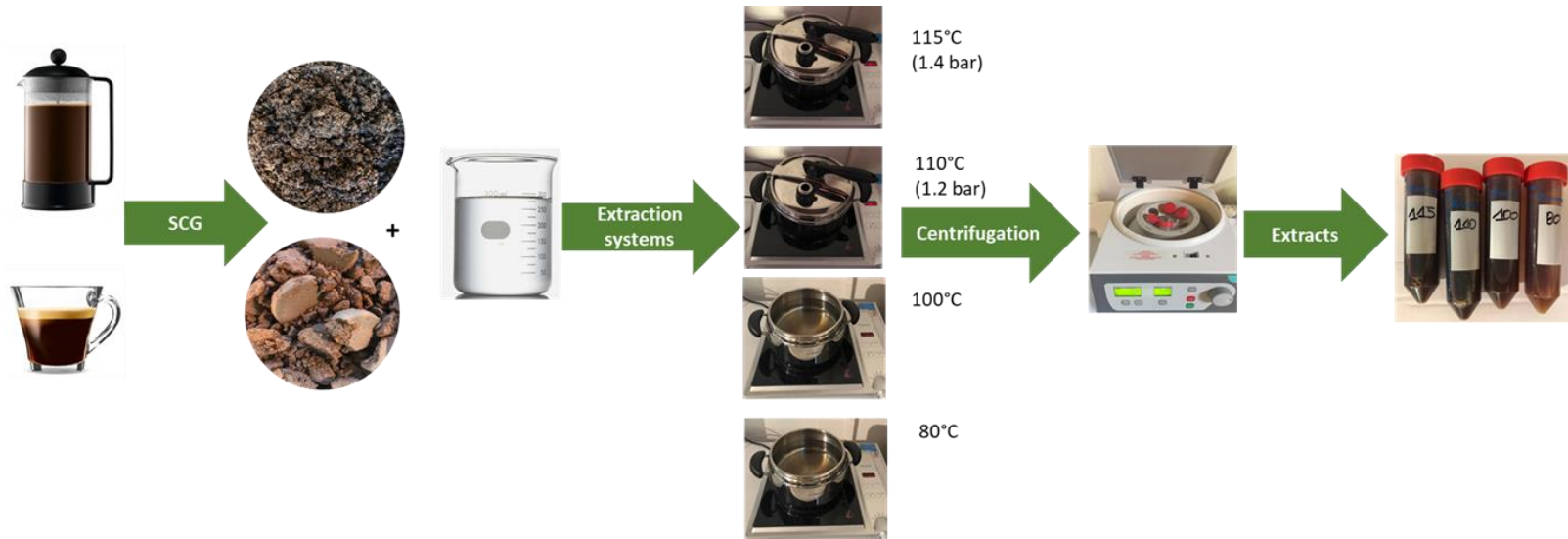
Figure 18: Samples preparation and selected factors for fully factorial scheme.

Table 15: Tested factors in full factorial scheme.

Trials	Source of SCG	Temperature	Time	SCG/ Water	Storage time	Water	SCG treatment
T1	Espresso	110°C	3 min	1/3	24 hours	Light mineral	No Treatment
T2	Espresso	115°C	3 min	1/3	24 hours	Light mineral	No Treatment
T3	Espresso	100°C	3 min	1/3	24 hours	Light mineral	No Treatment
T4	Espresso	80°C	3 min	1/3	24 hours	Light mineral	No Treatment
T5	French Press	110°C	3 min	1/3	24 hours	Light mineral	No Treatment
T6	French Press	115°C	3 min	1/3	24 hours	Light mineral	No Treatment
T7	French Press	100°C	3 min	1/3	24 hours	Light mineral	No Treatment
T8	French Press	80°C	3 min	1/3	24 hours	Light mineral	No Treatment

5.2.3 Physical analyses

A digital pH meter (GLP 21, Crison Instruments, Spain) was used to determine pH. Total dissolved solids (TDS) was measured using a refractometer (VST LAB Coffee III Refractometer, USA).

5.2.4 Analysis of compounds

For the determination of caffeine and total phenolic acids it has been used a spectrophotometric determination for the screening design. In the full factorial experiment, i.e. caffeine and CGAs were determined by HPLC

Measurement of caffeine with UV/vis spectrophotometer

For the characterization of caffeine de-ionized water was used. A mass of 0,04 g caffeine as external standard was dissolved in 250mL of de-ionized water (concentrate solution). From this concentrate solution, three different standard solutions were prepared. For the first, it was taken 10 mL of the concentrate and dissolved in 250 mL of de-ionized water (First Standard solution). The second standard solution it has been prepared with 20 mL taken from the concentrate solution and dissolved in 250 mL of de-ionized water. The third standard solution it was made with 10 mL and dissolved in 100 mL of de-ionized water.

The absorbance of the solutions was measured by an UV/vis spectrophotometer at room temperature at the wavelength 273 nm.

After the preparation of the standard curve and with the regression coefficient calculated, it has been possible to make a graphic with the concentration's values and the absorbance, and finally analysed the samples. 100mL of the SCG extract were dissolved in 250 mL of deionized water. Then, 10 mL of this solution was dissolved in 100 mL of deionized water.

Antioxidant Capacity by Folin−Ciocalteu (FC) Assay

The Folin−Ciocalteu reducing capacity of coffee was performed according to Bravo 2012. Spent coffee extracts were diluted 3:10 and 1:10, respectively, in demineralized water before analysis. A volume of 500 µL of Folin−Ciocalteu reagent was added to a mixture of 100 µL of the extracted sample and 7.9 mL of demineralized water. After a 2 min delay, 1.5 mL of a 7.5% sodium carbonate solution was added. Next, the sample was incubated in darkness at room temperature for 90 min. The absorbance of the sample was measured at 765 nm in a Lambda 25 UV−vis spectrophotometer (Perkin-

Elmer Instruments). Gallic acid (GA) was used as the reference, and the results were expressed as milligrams of GA per gram of spent coffee dry matter (mg GA/g spent coffee dm) or per gram of coffee (mg GA/g coffee).

HPLC-DAD analysis

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). An Inifinity Lab 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 one other work, (Angeloni 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 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 g; r2=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 g; r2=0.9994).

5.2.5 Statistical analyses

Conventional analysis of variance (ANOVA) was used to compare means determined for the different extraction methods. The tested factors were considered significantly different at $p < 0.05$. All statistical analyses were performed using R software (version 3.4.0 for Windows). In fully factorial scheme, in cases where the F-test was significant at the $p < 0.05$ level, multiple paired-means tests checked for significance using the post hoc Tukey Honest Significance Difference test ($p < 0.05$).

5.3 Results and discussion

The process variables used for extraction reactions, such as the reaction time, temperature and solid/liquid ratio, usually have great influence both on the kinetics of bioactive compounds release from the solid matrix as well as on the antioxidant activity of the produced extracts.

Therefore, this study aimed to evaluate the effect of different variables on the recovery of caffeine and phenolic compounds with high antioxidant activity, with the objective of selecting the conditions that maximize the extraction results in order to develop an eco-friendly recovery system.

5.3.1 Fractional Design

With the analysis of fraction design, it has been possible to establish the importance and the influence of the tested factors. Table 16 reported the results for every trial in relation to the measurements effectuated. In Figure 19 they are compared in terms of F-value from the out-put of the ANOVA.

ANOVA analysis of the effect of the seven variables on pH, clearly shows that water with different content of minerals had the main influence on the pH of the extract ($p = <0.05$), the mean values were 6.2 ± 1.1 for the water with high content of minerals, and 4.85 ± 0.6 for the other water.

The storage time has shown significant importance ($p = <0.05$) on the pH values (5.05 ± 1 for the powder stored for 48h, and 6.02 ± 0.6 for the fresh SCG), as also the time of extraction.

The extracts obtained with the lower time of extraction (3 minutes) have shown higher values of pH respect the extracts obtained in 10 minutes.

Table 16: Results of fractional design. Means and standard deviation are shown.

Trials	pH			TDS %			Caffeine (mg/g SCG)			Phenolic compounds (mg GAE/g SCG)		
T1	4.71	±	0.24	1.29	±	0.12	20.71	±	4.98	20.67	±	8.06
T2	5.45	±	0.07	0.98	±	0.32	13.00	±	3.77	10.85	±	6.97
T3	5.85	±	0.22	1.94	±	0.19	7.81	±	2.42	10.00	±	4.22
T4	5.44	±	0.21	2.61	±	0.21	23.31	±	5.17	17.60	±	5.81
T5	7.90	±	0.24	0.69	±	0.12	16.80	±	3.31	19.18	±	11.74
T6	5.71	±	1.04	0.75	±	0.16	17.11	±	9.68	16.10	±	2.26
T7	5.23	±	0.12	0.89	±	0.44	14.04	±	2.41	11.32	±	0.90
T8	3.98	±	0.37	1.48	±	0.06	22.86	±	2.30	22.80	±	13.10

The temperature has demonstrated significant effects on pH value, in fact, with a high temperature the extracts show a lower value of pH (5.14 ± 0.7), respect to the extracts obtained a temperature of 100°C in which there was an increment of pH value. The others factor tested had no significant effect on the pH value of the extracts ($p > 0.05$).

Concerning TDS %, the factors they have influenced the amount have been the time of extraction, with a significant value obtained in a long time of extraction (10 minutes TDS% = 1.72 ± 0.7).

The different source of SCG (from French Press or Espresso) and the different SCG/water have influenced the amount of total dissolved solid % significantly. As reported in several studies on the coffee beverages, TDS % seems to be related to the coffee/water ratio (Andueza 2007), and the brewing procedure (López-Galilea I 2007), (Angeloni G. 2018 b).

TDS % directly correlates with coffee strength: high TDS % is consistent with a potent 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 et al., 2007). In our case, being the tested treatment a second extraction, higher values of TDS are found in the extracts produced with the French Press.

Also, the Water and the Temperature of extraction have been a significant influence on the TDS%. ($P<0.05$).

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, 2008).

Concerning the concentration of caffeine, the factors that have been influenced his content were extraction temperature and source of SCG.

In general, the comparison of caffeine and the phytochemicals must take into consideration the fact that every operative condition regarding the extraction able to produce the SCG (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 critical factors is the ratio of ground coffee to the final volume of water (Andueza 2007).
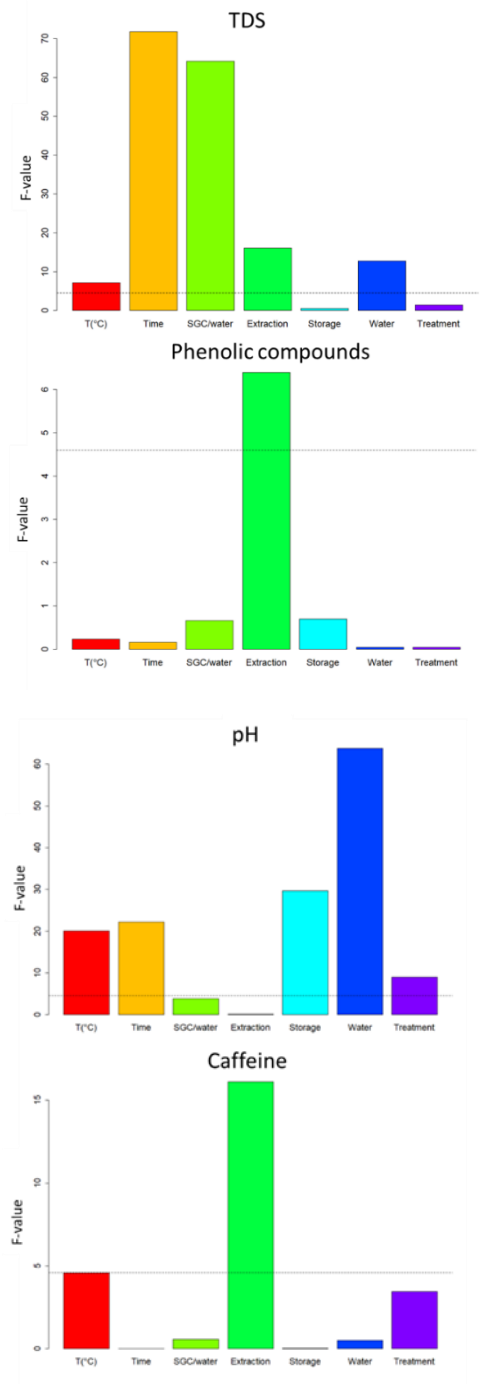
Figure 19: Bar charts of factors affecting pH, TDS, caffeine and phenolic compounds content. The dashed line represents the significance level (p<0.05).

In the second extraction from the SCG of the different source, espresso and french press, the operative conditions have been selected and controlled in order to reproduce the same for every trial.

Temperature has shown a significant effect on the recovery of caffeine from the extracts.

With higher Temperature of extraction (115°C) the amount of caffeine recovered was 19.07±6.7 mg/g against the 14.84±5.7 of the lower temperature (100°C).

A significant effect it was revelated for the different source of SCG, both for caffeine and phytochemicals compounds. The amount of compounds recovered from SCG it was higher in the SCG from French Press respect to the Espresso.

This situation it was predictable because from the studies carried out and previously described it was known that the Espresso it was a system with better efficiency of extraction respect the other methods.

This reverse situation with the recovery from the SCG, in which the number of compounds in French Press were higher respect Espresso, confirms the success of the whole extraction system to the recovery of compounds.

5.3.2 Fully factorial scheme

As previously mentioned, in the fully factorial scheme it has been considered two different extraction system (Espresso and French Press) and 4 levels of temperature (80°C, 100°C, 110°C and 115°C), for estimate how much caffeine and other bioactive compounds it's possible to recover with water as solvent.

Results of recover of Phenolic compounds

Concerning the concentration of phenolic compounds measured, some differences among the results were observed for the different extraction methods. This effect can be explained by the fact that the methods differ from each other regarding reaction mechanisms and, above all, the main reason is relative to the amount of bioactive compounds available for recovery. In a previous study it has been established that the extraction efficiency of several methods was different- Thus to cover/include a considerable yield range, have been selected these two different extractive methods.

In Table 17, are shown the values of phenolic compounds measured at different temperatures.

The content of phenolic compounds from espresso it was average 9.72 ± 4.77 mg $_{GAE}$/g $_{SCG}$, and no differences in concentrations between temperatures were found.

Equally for the French press method, no differences have been found in terms of concentration for the temperature of extraction. The average value of phenolic compounds recovered was higher than espresso, around 14.88 ± 4.93 mg $_{GAE}$/g $_{SCG}$.

Table 17: Phenolic compounds in spent coffee

Espresso				*French Press*		
Temperature (°C)	Phenolic compounds mg/GAE g			Temperature (°C)	Phenolic compounds mg/GAE g	
80°	9.79	±	5.57	80°	15.60	±	2.99
100°	9.87	±	5.19	100°	14.78	±	7.23
110°	9.48	±	3.31	110°	14.29	±	5.77
115°	9.84	±	5.02	115°	14.85	±	3.75

Conde and Mussatto compared different methods and extraction conditions, for the phenolic compound's recovery. They assert that the extraction with water temperatures around 60–65°C, for 90 min in ratio 40mL water/g SCG, was not efficient for phenolic compounds extraction from SCG. In these conditions, they have recovered 7.4 mg $_{GAE}$/g $_{SCG}$.

However, the use of water under higher temperature (120°C) was quite efficient for this purpose (Conde 2016). In fact, with this higher temperature, 20 mL/g SCG ratio for 20 minutes, the system it has been able to recover 32.92 mg $_{GAE}$/g $_{SCG}$. In that study is not reported the type of spent coffee ground, and the final concentration it seems more high respect to our content recovered. On the other hand, the liquid/solid ratio is very different, so that the same amount of phenolic compounds was recovered using amounts of water much higher than ours (about three times more).

Panusa and co-authors (Panusa 2013) reported value 17.4 mg $_{GAE}$/g $_{SCG}$ recovered in ratio 50mL water/3g SCG, for 10 min to 80°C. The value stated it was similar to our values revelated for French press, but for the same reason mentioned above, the amount of water it was about five times more than ours. Moreover, the ten minutes of extraction, was longer than ours.

In order to optimize a system to exploit byproducts from a food waste, we retain that our system has given satisfactory values for both espresso and French press extraction, using a reduced quantity of water and a shorter time of extraction, with respect to other studies present in scientific literature, with a similar range of temperature.

Analysis of caffeine and chlorogenic acids
The analyzed samples showed almost the same qualitative profile of bioactive substances found in HPLC/DAD profiles at 278 nm for monitoring caffeine, and at 330 nm for CGA detection.

A total of 15 CGAs were detected in the chromatographic profiles as in coffee beverage analyzed in previous studies and, in addition, 4 other compounds has been detected from HPLC/DAD profile.

In the others previous studies regarding the coffee beverages, it has been reported that the most abundant CGAs in the beverage were caffeoylquinic acids (CQAs), notably 5-O-caffeoylquinic (5-CQA) followed by its isomers 3- and 4-CQA. Follow by, Chlorogenic acid lactones (CQLs) Dicaffeoylquinic acid (3,4-, 3,5- and 4,5-diCQA), feruloylquinic acid (5-FQA), diferuloylquinic acid (dFQA) and p-coumaroylquinic acid (5-p-CoQA).

Concerning the extraction from SCGs, a similar trend has been detected, as shown in figure 20.

Differences emerge between the types of coffee grounds. In fact, for French press, the percentage of CQAs is higher than that in Espresso.

Regarding the class of unidentified compounds, it is possible that they belong to the class of diCQA. Subsequently they will be correctly identified.
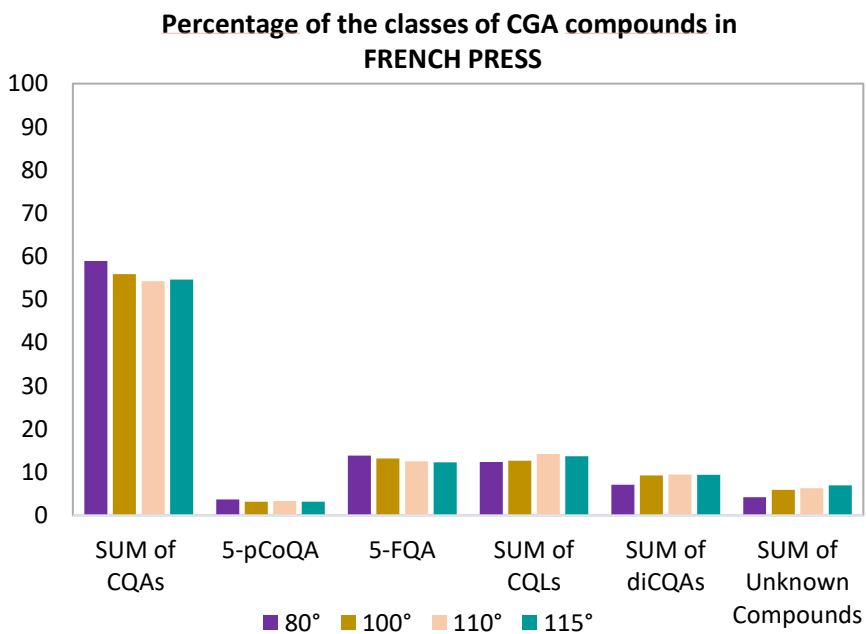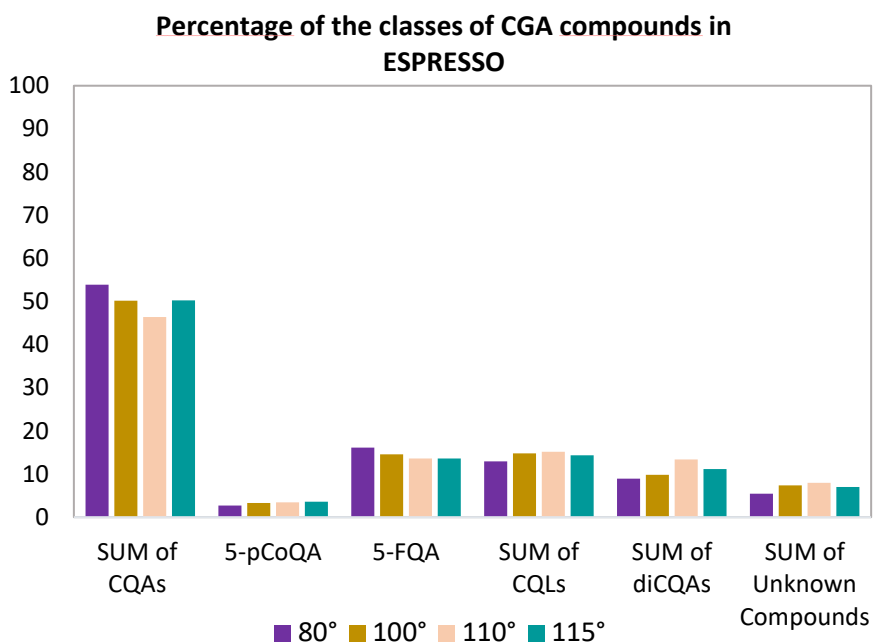
figure 20: Percentage (%) of the classes of CGAs in espresso and french press.

Concerning the recovery of caffeine, Table 18 showed the amounts (mg/g dry SCG) recovered for the different temperature for the two extractions from Espresso and French Press.

In particular, in espresso extraction (Table 18 a) the temperature did not show a significant effect on the recovery. The values of caffeine recovered attested in a range between 1.78 ±0.47 to 3.10 ± 1.98 mg/g SCG.

These amounts were in accord to the values reported in other studies (J. J. Bravo 2012), (Cruz 2012) where, for espresso coffee residues, they recovered 3.59 and 4.52 mg/g SCG, respectively. Moreover, in both these methods have been used higher amounts of water, but the similar time of extraction.

a

Extraction	Espresso								p
Temperature	80°		100°		110°		115°		
Caffeine	1.78±	0.47	2.88±	1.50	3.10±	1.98	2.83±	1.77	ns
	a		a		a		a		

b

Extraction	FrenchPress								p
Temperature	80°		100°		110°		115°		
Caffeine	6.72±	2.43	6.89±	1.67	10.44±	1.65	9.55±	1.76	***
	a		a		b		ab		

Table 18: Concentrations (mg/g) of caffeine as function of temperature

In the extract obtained from French Press coffee residues, caffeine concentration detected it was higher than espresso extract, as shown in the figure 22. Moreover, the temperature has been shown a significant effect (p=0.00033) on the recovery, as reported in the Table 18 b. At the extraction temperature of 110 °C, it has been registered the higher concentration recovered. At lower temperature, the values of caffeine were lower. The higher temperature, 115°C, does not show a significant difference in caffeine concentration with respect to the other temperature tested.
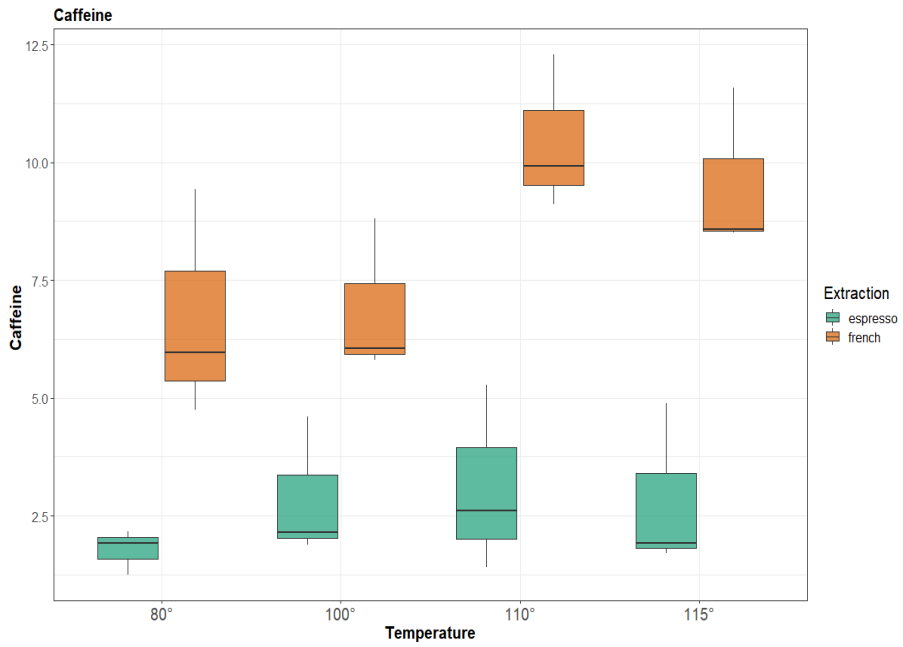
Figure 22: Comparison of the caffeine recovered between the two SCG, as function of temperature.

Regarding the recovery of CGAs, the

Table **19** showed the concentrations for espresso, as a function of temperature.

The group of caffeoylquinic acids (CQA, 3-CQA, CeQA, 5-CQA, 4-CQA) is the most abundant class in coffee, and as previously mentioned this occurrence has been confirmed in the present study. Their concentration varies as a function of temperature ($p< 0.05$) and the trend it was similar for all of them. In fact, at 80°C, it was revelated the lower concentration recovered (ranging from 0.02 to 0.45 mg/g SCG), while at 100°C for 3-CQA, 4-CQA, and 5-CQA it has been observed a significant increment of concentration (ranging from as low 0.60 to 1.10 mg/g SCG). For the higher temperatures, 110° and 115°C, the concentration values were similar for those found at 100 °C.

These amounts recovered are decidedly lower compared to the amounts in the coffee beverage.

The amounts of total caffeoylquinic acids per gram of espresso spent coffee ranged from 1.20 ± 0.37 at the lower temperature, to 2.96 ± 1.01 mg/g SCG at 110°C, as shown in Table 20.

Bravo and co-workers (J. J. Bravo 2012) reported higher values of total caffeoylquinic acids recovered, about 6.16 mg/ g, while Cruz and co-authors (Cruz 2012) reported a similar concentration range to ours for the 5-CQA.

For all the other compounds detected by HPLC/DAD, temperature has shown a significant effect ($p<0.05$). At the temperature of 80°C has shown a lower concentration of recovered compounds, while already at the temperature of 100°C the increment of concentration value it has been observed.

The other studies cited not reported a detailed identification of CGAs compounds.

Table 19: Concentrations (mg/g) of CGAs in espresso, as function of temperature[1].

Extraction Temperature	Espresso												p
	80°			100°			110°			115°			
CQA	0.02	±	0.02 a	0.06	±	0.02 a	0.08	±	0.03 a	0.07	±	0.04 a	ns
3-CQA	0.26	±	0.06 a	0.60	±	0.05 b	0.64	±	0.17 b	0.59	±	0.10 b	*
CeQA	0.05	±	0.01 a	0.12	±	0.06 ab	0.14	±	0.07 b	0.13	±	0.07 ab	*
CeQA	0.02	±	0.01 a	0.06	±	0.03 ab	0.07	±	0.03 b	0.07	±	0.04 ab	*
5-CQA	0.40	±	0.10 a	0.88	±	0.17 b	0.94	±	0.33 b	0.82	±	0.21 b	*
4-CQA	0.45	±	0.16 a	0.99	±	0.16 b	1.10	±	0.37 b	0.97	±	0.18 b	*
5-pCoQA	0.06	±	0.02 a	0.18	±	0.08 b	0.22	±	0.10 b	0.19	±	0.08 b	*
5-FQA	0.36	±	0.20 a	0.79	±	0.21 b	0.87	±	0.40 b	0.72	±	0.19 b	*
CQL	0.05	±	0.02 a	0.12	±	0.05 ab	0.16	±	0.07 b	0.12	±	0.06 ab	*
4-CQL	0.05	±	0.02 a	0.14	±	0.05 b	0.17	±	0.08 b	0.13	±	0.07 ab	*
CQL	0.09	±	0.04 a	0.25	±	0.10 b	0.30	±	0.15 b	0.23	±	0.12 b	*
CQL	0.10	±	0.04 a	0.28	±	0.11 b	0.34	±	0.14 b	0.28	±	0.11 b	*
1,4-diCQA	0.07	±	0.03 a	0.16	±	0.04 ab	0.30	±	0.14 b	0.20	±	0.08 b	**
3,5-diCQA	0.03	±	0.01 a	0.06	±	0.01 b	0.14	±	0.07 b	0.09	±	0.03 b	*
4,5-diCQA	0.11	±	0.07 a	0.31	±	0.13 ab	0.41	±	0.22 b	0.30	±	0.15 ab	*
Unknown Compound	0.04	±	0.02 a	0.14	±	0.06 b	0.18	±	0.11 b	0.13	±	0.05 b	*
Unknown Compound	0.03	±	0.00 a	0.09	±	0.09 a	0.12	±	0.13 a	0.07	±	0.07 a	ns
Unknown Compound	0.02	±	0.00 a	0.04	±	0.04 a	0.05	±	0.03 a	0.05	±	0.03 a	ns
Unknown Compound	0.04	±	0.00 a	0.13	±	0.02 b	0.15	±	0.08 b	0.11	±	0.02 b	*

1. data are expressed as mean ± standard deviation. letters indicate statistically significant differences between extraction methods. † indicates that the acylation position was uncertain.

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.

The data were summarized as concentrations of the classes of CGAs from espresso spent coffee, in which way was possible to show more easily and clearly the concentration trend at different extraction temperature.

Table 20: Concentrations of the classes of CGAs from espresso spent coffee

Extraction	Espresso			
Temperature	80°	100°	110°	115°
SUM of CQAs	1.20 ± 0.37	2.72 ± 0.49	2.96 ± 1.01	2.66 ± 0.64
5-pCoQA	0.06 ± 0.02	0.18 ± 0.08	0.22 ± 0.10	0.19 ± 0.08
5-FQA	0.36 ± 0.20	0.79 ± 0.21	0.87 ± 0.40	0.72 ± 0.19
SUM of CQLs	0.29 ± 0.13	0.80 ± 0.31	0.97 ± 0.43	0.76 ± 0.37
SUM of diCQAs	0.20 ± 0.11	0.53 ± 0.18	0.85 ± 0.42	0.59 ± 0.26
SUM of Unknown	0.12 ± 0.03	0.40 ± 0.20	0.51 ± 0.36	0.37 ± 0.17
SUM OF CGAs	**2.23 ± 0.83**	**5.41 ± 1.48**	**6.38 ± 2.73**	**5.29 ± 1.71**

Regarding the recovery of CGAs, the Table 21 showed the concentrations for French Press, as a function of temperature.

In particular, for the class of CQAs compounds at the temperature of 110°C, it has been registered the higher concentrations values.

At 110°C, the concentrations of 3-CQA, 4-CQA and 5-CQA were respectively 1.99 ± 0.27, 3.25 ± 0.36 and 3.29 ± 0.52 mg/g SCG.

Comparing with another study the values reported in our research were higher with respect other observed. Bravo and co-authors in their study have reported the concentrations of 1.10 ± 0.03 mg/g for 3-CQA, 1.75 ± 0.10 mg/g for 4-CQA and 2.48 ± 0.07 mg/g for 5-CQA.

Another study, (Ballesteros 2017), reported that the concentration of the chlorogenic compounds, referred to 5-CQA, was 2.25 ± 0.02 mg / g SCG, but the source of exhausted coffee was not indicated.

Although the concentration value is comparable to that found in our study, the methods of recovery were less sustainable about the extraction temperature (200 ° C), the liquid / solid ratio (15 ml/g) and extraction time (50 min).

The lower temperatures, 80°C and 100°C did not differ from each other in terms of concentration values, but at these temperatures the CQAs compounds have shown lower values.

The sum of CQAs recovered from French press spent coffee was shown in Table **22** and, it was higher than those reported for the espresso.

For all the other compounds detected by HPLC/DAD temperature has shown a significant effect ($p < 0.05$). In particular, for a large amount of compounds the temperatures of 80°C and 100°C have shown the lower concentrations, while at the temperature of 110°C the increment of concentration value it has been observed, but this temperature does not significantly differ from 115 ° C, as shown in

Table **22** .

Table 21: Concentrations (mg/g) of cgas in french press, as function of temperature.

Extraction Temperature	FrenchPress												p
	80°			100°			110°			115°			
CQA	0.10 ± a		0.06	0.16 ± ab		0.04	0.25 ± c		0.02	0.21 ± bc		0.01	**
3-CQA	0.89 ± a		0.49	1.24 ± a		0.05	1.99 ± b		0.27	1.92 ± b		0.07	*
CeQA	0.28 ± a		0.22	0.31 ± a		0.09	0.53 ± a		0.09	0.47 ± a		0.09	ns
CeQA	0.13 ± a		0.11	0.17 ± a		0.05	0.31 ± a		0.15	0.23 ± a		0.02	ns
5-CQA	1.57 ± a		0.92	2.11 ± a		0.18	3.29 ± b		0.52	2.91 ± b		0.34	*
4-CQA	1.53 ± a		0.93	2.04 ± a		0.24	3.25 ± b		0.36	3.00 ± b		0.34	*
5-pCoQA	0.28 ± a		0.26	0.34 ± a		0.18	0.59 ± a		0.11	0.51 ± a		0.10	ns
5-FQA	1.06 ± a		0.70	1.43 ± a		0.27	2.21 ± b		0.35	1.96 ± b		0.42	*
CQL	0.17 ± a		0.13	0.25 ± ab		0.11	0.39 ± b		0.08	0.40 ± b		0.13	**
4-CQL	0.19 ± a		0.16	0.27 ± ab		0.12	0.43 ± b		0.06	0.36 ± ab		0.08	*
CQL	0.26 ± a		0.24	0.45 ± ab		0.35	0.79 ± b		0.09	0.64 ± b		0.13	*
CQL	0.33 ± a		0.19	0.40 ± a		0.15	0.91 ± b		0.05	0.79 ± b		0.12	**
1,4-diCQA	0.16 ± a		0.10	0.28 ± b		0.08	0.50 ± c		0.08	0.50 ± c		0.11	***
3,5-diCQA	0.12 ± a		0.10	0.14 ± a		0.06	0.32 ± a		0.17	0.21 ± a		0.08	ns
4,5-diCQA	0.27 ± a		0.19	0.57 ± ab		0.23	0.86 ± b		0.18	0.78 ± b		0.27	**
Unknown Compound	0.08 ± a		0.05	0.18 ± b		0.04	0.31 ± c		0.04	0.35 ± c		0.10	**
Unknown Compound	0.07 ± a		0.05	0.11 ± a		0.04	0.23 ± a		0.14	0.19 ± a		0.05	ns
Unknown Compound	0.05 ± a		0.08	0.08 ± a		0.08	0.13 ± a		0.04	0.16 ± a		0.12	ns
Unknown Compound	0.12 ± a		0.03	0.27 ± b		0.02	0.43 ± c		0.10	0.41 ± c		0.05	***

1.   Data are expressed as mean ± standard deviation. Letters indicate statistically significant differences between extraction methods. † indicates that the acylation position was uncertain.

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 22: Concentrations of the classes of CGAs from French press spent coffee

Extraction	French Press											
Temperature	80°			100°			110°			115°		
SUM of CQAs	4.50	±	2.73	6.03	±	0.65	9.62	±	1.41	8.74	±	0.88
5-pCoQA	0.28	±	0.26	0.34	±	0.18	0.59	±	0.11	0.51	±	0.10
5-FQA	1.06	±	0.70	1.43	±	0.37	2.21	±	0.35	1.96	±	0.42
SUM of CQLs	0.95	±	0.72	1.37	±	0.74	2.52	±	0.28	2.19	±	0.46
SUM of diCQAs	0.54	±	0.39	1.00	±	0.37	1.68	±	0.43	1.50	±	0.46
SUM of Unknown	0.32	±	0.22	0.64	±	0.19	1.11	±	0.33	1.11	±	0.33
SUM OF CGAs	**7.65**	**±**	**5.02**	**10.81**	**±**	**2.50**	**17.73**	**±**	**2.91**	**16.01**	**±**	**2.65**

The concentrations of di-CQAs found in French Press were higher than those observed from the espresso, almost the double.

The barplot reported in figure 23 summarize the concentrations of the classes of CGAs from both spent coffee grounds. As show the bars plots, the concentrations recovered from the French press, for all the temperatures tested, were higher than of those registered for the espresso. In both cases at the temperature of 110°C it was found the highest concentration for all the classes of compounds. For the espresso however, 100°C it was a significant temperature, while at the temperature of 80°C it has been observed, for all classes of compounds and all sources of spent coffee, the lowest concentrations values.
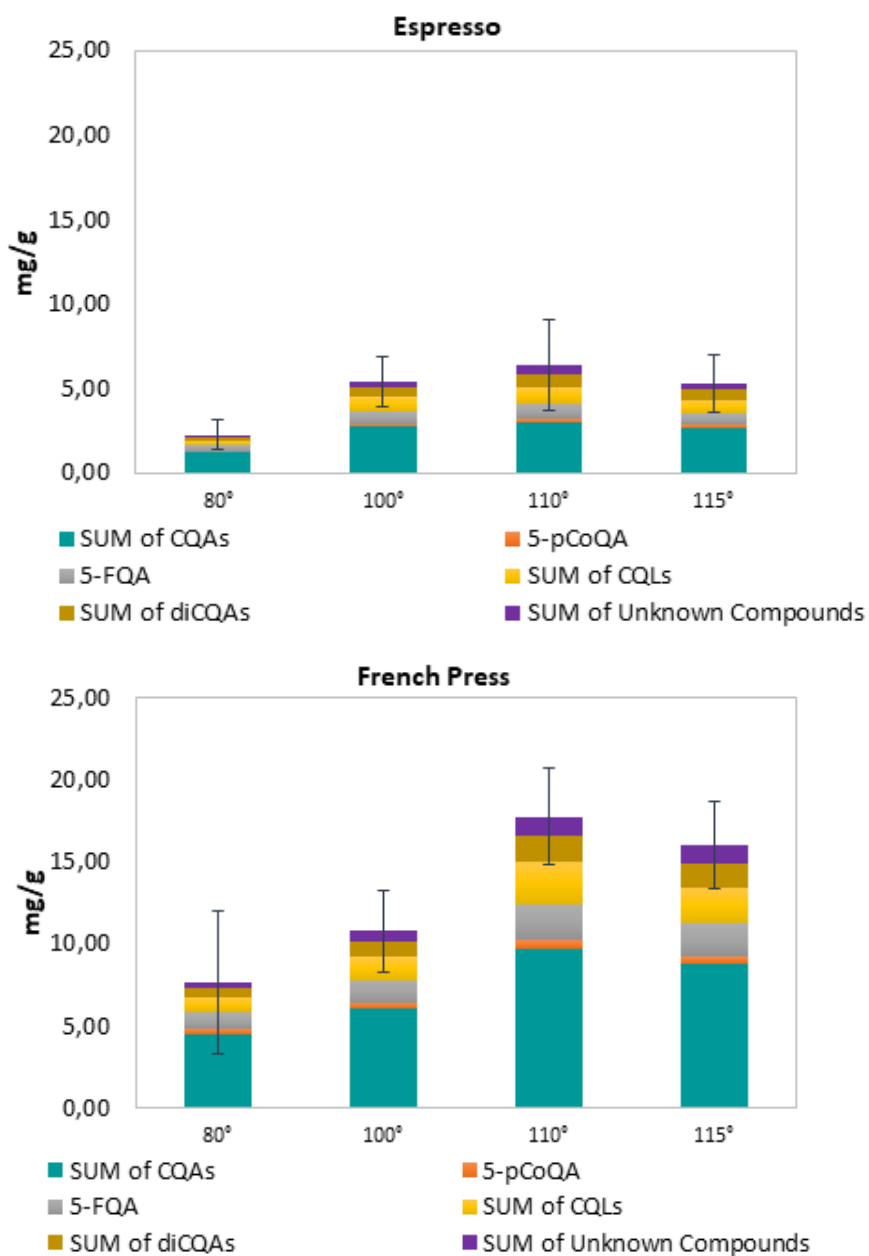
Figure23: Bar plot of the concentrations of the classes of CGAs from Espresso and French press spent coffee grounds.

5.4 Conclusions

Coffee by-products such as spent coffee grounds could be used as a source of new functional ingredients.

The results obtained by spectrophotometric measurements from the fractional design showed that the factors significant to recover phytochemicals were Temperature and type of SCG. In the second part of the experiments, the spent coffee grounds from Espresso and French press were extracted at 4 different temperature and analyzed by HPLC-DAD to estimate the recovery of phytochemicals in these different operative conditions. The concentrations of the compounds recovered from French Press's SCG were significantly higher with respect to those Espresso's SCG, in terms of caffeine CGAs and total phenolic compounds.

Temperature has shown a significant effect on the recovery of caffeine from the extracts. Moreover, at 110°C, a significant higher caffeine concentration was recovered for both extraction methods, 3.10 ± 1.98 mg/g from Espresso and 10.44 ± 1.65 mg/g by French Press.

At the same way, for the recovered of CGAs the temperature of 110°C it was able to recover a large amount of CGAs, 5.84 ± 2.32 mg/g and 16.42± 0.94 mg/g by French Press.

Only for phenolic compounds determined by spectrophotometric measurements, the temperature had not shown a significant effect.

These conditions of temperatures, as reported in Conde and Mussatto study (2016), could be considered mild conditions, which have proved to be effective, in terms of the concentration of recovered compounds and, compared to other methods involving the use of solvents or of a larger quantity of water.

The extracts produced by this method, which is an eco-friendly method that employs only water as the extraction solvent, have been presented the appropriate amount of phytochemicals and could be then of interest for application in the food, cosmetic, and pharmaceutical areas.

The experimental tests conducted have permitted to evaluate and estimate, among different operative variables, the condition able to optimize the extractions of phytochemicals, with a green method and in mild condition, comparable to other methods.

The results highlight the enormous potential of spent coffee grounds for use as the raw material for biotechnological processes, due to their antioxidant capacity and for the presence of phytochemicals, which have a wide application in food and pharmaceutical products.

Thus, a possible future study could be focused on the opportunity to reuse these natural phytochemicals extracted in mild condition and with a low-cost process.

Moreover, the optimization of the mild conditions used to extract the compounds is certainly an argument to be explored also in relation to the intended use of these recovered compounds.

6.General conclusions and future Perspectives

The research topic in this PhD program focuses on the process of extracting coffee and phytochemicals, under an engineering perspective. Hence, the engineering approach has been centered on the machines, on the extraction systems and on the operative variables that drive the extraction process. Moreover, part of work fits into the so-called green extraction of natural products, understood as the realization and design of extraction processes which will reduce energy consumption, allows use of alternative solvents and renewable natural products, and ensure a safe and high-quality extract/product.

The study of the extraction technologies showed how they significantly affect the, physical, chemical and sensorial properties of the beverage.

We have observed how the use of apparently similar extractive systems, i.e. between various Espresso's systems, or in the cold brewing methods, give beverage characteristics that are strictly dependent on process variables, such as the use of different pressures or slightly different temperatures.

All the differences emerged among the drinks were also found in the correspondent spent coffee grounds (SCGs) originated.

Extractions carried out on SCGs confirmed different extraction efficiencies already observed in previous studies. Adopting mild temperature, defined solid/liquid ratio conditions and simply water as solvent, it has been possible to re-extract a quantity of phytochemicals comparable to other studies which used different and more impacting techniques (e.g. chemicals as solvent or more water per unit of SCG).

Coffee is a very well-studied drink, both healthly and chemical aspects. Also, about the quality of the green and then roasted raw material, many studies have been carried out.

However, the topic of coffee extraction, either from the ground powder or from the exhausted powder, turns out to be a less studied theme at the moment, especially in terms of process development.

Future research activities will focus on this topic, trying to optimize the process phases of the extractive systems already studied and also trying to develop new extraction technologies based on the knowledge acquired.

Guidelines of green-extraction of natural products and the engineering approach will be central in these future studies.

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APPENDIX A - ORIGINAL PAPERS

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

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A R T I C L E   I N F O

A B S T R A C T

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

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, was 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 physicochemical 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 underextracted), 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).
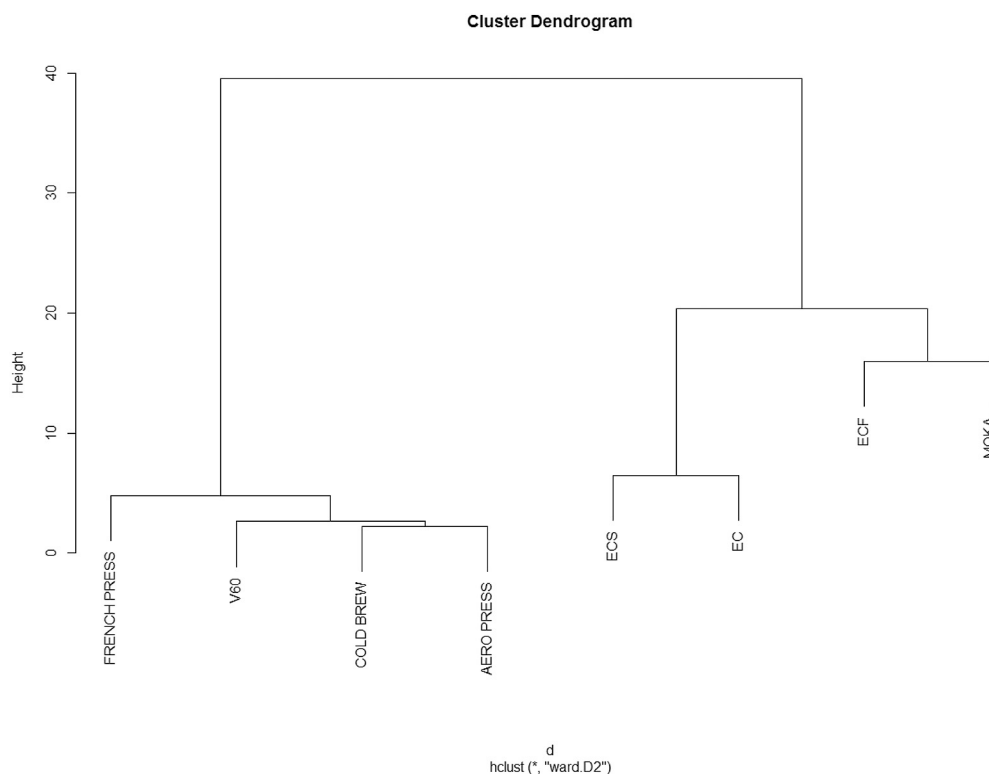
Cluster Dendrogram

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.

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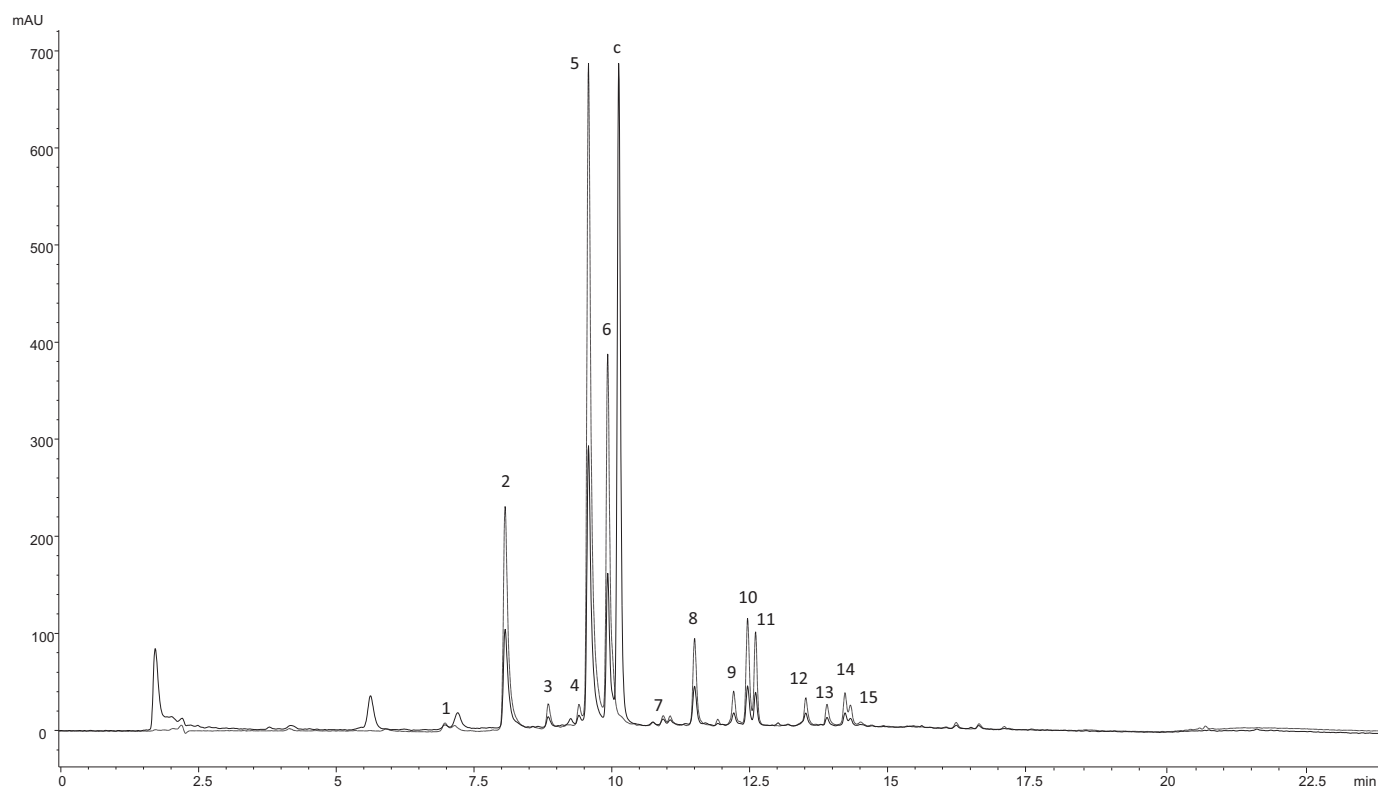
Fig. 2. Overlapping of HPLC/DAD chromatograms at 278 nm (whole line) and 330 nm (dotted line) for CGAs and caffeine monitoring of a representative coffee sample.

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.

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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 c	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
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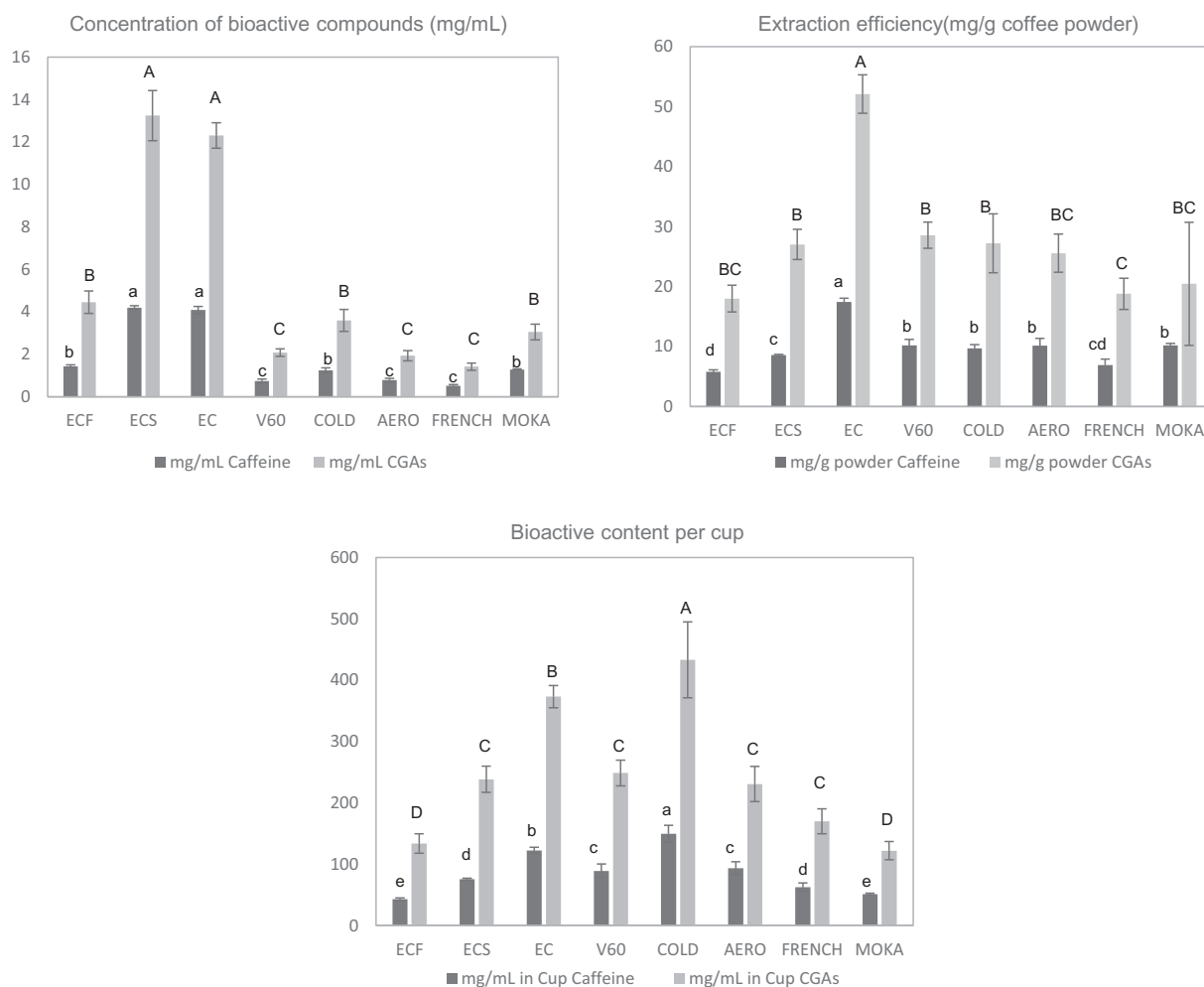
Fig. 3. Content per mL of extract (a), per gram of coffee powder (b), and per cup of coffee brew (c) of caffeine and of sum of CGAs. Letters indicate statistically significant differences between extraction methods. Capital letters indicate difference in CGAs while lowercase letters indicate differences 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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Characterization and comparison of cold brew and cold drip coffee extraction methods

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Abstract

BACKGROUND: Each region of the world has its own methods, protocols, instruments and procedures regarding how to brew coffee. The final result in the cup is strongly affected by the extraction method, and many studies have focused on this subject. However, few studies have investigated slow, cold extraction methods, despite their popularity among baristas. Therefore, the present study aimed to characterize and compare two cold extraction methods: cold brew and cold drip.

RESULTS: Physical and chemical analyses were used to describe coffee beverages in terms of pH, total solids, refractive index, density and viscosity. Caffeine and cinnamic acids were quantified using high-performance liquid chromatography (HPLC)/diode array detector and HPLC/mass spectrometry. A sensory evaluation included aroma, flavor and textural attributes.

CONCLUSIONS: Significant differences were found in the chemical and physical parameters, both between and within the two methods, as a function of the extraction temperature and contact time. Similarly, the sensory evaluation found differences in flavor profiles, as measured in terms of bitterness, sweetness, sourness and global intensity.
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Keywords: cold extraction; brewing; filtered coffee; CGAs; caffeine

INTRODUCTION

Coffee is one of the world's principal commodities and one of the most widely-consumed beverages, as a result of its pleasant taste and aroma, as well as its stimulating qualities. According to the International Coffee Organization,[1] more than nine million tons were consumed worldwide in 2016. Numerous different coffee brewing and extraction methods have been introduced in recent years and consumer preferences for a particular preparation mode are influenced by the geographical, cultural and social environment, not to mention personal preferences.[2]

Recent research has expanded knowledge of the chemical and sensory characteristics of coffee along the whole production chain, from bean to cup.

Furthermore, various studies have focused on the specific modes of coffee extraction, in particular espresso and coffee obtained by infusion. The literature has described the influence of the product (i.e. botanical variety and geographical origin of beans), process (roasting degree, and grinding) and extraction steps (i.e. contact time between coffee and water, temperature and pressure) on the physicochemical attributes and sensory profile of different methods.[3–7]

The brewing method affects the composition of the final coffee beverage: notably, considerable differences are found in polyphenol extraction, caffeine content, total solids, antioxidant activity and volatile profile.[8]

During extraction, soluble compounds are dissolved and, depending on the extraction methods, non-soluble compounds are washed with the extraction water, ending up in the extract as dissolved or suspended solids.[9,10]

Furthermore, the methods described in the literature vary widely because each method has a specific recipe. However, the temperature of the brewing water is usually consistent. Hot water is used to increase the extraction yield, whereas several chemical extraction studies have shown that different aromatic compounds are extracted at different temperatures.[3,11] Although consumers traditionally drink hot coffee, in recent times, the consumption of cold coffee has increased in northern European countries, the USA and Japan,[12] as a result of new preparation methods involving longer extraction times at colder temperatures (i.e. room temperature or less), rather than rapid exposure to high temperatures. This, cold brew method, indicates a coffee produced by cold extraction, and should not be confused with cold coffee, which is usually produced by a hot system and left to cool down.

Several recipes for the extraction of coffee powder with cold water have been developed. They differ with respect to apparatus configuration, contact time between powder and water, and water temperature, although they can be categorized into two broad methods: cold brew and cold drip. In the cold brew method, coffee powder is steeped in a volume of water at room temperature

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(or colder) for a long time (6 h or more) and then separated by pressing. In the cold drip method, water at room temperature (or colder) is slowly dripped onto a coffee panel supported by a filter and the beverage is recovered.

For these new methods, there are no specific and unequivocal recipes available with respect to times and extraction temperatures. Baristas rely on their perception and experience to set extraction parameters. However, there have been few empirical investigations of these slow, cold extraction methods that are designed to produce a *lungo* coffee.

The present study aimed to characterize and compare cold brew and cold drip extraction methods in terms of chemical composition, physical properties and a sensory evaluation of the coffee that is produced. The effects of the main process variables (temperature and contact time between coffee powder and water) were assessed in a full factorial experiment. To introduce a benchmark for beverage characterization, a third extraction method, the French press, was included in the experimental design.

MATERIALS AND METHODS
Experimental design
The experiment was designed to highlight differences between two cold extraction methods: cold brew and cold drip. Two temperatures (room temperature, 22 °C; refrigerator temperature, 5 °C) and two powder–water contact times were tested. Coffee preparation methods and operative conditions are shown in Table 1. Three replicates were performed for each sample. The order of beverage preparation was completely randomized. For the cold drip method, contact time between the powder and water was tested at two flow rates: one drop every 5 s and one drop every 10 s. For the cold brew method, extraction time was calculated from the two overall extraction times for the respective cold drip method.

In addition, the French Press extraction method was chosen as a benchmark.

Coffee samples and extraction methods
The same batch of coffee was used for all extractions (Illy Rosso 100% Arabica; illycaffè S.p.A. Trieste, Italy). Each pack of coffee beans (250 g) was opened immediately before brewing to avoid oxidative damage. Beans were coarse-ground using a professional coffee grinder (KE640; Ditting Maschinen AG, Bachenbülach, Switzerland).

The coffee was grinded 'coarse' as well as for all the other lungo and filter methods.[13] Water quality plays an important role in coffee beverage quality,[14] and so all samples were prepared using the same commercial brand of mineral water. The physical and chemical characteristics are shown in Table 2.

For the cold drip method, samples were prepared using a cold drip coffee equipment with 25 g of coffee powder and 250 mL of mineral water at different temperatures of extraction and times/flow rates. The equipment comprised three parts. An upper (glass) part, 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 entered from above, passed through a filter and into a lower carafe, where the final brew was collected. Used coffee grounds were retained in the filter. The average extraction time was 6.5 h for the slower times of extraction/flow rate and 3.3 h for the faster times of extraction/flow rate. Extraction was performed at room temperature (22 °C) and at refrigerator temperature (5 °C).

Table 1. Coffee preparation methods and operative conditions

Extraction procedure	Temperature	Time/flow rate
Cold drip	22 °C	1 drop/5 s
Cold drip	5 °C	1 drop/5 s
Cold drip	22 °C	1 drop/10 s
Cold drip	5 °C	1 drop/10 s
Cold brew	22 °C	3 h
Cold brew	5 °C	6 h
Cold brew	22 °C	6 h
Cold brew	5 °C	3 h
French press	95 °C	5 min

Table 2. The physico-chemical characteristics of mineral water

Analytical parameter	Values
pH	8.1
Electrical conductivity (20 °C)	249 μS cm^{-1}
Total dissolved solids	148 mg L^{-1}
Hardness	14 °F
Kubel oxydability	0.6 mg L^{-1}
Free carbon dioxide	3.3 mg L^{-1}
Calcium (Ca^{2+})	30.1 mg L^{-1}
Magnesium (Mg^{2+})	15.0 mg L^{-1}
Sodium (Na$^+$)	1.4 mg L^{-1}
Potassium (K$^+$)	0.5 mg L^{-1}
Hydrogen carbonate (view the MathML source)	157 mg L^{-1}
Sulfate (view the MathML source)	10.7 mg L^{-1}
Nitrate (view the MathML source)	5.0 mg L^{-1}
Chloride (Cl$^-$)	1.5 mg L^{-1}
Fluoride (F$^-$)	0.06 mg L^{-1}
Silicon dioxide (SiO$_2$)	6.6 mg L^{-1}

Cold brew coffee were prepared using 25 g of coffee powder and 250 mL of water. Cold brew extraction was performed under static conditions. Powder and water were in contact for the same amount of time as the cold drip method (6.5 h for the slower and 3.3 h for the faster). When extraction ended, the beverage was filtered through a paper filter. Extraction temperatures were the same as for the cold drip method.

French press coffee was prepared with coarse-ground coffee (25 g) and hot water (250 g at 95 °C) mixed in a brewer fitted with a mesh plunger. The mixture was brewed for 5 min and then the plunger was pressed to trap coffee grounds at the bottom of the container.

Physical analysis
All samples were brought to 20 °C before the selected parameters were analyzed and evaluated. A digital pH meter (GLP 21; Crison Instruments, Barcelona, Spain) was used to determine pH. Viscosity was measured with a capillary viscometer (Ostwald-type) fitted with an automatic optical reader (ViscoClock; Schott Instruments, Mainz, Germany) and expressed as mN s m^{-2}. Relative density was measured with a 25-mL pycnometer. Refractive index was measured with a portable digital refractometer (Refracto 30PX; Mettler Toledo, Milan, Italy) using the total internal reflection method. Total solids, expressed as mg mL^{-1}, were measured gravimetrically by drying ~10 mL (less than

±0.5 mL) of coffee at 100 °C for 24 h, until a constant weight was reached.[5]

Analysis of caffeine and chlorogenic acids

Coffee samples were centrifuged at 12 074 × g for 5 min and diluted 1:10 with water before high-performance liquid chromatography (HPLC) analysis. HPLC was carried out using a HP 1100 system (Agilent Technologies, Palo Alto, CA, USA) equipped with an autosampler, column heater module and quaternary pump, and coupled to a diode array detector (DAD) and a time-of-flight (TOF) mass spectrometer equipped with an electrospray interface (all from Agilent Technologies). HPLC was performed under the conditions: gas temperature 300 °C, nitrogen flow rate 12 L min⁻¹, nebulizer pressure 20 psi, capillary voltage 3800 V, fragmentors in the range 120–300 V, operating in negative ion mode for chlorogenic acids (CGA) and in positive ion mode for caffeine.

An InfinityLab 150 mm × 3 mm inner diameter, 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. The injection volume was 5 μL. Elution was performed at a flow rate of 0.4 mL min⁻¹ using water at pH 3.2 by formic acid (solvent A) and acetonitrile (solvent B). All solvents used were of HPLC grade. Based on our previous study,[6] the applied multistep linear gradient was modified to start from 95% A followed by a plateau for 5 min, then 15 min to 56% A and 2 min to 10% A, with a final plateau of 5 min at 10% A. The total analysis time was 24 min. Ultraviolet-visible (UV-vis) spectra were recorded in the range 220–600 nm and the detector was set at 330 nm for CGAs and 278 nm for caffeine.

CGAs and caffeine were identified by comparing their retention times, UV–vis and MS spectra with those of the respective standards. Identification of other CGAs was performed by electrospray ionization-tamdem mass spectroscopy (ESI-MS/MS) using an API 4000 triple quadrupole mass spectrometer equipped with a TurbolonSpray source (Applied Biosystems/Sciex, Toronto, Canada). The source was operated in negative ionization mode with a needle potential of −4500 V and a turbo gas flow rate of 10 L min⁻¹ of air heated to 150 °C (nominal heating-gun temperature). Mass calibration and resolution adjustments on the resolving quadrupoles were performed automatically using a 10^7 mol L⁻¹ polypropylene glycol solution introduced via a built-in infusion pump. The peak width was set on both resolving quadrupoles at 0.7 Th (measured at half height) for all MS and MS/MS experiments. Collision-activated dissociation MS/MS was performed in the LINAC Q2 collision cell (AB Sciex, Framingham, MA, USA), operating with nitrogen at 10 mTorr as the collision gas. The declustering potential and collision energy were automatically optimized for all species studied using Analyst, version 1.4 (AB Sciex). The acquired data were processed using Analyst, version 1.5.2, with the 'Explore' option for spectral interpretation.

CGAs were evaluated by HPLC/DAD using a five-point calibration curve of chlorogenic acid (purity 99%) (Extrasynthèse, Genay, France) at 330 nm ($r^2 = 0.999$) and caffeine content was determined by HPLC/DAD using a six-point calibration curve from Extrasynthèse (purity 95%) at 278 nm ($r^2 = 0.999$).

Sensory evaluation

Sensory evaluation was performed by a panel of eight trained sensory experts. Each brew was tested in duplicate in an air-conditioned room at 22 °C in independent sessions. The classification system developed by SCAA[15] was used. This includes

Table 3. Physical characterization of coffee beverages, comparing extraction method, temperature and flow rate

Extraction	Drip	Brew	Drip	Brew	Drip	Brew	Drip	Brew	French						
Temperature	22 °C	22 °C	5 °C	5 °C	22 °C	22 °C	5 °C	5 °C	press	Extraction	Temperature	Flow rate	Flow rate: extraction	Flow rate: temperature	Extraction: temperature
Flow rate	Fast	Fast	Slow	Slow	Slow	Slow	Fast	Fast							
	(mean ± SD)											P			
Refractive index	1.367 ± 0.503	0.767 ± 0.057	0.900 ± 0.624	1.200 ± 0.2	1.500 ± 0.1	1.133 ± 0.057	0.633 ± 0.351	0.667 ± 0.057	0.983 ± 0.186	NS	0.0192*	0.0248*	NS	NS	0.0248*
pH	5.5 ± 0.02	5.6 ± 0.3	5.8 ± 0.1	5.7 ± 0.04	5.4 ± 0.06	5.5 ± 0.09	5.7 ± 0.12	5.6 ± 0.11	5.237 ± 0.021	NS	0.0051**	NS	NS	NS	NS
Density 20° (g mL⁻¹)	1.046 ± 0.005	1.048 ± 0.008	1.018 ± 0.010	1.080 ± 0.014	1.071 ± 0.034	1.063 ± 0.027	1.051 ± 0.008	1.052 ± 0.029	1.061 ± 0.014	NS	NS	NS	NS	NS	NS
Viscosity (mN s m⁻²)	1.079 ± 0.062	1.075 ± 0.026	1.025 ± 0.106	1.135 ± 0.068	1.113 ± 0.024	1.117 ± 0.108	1.064 ± 0.048	1.036 ± 0.072	1.131 ± 0.075	NS	NS	NS	NS	NS	NS
Total solid (mg mL⁻¹)	19.411 ± 2.987	20.300 ± 2.128	14.023 ± 3.434	18.421 ± 0.686	18.004 ± 4.765	22.746 ± 0.687	18.892 ± 0.745	18.935 ± 0.100	27.354 ± 3.711	NS	0.033*	NS	NS	NS	NS

NS, $P > 0.05$; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.
The mean ± SD and P-values for each of the three variables, as well as their interactions, are reported. The mean ± SD for the French press extraction used as the benchmark is in reported a separate column. NS, not significant.
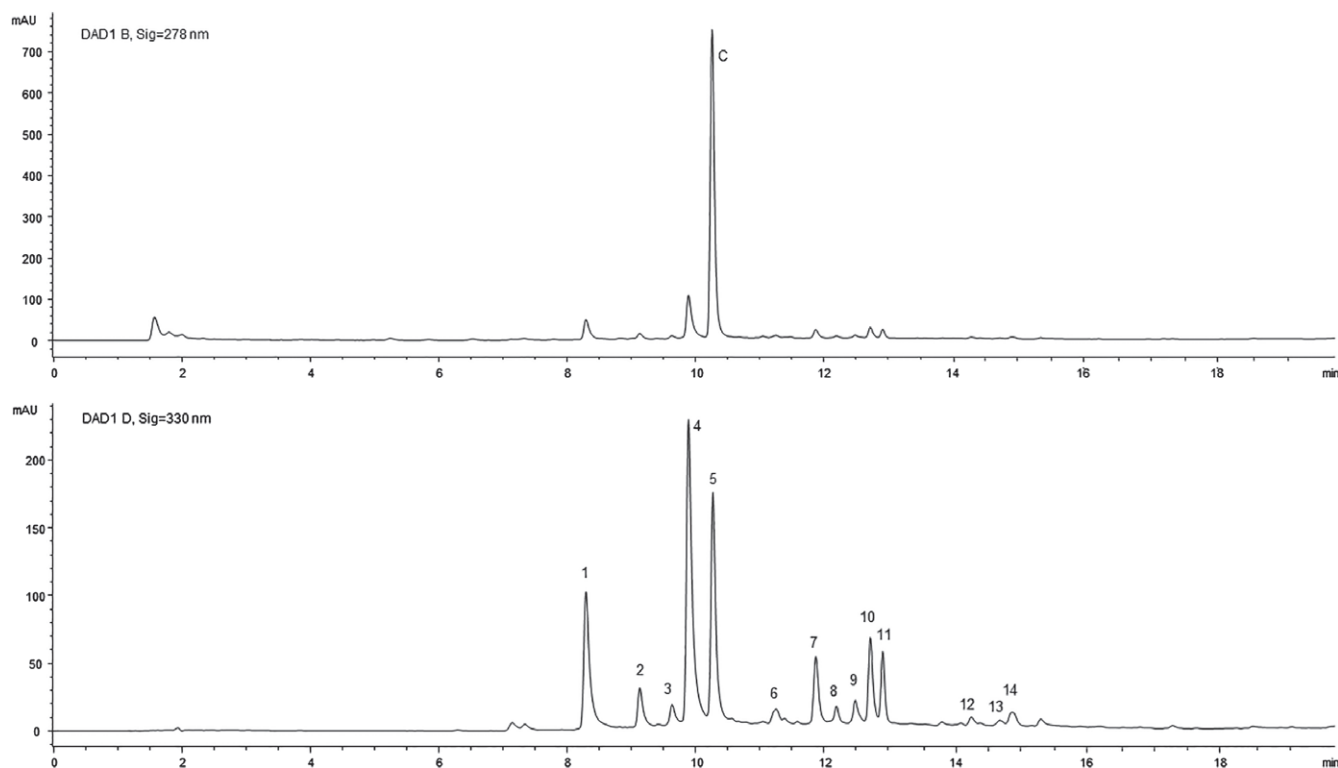
Figure 1. HPLC/DAD profiles at 278 nm for caffeine (C) and 330 nm for CGA detection (1 – 14) as listed in Table 4.

a standard list of attributes, divided into broad and specific categories.

For each sample, trained panelists were first asked to rate the intensity of odor descriptors perceived by the nose (aroma). Then, they were asked to sip the sample and rate the intensity of odors perceived retronasally. Finally, they took a second sip and rated taste and mouthfeel attributes. The odor attribute was Overall Intensity. Flavor attributes were Overall Intensity, Acidity, Sweetness, Bitterness, Enzymatic (flowery, fruity, herby), Sugar Browning (nutty, caramelly, chocolatey), Distillation (carbon, spicy, resinous) and Astringency. The perceived intensity of each sensation was rated on a nine-point scale ranging from 1 (extremely weak) to 9 (extremely strong).

Statistical analysis

Differences between means were assessed using a conventional analysis of variance (three-way analysis of variance) in a full factorial experiment. Three factors and all of their interactions were tested at two levels: extraction methods (Cold Drip and Cold Brew), temperature (5 and 22 °C) and times/flow rates (fast/slow). In cases where the F-test was significant at $P < 0.05$, multiple paired-means tests checked for significance using the post-hoc Tukey's honestly significance difference test ($P < 0.05$). All statistical analyses were performed using R, version 3.4.0 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS AND DISCUSSION

Physical analyses

The physical characterization of beverages is shown in Table 3, which compares preparation methods, temperature and flow rate conditions. For each parameter, the mean, SD

and P-values of the three variables and their interactions are reported. This analysis highlights differences between beverages prepared using different methods and under different conditions.

Significant differences were found for refractive index, pH and total solids. With respect to the refractive index, for the cold drip method, a higher temperature resulted in a higher refractive index, whereas no difference was found for the cold brew method (interaction extraction method × temperature; $P = 0.025$). Furthermore, the refractive index increased consistently as the time of contact between powder and water increased.

Similarly, temperature was found to influence pH ($P = 0.0051$). Infusion temperature is recognized as an important factor in coffee beverage preparation, and lower temperatures usually reduce the quantity of extracted beverage.[3] In the present study, temperature was a decisive influence on the measured physical parameters. Coffee prepared at lower temperature (5 °C) had a higher pH, regardless of the extraction method (drip or brew). pH varied from 5.5 ± 0.1 (at 22 °C) to 5.7 ± 0.1 (at 5 °C) (Table 3). These values were higher than the French press method (5.2 ± 0.1).

Nicoli *et al.*[16] showed that total solids are regulated by the brewing formula, coffee/water ratio, roast and percolation temperature. Similarly, in the present study, temperature was found to have a significant effect ($P = 0.033$), with more total solids in coffee prepared at 22 °C (20.115 ± 1.992 mg mL^{-1}) than in that prepared at 5 °C (17.56 ± 2.38 mg mL^{-1}). Total solids were lower compared to the benchmark French press method (27.358 ± 3.71 mg mL^{-1}), probably as a result of the lower extraction temperature.

However, a high variance in the total solids from different preparation methods has been reported previously.[4,8]

No significant difference was found for viscosity and density for either method, as well as for temperature or contact time. Furthermore, the values of these parameters were

Table 4. Negative ion MS^2 fragmentation data for CGAs

Compounds	Identity	Rt	Parent ion (m/z)	MS^2 base peak m/z	MS^2 ion m/z	Int	m/z	Int	Reference
1	3-CQA	8.1	353.2	190.9	179.0	61.6	173.0	2.9	Clifford et al.[30] (2005), Jaiswal et al.[23], Schutz et al.[31] (2004)
2	CeQA[a]	8.9	353.2	191.1	179.0	4.4	173.0	6.7	Jaiswal et al.[23]
3	CeQA[a]	9.45	353.2	179.0	190.7	85.7	173.2	28.6	Jaiswal et al.[23]
4	5-CQA (chlorogenic acid)	9.69	353.2	190.8	178.8	2.9	160.6	2.9	Clifford et al.[30] (2005), Jaiswal et al.[23], Schutz et al.[31] (2004)
5	4-CQA	10.07	353.2	173.0	178.9	81.2	191	37.3	Clifford et al.[30] (2005), Jaiswal et al.[23]; Schutz et al.[31] (2004)
6	5-p-CoQA	11.06	337.2	190.9	172.9	47	162.9	18	Jaiswal et al.[23]
7	5-FQA	11.63	367.2	191.2	173.0	72.1	193.0	18.8	Jaiswal et al.[23]
8	CQL[a]	11.94	335.3	160.6	173.1	94.7	178.8	30.7	Jaiswal et al. (2014)
9	4-CQL	12.23	335.3	160.6	178.9	34.7	172.8	27.1	Jaiswal et al. (2014)
10	CQL[a]	12.46	335.3	161.0	178.8	9.3	172.9	7.9	Jaiswal et al. (2014)
11	CQL[a]	12.64	335.3	161.0	179.1	8.8	172.8	3.4	Jaiswal et al. (2014)
12	1.4-diCQA	13.79	515.3	353.1	335.0	6.4	317.0	2.7	Clifford et al.[30] (2005)
13	3.5-diCQA	14.34	515.3	352.9					Clifford et al.[30] (2005)
14	4.5-diCQA	14.57	515.3	353.1	335.0	3.5	317.0	4.4	Clifford et al.[30] (2005)

[a] Acylation position is uncertain.
CQA, caffeoylquinic acid; CeQA, caffeoyl epi-quinic acid; p-CoQA, p-coumaroylquinic acid; FQA, Feruloylquinic acid; CQL, Caffeoylquinic acid lactone; diCQA, dicaffeoylquinic acid.

similar to measurements using the French press method. These parameters changed in different Espresso brewing techniques.[6]

Analysis of caffeine and chlorogenic acids

Qualitative results

CGAs and their derivatives are known to contribute to the acidity, astringency and bitterness of the final coffee beverage.[17,18] Chlorogenic acid lactones (CGLs) are formed from CGAs during roasting through a process that involves the loss of a water molecule from the quinic acid moiety and the formation of an intramolecular ester bond. Along with CGAs, CGLs contribute to coffee flavor and, despite their low concentrations, their impact on the final cup quality may be significant. CGLs have also been studied for their potential hypoglycemic effects, as well as their action on opioid and adenosine brain receptors.[19–21] The analyzed samples showed almost the same qualitative profile of bioactive substances found in HPLC/DAD profiles at 278 nm for monitoring caffeine, and at 330 nm for CGA detection (Fig. 1). In total, 14 CGA compounds were detected in coffee samples. Their peaks were identified on the basis of UV spectra and elution/retention sequences reported in the literature and confirmed by their mass spectrometric behavior (Table 4).

In the first set of experiments, coffee samples were analyzed by ESI-TOF mass spectrometry in negative ionization mode aiming to define the molecular weights of the different compounds. Five caffeoylquinic acids (CQAs), one feruloylquinic acids (FQA), one p-coumaroylquinic acids (p-CoQA), four caffeoylquinic acid lactones (CQLs) and three dicaffeoylquinic acids (diCQAs) were identified, according to Clifford and Jaiswal et al.[22,23]

In a second set of experiments, samples were subject to product ion scan measurement (MS2) in negative ionization mode using an ESI triple quadrupole mass spectrometer. Table 4 summarizes MS2 data for monoacyl and diacyl CGAs and CGLs.

Quantitative results

Caffeine content has been shown to vary substantially as a function of the variety and geographical origin of the coffee bean, as well as the extraction method.[24,25] Caffeine and CGA contents (mg mL^{-1}) for this experiment are shown in Table 5. Caffeine concentration was found to differ significantly as a function of both extraction method and temperature. Concentrations were higher in cold drip than cold brew beverages, as well as in beverages extracted at 22 °C (1.03 ± 0.19 mg mL^{-1} and 0.853 ± 0.15 mg mL^{-1}, respectively). This result was unsurprising because dynamic methods (drip) involve the continuous renewal of the extraction solvent. Because the matter transfer from the solid to the liquid phase is driven by the concentration gradient,[26] this is a more efficient way of extracting relevant molecules compared to a static system. In the latter case, coffee powder is in contact with the total volume of extractive solvent in a unique solution, leading to saturation. The highest caffeine concentration was measured using the drip extraction method at room temperature.

Caffeine content in French press coffees (1.09 ± 0.11 mg mL^{-1}) was similar to levels obtained with drip extraction at 22 °C. It may be that the longer brewing time used in the cold method (6 h compared to 5 min) compensates for the difference in temperature (~90 °C compared to 22 °C). No significant differences in caffeine content were found between the two contact times for the cold brew method.

Table 5. Concentrations (mg mL^{-1}) of caffeine, CQAs, CGA, FQA, 5-pCoQA, CQLs and diCQAs as a function of extraction method, temperature and flow rate

Extraction Temperature Flow rate	Drip 22°C Fast	Brew 22°C Fast	Drip 5°C Slow	Brew 5°C Slow	Drip 22°C Slow	Brew 22°C Slow	Drip 5°C Fast	Brew 5°C Fast	Extraction	Temperature	Flow rate	Flow rate: extraction	Flow rate: temperature	Extraction: temperature	French press
	(mean ± SD)								*p*						
Caffeine	1.140 ± 0.119	0.784 ± 0.108	0.795 ± 0.319	0.893 ± 0.207	1.267 ± 0.152	0.973 ± 0.124	0.938 ± 0.162	0.761 ± 0.170	0.026*	0.018*	NS	NS	NS	NS	1.094 ± 0.108
3-CQA	0.189 ± 0.020	0.138 ± 0.023	0.156 ± 0.053	0.138 ± 0.037	0.217 ± 0.036	0.175 ± 0.025	0.137 ± 0.023	0.159 ± 0.026	NS	0.0241*	NS	NS	NS	NS	0.199 ± 0.015
CeQA[a]	0.051 ± 0.009	0.036 ± 0.004	0.040 ± 0.016	0.034 ± 0.010	0.056 ± 0.006	0.045 ± 0.008	0.034 ± 0.007	0.043 ± 0.009	NS	0.0249*	NS	NS	NS	NS	0.051 ± 0.004
CeQA[a]	0.030 ± 0.007	0.019 ± 0.004	0.020 ± 0.006	0.017 ± 0.004	0.033 ± 0.002	0.023 ± 0.003	0.020 ± 0.004	0.106 ± 0.140	NS	NS	NS	NS	NS	NS	0.028 ± 0.003
5-CQA	0.398 ± 0.045	0.282 ± 0.041	0.328 ± 0.120	0.272 ± 0.074	0.447 ± 0.039	0.354 ± 0.045	0.285 ± 0.059	0.244 ± 0.020	NS	0.037*	NS	NS	NS	NS	0.399 ± 0.028
4-CQA	0.268 ± 0.027	0.193 ± 0.034	0.221 ± 0.073	0.201 ± 0.051	0.306 ± 0.032	0.252 ± 0.037	0.189 ± 0.040	0.220 ± 0.045	NS	0.0195*	NS	NS	NS	NS	0.228 ± 0.099
5-pCoQA	0.026 ± 0.004	0.019 ± 0.004	0.022 ± 0.006	0.018 ± 0.005	0.038 ± 0.010	0.024 ± 0.004	0.020 ± 0.006	0.024 ± 0.008	0.0413*	0.0397*	NS	NS	0.0478*	0.0423*	0.031 ± 0.005
5-FQA	0.083 ± 0.008	0.057 ± 0.012	0.066 ± 0.021	0.058 ± 0.015	0.099 ± 0.010	0.076 ± 0.013	0.058 ± 0.013	0.068 ± 0.013	0.0495*	0.0117*	NS	NS	NS	0.0398*	0.081 ± 0.009
CQL[a]	0.014 ± 0.004	0.007 ± 0.002	0.010 ± 0.005	0.058 ± 0.015	0.023 ± 0.003	0.011 ± 0.001	0.009 ± 0.003	0.029 ± 0.032	0.0315*	0.0255*	NS	NS	NS	0.0007***	0.019 ± 0.007
4-CQL	0.023 ± 0.003	0.014 ± 0.003	0.016 ± 0.006	0.008 ± 0.002	0.033 ± 0.006	0.017 ± 0.002	0.015 ± 0.003	0.013 ± 0.005	0.00017***	0.00013***	NS	NS	0.0298*	0.033*	0.028 ± 0.004
CQL[a]	0.072 ± 0.014	0.044 ± 0.006	0.051 ± 0.025	0.012 ± 0.005	0.086 ± 0.008	0.055 ± 0.004	0.046 ± 0.010	0.036 ± 0.022	0.00017***	0.0001***	NS	NS	NS	NS	0.087 ± 0.011
CQL[a]	0.055 ± 0.007	0.034 ± 0.006	0.038 ± 0.017	0.038 ± 0.014	0.068 ± 0.003	0.041 ± 0.004	0.034 ± 0.007	0.038 ± 0.008	0.0108*	0.005**	NS	NS	NS	0.0036**	0.067 ± 0.006
1,4-diCQA	0.012 ± 0.004	0.008 ± 0.002	0.010 ± 0.007	0.028 ± 0.010	0.016 ± 0.006	0.013 ± 0.003	0.008 ± 0.002	0.017 ± 0.013	ns	ns	NS	NS	NS	0.0068**	0.018 ± 0.004
3,5-diCQA	0.025 ± 0.007	0.017 ± 0.001	0.022 ± 0.012	0.006 ± 0.001	0.037 ± 0.004	0.024 ± 0.002	0.017 ± 0.005	0.010 ± 0.005	0.00018**	0.00006***	0.044*	NS	NS	NS	0.035 ± 0.012
4,5-diCQA	0.008 ± 0.003	0.005 ± 0.001	0.007 ± 0.004	0.014 ± 0.004	0.013 ± 0.002	0.005 ± 0.001	0.005 ± 0.001	0.011 ± 0.007	ns	ns	NS	NS	NS	NS	0.016 ± 0.009

[a] Acylation position is uncertain. NS, $P > 0.05$; *$P \le 0.05$; **$P \le 0.01$; ***$P \le 0.001$.
The mean ± SD and *P*-values for each of the three variables, as well as their interactions, are reported. The mean ± SD for the French press extraction used as the benchmark is in reported a separate column. NS, not significant.
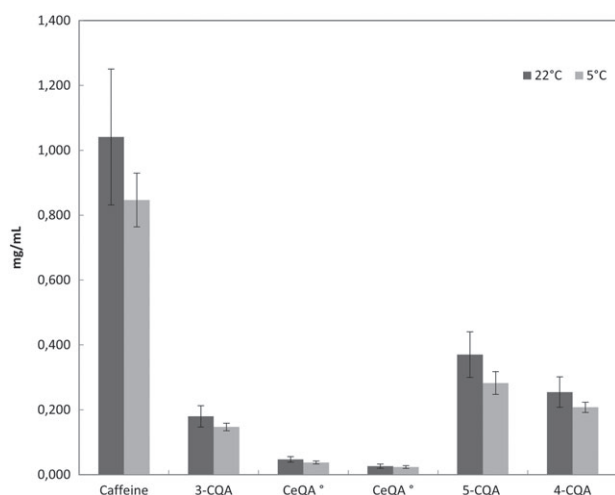
Figure 2. Concentrations (mg mL^{-1}) of caffeine, 3-CQA, CeQAs, 5-CQA and 4-CQA at different extraction temperatures.

CGAs are abundant phenolic compounds in coffee, whereas the literature reports that CQAs are the major subclass.[27] These compounds are known to influence flavor, contributing to acidity and conferring astringency and bitterness.[22]

Figure 2 shows that, in the present study, concentrations of 5-CQA (chlorogenic acid) and CQAs were significantly different at the two temperatures ($P = 0.037$ and 0.023, respectively).

The values of 5-CQA ranged from 0.37 ± 0.07 mg mL^{-1} for the extraction at 22 °C to 0.28 ± 0.03 mg mL^{-1} for the extraction at 5 °C, as well as from 0.51 ± 0.08 mg mL^{-1} (extraction at 22 °C) to 0.41 ± 0.03 mg mL^{-1} (extraction at 5 °C) for the sum of CQA. The French press values were 0.39 ± 0.03 mg mL^{-1} for 5-CQA and 0.51 ± 0.12 mg mL^{-1} for the sum of other CQAs. These values were consistent with the values reported in the literature for the filter coffees.[4,8,28]

Concentrations increase with temperature, regardless of the extraction method, flow rate or contact time. Significant differences are found for several compounds; notably, there are significant interactions between extraction method and temperature for 5-pCoQA ($P = 0.0423$), 5-FQA ($P = 0.0398$) and other classes of CQL compounds. More specifically, the higher temperature increases the concentrations of these compounds using the cold drip method, whereas this is not the case for the cold brew method. Concentrations were significantly higher in drip extraction at ambient temperature. A significant interaction between time and temperature was found for only two compounds (5-pCoQA and 4-CQLs). In this case, concentrations were highest for low flow rates/contact times, at ambient temperature. Finally, significant differences related to extraction and temperature were found for di-CQA compounds.

Sensory evaluation

Aromatic components are particularly important in coffee beverages because they are the main constituents of the sensory experience of coffee drinkers. Overall, the sensory evaluation found that *lungo* coffee is a little less intense that the typical Italian coffee. Significant differences ($P < 0.05$) were found for the Extraction method with respect to overall intensity of odor, bitterness, sugar caramelization and sweet taste (Table 6).

The cold brew method was characterized by a higher intensity of sugar caramelization attribute and sweet taste, whereas the cold

Table 6. Sensory evaluation n of coffee beverages, comparing extraction method, temperature and flow rate

Extraction															
Temperature °C	Drip 22°C	Brew 22°C	Drip 5°C	Brew 5°C	Drip 22°C	Brew 22°C	Drip 5°C	Brew 5°C							French press
Flow rate	Fast	Fast	Slow	Slow	Slow	Slow	Fast	Fast	Flow rate	Temperature	Extraction	Flow rate: extraction	Flow rate temperature	Extraction: temperature	
	(mean ± SD)								p						
O – global intensity	6.471 ±.21	4.94 ± 0.42	6.78 ± 0.19	4.64 ± 0.53	7.11 ± 0.84	5.31 ± 0.80	6.96 ± 0.40	5.11 ± 1.05	NS	NS	0.0002***	NS	NS	NS	5.45 ±0.63
F – global intensity	5.57 ± 0.49	5.69 ± 0.05	4.97 ± 0.55	5.17 ± 0.29	6.04 ± 0.63	4.89 ± 0.98	5.93 ± 0.61	5.17 ± 0.29	NS	NS	NS	NS	NS	NS	3.50 ±0.55
Enzymatic	3.58 ± 0.62	3.54 ± 1.70	4.42 ± 1.21	3.83 ± 1.66	3.81 ± 0.17	3.53 ± 1.32	4.58 ± 0.98	4.03 ± 0.59	NS	NS	NS	NS	NS	NS	3.08 ±0.58
Sugar caramelization	4.33 ± 0.58	6.17 ± 1.04	4.67 ± 0.58	5.06 ± 0.48	4.94 ± 0.59	4.90 ± 1.04	5.25 ± 0.25	5.38 ± 0.67	NS	NS	0.05	NS	NS	NS	5.66 ±0.61
Distillation	3.44 ± 1.39	4.03 ± 0.63	3.42 ± 0.72	4.03 ± 0.63	4.78 ± 0.38	3.74 ± 0.65	4.20 ± 0.35	3.80 ± 0.52	NS	NS	NS	NS	NS	NS	3.25 ±0.42
Bitter	7.44 ± 0.47	5.92 ± 0.14	6.93 ± 0.32	5.83 ± 0.29	7.89 ± 0.67	5.89 ± 1.01	7.43 ± 0.40	5.83 ± 0.29	NS	NS	0.0001***	NS	NS	NS	6.33 ±0.26
Sweet	2.58 ± 1.01	3.83 ± 0.29	2.67 ± 0.58	3.75 ± 0.25	2.89 ± 0.51	3.11 ± 0.19	3.19 ± 0.17	3.58 ± 0.52	NS	NS	0.002**	NS	NS	NS	2.16 ±0.41
Sour	6.06 ± 0.48	5.78 ± 0.69	4.83 ± 0.29	5.75 ± 0.43	6.94 ± 0.53	6.90 ± 0.74	5.64 ± 0.27	5.05 ± 0.52	0.039*	0.00081***	NS	NS	0.0237*	NS	1.75 ±0.42
Astringency	4.89 ± 1.51	4.53 ± 1.14	3.89 ± 1.39	3.42 ± 0.72	4.78 ± 0.38	3.63 ± 0.55	4.15 ± 0.25	3.39 ± 0.67	NS	NS	NS	NS	NS	NS	1.25 ±0.42

ns $P > 0.05$, * $P \le 0.05$, ** $P \le 0.01$, *** $P \le 0.001$.
The mean ± SD and P-values for each of the three variables, as well as their interactions, are reported. The mean ± SD for the French press extraction used as the benchmark is in reported a separate column. NS, not significant.

Figure 3. Spider plot of sensory attributes for the Drip, Brew and French press extraction methods.

drip method was characterized by a higher overall intensity of odor and bitterness.

In the drip method, the high intensities of the bitter attribute were also confirmed by a caffeine content greater than that of the cold brew. The concentration of caffeine is known to influence the perceived strength, body and bitterness of a brewed coffee.[4,29] Similar values were also found for the French press, which showed a value of bitter intensity that was slightly lower than the cold drip but higher then the cold brew system.

No significant differences were found for enzymatic and distillation attributes.

Temperature had a particularly dominant effect on the sour taste ($P = 0.000$, $F = 27.01$). Coffee extracted at temperatures of 22 °C was evaluated in terms of an intensity sourer than that obtained at 5 °C. On the other hand, coffee obtained with a French press shows a much lower value.

Temperature increased intensity and a significant interaction was found between this and flow rate/contact time ($P = 0.024$).

Coffees extracted slowly at 22 °C were more intense than those extracted at 5 °C.

The extraction method influences the intensity of sweet taste. Indeed, the coffee extracted by cold brew method was more sweet than that obtained by the drip method, as well as by the French press method.

Intensities for the cold brew, cold drip and French press methods are shown in the spider plot in Fig. 3, which reveals clear differences in the flavor profiles of the respective extraction methods in terms of bitterness, sourness, astringency and global intensity. Particularly, the increase in bitterness and astringency is consistent with a higher concentration of caffeine and

chlorogenic acids, as reported previously by Gloess *et al.*[4] for hot extractions.

CONCLUSIONS

Two extraction methods for preparing a cold coffee have been characterized: cold drip and cold brew.

The results obtained show that differences during cold coffee preparation lead to differences in physical parameters, the concentration of chemical compounds and the sensory profiles of coffees.

Cold drip coffees were recognized as being more bitter with greater contents of caffeine and chlorogenic compounds than cold brews.

Temperature was found to increase the concentrations of several compounds. Particularly, a higher temperature increases the total solid concentration of caffeine, CQAs and 5 CQA. However, refractive index and the remaining CGAs are increased by temperature only in cold drip coffee, whereas no difference was found for cold brew coffee.

Conversely, the contact time between the coffee powder and water has a limited effect on brew characteristics.

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