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Advanced experimental and analytical study for the optimization of the Espresso Coffee extraction

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To my Family

"…I am still learning…"

(Michelangelo)

"…Learning is better than house and land…"

("She stoops to conquer" by Oliver Goldsmith)

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Abstract

This PhD dissertation is the result of three years of research work, aimed at scientifically optimizing the espresso coffee extraction, particularly through a sustainable development approach to the process of brewing. For this purpose, a broad theoretical and applied research was carried out including experimental studies with espresso coffee supplementary tools, analytical methods and mathematical simulation programs. The research project has also produced four published articles, two submitted works, now under review, and some yet unpublished results.

In the first study, supplementary devices are applied in the research investigation for demonstrating the influence of different variables on espresso coffee extraction with the objective of lowering the amount of coffee powder used. Lowering the amount of roast and ground coffee can lead, in the long run, to a more sustainable consumption, by reducing the amount of the raw material and, in the end, producing less amount of waste, while the same quality of the beverage are maintained through the use of different tools and the proper calibration of the corresponding variables.

A follow-up study developed a new analytical method for the quantification of organic acids in espresso coffee, and its outcomes were compared with data from the sensory panel evaluation. By combining these two different assessments, it is possible to predict optimal conditions for the extraction of espresso coffee. A further follow up was the analysis of high molecular compounds in the extracted samples, implemented to research the correlation between various extractions of espresso coffee with respect to carbohydrates.

In the fourth study, interdisciplinary research was implemented through a mathematical simulation program with chemical analyses on espresso coffee extraction at various parametric conditions (temperature, pressure and tamping force). This interdisciplinary approach can give a preliminary prediction of the extraction process, which further facilitates the possible applications on espresso coffee brewing.

The fifth and final study was the consequent development of new cosmetic products from spent coffee. This research carried out the characterization of bioactive molecules, extracted with water and diluted solvents such as ethanol, proving the healthy effect of spent coffee on skin.

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Background and motivation

The worldwide popularity of coffee is directly associated with its pleasant flavour and aroma, stimulating effects on psychology, and many well-known bioactive substances (Petracco, 2005).

Coffee was found and cultivated in the Arabian Peninsula, between Yemen and northeast of Ethiopia. As a matter of fact, behind the famous legend there were the effects of caffeine, which led to discover the coffee in the 13th century. The legend tells that a shepherd noticed the sudden changes of goats' behaviour after chewing some cherries from a bush. Later the cherries were roasted and used by monks in order to stay more awake during the night prayers (Smith et al., 2001). A historical fact about the spreading of the coffee plant from Africa to Asia and Latin America was then the help of wanderers. An era of early consumption of coffee started during the Ottoman Empire. During the Renaissance, the consumption arrived to the port of Venice and, from there, the coffeehouse culture began to spread all over Europe and America (Folmer, 2017).

Coffee is one of the most consumed beverages in the world; it is an important agricultural product of the international trade, and backs an enormous industry, which can be seen both as a craft and science. In 2019, the world coffee production reached up nearly to 165 million bags of coffee (60 kg each), and the world consumption have increased of approximately 2.1% with respect to 2018 (Coffee Market Report , 2019). The American countries have been the largest consumers with about 62 million bags, with the North America the major (30 million bags). Europe came after America with a consumption about 54 million of 60 kg bags. In the last year, the main production countries have been the Brazil with about 63 million followed by Vietnam (31 million), Colombia (14 million) and Indonesia (9 million). The production of arabica coffee has slightly increased from 2017 to 2018 while the robusta showed a marked increment (International Coffee Organization, 2019b). In fact, some recent projections on coffee market have revealed a reduction of arabica production but an increment of robusta for the next year, 2019-2020 (International Coffee Organization, 2019a).

The properties of green coffee vary, depending on the geographical origin and the botanical species. The main two are *coffea arabica* (Arabica coffee) and *coffea canephora* (Robusta coffee), whilst two other species with minor scale production are *coffea liberica* (Liberica coffee) and *coffea excelsa* (Excelsa coffee). In addition, the method of roasting can generate more than 1000 volatiles, and the extraction process can modify the intrinsic characteristics due to the species, producing a coffee matrix in the cup and forming the chemical profile of the beverage with its sensory attributes (Petracco, 2005). Since coffee has become one of the favourite beverages in the world, also the

method of extraction underwent many advancements in relation to the consumers' demand, origin and culture. The methods of extraction are generally portrayed by the extraction devices, and grouped together with various key parameters influencing the final in-cup flavour profile. Well-known coffee extraction methods are espresso coffee (EC) in Italy, Moka pot/Stove-top in Southern Europe, drip filter and cold brew in Northern America and Northern Europe, and boiled coffee in Turkey. These different coffee extraction methods are based on various water interaction with coffee powder, which can alter the physical-chemical nature of coffee (structure, chemistry and molecular size), requiring different extraction techniques with varied parameters: temperature, pressure, particle size, flow rate, extraction time and coffee weight (Folmer, 2017).

Although producing a cup of coffee requires in most cases a quite simple activity performed usually as a daily routine, whether in bars or at home, when it comes to delve into the physical-chemical mechanisms that are behind the organoleptic description of the coffee beverage, this is a highly complex challenge. Coffee extraction is still in fact a process under ongoing analysis. The normal assessment is generally based on the sensory evaluation results, where the extraction phase can significantly influence. From the practical point of view, producing a cup of coffee depends also on the experience and ability of baristas, who can contribute to achieving different kinds of flavours, as for instance the "classic" flavour profile of splendid Ethiopia coffee (lemon-jasmine), or the blackberry sweetness of the wonderful central Kenya coffees (Folmer, 2017). These all appraisals are based on human perception. The baristas' impetus skills and their general approach to the brewing action make them able to extract an appropriate volume and flavour of coffee, exploiting also the particular manipulative settings of the machine (temperature and pressure) and the hydraulic resistance of the roast and ground (R&G) coffee by proper grinding and tamping. All their attempts in this sense, are still under investigations by scientists (Petracco, 2005).

A broad spectrum of chemical analyses of coffee indicates that a certain group of substances, such as caffeine, melanoidins, acids, minerals and sugar, have a direct impact on human health. The concentrations of bioactive compounds were calculated by long-lasting research in coffee science. Through those estimations of concentrations, scientists can recommend daily consumptions of this coffee beverage (Crozier et al., 2012; Cruz et al., 2014; Liang et al., 2015; Ribeiro et al., 2014; Sunarharum et al., 2014).

Extraction of coffee is traditionally regarded as an art, rather than as an authentic process of engineering unit, because for centuries it has implied the use of the small pots that are available in almost every home. The development of technologies in recent times had a knock-on effect on coffee extraction tools, although the extraction principles remained the same (solid-liquid interaction). The

motivation of this PhD research project has been guided and fostered by the ambitious goal of enhancing and optimizing the extraction process with the appropriate appurtenances.

The studies carried out through this PhD research are mainly focused on the influences of particle size distribution on the extraction of EC and, therefore, on the filter baskets and the heights of perforated discs used by the espresso coffee machines. Interdisciplinary approaches with mathematical and chemical analyses were implemented, and a new analytical method for quantification of organic acids was developed. Instrumental chemical analyses and industrial devices were used to assess the experimental results throughout the research.

Industrial relevance of the research

After a decade of strong collaborations between the University of Camerino and the Simonelli Group, “The International HUB for Coffee Research and Innovation” was established in 2016. The mission of this cooperation is to apply the scientific research in industrial applications and to enhance the coffee-related industrial processes, from the production to the consumption, involving the design and creation of the machines, paying particular attention to sustainability.

The Simonelli Group is an Italian company that produces coffee machines since 1936. The vast experience, the innovative approach and the high performing products, led to the international success of the company, bringing the business in 121 countries worldwide. The Simonelli Group, known as a cultural reference for excellent espresso coffee, has established on the market two prestigious brands, Nuova Simonelli and Victoria Arduino, which together express the family heritage of the company (simonelli group, 1936).

In order to remain successful in the highly ambitious environment of coffee, it is necessary to develop sustainable products in agreement with deep research and studies, which in the long term can impact effectively on the environment in a sustainable way.

Thesis layout

This first introductory section highlights the motivation and objective of the research, and the industrial relevance of the thesis.

Chapter 1 outlines a comprehensive overview of previous studies and presents cutting-edge developments of espresso coffee extraction techniques. It provides a literature review about the phases of coffee transformation through several processes, from the green beans, through the R&G coffee, up to the extraction of EC.

Chapter 2 describes the research methodology and objectives, providing detailed information, for each case study, on the analyses and extraction of the coffee samples, as well as on the applied industrial devices to optimize the brewing. In a similar vein, interdisciplinary approaches and a new method to analyse essential bioactive substances from green beans up to spent coffee are described.

Chapter 3 discusses the results obtained from different extractions of espresso coffee obtained with various particle size distribution, filter baskets and heights of perforated discs, and the analyses of caffeine, trigonelline, nicotinic acid, chlorogenic acids and volatile compounds with HPLC-VWD and GC-MS.

Chapter 4 provides the correlation between EC with various extraction conditions and the results of sensory evaluations, analysing and discussing the role of organic acids.

Chapter 5 discusses the preliminary mathematical model results about EC extraction, by comparing the virtual model with the real extracted espresso coffee substances, which are inserted in fact into the simulation program.

Chapter 6 examines the number of polysaccharides found in espresso coffee, extracted with various alteration of the variables in the brewing process.

Chapter 7 examines the number of bioactive compounds in spent coffee and its further application for the cosmetic industry. It also describes the extraction method for caffeine, trigonelline and nicotinic acid in spent coffee.

Chapter 8 draws the conclusions of this thesis and proposes directions to carry out future work in coffee science.

Publications and presentations

Publications:

1. Spent coffee grounds: a potential commercial source of phytosterols. Kamgang Nzekoue F., Khamitova G., Angeloni S., Sempere A.N., Tao J., Maggi F., Xiao J., Sagratini G., Vittori S., Caprioli G. Journal: Food Chemistry. Published: 17 April, 2020. https://doi.org/10.1016/j.foodchem.2020.126836
2. The impact of different filter baskets, heights of perforated disc and amount of ground coffee on the extraction of organics acids and the main bioactive compounds in espresso coffee. Journal Food Research International. Published: 02 April 2020. Khamitova G. Angeloni S., Fioretti L., Ricciutelli M., Torregiani E., Sagratini.G., Vittori S., Caprioli G. https://doi.org/10.1016/j.foodres.2020.109220
3. Water flow and transport in porous media for in-silico espresso coffee. International Journal of Multiphase Flow. Published: February, 2020. Giacomini J., Khamitova G., Maponi P., Vittori S., Fioretti L. https://doi.org/10.1016/j.ijmultiphaseflow.2020.103252
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Oral presentations:

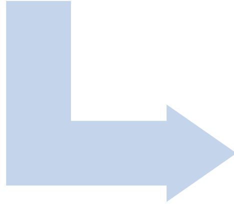
1. Extraction of espresso coffee by changing particle size distribution and evaluation of bioactive compounds through HPLC-VWD and HS-SPME/GC-MS. Gulzhan Khamitova, Simone Angeloni, Giovanni Caprioli, Lauro Fioretti, Gianni Sagratini, Sauro Vittori. 6th MS Food Day, Camerino 2019.
2. Development of a new quantification method for organic acids in espresso coffee. Gulzhan Khamitova, Giovanni Caprioli, Manuela Cortese, Massimo Ricciutelli, Gianni Sagratini, Sauro Vittori. Cibo & Nutreuceuticals, Camerino 2019.
3. Enhancing variables for espresso coffee extraction. Gulzhan Khamitova, Simone Angeloni, Giovanni Caprioli, Gianni Sagratini, Sauro Vittori. 6MSJDay, Rome, 2018.
4. Particle size dirstribution influences on espresso coffee extraction. Gulzhan Khamitova, Simone Angeloni, Giovanni Caprioli, Gianni Sagratini, Sauro Vittori. Cibo & Nutreuceuticals, Camerino 2018.
5. Effects of espresso machine variables on espresso coffee composition. “The quality of coffee: never ending research” International HUB for coffee research and innovation. Camerino, 2017.

Poster presentations:

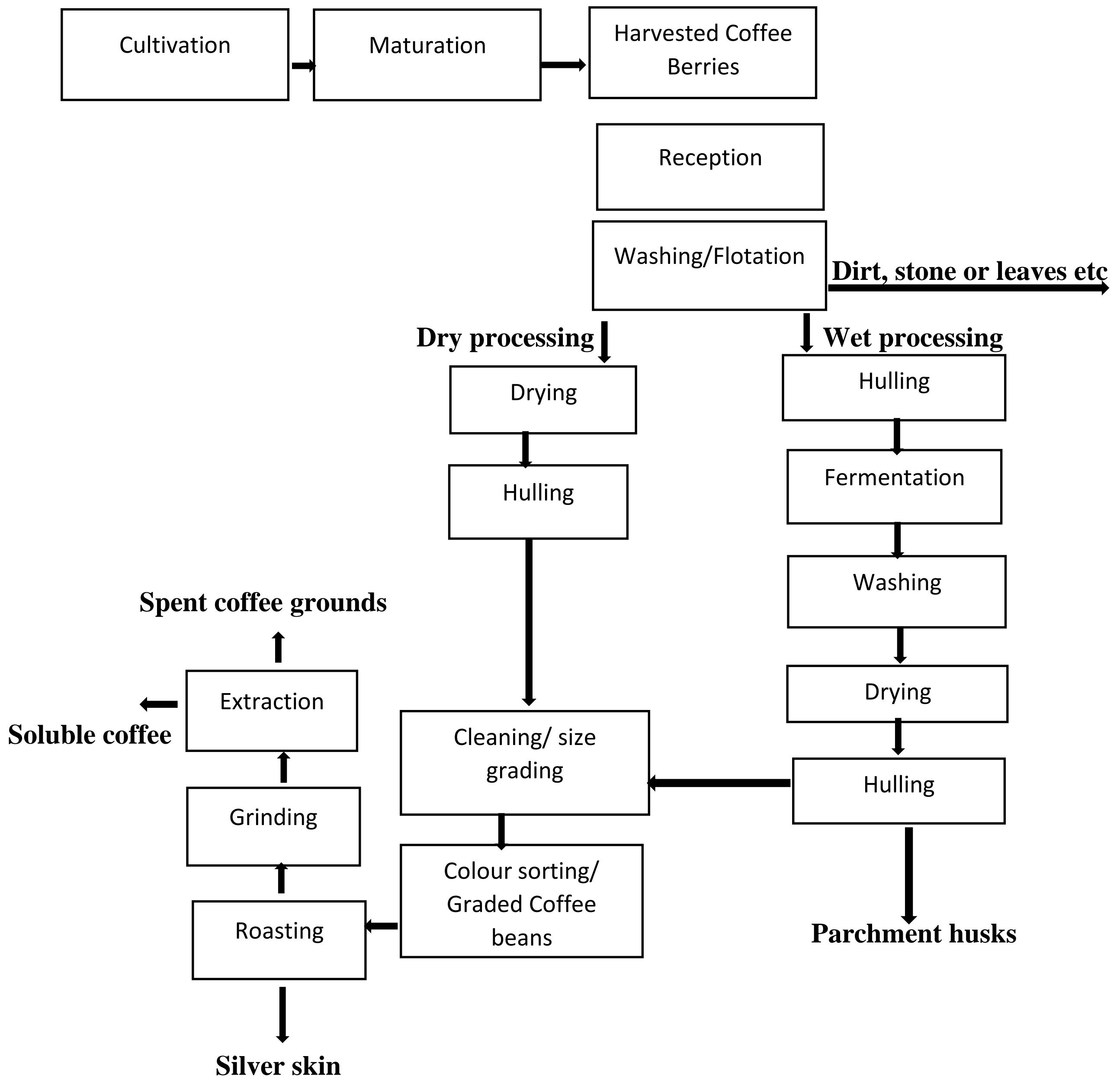
6. Reducing the use of ground coffee by decreasing the size of particles in espresso coffee extraction. Khamitova G., Angeloni S., Caprioli G., Sagratini G., Vittori S. COCOCTEA, Bremen, 2019.
7. Reducing the use of ground coffee by decreasing the size of particles in espresso coffee extraction. Gulzhan Khamitova, Simone Angeloni, Giovanni Caprioli, Gianni Sagratini, Sauro Vittori. Cibo & Nutreuceuticals, Camerino 2019.
8. Increase of bioactive compounds in espresso coffee by lowering the amount of ground coffee for extraction. Gulzhan Khamitova, Giovanni Caprioli, Manuela Cortese, Massimo Ricciutelli, Gianni Sagratini, Sauro Vittori. AiTuN conference, Camerino 2019.
9. How different particle sizes of ground coffee influence the extraction of a good espresso coffee. Gulzhan Khamitova, Simone Angeloni, Giovanni Caprioli, Gianni Sagratini, Sauro Vittori. Chimali conference, Camerino 2018.
10. Optimization of extraction variables for espresso coffee. Gulzhan Khamitova, Simone Angeloni, Giovanni Caprioli, Gianni Sagratini, Sauro Vittori. ASIC conference, Portland 2018.
11. Optimization of espresso coffee extraction with different particle size distribution and analysis through GC-MS and HPLC-VWD. XXII IMSC conference, Florence 2018.
12. Effects of espresso machine variables on espresso coffee composition. Gulzhan Khamitova, Simone Angeloni, Giovanni Caprioli, Gianni Sagratini, Sauro Vittori. 5th MS Food Day, Bologna, 2017.

CHAPTER 1.

LITERATURE REVIEW



COFFEE AND ITS PROCESSING PHASES



1.1 Green and roasted coffee

1.1.1 Coffee plant

The coffee plants belong to the *Rubiaceae* family, genus *Coffea*. More than 100 species are part of the *Coffea* genus (The Plant List 2013, 2019) but only two, i.e. *Coffea arabica* (arabica) and *Coffea canephora* Pierre ex A. Froehner (robusta) are responsible for 99% of the global coffee production (Pham, Reardon-Smith, Mushtaq, & Cockfield, 2019). The geographical origins of the *Coffea* genus is restricted to tropical humid regions of Africa and islands in the West Indian Ocean. Originally, arabica was a shrub living in the undergrowth of the forests of the southwestern Ethiopia and northern Kenya at an elevation between 1300 and 2000 m. Robusta originated from the humid lowland forests of tropical Africa (Folmer, 2017). In nature, coffee is a perennial evergreen plant. It has a prominent vertical stem with shallow root system, which in the arabica coffee penetrates relatively deeper into the soil, whereas in robusta concentrates very close to the soil surface. Coffee leaves are opposite decussate on suckers. The leaves appear shiny, wavy, and dark green in colour, with conspicuous veins. The inflorescence is a condensed cymose type subtended by bracts. Coffee is a short-day plant and, hence, the floral initiation takes place in short day conditions of 8-11 h of day light. Pollination takes place within 6 h after flowering. The fruit is a drupe, called cherry or berry, usually fleshy, containing generally two seeds (coffee beans) but sometimes only one and, in that case, the fruit assumes a rounder shape and is known as pea-berry (Ghosh et al., 2014). It varies in size but very little in shape. Its colour varies from yellow to black, though it is mostly orange to red. Seeds are elliptical or egg shaped and the seed coat is composed of a silver skin (Murthy & Madhava Naidu, 2012; Teketay, 1999).

1.1.2 Cultivation

Coffee plays an important role in the history of humanity since its discovery among the Arab peninsula. There is a widespread knowledge and passion for coffee, from cultivation to consumption. The evolution of the coffee industry has changed systems of cultivation and forms of consumption within centuries. Care of seeds has to start prior to the cultivation phase, in order to prevent future issues such as underdevelopment, phytosanitary problems, twisted tap roots and even plant death. The seedling-growth process applies different specific methods, depending on diverse factors (climate conditions, budget, technology and equipment), and in the long term it directly correlates with the profitability of the coffee production. The coffee-processing method is formed on three basic assumptions: the cost/benefit analysis of the production method, the need to adhere to environmental legislation, and the desired standard of quality of the coffee beans (Figueiredo, 2013).

1.1.3 Harvesting

Succeeding in the cultivation phase gives its fruit in a coffee tree within 4-7 years, which further allows to pass to the phase of harvesting. In this stage, essential flavours can considerably change owing to the gathering conditions applied to the coffee beans. Historically, the harvesting consisted in the manual gathering of coffee beans. With the development of technology, manual gathering of coffee beans became rarer due to cost and time. Up to the present time, both mechanical gathering and hand-picking are used methods, the choice of which one depends upon several factors (landscape, slope, coffee variety, labour cost, size of farm, distribution and cherry maturity) (Sanz-Uribe et al., 2017).

There are also other investigated harvesting methods such as stripping (pulling out all cherries from each branch), vibrating mechanical fingers (with ripe cherries falling more easily than unripe ones) and vacuum pumps to select ripe cherries from branches (but still this method has to be developed well). A new way of harvesting coffee is the *late harvest*. The principle of this practice is similar to a well-known one in the wine industry, in which grapes remain beyond the optimum ripeness and, as a consequence, wine become sweeter. Late harvesting has been mostly conducted in Gerrado region (Brazil) and in Tolima region (Colombia). The colour of cherries changes from red to dark or even black colour and, as a result, very unique and special flavour characteristics are obtained by this late harvesting method, producing sweet winey notes (Sanz-Uribe et al., 2017).

The harvesting process should be paralleled, from its very starting day, by a concurrent early processing of the green beans, in order to avoid undesired fermentation and the risk of mould contamination. There are three types of green bean processing: natural, pulped natural, and washed (Borém et al., 2014; Folmer, 2017), but in all types of processing it is important to pass through washer-separators where impurities and rocks are eliminated (Teixeira et al., 2005). The natural process is a common and simple method in which the whole coffee fruits, or unwashed coffee beans, are dried under the sun. In the wet processing method, the fruit skin (exocarp) and part of mucilage (mesocarp) are removed, and the rest of the mucilage that adheres to the parchment (endocarp) is removed by a subsequent washing, after which the bean can undergo the mechanical drying. Finally, pulped natural coffee is known as honey coffee and dried in slower process in a humid environment, where a combination of sun and mechanical drying are mostly applied (Folmer, 2017). Figure 1.1 displays a schematic overview of this first coffee processing.

a b c d

Figure 1.1 Main processing steps regarding the production and first transformation of the coffee bean: a – cultivation, b – harvesting, c – drying, d – roasting.

1.1.4 Separation and early processing methods

The separation process consists of several stages after the harvest gathering phase. This separation is complex because the harvesting brings in mixed cherries (fresh, ripe, over-ripe, semi-dry and dry). Initially, the coffee berry needs to pass the phases of winnowing, and then separation. These phases can help to dry the coffee berries and avoid the risk of quality loss caused by fungi and mycotoxins. The essential phase to remove light impurities, i.e. leaves, sticks or debris from the fruit is winnowing. The winnowing can be applied manually in small companies or mechanically in large companies. The following process is the separation phase: this is normally carried out through hydraulic separator with special sieves. In this phase, the contaminants are removed, the coffee berries are washed, and then separated according to their density (the low-density berry is known as *floaters* due to dry and overripe condition, while the dense berry is riper). After that, separated ripe berries are further divided by electronic separator, where coffee berries can be collected according to their colours by optical readers. After passing these phases, the coffee beans undergo processing methods that make cherries dry and homogenous. In any case, all these processing methods depend on several factors such as climate, consumers' demand and the availability of water treatment technologies (Illy et al., 2009).

1.1.5 Dry (natural) processing method

In the dry method, known as natural processing, the whole cherries (bean, mucilage and pulp) are dried on rocks under the sun or in mechanical dryers. By this method, the coffee cherries could generate strong "body" and pleasant "aroma". Moreover, this processing method has less environmental impact, with less wastewater and organic wastes (Borém et al., 2014). It is necessary to regulate the bed depth (under 40mm) and cherries should be regularly combed or raked in order to dry homogenously and to avoid unwanted fermentation (Ghosh & Venkatachalapathy, 2014), (A. Illy et al., 2009). Disadvantages of this processing method can be dust sediments or unexpected rainstorms that can damage the crop. The dried cherries are normally kept at certain conditions where moisture level should not exceed more than 12%. Sometimes, after the drying phase, cherries undergo the hulling and cleaning phases. At this point, the pericarp of cherries is hulled. The process of hulling is carried out mechanically with an air flow system. After the hulling phase, green coffee beans are

separated by their size, defects and density, which is the cleaning phase. This can be conducted manually or mechanically depending upon the farm size (Ghosh et al., 2014; Illy et al., 2009).

1.1.6 Washed (wet) processing method

The washed processing method is known as wet method, and is generally carried out in equatorial territories where the coffee harvesting season comes along with the regular raining season. The essential part of this method is the presence of fresh water for coffee processing. The wet processing requires a raw material composed of only ripe cherries that are handpicked thoroughly or separated mechanically in the process itself. Before fermentation, the coffee cherries should pass the pulping process, undergoing the removal of the mucilage from the parchment. Then, the containers with coffee cherries should remain in ambient temperature in the presence of microorganisms for 12-36 hours. The fermentation finishes when the parchment loses the slimy peel of the mucilage. The removal of mucilage is carried out mechanically by friction, and modern mucilage removers are advanced in terms of environmental pollution reduction (Illy et al., 2009).

1.1.7 Pulped natural processing method

The pulped natural processing is between natural and washed processes. In this process, the coffee cherries are pulped and beans in parchment dried with its mucilage. The remain mucilage is not removed by fermentation or other manners but is dried intact with the parchment coffee. The resulting coffee is called honey coffee for its resemblance with dry honey and caramel. Normally, pulped natural processing generates coffee flavours with stronger “body” than the washed method. In the preparation of pulped natural coffee, the pulp and mucilage can be used on the farm as a fertilizer (Illy et al., 2009).

1.1.8 Semi-washed processing method

The semi-washed process is quite similar to the washed processing method. The difference consists in avoiding the removal of the mucilage by fermentation. Instead, the mucilage removal is conducted mechanically. The advantages of the semi-washed processing method are the avoidance of using fermentation tanks and the reduction of the total amount of wastewater (Borém et al., 2014). The coffee beans after passing at processing method should be dried well (up to 10-12% of the moisture content). It is necessary to dry coffee beans immediately to avoid the development of unwanted flavour. The dried products will be then passed to the hulling and cleaning phases like in the natural method (Ghosh et al., 2014). Previous studies confirmed that post-harvesting methods have a direct influence on the final quality of the coffee beverage (de Melo Pereira et al., 2019; Gonzalez-Rios et al., 2007b; Joët et al., 2010). Among the different steps of coffee processing, microbial mucilage removal has great influence on the volatile composition and can affect the quality of the beverage (de Melo Pereira et al., 2019).

1.2 Coffee roasting

Roasting is a treatment with dry heat, with chemical and physical transformations occurring in the coffee beans during the process. While roasting, the raising temperature generates vast chemical reactions, dehydration and deep alterations of the microstructure. As a consequence of this operation, the green bean converts a hard texture into a porous and brittle texture, weak aroma turns into intense pleasant aroma, green colour transforms into a dark colour. Roasting develops the required changes to make coffee ready for the following grinding and extraction (Schenker et al., 2017).
The roasting is a complex process that requires heat and mass transfer superposed by endothermic and exothermic reactions. The main chemical processes involved in this thermic treatment are the Strecker degradation, the Maillard reaction and pyrolysis reactions (Fadai, et al., 2017; Flament, 2002). The application of heat to the coffee beans not only produces a temperature field, but also effects on the inner pressure and the re-circulation of moisture. The roasting equipment temperature can rise up to 160-250^{0}C depending on the desired roasting profile, and normally the process lasts between 8 to 20 minutes (Bonnlander et al., 2005). Accurate and small temperature gradients allow to achieve a homogenous roasting profile. The homogeneous distribution of temperature can be divided into these two main steps: the drying phase (20-130^{0}C), in which the green colour of beans changes into light yellow-brown, and the chemical reactions phase (130-250^{0}C), where Maillard reaction starts. At this point, sugar caramelization occurs in coffee beans – generating important flavour and aroma, involving polysaccharides, proteins, chlorogenic acid, and trigonelline – and begins to form the compounds responsible for the colour. In this stage, CO2 is released as a product of the reaction, contributing to the matrix expansion (Bottazzi et al., 2012; Bustos-Vanegas et al., 2018). Roasted beans have to be cooled immediately by water quenching or cold air, as soon as the desired degree of roasting is reached. Various industrial technologies are available in industrial operations of roasting, based on specific mechanisms of heating, such as hot air, infrared, microwave, superheated steam and others. Among them, the air system technology is widely used. Roasting machines operate by constantly moving the coffee beans inside the roasting chamber to deliver homogeneous heat transfer from the hot air to the coffee. Another heating mechanism occurs when heat is transferred from the hot walls of the roasting chamber to the beans. For large scale operation it is necessary to control the entire process. In this regard, normally, big roasting machines are equipped with sophisticated process control systems, which assure to set up and control the roasting profile (Folmer, 2017).

The constituents of coffee bean can be divided in volatile and non-volatile compounds. These vary from Arabica to Robusta cultivars, and from green to roasted coffee, where the latter features a higher number of them, depending on the roasting temperatures and on the grinding size, from coarse to fine. Figure 1.2 presents the group of chemical families of non-volatile species, highlighting the difference between the green and roasted Arabica coffee.

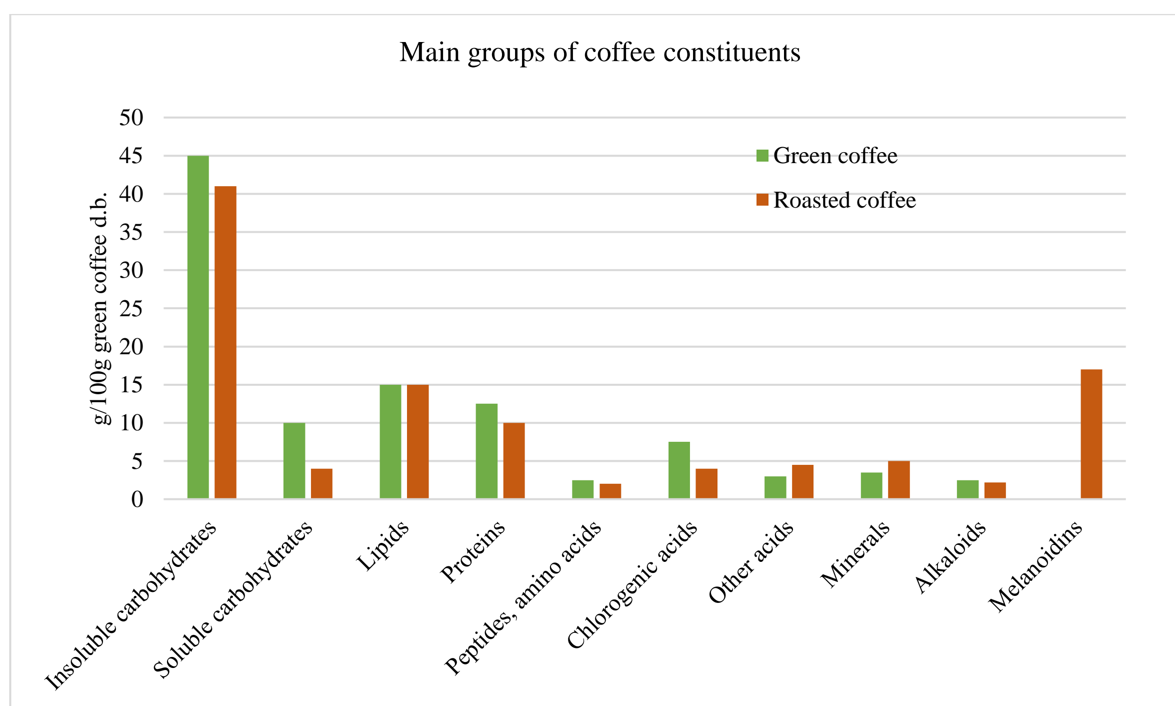


Figure 1.2 Typical composition of green and roasted Arabica coffee beans (Bonnlander et al. 2005)

As far as volatiles in roasted coffee are concerned, more than 800 volatile compounds are identified as aroma compounds, belonging to a wide range of chemical families. All these compounds are responsible for the flavour profile of the beverage, although the most significant influence on the perceived aroma profile is due to only 25-30 of them. These compounds are generally grouped in function of their characteristic flavour notes, such as caramel/chocolate, nutty/buttery, smoky/tobacco, fruity/flower or spicy. Since aroma is the result of a complex system of interactions and reactions, the full picture of the chemical mechanisms is not well studied yet. Another significant compound is carbon dioxide, formed during the roasting process in the Maillard and Strecker reactions. Although part of the generated gases is released from the beans while roasting, the vast majority could still remain in the beans. Carbon dioxide and other volatile compounds are able to rise the internal pressure of the bean, which can expand and eventually crack. The release of carbon dioxide from roasted beans passes a degassing process, which lasts around 4-14 hours depending on

the size of the bean. The remained part of the gases could leave during the grinding processes (Anderson et al, 2003). Degassing process is necessary in order to eliminate swelling of the package or explosion (Wang et al., 2014). The remained carbon dioxide in the bean might possibly interact with water penetration in the coffee particles during the brewing, eventually forming the foam (crema) on the surface of espresso coffee.

Regarding the non-volatile compounds, the main chemical changes in the roasting process can be confirmed by the significant production of melanoidins. Melanoidins are polymeric products of the Maillard reaction, which is the browning reaction between carbohydrates and constituents with a free amino group. These groups are identified as macromolecular and normally occur in all the heat-processed foods such as coffee, cocoa, malt and beer (Bekedam, 2008). Their concentrations in coffee beverages is estimated from the difference between the total dissolved solids and the mass of quantified compounds. The melanoidins are essential for their antioxidant capacity and their bind aroma molecules (Hofmann et al., 2001).

During the roasting, the coffee beans change their structure at macro and micro level. At the macrostructural level, the bean density can reduce from 1100-1300 to 500-700 kg/m3. This can be explained through the moisture, aroma and CO2 released and defined as roasting loss, while the bean volume expands (Geiger et al., 2002). At the microstructural level, the expansion of the bean volume and the mass loss results in the alteration of the bean porosity and pore size distribution. The roasting time determines the evolution of the coffee bean microstructure. For instance, a porosity of the green bean of 0.10 was altered after 5 minutes of roasting to 0.36 (Frisullo et al., 2012). Therefore, the combination of roasting time with temperature changes substantially the microstructure of the beans. Higher temperature at shorter times resulted in greater porosity and median pore sizes (0.528 and 13.45nm, respectively), if compared to lower temperature and longer times (0.474 and 11.22nm, respectively) (Schenker et al., 2000).

1.3 Coffee grinding and influence of particle sizes on EC extraction

Grinding is the process that converts the roasted beans into smaller particles and increases the surface area per unit of volume. This process can reduce the size of the bean structure from several millimetres up to few hundred microns due to the brittleness stemming from the roasting stage. The size of ground coffee can be obtained with different grinding techniques. Approximate size ranges from 100-150 μm for Turkish coffee, to 200-500 μm for Espresso coffee, 500-1000 μm for filtered coffee, up to 1000 μm for French press. To get the desired sizes of particles during the operation, grinding devices play a crucial role. Well-known devices are disc grinders and roller grinders. Disc grinders are

commonly designed for household and coffee shop uses at small scale, while roller grinders are for the industrial level. The particle size reduction can be achieved by the impact of blades at high speed (disc grinders) and the forces exerted by two rotating rollers (roller grinders) when the beans pass through these discs.

A certain desired flavour of the beverage could be reached by changing the particle size; therefore, an important part of the extraction process is actually to control in advance the grinding settings. Smaller particle size, with greater total surface area in contact with water, features a slower but more efficient extraction process (in terms of extracted compounds). In the case of larger particle size, the effect on the extraction process could be opposite.

A single particle of roasted and ground coffee can be defined by the characteristic dimension χ, which corresponds to the mesh size. During the grinding of coffee beans, the process generates various sizes and shapes. These ranges of size determine the particle size distribution (PSD), which is estimated with an accuracy of two to four decimal digits. PSD is an important feature for the kinetics of extraction and the hydrodynamics of coffee packed beds (Petracco, 1991 and 2005).

Commonly, PSD is defined with reference to the standard sizes χ_{10}, χ_{50} and χ_{90}, which are the characteristic dimensions or mesh sizes below 10%, 50% and 90% of all particles detected (Blittersdorff et al., 2017). In order to evaluate the effectiveness of the grinding machines, some classical testing can be done, such as the pinch of powder (assessing the coarseness). It is evident that size of ground coffee cannot be examined with much precision in this classical testing. However, there are several instruments developed throughout centuries. One of the well-known and earliest method is sifting. Putting the powder into a stack with graded-mesh sieves and shake until it passes to the following stacks. To measure the size of particles, several instruments are available, such as sieves (manual, vibrational, air and water flow), image analyses, laser and optical diffraction particle size analysers (Illy et al., 2009). The use of sieves has been applied for a long time, the process of sieving being simple. Particles can pass incrementally through the holes in sieve plates, with smaller particles passing before than larger ones. However, the sieving method alone cannot provide an accurate estimation, as it lacks a double-check of the quality of the final product. This method also contains another drawback that relates to the tendency of the particles of the coffee powder to easily agglomerate (Blittersdorff et al., 2017).

With the enhancement of new technology, the particle size analyses and estimation have been eased by laser diffraction (dry or wet method), broadly used in various fields, coffee science included. The main advantage of laser diffraction is the velocity in providing the whole particle size distribution of the samples. Laser diffraction is a non-destructive particle sizing method based on the Mie theory,

which describes the scattering of light by particles that constitute a region with a refractive index differing from the refractive index of its surroundings. Coffee particles pass through a beam producing a scattering that disperses an amount of light. The larger particles scatter the light intensely at a narrow angle in contrast to the smaller particles that scatter more widely at lower intensities. The limitation of laser diffraction remains the inability to distinguish between dispersed particles and agglomerates (Blittersdorff et al., 2017).

The target point of grinding is to generate a proper surface area in order to extract a good coffee. Through the contemporary development of technologies brought by various grinding machines, not only new developed burrs but also temperature control helps to avoid agglomeration, and sensor screen with balance can adjust the weight of grinded coffee in the portafilter. There is a variety of coffee grinders in the market.

The grinding process is an essential step for transforming the inedible coffee beans into coffee powder, namely, roast and ground coffee, ready for brewing. The grinding consists in converting roasted beans through the application of mechanical forces into roast and ground coffee, with the aim at increasing the specific extraction surface, or better to increase the extent of the interface between water and coffee. In this manner, the extraction of soluble and emulsifiable substances is facilitated and, at the same time, the rupture of coffee bean tissues and cells accelerates the release of carbon dioxide (CO_2) gas and volatile aroma, especially highly volatile compounds, and allows an easier extraction of the remaining aroma (Akiyama et al., 2003; Andueza et al., 2003; Folmer, 2017). Generally, the grinding process is empirically optimized by setting a specific distance between the grinding tools, to produce an adequate average of particle size for the desired coffee preparation. In fact, several studies have reported that particle sizes have an influence in coffee preparation, especially in espresso coffee (Andueza et al., 2003; Derossi et al., 2018; Kuhn et al., 2017; Severini et al., 2015; Severini et al., 2018).

In particular, Andueza et al. (2003) studied in detail the influence of grinding and torrefacto roasting on the chemical and sensorial characteristics of espresso coffee. They found that total solids (TS), caffeine, trigonelline and chlorogenic acids increased inversely with particle size but not significant differences on sensory attributes were reported. Instead, Severini et al., (2015) demonstrated that extraction time and grinding level significantly affect the overall aromatic profiles of EC, which was measured by using an electronic nose system. Another work (Kuhn et al., 2017a) showed that particle size significantly affects the extraction kinetics and confirmed that the smaller particles lead to a higher extracted amount of caffeine and trigonelline. Therefore, Derossi et al. (2018) reported that the use of different particle sizes for Turkish, American and espresso coffee brewing has the major impact on espresso extraction and in particular on pH changes of the beverage. Although studies

about the influence of PSD on EC extraction are reported, a lack of investigations occurs for other variables, i.e. filter basket, amount of roast and ground coffee and perforated disc, which influence the extraction process too. The filter basket is the conical support, drilled on the bottom, where the coffee powder is placed and tamped (coffee cake). The perforated disc is a metal plate, assembled under the shower of each serving group, which adjusts the distance between the coffee cake and the shower and assures an adequate empty space for cake swelling. To the best of our knowledge, only one paper studied the influence of filter basket and different particle sizes on espresso extraction. Specifically, this work investigated the effects of 1-cup and 2-cup filter basket on EC extraction at three grinding sizes (Severini et al., 2016). They found higher content of total solids and caffeine, in the first 8 s of coffee percolation per each range of particle sizes, when 2-cups filter was installed in the machine.

1.4 Extraction methods and techniques

The general increase of consumption in the globalized contemporary society has generated diverse demands on the quality of food products, and coffee has also witnessed new and growing consumers' requests. Nowadays, with this increasing demand for high-quality food products, coffee should be considered, and sometimes it is considered in fact, a high-quality artisanal food, like wine. This aspect has been strongly developed with the advent of specialty coffee, also called gourmet coffee, that coincided with both an increase in urban growth and the regular appearance of affordable luxuries (Lannigan, 2019).

Extraction is widely known as the process of interaction between two phases. The interaction of solid (ground coffee) and liquid (water) with certain methods and techniques can modify the physical-chemical characterization, chemical composition and sensory properties of the coffee beverage. Depending on the method, brewing techniques can be classified in various ways. At present, a number of extraction methods are available. They are diverse with regard to cultural habits and personal preferences. The effectiveness of an extraction technique could be defined by a balanced body, equilibrated aroma, taste and long-lasting mouthfeel (Folmer, 2017). The sensory attributes of coffee beverage normally correlates with quality identification, which directly depends on extraction yield and concentration of total soluble solids (Gloess et al., 2013). Up to now, a wide variety of preparation are determined, as for instance Turkish coffee, drip coffee, French press, espresso coffee, Moka pot, cold brew, etc, which are characterized by extraction tools and can also be grouped by various key parameters influencing the final flavour profile, such as pressure, brewing time, tamping and other criteria (Folmer, 2017).

Turkish coffee is brewed with fine particles of grinded coffee beans by pouring water in a pot. As soon as the water boils, the heating process ceases, and coffee is served in small ceramic cups along with its precipitants. This develops a strong coffee with a layer of foam on the top and sediments that settle on the bottom of the cup. Another well-known extraction is drip coffee; it could be called filter coffee or V60. The technique of this drip coffee is to pour the hot water on the course grinded coffee powder. The water passes by swallowing the particles (this process is known as *blooming*) into coffee by gravity, and the powder remains into a holder containing a filtering device. The water can be delivered manually or by automatic drip filter machine. This method produces a coffee milder than the Turkish one, with enhanced acidity and flavour. Coarse particles are also used for French press. The differences in this extraction technique are that the water and grinded coffee should be placed into a French press pot (*cafetière*). Other names are coffee press or coffee plunger, and the permeation process performs within 2-5 mins, the more the time the more the strength of the coffee. In that device, the role of plunger is to separate the liquid phase from the solid phase by a filter. The drawback of metal-mesh filtration is less separation of precipitants than the drip coffee. Another well-known and still popular brewer in household usage is moka pot. The moka pot is highly used in Southern Europe (Italy, Spain, Portugal and Greece). The design of moka consists of three chambers; the bottom chamber is filled with water, the middle chamber contains the coffee powder, and through boiling the hot water with steam passes the middle chamber by extracting the soluble substances from the coffee bed, and the extraction accumulates in the upper chamber. The air-vapor pressure generated in the bottom chamber drives the extraction process. (Caprioli et al., 2015; Folmer, 2017; Gloess et al., 2013; Sunarharum et al., 2014). Another example of technique that has experienced a recent surge in popularity is cold brew coffee. The cold brew consists in preparing coffee with cold water, usually at room temperature or lower, over a period longer than in the other coffee preparation methods. In fact, the brewing time ranges from 8 to 24 h. The main differences with the other techniques are the extraction temperature and brewing time. Temperature often significantly influences the aqueous solubility of compounds; hence, brewing temperatures significantly modify the composition of hot and cold brews. In addition, the longer brewing times of cold brew coffee may affect the content of numerous substances (Fuller et al., 2017; Lane et al., 2017). The most popular method that exploits the pressured and hot water for preparing a pleasant and short beverage is the espresso coffee.

1.5 Espresso coffee

The espresso coffee is the most commonly consumed beverage in southern Europe and is significantly becoming popular all around the world (Caprioli et al., 2015). "Espresso is a brew obtained by percolation of hot water under pressure through tamped/compacted roasted ground coffee, where the energy of the water pressure is spent within the cake" (Illy et al., 2009). This beverage is originally found in Italy and the meaning of the word *espresso* in Italian language defines *rapid*, which truly associates with espresso coffee that demands fast preparation and service after the consumer's order. The consumption culture of espresso coffee also confirms the extraction approach of this beverage, which is based on short percolation time under hot water and pressure. The extraction of espresso coffee has been broadly (Illy et al., 2009, Caprioli et al., 2013, Severini et al., 2018) and the outcomes of studies determined these following optimal criteria: water temperature (90 ± 5 °C), water pressure (9 ± 2 bar) and percolation time (30 ± 5 s), with preferred volume (15ml to 50ml according to the consumer's request) and dense crema (foam) on the surface of the beverage. These criteria can be varied according to preference and culture.

However, there are certain protocols and certifications for the espresso coffee extraction. In Italy, to follow the preparation of a Certified Italian EC, the beverage needs to perform strict production specifications. These specifications are issued by the Italian Espresso National Institute and approved by a Third-Party Body, protected and promoted through a product certification (certificate of product conformity Csqa n. 214: 24 September 1999, DTP 008 Ed.1). There are certain conditions to be followed for the production of the Certified Italian Espresso: roast and ground coffee: 7 ± 0.5 g, exit temperature of water from the unit 90 ± 2 °C, temperature of the drink in the cup 67 ± 3 °C, entry water pressure 9 ± 1 bar, percolation time 25 ± 2.5 s, viscosity at 45 °C > 1.5 mPa, total fat > 2 mg/ml, caffeine < 100 mg/cup, volume in the cup (including foam) 25 ± 2.5 ml (Caprioli et al., 2015; Odello et al., 2006).

Today, cutting-edge technologies are widely developed for the extraction of EC. The espresso machine is an essential instrument not only for the extraction of EC, but also for other types of coffee beverages with various modifications. In the market, numerous espresso coffee machines are commercially available, ranging from compact light ones for home use to professional computerized units with advanced design and technologies; all of them consist of three main parts: the pump, the extraction chamber and the heat exchanger. The extraction process starts as soon as the pump delivers water through to heat exchanger, then the water with the desired temperature reaches the extraction chamber through the heat exchanger. The extraction chamber consists in an upper block where a filter-holder cup is inserted. The coffee powder is tamped in the filter basket, which is in turn in the

filter-holder. After that, water passes through the perforated disc and the shower, which evenly spreads the liquid onto the surface of the coffee cake. As soon as water spreads on the coffee cake, a pre-infusion process (wetting and swelling) occurs in the first seconds. This allows the coffee surface to reach the required permeability (Caprioli et al., 2015), and then the extraction phase starts.

The process of EC extraction is very complex, as it is composed of different phases and mechanisms that involve different technological passages before having the beverage delivered into the cup. It is evident that the assessment of the beverage relies on the quality achieved in the cup, which directly correlates with the percolation stage. Percolation occurs when fluid flows through a porous medium. This percolation materializes in diverse features in daily life: a well-known example is when rainwater percolates through sand in the soil (Illy et al., 2009).

In hydrodynamics, the percolation process in the extraction of EC could be described by the following fluid-dynamics components: the fluid flow within the porous matrix, the dynamics of the dissolved compounds and the extraction of chemical substances by hot fluid from porous matrix, and the heat exchange between the fluid and the porous matrix (Vafai, 2015). This hydrodynamical phase influences the solubility of chemical substances that develops the flavour of the beverage. Hence, several phenomena occur, i.e. the dissolution of aqueous soluble compounds, the forced extraction of some less soluble compounds and physically entrapped molecules (e.g., arabinogalactans), the degradation reactions due to the heating that can affect the solubility of many substances (e.g., galactomannans), the migration of fine particles and coffee oil through the water flow, etc. (Chen et al., 2012).

Despite the influence of operational parameters on the extraction of EC, there are other variables such as the amount of R&G coffee, PSD, filter basket, perforated disc, water quality, percolation time, cake porosity, which can play each an essential role in the coffee extraction (Illy et al., 2009). Several studies have been trying to find the influence of water temperature, water pressure and particle sizes on the EC in cup (Andueza et al., 2002; Andueza et al., 2003; Caprioli et al., 2014, 2013; Derossi et al., 2018; Kuhn et al., 2017; Salamanca et al., 2017; Severini et al., 2015; Severini et al., 2018). Understanding the importance of those variables in the extraction of EC will help to implement a sustainable way of brewing a good quality of EC. Heretofore, to the best of our knowledge, none has investigated the possibility to reach a good quality of EC by lowering the amount of R&G coffee. Still, in fact, the mass of R&G coffee is empirically chosen on the base of filter basket design and other conditions determined from the experience. Therefore, the extraction of EC with different filter baskets could have an impact on coffee quality, though it has not been implemented yet. Drawing upon the extraction variables effects on the quality of EC, one of our objectives has been to investigate the quality of EC prepared by changing particle sizes, perforated disk heights and filter baskets, with the aim of lowering the amount of ground coffee used.

1.6 Bioactive compounds in coffee

Bioactive compounds are molecules produced by plants that preserves pharmacology and/or toxicology effects in human and animals. They are usually produced as secondary metabolites; vitamins are not part of the term "bioactive plant compounds". The secondary metabolites are substances derived from a side pathway with respect to the primary biosynthetic and metabolic routes of the most important compounds for the plant growth and development (sugars, carbohydrates, amino acids, proteins and lipids). Several aspects and criteria, i.e. biological effects, botanical categorization based on family and genera of plants, chemical classes, and biochemical pathways, are used for bioactive compounds classification (Bernhoft, 2010).

In the evaluation of the biochemical pathways and the chemical classes, coffee contains the following group of main bioactive compounds: alkaloids (caffeine and trigonelline), derivatives of phenolic acids (chlorogenic acids), diterpenoids (cafestol and kahweol), flavonoids (isoflavones) and phenylpropanoids (lignans). Cafestol and kahweol are the two most abundant unsaponifiable pentacyclic diterpene alcohols of the kaurene family found in coffee oil (Rafael et al., 2010). Diterpenes are present in the most important coffee species (arabica and robusta), but are more abundant in *Coffea arabica*; another two substances of the family, i.e. 16-O-methylcafestol and 16-O-methylkahweol, are specifically found in robusta coffee; and kahweol is reported to be specific in arabica. Therefore, the differences on diterpene contents can be exploited for coffee species identification (Illy et al., 2009; Novaes et al., 2019; Rafael et al., 2010). These molecules are extensively studied also for their association with health issues. For example, cafestol and kahweol can induce the degradation of toxic compounds and provide hepatoprotective effects against some toxicants, such as aflatoxin B1 and acrolein. Other studies reported the antioxidant, anti-inflammatory and anticarcinogenic activities of these lipids (Preedy, 2014).

1.6.1 Alkaloids: caffeine and trigonelline

Alkaloids are a class of bioactive organic compounds which contain nitrogen atoms defined by IUPAC as basic nitrogen compounds (mostly heterocyclic), mainly present in the plant kingdom. Some nitrogen-containing natural compounds such as amino acids, peptides, proteins, nucleotides, nucleic acids, amino sugars and antibiotics are not alkaloids. By extension, certain neutral compounds biogenetically related to basic alkaloids are included (McNaught & Wilkinson, 2014). Caffeine is a secondary metabolite of the alkaloid family, belonging to the purine alkaloid group. The purine alkaloids derive from purine nucleotides and are widespread in plant kingdom as they are found in nearly 100 species. The methylxanthines, e.g. caffeine (1,3,7-trimethylxanthine) and theobromine (3,7-dimethylxanthine), together with methyl uric acids, such as liberine and methylliberine, are the

most common purine alkaloids (Ashihara et al., 2008). Caffeine is the most well-known compound in coffee, and it is one of the most widely consumed active food ingredient in the world. It is present in beverages like coffee, tea, soft drinks, energy drinks as well as in products containing cocoa, chocolate and in a variety of dietary supplements and medications. The attractiveness and recognition of this molecule is due to the effect that has on the body and mind. In fact, caffeine stimulates the central nervous system aiding to stay awake and improving mental alertness after fatigue (Heckman et al., 2010). Moreover, other properties are attributed to caffeine, such as raising the blood pressure as a result of increases in total peripheral resistance, relaxation of bronchial muscle, increase of gastric acid secretion and diuresis, etc. (Caprioli et al., 2014; Grosso et al., 2017). It is known that robusta coffee contains an higher amount of caffeine than arabica; in fact, the levels of caffeine in green beans are respectively from 1.6 to 2.4% and from 0.9 to 1.2% of the dry weights (Illy et al., 2009; Preedy, 2014). During the roasting process, the content of caffeine does not dramatically change, but it has been reported that dark roasted beans can contain lower caffeine amount than light roasted beans (Hečimović et al., 2011). On the other hand, trigonelline level is intensely affected by thermal processes; after roasting, the content of this molecule noticeably decreases, due to a series of degradation mechanisms, which form volatile compounds responsible for the formation of flavour, and non-volatile molecules. (Casal et al., 2000; Illy et al., 2009). Trigonelline is a pyridine alkaloid, firstly isolated from *Trigonella foenum-graecum* L., which has been found in many plant and animal species (Zheng et al., 2004). In green beans, this pyridine is present in higher concentration in arabica (1-1.2% of dry weights) than robusta (0.6-0.8% of dry weights); during the roasting process, trigonelline can decompose via two major routes, i.e. decarboxylation and methyl rearrangement, to provide pyridines, and N-demethylation to give nicotinic acid. Trigonelline is also degraded by decarboxylation, generating the *N*-methylpyridinium cation, inductor of enzyme systems involved in detoxification of xenobiotics, activator of the Nrf2/ARE pathway, inducing cellular defence mechanisms, and novel phytoestrogen (Jeszka-Skowron et al., 2014; Stadler et al., 2002). In coffee beverages, the levels of caffeine, trigonelline and other bioactive compounds, such as chlorogenic acids, are influenced by the process used for the coffee preparation (Gloess et al., 2013) and, mainly in espresso coffee, by the chosen variables for the extraction, such as temperature and pressure of water, particle sizes, ratio of water and powder, etc. (Andueza et al., 2002; Caprioli et al., 2014; Severini et al., 2015; Severini et al., 2018). The analytical methods used for caffeine and trigonelline quantification are characterized by several extraction procedures and different analytical instruments chosen and optimized according to target matrix (green beans, espresso coffee…). A common quantitative approach is characterized by water extraction and quantitative analysis by high-performance liquid chromatography coupled with diode-array detection or mass spectrometry

(HPLC-DAD or HPLC-MS) (Caprioli et al., 2014; Casal et al., 2000; Jeszka-Skowron et al., 2014; Perrone et al., 2008).

1.6.2 Acids

Acids are important organoleptic parameters in coffee beverages. The formation of organic acids starts with the maturation phase of the green bean, continue through the post-harvest treatment, and depends on the roasting and brewing method too, which can modify in fact the acidity in the final brew (Avelino et al., 2005). In general, type of acids in coffee and coffee brews can be classified by these groups: aliphatic carboxylic acids, some alicyclic acids, and heterocyclic acids (Everaerts, 1983). The presence of acetic, malic, formic, citric and lactic acids, as well as quinic and chlorogenic acids, has been detected in the brew. A mineral acid (phosphoric) is also found in the brew (Illy et al., 2009). The major acids in high concentrations are present in green coffee beans, while during the roasting they decline or derivate.

The term flavour stands for the sum of taste and aroma, and the role of acidity is essential. The major organic compounds contain acidity by nature through their ionisable carboxylic groups (or any phenolic hydroxy groups). Therefore, the acidity is analysed through the hydrogen ion concentration (pH), which is based on the dissociation degree of a known acid present in an aqueous solution of acid or mixture of acids of a given concentration. The undissociated molecules of the acids could also impact on a specific flavour, by influencing the aroma through their volatility. There are various effects of undissociated molecules, together with hydrogen ions, on the taste and flavour that are perceived through the organoleptic mechanisms of the human body, in particular through mouth, tongue and nose all the way up to the brain by the nerve system. According to the perception of complex tangible taste (acid, bitter, salt, sweet or umami) and aroma (volatile compounds) sensations, the sensory evaluation is defined as a complex testing to carry out. Generally, sensory evaluators are required to pass through several training sessions in order to get a qualification. Considering the complex definition of flavour, the perceived acidity is recognized in coffee brews as an important attribute. The coffee beverages with light roasted coffee have “fine” acidity which “clean” the palate, while dark roasted coffee have little or almost no acidity, because the prevalent mouthfeel is in fact bitterness. The majority of aliphatic acids play a significant role for the development of the coffee flavours; quinic acid, for instance, can enhance the perception of acidity in foodstuffs just with a low concentration not exceeding 1% of the total acids (Everaerts, 1983; Illy et al., 2009).

1.6.3 Derivates of phenolic acids: chlorogenic acids

Phenolic compounds abundantly present in coffee, in particular the chlorogenic acids (CGAs), play a crucial role both in green and R&G coffee. CGAs are a large group of compounds derived from the esterification between certain *trans*-cinnamic acids, phenolic compounds, and (-)-quinic acid. CGAs

do not contain chlorine despite the "chloro" prefix. The historical nomenclature of chlorogenic acid in coffee was started in 1844 (Rochleder, 1844). The scientist observed that in green beans lead salts could precipitate caffeine with an acid, and that for instance the precipitation with sulphuric acid gave a yellow colour after addition of ammonia; then, the yellow solution turned green on exposure to oxygen. He proposed an empirical formula of $C_{16}H_9O_8$ for the free acid. Later, another scientist (Payen, 1846) reported the isolation of crystalline potassium caffeine chloroginate that formed some 3.5-5% of green coffee beans. These studies probably include the first usage of the term "chlorogenic acid" describing the production of green pigment on alkaline oxidation (Cllfford, 1985). This is most probably because of their green colour after oxidation reactions (Kremr et al., 2016). Chlorogenic acids are found in several plants; coffee is a rich source of these conjugated compounds. They can be formed by the binding of three different *trans*-phenyl-3-propenoic acids (cinnamic acids), varying in their ring substitution, with quinic acid. The widespread *trans*-cinnamic acids are caffeic (3,4-dihydroxycinnamic acid); ferulic (3-methoxy, 4-hydroxy), sinapic (3,5-dimethoxy, 4-hydroxy) and *p*-coumaric (4-hydroxy). CGAs can be classified by the identity, number and position of the acyl residues in the following subgroups: the relatively common mono-esters of caffeic acid (caffeoylquinic acids or CQA), *p*-coumaric acid (*p*-coumaroylquinic acids or *p*CoQA) and ferulic acid (feruloylquinic acids or FQA); di-esters (diCQA), tri-esters (triCQA) and the single tetra-ester of caffeic acid (tetraCQA); mixed di-esters of caffeic and ferulic acid (caffeoylferuloylquinic acids or CFQA), which are characteristic of robusta coffee and other minor groups (Clifford, 2000). Almost all the total CGAs in coffee is represented by mono-acyl (CQA, FQA and *p*CoQA) and di-acyl (di-caffeoylquinic acids and caffeoylferuloylquinic acids) compounds with at least three isomers per class (De Rosso et al., 2018). In green beans, the content of chlorogenic acids is higher in robusta than arabica; the presence ranges from 7 to 10% (robusta) and from 5.5 to 8% (arabica) of dry weights (Preedy, 2014). In the roasting process, these molecules chemically alter and generate the aroma compounds (Moon et al., 2010; Müller et al., 2006). In the dehydrating phase, there is the formation of chlorogenic lactones (CGL), associated with bitter taste (Farah et al., 2006; Farah et al., 2005). The widespread individual chlorogenic acid is 5-*O*-caffeoyiquinic acid (5-CQA), which sometimes is still called 3-*O*-caffeoylquinic acid (3-CQA) or just chlorogenic acid. This is because the IUPAC assigned the correct rules for nomenclature in 1976 and the current 5-CQA was in fact 3-CQA. The use of these two historical names should be discouraged (Clifford, 2000; Kremr et al., 2016). Some studies reported that chlorogenic acids perform antioxidant, anti-inflammatory and anticarcinogenic activities; numerous epidemiologic studies have analyzed these properties (Baeza et al., 2014; Liang & Kitts, 2015; Rocha et al., 2012; Sato et al., 2011). They can have positive effects on type-2 diabetes, obesity, Alzheimer's disease, stroke, endothelial function and blood pressure (Tajik et al., 2017). Likewise, the coffee beverages is the richest source of CGAs in human diet: the daily intake of CGAs

in modest and heavy coffee drinkers ranges from 0.1 to 2 g (De Rosso et al., 2018). Many variables could impact on the levels of chlorogenic acids in the cup of coffee, such as the extraction methods, the degree of roasting, and the type of coffee blend (Fujioka et al., 2008; Gloess et al., 2013; Tfouni et al., 2014). Numerous studies on quantitative and qualitative analysis of CGAs in dietary sources were carried out on HPLC-UV-VIS and HPLC-MS (Caprioli et al., 2013; Craig et al., 2016, Farah, 2019).

1.6.4 Carbohydrates

Carbohydrates are an important class of bioactive compounds in coffee. They are divided in water-soluble and water-insoluble carbohydrates. The presence of carbohydrates in green beans are about 40-65%. The composition is complex with various mono-, poly- and oligosaccharides (flament, 2002). The water-soluble species are divided in two groups, i.e. high molecular weight (HMW) and low molecular weight material (LMW). Since the carbohydrate's composition is complex, the quantifications are more sophisticated and outcomes of analyses are hard to compare. In fact, their composition is modified by the processes of decaffeination of green coffee beans, roasting and even more the extraction of beverage (Lopes et al., 2016; Lopes et al., 2019; Moreira et al., 2017; Nunes et al., 1998, 2007a, 2007b).

1.6.4.1 Water-soluble LMW and HMW

The water-soluble LMW compounds contain important flavour constituents such as sugar, trigonelline, CGA, mono- and disaccharides. They are the main responsible groups, particularly LMW, for the formation of aroma classes due to the Maillard reaction and caramelization process. Disaccharides are composed of glucose and fructose, the most abundant in green coffee with 8% in Arabica and 3-6% in Robusta. During the roasting, the concentration of glucose and fructose increases at the initial stage because of the sucrose degradation phase. All free sugars are degraded to carbon dioxide, water, colour, aroma and taste on roasting phase due to the Maillard reaction and caramelization (Illy et al., 2009).

The water-soluble HMW polysaccharides are galactomannans and arabinogalactans. In the green coffee, arabinogalactans are covalently linked to proteins and form arabinogalactan proteins (AGPs). During the roasting, the structure of AGPs modifies and releases a free arabinose that is essential sugar precursor. Hence, the arabinose effects on the formation of melanoidins, and the arabinose residues of arabinogalactans could impact on acid formation (formic and acetic acid) (Esquivel et al., 2012).

1.6.4.2 Water-insoluble HMW

The water-insoluble HMW consist of polymeric compounds. These polysaccharides are normally found in thick, dense coffee cell wall complex, consisting of three polymers, i.e. mannans,

hemicellulose and cellulose. The content of these polymers is lower in Robusta than in Arabica coffee. The most abundant polysaccharides in green coffee is galactomannans (nearly 19% of its mass). They play the role of a dietary fiber complex and the content of dietary fiber in coffee brews ranges from 0.14g to 0.65g per 100ml depending on the type of coffee, roasting, grinding and brewing procedure (Farah, 2019; Folmer, 2017). In addition, several polysaccharides are identified after hydrolysis by their monosaccharide units. Quantified units as D-mannose, D-galactose, D-glucose and L-arabinose are found in green coffee with ratios 6:2:2:1 by using 10% potassium hydroxide (Flament, 2002).

1.6. 4.3 Melanoidins

Melanoidins are heterogenous, brown-coloured, nitrogen-containing, HMW products formed during the roasting of coffee (Maillard reaction). The general reaction model is:

Reducing sugar + amine + heat → *N*-substituted glycosylamine (Schiff base) + water

This reaction is responsible for the formation of hundreds of chemical compounds, which give the flavour of coffee (Dorsey et al., 2017). In coffee brew the presence of melanoidins accounts for more than 25% of the total solids. The amount of melanoidins in a human diet with consumption of coffee varies between 0.5 to 2.0 g per day depending on consumers (Illy, 2002; Petracco, 2008; Sanchez et al., 2012; Sunarharum et al., 2014).

1.7 Coffee aroma. Volatile compounds in coffee

Aroma is an important flavour characteristic not only in roasted coffee, but also in the coffee beverage. In particular, espresso coffee generates the most intense aroma. Flavour is a complex sensation which describes the combination of aroma, taste, mouthfeel and texture. The aroma or odour, in some bibliographic resources is not considered the most important component of the coffee flavour (Sunarharum et al., 2014). However, other studies have investigated the aroma fraction of coffee beans and coffee beverages (Bicchi et al., 1997; Blank et al., 1991; Caprioli et al., 2012; Czerny et al., 2000; Risticevic et al., 2008).

The characteristics of coffee aroma and taste derive from a complex combination of physical and chemical changes during the roasting. More than 1,000 volatile compounds, produced by coffee roasting, have been identified, but just 20-30 compounds play an important role for coffee aroma and could be responsible for the overall coffee odour (Blank et al., 1991; Czerny et al., 1999; Czerny et al., 2000; Semmelroch et al., 1996). The coffee quality and its aroma can vary owing to some factors such as species and plant cultivation, harvesting and processing methods, roasting, grinding and preparation techniques (Blank et al., 1991; Gonzalez-Rios et al., 2007a; Sanz et al., 2002; Sunarharum et al., 2014). The aroma of green coffee is similar to green, hay-, pea-like and the taste is astringent

and sweet (Folmer, 2017); as soon as coffee beans pass the roasting process, the aroma and taste become more pleasant (Czerny et al., 1999; Czerny et al., 2000). The roasting process is described through the Maillard reaction, which comprehends several chemical reactions occur between sugars and amino acids. Then, Strecker degradation takes place, sugar and minor lipid degrade and, meanwhile, intermediate decomposition products interact to each other, further leading to an increment of volatile compounds (Buffo et al., 2004; Caprioli et al., 2015; Sunarharum et al., 2014). The aroma-impact compounds belong to various chemical classes such as thiols, sulphides, aldehydes, pyrazines, phenols, furanones and dicarbonyls (Folmer, 2017). From a quantitative point of view, furans and pyrazines are the most important volatile classes, while qualitatively, sulfur-containing compounds and pyrazines are the most relevant (Sunarharum et al., 2014).

Furans are ubiquitous compounds found in thermally processed foods and their importance has been rapidly boosted since 1995, when they have been classified as "possibly carcinogenic to humans" (group 2B) from the International Agency for Research on Cancer (IARC) (Rahn et al., 2019; IARC (Ed.), 1995). These compounds possess sensory thresholds that are relatively high compared to other groups of coffee volatiles, but they are present in high concentrations and therefore are relevant for coffee aroma quality (Sunarharum et al., 2014).

Pyrazines are an important group of compounds, which is found in different foodstuff including coffee. Several alkyl pyrazines, such as 3-isopropyl-2-methoxypyrazine, 3-isobutyl-2-methoxypyrazine, 2-ethyl-3,5-dimethylpyrazine, and 2,3-diethyl-5-methylpyrazine, characterized as nutty, earthy, roasty, green aromas, are reported to be potent odorants of coffee aroma (Blank et al., 1991; Grosch, 1998).

2-Furfurylthiol is a well-known sulphur-containing compound present in coffee with a roasted, pungent and coffee-like aroma. It possesses one of the highest Odour Activity Values (OAV) and a low level of odour threshold. Therefore, it is considered a key aroma compound of coffee brew (W. Grosch, 1998; Semmelroch & Grosch, 1996; Sunarharum et al., 2014).

Phenolic compounds also play a key role in coffee aroma, such as guaiacol, 4-vinylguaiacol, 4-ethylguaiacol and vanillin. These phenols originate from thermal degradation of chlorogenic acids providing a flavour discrimination between arabica and robusta, as they contain significantly inequal contents of CGA (Sunarharum et al., 2014). Different techniques are developed to analyse volatile compounds in coffee. One of the most recently adopted is the headspace solid phase microextraction (HS-SPME) (Bressanello et al., 2017a; Caprioli et al., 2012; Mondello et al., 2005; Risticevic et al., 2008), a rapid preparation technique that extracts volatile analytes prior to analyse them, for example through GC-MS. The choice of a SPME fiber is dependent on the specific physico-chemical characteristics of the target solutes to be extracted (Mondello et al., 2005), and for coffee it is commonly used the Divinyl-benzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS)

(Bressanello et al., 2017a; Caprioli et al., 2012; Risticevic et al., 2008). Another approach is to characterize the aroma of coffee by the Aroma Extract Dilution Analysis (AEDA) and, as analytical instrument, to exploit a gas chromatographer-olfactometer (GC-O). This technique, coupled with an olfactometric instrument, which commonly possesses another detector (e.g., flame ionization detector (FID), MS), permits to assign an odour description for each studied compound and measures the potency of odorants by calculating the Flavour Dilution (FD) factor (Werner Grosch, 1994). These analyses are part of a more complex concept called sensomic approach that led to improve the knowledge about key aroma compounds in coffee (Blank et al., 1991; M Czerny et al., 1999).

1.8 Coffee by-products as source of bioactive compounds

1.8.1 Coffee by-products. Coffee husks and pulp

Coffee is one of the most important agricultural commodities in the world. However, its processing generates significant amounts of by-products whose content is rich in bioactive compounds. Coffee by-products are a major concern among producing and consuming countries for environmental, social and economic reasons. Considering these great concerns, various implementations as recycling, biogas production, animal feed, more recent by extraction of value-added fractions based on bioactive compounds, are utilized (Cruz et al., 2014). For this reason, in the latest years, several authors have proposed original mechanisms to reuse the coffee by-products in order to manage and reduce its disposal (Hijosa-Valsero et al., 2018; Machado et al., 2018; Martinez-Saez et al., 2014; Mussatto et al., 2011; Narita et al., 2014; Scully et al., 2016).

Coffee husk is the outer skin of berries. Coffee husk contains high number of bioactive compounds: carbohydrates (35-85%), soluble fibers (30.8%), minerals (3-11%) and proteins (5-11%); also, insoluble dietary fiber such as tannins (5-9%) and cyanidins (20%). This valuable chemical content of coffee husk, has a great potential to be utilized as a food ingredient rich in natural nutrients and bioactive compounds (Iriondo-DeHond et al., 2019). Coffee pulp is the main residue that is obtained from the wet or semi-wet processing. Coffee cherries contain nearly 50% of cherry pulp, and its presence in dry matter (DM) reaches up to 30%. Coffee pulp also contains a high amount of bioactive compounds: carbohydrates (50%), protein (20%), fibers (20%), fat (2.5%), caffeine (1.3%) and phenolic compounds (Cantergiani et al., 2017).

1.8.2 Coffee silverskin

Coffee silverskin (CS) is the major residue that is obtained as a by-product of the roasting process. It is a thin tegument that directly covers coffee seeds; during roasting, coffee beans expand and this thin layer is detached (Bessada et al., 2018). Although CS accounts for only a minimal fraction of the whole coffee cherry (1-2%), it contains high level of dietary fiber, bioactive compounds and features

antioxidant activities (Janissen et al., 2018; Mussatto et al., 2011). That is why, in recent years, some authors proposed the use of CS as raw material for the recovery of functional compounds of potential interest. Indeed, CS is a rich source of soluble and insoluble dietary fibers (3.7 and 64%, respectively), which can be used for food enrichment (Iriondo-DeHond et al., 2019). Moreover, recent studies have evidenced that CS is a valuable source of bioactive compounds such as melanoidins, caffeine and polyphenols, which allow potential applications of CS extracts as functional ingredient in cosmetic and nutraceutical formulations (Bertolino et al., 2019; Bessada et al., 2018). Other authors have suggested to apply this coffee residue as feedstock in biofuel production (Hijosa-Valsero et al., 2018), as adsorbent material to remove potential toxic metals in water (Malara et al., 2018), as a source of cellulose for paper production (Mussatto et al., 2011), and as an ingredient to be exploit in food industry. Indeed, Martinez-Saez et al. (2014) have proposed the use of CS for a novel beverage production aimed at body weight control. Recapitulating, several studies have reported the nutritional composition of coffee silverskin and the content of bioactive compounds such as caffeine, chlorogenic acids, melanoidins and other polyphenols (Janissen et al., 2018; Narita et al., 2014; Toschi et al., 2014).

1.8.3 Spent coffee

The solid residue after consumption of coffee beverages is described as spent coffee. The daily consumption of million cups per day around the world produces enormous amount of spent coffee. However, the spent coffee also contains a number of bioactive compounds: carbohydrates, proteins and phenolic compounds. Considering the health and environmental benefits of bioactive compounds in spent coffee, several analytical methods have been applied to extract those bioactive compounds: for instance, solid-liquid extraction or spray drying to extract antioxidant phenolic compounds (Mussatto et al., 2011), and microwave superheated water extraction to extract carbohydrates (Passos et al., 2014; Passos et al., 2019a). The fields of usage of the bioactive compounds from spent coffee are diverse. However, several industrial applications have been developed for particular fields such as energy, nutraceutical, material production and pharmaceuticals (McNutt & He, 2019a).

CHAPTER 2.

RESEARCH DESCRIPTION AND OBJECTIVES

The research work is developed with a close collaboration between industry and university for the enhancement and optimization of the extraction of espresso coffee. The industrial performance was carried out together with Simonelli Group S.p.A, one of the founders of the "International Hub for Coffee Research and Innovation" (Coffee HUB). A preliminary optimization of espresso coffee extraction was implemented by modifying variables of the espresso machine, aiming at producing an espresso coffee of good quality, lowering the amount of coffee powder used for obtaining the espresso. The variables under study were the particle size distribution (PSD) of R&G coffee, the design of the filter baskets and the height of the perforated disc. For this purpose, specific particle size distribution (200-1000 μm) of R&G coffee in three different designed filter baskets (A, B and C) were used to prepare espresso coffee, utilizing standard and lower amount of powder for a double EC extraction (14 and 12 g, respectively). Moreover, various heights of perforated disc (4-7 mm) were assembled into the machine, and espresso coffee was extracted with 14 g and 12 g. The EC quality was investigated from a chemical point of view by studying and comparing the content of some well-known compounds in coffee, brewed at different conditions. In particular, chemical studies concerned the total solids (TS) analysis and the quantification of caffeine, derivatives of chlorogenic acids and trigonelline (chemical structures shown in Figure 2.1). In addition, some analyses on the volatile fraction were performed.

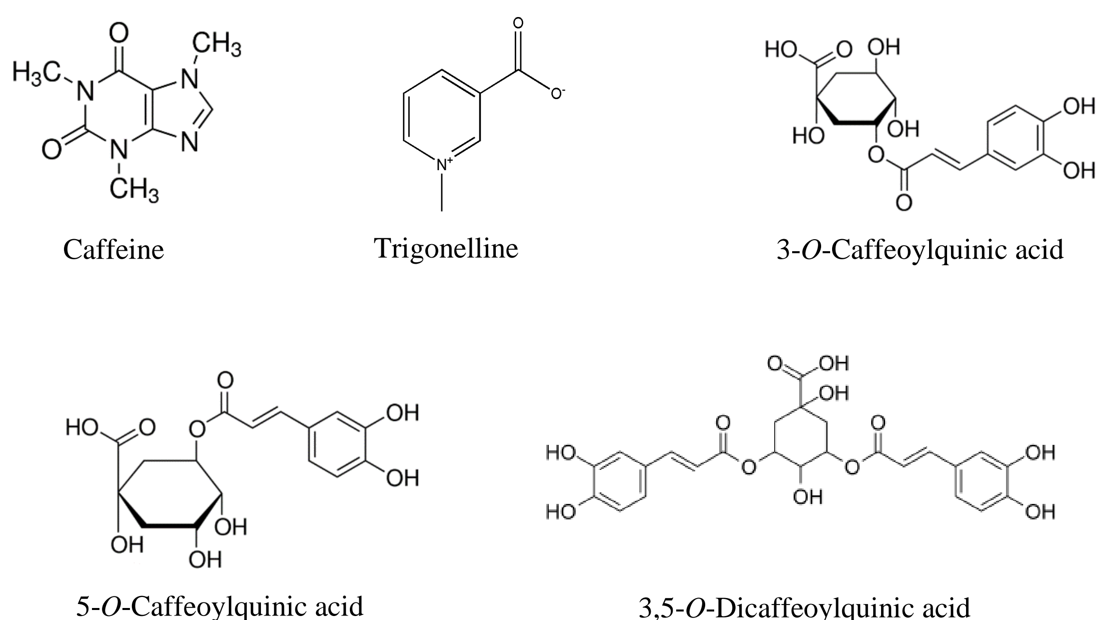


Figure 2.1. Chemical structures of target molecules quantified in ECs.

Following these supplementary tools and variables in the espresso machine (PSD and heights of perforated disc), the low molecular organic acids had been quantified with a new developed method by high performance liquid chromatography - variable wavelength detector (HPLC-VWD). Then, the outcomes were compared with the sensory evaluation results. Various heights of perforated disc (4-7 mm) were assembled into the machine and espresso coffee was extracted with 14 g and 12 g by using two filter baskets (named as A and B). Detailed chemical studies concerned the total solids (TS) analysis and the quantification of acetic acid, caffeine, caffeic acid, citric acid, chlorogenic acid, malic acid, nicotinic acid, tartaric acid and trigonelline (chemical structures shown in Figure 2.2).

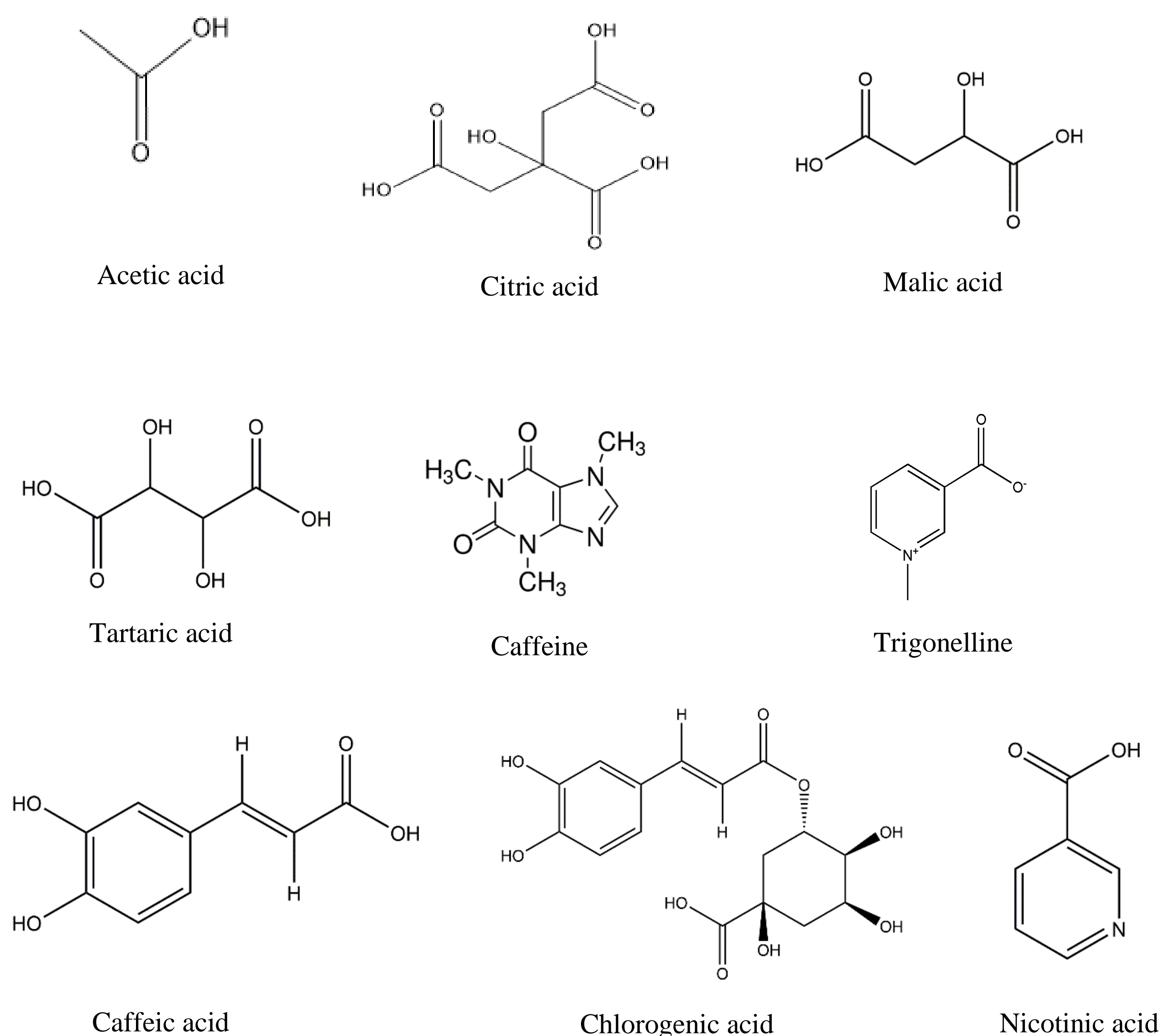


Figure 2.2. Chemical structures of compounds quantified in ECs.

This research work therefore intends to determine the physicochemical details of the extraction of espresso coffee by a numerical study applied through a mathematical model. An important component of this research is an in-depth knowledge of the physical-chemical processes occurring during the espresso coffee preparation. The extraction of espresso coffee was carried out under different

conditions such as four tamping forces (10kgF, 15kgF, 20kgF and 30kgF), different pressure (7 bar, 9 bar and 11 bar) and temperature (88C, 93C, and 98C). The research studies used a mathematical model for describing the percolation processes occurring during the espresso coffee extraction by exploiting fluid-dynamics components. The validity of this water percolation model into porous media is demonstrated on an experimental basis.

In addition, those espresso coffee samples were used to analyse high molecular weight compounds and in particular an alteration of carbohydrates. The experimental part was conducted at the Department of Chemistry in the University of Aveiro (Portugal) under the supervision of Prof. Manuel A. Coimbra. The characterization of carbohydrates was carried out through gas chromatography-flame ionization detector (GC-FID), and by comparing the mono- and polysaccharides found in espresso coffee samples with reference compounds by internal standard, using capillary columns. More in detail, chemical studies on carbohydrates, in particular the quantification of rhamnose, arabinose, mannose, galactose and glucose (chemical structures shown in Figure 2.3) were carried out.

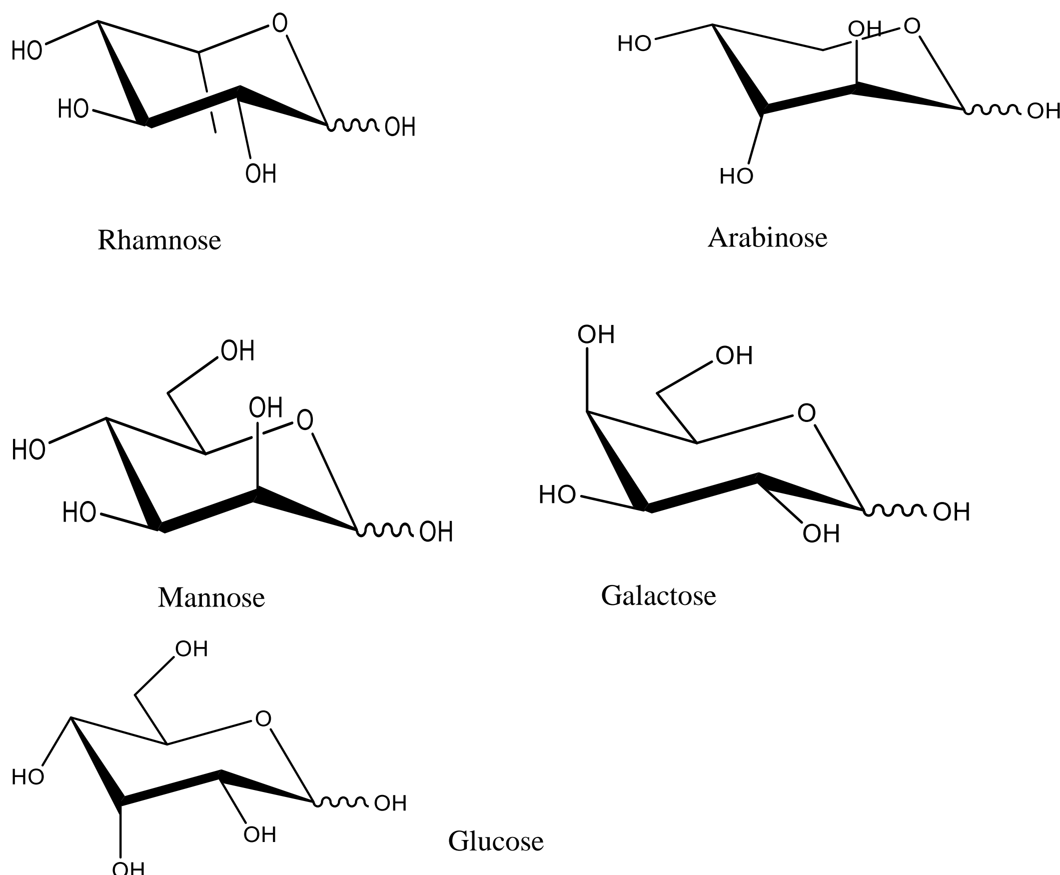
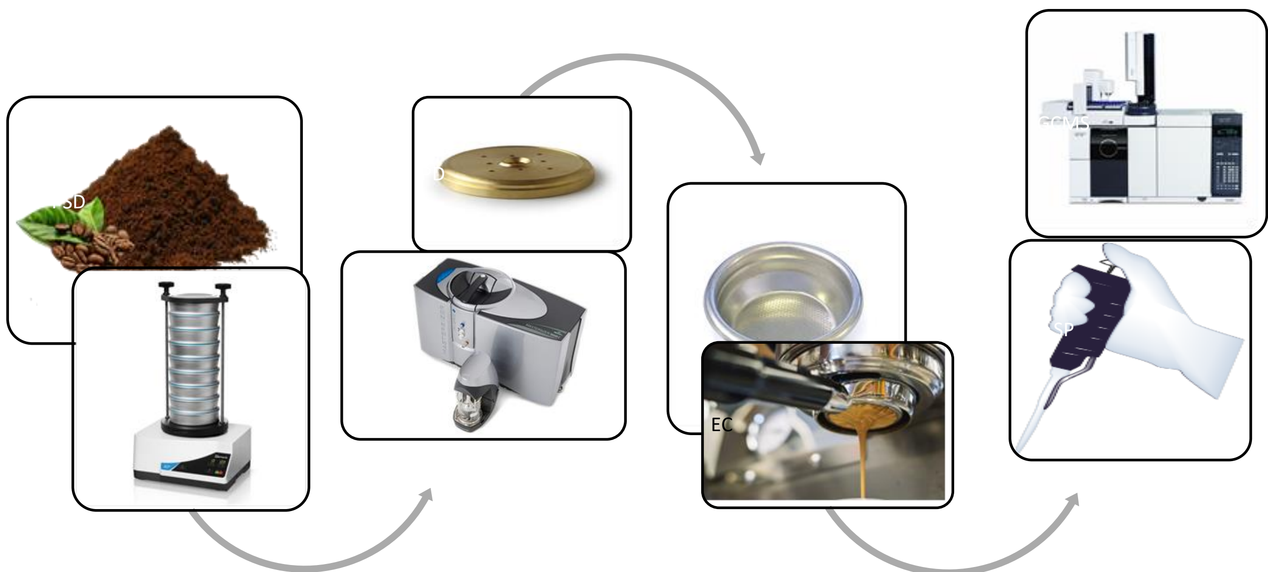


Figure 2.3. Chemical structures of compounds quantified in ECs.

CHAPTER 3.

STUDY 1. OPTIMIZATION OF ESPRESSO COFFEE EXTRACTION BY LOWERING THE AMOUNT OF GROUND COFFEE

In this study, the extraction of espresso coffee is investigated through the implementation of various supplementary tools and the alteration of some relevant variables. A reduction in the amount of roast and ground coffee during the extraction process was conducted. As a result, an increase of bioactive compounds had occurred at finer particle sizes.



3.1 Extraction of espresso coffee through variation of particle sizes, filter basket and perforated disc

3.1.1 Introduction

Extracting EC with tantalizing aroma and rich mouthfeel requires certain optimal conditions with regard to the roasted coffee, temperature and water pressure, the amount of grinded coffee, the tamping pressure and the impetus skills of barista (Blittersdorff et al., 2017; Corrochano et al., 2015). EC extraction has in fact great influence on trigeminal sensation (e.g., cooling, hot and tingling). Many different techniques for EC extraction and optimal parameters of brewing has been studied in depth (Andueza et al., 2002; Caprioli et al., 2012; Illy et al., 2011; Kuhn et al., 2017a; Parenti et al., 2014; Severini et al., 2015; Vittori et al., 2014; Wellinger et al., 2017). A sweet, clean, and intense taste and aroma in the cup is also dependent to a great extent on different procedures and protocols, and in particular on the grinding process, which can generate the best out of the bean, producing very different types of ground coffee. Some studies on the coffee extraction show that different brewing methods (e.g. Turkish, French, or filtered coffee extraction) demand certain particle size distribution (PSD), preferably with similar average of the particle sizes, and this is also true for the EC extraction (C. Severini et al., 2015). Due to the short extraction time of EC, beans grinded for espresso should contain at least a certain percentage of fines to achieve enough pressure in the coffee cake, as well as to produce a body and delicate cream (Labbe et al., 2016a). However, researchers have not studied yet in depth how different tools can be adjusted complementarily in coffee extraction, and how different filter baskets could be chosen according to the grinding burr that produces different particle size of ground coffee. The present study is developed through three case studies in order to investigate grinded beans at specific particle sizes (from 200 µm to 1000 µm) in three variously designed filter baskets (Figure 3.1), and to compare the concentration of bioactive compounds while decreasing the amount of ground coffee of a double EC extraction (14-12 g).

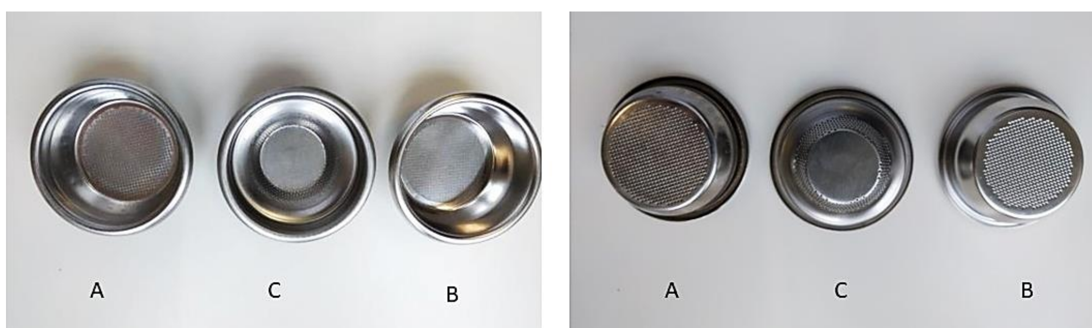


Figure 3.1. Filter baskets

3.1.2 Materials and Standards

Standards of caffeine, trigonelline, 5-*O*-caffeoylquinic acid (5-CQA), 3-*O*-caffeoylquinic acid (3-CQA) and 3,5-di-*O*-caffeoylquinic acid (3,5-diCQA) were purchased from Sigma-Aldrich (Milano, Italy). Divinylbenzene-carboxen-polydimethylsiloxane (DVB-CAR-PDMS) Stable Flex fiber of 50/30 μm was acquired (Supelco, USA) and used for the HS-SPME. HPLC-grade methanol was supplied by Sigma-Aldrich (Milano, Italy) and HPLC-grade formic acid (99%) was supplied by Merck (Darmstadt, Germany). Individual stock solutions of caffeine, trigonelline, 5-CQA, 3-CQA and 3,5-diCQA were prepared by dissolving 10 mg of each compound in 10 ml of methanol (HPLC-grade, 99.9%; Sigma-Aldrich, Milano, Italy) and stored in glass-stoppered bottles at 4°C. Standard working solutions, at various concentrations, were daily prepared by appropriate dilution of aliquots of the stock solutions in methanol. Deionized water (> 8 MΩ cm resistivity) was obtained from the Milli-Q SP Reagent Water System (Millipore, Bedford, MA). All solvents and solutions were filtered through a 0.45 μm polyamide filter from Sartorius Stedim (Goettingen, Germany) before use. Before HPLC analysis, all samples were filtered with Phenex™ RC 4 mm 0.45 μm syringeless filter, Phenomenex (Castelmaggiore, BO, Italy).

3.1.3 Total solids (TS)

TS were measured by following a developed procedure (Caprioli et al., 2012; Parenti et al., 2014), with some modifications. Briefly, 1 ml of espresso coffee was collected and oven-dried until constant weight was reached (12 h, 100 ± 2 °C). TS was defined as ratio between dry coffee residue and the volume of EC (w/v) expressed in mg mL^{-1}.

3.1.4 Analysis of caffeine, chlorogenic acids and trigonelline

The analysis of caffeine, trigonelline and chlorogenic acids were performed following previous developed methods (Caprioli et al., 2014, 2013). 1 mL of espresso coffee was diluted 50 times in mobile phase and an aliquot of supernatant was collected and centrifuged at 13300 rpm for 10 min. Before HPLC-variable wavelength detector (VWD) injection, the sample was filtered using a 0.45 μm syringeless filter. For caffeine and trigonelline analysis the analytical column was a Gemini C18 110 Å (250 x 3 mm I.D., 5 μm, Phenomenex, Cheshire, U.K.). The mobile phase was composed of water with 0.3% of formic acid (A) and methanol (B). The flow rate was 0.4 mL min^{-1} with this gradient elution: 0 min, 25% B; 0–10 min, 60% B; 10–15 min, 60% B; 15–20 min, 25% B; and B was kept constant until the end of the run (25 min). The injection volume was 10 μL and HPLC-VWD experiments were carried out using a Hewlett Packard (Palo Alto, CA, USA) HP-1090 Series II, made of an autosampler and a binary solvent pump, equipped with a variable wavelength detector (VWD). HPLC-VWD analyses were performed at two different wavelengths in the same run: 265 nm

for trigonelline and 270 nm for caffeine. The quantification of chlorogenic acids, such as 3-*O*-caffeoylquinic acid (3-CQA), 5-*O*-caffeoylquinic acid (5-CQA) and 3,5-*O*-dicaffeoylquinic acid (3,5-diCQA), was carried out through the same instrument but using as analytical column a Polar-RP 80 Å (150 x 4.6 mm I.D., 4 µm) from Phenomenex (Chesire, U.K.). The mobile phase was composed of water (A) and methanol (B), both containing 0.1% of formic acid and the flow rate was 1 mL min^{-1}. The solvent composition varied from 0-5.5 min: 25% B (v/v); 5.5-8 min: 50% B (v/v); 8-13.5 min: 50% B (v/v); 13.5-18 min: 25% B (v/v). The injection volume was 5 µL. HPLC-VWD analyses were performed monitoring 325 nm for all chlorogenic acids.
In this study, total solid analyses and the quantification of caffeine, trigonelline, nicotinic acid and chlorogenic acids on HPLC-VWD were carried out in the samples of each case study.

3.1.5 Validation of HPLC-VWD methods

The validation parameters studied were linearity, limit of detection (LOD), limit of quantification (LOQ), repeatability and specificity (Caprioli et al., 2014, 2013). Linearity was tested by injecting five different concentrations (from 10 to 250 mg L^{-1}) of the three chlorogenic acids, caffeine and trigonelline, in triplicate, then plotting and calculating calibration curves with the respective determination coefficients (R^2). All target molecules showed good linearity, since the R^2 equalled or exceeded 0.9972. The LODs and LOQs of target compounds (estimated in matrix and expressed in mg L^{-1}) were calculated through signal to noise ratio (SNR) of 3:1 and 10:1. For chlorogenic acids LODs and LOQs were in the range of 0.06-0.08 mg L^{-1} and 0.18-0.24 mg L^{-1}. Method repeatability was tested by injecting 5 replicates for each standard concentration over the course of five days. The intra-day repeatability or run-to-run precision and inter-day repeatability or day-to-day precision were expressed by Relative Standard Deviation (RSD) percent. The RSD ranged from 0.85 to 2.86% for run-to-run precision and from 1.25 to 3.79% for day-to-day precision. The method specificity was evaluated by measuring retention time stability for each molecule. The retention time stability was studied 5 times over a period of 5 days (n=25) and expressed by RSD. RSDs were in all cases lower than or equal to 1.68%.

CASE STUDY I.

3.2 Particle size distribution and filter baskets

Particle size of ground coffee plays a crucial role in EC extraction. In general, particle size of grinded coffee is divided in three big categories: fine, medium and course. According to this grinding classification, grinding machines normally can produce coffee particles from fine to course. Previous researches had demonstrated that the size of particles greatly influence the extraction kinetics (Kuhn et al., 2017b). In fact, some studies on comminution of particles had highlighted that bigger particles can ease the percolation during the brewing process. However, the fine particles generate intensity of taste, the only issue of fines being the possible clogging of the filter baskets (Blittersdorff et al., 2017; Kuhn et al., 2017b). Besides many independent variables, such as the ratio solid/water, and besides intrinsic factors associated with the quality of the coffee bean, such as coffee cultivation and roasting, even to customers it is evident that particle size of ground coffee can bring significant alteration on the coffee extraction process and influence therefore the quality of EC (Petracco, 2008). A few research studies have been investigating the effect of the grinding size on the extraction of soluble compounds, in which the outcomes stated that finely ground coffee generates significantly higher caffeine while the coarsely ground coffee generates lower caffeine values (Bell et al., 1996a). These results have similar pattern in terms of volatile compounds and soluble compounds concentrations (Andueza et al., 2002). Based on the size of particles, a certain extraction accessory (e.g. filter baskets, distributor and tamping), should be used and tuned in a specific way, in order to extract a good EC (Spiro et al., 1985).

In this case study, variation in particle size distribution and three different filter baskets are applied. These variously designed filter baskets are named in alphabetical order: A (standard filter basket), B (A filter basket covered with 180-micron holes of net) and C (radial filter basket). Vibrational sieve was used to get each range of particle size distribution. Different particle sizes collected from the sieve were used to extract EC with 14g and 12g. In details, they were divided into five categories (particles below 200 microns, particles of 200-300 microns, particles in the range of 300-400 micron, particles from 400 microns and above), and compared with the results obtained using mix particles (namely, all particles of ground coffee from the grinding machine without sieve separation, which were collected and put directly into the filter baskets).

The coffee beans 100% *Coffea arabica* (arabica) from "Le Piantagioni del Caffè srl" (Livorno, Italy) roasting company, supplied by Simonelli Group S.p.A., were used for this case study. The coffee, of certified geographical origin from America, was suggested by certified roasters for EC preparation. The arabica beans were kept under sealed packages at room temperature and were opened just before grinding. Coffee beans were milled between fine and medium sizes using a Mythos 1 grinder from Simonelli Group S.p.A. (Belforte del Chienti, Italy) and were separated by AS 200 Control vibrational sieve machine from Retch (Germany) using various sieve plates. The grinded coffee was separated into sieve plates with 200-300 µm, 300-400 µm, 400-500 µm and 500-1000 µm. A Crystal Series analytical scale from Gibertini, (Italy) was used to weigh 12 and 14 g of separated ground coffee.
Espresso coffee was extracted using a VA388 Black Eagle espresso coffee machine from Victoria Arduino (Simonelli Group S.p.A., Italy). Preliminarily, for each filter basket (A, B and C), the grinding machine has been tuned and calibrated to obtain optimal EC (for two espresso: 14±0.01 g in filter basket, 50 ± 2 ml in the cup and 25 ± 1 s of extraction) without separating ground coffee by sieves. After calibration, grinded coffee was separated by a vibrational sieve. Separated microns (200-300 µm, 300-400 µm, 400-500 µm and 500-1000 µm) and weights (12 and 14 g) of ground coffee were transferred in three different filter baskets (A: standard, around 300 µm sized; B: 180 µm sized filter basket; C: net designed on the boundary of filter basket) to prepare different espresso coffee samples which were analysed for content of TS, bioactive compounds and aroma compounds. To prepare the EC samples, the machine was set at the following constant conditions: 25 s, 93 °C, 9 bars and 5 mm of perforated disc.
The utilized water was the same production batch of a minimally mineralized water (Blues, Acqua Minerale Naturale, minimamenta mineralizzata, Italy). This water is commercially available and its mineral contents was: total solids at 180 °C (22.0 mg/L); HCO_3^- (9.5 mg/L); Ca^{2+} (2.8 mg/L); Mg^{2+} (0.45 mg/L); SiO_2 (7.3 mg/L); NO_3^- (1.0 mg/L); Na^+ (1;8 mg/L); SO_4^{2-} (3.6 mg/L); Cl^- (0.21 mg/L); K^+ (0.20 mg/L); F^- (< 0.10 mg/L). All extracted EC samples were performed in triplicate and immediately collected from the portafilter of the espresso machine in a ceramic espresso cup, and the weight of the extracted EC samples was measured by Hario and Acaia balance.

3.2.1 Particle size analysis

Coffee beans, after passing the comminution process, were analysed by Mastersizer 3000 Aero Series dry dispersion unit (Malvern PANalytical Ltd., UK), which uses a laser diffraction to measure the size of particles (from 0.01 to 3500 µm). The instrument operates with continuous air flow, generated by industrial compressor at 6.5 bar, which penetrates into the Aero dry dispersion unit, which in turn transfers the particles at 2-3 bar to laser diffraction. In this way, the particles move in laminar flow and the vacuum extraction unit (KARCHER Professional NT 45/1 Tact, Germany) removes samples from the Aero dry. The grinded and separated coffee powder with various particle sizes were

collected: a portion for Mastersizer 3000 and the rest for the extraction of espresso coffee. The size of particles for each sample were examined in fivefold and the mean value was used for comparison.

3.2.3 Analysis of volatile compounds

Just after brewing, 2 ml of EC was placed in 20 mL screw top vials for the analysis of volatile compounds, using headspace solid-phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GS-MS). The instrument was equipped with PAL3 auto sampler system. DVB/CAR/PDMS fiber (Sigma Aldrich, Milan, Italy; 100 µm thickness) was used according to previous works (Bressanello et al., 2017b; Caprioli et al., 2012; Risticevic et al., 2008). The vial was tightly screwed on magnetic cap with PTFE-silicon septum and the system was set for automatic functioning mode. The sample was placed into a stirrer, incubated at 60° C, and stirred at 250 rpm for 20 mins. Then, HS-SPME automatically was inserted in the sample and remained 20 mins for the extraction. After adsorption, HS-SPME automatically injected the analytes into the gas-chromatographic system. A desorption time of 5 min was sufficient to desorb analytes from the fiber. Cleaning was automatically performed with PAL system by inserting the fiber in the conditioning port at 230° C for 20 min after each process. A gas chromatograph/mass selective detector (GC/MSD – Agilent, Santa Clara, CA, USA, Agilent 7890B GC Hardware with Agilent 5977 Series MSD and Mass Hunter GC/MSD Data Acquisition) was used. The column used for separation was DB-WAX (0.25 mm x 60 m x 0.25 µm - Agilent 122-7062, CA, USA). The workstation in the GC-MS system was an Agilent Chem. The flow rate (He) was 1.2 mL min^{-1} under spitless mode. The temperature of the injector was 260 °C. The temperature for the column was programmed: from 35 °C (4 min) to 120 °C (2.5 °C per min), and from 120 °C to 250 °C (15 °C per min); then, 240 °C for 3.33 min remained plateau and the total run time was 49.3 min. Data were acquired through the electron impact (EI) mode and full-scan acquisition mode by monitoring from 25 to 500 m/z. Each sample was injected three times and values were expressed as the means of three replicates.

3.2.4 Statistical analysis

Data on selected volatile compounds were examined by principal component analysis (PCA) using Statistica v.7.1 (Stat Soft Italia, Vigonza, Italy). PCA was applied in order to visualize information at various particle sizes that were used for EC extraction in the three filter baskets.

3.2.5 Extraction of espresso coffee with particle sizes 200-500 micron and filter baskets (A-B-C)

The extraction process is highly dependent on multiple variables, namely, water volume, water temperature and pressure, grinding size, the porosity of the ground coffee matrix, the pore network between coffee grinded particles, and the amount of coffee cake and brewing time (Cordoba et al., 2020). From the literature, the particle size for espresso coffee extraction is recommended to stay between 200-500 microns. However, this particle size range is actually in a mixture of sizes. If the

particle sizes can be separated exactly at each particle size range (between 100 and 200 microns, 200 and 300 microns, 300 and 400 microns, 400-500 microns, and above 500 microns), the bioactive compounds species in espresso coffee could be better studied and assessed. In order to achieve these more precise microns ranges, there are certain instruments needed to use. A well-known method for separation of particles is represented by the sieving process. A sieve has certain plates that can hold different particles. The operation of sieves can be done mechanically, vibrationally or with air flow. In these experimental studies, the used sieve was a vibrational sieve (Retch A200, Germany). The operation of the vibrational sieve was easy, however, a big issue occurred with agglomeration of particles. Due to the fact that coffee particles have complex organic compounds with different levels of lipids, carbohydrates and other, an agglomeration problem can occur indeed. In order to avoid this issue, during the separation process, special rubbers are used. All the separated particles are applied directly to the espresso machine for the EC extraction stage.

As regards the espresso coffee extraction, an Italian espresso coffee extraction protocol was applied. This protocol is based on a specific perforated disc height and a certain basket dimension (PD is 5 mm high; the basket is 2.5 cm high). The amount of ground coffee into the basket is 14g at 25 seconds with 25 ml of espresso coffee in the cup. During this extraction process, the amount of R&G coffee was reduced from 14 g to 12 g; two species of coffee were Arabica (Le Piantagioni) and Robusta (Bali); and the conditions of pressure and temperature were kept at 9 bars and 92±2^{0}C, respectively (Caporaso et al., 2014; Caprioli et al., 2013). The extraction time was constant, so the volume of the extracted coffee slightly changed. According to results obtained from the extraction, the volume of the coffee in the cup determines actually the concentration of total solids (TS). Furthermore, the extracted EC was prepared for analytical instrumental analysis on HPLC-VWD (Agilent, USA) and applied into the developed method shown at 3.1.4.

3.3 Outcomes of case study I

The analysis of particle size distribution was performed by using Mastersizer 3000 instrument. The process of analysis was rapid due to the laser diffraction. Measurements were repeated fivefold for single sample and the mean value was used to create the Gaussian graph. The graphs (Figure 3.2 a and b) deliver information of particles through percentage of volume density. The percentage of volume density comes through scattered light, because large particles scatter light at small angles with the laser beam, whereas small particles scatter light at large angles. This angular scattering intensity data are used to calculate the size of particles. Data analysis is based on the Mie theory (Do et al., 2007). Figure 3.2 a and b highlight those sizes of particles separated through vibrational sieve

that are distinguishable. These results show differences of particles (from 200 µm up to 1000 µm) used to extract EC with 14 g and 12 g, using arabica coffee and the standard filter basket (A). The two figures show the consistency of the grinding machine settings, and its efficacy in grinding at different particle sizes. Therefore, different PSDs were employed for brewing coffee with 14 g and 12 g. Afterwards, the grinding condition was kept constant and the grinded coffee was filtered through vibrational sieve; the separated particles of different dimensions were utilized for extraction of EC. The average size of particles between 200-300 µm was 256 µm, 300-400 µm was 325 µm, 400-500 µm was 440 µm and 500-1000 µm was 580 µm.

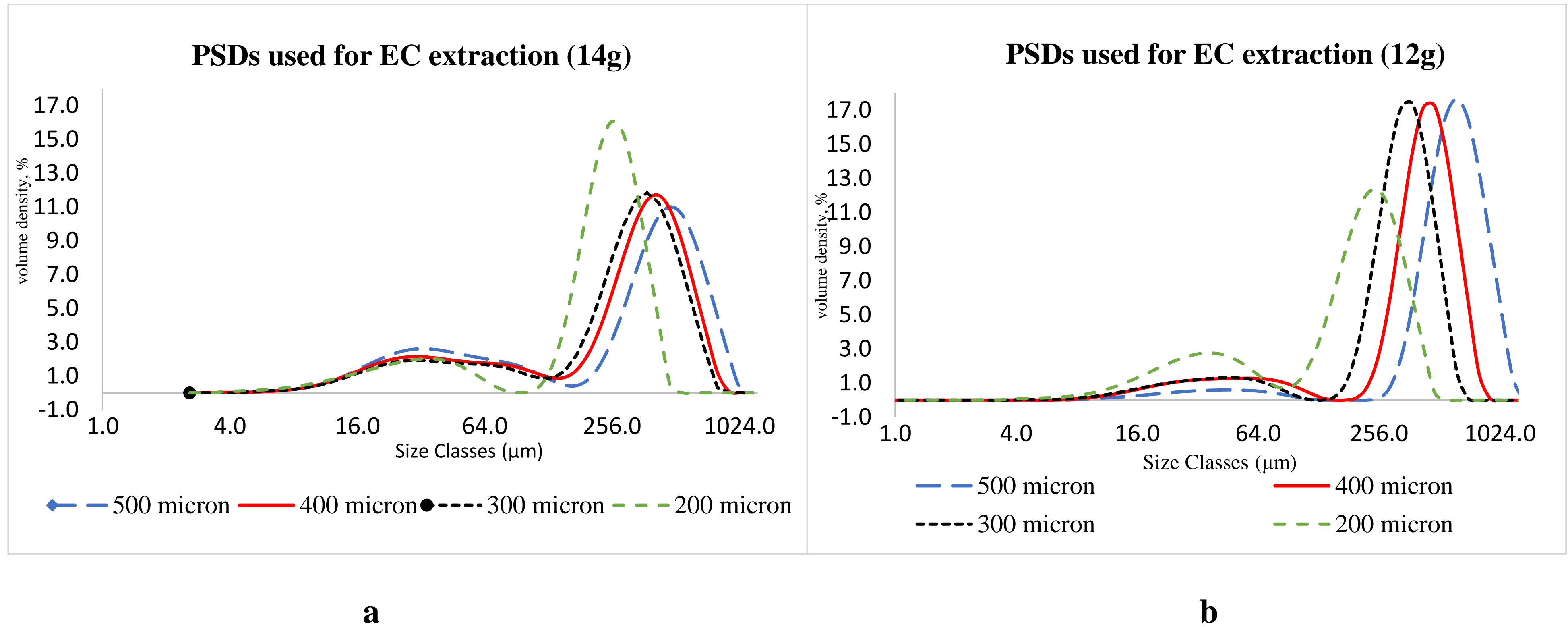


Figure 3.2 a and **b**. Particle size distribution measured through Mastersizer 3000. PSDs of arabica ground coffee used for extraction of EC with 14 g (**a**) and 12 g (**b**).

3.3.1 Total solid (TS): influence of particle sizes and filter baskets

Total solids, also called dry matter, measure the strength or concentration of coffee brew which is the first indicator of coffee extraction efficiency. TS can be measured by drying a portion of espresso coffee and weighting the remaining solids. The remaining dry matter, in correlation with the volume of coffee, measures the strength of the beverage (Folmer, 2017). Three filter baskets, A (standard), B (180 µm sized) and C (with radial holes on the side part) and four size ranges of particles (200-300 µm, 300-400 µm, 400-500 µm and 500-1000 µm) using 12 and 14 g of ground coffee were evaluated to study their influence on the coffee extraction.

Table 3.1 shows the total solids, expressed in mg mL^{-1}, for 30 different EC samples, prepared with the different variables under study. Before separating the various ranges of particle size, mixed particles of ground coffee were put in each filter basket directly from the grinding machine, as a calibration of the EC extraction. The resulting EC samples were used as a reference for the rest of the samples, extracted with different sizes of particles. Starting from the standard condition on the top (filter basket A, 14 g and mixed particles), and reading down through the rows of Table 3.1, it is possible to notice that the highest content of total solids (53.7 mg mL^{-1}) was found using particles at 200-300 µm and a growing trend from 500-1000 to 200-300 µm (from 29.15 to 53.70 mg mL^{-1}) was obtained. These results are in agreement with those reported by Andueza et al., (2003) and Severini et al., (2015), i.e., higher TS levels were found when finer particles were used. Moreover, looking at the averages of cup volumes, it is evident that by using course particles, long espressos with low levels of TS were obtained (volume: 42.5 mL, TS: 29.15 mg mL^{-1} for 500-1000 µm; volume: 43.5 mL, TS: 35.15 mg mL^{-1} for 400-500 µm; volume: 44 mL, TS: 32.10 mg mL^{-1} for 300-400 µm). This suggests that extracting coffee with coarse particles could decrease extraction efficiency, yielding under-extracted coffee because the volume specific surface would be too small to retain enough water and allow a good coffee compounds solubilization and emulsification (Andueza et al., 2003). On the other hand, when fine particles were used to extract coffee, we obtained a comparable level of volume (28.5 mL) with respect to the reference one, but with higher levels of TS, 53.70 mg mL^{-1}.

14 g of ground coffee						
	Filter A		***Filter B***		***Filter C***	
Particle sizes (μm)	**Volume per cup (ml)**[a]	**TS**[b]	**Volume per cup (ml)**	**TS**	**Volume per cup (ml)**	**TS**
Mixed particles	26	44.60	26	52.20	24	35.96
500-1000	42.5	29.15	17.5	69.80	37	29.20
400-500	43.5	35.15	20.5	66.10	45.5	25.10
300-400	44	32.10	44	40.10	47	23.70
200-300	28.5	53.70	45	35.16	38	34.15
12 g of ground coffee						
	Filter A		***Filter B***		***Filter C***	
Particle sizes (μm)	**Volume per cup (ml)**	**TS**	**Volume per cup (ml)**	**TS**	**Volume per cup (ml)**	**TS**
Mixed particles	26	46.50	26	44.30	25	43.00
500-1000	54	15.50	46	13.00	54	24.35
400-500	52	16.80	50	17.85	55	20.75
300-400	50	22.75	52	23.35	56	22.10
200-300	46	29.05	49	29.85	57	24.35

Table 3.1. Total solids (TS), expressed in mg mL^{-1}, in arabica EC samples, by using 14 and 12 g of ground coffee with various particle size distributions in three different filter baskets.

[a] RSD for EC volumes were 1.2-4.8 % for filter basket A, 2.2-5.7 % for B and 1.3-8.2% for C. [b] RSD for TS were 2.3-7.7 % for filter basket A, 3.6-9.2 % for B and 4.5-10.8 % for C.

Decreasing the amount of ground coffee from 14 to 12 g, determined similar levels of TS in the reference samples (44.60 and 46.50 mg mL^{-1}, respectively), and a decrease of TS in the various size ranges. Moreover, when using 14 g, a growing trend in TS from 500-1000 to 200-300 μm was present (from 15.5 to 29.05 mg mL^{-1}, and volume: from 54 to 46 mL). Investigating the three prototypes of the filter baskets, the highest TS content, for mixed particles using 14 g, was obtained in the B prototype (52.20 mg mL^{-1}). With filter basket B, using 14 g of ground coffee, the highest results were obtained also for all the other various size ranges (500-1000 μm: 69.80 mg mL^{-1}, 400-500 μm: 66.10 mg mL^{-1}, 300-400 μm: 40.10 mg mL^{-1} and 200-300 μm: 35.16 mg mL^{-1}). In this case, in contrast with

filter A, a decreasing trend was evident. Decreasing the amount from 14 to 12 g, lower levels of TS were found, in particular in reference samples (14-12 g: 52.20-44.30 mg mL^{-1}) and in 200-300 µm (14-12 g: 35.16-29.85 mg mL^{-1}, volume: 45-49 mL). Studying the prototype C, which possessed the radial holes on the side surface of the basket, we can observe a lower level of total solids, especially in the reference and in 200-300 µm samples. Decreasing the amount of R&G coffee, the samples showed good results for reference EC (from 35.96 to 43.00 mg mL^{-1}), but changing the sizes, no large differences were noted (500-1000 µm: 24.35 mg mL^{-1}, volume: 54 mL; 400-500 µm: 20.75 mg mL^{-1}, volume: 55 mL; 300-400 µm: 22.10 mg mL^{-1}, volume: 56 mL; 200-300 µm: 24.35 mg mL^{-1}, volume: 57 mL).

3.3.2 Bioactive compounds in EC: influence of particle sizes and filter baskets

The effect of particle sizes on EC extraction using 14 and 12 g in different filter baskets has been studied. EC samples (30 samples of arabica) were analysed for detecting caffeine, trigonelline and chlorogenic acids (3-CQA, 5-CQA and 3,5-diCQA). Results, expressed in mg L^{-1}, are shown in Table 3.2 for caffeine and trigonelline and Table 3.3 for chlorogenic acids. Starting from standard conditions, i.e. filter basket A and 14 g, we found that the content of caffeine was higher in 200-300 µm (3005.68 mg L^{-1}) than in mixed particles (2597.01 mg L^{-1}) and other sizes (Table 3.2). Similar behaviour was noticed for trigonelline (1557.51 mg L^{-1} in 200-300 µm particle sizes and 1408.68 mg L^{-1} in mixed particles). The inverse increment of caffeine and trigonelline levels, with respect to particle sizes, were found also in other scientific papers (Andueza et al., 2003; Severini et al., 2018).

Particle sizes (μm)	*Filter A*		*Filter B*		*Filter C*	
	Caffeine	**Trigonelline**	**Caffeine**	**Trigonelline**	**Caffeine**	**Trigonelline**
Mixed particles	2597.01	1408.68	2811.88	1522.45	2034.14	1085.93
500-1000	1722.34	938.02	3536.42	2048.31	1740.54	942.77
400-500	2028.19	1080.76	3540.03	1907.57	1681.56	875.16
300-400	1881.10	971.36	1949.21	951.37	1473.81	796.97
200-300	3005.68	1557.51	2113.71	1013.76	2013.63	1076.15
			12 g of ground coffee[a]			
Particle sizes (μm)	*Filter A*		*Filter B*		*Filter C*	
	Caffeine	**Trigonelline**	**Caffeine**	**Trigonelline**	**Caffeine**	**Trigonelline**
Mixed particles	2894.63	1586.49	2668.92	1480.31	2420.59	1420.74
500-1000	695.87	421.36	717.87	430.54	594.32	397.80
400-500	954.59	550.42	1032.88	583.03	1182.29	709.79
300-400	955.28	541.38	1239.36	707.78	1146.04	667.80
200-300	1391.95	683.90	1490.18	795.91	1310.12	731.37

Table 3.2. Influence of particle sizes and filter baskets on EC extraction, in terms of caffeine and trigonelline content (mg L^{-1}), using 14 and 12 g of arabica ground coffee.

[a] RSD were 0.1-12.5 % for caffeine, 0.2-14.1 % for trigonelline using 14 g and 2.3-13.3 % and 1.8-12.6 % using 12 g.

The results obtained by decreasing the amount of ground coffee from 14 to 12 g, showed an increase in caffeine and trigonelline levels in mixed particles (from 2597.01 to 2894.63 mg $L^{-1}$and from 1408.68 to 1586.49 mg L^{-1}, respectively) and lower content for other sizes. Comparing the reference ECs extracted with different filter baskets, using 14 g, the highest levels of caffeine and trigonelline were found in B (2811.88 and 1522.45 mg L^{-1}). When filter basket B was used, the same high levels

of caffeine and trigonelline were found in all different particle sizes (from 500-1000 µm to 200-300 µm: 3536.42-2113.71 mg L^{-1} for caffeine and 2048.31-1013.76 mg L^{-1} for trigonelline). When filter basket C was assembled into the filter holder, the lowest content of caffeine and trigonelline, in reference samples, were obtained. Moreover, we noticed a decrease of detected compounds in all particle sizes, if compared with those found in the other two filter baskets; quite good results were obtained, anyway, with 200-300 µm.

Table 3.3 shows the concentration (mg L^{-1}) of chlorogenic acids (3-CQA, 5-CQA and 3,5-diCQA) in EC samples extracted with three filter baskets, using 12 and 14 g of different particle sizes. According to previous research studies (Labbe et al., 2016b; Severini et al., 2015) particle size influences the amount of the extracted bioactive components. Therefore, also chlorogenic acids level can be affected by the particle sizes; our results showed that in standard conditions (A, 14 g), by modifying the particle sizes, the highest concentration of total CQAs was found in 200-300 µm (1507.23 mg L^{-1}). Hence, also for chlorogenic acids a greater extraction efficiency was obtained with fine particles, as already reported in literature (Andueza et al., 2003). Moreover, this concentration was higher than that in reference sample (1250.63 mg L^{-1}). Using 12 g, the highest concentration of total CQAs was found in mixed particles (1415.42 mg L^{-1}). In reference samples the highest content of total CQAs were obtained in filter basket B (1351.76 mg L^{-1}). As already noticed for caffeine and trigonelline, the ECs prepared with prototype B showed also the highest concentration of total CQAs in ranges 500-1000 and 400-500 µm (1829.23 and 1750.94 mg L^{-1}). Decreasing the amount of R&G coffee (filter basket B), we found lower concentration of total CQAs than in 14 g. Studying finally the extraction efficiency of filter basket C, the lowest levels were recorded in reference samples, rather than in those obtained with other filter baskets. The highest levels, with filter basket C, of total CQAs were found in mixed particles (12g) (1256.36 mg L^{-1}) and in 200-300 µm (14 g) (1039.36 mg L^{-1}).

Particle sizes (μm)	14 g of ground coffee[a]											
	Filter A				*Filter B*				*Filter C*			
	5-CQA	**3-CQA**	**3,5-diCQA**	**Total CQA**	**5-CQA**	**3-CQA**	**3,5-diCQA**	**Total CQA**	**5-CQA**	**3-CQA**	**3,5-diCQA**	**Total CQA**
Mixed particles	756.80	427.98	65.85	1250.63	825.03	465.01	61.72	1351.76	596.03	340.45	43.53	980.01
500-1000	499.17	287.98	41.18	828.33	1119.64	627.63	81.96	1829.23	499.55	287.38	35.08	822.01
400-500	591.25	343.74	49.59	984.58	1065.83	609.37	75.74	1750.94	479.52	276.60	34.60	790.72
300-400	553.76	318.45	42.89	915.1	536.29	317.99	43.53	897.81	436.64	256.88	30.47	723.99
200-300	922.54	511.85	72.84	1507.23	602.73	348.76	49.63	1001.12	631.26	360.93	47.17	1039.36

Particle sizes (μm)	12 g of ground coffee[a]											
	Filter A				*Filter B*				*Filter C*			
	5-CQA	**3-CQA**	**3,5-diCQA**	**Total CQA**	**5-CQA**	**3-CQA**	**3,5-diCQA**	**Total CQA**	**5-CQA**	**3-CQA**	**3,5-diCQA**	**Total CQA**
Mixed particles	863.35	479.19	72.88	1415.42	791.20	440.68	66.93	1298.81	767.69	426.80	61.87	1256.36
500-1000	198.83	116.53	13.91	329.27	195.45	115.26	14.36	325.07	177.67	106.08	12.13	295.88
400-500	279.64	161.31	21.39	462.34	303.27	171.84	24.96	500.07	359.37	207.74	28.20	595.31
300-400	282.76	163.72	22.21	468.69	371.78	216.57	31.70	620.05	350.49	200.91	27.27	578.67
200-300	409.84	224.63	39.06	673.53	467.53	259.93	42.49	769.95	413.99	233.79	34.15	681.93

Table 3.3. Influence of particle sizes and filter baskets on EC extraction, in terms of chlorogenic acid content (mg L^{-1}), using 14 and 12 g of arabica coffee.

[a] RSD were 2.2-11.6 % for 5-CQA, 3.4-9.2 % for 3-CQA and 5.7-12.1 % for 2,5-diCQA using 14 g and 2.8-8.3 %, 1.6-9.1 % and 3.7-11.7 % using 12 g.

3.3.3 Volatile compounds analysis

The volatile fraction of ECs was analysed through HS-SPME-GC-MS. Just after extraction, ECs were collected into screw top vial and injected using PAL autosampler. Volatile compounds were identified by comparing the mass spectra of the analytes with those of NIST 17 Mass Spectral Library with a similarity above 85%, and then the identified compounds' areas were integrated. Relative peak area percentage of individual components was expressed as percent peak areas relative to total peak areas (RPA%) (Thammarat et al., 2018; Zhang et al., 2007). Table 3.4 provides information about odour description and RPA% of identified volatiles, which are divided into chemical classes (Caporaso et al., 2018; Thammarat et al., 2018). Between 3 and 40 key odorants are typically composing a specific odour code of a food, which could feature in fact more than 10.000 volatiles (Dunkel et al., 2014). Furfural, furfuryl acetate, methylpyrazine, 2,5-dimethylpyrazine, 2-ethyl-6-methylpyrazine, 3-ethyl-2,5-dimethylpyrazine, pyridine and 1-hydroxy-2-propanone were found with the highest means of RPA in all samples. Signals at retention time 22.315 and 33.144 min are identified as member of Pyridine group; mean RPA of single volatiles found in this group corresponds to 7.55% of pyridine and 0.46% of 3-ethylpyridine. A key odorant of this group presents bitter, astringent, roasted and burnt aroma notes. In fact, coffee roasting defects can be associated to the presence of compounds of the pyridine family (Yang et al., 2016). The members of pyrazine group are found in extracted EC at the following mean RPA: 5.62% methylpyrazine, 4.69% 2,5-dimethylpyrazine, 3.61% ethyl pyrazine, 4.41% 2-ethyl-6-methylpyrazine, 3.95% 2-ethyl-5-methylpyrazine, 3.71% 2-ethyl-3-methylpyrazine, 0.41% 2-(n-propyl)-pyrazine, 4.13% 3-Ethyl-2,5-dimethylpyrazine, 1.49% 2-Methyl-5-propylpyrazine and 0.46% 3,5-Diethyl-2-methylpyrazine; the retention times of these compounds were within 26.816 - 37.555 min. They generate nutty, roasted, popcorn, earthy, grassy and hazelnut-like aroma notes. The components of furan group in EC were obtained at different mean RPA%: 0.78% 2-(methoxymethyl)-furan, 0.98% dihydro-2-methyl-3 (2H)-furanone, 0.99% 2-furanmethanol, 0.96% 5-ethylmethyltetrahydro-2-furanmethanol, 3.55% 1-(2-furanyl)-ethanone, 1.44% 2-N-Butylfuran, 4.56% furfuryl acetate, 3.84% 5-methylfurfural, 1.13% 2-furanmethanol propanoate, 2.77% 2-propionylfuran, 2.05% furan, 2,2'-methylenebis; these compounds were detected in the range 25.165 - 45.252 min. The general key odorants of furan groups are caramel, ethereal, rum, cocoa note, and nutty. The fluctuation in detecting the furan groups shows that EC aroma is quite dense and complex. Notably, the furan group components are higher in Arabica than in Robusta (Cordeiro et al., 2018). A derivative group of furans is furfural, found with 5.66% in EC aromas, and its detected retention time is 37.261 min. The generated aroma notes are almond, sweet,

toasted odour and burnt. Chemicals of phenol group were 1.60% for 4-ethyl-2-methoxyphenol and 1.91% for 2-methoxyphenol, detected at retention times 45.587- and 47.139-min. Key-odorants that come with this group are mostly associated with spicy, clove-like and smoky description. RPA values for ketone and aldehyde groups were: 4.36% for 1-hydroxy-2-propanone, 0.92% for 3-hydroxybutanone and 1.89% for benzaldehyde. Normally, the aroma of these groups is close to buttery, alcoholic-fruity, almond, and fruity (Niu et al., 2011). Terpene alcohol groups, as linalool with 0.48%, mostly present sweet, fruity and citrus notes. Components of pyrrole groups, as 2-formyl-1-pyrrole (0.68%), 2-acetylpyrrole (1.04%) and 1-furfurylpyrrole (2.05%) in EC aroma, release nutty, musty, hay-like, mushroom-like, and herbaceous notes (Yang et al., 2016). They are detected from 42.028 to 46.652 min. Volatile compounds found in different EC samples were compared by extraction conditions and PCA was applied to evaluate the relationship among the studied variables.

Tentative compounds	A 500-400 µm, 14g	A 500-400 µm, 12g	A 300-200 µm, 14g	A 300-200 µm, 12g	B 500-400 µm, 14g	B 500-400 µm, 12g	B 300-200 µm, 14g	B 300-200 µm, 12g	C 500-400 µm, 14g	C 500-400 µm, 12g	C 300-200 µm, 14g	C 300-200 µm, 12g	Mean RPA, %	Rt (m)	OD
Furans															
2-(methoxymethyl)-furan	0.84	1.25	0.84	1.01	0.68	0.55	0.61	0.5	0.54	1.20	0.57	0.79	0.78	25.165	Coffee
Dihydro-2-methyl-3 (2H)-furanone	0.49	1.14	1.40	1.16	1.08	1.13	1.06	0.80	0.84	0.94	0.86	0.82	0.98	26.591	Bready
2-furanmethanol	0.45	0.96	1.05	1.01	1.06	1.32	1.12	1.01	0.96	1.00	0.98	0.98	0.99	36.408	Chocolatey
Furfural	5.97	5.45	5.83	5.53	5.18	3.03	4.85	5.76	5.95	6.96	6.22	7.23	5.66	37.261	Bready
5 ethylmethyltetrahydro-2 furanmethanol	0.92	0.99	1.08	1.07	1.02	1.03	0.92	0.94	0.91	0.90	0.91	0.77	0.96	37.868	-
1-(2-furanyl)- ethanone	3.53	3.36	3.53	3.43	3.42	3.75	3.30	3.68	3.79	3.65	3.51	3.60	3.55	39.158	Balsamic
2-N-Butylfuran	1.53	1.50	2.10	1.92	1.42	1.39	1.26	1.21	1.09	1.38	1.19	1.25	1.44	39.663	-
Furfuryl acetate	4.42	4.39	4.54	4.39	4.46	4.91	4.10	4.67	4.79	4.87	4.41	4.75	4.56	40.224	Fruity
5-methylfurfural	3.73	3.87	4.12	5.04	3.64	3.62	3.03	3.95	4.09	4.09	4.67	2.25	3.84	41.197	Caramelly
2-Propionylfuran	3.06	3.17	3.56	3.36	2.85	2.20	1.93	2.69	2.66	2.75	2.44	2.54	2.77	41.715	-
2-Furanmethanol propanoate	1.30	1.70	1.93	1.68	1.22	1.01	0.45	0.72	1.16	1.13	0.66	0.56	1.13	41.981	Fruity

Furan, 2,2'-methylenebis	1.95	1.98	2.47	1.92	2.02	2.21	1.78	2.06	1.97	2.24	1.95	2.03	2.05	45.252	Roasted
Total percentage (furans)	28.19	29.76	32.45	31.52	28.05	26.15	24.41	27.99	28.75	31.11	28.37	27.57	28.69		
Pyrazines															
Methylpyrazine	6.77	5.54	6.38	6.57	5.03	5.69	4.86	5.27	5.62	5.20	5.28	5.26	5.62	26.816	Nutty
2,5-Dimethylpyrazine	4.85	4.51	4.86	4.57	5.74	4.94	4.43	4.51	4.56	4.65	4.37	4.33	4.69	29.954	Chocolatey
Ethyl pyrazine	3.67	3.46	3.53	3.67	3.49	3.83	3.44	3.37	3.69	3.59	3.15	4.37	3.61	30.577	Nutty
2,3-Dimethylpyrazine	0.97	0.92	1.04	0.98	0.90	0.98	0.91	0.83	0.88	0.89	0.83	0.81	0.91	31.249	Nutty
2-Ethyl-6-methylpyrazine	4.44	4.41	4.40	4.37	4.37	4.80	4.13	4.33	4.51	4.55	4.34	4.24	4.41	33.379	Potato
2-Ethyl-5-methylpyrazine	3.80	4.06	4.18	4.08	4.06	4.10	3.64	3.62	4.08	3.95	3.66	4.14	3.95	33.702	-
2-Ethyl-3-methylpyrazine	3.78	3.72	4.02	3.77	3.70	3.92	3.51	3.63	3.70	3.70	3.64	3.43	3.71	34.392	Nutty
2-(n-propyl)-pyrazine	0.40	0.45	0.49	0.45	0.41	0.46	0.41	0.34	0.40	0.39	0.37	0.37	0.41	35.082	-
3-Ethyl-2,5-dimethylpyrazine	4.59	4.12	4.18	4.13	4.05	3.88	3.90	4.10	4.20	4.28	4.12	4.06	4.13	36.527	Roasty
2-Methyl-5-propylpyrazine	1.59	1.59	1.66	1.69	1.33	1.54	1.38	1.30	1.09	1.62	1.58	1.46	1.49	37.408	-
3,5-Diethyl-2-methylpyrazine	0.48	0.51	0.50	0.48	0.50	0.51	0.48	0.44	0.41	0.37	0.48	0.37	0.46	37.555	Nutty
Total percentage (pyrazine)	35.34	33.29	35.24	34.76	33.58	34.65	31.09	31.74	33.14	33.19	31.82	32.84	33.39		
Pyrroles															

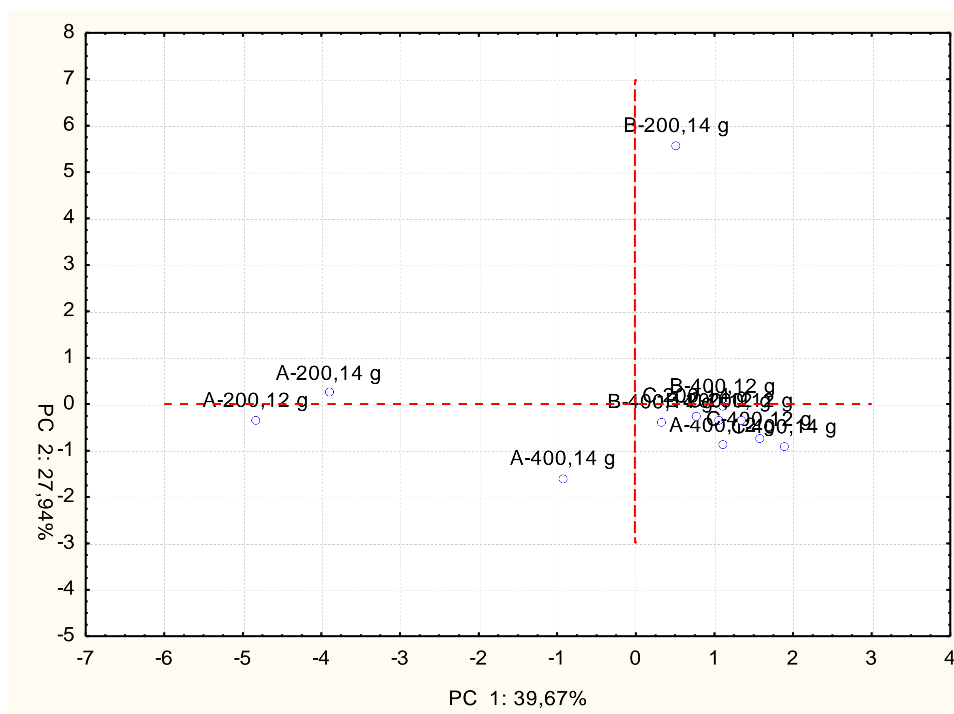
2-Formyl-1-pyrrole	0.30	0.54	0.73	0.64	0.60	0.85	1.37	0.62	0.74	0.59	0.61	0.55	0.68	42.028	Roasted
2-Acetylpyrrole	1.48	1.21	1.55	1.06	0.91	0.87	0.78	0.98	0.93	0.96	0.84	0.96	1.04	46.652	Musty
1-Furfurylpyrrole	1.95	1.98	2.47	1.92	2.02	2.21	1.78	2.06	1.97	2.24	1.95	2.03	2.05	45.252	Vegetable
Total percentage (pyrroles)	3.73	3.73	4.75	3.62	3.53	3.93	3.93	3.66	3.64	3.79	3.4	3.54	3.77		
Pyridines															
Pyridine	7.98	7.24	9.27	8.83	7.54	7.50	7.18	7.84	6.52	5.89	6.36	8.39	7.55	22.315	Fishy
3-Ethylpyridine	0.49	0.40	0.49	0.52	0.42	0.55	0.41	0.42	0.42	0.50	0.40	0.45	0.46	33.144	-
Total percentage (pyridine)	8.47	7.64	9.76	9.35	7.96	8.05	7.59	8.26	6.94	6.39	6.76	8.84	8.00		
Phenolic compounds															
4-Ethyl-2-methoxyphenol	0.90	1.89	1.37	1.40	1.38	1.34	1.28	1.78	1.98	1.96	1.93	1.97	1.60	45.587	Spicy
2-Methoxyphenol	0.85	1.42	1.75	1.35	1.31	1.42	7.02	1.60	1.36	1.59	1.45	1.74	1.91	47.139	Phenolic
Total percentage (phenolic compounds)	1.75	3.31	3.12	2.75	2.69	2.76	8.3	3.38	3.34	3.55	3.38	3.71	3.50		
Esters															
Isobutyl acetate	0.43	0.65	0.15	1.19	0.90	0.35	0.15	0.30	0.22	0.16	0.17	0.14	0.40	37.038	Fruity
Total percentage	0.43	0.65	0.15	1.19	0.90	0.35	0.15	0.30	0.22	0.16	0.17	0.14	0.40		
Ketones															

1-Hydroxy-2-propanone	4.67	5.89	0.92	0.63	4.64	5.54	3.69	5.38	6.08	5.25	4.19	5.42	4.36	35.491	Caramelly
3-Hydroxybutanone	1.01	1.04	1.00	1.14	0.98	0.97	0.93	1.27	0.83	0.87	0.88	0.91	0.99	40.072	Buttery
Total percentage	5.68	6.93	1.92	1.77	5.62	6.51	4.62	6.65	6.91	6.12	5.07	6.33	5.34		
Aldehydes															
Benzaldehyde	1.98	1.93	1.69	1.78	1.82	1.89	1.48	1.82	1.91	2.25	1.97	2.19	1.89	39.779	Almond
Total percentage	1.98	1.93	1.69	1.78	1.82	1.89	1.48	1.82	1.91	2.25	1.97	2.19	1.89		
Terpenes															
Linalool	0.31	0.46	0.43	0.42	0.49	0.56	0.48	0.52	0.53	0.50	0.57	0.44	0.48	40.541	Fruity
Total percentage	0.31	0.46	0.43	0.42	0.49	0.56	0.48	0.52	0.53	0.50	0.57	0.44	0.48		
Total percentage of all compounds	**85.88**	**87.70**	**89.51**	**87.16**	**84.64**	**84.85**	**82.05**	**84.32**	**85.38**	**87.06**	**81.51**	**85.60**	**85.47**		

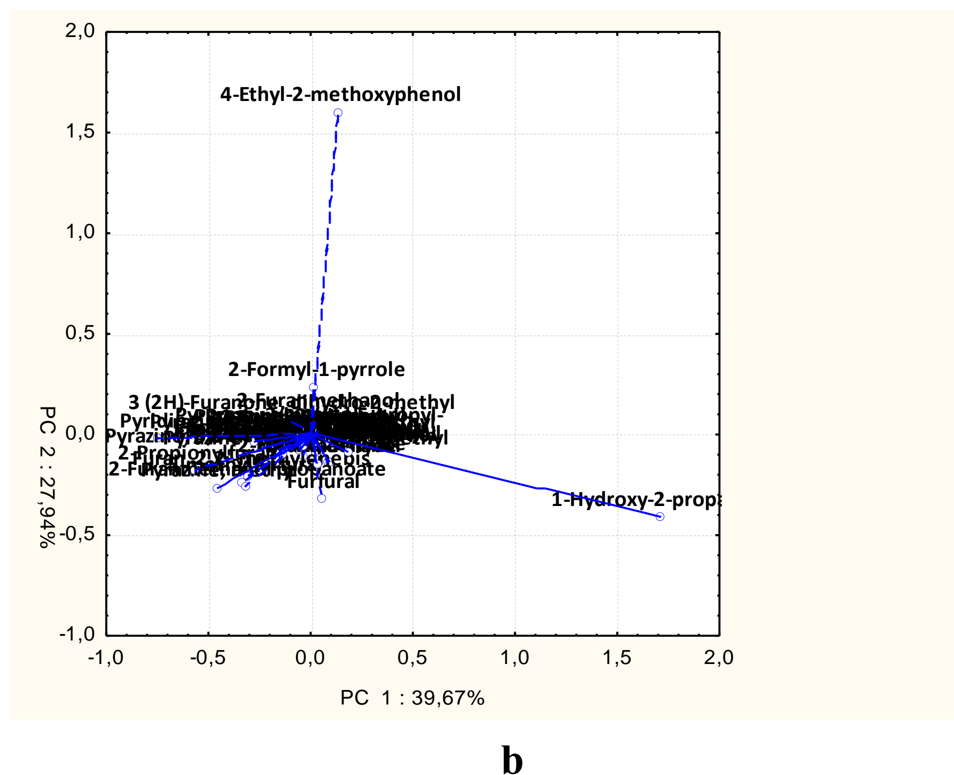
Table 3.4. Tentative volatile compounds of EC samples by using 14 and 12 g of ground coffee in various filter baskets at 200-300 µm and 400-500 µm obtained by HS-SPME-GC-MS, mean relative peak area (RPA) percentage, retention time (Rt) of volatiles and odour description (OD).

3.3.4 Principal component analysis (PCA)

The principal component analysis was applied to evaluate the relationship between the different particle sizes (200 µm and 400 µm) on the one hand, and the three different filter baskets (A, B and C) on the other, by reducing the amount of ground coffee in the filter baskets from 14 to 12 g (Figure 3.3a). Statistical data analysis indicates that filter baskets play a key role in brewing coffee. Three filter baskets, named A, B and C, proved that lowering the amount of ground coffee at the same particle size had little impact on the release of volatile compounds. With filter basket A, the left side of Figure 3.5a, 14 grams of coffee can generate nearly the same percentage of volatile compounds produced by 12 grams at the same size of particles. Indeed, the two filter baskets B and C show no significant percentage variance of volatile compounds with different particle sizes at 12 and 14 g of ground coffee. Figure 3.5b illustrates the percentage variance of volatiles in PCA. The variables most contributing to data variability were 1-hydroxy-2-propanone (caramelly) on the first principal component and 4-ethyl-2-methoxyphenol (spicy) on the second principal component. They explained 67.61% of variance. Most of samples bearing baskets B and C were positively correlated with toasty caramel aroma, whereas only one sample with 200 µm mesh size and equipped with basket B was characterized by spicy aroma. Finally, the samples on the left side of Figure 3.5b were those from two samples with 200 µm mesh size and equipped with basket A; they were correlated with several compounds such as pyrazine, furan, pyridine, carboxylic acid, aldehyde, alcohol and pyrrole groups.



a



b

Figure 3.3 a and **b.** Principal component analysis (PCA) of different filter baskets with 12 and 14 g of ground coffee used for extraction of EC.

3.3.5 Results of bioactive compounds in espresso coffee extracted with particle sizes 200-500 micron and filter baskets (A-B-C)

The results obtained from the analyses are applied to *minitab* statistical software (minitab, n.d.), where the global trend of alteration is relatively straightforward to portray a clear picture of the PSD and filter baskets effect on EC. The preliminary observation was done with TS in Figure 3.4, which shows that with more R&G used (14g), the TS value was slightly higher in Arabica than in Robusta. However, an interesting trend can be rather seen in the three different filter baskets: the highest TS was obtained with filter basket B (with quite similar value for basket A), while filter basket C had significant lower TS value. Considering PSD effect on EC, the mixed particles produce a significant higher TS value than the particles separated into the precise ranges. Excluding mix PSD, particles below 200 microns produced considerably higher TS values and, following this trend, PSD between 400 and 500 microns showed moderately high values, while PSD between 300 and 400 microns generated the lowest TS values.

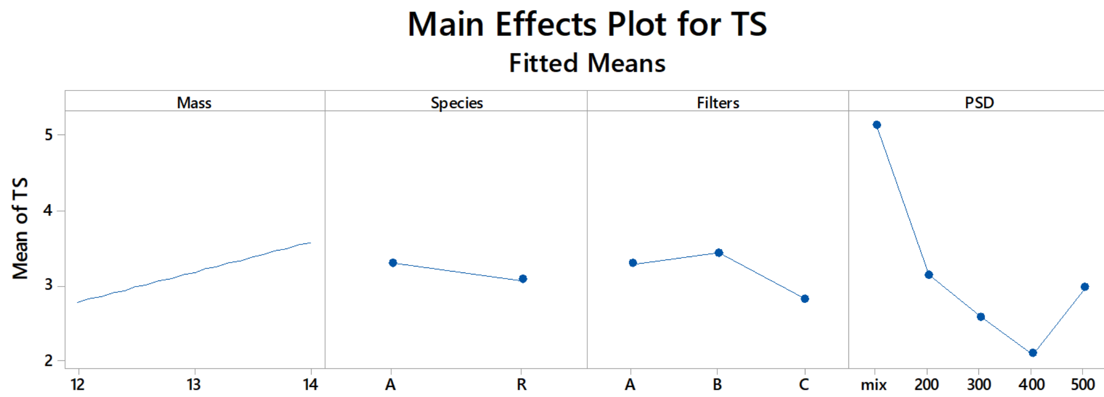


Figure 3.4 Main effects plot for total solids (TS) with mean value presented for each different case: R&G coffee mass (12-14g), species (Arabica and Robusta), filter baskets A, B and C, PSD mixed, 200, 300, 400 and 500 microns.

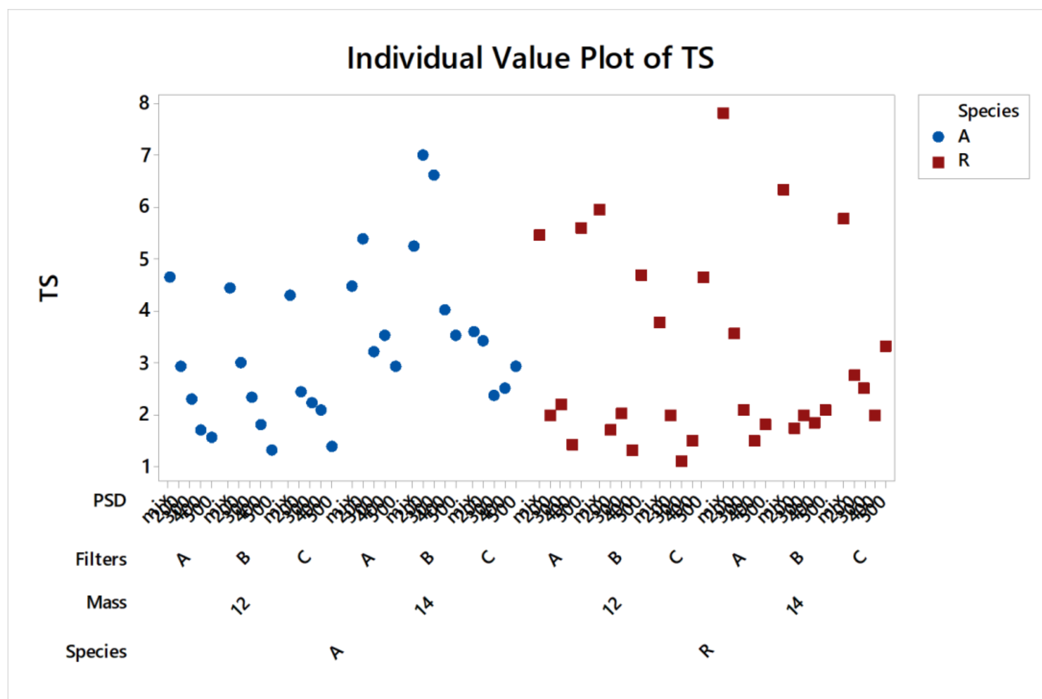


Figure 3.5 Individual value plot of TS with mean value presented for each different case: R&G coffee mass (12-14g), species (Arabica and Robusta), filter baskets A, B and C, PSD mixed, 200, 300, 400 and 500 microns.

The analyses of EC samples extracted with various PSD and filter baskets are conducted with HPLC-VWD to quantify the content of trigonelline, nicotinic acid, caffeine and derivatives of chlorogenic acids (3-CQA, 5-CQA and 3,5-di-CQA). Figure 3.6 presents the mean values of caffeine (mg/L) in the statistical analysis, provided by the results of EC extraction. It is evident that the two different amounts of R&G coffee (12 and 14g) produce quite different values due to the different surface area in contact with water, and, as many previous research studies confirmed, Robusta generates in general a higher content of caffeine than Arabica (Belguidoum et al., 2014; Calamai et al., 2013; Caprioli et

al., 2015). With regard to the filter basket effect on caffeine content, the study shows that A and B filter baskets are nearly in similar trend, whereas C filter basket produced almost the half of caffeine content than the other two baskets. In addition, the mix PSD and the range below the 200 microns have very close results compared to other microns ranges. This means that the finer the particles, and the more R&G coffee in the filter baskets, the higher concentration of caffeine will turn out, fact due to the solid liquid interaction at high temperature and pressure in short contact time (Blittersdorff et al., 2017).

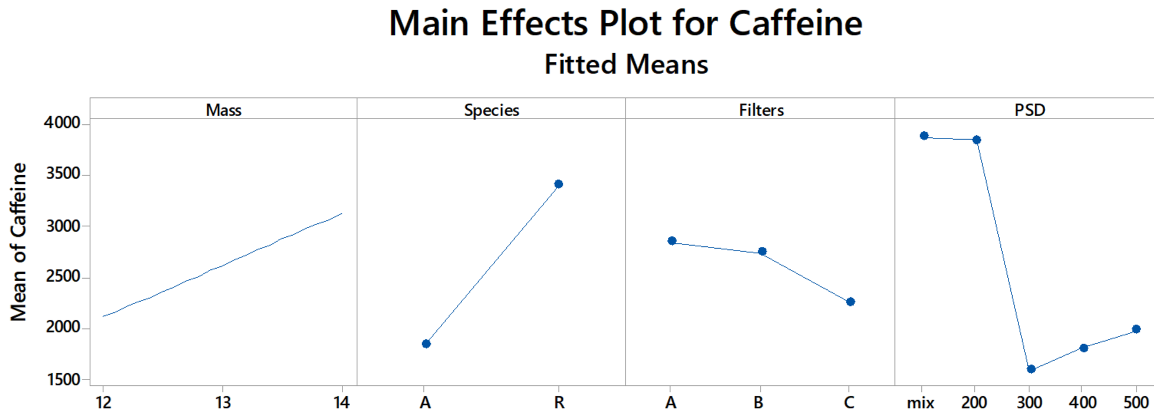


Figure 3.6 Main effects plot for caffeine with mean value presents for each different cases, R&G coffee mass (12-14g), species (Arabica and Robusta), filter baskets A, B and C, PSD mixed, 200, 300, 400 and 500 microns.

The interaction plot for caffeine in Figure 3.7 provides more detailed information about the effect of the PSD and filter baskets on EC. It can be seen that the increase of the mass plays no significant role in the increase of the caffeine level if compare to the role played by the filter baskets. However, the PSD value can impact on the concentration of caffeine, and it is also evident the significant differences between the species of coffee. Considering the three filters with various PSD, it is notable that mix particles and below 200 microns generate higher level of caffeine concentration in A and B filter baskets, while PSD value above 300 microns showed similar pattern for the three filter baskets. In contrast to earlier studies, the concentration of caffeine significantly vary within the species between Arabica and Robusta (Albanese et al., 2009; Caprioli et al., 2014; Crozier et al., 2012), which can be seen in the Figure 3.8. In the same way, the statistical results for trigonelline, nicotinic acid and derivatives of chlorogenic acids (3-CQA, 5-CQA and 3,5-di-CQA) are shown in the Appendix part. The statistical outcomes of the analyses clearly show that the studies with filter baskets need to be separated between A, B and C, due to the different design. Case study II presents six various C filter baskets radially holed in different ways. Filter basket A is used as benchmark. In this II case study, the extraction time, first, and then the volume of coffee, was set as constant parameter.

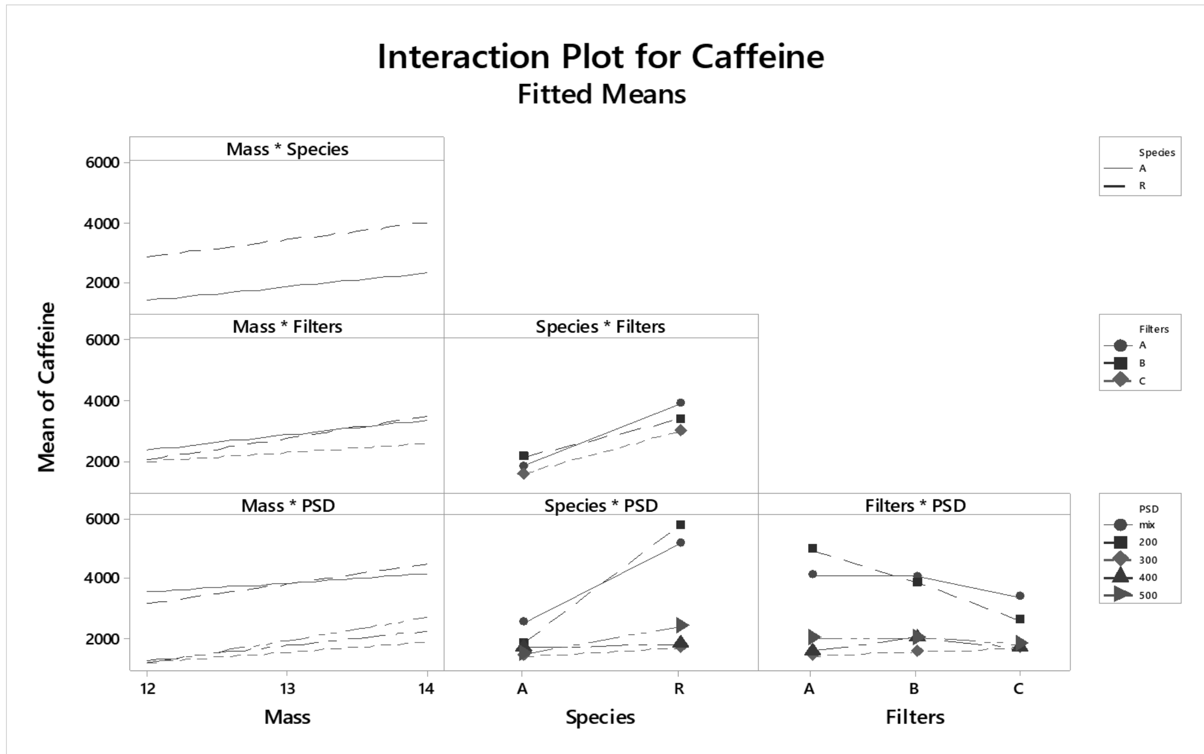


Figure 3.7 Interaction plot for caffeine with mean value presented for each different case: R&G coffee mass (12-14g), species (Arabica and Robusta), filter baskets A, B and C, PSD mixed, 200, 300, 400 and 500 microns.

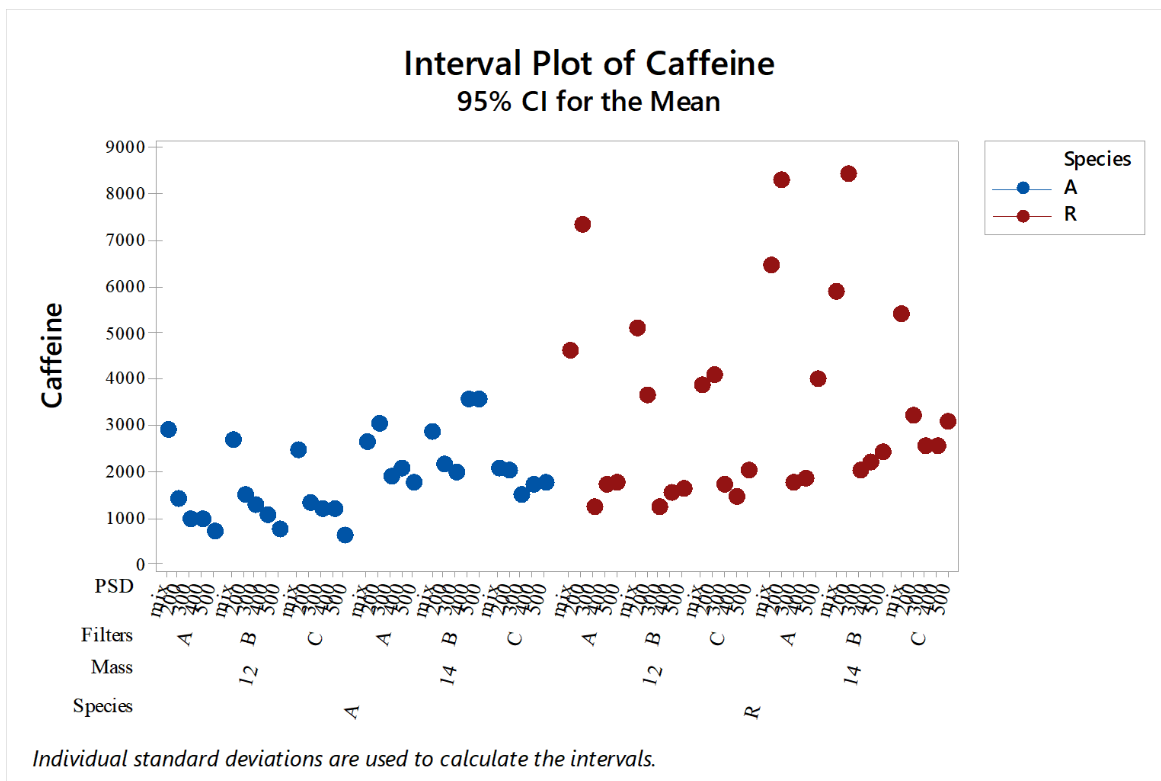


Figure 3.8 Interval plot for caffeine with mean value presented for each different case: R&G coffee mass (12-14g), species (Arabica and Robusta), filter baskets A, B and C, PSD mixed, 200, 300, 400 and 500 microns

CASE STUDY II.

3.4 Radially holed designed filter baskets

The present case study carried out detailed research on filter basket C, the basket with radial holes located on the boundary part of the filter. This study focuses on how the design of the filter basket impacts on EC. The analytical procedure on HPLC-VWD is also conducted in case study II by following the developed method shown at 3.1.4. In the classical filter baskets, where the holes are made on the bottom part, there are gravitational and conventional forces that affect the solid and liquid interaction, which here are less important. Considering the short percolation time and other physical variables during EC extraction, these special filter baskets might influence on bioactive compounds concentrations, so that there might be an opportunity to reduce the amount of coffee cake and enhance the brew.

Figure 3.9 Filter basket C types: the number and rows of the radial holes on the boundary increments from left to right.

3.4.1 Different holed area of filter basket C used to extract EC

Basket C has radial holes that are located within different lines on its boundary. The basket was designed with six different lines with holes as displayed by Figure 3.9. All holes lie on the boundary part of the basket. The objective of using this kind of baskets is to find similar extraction results with reference to those produced by filter basket A. Particularly, when all parameters of espresso machine and size of ground coffee are kept the same, the concentration and quantity of bioactive compounds are desired to be the same, even though the amount of ground coffee is reduced, by exploiting the different arrange of lines with holes in the filter baskets.

3.4.2 Extraction of EC with filter basket C with constant time and constant volume

Selecting filter basket C, the extraction method has been set according to Italian standard for extraction of EC. The parameters of water pressure and temperature were maintained constant, brew ratio, time and volume varied depending on the extraction settings. The following settings were applied to filter basket C:

Setting 1: constant time for extraction, 25 seconds. The ground coffee for all baskets is decreased from 12 grams to 7 grams, due to the reduction of the boundary holes. Temperature and pressure, 94 ± 1^0C and 9 bar, are constant. The volume is variable, and filter basket A, used as a benchmark, is filed in with 14 grams.

Setting 2: constant volume for extraction, 25 ml. The ground coffee for all baskets is decreased from 12 grams to 7 grams, due to the reduction of the boundary holes. Temperature and pressure, 94 ± 1^0C and 9 bar, are constant. The time is variable, and filter basket A, used as a benchmark, is filled in with 14 grams. The extraction of EC samples with six filter basket C and one filter basket A (as reference) are shown in Table 3.5.

Sample name, filters	P, bar / Temp, C	Initial mass, g	**Time constant**			**Volume Constant**		
			Final volume, ml	Time, sec*	Brew ratio	Final volume, ml	Time, sec*	Brew ratio
A	9/94	14	23.5	7/26	0.6	27	7/25	0.5
C1	9/94	12	23	6/25	0.5	27.9	6/29	0.4
C2	9/94	11	16.5	6/25	0.67	27.8	6/32	0.4
C3	9/94	10	12.5	6/25	0.8	27.9	6/31	0.35
C4	9/94	9	11.5	7/25	0.78	27.5	6/37	0.33
C5	9/94	8	6.5	7/25	1.2	26.7	6/61	0.29
C6	9/94	7	6	8/25	1.2	26.2	7/54	0.27

Table 3.5 Extraction of EC with time and volume as constant conditions. *Time shows first drop/final drop of EC in seconds.

Subsequently, the extracted espresso coffee is analysed for TS, caffeine, trigonelline, nicotinic acid and derivatives of chlorogenic acids (3-CQA, 5-CQA and 3,5-CQA). The instrumental analysis for liquid was HPLC-VWD.

3.5 Outcomes of EC extraction with filter basket C and A.

Experimental results showed manifold outcomes in relation to the different settings of this case study. The extracted EC with filter basket A is named ***At*** for time constant and ***Av*** for volume constant. Following these naming, EC samples conducted with filter basket C where numbered according to the number of holes on the boundary, from C1 to C6. Therefore, if the time remained constant, ***Ct1-Ct6***, and if the volume was constant, ***Cv1-Cv6*** are used for the identification of analyses on HPLC-VWD.

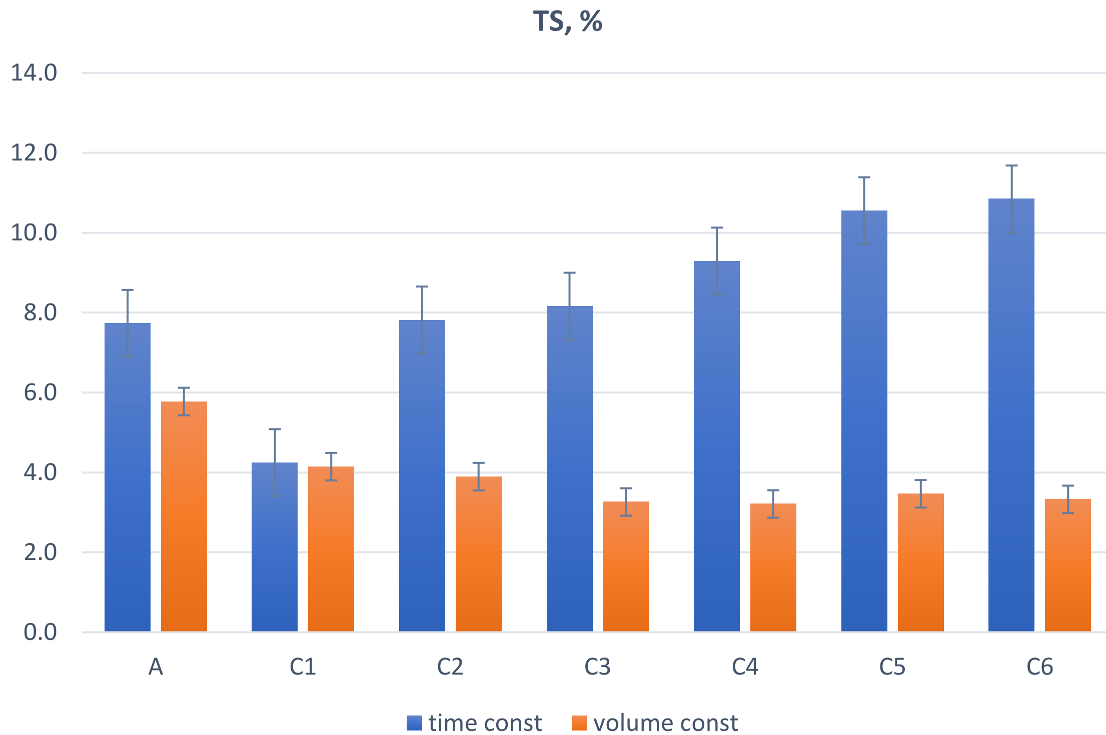
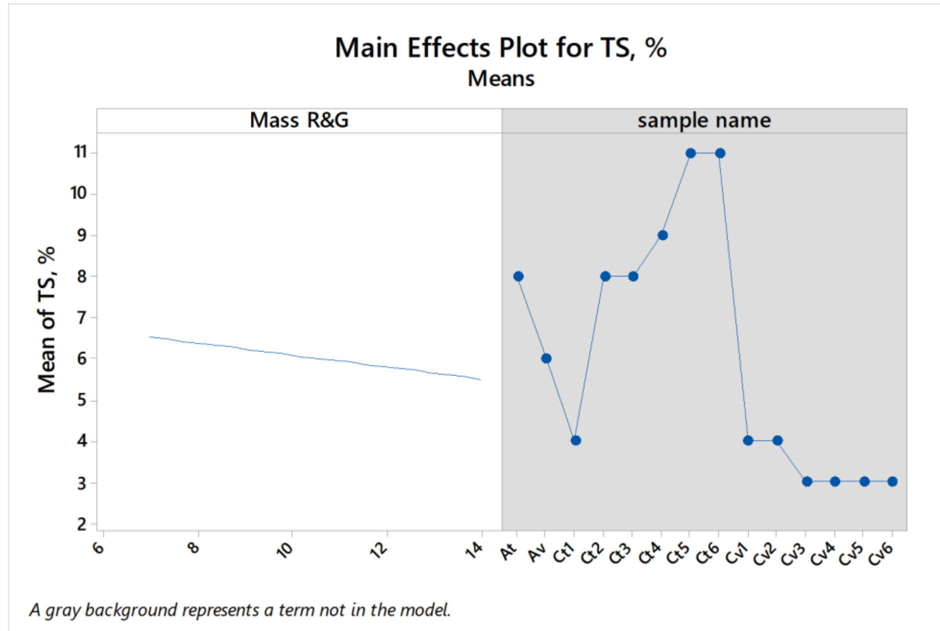
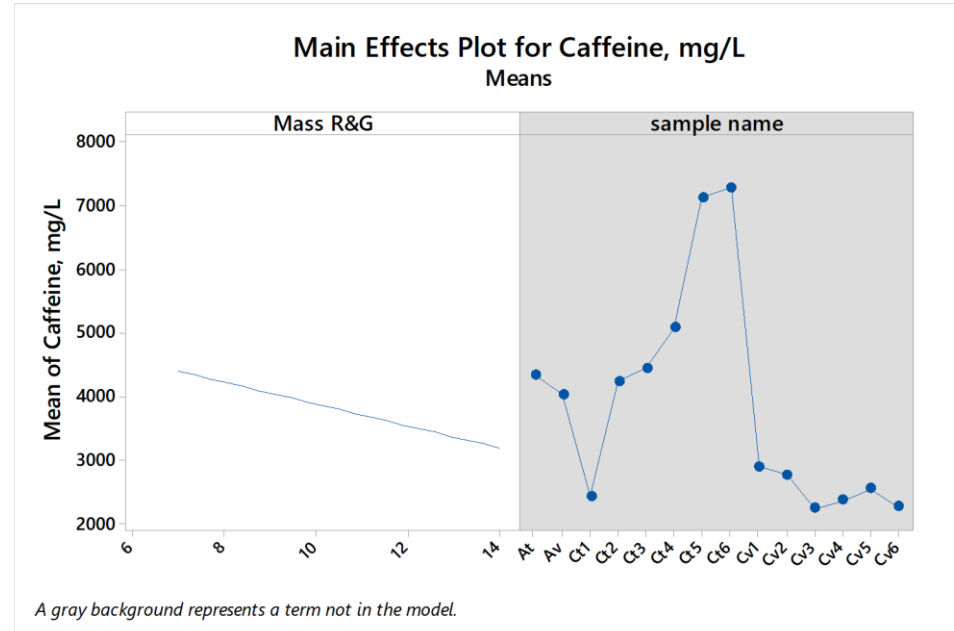
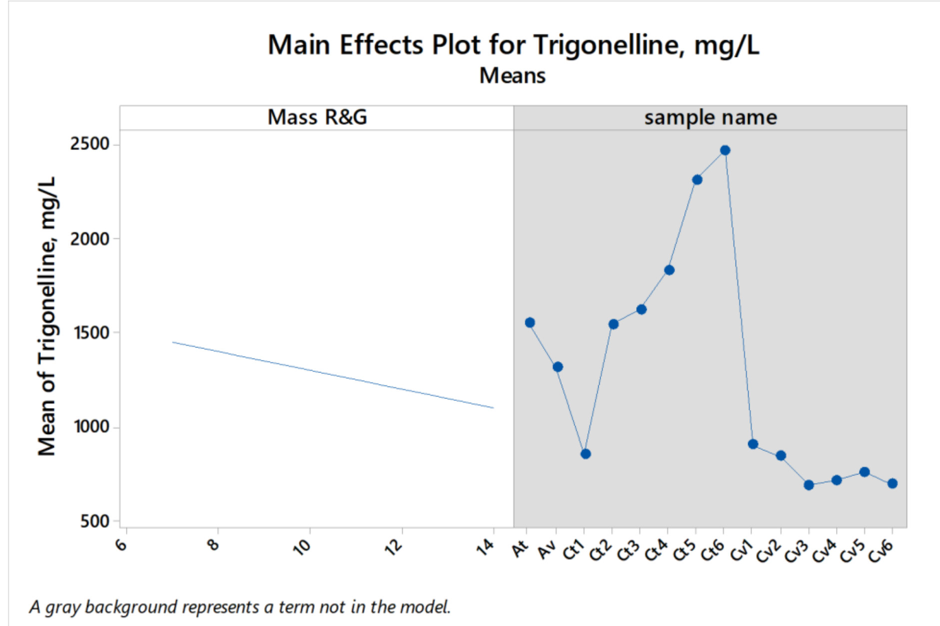
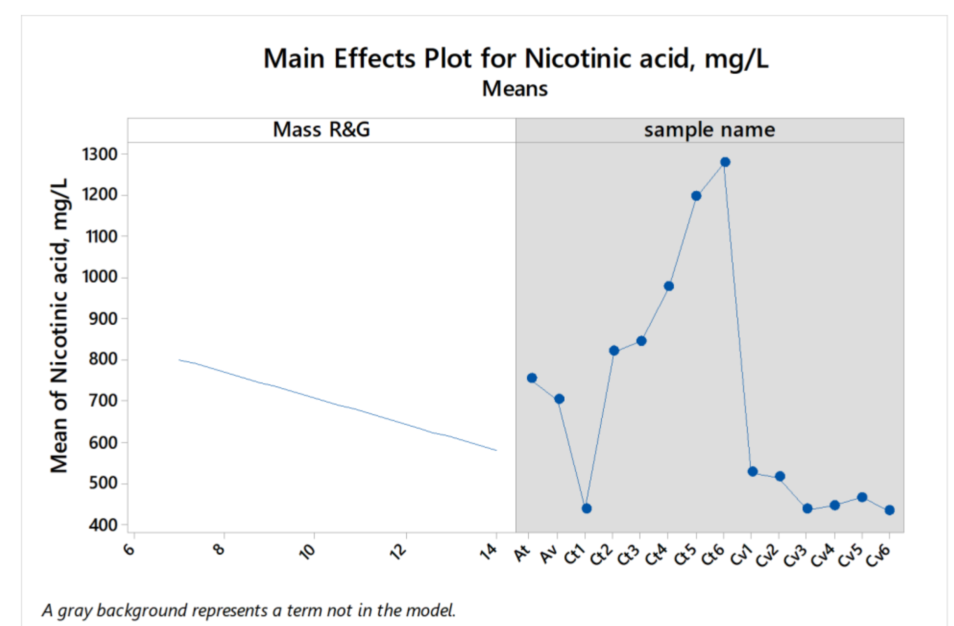


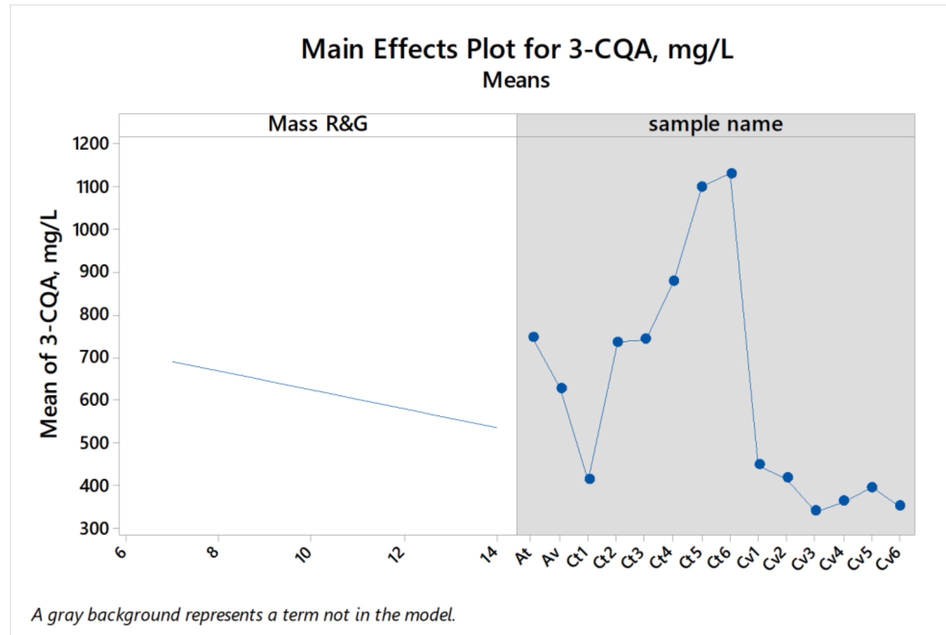
Figure 3.10 Total solids obtained from samples that were extracted with filter basket A and C.

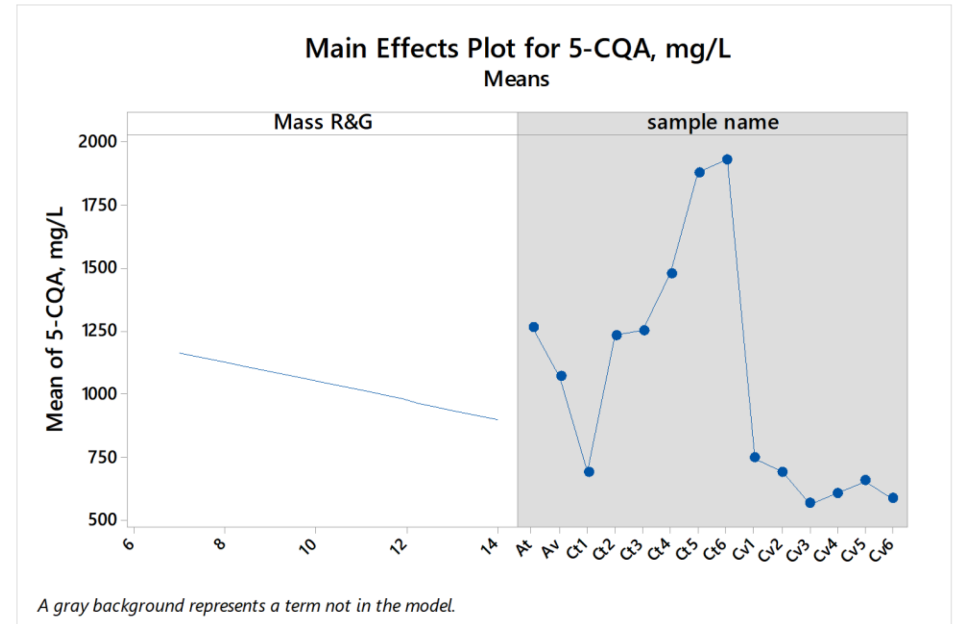

Main Effects Plot for TS, %
Means
Mass R&G
sample name
Mean of TS, %
11
10
9
8
7
6
5
4
3
2
6
8
10
12
14
At Av Ct1 Ct2 Ct3 Ct4 Ct5 Ct6 Cv1 Cv2 Cv3 Cv4 Cv5 Cv6
A gray background represents a term not in the model.


Main Effects Plot for Caffeine, mg/L
Means
Mass R&G
sample name
Mean of Caffeine, mg/L
8000
7000
6000
5000
4000
3000
2000
6
8
10
12
14
At Av Ct1 Ct2 Ct3 Ct4 Ct5 Ct6 Cv1 Cv2 Cv3 Cv4 Cv5 Cv6
A gray background represents a term not in the model.


Main Effects Plot for Trigonelline, mg/L
Means
Mass R&G
sample name
Mean of Trigonelline, mg/L
2500
2000
1500
1000
500
6
8
10
12
14
At Av Ct1 Ct2 Ct3 Ct4 Ct5 Ct6 Cv1 Cv2 Cv3 Cv4 Cv5 Cv6
A gray background represents a term not in the model.


Main Effects Plot for Nicotinic acid, mg/L
Means
Mass R&G
sample name
Mean of Nicotinic acid, mg/L
1300
1200
1100
1000
900
800
700
600
500
400
6
8
10
12
14
At Av Ct1 Ct2 Ct3 Ct4 Ct5 Ct6 Cv1 Cv2 Cv3 Cv4 Cv5 Cv6
A gray background represents a term not in the model.





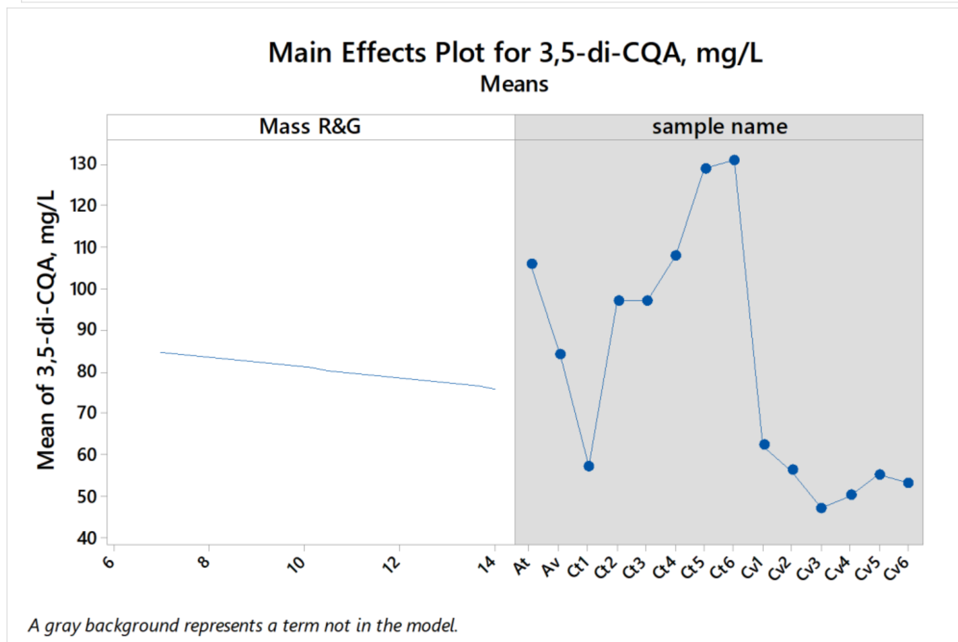


Figure 3.11 The *minitab* statistical software portrayed the overall main effects plot for each compound by its alteration of concentration within samples extracted with time and volume constant. Samples ***Ct1***-***Ct6*** are six samples with time constant and ***Cv1***-***Cv6*** are six samples with volume constant. ***At*** is filter basket A with time constant and ***Av*** is filter basket A with volume constant.

The sought-for comparison was between the extraction made with filter basket C and that made with filter basket A, taking into consideration the amount of ground coffee used in the basket and the quantity of coffee obtained at different extraction time and volume. This comparison showed the following main result: the samples with constant time showed an increase in the concentrations of bioactive compounds when using filter baskets with fewer holes. On the contrary, the samples with constant volume showed approximately similar trend with all used filter baskets. These results can be explained by the fact that fewer holes increase the density of the packed bed in the basket when the solid liquid interaction occurs (Corrochano et al., 2015; Moroney et al., 2015; Kevin M. Moroney et al., 2019). So, although the holes in the filter baskets and the amount of R&G coffee changed according to the used basket, the maintenance of time and volume constant resulted in significantly different outcomes. These outcomes clearly prove the importance of the solid and liquid contacting phase when lowering the amount of R&G coffee in filter baskets. Case study III is focused therefore right on the water distribution in the coffee cake inside the filter baskets.

CASE STUDY III.

3.6 Heights of the Perforated disc with different filter baskets

In each case study, the filter baskets proved their meaningful impact on the extraction. Nowadays, in normal bar espresso machines, not only the ergonomic design has been enhanced, but also the complex operational system. Indeed, thanks to these developments, the present manufactures are able to maintain a homogenous distribution of water into the coffee cake inside the filter baskets. Among those improvements, one of the most influencing parts of the machine for the extraction process is the perforated disk (PD). It is located above the shower part of the group (see Figure 3.12). The main function of PD is to regulate the water distribution into the basket in an even way, and avoid channelling on coffee cake. Due to the constant distribution of water during the surface contacts in the cake, coffee particles can generate the desired flavour and aroma in the cup. In this part of case study III, Simonelli Group S.p.A. provided different heights of PD (4mm, 5mm, 6mm and 7mm).

The experiments made use of various heights of perforated disc under the shower (4-7 mm), and extracted coffee with 14 g and 12 g of ground coffee, using filter basket A and B. By combining these operations, it was possible to optimize the extraction of EC. To conduct EC extraction, carry out experiments and fulfil the objectives of the research, specific analytical methods (see 3.1.4). The methods were applied to evaluate the differences between Arabica and Robusta cultivars that were used to extract EC samples, in terms of the contents in caffeine, trigonelline and chlorogenic acids.

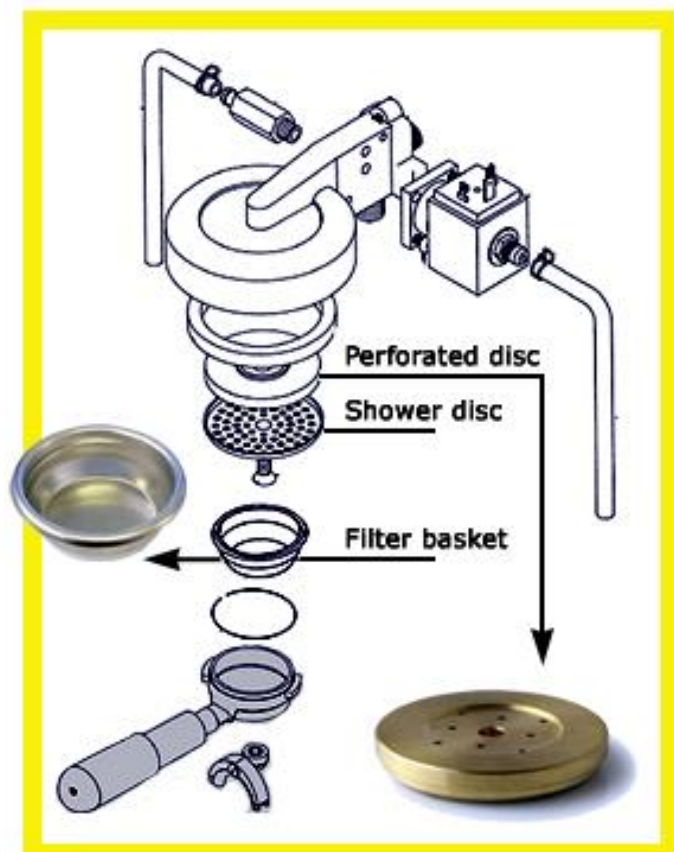


Figure 3.12 Scheme of the general group with perforated disc and filter basket.

3.6.1 Espresso coffee preparation

The espresso coffee preparation was the same of case study I. Here, the influence of different heights of the perforated disc was evaluated using the standard filter basket (A). After calibration, the EC extraction was performed using 12 and 14 g of ground coffee by modifying the height of the perforated disc (4, 5, 6 and 7 mm). The perforated disc was assembled under the shower to adjust the distance between the coffee cake and the shower. The extraction of ECs for each perforated disc (4-7 mm with 12 and 14g) was implemented in two ways: first, the extraction time was kept constant, and second, the extracted volume was maintained constant. These parameters were automatically adjusted by the program of the espresso machine (25 ± 1 seconds and 50 ± 2 ml for two espressos).

3.7 Outcomes of case study III

3.7.1 Total solid (TS): influence of perforated discs

Table 3.6 shows the total solids measured in different EC samples extracted with filter basket A using 12 and 14 g of arabica ground coffee, modifying the height of the perforated disc (4, 5, 6, 7 mm). After calibration, the particle sizes were kept constant and the experiment was applied in two ways: at first, maintaining time constant (25 s) and then maintaining volume constant (50 ml). Eventually, a total of 16 samples were analysed. The highest levels of total solids were found with perforated disc 5 mm at time constant (97 mg mL^{-1}) and perforated disc 4 mm at volume constant (86 mg mL^{-1}), when 14 g were used. On the other hand, using 12 g, high amount of TS was found in 6 mm at time constant (86.3 mg mL^{-1}) and 7 mm at volume constant (83.8 mg mL^{-1}). Considering both constants for each perforated disc, the highest content of total solids were in perforated disc 4 mm (time constant: 84 mg mL^{-1}; volume constant: 86 mg mL^{-1}) and 5 mm (time constant: 97 mg mL^{-1}; volume constant: 83.5 mg mL^{-1}), when 14 g where used. Decreasing the amount of ground coffee from 14 to 12 g, the best results were observed with perforated disc 5 mm (time constant: 84.5 mg mL^{-1}; volume constant: 77 mg mL^{-1}) and 6 mm (time constant: 86.3 mg mL^{-1}; volume constant: 74.5 mg mL^{-1}). Comparing perforated discs 6 and 7 mm when 14 and then 12 g were used, higher TS were found extracting coffee with less amount of coffee powder. Concluding, the best results for 14 g were obtained using 4 and 5 mm heights of the perforated disc, while for 12 g using 5 and 6 mm. Moreover, our results could suggest that decreasing the amount of coffee allows to obtain similar amount of TS when increasing the heights of the perforated disc.

Filter A		Total solids (TS) (mg mL^{-1})	
Mass of ground coffee[a]	Perforated disc (mm)	Time constant (25 s)	Volume constant (50 mL)
14 g	4	84.0	86.3
14 g	5	97.0	83.5
14 g	6	79.0	74.0
14 g	7	88.0	78.5
Mass of ground coffee	**Perforated disc (mm)**	**Time constant (25 s)**	**Volume constant (50 mL)**
12 g	4	74.0	73.8
12 g	5	84.5	77.0
12 g	6	86.3	74.5
12 g	7	73.5	83.8

Table 3.6. Total solids (mg mL^{-1}) in EC samples prepared by brewing 14 and 12 g of R&G coffee and assembling different height of perforated discs. The extractions were performed setting time constant (25 s) and then volume constant (50 mL for two cups).

[a] RSD were 2.3-6.8 % for ECs extracted with 14 g and 1.8-7.2 % for ECs extracted with 12 g.

3.7.2 Bioactive compounds in EC: influence of the perforated disc

A total of 16 EC samples were tested to evaluate the influence of different heights of the perforated disc on coffee extraction. After calibration, the ECs were prepared by maintaining constant the particle sizes but modifying the perforated disc and working in two ways: fixing constant time of extraction (25 s) and fixing constant the final volume in the cup (50 mL for two espressos). The perforated disc is a metallic disc that is assembled under the shower in order to adjust the distance between the coffee cake and the group shower. Table 3.7 shows the content, expressed in mg L^{-1}, of caffeine, trigonelline and chlorogenic acids, in ECs prepared using 14 and 12 g of arabica ground coffee. With constant time, the highest levels of caffeine, trigonelline and total chlorogenic acids were found using 5 mm perforated disc (5723.00, 3485.78, 2717.37 mg L^{-1}, respectively). The same results were obtained when the volume was kept constant (caffeine: 5500.00 mg L^{-1}, trigonelline: 3194.09

mg L^{-1} and total CQAs: 2492.57 mg L^{-1}). The highest concentrations of bioactive compounds were found in 6 mm perforated disc using 12 g of ground coffee and maintaining time constant. Specifically, caffeine was 5619.39 mg L^{-1}, trigonelline 3227.27 mg L^{-1} and total CQAs 2553.76 mg L^{-1}. With constant volume, the highest levels were obtained in 6 and 4 mm. Considering both constants, the best results, in quantitative terms, were found in 5 mm perforated disc using 14 g and in 6 mm perforated disc using 12 g. These results confirm what we already found for the total solid analysis: using a lower amount of ground coffee to obtain similar extraction efficiency, in terms of TS and bioactive compounds, requires a higher (namely, thicker) perforated disc.

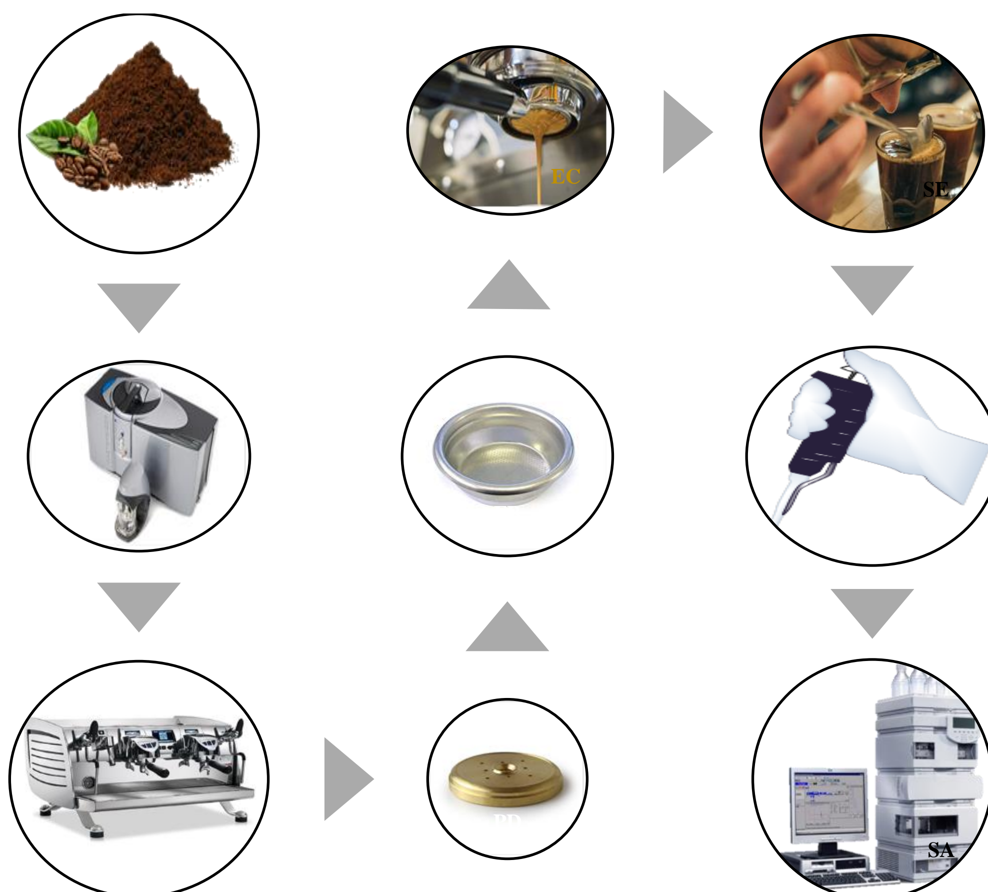
14 g of R&G coffee	*Constant time*					
Perforated disc (mm)	**Trigonelline**[a]	**Caffeine**	**3-CQA**	**5-CQA**	**3,5-diCQA**	**Total CQA**
4	3270.53	5410.67	870.04	1481.90	104.35	2456.29
5	3485.78	5723.00	921.73	1665.62	130.02	2717.37
6	2901.46	5013.90	770.35	1355.20	94.49	2220.04
7	3168.25	5715.86	825.75	1482.89	125.74	2434.38
14 g of R&G coffee	*Constant volume*					
Perforated disc (mm)	**Trigonelline**	**Caffeine**	**3-CQA**	**5-CQA**	**3,5-diCQA**	**Total CQA**
4	3195.26	5150.15	843.16	1459.85	99.52	2402.53
5	3194.09	5500.00	847.82	1517.15	127.60	2492.57
6	2679.81	4689.42	715.23	1261.86	88.17	2065.26
7	2673.72	4990.81	716.19	1281.75	112.17	2110.11
12 g of R&G coffee	*Constant time*					
Perforated disc (mm)	**Trigonelline**	**Caffeine**	**3-CQA**	**5-CQA**	**3,5-diCQA**	**Total CQA**
4	2832.99	5022.70	774.05	1373.80	101.75	2249.60
5	2985.79	5074.84	788.17	1397.53	109.19	2294.89
6	3227.27	5619.39	881.14	1548.36	124.26	2553.76
7	2569.56	4567.07	692.88	1244.54	112.17	2049.59
12 g of R&G coffee	*Constant volume*					
Perforated disc (mm)	**Trigonelline**	**Caffeine**	**3-CQA**	**5-CQA**	**3,5-diCQA**	**Total CQA**
4	2910.99	4964.10	804.63	1420.47	121.09	2346.19
5	2583.93	4448.27	687.39	1233.12	98.21	2018.72
6	2906.54	5163.92	807.92	1415.06	118.49	2341.47
7	2750.00	5048.21	725.10	1308.43	121.65	2155.18

Table 3.7. Content (mg L^{-1}) of caffeine, trigonelline and chlorogenic acids (3-CQA, 5-CQA and 3,5-diCQA) by brewing 14 and 12 g of R&G coffee by changing the heights of perforated disc in two condition: constant time (25 s) and constant volume (50 mL for two cups).

[a] RSD were 0.2-13.7 % for trigonelline, 0.5-11.3 % for caffeine, 0.3-11.1 % for 3-CQA, 0.7-10.5 % for 5-CQA and 1.0-10.9 % for 3,5-diCQA.

Study 2. Organic acids in espresso coffee and sensory evaluation

In the present research study, the influence of different supplementary devices (two filter baskets and four perforated discs) on EC has been analysed while lowering the amount of R&G coffee. The results were evaluated by sensory panellists. A new HPLC-VWD method has been developed for organic acids and bioactive compounds.



4.1 Introduction

The brewing of EC has been analysed in scientific experiments using various devices (e.g. espresso and capsule machines, etc.) and methodologies (for instance, by maintaining constant the water pressure and temperature), in order to simplify the process of extraction. Different extraction processes are continuously analysed and benchmarked with quantified data of compounds, fostering further investigations for the development and optimization of the brewing process (Andueza et al., 2002; Bell et al., 1996b; Farah, 2019; Illy, 2002; Parenti et al., 2014, 2014; Petracco, 2008). Another variable that can influence the extraction is the possible variations in particle size of the R&G coffee. Based on the size of particles, certain extraction accessories (e.g. filter baskets, distributor and tamping), should be used and tuned in a specific way, in order to extract a good EC (Petracco, 2008; Spiro et al., 1985). However, researchers have not yet studied in depth how different tools can be adjusted complementarily in coffee extraction, as for instance how different filter baskets could be chosen according to the burr that produces different particle size of ground coffee (Illy, 2002; Salamanca et al., 2017a).

An appreciated characteristic of EC is the flavour associated to acidity (or sourness). The major responsible compounds for acidity in coffee are non-volatile organic acids and low-molecular weight organic acids, which give a double contribution, both to aroma and flavour (Sunarharum et al., 2014; Vitzthum et al., 1975). The formation of organic acids starts with the maturation phase of the green bean, continue through the post-harvest treatment, and depends on the roasting and brewing method too, which can modify in fact the acidity in the final brew (Avelino et al., 2005). A number of published studies describes how the acidity of EC depends not only on the pH of the liquid, but also on the extraction variables (e.g. temperature and pressure), on partial dissociation of weak organic acids, and on the interaction with the taste buds of the tongue; these complex combinations substantially influence the acidic perception of the consumer (perceived acidity) (Salamanca et al., 2017a). There are two interrelated concepts in food analysis that relates to acidity, namely, pH and titratable acidity. Each of these quantities is analytically determined in separate way. Also, each has its own particular impact on food quality. Titratable acidity is connected to the measurement of the total acid concentration contained within a food (also called total acidity). This quantity is determined by exhaustive titration of intrinsic acids with a standard base. Titratable acidity is a better predictor of acid's impact on flavour than pH (Sadler, 2010). The measured pH values of EC range between 4.5-5.5, while the perceived acidity is defined by the total acidity, which in turn is determined by

organic acids. These bioactive compounds cause, in sensory analysis of EC, notes of bitterness and acidity (Parenti et al., 2014). The organic acids found in coffee fall normally into four main categories (aliphatic, alicyclic, chlorogenic acids and phenolic acids), although some authors highlighted that there are in fact more typologies of organic groups to take possibly into account, such as amino acids and fatty acids (Clifford, 2000). Relatively few historical studies exist in the area of organic acids in EC. The EC contains organic acids such as citric, malic, tartaric, acetic and caffeic acids, which are well-defined in the content of juice or wine. These acids generally highlight the combination of flavours, such as sweet, clean, and intense taste. Most studies on organic acids are still focused particularly on wine and grape juice (Mato et al., 2005). Outcome data from several studies for the determination of sugars and organic acids (acetic, citric, formic, malic, pyruvic, quinic and succinic) in wines and grape juices resulted from several simultaneous methods that were carried out with HPLC-DAD or HPLC-UV, with direct injection through a solid-phase extraction (SPE) separation method (Mesquita et al., 2018).

The vast majority of studies on organic acids in coffee science regards mainly the green or roasted coffee beans, but relatively few historical studies were carried out with regard to EC. Thus far, the existing research studies on organic acids in coffee have revealed a precise correlation between the outcomes of analytical method for quantification of volatile and non-volatile compounds (e.g. by HPLC or HS-SPME/GCMS), and those of the panel tasters of coffee (Bressani et al., 2019). The acidity of brewed coffee, an essential feature that influences to great extent the consumers' appreciation of the drink, depends also on the type of roasted beans used for the beverage. The method to extract organic acids from green coffee, roasted and different brewed coffee through SPE-RP/UV-HPLC has provided a good precision data for seven organic acids (acetic, citric, formic, malic, pyruvic, quininic and succinic acids) and caffeine. The outcomes of this research study showed that the content of acetic, citric, succinic acids and caffeine was higher in Robusta than in Arabica cultivar. This in fact could contribute to a far-reaching impact on further research possibly aimed at identifying the best roasting conditions for developing balanced acidity and sweetness profile in the brewed coffee (Rodrigues et al., 2007; Cordeiro et al., 2018). This experimental study provides in fact an important opportunity to advance the understanding of the effect of supplementary tools, in particular of two variously-designed filter baskets (named as A and B), combined with perforated discs of different heights (4-7mm) on the extraction of espresso coffee, while decreasing the amount of ground coffee used for a double EC extraction (from 14 g to 12 g).

Drawing upon the scientific research in analytical chemistry to quantify organic acids and bioactive compounds, this study applies HPLC-VWD analysis in EC, and compare the outcomes with data

from the sensory panel evaluation. By combining these two performances, it is possible therefore to predict optimal conditions for the extraction of EC. To conduct EC extraction, carry out experiments and fulfil the objectives of the research, a new developed analytical method and particular instruments have been used (VA388 Black Eagle espresso machine and Mythos 1 grinder provided by Simonelli Group SpA., Belforte del Chienti, Italy). For the evaluation of Arabica and Robusta cultivars that were used to extract EC samples, the newly developed method is applied in particular to quantify acetic acid, caffeine, caffeic acid, citric acid, 5-caffeoylquinic acid, malic acid, nicotinic acid, tartaric acid and trigonelline.

4.2 Materials and Standards

4.2.1 Chemicals

Standards of acetic acid, caffeic acid, caffeine, citric acid, malic acid, nicotinic acid, tartaric acid, trigonelline and chlorogenic acid, as well as acetonitrile (CH3CN – HPLC gradient) and phosphoric acid (H3PO4-85%) were purchased from Sigma-Aldrich (Milano, Italy).

4.2.2 Coffee samples and espresso machine

Roasted coffee samples (Arabica 100% and Robusta 100%) from two production areas of different geographical origin (America and Asia) were suggested by certified roasters for EC preparation, while the semi-automatic espresso machine (Vittoria Arduino, VA388 Black Eagle) and the grinding machine (Mythos 1) were provided by the espresso machine manufacturing company Simonelli Group SpA. (Belforte del Chienti, Italy). From now on, the two different coffee cultivars used for the EC samples, are simply referred to as Arabica and Robusta.

4.2.3 Particle size analysis

Particle size analysis is described in *Chapter 3 (3.2)*.

4.2.4 EC sample extraction

Ground coffee was weighed (12-14 grams) and transferred into two different filter baskets (A – standard, less than 300 µm sized filter basket for Italian espresso coffee, B – 180 µm sized filter basket) and the resulting EC samples were used to test the concentration of bioactive compounds. The extraction conditions of EC at 12 and 14 grams were maintained constant, especially with regard to the distance between the coffee cake and the shower, by changing the height of the perforated disc (4 mm-7 mm, *Figure 3.15*). The perforated disc is a perforated metal plate, assembled under the shower of each serving group and assuring a homogeneous water diffusion over the coffee cake

surface. This allows to evenly wet the coffee cake and avoid under or over-extraction phenomena in the cake. Moreover, the perforated disc adjusts the distance between the coffee cake and the shower and ensures an adequate empty space for cake swelling. This allows the coffee surface to reach the required permeability (Caprioli et al., 2015). Hence, for all these aspects, the perforated disc can influence the extraction phase, even if no study has been reported yet. Perforated discs of four different heights have been used (4, 5, 6 and 7mm), each tested with different distance between the shower and the coffee cake. The espresso machine parameters were 93±2 0C and 9 bar.

The extraction of EC for each filter basket was performed in triplicate (14-12g) and was implemented in the extraction at time constant for all different heights of the perforated disc and different amounts of ground coffee in the filter baskets. The brew ratio between grams of coffee cake and ml of EC was kept 1:2±0.2. All extracted EC samples were immediately collected from the portafilter of the espresso machine in a ceramic espresso cup, and the weight of the extracted EC samples was measured by Hario balance.

4.2.5 Sample extraction and HPLC-VWD analysis

The sample preparation was performed with 1 ml of each EC sample, which was diluted 50 times in the mobile phase. The diluted solution was centrifuged at 13,000 rpm for 10 min and filtered at 0.45 µm (PTFE filter) before HPLC-VWD analysis. The analytical column Gemini C18 110A (250 x 3 mm I.D., 5 µm, Phenomenex, Cheshire, U.K.) was used for the separation. The flow rate was 0.4 ml min-1 with gradient elution. The mobile phases were A) aqueous-phosphoric acid solution (pH 2.5) and B) Acetonitrile, in gradient mode (0-5 min, 2% B; 5-10 min, 15% B; 10-15 min, 20% B; 15-20 min, 50% B; 20-30 min, 100% B; 30-40 min,100% B; 40-50 min the flow was held at 2% B). The injection volume was 5 µL and HPLC-VWD experiments were carried out using a Hewlett Packard (Palo Alto, CA, USA) HP-1090 Series II, made of an autosampler and a binary solvent pump, equipped with a variable wavelength detector (VWD). HPLC-VWD analyses were performed at two different wavelengths in the same run: 200 nm for 10 min after 240 nm for the rest run time.

4.2.6 Sensory evaluation of EC

The sensory evaluation was carried out soon after the extraction process of EC. The panel tasters were long-experienced professionals with strong background in coffee industry and cupping, with three of them holding an official qualification in coffee tasting. They were in fact six experts from the Simonelli Group (Belforte Del Chienti, Italy) and the International Hub for Coffee Research and Innovation.

4.3 Results and discussion

In the reviewed literature, no data were found on the association between the heights of the perforated discs and the filter baskets used to brew EC. The present study was designed to determine the effect of supplementary devices to the extraction of espresso with two different weights (14g to 12g) of ground coffee in the filter baskets. The experimental results are divided and discussed according to the different nature of the acquired data, whether quantitative or qualitative. The experiments were performed in triplicates and the reported conversion values are averaged.

4.3.1 Particle size distribution, and instrumental analysis of ground coffee

The analysis of particle size distribution was implemented with Mastersizer 3000 instrument. The process of analysis was rapid due to the laser diffraction method. Measurements were repeated five times for each sample and the mean value was used to create the Gaussian graph and cumulative sum. The volume density is relevant for many samples as it reflects the size of those particles which constitute the bulk of the sample volume. It is influenced very much by the presence of large particulates in the size distribution. The particle size distribution curve was drawn from the data displayed on the program developed by Malvern Panalytical, which was connected directly on the Mastersizer 3000 instrument. Each sample, which was injected in fivefold, could generate its mean value, in which the data were obtained in the format of tables, graphs, diagrams or in the format of pictures. In each format, the particle size curve is shown anyway in the form of a Gaussian graph or table. The data provide information about the correlation between particle size classes and volume density; thus, the cumulative sum can be estimated. The graphs (Figure 4.1 and 4.2) deliver information about the particles through percentage of volume density. The percentage of volume density comes through scattered light, because large particles scatter light at small angles with the laser beam, whereas small particles scatter light at large angles. The maximum volume density in scattered light is 10. This angular scattering intensity data are used to calculate the size of particles. Data analysis is based on the Mie theory (Do et al., 2007)

Figure 4.1 and 4.2 highlight that size of particles grinded through Mythos 1 are nearly equal with small variations. These results provide the inter-correlations among the five times measurements of ground coffee, which were used to extract EC with 14 grams and 12 grams. The various percentages of volume density in both figures in similar micron are ranged in the same pattern, where particles are used for brewing at 25 seconds with 25 ml of EC. During the extraction operation, the ground condition was maintained constant.

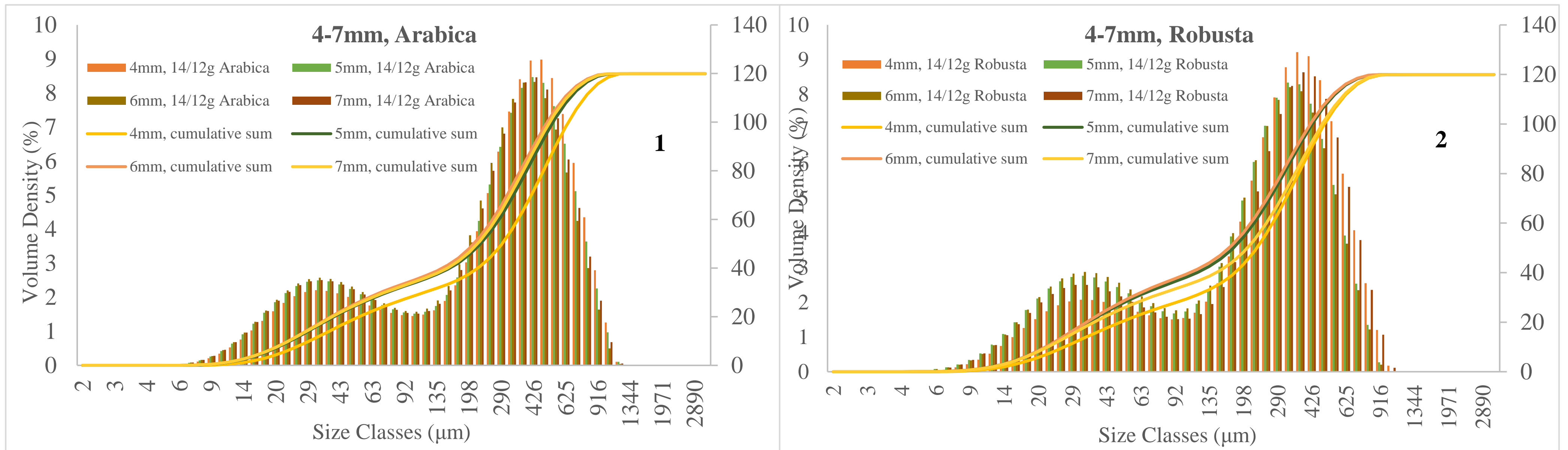


Figure 4.1 and **4.2**. Analysis of particle sizes of Arabica and Robusta ground coffee used to extract EC with different heights of perforated disc

4.3.2 Optimization of Chromatographic conditions

The determination of the chromatographic conditions was guided by the need to obtain chromatograms with good resolution of adjacent peaks in the analysis of selected compounds. Prior to analyse all selected compounds, the pH of the mobile phase on each compound, the chromatogram indication and the retention time were checked. The methanol as a mobile phase was eliminated and acetonitrile was a good replacement solvent. The use of a pH value higher than 3 for the mixture of aqueous-phosphoric acid solution yielded poor separation and co-elution of most peaks, whereas the pH value 2.5 of the mixture of aqueous-phosphoric acid solution showed a good separation without coelution of peaks and led to good peak shape. Once the effects of the pH of mobile phase on the compounds were defined, it was first necessary to examine the organic solvents ratio for the separation of compounds (i.e. aqueous-phosphoric acid solution at pH 2.5 and acetonitrile). Different ratio were checked: 75:25, 80:20 and 90:10. The optimum separation of mix compounds (acetic acid, caffeic acid, caffeine, chlorogenic acid, citric acid, malic acid, nicotinic acid, tartaric acid and trigonelline) was achieved when the ratio was 98:2. In addition, gradient elution was used to achieve excellent separation (0-5 min, 2% B; 5-10 min, 15% B; 10-15 min, 20% B; 15-20 min, 50% B; 20-30 min, 100% B; 30-40 min,100% B; 40-45 min, 2% B; and then kept until the end of the run at 50 mins). A chromatogram of all monitored compounds is shown in Figure 4.3.

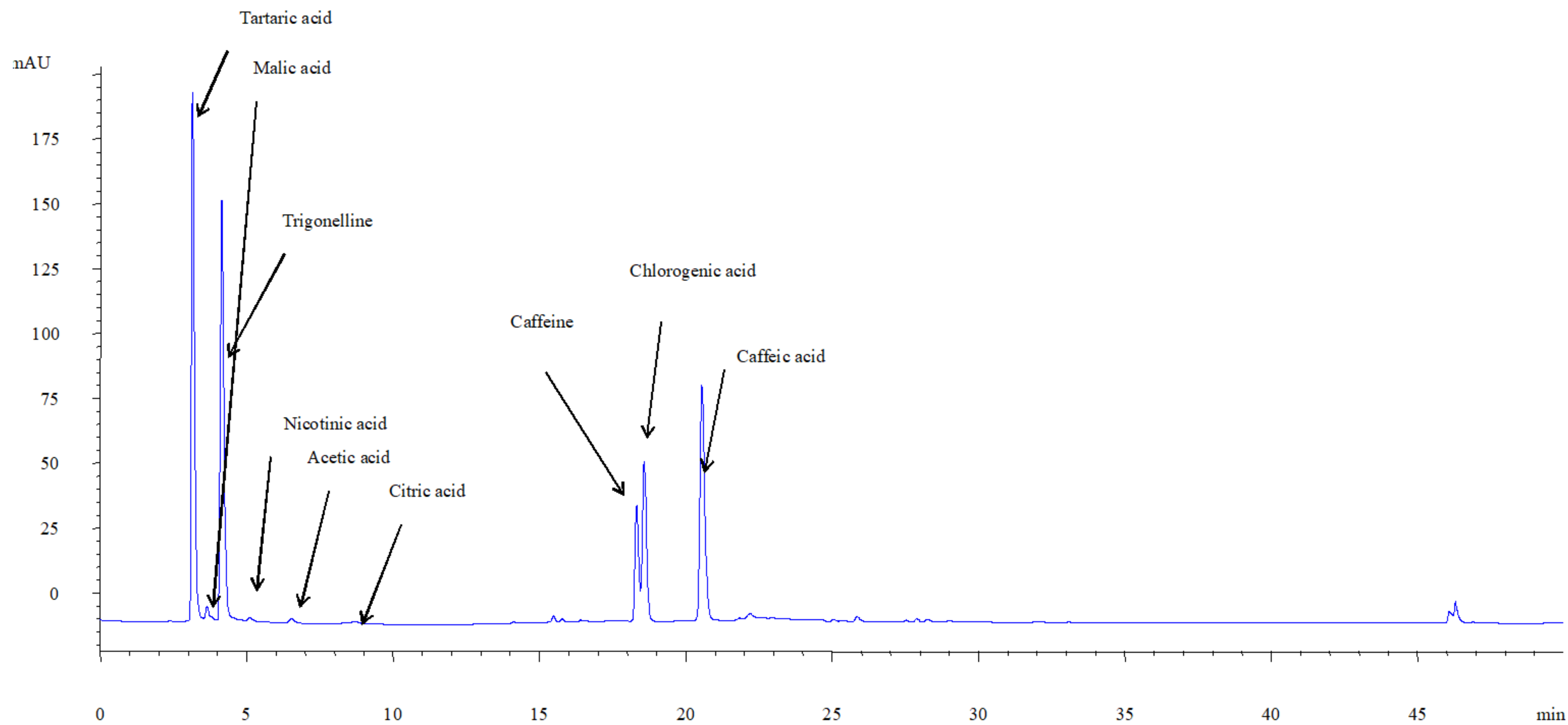


Figure 4.3. Chromatograph of the nine standard compounds.

4.3.3 Method validation for HPLC-VWD

The validation of the method has been carried out with respect to linearity, repeatability and with-in reproducibility, limits of detection (LODs) and limits of quantification (LOQs) (Table 4.1). Prior to the analysis, calibration curves of identified compounds at five different concentrations, i.e. 5, 10, 25, 50 and 100 mg L-1, were prepared by injecting 5 µL from standard solutions. Relative standard deviations (RSDs) of each concentration range from 0. 6 to 5.1 %. A correlation curve for each analysed compound indicates a value greater than or equal to 0.9863, except malic acid 0.9281. In the HPLC-VWD analysis of espresso coffee samples, the recoveries, obtained by spiking in the beverage solution a final concentration of 100, 500 and 1000 mg L^{-1} with a standard mixture of the nine compounds, were in the range 71-100% for all analysed compounds except tartaric acid, which displayed satisfactory recovery only at 500 and 1000 mg L^{-1} . The repeatability of the method was calculated on fortified samples at 100, 500 and 1000 mg L^{-1} (n=3), giving RSD% that were in a range 3.53–11.01%, 2.56-7.88% and 0.74–4.44%, respectively, for all compounds. The LODs and LOQs of nine compounds (estimated in matrix and indicated in mg L^{-1}) are calculated in signal to noise ratio of 3:1 and 10:1 S/Ns. For nitrogenous compounds, LODs and LOQs are in the range of 0.02-1.7 and 0.05-2.5 mg L^{-1}. The specificity and validity of the method is verified by the retention time stability.

Table 4.1. Analytical characteristics of the developed HPLC-VWD method

Compounds	Linear range mg/L	R^2	LOQ (mg/L)	LOD (mg/L)	Recovery %, mg/L (RSD %)		
					100	500	1000
Acetic acid	5-100	0.9996	2.5	0.8	71 (1.41)	93 (1.08)	97 (2.15)
Caffeic acid	5-100	0.9945	0.05	0.02	97 (2.59)	99 (2.53)	100 (2.51)
Caffeine	5-100	0.9963	0.3	0.10	81 (2.47)	91 (2.28)	99 (1.54)
Citric acid	5-100	0.9999	5	1.7	68 (2.94)	91 (2.53)	96 (8.74)
5-CQA	5-100	0.9996	0.2	0.07	71 (3.53)	92 (2.27)	96 (0.35)
Malic acid	5-100	0.9981	0.7	0.25	83 (1.26)	96 (5.36)	98 (3.59)
Nicotinic acid	5-100	0.9976	1	0.3	92 (1.02)	98 (2.81)	99 (6.59)
Tartaric acid	5-100	0.9995	0.08	0.03	16 (3.53)	52 (2.32)	53 (1.10)
Trigonelline	5-100	0.9976	0.1	0.03	80 (1.30)	96 (0.74)	98 (2.35)

4.3.4 Organic acids, caffeine, trigonelline, nicotinic acid and chlorogenic acid in EC

The higher content of organic acids can be found in the green beans, while the role of roasting and brewing is pivotal in flavour development. Throughout roasting, the content of organic acids passes through degradation, which turns them into other forms of compounds: as an example, part of malic acid can form fumaric or maleic acid, while citric acid may remain intact (Rodrigues et al., 2007). The causes of acidity have been the subject of intense debate within roasting and brewing operations. However, these operations are not only resulting from the profile of roast and ground coffee, but also immensely depend on other factors such as water temperature and pressure, percolation time, and the method of preparation (Parenti et al., 2014; Jeon et al., 2017; Wang et al., 2019). The case-study approach was chosen to obtain further in-depth information on the effect of various supplementary tools, such as heights of the perforated discs and filter baskets, on the extraction of EC. EC samples were analysed for detecting caffeine, trigonelline, nicotinic acids, 5-caffeoylquinic acid and organic acids (acetic acid, caffeic acid, citric acid, malic acid and tartaric acid). Extraction of EC was triplicated in the same conditions for each filter and each perforated disc (Table 4.2).

Preliminarily, for each height of perforated disc, the grinding machine has been tuned and calibrated so as to obtain optimal EC. To extract EC with 14 and 12 grams of ground coffee, the variables of the espresso machine were set at the following conditions: 25 seconds at 93±2 °C and 9 bar. After calibration, ground coffee was collected for the further analysis of Mastersizer. Following the grinding process, 14 and 12 grams of ground coffee were used for extraction. According to previous research studies (Gloess et al., 2013; Masella et al., 2015; Labbe et al., 2016) particle size influences on the amount of the extracted bioactive components. Regression analysis was used to predict the concentration of organic acids with combination of caffeine, nicotinic acid, trigonelline and 5-caffeoylquinic acid in EC. As can be seen from Table 4.2, the group of organic acids (acetic, caffeic, citric, malic, and tartaric) are significantly less than caffeine, trigonelline, nicotinic and 5-caffeoylquinic acid. This trend can be explained with the effect of various factors such as origin, post-harvest treatment and roasting, which highly impacts on the declination of organic acids during the process. On the other hand, the distance between the coffee cake in the filter basket and the perforated disc plays a significant role during the solid-liquid interaction for the concentration of bioactive compounds in the brew, even though the amount of coffee is similar in the extraction process with 14g or 12g for each 4mm-7mm. From this data, it can be seen that 4mm resulted the lowest value of concentration of nearly all introduced compounds in Table 4.2. The differences between 14g and 12g with respect to different filter baskets and heights of perforated disc are highlighted in this study,

which aims in fact to quantify, interpret and discuss the resulting concentration of bioactive compounds in each EC case. The correlation between 14g and 12g is interesting because supplementary tools can produce apparently closer trend.

The most studied bioactive compounds in coffee are caffeine, trigonelline, nicotinic and 5-caffeoylquinic acid (Navarini 2004; Caprioli 2014). Table 4.2 shows the concentrations of these bioactive compounds in EC. The results with 14 g (Arabica) in filter basket A with different heights of perforated discs (4 mm-7 mm) prove that the concentration of caffeine had a stable increase at 4 mm 3247 mg L^{-1}, 5 mm 4206 mg L^{-1}, 6 mm 4299 mg $L^{-1}$and 7 mm 5766 mg L^{-1}. This trend is also recorded for nicotinic acid at 4 mm 110 mg L^{-1}, 5 mm 117 mg L^{-1}, 6 mm 191 mg L^{-1}, except for 7 mm height of the perforated disc (179 mg L^{-1}). On the contrary, trigonelline concentrations have demonstrated an opposite trend: at 4 mm height of the perforated disc, it is found a concentration of 149 mg L^{-1}, at 5 and 6 mm a similar concentrations of 122 mg L^{-1} and 126 mg L^{-1}, and at 7 mm height of the perforated disc there was a significant high concentration of 206 mg L^{-1}. The concentration of chlorogenic acid has notably grown in almost each height of the perforated disc: at 4 mm 1894 mg L^{-1}, 5 mm 3912 mg L^{-1}, and 7 mm 5047 mg L^{-1}, but not at 6 mm 3589 mg L^{-1}.

Organic acids represent another important quantified compound in EC. Their concentrations vary with respect to the heights of the perforated discs. It can be seen that the concentrations of all organic acids (acetic: 4 mm-537 mg L^{-1}, 5 mm-971 mg L^{-1}, 6 mm-1135 mg L^{-1}, 7 mm-1669 mg L^{-1}; caffeic: 4 mm- 27 mg L^{-1}, 5 mm-53 mg L^{-1}, 6 mm-54 mg L^{-1}, 7 mm- 71 mg L^{-1};, citric: 4 mm- 511 mg L^{-1}, 6 mm- 608 mg L^{-1}, 6 mm- 939 mg L^{-1}, 7 mm-1033 mg L^{-1}; malic: 4 mm-92 mg L^{-1}, 5 mm-233 mg L^{-1}, 6 mm-246 mg L^{-1}, 7 mm-359 mg L^{-1}; and tartaric acids: 4 mm- 258 mg L^{-1}, 5 mm-533 mg L^{-1}, 6 mm-512 mg L^{-1}, 7 mm-704 mg L^{-1}) have a gradual rise following the increase in the height of the perforated disc, from 4 mm to 7 mm. The results obtained from the EC with 12 g (Arabica) in the filter basket A indicate the increase of concentrations from 4 mm to 6 mm. However, it can be seen that remarkable lower concentrations are obtained at 7 mm with respect to all bioactive compounds.

On the other hand, the results obtained from the EC with 14 g (Arabica) in filter basket B with different heights of the perforated discs (4 mm-7 mm) present an increase in the concentration of caffeine between 4 mm 3812 mg L^{-1} and 5 mm 5076 mg L^{-1}, but a slight decrease between 6 mm 3786 mg L^{-1} and 7 mm 4852 mg L^{-1}. This trend is also found for trigonelline, 5-caffeoylquinic acid and organic acids. The concentration of nicotinic acid rises gradually from 4 mm to 7 mm (84 mg L^{-1}, 148 mg L^{-1}, 164 mg L^{-1} and 167 mg L^{-1}). A very similar trend of fluctuation in the concentrations of the compounds is found for the EC with 12 g (Arabica) in filter basket B.

As regards the EC samples with Robusta cultivar, using 14 g in filter basket A, it can be seen that at 4 mm and 7 mm the concentration of caffeine is lower (6833 mg L^{-1} and 8805 mg L^{-1}) than at 5 mm and 6 mm (11537 mg L^{-1} and 10370 mg L^{-1}). This pattern is also recorded for 5-caffeoylquinic acid (4 mm 3434 mg L^{-1}, 5 mm 5827 mg L^{-1}, 6 mm 5422 mg L^{-1}, 7 mm 3689 mg L^{-1}) and organic acids (acetic, caffeic, citric, malic and tartaric). The concentration of trigonelline and nicotinic acid have instead a similar pattern (trigonelline: 4 mm 188 mg L^{-1}, 5 mm 266 mg L^{-1}, 6 mm 207 mg L^{-1}, 7 mm 200 mg L^{-1}; nicotinic acid: 4 mm 136 mg L^{-1}, 5 mm 140 mg L^{-1}, 6 mm 111 mg L^{-1}, 7 mm 141 mg L^{-1}). This scenario is found also in the EC with 12g in filter basket A.

The results from EC with 14 g (Robusta) in filter basket B with different heights of the perforated discs (4 mm-7 mm) show a gradual increase in the concentration of caffeine from 4 mm 5989 mg L^{-1} to 5 mm 12108 mg L^{-1}, but a slight decrease from 6 mm 9868 mg L^{-1} to 7 mm 8695 mg L^{-1}. This trend is found for trigonelline 5-caffeoylquinic acid and organic acids too. The concentration of nicotinic acid oscillates between 4 mm and 7 mm (140 mg L^{-1}, 296 mg L^{-1}, 116 mg $L^{-1}$and 108 mg L^{-1}). The EC with 12 g (Robusta) in filter basket B has demonstrated that at 4 mm and 7 mm the concentration of caffeine is lower (4859 mg L^{-1} and 5494 mg L^{-1}) than at 5 mm and 6 mm (6403 mg L^{-1} and 6115 mg L^{-1}). A similar oscillating concentration is shown also for 5-caffeoylquinic acid, whereas the concentration of trigonelline is gradually decreasing (4 mm 157 mg L^{-1}, 5 mm 142 mg L^{-1}, 6 mm 131 mg L^{-1}, 7 mm 128 mg L^{-1}). A slight increase of concentrations for acetic acid (4 mm 395 mg L^{-1}, 5 mm 526 mg L^{-1}, 6 mm 678 mg L^{-1}, 7 mm 860 mg L^{-1}), citric acid (4 mm 410 mg L^{-1}, 5 mm 631 mg L^{-1}, 6 mm 683 mg L^{-1}, 7 mm 835 mg L^{-1}) and malic acid (4 mm 113 mg L^{-1}, 5 mm 189 mg L^{-1}, 6 mm 195 mg L^{-1}, 7 mm 207 mg L^{-1}) is identified at different heights of the perforated discs.

Therefore, the height of the perforated discs (4 mm -7 mm) and the weight of ground coffee (14 g - 12 g) in the filter basket may gradually increase or decrease the concentrations of compounds in EC, also in relation to the distance between the solid (coffee cake) and the liquid (shower). The resulting scenarios clearly demonstrate an increase of concentrations when the height of the perforated disc is 7 mm and the weight of ground coffee is 14 g, and a decrease of concentrations when the height of the perforated disc is 4 mm and the weight of ground coffee is 12 g. This phenomenon is due to the fact that the relative distance between the coffee cake and the shower is closer at 7 mm with 14 g than at 4 mm with 12 g. Depending on this distance, the water could pass more or less easily (the higher is the distance, the easier is the water flow).

In fact, a significant alteration in the number of bioactive compounds was also highlighted by using different filter baskets. Filter basket B, if compared to basket A, allowed for a clear increase of all

biocomponents in most cases and samples. In particular, the significant variations can be seen in Arabica with 14g at 4mm for acetic acid (filter basket: A - 537 mg L^{-1} and B - 825 mg L^{-1}), caffeic acid (filter basket: A - 27 mg L^{-1} and B - 46 mg L^{-1}), chlorogenic acid (filter basket: A -1894 mg L^{-1} and B -3358 mg L^{-1}), malic acid (filter basket: A - 92 mg L^{-1} and B - 186 mg L^{-1}) and tartaric acid (filter basket: A - 258 mg L^{-1} and B - 474 mg L^{-1}). On the other hand, the outcomes for Arabica with 12g at 4mm showed contrary results: acetic acid (filter basket: A – 783 mg L^{-1} and B - 723 mg L^{-1}), caffeic acid (filter basket: A – 44 mg L^{-1} and B - 35 mg L^{-1}), 5-caffeoylquinic acid (filter basket: A – 2815 mg L^{-1} and B - 2711 mg L^{-1}), malic acid (filter basket: A – 207 mg L^{-1} and B - 160 mg L^{-1}) and tartaric acid (filter basket: A – 409 mg L^{-1} and B - 384 mg L^{-1}) slightly decreased. The caffeine concentration differs with respect to filter baskets A and B: in particular, there is an increase in the levels of caffeine detected after extracting with filter B for Arabica with 14 g at 4mm (filter basket: A – 3247 mg L^{-1} and B – 3812 mg L^{-1}) and at 5mm (filter basket: A – 4206 mg $L^{-1}$and B - 5076 mg L^{-1}); and a decrease at 6mm (filter basket: A – 4299 mg L^{-1} and B – 3786 mg L^{-1}) and at 7mm (filter basket: A – 5766 mg L^{-1} and B - 4852 mg L^{-1}). Different trend occur when the weight of ground coffee is reduced to 12 g, with a decrease at 4 mm (filter basket: A – 3368 mg L^{-1} and B - 3113 mg L^{-1}) and 6 mm (filter basket: A – 3844 mg L^{-1} and B - 3407 mg L^{-1}); and a decrease at 5 mm (filter basket: A – 3450 mg L^{-1} and B - 4120 mg L^{-1}); and at 7 mm (filter basket: A – 3851 mg L^{-1} and B - 4782 mg L^{-1}).

In Robusta, it can be seen that the concentration of caffeine had a considerable increase. More than a third of bioactive compounds at 12 g with the heights of the perforated disc at 4 mm-6 mm have been increased in fact their concentration of approximately 20%.

Therefore, filter baskets and perforated discs have a different effect on the contents of bioactive compounds, while reducing the weight of ground coffee used for the extraction. The aim of this investigation is to obtain closer data after reducing 14 g of ground coffee to 12 g, by combining various auxiliary devices and different models of filter baskets.

Table 4.2. Concentration (mg/L) of acetic acid, caffeine, caffeic acid, citric acid, 5-caffeoylquinic acid, malic acid, nicotinic acid, tartaric acid and trigonelline, obtained in EC samples of Arabica (n=3, RSD%, 0.29-28.9%) and Robusta (n=3, RSD%, 0.26-29.5%). Extractions of EC were performed by using 12 and 14 grams of ground coffee with various heights of the perforated disc (4mm-7mm), and two filter baskets with different percolation constraints (different size of the holes).

Arabica Mass of ground coffee	Filter A Perforated disc, mm	Acetic acid mg/L±RSD	Caffeine mg/L±RSD	Caffeic acid mg/L±RSD	Citric acid mg/L±RSD	5-Caffeoylquinic acid mg/L±RSD	Malic acid mg/L±RSD	Nicotinic acid mg/L±RSD	Tartaric acid mg/L±RSD	Trigonelline mg/L±RSD
14 g	4	537±26.12	3247±0.97	27±2.40	511±5.12	1894±2.29	92±8.59	110±17.33	258±0.38	149±3.36
14 g	5	971±5.45	4206±2.36	53±11.0	608±1.77	3912±3.03	233±21.3	117±11.05	533±1.46	122±3.19
14 g	6	1135±8.85	4299±0.53	54±8.54	939±1.76	3589±0.38	246±1.85	191±6.05	512±2.65	126±17.17
14 g	7	1669±1.46	5766±1.22	71±2.45	1033±3.27	5047±0.70	359±1.35	179±6.21	704±4.18	206±11.4
12 g	4	783±9.38	3368±3.55	44±11.11	528±4.90	2815±6.49	207±8.66	107±3.75	409±5.98	109±4.35
12 g	5	761±5.77	3450±4.49	43±4.54	573±15.5	2950±2.25	226±4.57	109±10.19	420±2.42	81±28.49
12 g	6	1243±2.25	3844±8.50	47±3.32	904±0.71	3397±4.39	210±0.29	131±7.48	475±8.30	115±15.18
12 g	7	749±4.39	3851±3.16	47±3.39	820±9.80	3217±2.76	263±3.70	148±12.31	467±2.42	147±0.41

Robusta Mass of ground coffee	Filter A Perforated disc, mm	Acetic acid mg/L±RSD	Caffeine mg/L±RSD	Caffeic acid mg/L±RSD	Citric acid mg/L±RSD	5-Caffeoylquinic acid mg/L±RSD	Malic acid mg/L±RSD	Nicotinic acid mg/L±RSD	Tartaric acid mg/L±RSD	Trigonelline mg/L±RSD
14 g	4	585±8.22	6833±3.01	118±4.06	693±6.94	3434±2.36	171±5.13	136±9.78	332±3.02	188±12.82
14 g	5	1127±3.90	11537±4.22	215±0.30	1212±3.55	5827±3.26	353±1.55	140±3.95	593±4.25	266±20.55
14 g	6	1166±4.26	10370±0.12	185±0.72	1116±4.38	5422±1.48	266±6.27	111±7.99	513±0.55	207±1.21
14 g	7	1402±3.39	8885±0.45	120±0.58	1311±6.46	3689±1.19	314±4.44	141±13.60	460±0.24	200±1.94
12 g	4	440±1.80	5057±3.28	90±5.77	463±17.4	2474±6.74	119±7.87	111±16.07	243±3.16	144±6.20
12 g	5	546±0.95	5703±0.44	105±3.58	523±9.74	2880±1.22	168±5.05	71±1.87	282±0.64	165±12.41
12 g	6	1053±11.15	9575±1.77	163±1.38	1012±5.25	4839±1.43	271±6.61	107±2.09	462±2.63	189±3.02
12 g	7	804±0.59	5193±0.81	71±1.95	724±8.13	2107±0.26	212±8.86	85±9.43	267±1.77	122±1.93

Arabica Filter B

Mass of ground coffee	Perforated disc, mm	Acetic acid mg/L±RSD	Caffeine mg/L±RSD	Caffeic acid mg/L±RSD	Citric acid mg/L±RSD	5-Caffeoylquinic acid mg/L±RSD	Malic acid mg/L±RSD	Nicotinic acid mg/L±RSD	Tartaric acid mg/L±RSD	Trigonelline mg/L±RSD
14 g	4	825±0.16	3812±0.96	46±9.32	575±6.50	3358±3.18	186±6.04	84±1.06	474±0.42	127±28.94
14 g	5	1243±2.25	5076±4.91	61±3.45	999±14.87	4566±6.81	273±20.58	148±10.83	642±5.36	143±5.11
14 g	6	1035±3.12	3786±3.05	46±16.57	741±8.91	3341±2.43	209±0.73	164±8.70	465±3.02	115±11.56
14 g	7	1451±5.83	4852±3.60	62±13.18	1095±7.47	4267±1.60	271±8.06	167±13.82	577±1.21	168±9.52
12 g	4	723±0.44	3113±1.42	35±5.53	470±10.4	2711±4.01	160±4.54	87±17.87	384±0.28	110±2.92
12 g	5	749±4.10	4120±6.72	58±9.27	644±3.45	3280±6.44	189±2.57	105±9.32	434±5.09	136±24.27
12 g	6	852±0.68	3407±3.06	43±15.05	607±2.60	2717±1.46	191±5.56	142±22.8	389±2.07	93±9.53
12 g	7	1323±13.07	4782±9.38	58±10.95	1003±21.3	4216±5.14	298±17.32	164±10.84	578±8.32	147±14.96

Robusta Filter B

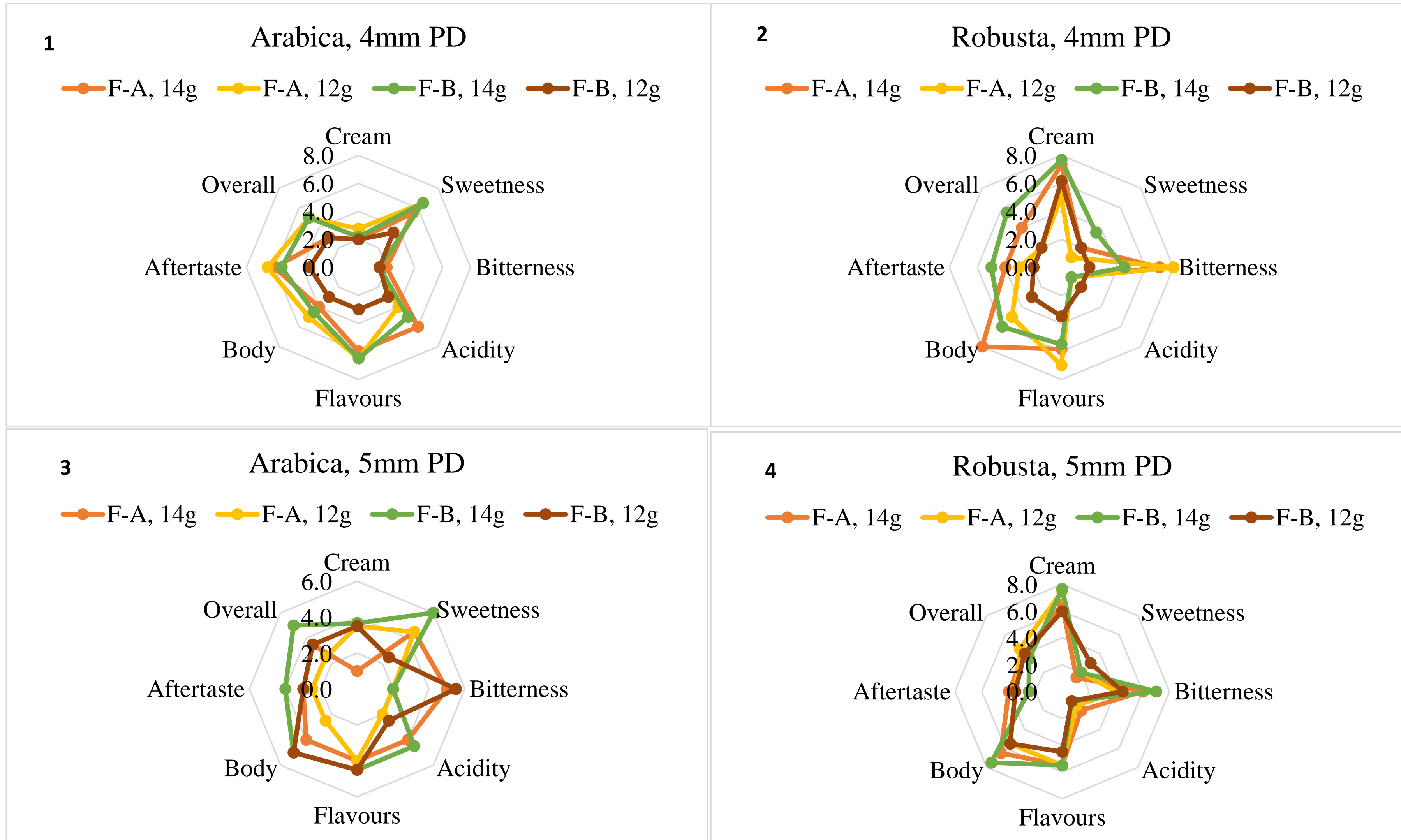
Mass of ground coffee	Perforated disc, mm	Acetic acid mg/L±RSD	Caffeine mg/L±RSD	Caffeic acid mg/L±RSD	Citric acid mg/L±RSD	5-Caffeoylquinic acid mg/L±RSD	Malic acid mg/L±RSD	Nicotinic acid mg/L±RSD	Tartaric acid mg/L±RSD	Trigonelline mg/L±RSD
14 g	4	504±2.52	5989±3.71	107±6.01	535±7.12	2897±7.03	152±15.4	140±15.85	281±4.92	161±3.55
14 g	5	1234±22.6	12108±7.05	216±5.93	1602±15.3	6151±4.67	327±3.99	296±9.61	596±5.25	284±29.3
14 g	6	1085±2.73	9868±3.42	173±3.74	1272±9.26	4988±0.90	299±21.4	116±13.0	475±1.80	201±6.92
14 g	7	1337±1.98	8695±0.25	121±1.60	1262±9.56	3641±0.80	303±7.91	108±11.13	442±1.26	185±5.27
12 g	4	395±3.75	4859±2.97	82±6.24	410±2.10	2180±1.07	113±11.29	106±12.72	219±2.28	157±15.77
12 g	5	526±17.58	6403±5.36	115±6.33	631±5.46	3099±4.67	189±6.25	124±1.81	306±5.11	142±4.79
12 g	6	678±1.09	6115±0.25	115±0.85	683±0.31	3118±5.97	195±16.68	64±9.71	299±0.78	131±2.66
12 g	7	860±4.74	5494±5.14	76±5.45	835±5.07	2176±2.98	207±8.8	93±3.58	281±6.21	128±16.10

4.3.5 Sensory evaluation outcomes

In general, the acidity of food indicates the presence of some organic anions (e.g. citrate, malate, lactate) together with inorganic anions (e.g. phosphate) (Demigne et al.,2004). The correlation of acidity with bitterness has been accepted as an essential sensory attribute in coffee, describing in fact the quality of the beverage, regardless it is favoured or not by consumers. Commonly, consumers do not please the acidity notes, which are not considered as a good quality. It can be explained that the content of acid may interact with taste buds of the tongue and it may vary between individuals (e.g. depends on receptors located in the individual tongue) (Lingle, 2011; Dunkel et al., 2014). In sensory evaluation, flavour notes are described in association with certain fruits or vegetables. A well-known example is the one of citric acid fresh flavour correlated with lemon and orange. Another one is instead the malic acid, associated with many fruits, but mostly responsible for the flavour notes of green apple, plum or peach in the beverage. On the other hand, the excess of acids present negative flavour. As an example, high concentration of tartaric acid can give more sour taste, while low concentration of tartaric acid can present grape-like or winey notes. (Theron et al., 2007).

In this study, the samples are evaluated by professional tasters from Simonelli Group S.p.A. The evaluation results followed the coffee tasting protocol, in which the sections of protocol are thoroughly controlled by panellists. In the protocol tables, there are also sub-sections: for instance, the “cream” section consists of colour, texture and persistency, the “flavours” section is divided into sour/fermented, fruity, floral, sweet, nutty/cocoa, spices, roasted, other and green vegetables, while the rest (sweetness, bitterness, acidity, body, aftertaste and overall) is without sub-sections. The final scores are combination of sub-sections (the maximum score is 9), and the average scores are presented in the Figure 4.4 (1-8). The radar charts are divided by the cultivars and heights of the perforated disc. Each radar chart displays two different filter baskets with two different amounts of coffee used in the extraction of EC (14 g and 12 g). The results of this study indicate that higher sweetness notes are in Arabica, whereas the dense cream and more bitterness are in Robusta. Arabica samples with different heights of the perforated disc (4 mm-7 mm) highlight that the highest sweetness and aftertaste are found particularly at 4mm, in filter basket B with 14 g, and in filter basket A with 12 g. In the sensory attributes for Robusta with two filter baskets and different heights of the perforated disc, the results are higher in the sections of flavours and body. This finding supports the work of other studies (Kreum et al., 2003), which have demonstrated that Robusta coffee beverages have generally higher bitterness notes, while Arabica has higher perceived acidity and aroma.

Figure 4.4 (1 and 2) shows a few significant low scores of EC samples prepared with Arabica and Robusta. These scores can be read in connection with filter basket B and weight of ground coffee (12 g), where the sensory results have shown similar alterations in the sensory notes, except at 4 mm with 12 g in filter basket B (F-B, 12 g) for Arabica and Robusta. This inconsistency may be due to the distance between the solid (coffee cake) and the liquid (shower), which might also impact on EC flavour. This interpretation is partially consistent with the concentrations found with instrumental analyses in Table 4.2. The concentrations of samples at 4 mm with 12 g for filter basket B used for Arabica and Robusta are approximately 2-5 % different from those of samples at 5 mm with 12 g used for Arabica and Robusta. In the sensory report, the outcomes of 5 mm with 12 g in filter basket B (F-B, 12 g) were assessed as in line with the average found with other filter baskets. Multiple regression analysis revealed that the amounts of coffee, 12 g and 14 g, used in filter baskets to extract EC determined the physical characteristics of the packing bed by the modification of the heights of the perforated disc (4-7 mm). Excessive amount of coffee normally does not allow sufficient expansion of coffee grounds during wetting, which might cause over-compaction, disrupted percolation, and solid deposition in the cup. This study has observed, with regard to 14 g in the 7mm of the perforated disc, the reduction of bed permeability, while the 12g in 4mm of the perforated disc has performed slightly different scenario in terms of physical and chemical analyses. Although the present research has combined very different approaches, namely, the quantification of compounds in EC and the sensory evaluation of EC, scientific repetitions could be done, and the findings, both in quantitative and qualitative terms, will not likely be affected neither with respect to the total sensory attributes of EC, nor with regards to the concentration of compounds obtained through analytical instruments.


1
Arabica, 4mm PD
F-A, 14g
F-A, 12g
F-B, 14g
F-B, 12g
Cream
Sweetness
Bitterness
Acidity
Flavours
Body
Aftertaste
Overall
8.0
6.0
4.0
2.0
0.0
2
Robusta, 4mm PD
F-A, 14g
F-A, 12g
F-B, 14g
F-B, 12g
Cream
Sweetness
Bitterness
Acidity
Flavours
Body
Aftertaste
Overall
8.0
6.0
4.0
2.0
0.0
3
Arabica, 5mm PD
F-A, 14g
F-A, 12g
F-B, 14g
F-B, 12g
Cream
Sweetness
Bitterness
Acidity
Flavours
Body
Aftertaste
Overall
6.0
4.0
2.0
0.0
4
Robusta, 5mm PD
F-A, 14g
F-A, 12g
F-B, 14g
F-B, 12g
Cream
Sweetness
Bitterness
Acidity
Flavours
Body
Aftertaste
Overall
8.0
6.0
4.0
2.0
0.0

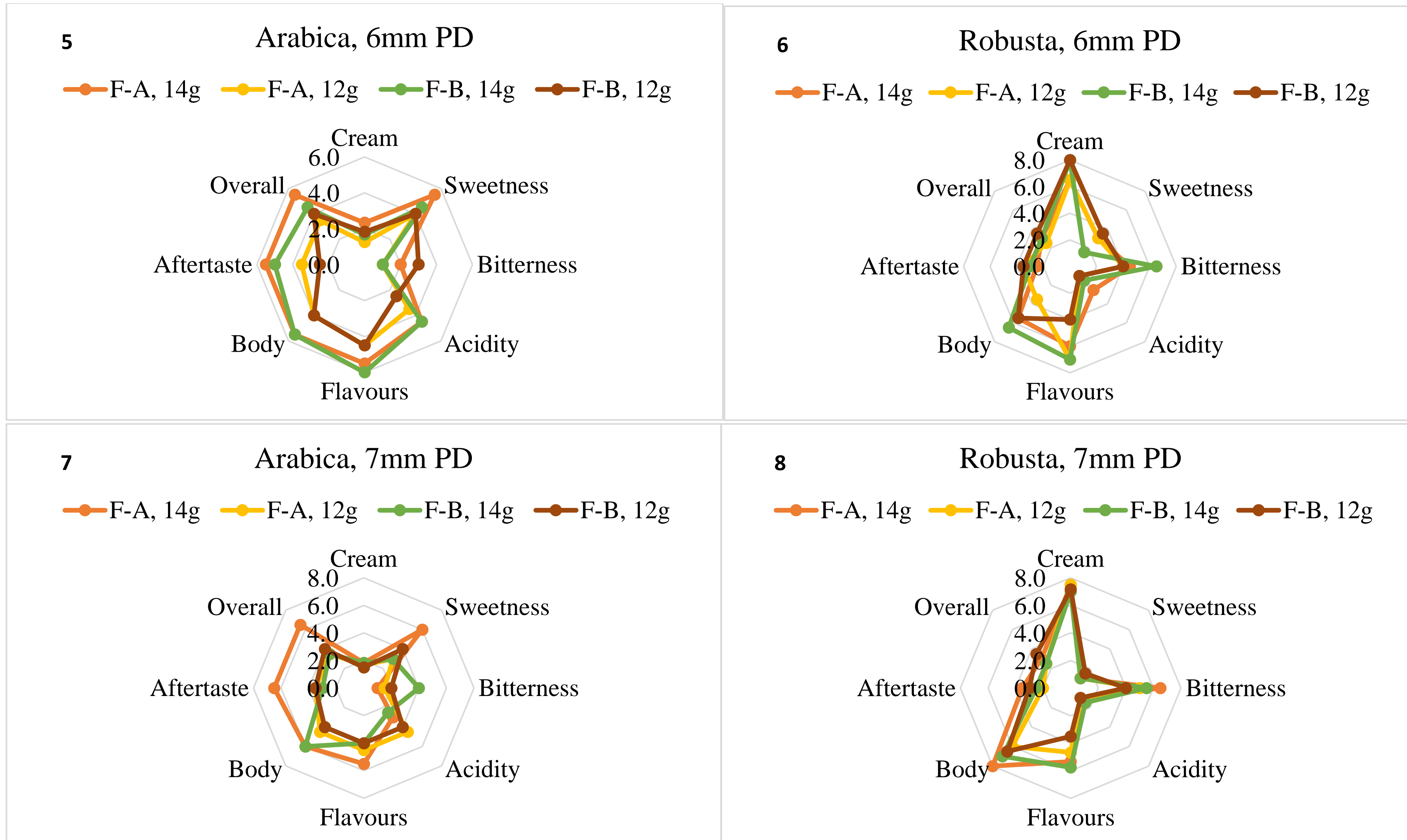
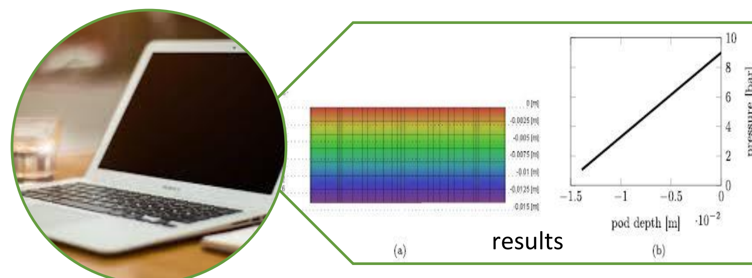


Figure 4.4 (1-8) shows the results of sensory evaluation of EC extracted with different heights of perforated discs (4mm-7mm), different amount of ground coffee (14-12 g), and different filter baskets (A-B, with different percolation constraints, namely, different size of the holes).

Study 3. Mathematical simulation model

This study is the result of an interdisciplinary collaboration with mathematicians for making a step forward in the optimization of the EC extraction. The research work is based on extraction of espresso coffee with different temperature (88C, 93C, 98C), pressure (7bar, 9bar, 11bar) and tamping force (10kgF, 15kgF, 20kgF, 30kgF). The experimental results obtained through chemical analyses are then applied, with an interdisciplinary approach, into the mathematical model.

$$\frac{\partial}{\partial t}\left(\sum_j B_j\phi_j\right)d\Omega^e + \sum_{e=1}^{N_e}\int_{\Omega^e} B_i\mathbf{q}\cdot\nabla\left(\sum_j B_j\phi_j\right)d\Omega^e$$
$$+\sum_{e=1}^{N_e}\int_{\Omega^e}\nabla B_i\cdot\left(\mathbf{D}\cdot\nabla\left(\sum_j B_j\phi_j\right)\right)d\Omega^e$$
$$-\sum_{e=1}^{N_e}\int_{\Omega^e} B_i\left(\nabla\cdot\mathbf{q}\left(\sum_j B_j\phi_j\right)-Q\right)d\Omega^e$$
$$\sum_{e=1}^{N_e}\int_{\Gamma_N^e} B_i q_N\, d\Gamma^e - \sum_{e=1}^{N_e}\int_{\Gamma_C^e} B_i\Phi\left(\phi_C-\left(\sum_j B_j\phi_j\right)\right)d\Gamma^e$$
$$1\le i,j\le N, \qquad (11)$$



5.1 Introduction

Many physical and chemical-characterization studies have been focused on different coffee brews. Among them, the extraction of espresso coffee (EC) is peculiar, because EC is a complex brew, less stable in terms of volatiles and foam. The fast alteration after brewing demands a proper understanding of the effect on the beverage of the physical and chemical variables during the extraction (Illy et al., 2009). The peculiarity of EC is due in fact to various concentrated substances such as acids, sugars, salts, caffeine and other hydrophilic compounds (Illy et al., 2009). Each group of substances has a considerable impact on the final cup. In order to assess the constituents of EC, accurate chemical methods can identify the precise content of substances in EC (Caprioli et al., 2014). The large variety of studies in food chemistry dealing with coffee brewing has stimulated the feasibility study of a mathematical model able to describe the complex process of coffee extraction from the physical and chemical points of view. A low number of studies has been developed in such physicochemical modelling of coffee extraction. One of the first models has been provided in Fasano, Talamucci, and Petracco (2008). It shows three levels of difficulty that reflect three levels of model formulation with increasing refinements. The first model contains the peculiar features of the extraction process, such as the fluid flow and the removal and transport of a single solid particle for a specific compound; the second one adds the transport of more solid particles, each representative of a different compound; the third one considers the dissolution process and the formation of a compact layer. All of them are one-dimensional and theoretical; in fact, they involve functions with precise formal properties but not showing a full definition. Another model has been provided in Moroney et al. (2016), where some operational assumptions are used to simplify the model formulation. This last version is also used to show some numerical simulations. A recent application of this one-dimensional model considering only one soluble compound can be found in Moroney et al. (2019), where it is coupled with a spatially resolved fluid model. In particular, this model is used to investigate the coffee extraction uniformity; the numerical results from the model are compared with the experimental data, with a major focus on the influence of non-uniform flow on the extraction uniformity.

In this study, we propose a research on the modelling of the EC extraction process, in order to identify the organoleptic and nutritional characteristics of an espresso beverage. The proposed model considers the dynamics of the fluid flow coupled with the dissolution/erosion, diffusion and transport of the relevant soluble compound in a cup of coffee and of solid fine particles. In addition to the fluid-

fluid and fluid-solid flow, the model also considers the energy transport and diffusion. Moreover, each selected compound is modelled by a dedicated equation, since it has its own chemical and physical characteristics that cannot be clustered with those of other compounds. In particular, a detailed physicochemical model of water percolation into a porous medium is considered. Starting from the knowledge of some physical parameters of the extraction process and the concentration of some relevant chemical compounds in the coffee powder, such model computes the chemical characteristics of the EC in terms of the final concentration of those compounds in the coffee beverage.

The reliability of the proposed model is assessed on an experimental basis, by comparing the results of the simulation model with chemical laboratory analyses on espresso coffee samples collected under different extraction conditions (water temperature, water pressure and powder tamping). The first results show that the proposed model is a good starting point in the physicochemical formulation of the coffee extraction process. However, the complete description of the coffee percolation requires further investigations to enlarge the number of chemical compounds taken into account, to refine the choice of modelling parameters and to analyse the soundness of the mathematical formulation of the percolation process.

The study is organised as follows. Section 5.2 presents the mathematical model of water percolation into the porous media. Section 5.3 describes the methodologies used for the chemical laboratory analyses on the ground coffee samples and the espresso coffee samples, providing also the laboratory results. Section 5.4 shows the results of the numerical simulations with the proposed model and compares these numerical results with the laboratory data reported in the previous sections; these preliminary tests over real data allow to obtain a degree of reliability of the model.

5.2 The percolation modelling

The extraction of EC consists in these phases: pressurised hot water enters the basket filled with the roast and ground coffee suitably tamped, it flows through the void spaces among coffee grains and, at the same time, it dissolves various chemical substances from the wetted coffee grains, while removing some amount of fine particles from the coffee powder. This extraction process is referred to as a percolation process, which can be described by means of the following fluid-dynamics components: the fluid flow within the porous matrix, the dynamics of the dissolved chemical compounds and oily substances extracted by the warm fluid from the porous matrix, the dynamics of the fine particles removed by the fluid from the porous matrix, and the heat exchange between the fluid and the porous matrix. So, a general percolation model is a system of these equations: the mass

balance equation, the transport equation, and the momentum balance equation for the fluid phase. A general formalisation of percolation processes can be found in Bear and Bachmat (1990) and Vafai (2015). In the proposed model, we divide the substances taking part in the percolation process into fluid compounds and solid compounds. The fluid compound consists in the carrier fluid, i.e. the water, together with the solutes and the oily substances. They form the fluid phase, which in the particular case considered here can be assumed as the liquid phase since no gaseous phase is imagined into the pores. The solid compound encompasses all the particles that are not fluid and belong to the porous medium. In this model, we consider some simplifying hypotheses that seem appropriate in the case of the coffee extraction, also in relation to analogous phenomena like water infiltration in soils (Hasson et al., 2018) and the transport of contaminants (Pinder et al., 2006). So, the most important assumptions for our model are (Pinder et al., 2006; Nield et al., 2006; Diersch et al., 2013):

• the porous medium is considered homogeneous and isotropic. The property of homogeneity has to be intended in this way: the ground coffee is a packing of particles with different size but their physical and chemical characteristics remain constant among the grains independently of size and position in space;

• the motion of the fluid flow into the porous medium is slow, so the inertia effects appearing in the momentum equation for the fluid are negligible; besides, being the pore Reynolds number (*Re*) sufficiently small (it is possible to estimate the Reynolds number using the medium size of the grains in the porous medium and the fluid velocity in this case study as $Re \approx 10^{-1}$), the momentum equation for the liquid phase is simplified by the Darcy equation. In fact, when the length scale of the problem is greater than $(\mathrm{k}\mathbf{K}\mathrm{k}/\varepsilon)^{1/2}$ the Brinkman term becomes negligible in comparison to the Darcy term (Diersch, 2013); in our study cases $(\mathrm{k}\mathbf{K}\mathrm{k}/\varepsilon)^{1/2} \leq 7 \cdot 10^{-4}$ and the length scale of the problem is $\approx 10^{-2}$. Also the Forchheimer term, which provides a quadratic drag term due to surface friction (Diersch, 2013), is discarded in this model since the pore Reynolds number is sufficiently small; we note that such term is usually considered when $Re > 10$ in order to improve the approximation of the flow into the porous medium provided by the laminar flow regime (Diersch, 2013);

• liquid mass balance equation is coupled with solid mass balance equation by the divergence of the solid mass velocity; this can be seen by substituting in the first equation the divergence term of the fluid mass velocity with a divergence term involving the solid mass velocity. Assuming a slowly deformable porous medium and a slightly compressible fluid, such solid-divergence term can be written as function of porosity, exploiting the solid mass balance equation; in turn, porosity can be considered as depending only on liquid pressure by introducing a compressibility coefficient of the

porous medium. In this way, the original solid-divergence term can be rewritten using only liquid phase variables, so the liquid and solid equations become decoupled. As a consequence, there is no need to solve explicitly the momentum and mass equations for the solid phase, so the solid phase is modelled only implicitly by its compression. The complete procedure is reported in Diersch (2013);

• some compounds can occur in the model both in liquid and solid phases; moreover, a compound appears in the liquid or solid phase independently of its actual physical state but on the base of its involvement or not in the transport process; in particular, a fine solid particle, which is clearly a solid compound from the physical point of view, is considered liquid if it is involved in the mass transport by the fluid flow; in the same way, a soluble compound is considered solid if it is bounded to the porous medium during the percolation process;

• Oberbeck-Boussinesq approximation is assumed; it consists in considering all the thermo-physical properties constant except for the density of the fluid in the buoyancy term (ρg in the momentum equation) which depends linearly on the local temperature;

• the percolation process has a total duration of about 25 s (seconds), water pours out after a short initial time lapse (5 s) necessary for the imbibition of the porous medium, thus we assume that, after the imbibition, the medium is saturated and the gaseous phase definitely does not interfere with the process; unsaturated flow during imbibition is not considered;

• expansion of the porous medium due to the swelling of coffee particles when wetted by water is discarded;

• local thermal balance between the liquid and solid phases is assumed after the initial imbibition.

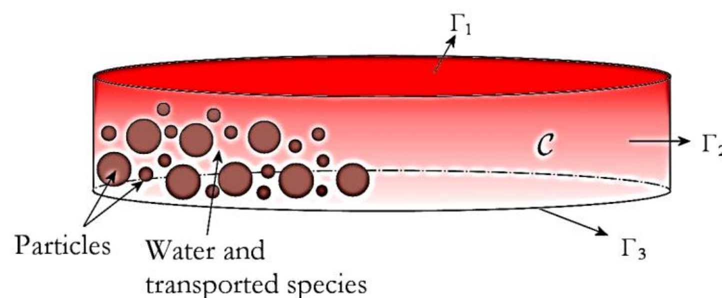


Figure 5.1 Scheme of the geometry of the espresso pod.

Symbols occurring in the percolation model with a brief description.

Symbol	Description
h	hydraulic head
p	pressure
$\mathbf{q}$	fluid flux
C_k	mass concentration of liquid/solid species k
C^s_m	mass concentration of solid species m
ε	porosity
ε_s	solid volume fraction
S_0	specific storage coefficient
$\mathbf{K}$	hydraulic conductivity tensor
f_μ	viscosity relation function
χ	buoyancy coefficient
$\mathbf{e}$	$(0, 0, 1)^T$
$\mathbf{j}_k$	hydrodynamic diffusion/dispersion vector of species k
$\mathbf{D}_k$	hydrodynamic diffusion/dispersion tensor of species k
D_k	molecular diffusion coefficient of species k
β^k_L, β^k_T	longitudinal and transverse dispersion coefficients of species k
Q	sources/sinks of water
R_k	total reaction rate of the species k
R^s_m	total reaction rate of the species m
T	temperature
T_0	reference temperature
ρc	volumetric heat capacity of the fluid
$\rho^s c^s$	volumetric heat capacity of the solid
$\mathbf{\Lambda}$	thermal hydrodynamic conductivity
Λ, Λ^s	thermal conductivities of fluid and solid phases
γ_L, γ_T	thermal longitudinal and transverse dispersion coefficients
H_e	sources/sinks of internal energy

Table 5.1 Description of symbols in the percolation model

The spatial domain of our problem, sketched in Figure 5.1, is a circular cylinder C whose symmetry axis is along the z-axis of a Cartesian three-dimensional coordinate system and whose top circular face Γ_1 lays on the plane $z = 0$ and has radius R. The height of the cylinder is $H > 0$, thus the bottom circular face Γ_3 lays on the plane $z = -H$. On the base of the previous assumptions, the model that we propose for the physicochemical description of the EC percolation is:

$$\begin{cases} S_0 \frac{\partial h}{\partial t} + \nabla \cdot \mathbf{q} = Q \\ \mathbf{q} = -\mathbf{K} f_\mu \cdot (\nabla h + \chi \mathbf{e}) \\ \varepsilon \frac{\partial C_k}{\partial t} + \mathbf{q} \cdot \nabla C_k + \nabla \cdot \mathbf{j}_k = R_k - C_k Q, & k = 1, \ldots, N_{l\text{-}s}, \\ \varepsilon_s \frac{\partial C^s_m}{\partial t} = R^s_m, & m = 1, \ldots, N_s, \\ (\varepsilon \rho c + \varepsilon_s \rho^s c^s) \frac{\partial T}{\partial t} + \rho c \mathbf{q} \cdot \nabla T - \nabla \cdot (\mathbf{\Lambda} \cdot \nabla T) = H_e - \rho c (T - T_0) Q, \end{cases} \quad (1)$$

where each equation is prescribed in C, $t \in (0, \tau)$, where $\tau > 0$ is the percolation duration. The symbols appearing in formula (1) and in the forthcoming formulas are reported in Table 5.1. The unknowns of formula (1) are the hydraulic head, h, directly connected to the Darcy flux $\mathbf{q}$, the mass concentrations of the chemical compounds C_k, C^s_m, the temperature T of the fluid and the solid matrix.

Models similar to (1) are widely used for simulating geological phenomena in (saturated) confined aquifers with transport of relevant substances, such as contaminants. The first equation in formula (1) is the Richards equation for the fluid flow in the saturated porous medium, where the flux term is given by the Darcy law (second equation). The third equation gives the transport rule for the compound k with concentration C_k that can be liquid or solid; in other words, it does not make any difference if the compound is a solute or a fine solid particle; the characteristic for a compound to appear in this equation is to undergo transport and diffusion processes. There are N_{l-s} equations of this type, one for each liquid-solid chemical compound to be traced. Conversely, the fourth equation describes the mass conservation for the compound m with concentration C_m^s that can be only solid and bounded to the porous medium, as highlighted by the superscript s; these compounds are not involved in the transport and diffusion phenomena. There are N_s equations of this type, one for each solid chemical compound to be monitored into the porous medium. The last equation is the heat equation in the porous medium where both the convective and diffusive processes are considered.

In particular, in the Richards equation, the hydraulic head is defined as $h = \psi + z$, with $\psi = p/\rho_0 g$ the pressure head, p the pressure, ρ_0 a reference mass density of the fluid, and g the gravity acceleration; S_0 is the specific storage coefficient defined as $S_0 = \rho_0 g(\varepsilon\gamma + \mathrm{V})$ with γ the fluid compressibility and V the porous matrix compressibility, ε is the porosity that is defined as the ratio between the volume of empty spaces and the total volume of the porous medium; the term Q represents sources/sinks of liquid mass. In the Darcy law, **K** is the hydraulic conductivity tensor; f_μ is the viscosity relation function; χ is the buoyancy coefficient, $\mathbf{e} = (0,0,1)^T$. Here, the buoyancy coefficient χ and the viscosity relation function f_μ gather the effects of the actual pressure, compound concentration and temperature on the liquid density and the liquid viscosity. In the third equation, $\mathbf{j}_k$ is the hydrodynamic diffusion/dispersion vector defined by the Fick's law:

$$\mathbf{j}_k = -\mathbf{D}_k \cdot \nabla C_k.$$

where the hydrodynamic dispersion tensor, $\mathbf{D}_k$, consists of the molecular diffusion part $\varepsilon D_k I$ and the mechanical diffusion part $\mathbf{D}_{\mathrm{mech}}$, that is:

$$\begin{aligned}\mathbf{D}_k &= \varepsilon D_k I + \mathbf{D}_{\mathrm{mech}} \\ &= \left(\varepsilon D_k + \beta_T^k \|\mathbf{q}\|\right) I + \left(\beta_L^k - \beta_T^k\right) \frac{\mathbf{q} \otimes \mathbf{q}}{\|\mathbf{q}\|},\end{aligned} \tag{2}$$

where $\otimes$ is the tensor product, I is the identity matrix, D_k is the molecular diffusion coefficient, $\beta_T{}^k, \beta_L{}^k$ are the transverse and longitudinal dispersion coefficients. In turn, the molecular diffusion can be estimated from the diffusion coefficient in an open fluid body D_k, taking into account the tortuosity T_* of the porous medium. In our case, being the porous medium isotropic, T_* is a scalar, and its estimation is obtained by Millington formula in Bear and Cheng (2010): $T_* = \theta^{7/3}/\varepsilon$, where θ denotes

the liquid volume fraction; besides, being the porous medium saturated, θ can be naturally approximated by the porosity ε and we obtain, $D_k = T_* D_k \approx \varepsilon^{4/3} D_k$. We note that, in the case of multicomponent mixtures, the Maxwell-Stefan equation (Boudin, Grec, and Salvarani, 2012) provides a more rigorous approach to define $\mathbf{j}_k$. However, since the direct proportionality between flux and concentration gradient provides a reasonable approximation of the diffusion process in many common situations, we choose the classical Fick's law for the description of the diffusion vector. Finally, R_k is the total reaction rate of the compound k (Diersch, 2013). In the fourth equation, $\varepsilon_s = 1 - \varepsilon$ is the solid volume fraction; and R^s_m is the total reaction rate of the solid compound m. In the last equation in (1), $\rho c, \rho^s c^s$ are the volumetric heat capacity of the fluid and solid, respectively; T_0 is a reference temperature; $\mathbf{\Lambda}$ is the thermal hydrodynamic conductivity tensor defined similarly to $\mathbf{D}_k$. In fact:

$$\begin{aligned}\mathbf{\Lambda} &= (\varepsilon\Lambda + \varepsilon_s\Lambda^s)\boldsymbol{I} + \rho c\tilde{\mathbf{D}}_{\text{mech}} \\ &= (\varepsilon\Lambda + \varepsilon_s\Lambda^s + \rho c\gamma_T\|\mathbf{q}\|)\boldsymbol{I} + \rho c(\gamma_L - \gamma_T)\frac{\mathbf{q}\otimes\mathbf{q}}{\|\mathbf{q}\|},\end{aligned} \tag{3}$$

where $\tilde{\mathbf{D}}_{\text{mech}}$ is the thermal mechanical diffusion tensor, Λ, Λ^s are the thermal conductivities of the fluid and solid phases, respectively, and γ_T, γ_L are the thermal transverse and longitudinal dispersion coefficients, respectively. Finally, H_e describes all the internal sources/sinks of energy.

Formula (1) must be endowed with constitutive relations for the closure of the problem, i.e. expressions that define χ, f_μ. Details on the constitutive relations for this model can be found in Diersch (2013).

Formula (1) must be equipped with boundary and initial conditions. The conditions for the hydraulic head are:

$$\begin{cases} h = h_{z0}, & \text{on } \Gamma_1, t > 0, \\ \frac{\partial h}{\partial r} = 0, & \text{on } \Gamma_2, t > 0, \\ \mathbf{q}\cdot\mathbf{n} = -\Phi_h \min\{h_C - h, 0\}, & \text{on } \Gamma_3, t > 0, \\ p = p_0(z), & \text{in } C, t = 0, \end{cases} \tag{4}$$

where p_0 is a prescribed initial pressure that automatically defines an initial condition for h, $h_{z0} = p_0(z = 0)/\rho_0 g$ so that compatibility condition holds, $\mathbf{n}$ is the outward unit normal vector, h_C is a prescribed value for the hydraulic head and Φ_h a prescribed transfer coefficient such that, when $h > h_C$, an outward flux starts with rate $\Phi_h(h - h_C)$. Thus, Φ_h plays the role of the admittance of the filter at the base of the coffee pod. In more detail, the fluid at the bottom of the porous medium flows through this filter and subsequently drops into the cup, and, when passing through the filter, the fluid encounters a resistance mainly due to the geometric structure of the filter (the micrometric pores).

So, we simulate the filter through the action of a resistance that fully operates when a sufficient amount of fluid accumulates on the bottom of the medium. The conditions for the concentration C_k of the liquid/solid compound k are:

$$C^s_m = C^s_0, \quad \text{in } \mathcal{C}, t = 0, \qquad (6)$$

where C_{kC} is a prescribed value of concentration that, once reached, i.e. $C > C_{kC}$, activates an outward-directed mass flux with rate $\Phi_k(C - C_{kC})$. Again, Φ_k can be interpreted as the admittance of the filter for the compound k, as discussed above for the hydraulic head. Besides, we prescribe a Neumann condition on the top and lateral faces as usual when no further changes in the solution have to be taken into account.

The solid compound m needs only the initial condition:

$$\begin{cases} T = T_{z0}, & \text{on } \Gamma_1, t > 0, \\ \nabla T \cdot \mathbf{n} = 0, & \text{on } \Gamma_2, \Gamma_3, t > 0, \\ T = T_0, & \text{in } \mathcal{C}, t = 0, \end{cases} \qquad (7)$$

where C^s_0 is the concentration of the compound m into the porous medium (coffee powder) before the extraction process.

Finally, the conditions for the temperature are:

$$\begin{cases} \nabla C_k \cdot \mathbf{n} = 0, & \text{on } \Gamma_1, \Gamma_2, t > 0, \\ -(\mathbf{D}_k \cdot \nabla C_k) \cdot \mathbf{n} = -\Phi_k \min\{C_{kC} - C_k, 0\}, & \text{on } \Gamma_3, t > 0, \\ C_k = 0, & \text{in } \mathcal{C}, t = 0, \end{cases} \qquad (5)$$

where the T_{z0} is the temperature of the incoming water and T_0 is the initial temperature (after the imbibition of the porous medium) of the system water-porous matrix. The Neumann condition at the lateral and bottom faces may seem to complicate the problem but it is due to the lack of knowledge of the real temperature profile on those faces.

5.3 Chemical analyses

Caffeine, the most studied compound in coffee due to its stimulating effect on humans, becomes easily soluble in water at certain temperatures (Kurzrock et al., 2001). Caffeine extraction from ground coffee into EC ranges usually from 75 up to 85% of the total content in the coffee powder. This is due to the short time of the EC extraction, which does not allow to releasing all the caffeine withheld in the cellular structure of the ground coffee. Therefore, caffeine amount in the

coffee brew ranges from 60 mg (milligrams) for cultivars of pure Arabica blends up to 130 mg for pure Robusta blends (Caprioli et al., 2015).

Coffee features also one of the highest concentrations of chlorogenic acids among food items for human consumption. Chlorogenic acids generate the bitterness flavour of coffee due to their decomposition in phenolic compounds during the roasting process. When roasting the coffee beans, chlorogenic acids turn into caffeoylquinic acids (CQA), dicaffeoylquinic acids (diCQA) and feruloylquinic acids (FQA) (Jeon et al., 2017). The total amount of chlorogenic acids can therefore significantly decrease from the green to the roasted coffee (Bicho et al., 2013).

In the EC, the relative number of chlorogenic-acid derivatives vary significantly. However, the total amount of different derivatives is a reliable measure to estimate the chlorogenic acids. The experiments of this study are aimed at the quantification of caffeine and chlorogenic acid concentration by the method developed by Caprioli (2014).

5.3.1 Materials and methods

1. Chemicals: standards of caffeine, trigonelline, nicotinic acid, 5-O-caffeoylquinic acid (5-CQA), 3-O-caffeoylquinic acid (3-CQA) and 3,5-di-O-caffeoylquinic acid (3,5-diCQA), formic acid (HCOOH - 99%), methanol (CH4OH – HPLC gradient) were purchased from Sigma-Aldrich (Milano, Italy).
2. Coffee samples and espresso machine: coffee is 100% Arabica, named Cibao Altura (HMC): origin – Dominican Republic; region – Juncalito Montains; producer – Finca Nunez; variety– typica, cattura; process – fully washed; altitude – 900 masl (metres above sea level); descriptive notes – cashew nuts, cane sugar, dried apricot, were suggested by certified roasters for EC preparation, while the fully automatic espresso machine (Vittoria Arduino, VA388 Black Eagle), the grinding machine (Mythos 2) and tamping machine were provided by the espresso machine manufacturing company Simonelli Group SpA. (Belforte del Chienti, Italy).
3. Ground coffee: the ground coffee was prepared to analyse chlorogenic acids, caffeine, trigonelline and nicotinic acids. 1 g (gram) of ground coffee was mixed with 10ml of water and the solution was stirred 30 min (minutes) at 80°C. Then, 0.5 ml of aliquot and 0.5 ml of mobile phase were transferred into 1 ml vial. The sample was centrifuged at 13000 rpm (revolutions per minute) for 10 min and filtered 0.45 µm (micrometres, PTFE filter) before HPLC-VWD analysis (High Performance Liquid Chromatography with Variable Wavelength Detector).
4. EC sample extraction: 20 g of ground coffee was filled into 20 g VST [c] Competition filter basket (produced by VST Inc. for use in World Coffee Events, especially in World Barista Championship)

and inserted into the portafilter. The mass of extracted EC was kept constant at 40g (unless measurement error within 2 g) and time for extraction was 25±1 s. In accordance to research studies about water influence on EC quality (Navarini et al., 2010), the utilised water was taken always from the same source which was chosen for EC. The extraction of EC was performed with different values of extraction temperature, extraction pressure and tamping pressure, by changing one at a time these settings within a set of prescribed values; the sample for each extraction configuration has been duplicated. These parameters were properly arranged in the tamping machine and in the espresso machine by a skilled professional barista. In all cases, the brew ratio between grams of coffee cake and mass of EC was kept 1:2±0.02 (20 g in and 40±2 g out). All extracted EC samples were immediately collected from the portafilter of the espresso machine in a ceramic espresso cup, and the weight of the extracted EC samples was measured by Acaia balance. The total extracted samples of EC were 72.

5. Sample extraction and HPLC-VWD analysis are described (*Chapter 3.1.4*).

5.3.2 Results of chemical analyses

Five compounds of bioactive components, namely, derivatives of chlorogenic acids (total chloroquine acids (CQA)), caffeine, trigonelline, and nicotinic acid, were quantified in ground and EC. Analytical results for the selected compounds are highlighted in Tables 5.2-5.6, which were used for simulation program of the mathematical model. In Tables 5.2-5.6, the highest and lowest amount of the analysed compound in cup have been highlighted for each tamping pressure when varying water pressure and temperature. In order to determine the highest and lowest amount of the components in EC, the extraction of EC was performed at three different pressures and temperatures (7 bar, 9 bar, 11 bar and 88˚C, 93˚C, 98˚C) and four different tamping forces (10 kgF, 15 kgF, 20 kgF and 30 kgF). [The method validation for HPLC-VWD is described in Chapter 3.1.5]

5.3.3 Caffeine, trigonelline, nicotinic acid and chlorogenic acids in EC

The effect of water temperature, water pressure and tamping pressure on EC extraction has been studied. EC samples (36 samples of EC in duplicate) were analysed for detecting caffeine, trigonelline and derivatives of chlorogenic acids (3-Caffeoylquinic acid, 5-Caffeoylquinic acid and 3,5-di-Caffeoylquinic acid). The extraction of EC was repeated using different values of the three variables (temperature, pressure and tamping), but ground coffee in filter baskets (20 g) and EC in the cup (40g) were constant at 25±1s.

For each variable, the espresso, grinding and tamping machines were tuned manually and calibrated to obtain the exact amount of mass and volume in the cup. The samples were prepared for HPLC-

VWD. The results have showed that the average quantities (in milligrams in the cup) of caffeine, trigonelline and nicotinic acid, and of the total chlorogenic acids slightly vary depending on the different values of the three physical variables. The previous studies (Gloess et al., 2013; Caprioli et al., 2014; Petracco, 2008) confirmed that the chemical composition of coffee can vary considerably depending on the roasting process and the brewing method. As showed in literature by Illy and Viani (2005), the amount of caffeine in arabica is approximately 2.6 mg/ml in 30ml/30s, and on the average, the variation of caffeine in beverages changes between 1.2 mg/ml up to 4 mg/ml depending on preparation, size of cup and blend composition (Navarini et al., 2004). In the experimental data this amount ranges on average 234±15mg per 40ml/26s, which is approximately 5.84±0.4 mg/ml in 40ml/26s.

Table 5.2. Total CQA concentration for EC extraction conditions depending on pressure (in bar), temperature (in ^{0}C) and tamping force (in kgF): for each tamping force, the first column shows the concentration in mg/40 ml, the second column concentration in mg/ml and third column the percentage RSD value.

Conditions	**10kgF**			**15kgF**			**20kgF**			**30kgF**		
P, T	**mg/40ml**	**mg/ml**	**RSD**	**mg/40ml**	**mg/ml**	**RSD**	**mg/40ml**	**mg/ml**	**RSD**	**mg/40ml**	**mg/ml**	**RSD**
7 bar, 88^{0}C	131.84	3.29	2.9	144.31	3.61	1.9	140.67	3.52	3.9	138.49	3.46	5.1
9 bar, 88^{0}C	133.30	3.33	1.6	133.22	3.33	2.9	136.17	3.40	1.6	134.38	3.36	5.7
11 bar, 88^{0}C	128.09	3.20	2.0	131.64	3.29	3.0	128.56	3.21	7.7	125.94	3.15	2.5
7 bar, 93^{0}C	137.54	3.44	2.9	143.14	3.58	3.4	134.98	3.37	0.6	142.65	3.57	0.9
9 bar, 93^{0}C	150.24	3.76	4.2	138.92	3.47	3.5	150.75	3.77	4.1	141.00	3.53	1.7
11 bar, 93^{0}C	136.31	3.41	2.9	134.27	3.36	1.5	139.92	3.50	3.6	138.43	3.46	4.1
7 bar, 98^{0}C	141.60	3.54	1.3	129.41	3.24	6.1	141.94	3.55	1.4	136.02	3.40	0.8
9 bar, 98^{0}C	139.52	3.49	3.8	138.56	3.46	2.0	137.94	3.45	3.0	134.26	3.36	3.9
11 bar, 98^{0}C	138.01	3.45	3.9	137.50	3.44	4.5	133.12	3.33	0.9	134.66	3.37	0.6

Table 5.3. Caffeine concentration for EC extraction conditions depending on pressure (in bar), temperature (in ^{0}C) and tamping force (in kgF): for each tamping force, the first column shows the concentration in mg/40 ml, the second column concentration in mg/ml and third column the percentage RSD value.

Conditions	**10kgF**			**15kgF**			**20kgF**			**30kgF**		
P, T	**mg/40ml**	**mg/ml**	**RSD**	**mg/40ml**	**mg/ml**	**RSD**	**mg/40ml**	**mg/ml**	**RSD**	**mg/40ml**	**mg/ml**	**RSD**
7 bar, 88^{0}C	211.67	5.29	1.7	227.66	5.69	1.6	224.85	5.62	4.3	223.19	5.58	4.0
9 bar, 88^{0}C	234.53	5.86	1.0	232.19	5.80	1.0	236.56	5.91	3.5	226.85	5.67	3.7
11 bar, 88^{0}C	227.24	5.68	0.8	233.24	5.83	6.0	222.95	5.57	4.5	217.60	5.44	0.8
7 bar, 93^{0}C	236.49	5.91	3.1	248.11	6.20	2.2	235.41	5.89	0.4	248.08	6.20	0.2
9 bar, 93^{0}C	255.87	6.40	0.8	245.04	6.13	0.7	253.17	6.33	1.5	240.68	6.02	1.2
11 bar, 93^{0}C	231.91	5.80	2.2	236.14	5.90	1.8	240.35	6.01	0.1	243.17	6.08	1.9
7 bar, 98^{0}C	225.15	5.63	1.3	207.33	5.18	7.2	226.45	5.66	0.9	215.36	5.38	0.6
9 bar, 98^{0}C	245.51	6.14	3.9	238.54	5.96	2.0	235.94	5.90	3.6	234.92	5.87	3.9
11 bar, 98^{0}C	241.35	6.03	4.5	237.23	5.93	3.0	230.25	5.76	1.7	234.41	5.86	1.5

Table 5.4. Trigonelline concentration for EC extraction conditions depending on pressure (in bar), temperature (in ^{0}C) and tamping force (in kgF): for each tamping force, the first column shows the concentration in mg/40 ml, the second column concentration in mg/ml and third column the percentage RSD value.

Conditions	**10kgF**			**15kgF**			**20kgF**			**30kgF**		
P,T	**mg/40ml**	**mg/ml**	**RSD**	**mg/40ml**	**mg/ml**	**RSD**	**mg/40ml**	**mg/ml**	**RSD**	**mg/40ml**	**mg/ml**	**RSD**
7 bar, 88^{0}C	120.95	3.02	0.1	130.30	3.26	0.6	126.96	3.17	3.9	127.76	3.19	4.0
9 bar, 88^{0}C	123.05	3.08	1.9	123.81	3.10	0.2	124.45	3.11	3.5	123.34	3.08	0.8

11 bar, 88^{0}C	118.52	2.96	1.0	122.93	3.07	2.9	118.49	2.96	5.0	115.39	2.88	1.5
7 bar, 93^{0}C	124.89	3.12	2.6	130.63	3.27	2.4	125.21	3.13	0.5	129.03	3.23	0.2
9 bar, 93^{0}C	131.18	3.28	2.4	127.26	3.18	1.7	134.13	3.35	1.0	128.95	3.22	0.2
11 bar, 93^{0}C	122.74	3.07	3.6	124.27	3.11	1.9	126.45	3.16	0.1	128.23	3.21	3.3
7 bar, 98^{0}C	125.78	3.14	0.4	117.76	2.94	8.2	129.92	3.25	0.9	122.14	3.05	0.5
9 bar, 98^{0}C	129.22	3.23	1.4	127.17	3.18	2.1	124.17	3.10	1.5	121.59	3.04	2.7
11 bar, 98^{0}C	126.66	3.17	2.8	123.56	3.09	3.6	119.18	2.98	0.3	121.41	3.04	1.3

Table 5.5. Nicotinic acid concentration for EC extraction conditions depending on pressure (in bar), temperature (in ^{0}C) and tamping force (in kgF): for each tamping force, the first column shows the concentration in mg/40 ml, the second column concentration in mg/ml and third column the percentage RSD value.

Conditions	**10kgF**			**15kgF**			**20kgF**			**30kgF**		
P,T	**mg/40ml**	**mg/ml**	**RSD**	**mg/40ml**	**mg/ml**	**RSD**	**mg/40ml**	**mg/ml**	**RSD**	**mg/40ml**	**mg/ml**	**RSD**
7 bar, 88^{0}C	2.72	0.07	11.7	3.17	0.08	9.7	2.78	0.07	5.1	3.00	0.08	6.5
9 bar, 88^{0}C	3.35	0.08	5.8	3.12	0.08	6.8	3.23	0.08	11.4	3.20	0.08	10.0
11 bar, 88^{0}C	3.29	0.08	8.8	3.05	0.08	0.1	3.64	0.09	6.1	3.05	0.08	21.5
7 bar, 93^{0}C	5.24	0.13	0.9	6.41	0.16	16.6	4.65	0.12	3.8	5.21	0.13	6.3
9 bar, 93^{0}C	7.68	0.19	0.2	6.70	0.17	3.9	7.44	0.19	2.8	6.67	0.17	4.5
11 bar 93^{0}C	4.45	0.11	2.2	4.51	0.11	0.1	4.77	0.12	0.9	4.66	0.12	10.8
7 bar, 98^{0}C	2.68	0.07	2.4	2.72	0.07	18.7	3.15	0.08	2.8	2.78	0.07	3.3
9 bar, 98^{0}C	2.84	0.07	3.2	2.83	0.07	2.7	2.23	0.06	10.8	1.83	0.05	6.1
11 bar, 98^{0}C	2.55	0.06	15.9	2.74	0.07	14.8	2.22	0.06	8.0	2.06	0.05	6.7

These results are relatively higher than those highlighted in literature. This is due to the different volume of extraction, and to the method of extraction. The amount of ground coffee used in published data describes "traditional" EC (single shot), which should be made with 7.5±1 g of ground coffee, whereas in the present experimental analyses 20 g of ground coffee have been used. The caffeine, as shown in Table 5.4, presents amounts that are almost 3 times higher than in the "traditional" cup. The highest content of caffeine occurred at the temperature of 93◦C independently of tamping force at either 7 or 9 bar. The outcomes of the experiments show therefore that the content of bioactive components is dependent on the relative changes of water temperature and tamping pressure, as well as on the water pressure used in the espresso machine (see Tables 5.2-5.5); anyway, variations are in a range quite narrow. Nevertheless, briefly comparing the variability of the bioactive components among varying extraction conditions (for instance, calculated as the percentage dispersion) with the mean RSD for each component, we can conclude that even small variations among different extraction conditions are appreciable being approximately two/three times the RSD. In Table 5.6, the ground coffee was analysed to figure out the number of bioactive compounds in 1 g, which is extracted in 10 ml of water. It was clarified that the extraction of bioactive compounds through an espresso machine can reach up to 80% of all soluble compounds from the ground coffee. This percentage is due to the short percolation time. All bioactive compounds in EC, extracted with various tamping forces, pressures and temperatures, were nearly 20% lower than in ground coffee, while keeping constant mass (20 g in, 40 g out), and time (25±1 s).

Table 5.6. Ground coffee: mg/ml of total CQA, caffeine, trigonelline and nicotinic acid and the percentage RSD in 1g of ground coffee.

Ground coffee	**Total CQA**		**Caffeine**		**Trigonelline**		**Nicotinic acid**	
	mg/10ml	**RSD**	**mg/10ml**	**RSD**	**mg/10ml**	**RSD**	**mg/10ml**	**RSD**
1 g	37.17	0.1	16.24	7.0	8.55	9.9	0.36	2.6

5.4 Numerical results

The computer implementation of the model presented in Section 5.2 can be used to obtain in-silico EC by running a computer program, with the consequent capability to optimise the extraction result in terms of the consumer preferences or the nutritional properties of the coffee beverage. So, an

accurate model for the coffee percolation can be a powerful tool for the coffee industry. We describe the results of a numerical experiment with the proposed model, where the in-silico EC is obtained under the same extraction conditions of Section 5.3; so, the numerical results can be compared with the laboratory measurements reported in that section.

5.4.1 The complete system

The system of equations we are actually solving takes into account two relevant compounds among those analysed in Section 5.3: caffeine (C) and chlorogenic acids (CQA); note that CQA accounts for the total amount of the various chlorogenic acids derivatives. We also consider the erosion and transport of fine particles (Fines). As usual in coffee research, Fines encompass all the particles in the coffee powder below 100 μm. The complete system is:

$$\begin{cases} S_0 \frac{\partial h}{\partial t} + \nabla \cdot \mathbf{q} = 0 \\ \mathbf{q} = -\mathrm{K} f_\mu \cdot (\nabla h + \chi \mathbf{e}) \\ \varepsilon \frac{\partial C_C}{\partial t} + \mathbf{q} \cdot \nabla C_C + \nabla \cdot \mathbf{j}_C = R_C, \\ \varepsilon \frac{\partial C_{CQA}}{\partial t} + \mathbf{q} \cdot \nabla C_{CQA} + \nabla \cdot \mathbf{j}_{CQA} = R_{CQA}, \\ \varepsilon \frac{\partial C_{Fines}}{\partial t} + \mathbf{q} \cdot \nabla C_{Fines} + \nabla \cdot \mathbf{j}_{Fines} = R_{Fines}, \\ \varepsilon_s \frac{\partial C_C^s}{\partial t} = R_C^s, \\ \varepsilon_s \frac{\partial C_{CQA}^s}{\partial t} = R_{CQA}^s, \\ \varepsilon_s \frac{\partial C_{Fines}^s}{\partial t} = R_{Fines}^s, \\ (\varepsilon \rho c + \varepsilon_s \rho^s c^s) \frac{\partial T}{\partial t} + \rho c \mathbf{q} \cdot \nabla T - \nabla \cdot (\Lambda \cdot \nabla T) = H_e, \end{cases} \quad (8)$$

where each equation is prescribed in C, $t \in (0, \tau)$, and the right-hand-side terms are simplified with respect to those in formula (1) since there are no internal sinks or sources of water, so $Q = 0$. In formula (8), each compound belongs to the liquid phase as well as to the solid one, because the liquid component accounts for the amount of that compound which is in solution or in suspension in the water and is involved in the transport and diffusion phenomena, while the solid component accounts for the amount of the same compound that is bounded to the porous matrix. We note that all the three compounds C, CQA and Fines are considered to be distributed uniformly in the whole porous medium with initial concentrations properly defined in Section 5.3, based on the chemistry analyses of coffee samples. This does not mean that the coarser particles of ground coffee are discarded; in fact, fine and coarser particles compose the porous medium but only the movement of fines must be analysed in the percolation process. Finally, the initial and boundary conditions to equip this system with, have been already illustrated in Section 5.2; in particular, the hydraulic head h satisfies (4); the caffeine concentration C_C, the chlorogenic acids concentration C_{CQA} and the fines concentration C_{Fines}

for the liquid phase satisfy (5); while the concentrations for the solid phase C_C^s, C_{CQA}^s, C_{Fines}^s satisfy (6); and the temperature T satisfies (7). The parameters necessary for their definition will be given in Section 5.4.3.

5.4.2 Scheme of the numerical approximation

The numerical approximation of the model is based on the finite element method (FEM). We briefly illustrate the approximation scheme by taking into account a partial differential equation that is representative for the flow and transport processes under consideration, i.e. the advection-dispersion equation for the scalar state variable φ:

$$\begin{cases} \frac{\partial \phi}{\partial t} + \mathbf{q} \cdot \nabla \phi - \nabla \cdot (\mathbf{D} \cdot \nabla \phi) + \phi \nabla \cdot \mathbf{q} - Q = 0, & \text{in } \Omega, t \in [0, \tau], \\ \phi = \phi_D, & \text{on } \Gamma_D, t \in [0, \tau], \\ \nabla \phi \cdot \mathbf{n} = q_N & \text{on } \Gamma_N, t \in [0, \tau], \\ -(D \cdot \nabla \phi) \cdot \mathbf{n} = -\Phi \min\{\phi_c - \phi, 0\}, & \text{on } \Gamma_C, t \in [0, \tau], \\ \phi = \phi_0, & \text{on } \Omega, t = 0, \end{cases} \tag{9}$$

where **Ω** is a generic spatial domain, Q is a generic source/sink term, φ_D is the value of φ on the Dirichlet boundary Γ_D, q_N is the flux prescribed on the Neumann boundary Γ_N, Φ, φ_c are the transfer coefficient and the reference value for φ, respectively, prescribed on the Cauchy boundary Γ_C, φ_0 gives the initial condition for φ. The spatial domain **Ω** is discretised by an unstructured mesh of N_e prisms with triangular basis Ω^e, $e = 1., Ne$. The solution φ is approximated by $\hat{\varphi}$, having the following form:

$$\phi(\mathbf{x}, t) \approx \hat{\phi}(\mathbf{x}, t) = \sum_{i=1}^{N_D} B_i(\mathbf{x}) \phi_i^D(t) + \sum_{i=1}^{N} B_i(\mathbf{x}) \phi_i(t), \tag{10}$$

where B_i, $i = 1,...,N,$ are the basis functions, φ_i, $i = 1,...,N,$ are the unknown coefficients, N is the number of nodes in the domain, including the boundaries except for the Dirichlet boundary where the function φ is known, $\phi_i^D = \phi_D(\mathbf{x}_i, t)$ are the values of φ on the node $i = 1,...,N_D,$ with coordinate $\mathbf{x}_i$ belonging to the Dirichlet boundary Γ_D. This variable separation procedure is termed as Kantorovich semidiscrete method and allows first the discretisation in space of the differential problem and then a time marching procedure for the temporal discretisation. We apply the method of weighted residuals (Quarteroni et al., 1994; Grossmann et al., 2007): the problem is reformulated in its weak form by means of weighting functions B_i, $i = 1,...,N$; the resulting scheme is usually called Galerkin method (Egidi et al., 2018). So, for Eq. (9), by using the integration by parts, we obtain:

$$\sum_{e=1}^{N_e}\int_{\Omega^e} B_i \frac{\partial}{\partial t}\left(\sum_j B_j\phi_j\right) d\Omega^e + \sum_{e=1}^{N_e}\int_{\Omega^e} B_i \mathbf{q}\cdot\nabla\left(\sum_j B_j\phi_j\right) d\Omega^e$$
$$+\sum_{e=1}^{N_e}\int_{\Omega^e} \nabla B_i\cdot\left(\mathbf{D}\cdot\nabla\left(\sum_j B_j\phi_j\right)\right) d\Omega^e$$
$$+\sum_{e=1}^{N_e}\int_{\Omega^e} B_i\left(\nabla\cdot\mathbf{q}\left(\sum_j B_j\phi_j\right) - Q\right) d\Omega^e$$
$$+\sum_{e=1}^{N_e}\int_{\Gamma_N^e} B_i q_N d\Gamma^e - \sum_{e=1}^{N_e}\int_{\Gamma_C^e} B_i\Phi\left(\phi_C - \left(\sum_j B_j\phi_j\right)\right) d\Gamma^e$$
$$= 0,\quad 1 \le i, j \le N, \tag{11}$$

where Γ^e_N, Γ^e_C are the Neumann and Cauchy boundaries, respectively, of the element Ω^e. We note that the boundary with Dirichlet condition does not appear, since there the basis functions B_i, $i = 1,...,N$ vanishes. Since in (9) the only unknown function is φ, we have chosen only one set of basis functions B_i satisfying the request of C_0-continuity. However, there is the need to calculate the Darcy velocity as well as the mass and heat flux during the solving procedure. Considering for instance the (mass or heat) flux $\mathbf{j} = -\mathbf{D}\cdot\nabla\varphi$, the discrete flux is:

$$\hat{\mathbf{j}} = -\sum_{i=1}^{N} \mathbf{D}\cdot\nabla B_i(\mathbf{x})\phi_j(t),$$

where the first derivatives in $\nabla B_i(\mathbf{x})$ are no longer continuous since the shape function B_i is continuous in Ω and continuously differentiable only in each element Ω^e. Thus, the elemental fluxes become discontinuous between elements, and no unique flux results at nodal points, which creates drawbacks in the simulation results, for instance in the calculation of balance errors. This fact can be avoided by reformulating the Eq. (9) where both φ and $\mathbf{j}$ are chosen as unknowns. Such a choice yields the mixed finite element formulation, where the advantage is given by considering different basis functions for the representation of the two unknowns. Usually, the flux $\mathbf{j}$ is interpolated by piecewise quadratic shape functions and φ by piecewise linear shape functions. This choice ensures the stability of the method, since the Babuška-Brezzi condition is satisfied (Babuška, 1973; Cuvelier et al., 1977). However, since this approximation procedure significantly increases the computational effort, it is usually avoided; for example, FeFlow implements (global or local) smoothing strategies to obtain accuracy in the fluxes reconstruction (Diersch, 2013).

The semidiscrete Galerkin approximation applied to formula (1) leads to a system of ordinary differential equations, which is solved by a time marching scheme. In particular, the 2nd-order accurate predictor-corrector (Adams-Bashforth/Crank-Nicolson) method is used, primarily because

it allows an adaptive time-step selection strategy, based on the physics of the simulated processes. Besides, when computing flow and transport processes, usually the advection dominates over the diffusion/dispersion, causing wiggles, i.e., oscillatory behaviours of the solution, especially on coarse meshes. To avoid such shortcomings, the weighting functions can be modified adding an artificial diffusion along proper directions in accordance to some upwinding strategy (Brooks et al., 1982; Diersch, 2013), like Petrov-Galerkin method (or full upwind), streamline upwind, Petrov-Galerkin least-square method. However, our simulation cases do not show similar shortcomings so we avoid any upwinding strategy to avoid an excessive computational load. Once the spatial and time approximations are done, we obtain a linear system of algebraic equations for each time step, where the coefficient matrix is sparse, and it is solved by a standard iterative method.

5.4.3 Numerical simulation setting

The real coffee extraction, as described in Section 5.3.1, has been made with a filter basket widely used in the specialty coffee world, the VST c filter basket, having cylindrical shape with inner radius R = 29.25 mm, height 26 mm, and capacity of 20 g. Thus, the domain in the numerical simulations has always the same radius R, but the height H depends on the tamping of the coffee pod. The height of three coffee pod samples for each tamping were measured. The mean values are: 14.14 mm for tamping 10 kgF, 13.85 mm for 15 kgF, 13.77 mm for 20 kgF, 13.74 mm for 30 kgF, showing a trend in exponential decrease. However, very similar results, with respect to the ones reported below, can be obtained by using a constant height H equal to the mean value 13.88 mm. We note that the actual height of the coffee pod is also influenced by consolidation and grain swelling due to the wetting of ground coffee, but, as already mentioned above, this is neglected. The spatial domain C having the above-mentioned dimensions has been discretised by a mesh consisting in Ne = 3486 triangular prisms and 2160 total nodes, with 270 nodes on each of the 8 circular cross sections. The different settings in EC extractions are 36, and they are carried out by varying the following three variables in all the possible combinations: the temperature of the incoming water Tz0, 88, 93, 98◦C; the pressure of the incoming water pz0, 7, 9, 11 bar; the tamping of the coffee pod, 10, 15, 20, 30 kgF. The other relevant physical and chemical variables influencing the extraction, such as chemical composition of the coffee powder and granulometry, have been kept constant, which means that the type of coffee, the grinding settings and the extraction equipment are the same for all the extractions (see Section 5.3 for details).

Boundary and initial conditions terms in (4) of the hydraulic head depending on the extraction pressure.

	p [bar]		
	7	9	11
h_{z0} [m]	71.38	91.76	112.17
Φ_{π} [1/s]	7×10^{-5}	7×10^{-5}	7×10^{-5}
h_C [m]	0	0	0

Table 5.7. Boundary and initial conditions terms in (4) of the hydraulic head depending on the extraction phase

The simulation settings are based on such real data. In particular, the boundary and initial conditions (4) for the hydraulic head depend on the extraction pressure that is imposed at the top of the coffee powder by the incoming pressurised water. Besides, we assume that the initial pressure profile inside the coffee powder decreases linearly from the top to the bottom of the coffee pod till the value of the atmospheric pressure, that is:

$$p_0(z) = \frac{z}{-H}(1 - p_{z0}) + p_{z0},$$

where p_{z0} is the pressure of the incoming water.

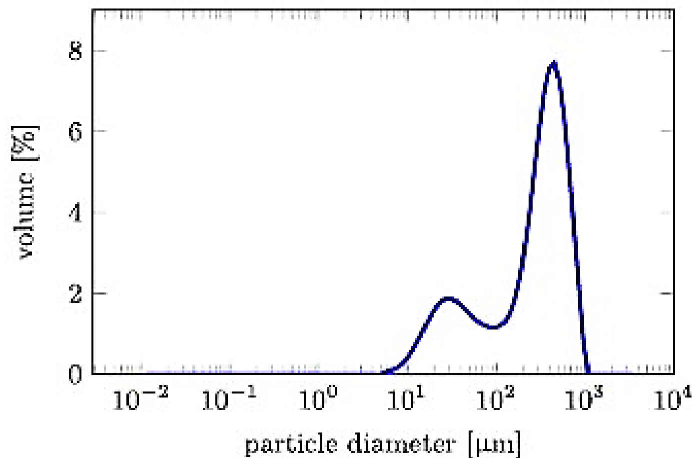


Figure 5.2. Particle size distribution curve of the ground coffee used in the percolation experiment.

The other parameters for the definition of the boundary conditions (4) are reported in Table 5.7. Regarding the temperature, the boundary conditions in (7) only need the knowledge of the temperature of the incoming water T_{z0}, whose values have been reported above when describing the various extraction instances, while the initial condition in (7) has been considered constant for all the cases, i.e., T_0 = 70°C. Regarding the chemical compounds, the parameters for the definition of the boundary conditions in (5) and the initial condition (6) are reported in Table 5.8. The initial

concentration of the solid compound C_0^s has been calculated exploiting the amount of each chemical compound in the ground coffee reported in Table 5.6 multiplied by 20 g, which is the total amount of coffee powder used, and divided by the actual volume of the coffee pod into the filter basket. We note that the exact initial concentration of the solid compound should be obtained dividing the total amount by the solid volume in the pod and not by the pod volume, but this value is extremely hard to be obtained. In other words, the sum of the particle volumes should be taken discarding the empty spaces between them. Indeed, this is the main difficulty in every experiment aimed at giving a porosity measurement, unless such porosity measurement is done wetting the coffee powder. Thus, for our aims of preliminary test of the model, such approximation of the solid volume seems reasonable. Fines are analysed by using the particle size distribution of the ground coffee by means of the laser diffraction device Mastersizer 3000, Malvern Instruments (Mastersizer - Malvern) and we obtained the granulometry curve in Figure 5.2. This device analyses the volume of particles into the sample and calculate the diameter of equivalent spheres, so the particles are classified by a unique value, i.e. the diameter, regardless of their actual shape. The result of this analysis (see Figure 2) gives for each diameter the percentage in volume covered by all the particles having that diameter. The volume of the analysed sample covered by particles below 100 µm is 26%, hence with the previous procedure we calculated the initial concentration C_0^s for Fines, considering that the percentage in volume remains the same percentage value in mass. This is feasible because we considered the mass density in fine particles equal to the mass density in bigger particles, meaning that we assumed homogeneous ground coffee.

Boundary and initial conditions terms in (5),(6) of the chemical species, calculated exploiting the chemical laboratory analyses.

	C	CQA	Fines
Φ_k [mm/s]	10	2	0.1
C_{kC} [mg/l]	500	500	10000
C_0^s [mg/l]	8706.13	19926.52	139383.79

Table 5.8. Boundary and initial conditions terms in (5), (6) of the chemical species, calculated exploiting the chemical analyses.

An essential characteristic in the simulation of percolation processes is the modelling of the dissolution/erosion of chemical compound from the porous medium, which in model (1) is addressed by the reaction terms R_k, R_m^s. Relying on the dissolution processes occurring in groundwater flow, we defined such terms as follows:

where the index m is replaced by k due to the one-to-one correspondence between the liquid and solid compound as made clear in system (8); the dissolution rate α_k has been modelled as follows:

$$\alpha_k = \frac{\varepsilon}{0.32}\left(B + A\exp\left(-0.5\left(\frac{T_{z0}-\bar{T}}{w_1}\right)^2 - 0.5\left(\frac{p_{z0}-\bar{p}}{w_2}\right)^2\right) + aT_{z0} + bp_{z0} + cT_{z0}^2 + dp_{z0}^2 + fT_{z0}p_{z0}\right), \quad (13)$$

where the coefficients depend on the compound into account and are listed in Table 5.9. For the fine particles, a constant dissolution rate has proved sufficient to reproduce their qualitative behaviour, namely the formation of a compact layer, since no quantitative information are available, e.g. the height of the compact layer or the amount of fine particles dropped into the cup. The procedure for the tuning of the caffeine and chlorogenic acids dissolution rates consisted in searching for dissolution rates depending on the compound for each couple of temperature and pressure, once the main parameters about the flux and the transport were fixed. Such values of dissolution rates have been fitted over temperature and pressure by a second order polynomial for caffeine and a gaussian law for chlorogenic acids.

Coefficients for modelling the dissolution rate α_k of caffeine, chlorogenic acids, and fine particles.

	C	CQA	Fines
B	9135.77	368.84	500
A	0	313.08	0
$\bar{T}$	-	94.55	-
$\bar{p}$	-	8.66	-
w_1	-	3.08	-
w_2	-	1.46	-
a	99.47	41.82	0
b	90.33	24	0
c	−0.5	−0.22	0
d	−2.5	−1.38	0
f	−0.5	0	0

Table 5.9. Coefficients for modelling the dissolution rate.

Another important characteristic when simulating flow and transport processes in porous media is the porosity ε. It is influenced by both the granulometry of the ground coffee and the tamping of the pod. On the base of an experimental study we are carrying out and that will be available in a forthcoming paper, the porosity of ground coffee tamped with 10 kgF is in the range 0.30 − 0.35, so we set $\varepsilon_{10} = 0.32$. In addition, we assumed that the porosity varies with tamping with the same trend

of the volume of the coffee pod, which means a proportional law of this kind $\varepsilon_t = \varepsilon_{10} V_t/V_{10}$, where V_t is the volume of the coffee pod with tamping t and ε_t is the corresponding porosity. It yields: $\varepsilon_{15} = 0.314$, $\varepsilon_{20} = 0.305$, $\varepsilon_{30} = 0.297$. Besides, the fluid flow is largely influenced by the hydraulic conductivity tensor **K** that we have chosen as a constant diagonal matrix with elements value:

$$k(p) = -1.21 \times 10^{-8} p^2 + 2.12 \times 10^{-7} p - 7.6 \times 10^{-7}.$$

$$R_k = -\alpha_k (1-\varepsilon) C_k^s,$$
$$R_k^s = \alpha_k (1-\varepsilon) C_k^s, \qquad (12)$$

We note that this value of **K** is calculated to match the flow rate observed in the experiment. Finally, other parameters used in simulations are listed with their values in Table 5.10.

Parameters of model (1)-(7) necessary in simulations. The superscript C, CQA specifies the species which the parameter refers to, and F to Fines.

S_0	10^{-5} 1/m
β_L^C, β_T^C	5,0.5 m
$\beta_L^{CQA}, \beta_T^{CQA}$	0.01,0.001 m
β_L^F, β_T^F	0.01,0.001 m
$\overline{D}^C, \overline{D}^{CQA}, \overline{D}^F$	10^{-9} m²/s
T_0	100 °C
ρc	4.18 MJ/m³ K
$\rho^s c^s$	3.184 MJ/m³ K
Λ	0.673 W/m K
Λ^s	0.337 W/m K
γ_L, γ_T	0.5,0.05 m
τ	20 s

Table 5.10. Parameters of model (1)-(7) necessary in simulation.

The specific storage coefficient S_0 and the molecular diffusivities D have standard values for transport processes in hydrogeology (Diersch, 2013). Also the longitudinal and transverse dispersion coefficients β_L,β_T belong to the range than can be found in literature for the transport of contaminant through underground water (Diersch, 2013); in addition, their variability between longitudinal and transverse coefficient for each compound follows the empirical law that the longitudinal dispersivity is greater than the transverse dispersity of 1 or 2 magnitude orders (Bear et al., 1987). Besides, the dispersion coefficients of caffeine have been chosen near those of the soils with high mechanical dispersion, since from chemical laboratory results the caffeine has revealed the highest amount in the cup with respect to the other tracked compound even if its initial amount in the ground coffee is not

the highest; thus, it is reasonable to assume that solubility and/or transport are more efficient for caffeine. We note that the values of admittance Φ_k, concentration threshold C_{kC}, k = C,CQA, Fines, in Table 5.8, the coefficients of dissolution rate α_k, k = C,CQA, Fines, in Table 5.9, and the variability between the dispersion coefficients of caffeine and chlorogenic acids in Table 5.10 have been estimated by means of a trial-and-error calibration made on experimental data similar to the ones described in Section 5.3, which are not reported here for the sake of brevity. Finally, among the parameters involved in the heat balance equation, we note that the volumetric heat capacity of the solid $\rho^s c^s$ exploits the density value 0.8 that has been estimated in the above-mentioned experimental study focused on the porosity measurement, whereas the fluid volumetric heat capacity ρc refers to the water. Analogously, the thermal conductivity of the fluid Λ is the one of the water, while we assumed the solid thermal conductivity Λ^s is half the fluid one. However, the calibration of these last parameters requires further study, so the values in Table 5.10 need probably some refinement.

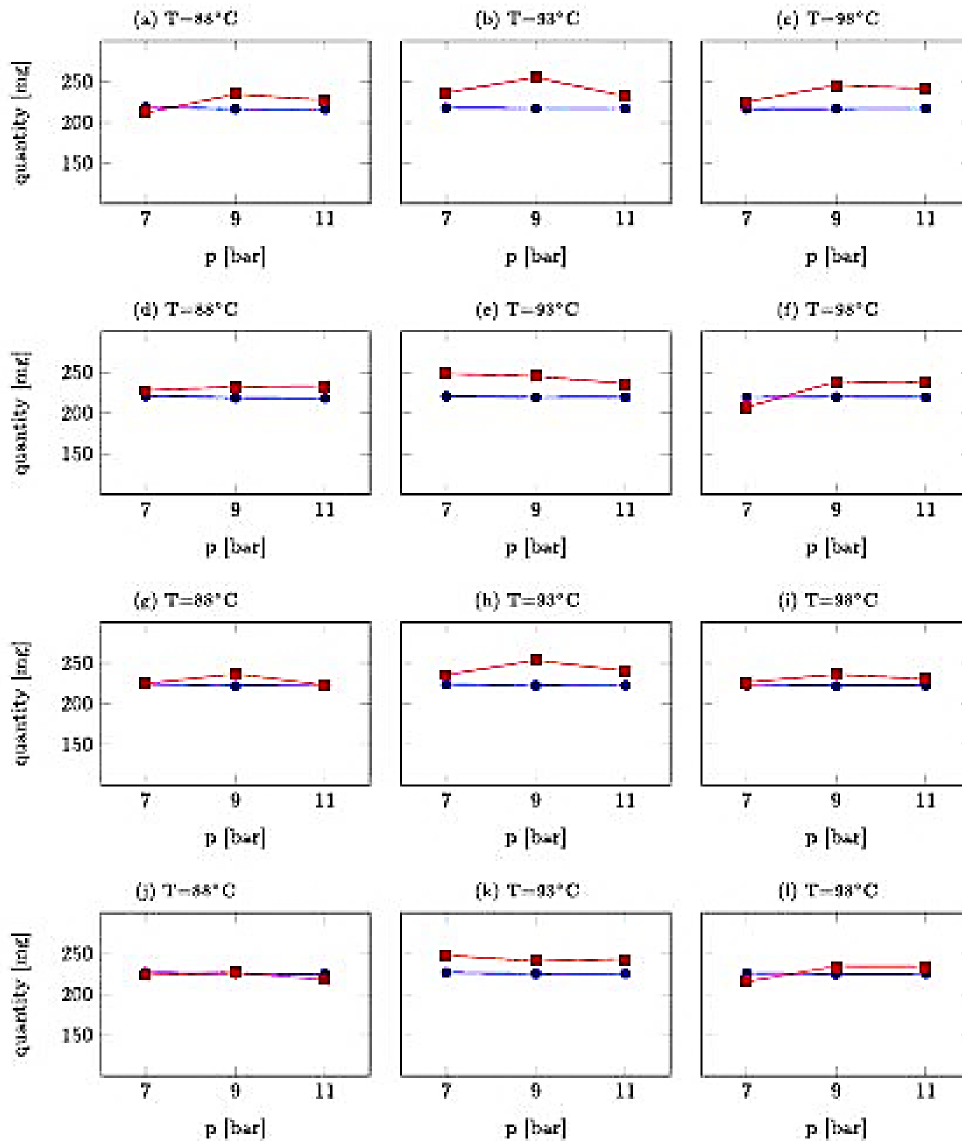


Figure 5.3. Extracted amount of caffeine under varying water temperature, water pressure and tamping conditions: comparison between the numerical results (blue line with circular markers) and the laboratory analyses (red line with squared markers). (a)-(c) show results for tamping 10kgF, (d)-(f) for tamping 15kgF, (g)-(i) for tamping 20kgF, (j)-(l) for tamping 30kgF.

5.4.4 Results: comparisons and discussion

Figures 5.3 and 5.4 show the extracted amounts of caffeine and chlorogenic acids for all the different extraction cases considered. In these figures, the blue lines with circular markers refer to the results of numerical simulations based on the model (1)-(7), while the red lines with squared markers refer to the results of laboratory analyses already reported in Section 5.3 (see Tables 5.3 and 5.4 for details). In each figure, tamping varies along rows, temperature varies along columns, and in each subfigure

the trend is shown as function of pressure. A good agreement between the red and blue profiles can be detected at a first glance. In particular, in Figure 5.4 the caffeine content predicted by the model is almost constant since in the reaction terms (12), the reaction rate (13) has the maximum order term constant, and the exponential dependence vanishes as reported in Table 5.9. Also, we can observe in Table 5.11 that the average error for caffeine calculated over all the cases is around 6%. In Figure 5.4, the numerical profile of chlorogenic acids is near to the experimental profile for all the cases. In many cases the correspondence is almost complete, in others the trend of the blue profile is the same of the red one and they are close to each other. This fact is confirmed by the average error in Table 5.11, that is around 3.6%. The case T = 93°C, p = 9 bar shows a peak for the tamping value 10 kgF that disappears at higher tamping forces; this is due to fact that the fitting procedure has been calibrated on the smallest tamping force. In general, the proposed model manages to approximate the available chemical data up to a satisfactory level. Even if in Figure 5.3, and also in Figure 5.4 less frequently, the variability of the model results with respect to pressure is almost inappreciable, the model catches the correct order of magnitudes of all the extraction conditions and in some of them the correspondence can be found on the magnitude value itself.

	Max		Min		Average
	err [%]	p [bar], T [°C], tamp [kgF]	err [%]	p [bar], T [°C], tamp [kgF]	err [%]
C	15	9,93,10	0.4	7,88,20	6
CQA	8.7	11,88,30	0.06	9,98,15	3.6

Table 5.11 Percentage error calculated as the ratio of the difference between the compounds obtained by laboratory data and the corresponding result of numerical simulations, over the laboratory data.

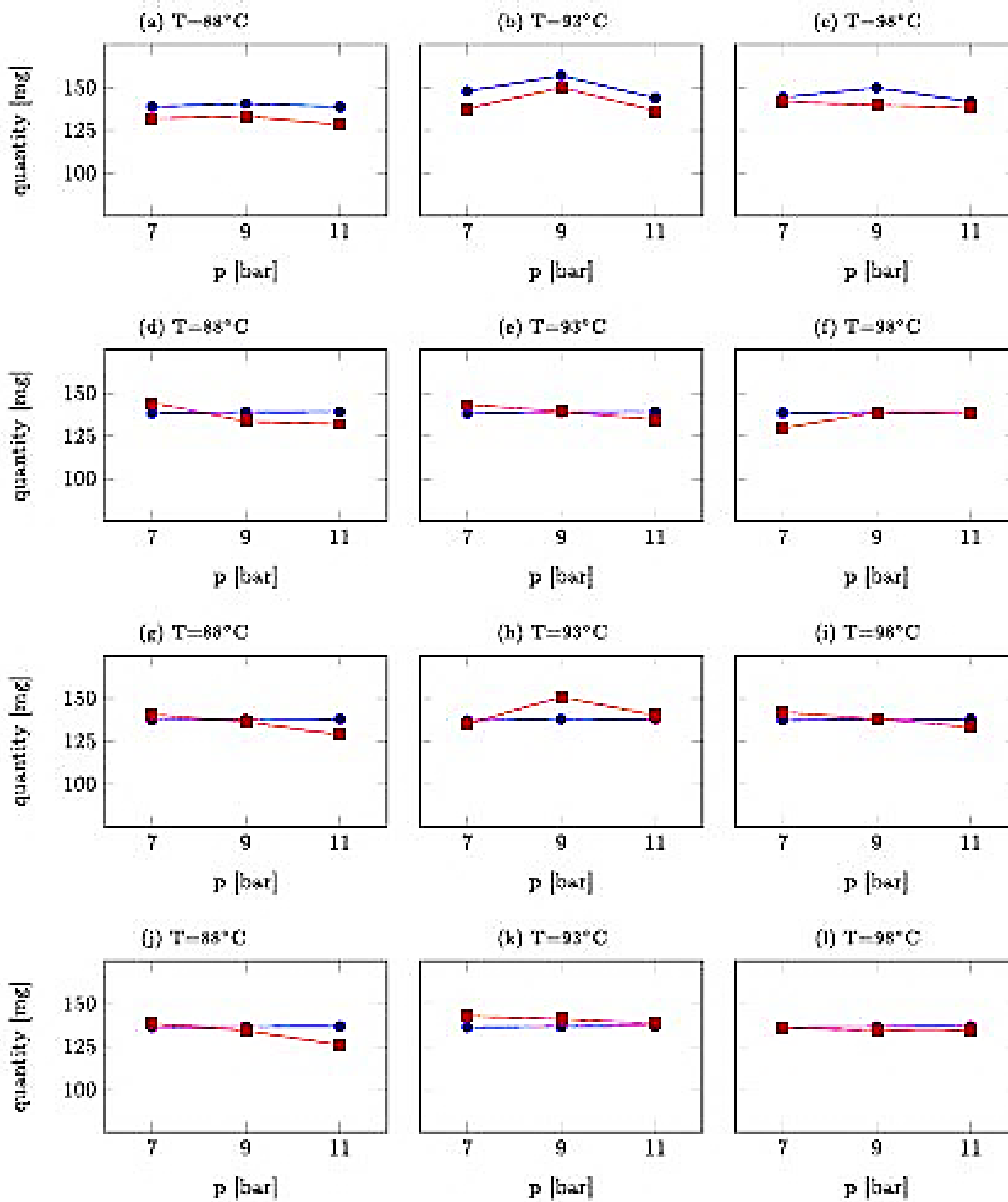


Figure 5.4. Extracted amount of chlorogenic acids under varying water temperature, water pressure and tamping conditions: comparison between the numerical results (blue line with circular markers) and the laboratory analyses (red line with squared markers). (a)-(c) show results for tamping 10kgF, (d)-(f) for tamping 15kgF, (g)-(i) for tamping 20kgF, (j)-(l) for tamping 30kgF.

Such fact has been considered a good preliminary result in the first calibration and tests of the proposed model. In addition, we avoided the usage of average values among all extraction conditions, even if the prediction accuracy of the model could have been better, since the variability due to the three varying physical parameters (water pressure and temperature, tamping pressure) is essential to go in the direction of EC customisation.

Regarding the quantity of the liquid at the end of the simulation, we obtain volumes in the range of 39.5−41.7 cm^3 for all the cases, which is in complete agreement with what expected from real extraction, i.e. 40 cm^3 of coffee in cup, having assumed the equivalence between mass and volume. In addition, Figure 5.5 shows the pressure, temperature and fine particle transport on the vertical cross section of the domain at the final time $t = 20$ s. We show the case $T = 93^{\circ}$C, $p = 9$ bar, tamping 10 kgF because these temperature and pressure are the usual condition for espresso extraction, while this qualitative behaviour is almost unaffected by the tamping. Figures 5.5(a) and 5.5(b) show the pressure profile that keeps the initial trend of linear decrease.

Figures 5.5(c) and 5.5(d) show the temperature profile that at the final time converges to the temperature of the incoming water. Figures 5.5(e) and 5.5(f) show the final distribution of the moving fine particles. Here, the creation of a compact layer at the bottom of the pod is clearly visible. In fact, in the last two layers of the numerical coffee pod the concentration of these moving particles is at least three times higher than the concentration on the first layers of the pod and the profile along the z direction has a sublinear trend with a growth at the final depth. This means that the fine particles are transported by the water flow towards the bottom and they start to accumulate there since only a small amount of them can pass through the filter. Nevertheless, this is a preliminary modelling of fine particles that needs to be refined. In fact, fines take part in the dynamics of the percolation process appearing into the system of differential equations in both liquid and solid phases, so they are considered as the other dissolved compound, either caffeine or chlorogenic acids, without defining their characteristic size. Hence, up to now it is possible to observe their accumulation at the bottom face which surely means an increased resistance to fluid flow, but phenomena as clogging of the filter or of water pathways cannot be observed.

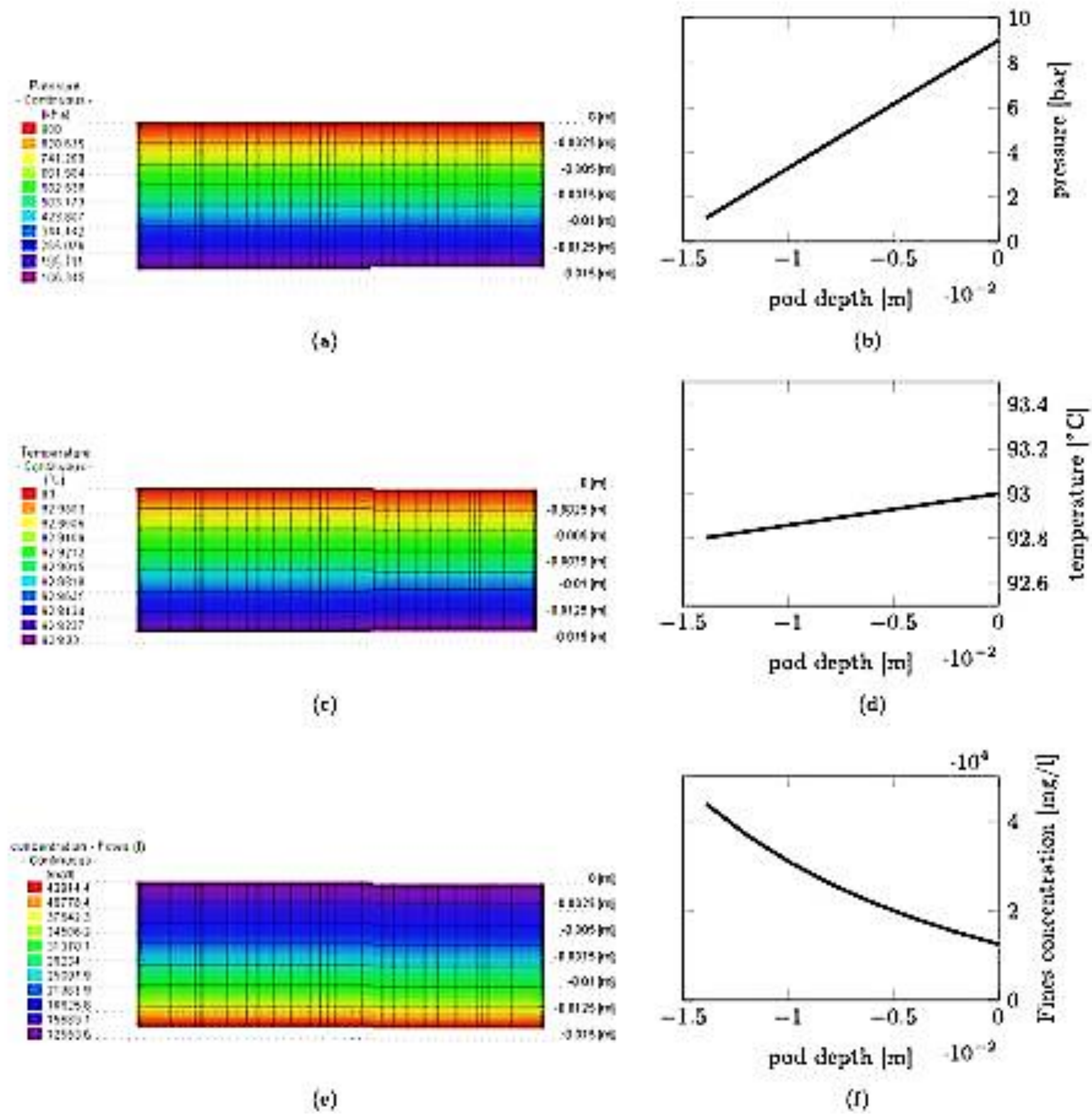


Figure 5.5: Pressure (a), temperature (c) and fine particles (e) on a cross section and the respective plots along the z direction (b),(d),(f) at the final time $t = 20$ s for the case with $T = 93$°C, $p = 9$ bar, tamping 10 kgF.

Study 4. Carbohydrates in espresso coffee

The present work is the follow-up of the previous studies in which the influence of different supplementary devices (two filter baskets and four perforated discs) on EC samples has been analysed while lowering the amount of R&G coffee. The experiments were conducted to analyse the carbohydrates content. The results were compared with the previous outcomes from the sensory evaluation of EC carried out by sensory panellists.

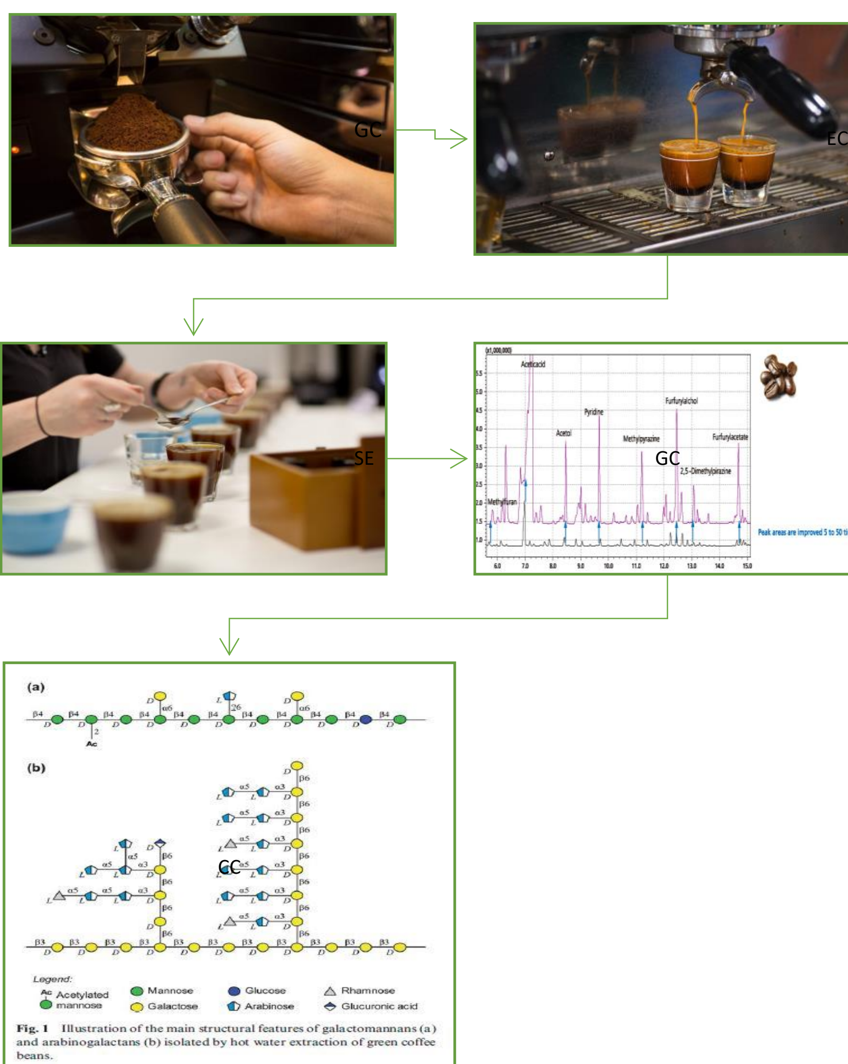


Fig. 1 Illustration of the main structural features of galactomannans (a) and arabinogalactans (b) isolated by hot water extraction of green coffee beans.

6.1 Introduction

Coffee consumers are aware of the caffeine presence in coffee beverages. However, coffee also contains complex mixture of different compounds such as low molecular weight and high molecular weight carbohydrates. Carbohydrates are nearly 60% in green coffee beans and during the roasting undergo structural changes. Among high molecular weight carbohydrates, the most known are polysaccharides, which are familiar as carbohydrate polymers. Polysaccharides are composed of monosaccharide units bound together with glycosidic bonds. Polysaccharides highly influence the organoleptic properties of coffee beverages, mainly for the viscosity and foam stability of espresso coffee (Nunes et al., 1997), and they also effect on the retention of volatile compounds (Sunarharum et al., 2014). In addition to the effects on the properties of the coffee beverage, some research reports have shown that polysaccharides in coffee and their products formed during roasting are involved in different biological activities that potentially affect human health. It has been demonstrated that the majority of these polymers interacts with several cells of the immune system, which confirmed their potential immunostimulatory activity as anti-microbial, antiviral, antitumoral, anti-septic and even as therapeutic agents (Ferreira et al., 2018). Coffee polysaccharides, namely galactomannans, arabinogalactans, and glucose are present in the coffee brew. Besides polysaccharides, melanoidins, lipids, protein, minerals, chlorogenic acids, caffeine, other nitrogenous compounds, and volatiles are part of the complex matrix of roasted coffee and coffee brew (Lopes et al., 2020; Nunes et al., 1998, 2001a, 2001b; Nunes et al., 1997; Passos et al., 2013).

From the nutritional point of view, coffee polysaccharides are soluble dietary fibers, because by human intestinal enzymes these compounds are not depolymerized. Compared to other types of coffee beverages, the highest values of polysaccharides are found in espresso coffee, due to the percolation time, temperature and pressure (Lopes et al., 2016). Several studies estimated that 150 mL of coffee brew contains about 0.705-1.125 g of soluble dietary fiber, nearly 65% of which is polysaccharides; they have been formed as Maillard reaction products. The dietary fiber from coffee beverages can contribute directly or indirectly to the intestinal bacteria, which leads to the production of *short-chain fatty acids*, and influence positively in terms of physiological effects (Petkowicz, 2015). A recent study aimed at the reduction of cholesterol by promoting the interaction of coffee-water extracts with bile salt, through a model of the intestine. This process is partially favoured by the presence of polysaccharides, in particular the high number of galactomannans and arabinogalactans. According to preliminary results, the study might recommend for consumers certain amount of espresso coffee

consumption per day after major daily meals, in order to reduce cholesterol levels in the intestine (Coreta-Gomes et al., 2020).

In the present study, espresso coffee samples extracted with various supplementary tools, namely two filter baskets and four perforated discs, by lowering the amount of ground coffee, are analysed. These samples are studied to evaluate the levels of carbohydrates in various coffee brews. The outcomes of the research have been compared with the results of the sensory evaluation, which further proves the correlations between carbohydrates and taste attributes.

6.2 Material and methods

6.2.1 Samples and espresso coffee preparation

Espresso coffee extracted with various supplementary tools are described in Chapter 4 (4.2.4 EC sample extraction).

6.2.2 Chemicals

Chemicals: acetic acid (CH_3COOH), acetic anhydride ($(CH_3CO)_2O$), 2-detoxy-glucose ($C_6H_{12}O_5$), 72% of sulfuric acid (H_2SO_4), ammonia 25% NH_3 and 3M NH_3, 15% $NaBH_4$, 1-methylimidasole ($CH_3C_3H_3N_2$), dichloromethane (CH_2Cl_2) were supplied by Merck (Darmstadt, Germany).

6.2.3 Sample preparation for sugar analysis

All freeze-dried EC samples were analysed for the carbohydrates content and composition. They were analysed through the sum of the amount of individual sugars after acid hydrolysis, derivatisation to alditol acetates and analyses by GC-FID (Lopes et al., 2016; Nunes & Coimbra, 2007; Passos et al., 2017, 2017, 2014, 2019b, 2019a).

The first phase is hydrolysis. To carry out hydrolysis, about 1-2 mg of samples were weighed in a tube (10mL) and 200 µL of 72% H_2SO_4 were added. After incubation for 3 hours at room temperature with occasional stirring, 1 mL of distillate water was added and incubated for another 1 hour at 120^0C (final concentration H_2SO_4 2M). The tubes were cooled down in a cold-water bath.

The second phase is reduction and acetylation. For the reduction phase: in cooled tubes 200 µL of internal standard (2-deoxy-glucose 1 mg/mL) was added and 0.5 mL of sample was transferred to other tubes. Then, the sample with 200 µL of 25% NH_3 solution was neutralized. The reduction was performed adding 100 µL of 15% (m/v) $NaBH_4$ in 3M NH_3 to the samples, and incubating for 1 hour at 30^0C. After cooling down the tubes in a cold-water bath and adding 2 x 50 µL of acetic acid, 300 µL of samples were transferred to *sovriel* tubes. The acetylation phase was performed by adding 450

μL of 1-methylimidazol and 3 mL of acetic anhydride. After mixing on vortex, the samples were incubated for 30 minutes at 30^0C. The resulting alditol acetates were extracted to an organic phase by adding 3 mL of distillate water and 2.5 mL of dichloromethane, followed by vigorous stirring and separation by centrifugation (30s at 3000 rpm). Afterwards, the aqueous phase was removed by suction with vacuum. Addition of 3 mL of distillate water and 2.5 mL of dichloromethane, stirring, centrifuging and removing, were repeated as described previously. Soon after, the organic phase was washed twice with 3 mL of distilled water, mixed, and centrifuged, whereupon the aqueous phase was completely removed. The organic phase was transferred to specific *speedvac* tubes and the dichloromethane evaporated. Afterwards, 1 mL of anhydrous acetone was added and evaporated twice.

6.2.4 Sugar analysis by GC-FID

GC-FID analysis was performed using 2-deoxyglucose as internal standard in a Perkin Elmer – Clarus 400 chromatograph (PerkinElmer, Massachusetts, USA) equipped with FID detector and a capillary column (DB-225 column; 30m length, 0.25 mm internal diameter, 0.15 μm film thickness). The samples were dissolved with the alditol acetates in 50 μL of anhydrous acetone; then, 2 μL were injected in the GC-FID. The conditions of GC-FID and the relative method were as follows: $T_{injector}$ was 220^0C; $T_{detector}$ was 230^0C; range was 1; attenuation was 6; split was 10mins. Temperature program: t_{total} 9 minutes, $T_{initial}$ 200^0C; rate 1= 40^0C/min until 220^0C and remains for 7 minutes; rate 2 = 20^0C/min until 230^0C, T_{final} 230^0C for 1 minute. The carrier gas (H_2) had a flow rate of 1.7 mL min^{-1}. The total sugars content was determined by the sum of the amount of the individual sugars. All determinations were performed in duplicate.

6.3 Outcomes of the study

6.3.1 Total solid content

Prior to the analysis of carbohydrates, dietary fiber or melanoidin content, it was necessary to calculate the total solid content obtained for the espresso coffee. The espresso coffee samples were frozen in -80^0C and transferred into freeze-drying until they became dehydrated. The yield of espresso coffee samples was calculated before and after lyophilization, the differences accounted between 1.0 g and 1.5 g. The range of total solids obtained for the espresso coffee was in a range consistent with those drawn from scientific literature (Bell et al., 1996; Gloess et al., 2013; Navarini et al., 2004; Nunes et al., 1997; Parenti et al., 2014; Petracco, 2008; Severini et al., 2016), where the total solids varied from 1.36 to 1.81g. This explains why the higher coffee/water ratio generates higher total solid

amount in the brew (Andueza et al., 2003). Therefore, the amount of R&G coffee plays a crucial role in the variation of total solids. For instance, previous reports observed that single-dose capsule system gives 0.15-0.24g g^{-1}, where single-dose system formed lower total solids because of lower amount of coffee powder used in capsule (Gloess et al., 2013). This was also reinforced by Parenti et al., (2014), who determined that a certain amount of coffee powder used for single-dose capsule systems may give similar or higher total solids in the brew than the same amount used in a conventional espresso machine. The content of coffee powder in the filter basket is thus a variable that should be considered for the overall quality of espresso coffee.

6.3.2 Recovery of low and high molecular weight materials (LMW and HMW) by dialysis.

Among the various methods to isolate or purify HMWs from coffee brew, the most well-known is dialysis (Moreira et al., 2012b). The lyophilized EC samples are diluted in water with 1:1 ratio (mg/ml) and transferred into membranes with a molecular weight cut-off 10 kDa. The amount of coffee material, obtained as high and low molecular weight materials, is estimated after a dialyses process (enrichment of HMW). During the dialyses process HMW remains as retentate in the membrane, and LMW as permeate crosses the membrane. The dialyses process remains between 48-72 hours until an equilibrium is reached with regard to nearly complete transition of LMW from HMW through the membrane. The samples collected from membrane are lyophilized, and then the yields of the HMW are calculated. They presented approximately 20% of HMW before the dialyses process, while after that it reached nearly 45%. Figure 6.1 presents the initial and dialyses sugar data that represents the HMW for different extractions, namely, the weight of ground coffee, the heights of the perforated discs, the cultivars and the filter baskets. LMW is significantly high in the initial case, reaching 80%, and after dialyses it declines over 55%. It was already described in the literature that phenolic compounds are retained by polysaccharides. This result is in accordance with literature reports, which pointed out 15-20% as the retentate yield for espresso coffee (Lopes et al., 2016).

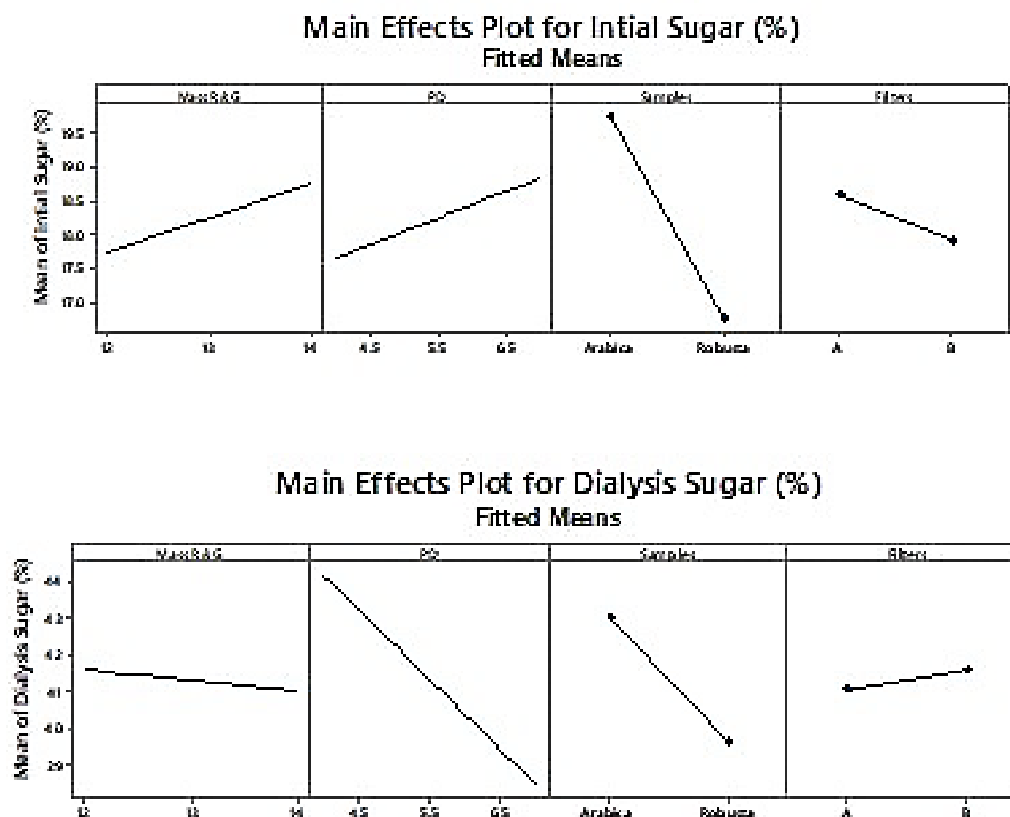


Figure 6.1. Main effects plot for initial and dialyses sugar

6.3.3 Carbohydrates composition

The carbohydrate compositions of the HMW quantified before and after dialysis were from double EC samples extracted with different supplementary devices, with different weights of ground coffee (12g and 14g) and with two cultivars. Galactose and mannose are the main sugar residue constituents of espresso coffee polysaccharides. Arabinose, rhamnose and glucose are the sugar residue constituents of *galactomannans*. Galactomannans from coffee (Figure 6.2 (a), (Moreira et al., 2012b)) are composed by a linear (β1→4)-D-Man*p* residues backbone substituted at O-6 with single residues of α-D-Gal*p* residues (Simões et al., 2010). They also contain single arabinose residues as side chains and (β1→4)-Glcp residues interspersed in the main backbone (Nunes et al., 2005). These galactomannans are acetylated polysaccharides, and as acetyl groups have been observed at the O-2 or O-3 of mannose residues (Oosterveld et al., 2004).

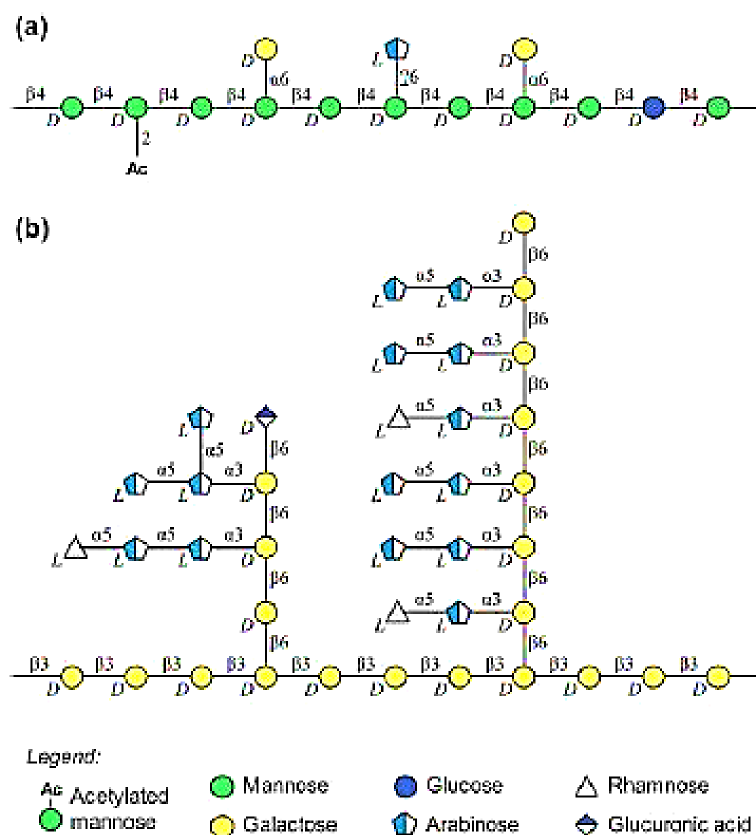


Figure 6.2 Illustration of the main structural features of galactomannans (a) and arabinogalactans (b) (Moreira et al., 2012a)

Galactose and arabinose are constituents of *arabinogalactans*. Coffee type II arabinogalactans are polysaccharides (Figure 6.2 (b), (Moreira et al., 2012b)), usually covalently bonded to proteins (Redgwell et al., 2002). They have a main backbone of (β1→3)-D-Gal residues, with substitutions at the O-6 position with short chains of (β1→6)-D-galactose residues (Merwe et al., 1990). The galactose residues of (β1→6)-D-Gal side chains can be substituted at the O-3 position with single α-arabinose residues, (1→5)-linked arabinose disaccharides (Redgwell et al., 2002), rhamnoarabinose disaccharides or rhamnoarabinoarbinose trisaccharides (Nunes et al., 2008). Terminally linked to (β1→6)-D-galactose side chains can be GlcA residues (Redgwell et al., 2002). Therefore, arabinogalactans are heterogeneous both with regard to the degree of branching and the degree of polymerisation of their side chains.

The carbohydrate composition in espresso coffee samples is in concordance with the reported case of galactomannans and arabinogalactans in coffee beverages (Nunes et al., 1997). The espresso samples,

in total thirty two in duplicate for two species (Arabica and Robusta), were analysed for the carbohydrate compositions; then the results were analysed by “metaboanalyst” statistical software (Metaboanalyst, n.d.). The carbohydrate composition (mol%) was inserted into the statistical software: initial total sugar, initial rhamnose, initial arabinose, initial mannose, initial galactose, initial glucose, initial galactomannans, initial arabinogalactans, HMW yield, dialyses total sugar, dialyses rhamnose, dialyses arabinose, dialyses mannose, dialyses galactose, dialyses glucose, dialyses galactomannans, dialyses arabinogalactans, the ratio between initial galactomannans and arabinogalactans, the ratio between dialyses galactomannans and arabinogalactans, LMW; extraction yield (%) and chlorogenic acid (mg/ml). At the same time, the sensory evaluation score was inserted into the statistical software: acidity, aftertaste, bitterness, body, cream, flavours, sweetness, and overall. The results of the statistical data presented are auto scaled and normalized: in Figure 6.3, it can be seen that the two species of EC samples are clearly separated.

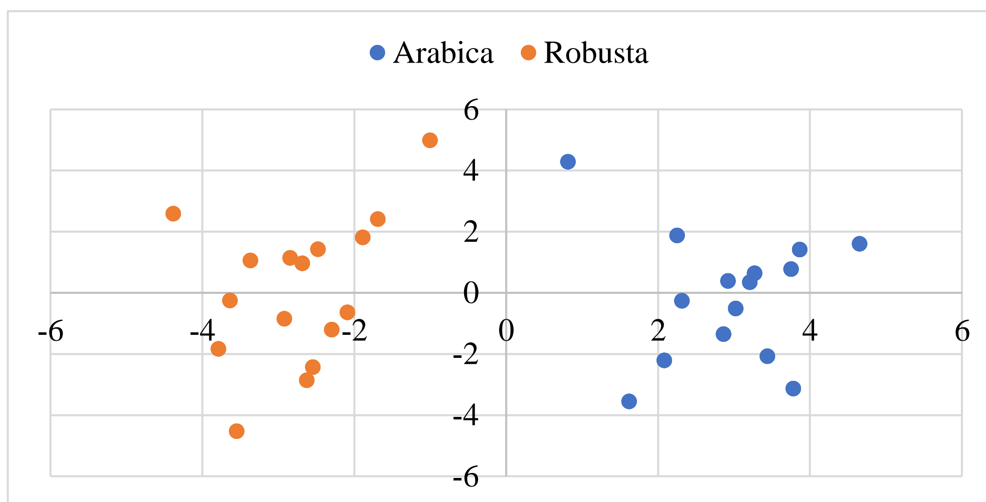


Figure 6.3 Total EC samples applied in the statistical program, with a clear separation of cultivars (Arabica and Robusta).

The differences between Arabica and Robusta in EC extraction could appear more in chemical compounds variations such as caffeine, chlorogenic acids, lipids and also carbohydrates content (Navarini et al., 2004). Apart from the chemical groups, the differences between the two cultivars in the EC are also evident in terms of flavour and aroma (Albanese et al., 2009). The presence of high molecular weight materials in EC lead to determine important characteristics of the beverage such as foamability and foam stability. Foamability of EC is correlated with the amount of protein in the extracted beverage and the degree of roasting. On the other hand, foam stability of EC as a function

of the degree of roasting is depended upon the amount of galactomannan and arabinogalactan (Nunes et al., 1997). Since the behaviour of the two cultivars is quite diverse, the detailed results of Arabica and Robusta are divided and the statistical analysis is separately performed for a deeper understanding of the EC extraction variables (Figure 6.4).

Figure 6.4 presents the principal component analyses (PCA) of the two cultivars, where the samples are clearly differentiated in accordance with species. Therefore, some variables have more impact on the statistical data, which is shown in biplot graphs (Figure 6.5). The results are displayed in Figure 6.4 and chart 6.1, where the samples extracted with different heights of perforated disc, filter baskets and amount of ground coffee are integrated. In fact, the initial carbohydrate analyses and samples after dialyses are shown separated in two sides. The correlation between these results and the sensory data are given in Figure 6.6.

The outcomes of the analysis for specific cultivar demonstrated that the samples of EC with Arabica have more separation in the case of lowering ground coffee from 14g to 12g. This picture was not true with EC samples of Robusta cultivar, where the heights of perforated disc had a great effect in the separation of Robusta. These case studies can be explained by the fact that the soluble compounds of the two cultivars are different (Andueza et al., 2003; Calamai et al., 2013; Masella et al., 2015; Nunes et al., 1997; Salamanca et al., 2017a).

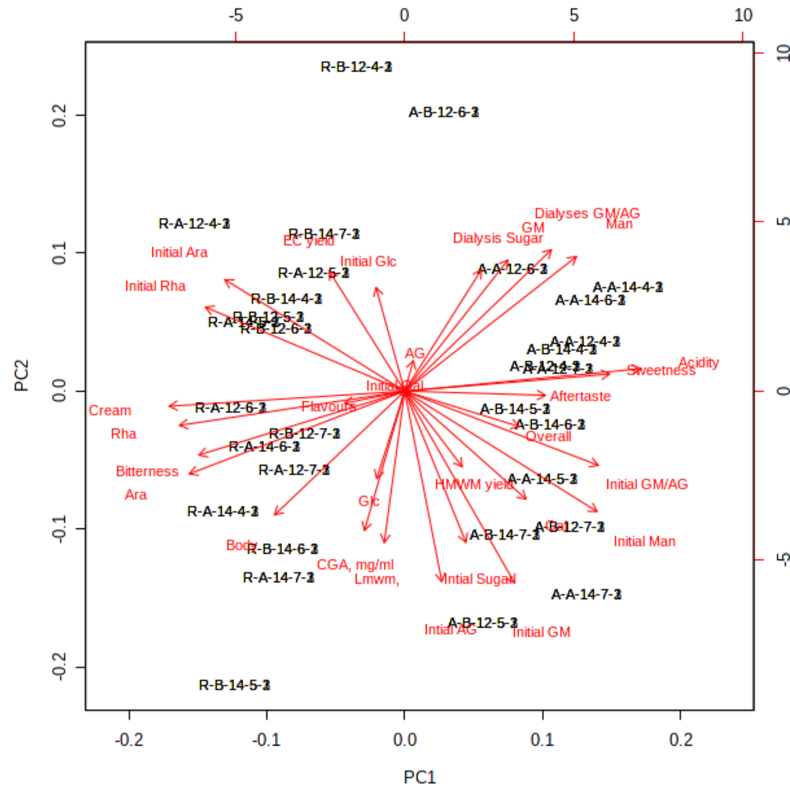


Figure 6.4. Total EC samples applied in the statistical program, with a clear separation of cultivars (Arabica and Robusta).

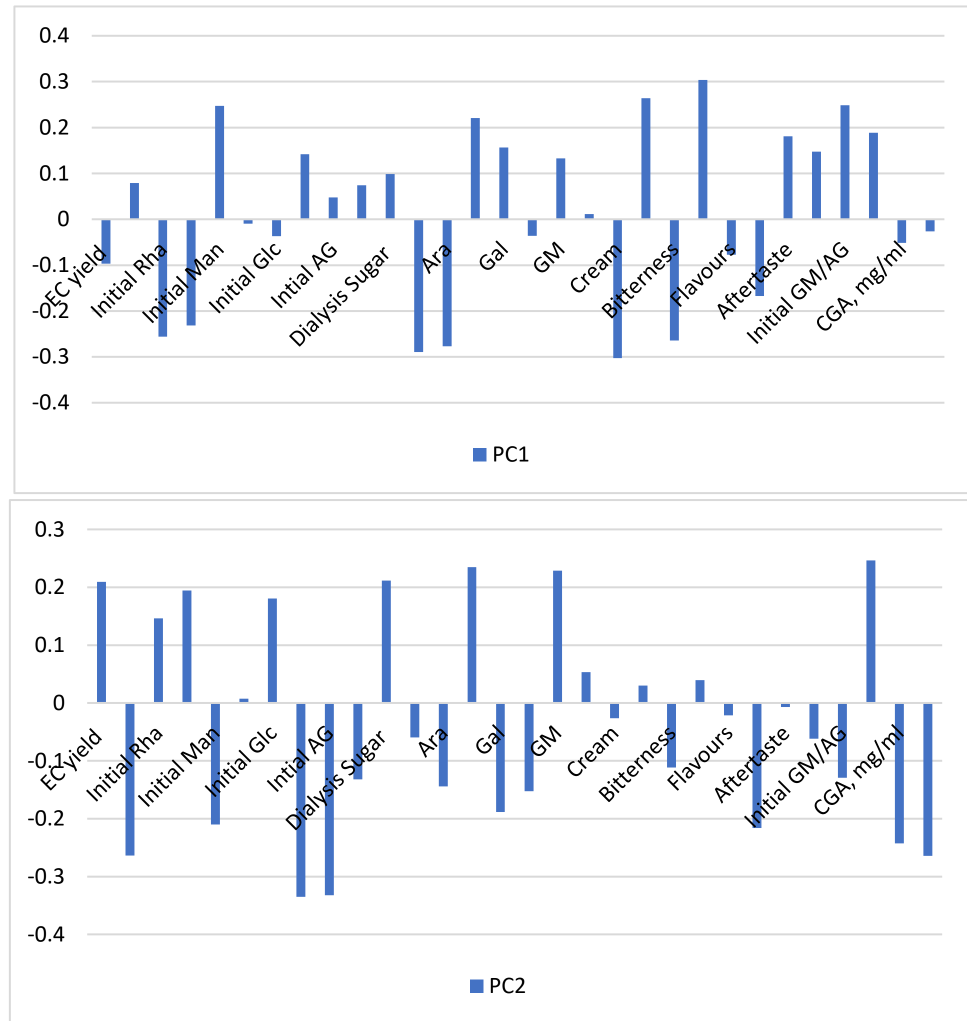


Chart 6.1. Total EC samples applied in the statistical program, with a clear separation of cultivars (Arabica and Robusta).

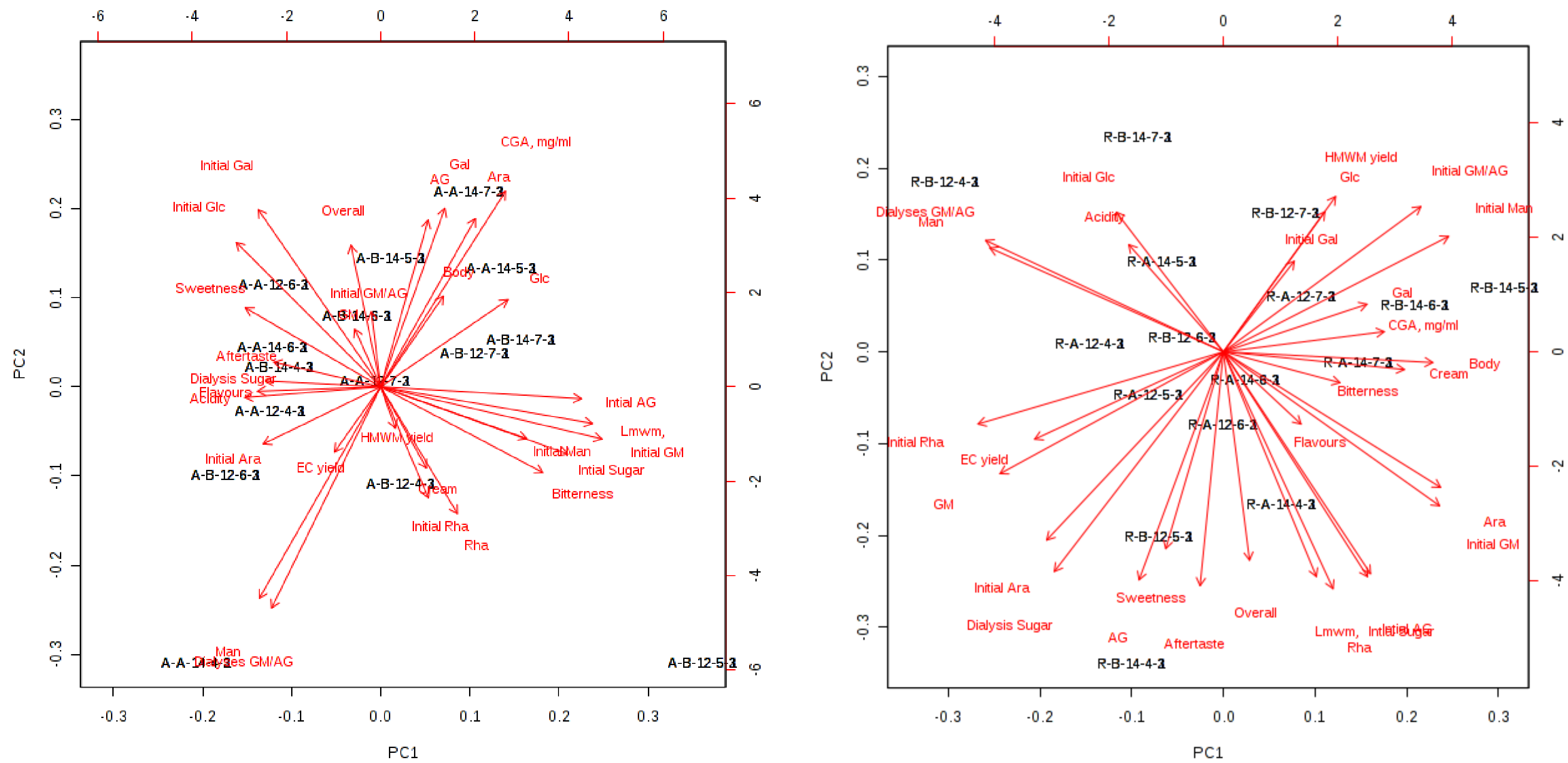


Figure 6.5. Biplot graphs of EC samples extracted with different PDs and filter baskets with Arabica and Robusta cultivars.

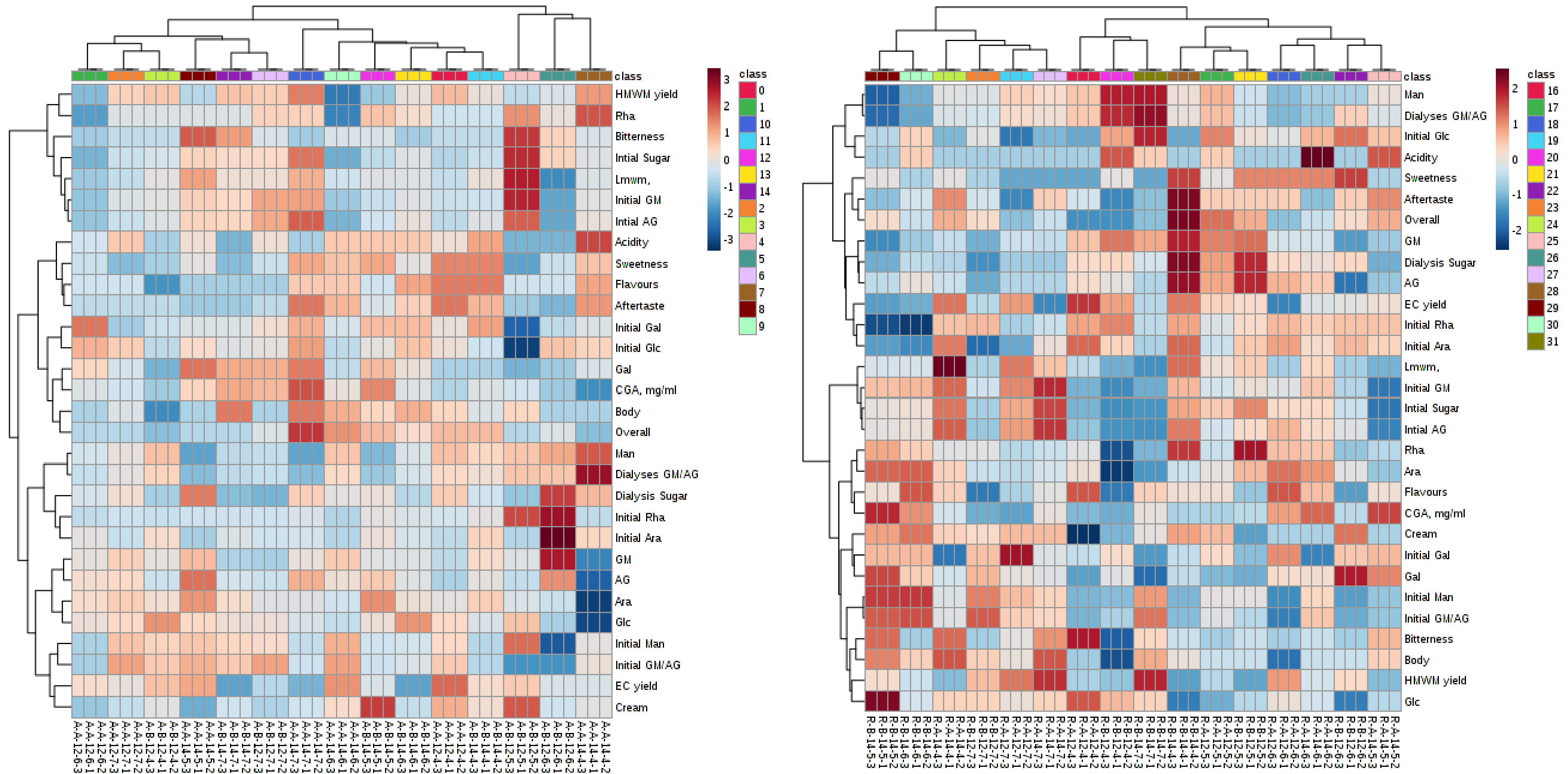


Figure 6.6. Correlation map of EC samples extracted with different PDs and filter baskets with Arabica and Robusta cultivars.

Previous studies described the extraction phase as a solid-liquid interaction where the initial phase favours the increase of the surface per unit volume of the solids, which need to be leached or decreased by a radial distance that is traversed by the liquid. Therefore, they both mutually contribute to the decrease of the particle size (Petracco, 2008). In the present study, experimental extractions with various supplementary tools were applied to reach the equilibrium level of solid liquid interaction by lowering the weight of ground coffee and increasing the height of the perforated discs. Considering the lowering amount of ground coffee from 14g to 12g, the outcomes of Arabica cultivar provided an interesting statistical data. A significant difference is found in 4mm with 12g of ground coffee versus 7mm with 14g of ground coffee. In fact, the initial total sugar results are separated from dialyses total sugars, which ascertains that the HMW compounds are enriched with the dialyses procedure. Arabinogalactans and galactomannans are dependent on the amount of ground coffee; in particular, arabinogalactans can be increased by the lowering the amount of R&G coffee (Reis et al., 2015).

In contrast, the Robusta samples provided outcomes more significant in relation to the changes in the heights of the perforated discs while lowering the amount of ground coffee (Figure 6.7). The heights of the perforated discs, from 4mm to 7mm, are significantly influent with respect to the extraction of arabinogalactans and galactomannans. The arabinogalactans are higher in the samples at 7mm of the perforated disc with 14g, resulting in an increased foamability that is expressed by the cream produced on the surface of the EC. The galactomannans are higher instead in the samples at 4mm of the perforated disc with 12g, resulting in an increased foam stability. Therefore, the findings are consistent with previous research, which proved that galactomannans can be extracted under a longer wetting process with hot water (Nunes et al., 1997), whereas arabinogalactans linked by chlorogenic acids are extracted easier with shorter percolation (Moreira et al., 2015). Figure 6.8 demonstrates the statistical data of arabinogalactans and galactomannans ratio of initial and dialyses for two cultivars: samples of EC extracted with Arabica by lowering the amount of ground coffee in filter baskets, and with Robusta by changing the heights of the perforated discs. Finally, in Figure 6.9, the results of the sensory evaluations are also applied to the statistical software as a comparison with the outcomes of Figure 6.8.

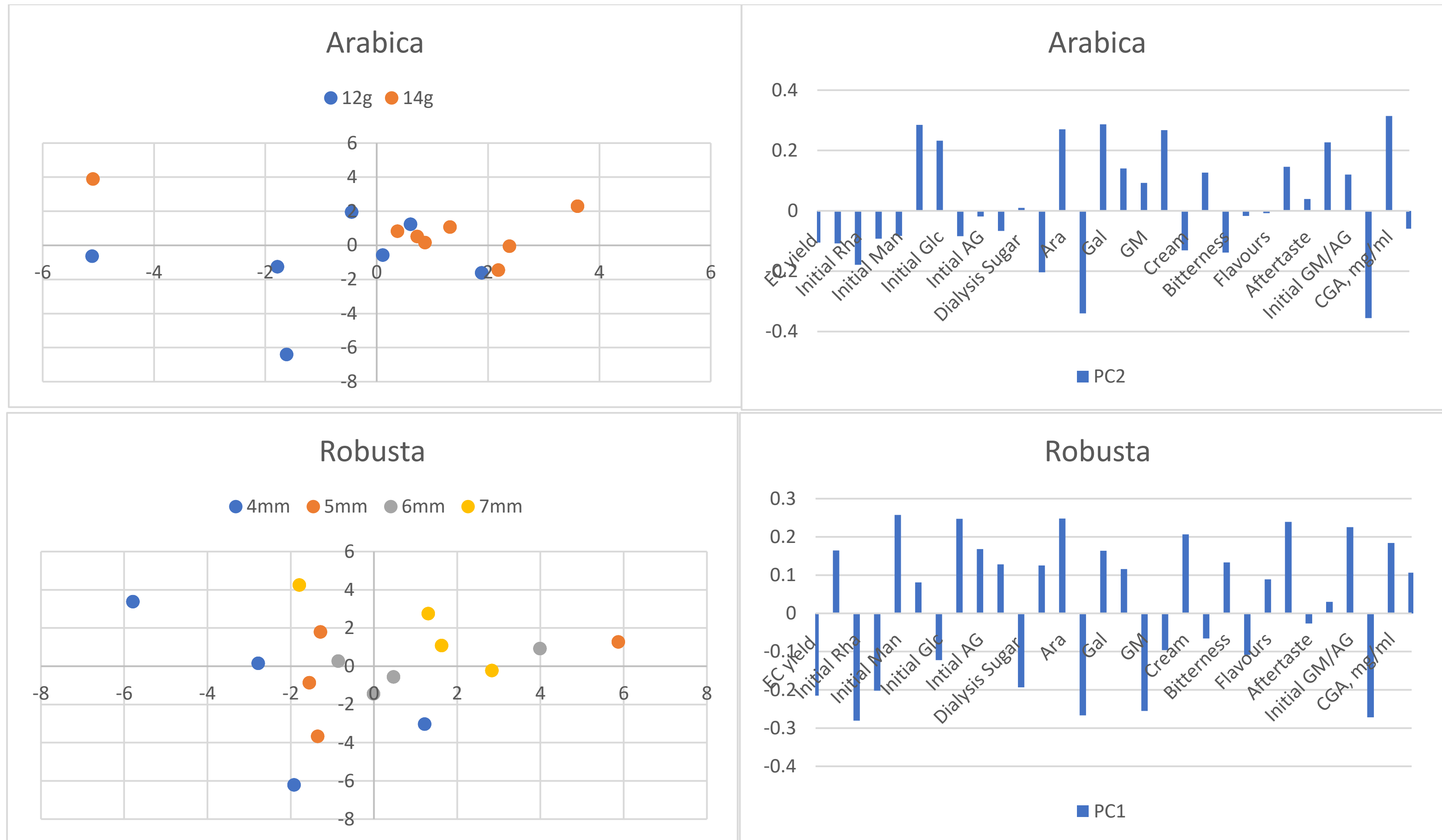


Figure 6.7 Total sugar results of EC samples are applied in the statistical program separately for the two cultivars (Arabica and Robusta).

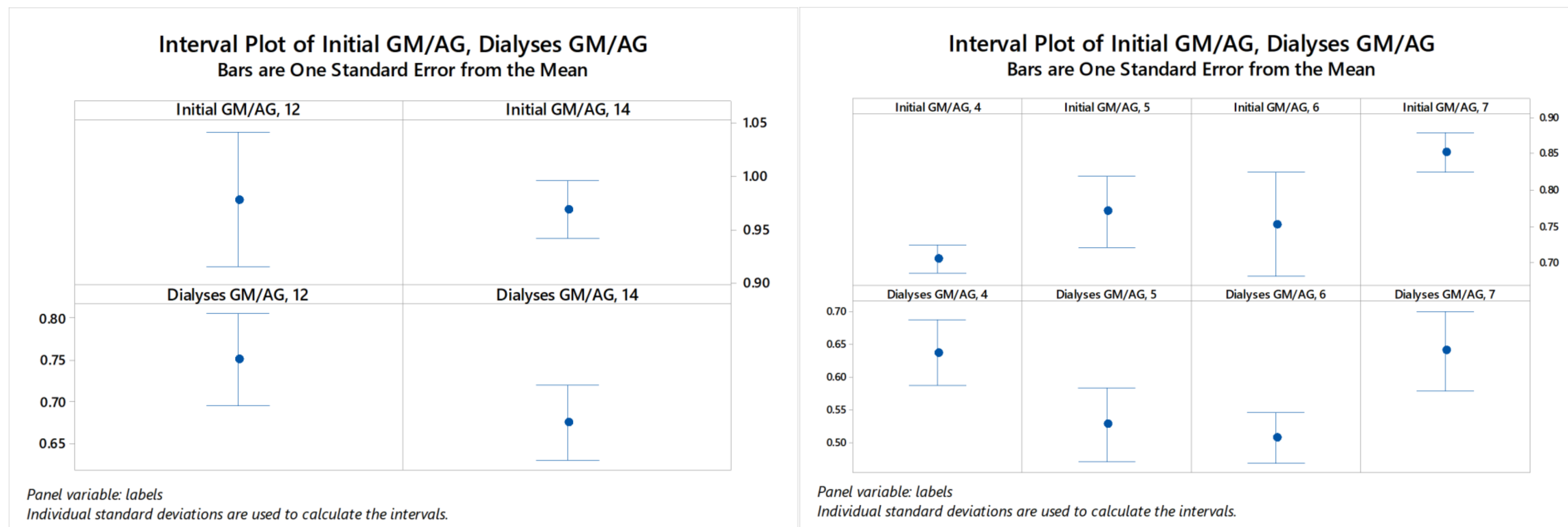


Figure 6.8 Total sugar results of EC samples are applied in the statistical program separately for the two cultivars (Arabica and Robusta).

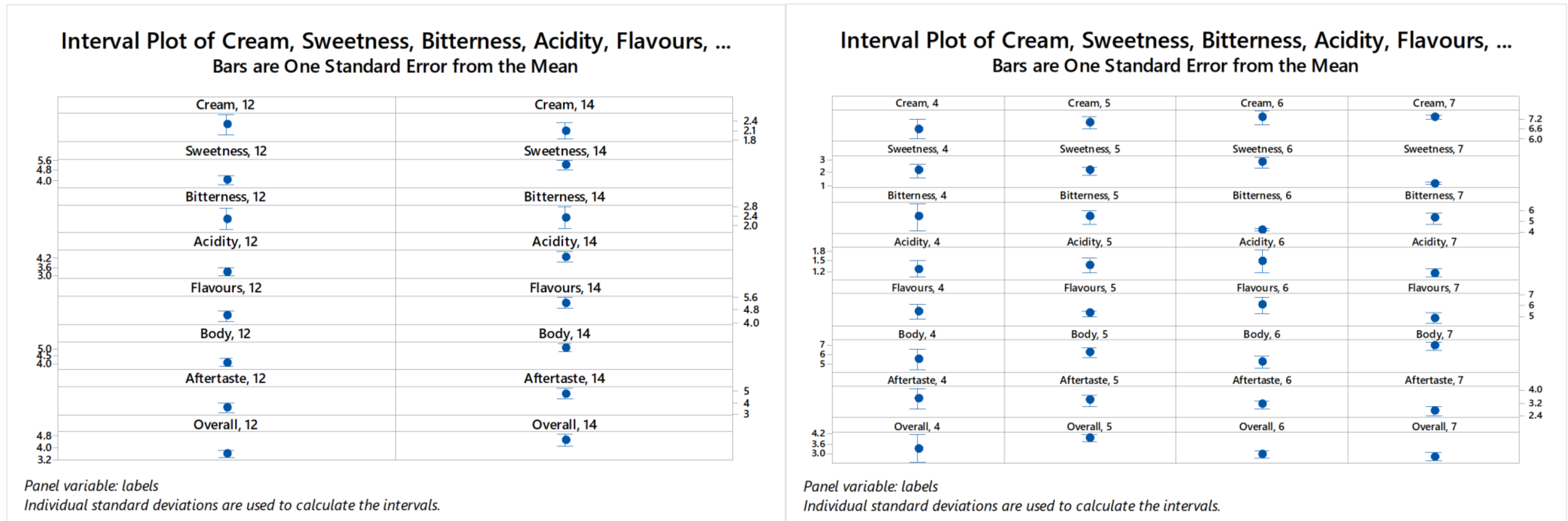


Figure 6.9 EC samples sensory evaluation results are applied in the statistical program separately for the two cultivars (Arabica and Robusta).

Study 5. Spent coffee from EC samples

The present work is another follow-up of the previous studies, putting into perspective the potential of the EC science. The accumulated residue from EC samples was re-used and processed in fact to re-extract bioactive compounds for cosmetic purposes. The experiments were conducted to analyse caffeine, trigonelline and nicotinic acid content in spent coffee. The results were proposed to examine antioxidant properties for cosmetic products. The motivation behind this effort is to apply a zero-waste approach for a more sustainable development of EC consumption.

7.1. Introduction

Worldwide, around 10 million tons of coffee are consumed each year producing millions of tons of coffee by-products such as coffee silverskin (CSS) and spent ground coffee (SGC). SGC is the residue obtained after coffee brewing and soluble coffee production. Indeed, 100 kg of ground coffee can produce around 90 kg of SGC (Banu et al., 2020). This huge amount of coffee waste needs to be properly managed considering the global environmental awareness and the existing regulations (Kavitha et al., 2020).

Therefore, various ideas have been proposed to provide SGC with an added-value to be exploited in possible applications, in order to reuse it as, for instance, fertilizer, sorbent for metal ions removal and feedstock for biofuel production (Iriondo-DeHond et al., 2019; Psota et al., 2011; Salazar-López et al., 2020). Moreover, SGC showed a potential as functional ingredient for the food, pharmaceutical, and cosmetic industries. Indeed, various studies showed SCG is a good source of bioactive compounds such as soluble dietary fiber, protein, caffeine and polyphenols (Iriondo-DeHond, Ramírez, et al., 2019; McNutt & He, 2019; Nguyen et al., 2020; Salazar-López et al., 2020; Severini et al., 2020). In the same direction, the food industry tends to exploit different by-products and to reuse ingredients to add economic value to waste (Campos-Vega et al., 2015a). Among them, SGC has been widely considered for its reuse (McNutt & He, 2019). One of its potential is the antioxidant activity ascribed to polyphenols, particularly chlorogenic acids such as caffeic acid (Jeszka-Skowron et al., 2016, Panusa et al., 2013), nicotinic acid (Campos-Vega et al., 2015), and trigonelline that is converted to nicotinic acid under thermal treatment (Taguchi et al., 1985). Several extraction methods have been envisaged to optimise the recovery of active substances, in particular polyphenols: ethanol or ethanol/water based extraction methods (Campos-Vega et al., 2015b; Duque-Acevedo et al., 2020; Zuorro, 2015) ethanol extraction with microwave assistance (Ranic et al., 2014), ultrasound assisted ethanol extraction (Al-Dhabi et al., 2017), ultrasound-assisted methanol extraction (Severini et al., 2017), subcritical water liquefaction, which has been proved to produce high yields of polyphenolic compounds (Getachew et al., 2017; Xu et al., 2015), microwave pre-treatment before subcritical water liquefaction (Getachew et al., 2017), water boiling of SCG (Sant'Anna et al., 2017), and ultrasound assisted ethanol extraction (Andrade et al., 2012).

The present study aims to optimize a very simple extraction method based on the optimization extraction of three different molecules: caffeine, trigonelline and nicotinic acid. These molecules have been selected because they are among the most interesting active molecules contained in the SCG for

cosmetic purposes. In particular, trigonelline is beneficial for its antimicrobial properties, and thus it could prevent the microbial contamination of the extracts and cosmetic formulations (Mohamadi et al., 2018). Niacine possesses a stabilizing effect on epidermal barrier function, reducing the transepidermal water loss and thus favouring the skin hydration. Moreover, niacinamide stimulates the protein synthesis such as keratin, as well as ceramide synthesis, and favours the keratinocyte differentiation, with benefits on aged skin (Gehring, 2004). For this purpose, a plan of experiments was designed in order to recover the best extract with few steps. The best extract was thus characterized for antioxidant activity. In this study, the best characterization for antioxidant activity is described.

7.2 Materials and methods

Pure standards of caffeine, trigonelline, and nicotinic acid were purchased from Sigma Aldrich (Stenheim, Germany). HPLC-grade methanol was supplied by Sigma-Aldrich (Milano, Italy) and HPLC-grade formic acid (99%) was supplied by Merck (Darmstadt, Germany). Methanol as extraction solvent was also purchased from Sigma Aldrich (Stenheim, Germany). Ultrapure water (resistivity > 8MΩ cm) was produced in house by Gradient Milli-Q® (Millipore, Molsheim, France). The starting coffee consisted of a 100% grain Arabica from India and was provided by Illy Caffè S.p.A. (Trieste, Italia).

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (TROLOX), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (98%TLC) (ABTS, gallic acid, sodium carbonate monohydrate acs reagent, sodium acetate and ethanol (ethanol absolute grade) were purchased from Sigma-Aldrich (Stenheim, Germany). Manganese (IV) oxidize activated (≥90%) and Folin Ciocalteu's phenol reagent were purchased from Fluka (Buchs, Switzerland). Anhydrous sodium acetate, Iron(III) anhydrous hydrochloride were purchased from J.T. Baker Analyzed (Center Valley, PA, USA) and sodium carbonate anhydrous was purchased from Carlo Erba (Milano). All solvents and reagents were of analytical grade. The ultrapure water was produced from the Millipore system (Millipore Sigma, Darmstadt, Germany), and filtered with a 0.20 µm Sartolon polyamide filter (Sartorius Stedim Biotech, Göttingen, Germany). Before HPLC analysis, all samples were filtered with Phenex™ RC 4 mm 0.45 µm syringeless filter, Phenomenex (Castelmaggiore, BO, Italy).

7.2.1 Sample extraction

Coffee beans, 100% Coffea arabica were milled through coffee grinder (Mythos 1, Simonelli Group S.p.A., Belforte del Chienti, Italy). The espresso coffee was produced through a professional machine (VA388, Black Eagle, Victoria Arduino, Simonelli Group S.p.A., Belforte del Chienti, Italy) following these conditions: 7.5 g of the finely ground coffee for each cup; 9 bar of water pressure and 92 °C of water temperature; 25 ± 1 mL of product per cup; 25 ± 1 s of extraction.

Exactly weighted coffee grounds were desiccated in an oven (Heraeus, Hanau, Germania) at 50 °C until reaching a constant weight, that was approximately for 48 h.

Extractions were carried out starting from 1 g of ground coffee exposed to different solvents (water, water-ethanol, or water-glycerol) for a same extraction time (30 minutes) in a water bath (Arex Heating mag. Stirrer, 230 V-50/60 Hz, code F20500413, Velp Scientifica Srl, Monza Brianza, Italia Velp Scientifica Srl, Monza Brianza, Italia) at different temperatures, and in different extraction volumes according to the experimental plan described in the further paragraphs.

Once extraction ended, samples were centrifuged at 4000 rpm (IEC CL 10 centifuge, 230 V, 310 W, max speed 6500 rpm, model n°11210900 Thermo Scientific, Monza, Italia) to recover the limpid extract, to be used for further studies. Extracts were stored after freeze drying at –50 °C and a pressure of 0.03 bar (FreeZone 1 Liter Benchtop Series 77400 freeze-dryer, LABCONCO, Kansans City, MO, USA) in 50 mL polyethylene vials with screw cap (BD Falcon ™, BD Biosciences, Bedford, MA, USA).

7.2.2 HPLC determination of unknown solutions

The determination of unknown constituents of solutions of the extract was provided by injecting 10 μL in a HPLC-VWD; details of the method with HPLC-VWD are described in Chapter 3 (3.1.4).

7.2.4 Optimization of the extraction conditions

For this optimization study a methodological approach based on experimental design and statistical analysis was chosen. The objective was to identify the best extraction conditions allowing simultaneously for the highest content of caffeine, trigonelline, and nicotinic acid. Within this objective, and according to preliminary essays, two dependent variables were identified: the extraction volume and the extraction temperature. For each of these two variables, the corresponding ranges were also identified and are reported in Table 7.1. For the determination of the best extraction conditions, three independent variables – determined by HPLC-VWD analysis – were taken into account: the content of caffeine (Y_1), trigonelline (Y_2), and nicotinic acid (Y_3).

Process variables		Coded variable	Original units	Coded units	Response variables	
U_1	volume (ml)	X_1	10 30	-1 +1	Y_1	Caffeine content
					Y_2	Trigonelline content
U_2	temperature (°C)	X_2	60 100	-1 +1	Y_3	Nicotinic acid content

Table 7.1 Process variables, along with the corresponding tested levels (lower an upper level) and the three response variables under study for the extraction optimization from spent coffee.

From the set of variables, it was possible to build the experimental plan based on a Central Composite Design (CCD) consisting of a full-factorial two-level design (runs 1-4) with centre points (runs 9 and 10) and the star or axial points (runs 5-8) given in Table 7.2. For this optimization study, to the classical CCD points three more experiences (test or check points) were added (runs 11-13) to assess the adequacy of the postulated model.

Experiment number	Design (coded variables)		Run order	Plan (natural variables)	
	X_1	X_2		Volume (ml)	temperature (°C)
1	-1.00	-1.00	13	10	60
2	+1.00	-1.00	11	30	60
3	-1.00	+1.00	6	10	100
4	+1.00	+1.00	12	30	100
5	-1.414	0.00	8	6	80
6	+1.414	0.00	9	34	80
7	0.00	-1.414	4	20	52
8	0.00	+1.414	2	20	108
9	0.00	0.00	7	20	80
10	0.00	0.00	3	20	80
11	-0.61	-0.35	1	14	73
12	+0.61	-0.35	10	26	73
13	0.00	+0.70	5	20	94

Table 7.2 The Central Composite Design for spent coffee extraction optimization: coded and natural variables under study.

The CCD is also known as a second-order design because it allows fitting and checking the second-degree polynomial model – including linear terms and squared terms for all factors, and products of all pairs of factors – given in equation (1):

$$\eta = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_{ii}^2 + \sum_{i=1}^{k-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j \quad (1)$$

7.2.5 Total Phenol Content determination

The Total Phenol Content (TPC) of the samples was determined according to the Folin-Ciocalteu spectrophotometric method (Singleton, Rossi, & Jr, 1965) with some modifications (Zorzetto et al., 2015). Briefly, all the samples were used to prepare water limpid solutions at a concentration of 10 mg ml^{-1}. A 50 µl aliquot of this solutions was added to 150 µl of Folin-Ciocalteu's phenol reagent, diluted 1:4 with water. Then, 50 µl of Na2CO3 saturated solution were added. After incubation at room temperature for 10 min, the absorbance of each well microtitre plate was determined at 765 nm using a microplate reader (FLUOstar Omega, BMG Labtech GmbH, Ortenberg, Germany). The measurement was compared to a calibration standard solution of gallic acid (GA), and results were expressed as milligrams of gallic acid equivalents (GAE) per grams of by-product (mg GAE/g).

7.2.6 Evaluation of the antioxidant activity

The antioxidant activity of the extracts was evaluated by measuring 1,1-diphenyl-2-picrylhydrazyl (DPPH•) radical scavenging activity, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS•+) radical cation scavenging capacity, and Ferric Reducing Antioxidant Capacity (FRAP). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as calibration standard. Values were expressed as IC50, defined as the concentration of the tested material required to cause a 50% decrease in initial DPPH, ABTS or iron concentration, as well as µmol Trolox equivalent/g of sample. DPPH free radical scavenging activity was evaluated on a microplate analytical assay according to the previously published methods (Brand-Williams et al., 1995) with some modifications (Venditti et al., 2015). Briefly, a 50 µl aliquot of the sample (concentration of 10 mg ml-1) and standard were added to 150 µl of DPPH in absolute ethanol in a 96-well microtitre plate (BD FalconTM). After incubation at 37 °C for 20 min, the absorbance of each well was determined at 517 nm using a microplate reader (FLUOstar Omega, BMG Labtech GmbH, Ortenberg, Germany). The ABTS assay was performed following the procedures applied to a 96-well microliter plate assay (Venditti et al., 2015). The ABTS•+ solution (5 mM) was prepared by oxidizing ABTS with MnO2 in water for 30 min in the dark. A 50 µl aliquot of the different concentrations of sample and standard (trolox) were added to 150 µl of ABTS•+ solution in a 96-wells microtitre plate (BD FalconTM).

After incubation at room temperature for 10 min, the absorbance of each well was determined at 734 nm using a microplate reader.

The FRAP values of extracts were determined according to previously published method (Benzie & Strain, 1996) with some modifications (Ornano et al., 2013). The FRAP reagent was prepared by mixing the following three solutions:

1. 50 ml 0,3M acetate buffer pH 3.6 (1.23 g of sodium acetate in 50 ml of water acidifying with acetic acid);
2. 5 ml of stock solution of 5 mM TPTZ (2,4,6-tripyridyl-s-triazine) (15.6 mg) in 40 mM HCl;
3. 5ml of 5 mM FeCl3·6 H2O (16.2 mg) in 40 mM HCl.

The FRAP reagent was heated at 37 °C before use. Aliquots of 25 µl sample (solutions at the concentration of 10 mg ml-1) were added in triplicate onto wells of a 96-well plate (BD FalconTM). The assay was started by adding 175 µl of FRAP reagent to each well. The plate was immediately shaken in a FLUOstar Omega plate reader for 30 seconds and the reaction was allowed to run for 10 min after which the plate was read on a plate reader (593 nm). A reference solution of Trolox was run simultaneously and used to generate the calibration curve by linear regression. The standard curve was linear between 25 and 800 µM Trolox (TE). Results were expressed in µM trolox equivalent (TE) g-1 sample.

7.2.7 Statistical analysis

The design experiments, all data processing, along with plots and contour surfaces here presented, were carried out using Nemrod-W® software developed by NemrodW SAS (Marseille, France).

7.3 Results of the study

7.3.1 Optimization of the extraction from spent coffee grounds using the desirability approach

On the basis of some preliminary results obtained from a previous HPLC-VWD based screening study on the extraction process from spent coffee, the most significant factors identified were the extraction volume and temperature. Consequently, for the process optimization, a quadratic domain was experimentally investigated with a Central Composite Design to estimate the second-order polynomial model of Equation 1, using the response surface design methodology.

The results of ANOVA (Analysis of Variance) for the model of the three variable responses under study – the content of caffeine (Y_1), trigonelline (Y_2) and nicotinic acid (Y_3) are listed in Table 7.3. In particular, the Lack of Fit (LOF) test results are here reported to assess whether the models accurately

fit the measured experimental data. The LOF test procedure consists in portioning the error (residual sum of squares - SS_E) into a component due to "pure" error (pure error sum of squares - SS_{PE}) computed on the basis of repeated measurements, and a component due to lack of fit (lack of fit sum of squares - SS_{LOF}). If the F-ratio for lack of fit is not significant, it is possible to accept the hypothesis that the model adequately describes the data. In this case, for all the three response variables under study, there is no evidence that the quadratic model does not fit the data. The goodness of fit of the proposed model can be also positively assessed according to the values of the coefficients of determination R-squared (R^2) and R-squared adjusted (R^2_A) for the degree of freedom (*d.f.*), which were all higher than 0.90.

The estimated coefficients of the second-order polynomial model of equation (1) for the three response variables are listed in Table 7.4. The experimental and predicted values for the contents of caffeine, trigonelline and nicotinic acid are instead given in Table 7.5, along with their residuals.

As previously mentioned, for the validation of the postulated model, in the experimental design three test points (runs 11, 12 and 13) were also included, in order to assess whether the model well represents the response variables in the experimental domain of interest. The two replicated experiments at the centre of the experimental domain allow to have an estimation of the repeatability and hence of the size of the experimental error that can be compared to the difference (the residual) between the observed value of the dependent variable, $Y_{i,exp}$, and the predicted value $Y_{i,,calc}$ obtained at the test points. In this case, the estimated experimental repeatability for the three variable responses were: ± 9.1648 for Y_1, ± 4.3707 for Y_2 and ± 0.0424 for Y_3. It was therefore possible to validate the models, since all the differences between the values calculated by the models for the test points and the experimental values measured in the same points were of the order of magnitude of the repeatability of the replicate points at the centre of the experimental domain.

Once validated the models, the experimental data obtained at the check points may be included for a new estimation of the model coefficients to improve the fit. The model coefficients and their significance level estimated with complete set of the 13 data values are listed in Table 7.4. In the end, Table 7.6 reports the new ANOVA table.

		Caffeine				Trigonelline				Nicotinic acid			
Source	*d.f.*	Sum of Squares	Mean Squares	*F*-ratio	Signif.	Square sum	Square mean	*F*-ratio	Signif.	Square sum	Square mean	*F*-ratio	Signif.
Regression	5	2.70279E+0004	5.40557E+0003	38.4979	0.178 **	5.09583E+0003	1.01916E+0003	42.1626	0.149 **	0.2950	0.0590	17.4671	0.803 **
Residual SS_E	4	5.61648E+0002	1.40412E+0002			9.66891E+0001	2.41723E+0001			0.0135	0.0034		
Lack of fit SS_{LOF}	3	4.77654E+0002	1.59218E+0002	1.8956	48.0	7.75867E+0001	2.58622E+0001	1.3539	54.7	0.0117	0.0039	2.1688	45.4
Error SS_{PE}	1	8.39938E+0001	8.39938E+0001			1.91024E+0001	1.91024E+0001			0.0018	0.0018		
Total SS	9	2.75895E+0004				5.19252E+0003				0.3085			

Table 7.3 ANOVA for the quadratic experimental models for caffeine, trigonelline and nicotinic acid

	Model Fitting without test points						Model Fitting with the complete set of 13 data values					
	Caffeine		Trigonelline		Nicotinic acid		Caffeine		Trigonelline		Nicotinic acid	
	Coefficient	Signif. %	Coefficient	Signif. %	Coefficient	Signif. %	Coefficient	Signif. %	Coefficient	Signif. %	Coefficient	Signif. %
b_0	118.98	0.0143 ***	30.33	0.0950 ***	0.39	0.0697 ***	116.89	< 0.01 ***	30.89	< 0.01 ***	0.37	< 0.01 ***
b_1	-54.60	0.0204 ***	-19.70	0.0353 ***	0.01	82.0	-54.24	< 0.01 ***	-19.78	< 0.01 ***	0.01	75.6
b_2	17.18	1.51 *	14.70	0.109 **	0.10	0.783 **	17.78	0.0888 ***	14.61	< 0.01 ***	0.10	0.0338 ***
b_{11}	-2.28	70.5	6.31	5.4	-0.06	8.1	-1.52	71.1	6.09	0.614 **	-0.05	2.44 *
b_{22}	-14.35	6.3 *	-0.22	93.0	0.04	25.8	-13.16	1.26 *	-0.51	75.5	0.04	5.2
b_{12}	4.66	47.5	-4.73	12.7	0.21	0.203 **	4.41	37.8	-4.67	4.09 *	0.21	< 0.01 ***

Table 7.4 Estimated coefficients of the second-order polynomial model of Equation 1 for the three response variables, along with the significant values where the symbol * stands for a p-value (p) ≤ 0.05, ** for $p \leq 0.01$, and *** for $p \leq 0.001$

	Caffeine			Trigonelline			Nicotinic acid		
N° Exp	$Y_{1,exp}$	$Y_{1,cal}$	Difference	$Y_{2,exp}$	$Y_{2,cal}$	Difference	$Y_{3,exp}$	$Y_{3,cal}$	Difference
1	145.860	144.4302	1.4298	35.692	36.6957	-1.0037	0.5080	0.4616	0.0464
2	35.0080	25.9049	9.1031	10.7200	6.7551	3.9649	0.0760	0.0561	0.0199
3	157.822	169.4687	-11.6467	70.9080	75.5516	-4.6436	0.3010	0.2500	0.0510
4	65.6290	69.6024	-3.9734	27.0300	26.7050	0.3250	0.7000	0.6756	0.0244
5	197.722	190.9433	6.7787	74.1740	70.2787	3.8953	0.2010	0.2561	-0.0551
6	33.8860	38.0692	-4.1832	11.9250	15.1277	-3.2027	0.2530	0.2702	-0.0172
7	58.7620	66.8046	-8.0426	7.0710	9.3247	-2.2537	0.2840	0.3169	-0.0329
8	125.558	114.9199	10.6381	53.4350	50.4888	2.9462	0.5630	0.6024	-0.0394
9	125.414	118.9854	6.4286	33.4090	30.3324	3.0766	0.4200	0.3886	0.0314
10	112.453	118.9854	-6.5324	27.2280	30.3324	-3.1044	0.3600	0.3886	-0.0286
11	134.492	144.1289	-8.6369	40.1900	38.2579	1.9321	0.3400	0.3749	-0.0449
12	73.3240	76.6523	-3.3283	17.3240	38.2579	0.7171	0.2600	0.2937	-0.0437
13	125.544	123.9834	1.5606	40.6470	40.5170	0.1300	0.4520	0.4777	-0.0257

Table 7.5 Experimental values ($Y_{i,exp}$), predicted values ($Y_{i,cal}$) for the contents of caffeine (Y_1), Trigonelline (Y_2) and Nicotinic acid (Y_3), along with and residuals

		Caffeine				Trigonelline				Nicotinic acid			
Source	*d.f.*	Sum of Squares	Mean Squares	*F*-ratio	Signif.	Square sum	Square mean	*F*-ratio	Signif.	Square sum	Square mean	*F*-ratio	Signif.
Regression	5	2.92657E+0004	5.85315E+0003	65.2968	< 0.01 ***	5.46235E+0003	1.09247E+0003	76.8001	< 0.01 ***	0.3126	0.0625	28.9179	0.0015 ***
Residual SS_E	7	6.27473E+0002	8.96391E+0001			9.95740E+0001	1.42248E+0001			0.0151	0.0022		
Lack of fit SS_{LOF}	6	5.43480E+0002	9.05800E+0001	1.0784	62.7	8.04716E+0001	1.34119E+0001	0.7021	72.2	0.0133	0.0022	1.2345	59.7
Error SS_{PE}	1	8.39938E+0001	8.39938E+0001			1.91024E+0001	1.91024E+0001			0.0018	0.0018		
Total SS	12	2.98932E+0004				5.56192E+0003				0.3277			

Table 7.6 ANOVA for the quadratic experimental models for caffeine, trigonelline and nicotinic acid estimated on the complete experimental data set

To identify an optimal compromise area where the highest response for all the three variables under study are obtained, the desirability approach was considered (Lewis et al., 1999). This method is developed in two steps:

step 1: transformation of every response (Y_i) as a function of the objectives under the form of an individual or elementary desirability function (d_i) by the definition of specific target values, T_i

$$d_i = Ti\ (Y_i, \text{objectives}) \quad (2)$$

The desirability function assigns number between 0 and 1 to the possible values of Y_i, where 0 identifies a completely undesirable value of Y_i and 1 the optimal response value.

step 2: determination of the overall desirability function (D) giving the global optimal compromise obtained computing the geometric mean (G) of the desirability functions

$$D = G\ (d_1, d_2,..., d_m) \quad (3)$$

The compromise solution is 'favourable' with increasing positive of D and becomes 'perfect' when D = 100%.

In Figure 7.1 the three individual desirability functions, one for each of the response variables to optimise, are graphically represented.

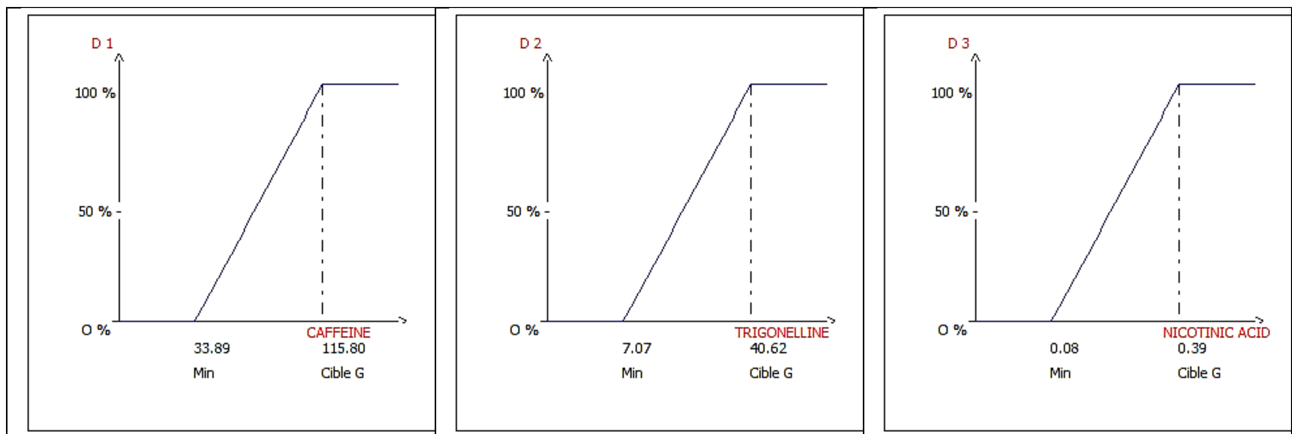


Figure 7.1 The elementary desirability function (d_1, d_2, and d_3) for maximization of the three response variables Y_1 = caffeine, Y_2 = trigonelline and Y_3 = nicotinic acid

In order to determine which is the zone, inside the experimental domain of interest, where the estimation of all the responses can be calculated with a probability ≤ α% of not respecting predefined constraints for each response, the following constraints were given:

$$\text{Prob (Caffeine content} > 33.89) \geq 0.95$$

$$\text{Prob (Trigonelline content} > 7.07) \geq 0.95 \quad \text{(Eq.2)}$$

$$\text{Prob (Nicontinic acid content} > 0.08) \geq 0.95$$

In this way, it will be possible to assess the reliability of the desirability function, or in other terms, where, inside the optimal zone, it is possible to find solutions respecting the given constraints with a probability ≥ (1-α).

Figure 7.2 shows the bidimensional contour plot of the overall desirability function respecting the given constraints (Eq. 2) with a probability ≥ 5%. Here, the variations in *D* values in the experimental domain of interest are represented graphically through the response surface model by determining operating parameter ranges satisfying the predefined objectives and constraints.

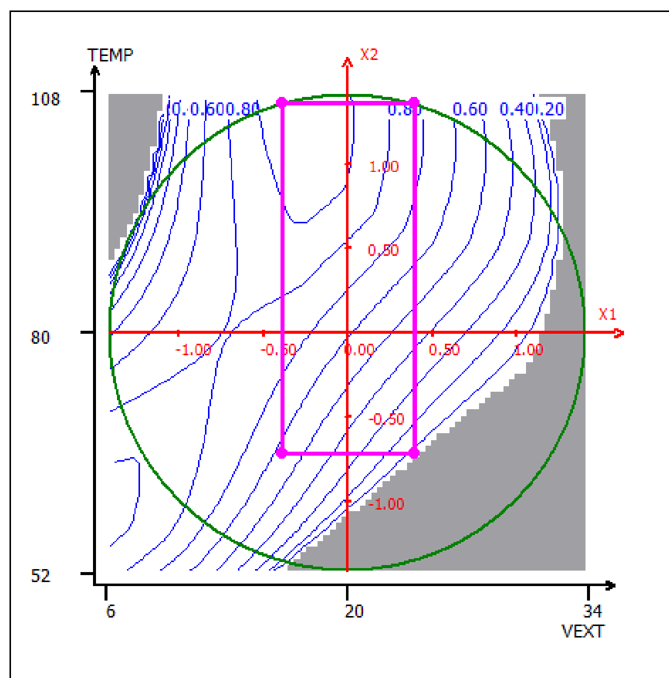


Figure 7.2 Contour plot of the global desirability function and the design space given by the operating parameter ranges satisfying the constraints given in Eq. 2.

In Figure 7.2 is also identified the "design space", in other words the experimental domain where the constraints of Eq. 2 are satisfied, given by the following operating parameter ranges:

$$16.1 < \text{Extraction Volume} < 23.9$$

$$65.7 < \text{Extraction Temperature} < 107.2$$

7.3.2 Evaluation of total phenol content and antioxidant capacity of spent coffee extracts

According to the experimental plan, an extract was produced in a volume of 20 ml and at a temperature of 80 °C for 30 minutes. In Table 7.7, the Total Phenol Content (TPC) and the antioxidant capacities (ABTS, FRAP, and DPPH) of spent coffee extract are reported.

It must be noted that the total phenol content and antioxidant capacity may depend not only on caffeic and nicotinic acids, but also on other antioxidant molecules that could be present inside the extract, but that have not been quantified in the present study. If one compares these results with those reported in the literature and carried out in hot water, it is possible to note that TPC and antioxidant capacity are approximately of the same order of magnitude (McNutt & He, 2019). In general, water extracts are characterized by higher values than ethanol extracts, and among the water extracts the highest value was obtained under subcritical water extraction at 179 °C for 36 minutes (TPC: 88.34 mg GAE/g, ABTS: 88.65 mmol TE/100g, DPPH: 38.28 mmol TE/100g) (Xu et al., 2015).

TAC	**FRAP**	**ABTS**	**DPPH**
mg GAE/g	**TEAC (µmol TE/g)**	**TEAC (µmol TE/g)**	**TEAC (µmol TE/g)**
61.49 ± 1.36	311.62 ± 22.65	735.47 ± 0.60	324.51 ± 13.58

Table 7.7 Total Antioxidant Capacity (TAC) and antioxidant activities determined according to the FRAP, ABTS, and DPPH methods of spent coffee extract obtained according to the experimental plan

CHAPTER 8.

Concluding remarks

8.1 Optimization of espresso coffee extraction (study 1).

The project on the optimization of espresso coffee extraction aimed to enhance the extraction efficiency in order to prepare the same high quality of EC using a lower amount of R&G coffee. We studied different variables, i.e. particle sizes, filter baskets and perforated discs, by brewing coffee with standard and reduced amount.

The results of our experiments with particle size distribution demonstrated that extracting coffee with different particle sizes has a great influence on total solids (TS) and bioactive compounds found in the beverage. In particular, when the standard filter (A) was set in the machine, we found certain levels of TS and target molecules that we used as reference data (namely, from EC samples extracted with mixed particles). Decreasing the amount of R&G coffee from 14 g (standard) to12 g, similar TS levels and contents of bioactive compounds were obtained at 200-300 µm mesh size. The filter basket played also a key role on coffee extraction, and results demonstrated that, with regard to the quantity of the reference compounds (TS and bioactive ones), filter baskets A and B performed better than the prototype C. This was probably diverse for its own design, characterized by the holes on the boundary surface rather than on the bottom. Data on aroma compounds showed that lowering the amount of ground coffee at the same particle size had little impact on the release of volatile compounds, especially in the standard filter basket. Another important variable to be optimized during the EC extraction is the distance between the coffee cake and the shower. By installing different perforated discs with their specific heights, it is possible to adjust that distance. The study on the perforated discs demonstrated that when using 14 g, 5 mm height was the optimal with regard to the quantity of the reference compounds, while a higher height (6 mm) was necessary in using 12 g in order to obtain similar content of bioactive compounds and TS levels. Our data suggest in fact that using a lower amount of ground coffee requires generally a proportional increase in the height of the perforated disc to obtain the same extraction yield. The implementation of different filters for smaller particle sizes, and of different heights of perforated discs for reducing the amount of ground coffee, are both easy adjustments to apply in coffee houses (bars, coffee shops etc.). Simple and feasible as it is, this optimization of the coffee brewing process could lead in fact, in the long run, to a more sustainable

consumption of EC, by reducing the amount of the raw material and, in the end, producing a lower amount of spent coffee while maintaining the same quality of beverage.

8.2 Organic acid impact on the EC and comparison with sensory data (study 2).

This second research was designed to determine the effect of supplementary tools on the extraction of EC and sensory perception. The new developed method used for this study may be applied to other types of beverages to quantify the organic acids that have a great impact on the taste of the beverage. The comparison between different extractions of EC with two different filters while reducing the amount of ground coffee from 14 g to 12 g provides information and results about the influence of various heights of perforated discs in the extraction process. Two filter baskets were used to extract EC with 14 g and 12 g of ground coffee and with 4 mm -7 mm heights of perforated discs. The results demonstrated again, as in study 1, that similar concentrations of bioactive compounds and organic acids were found when the decrease in the amount of ground coffee used was matched with a proportional increase in the height of the perforated disc. In other words, this means that the changes in the structure of the perforated disc can help generate almost equal concentration of bioactive compounds when the mass of ground coffee in the basket is only 12 g. These findings provide insight and prospects for future research based on the analysis of high molecular weight compounds in EC, aimed likewise at possible further reduction of ground coffee in the filter basket. There is, therefore, a definite need for implementation of different filters and different heights of perforates discs for the desirable reduction in the amount of ground coffee. These tools and devices represent easy adjustments to apply in the fields of coffee industry and consumption.

8.3 Mathematical model on EC extraction process (study 3)

The third study deals with the mathematical modelling and formulation of the water percolation process in a porous medium, in the particular case of EC extraction. The proposed model tries to obtain a simplified version of the relevant fluid dynamics phenomena occurring in the physico-chemical process of coffee extraction. The resulting model allows the evaluation of the water flow and the transport of the dissolved substances, the dynamics of the fine particles, and the energy balance between the fluids and the porous matrix. As a result of the numerical solution of such a model, we can compute the amount of the chemical compounds in the extracted coffee. The paper provides also a preliminary test of the proposed model by considering 36 extraction cases that differentiate for the relevant extraction settings: water temperature, water pressure and tamping pressure. In particular, two kinds of investigations have been performed: a numerical simulation of

the extraction process and chemical laboratory analyses on the real extraction product. We primarily focused on two relevant chemical compounds that can be found in a cup of EC: caffeine and chlorogenic acids. The proposed validation consisted in the comparison of the results from the numerical simulations with the results of the chemical analyses. A good agreement between them turned out, with low percentage error in most of the cases. Moreover, we also showed a qualitative analysis of the fine particles, which are of particular importance since they create the "crema" of the espresso cup. On the other hand, however, they bring a new challenge to the extraction process, since they are responsible of the compact layer at the bottom of the coffee pod, which might clog the filter.

Future studies have to complete the test and the validation of the model by extending the set of the extraction settings (taking into account also different coffee varieties and different granulometries) as well as the chemical compounds considered in the extracted coffee. In particular, the measurement of temperature and pressure profiles into the coffee pod during the extraction can support an in-depth quantitative test of the model.
After the validation phase, the proposed model can become a valuable tool for the coffee industry. In fact, it can concretely support future challenges in the coffee market such as the customisation of the coffee beverage.

8.4 The effect of supplementary tools on carbohydrates in EC (study 4)

This fourth study analysed the carbohydrates content in EC following-up on study 2, in which the influence of different supplementary devices (two filter baskets and four perforated discs) on EC samples was demonstrated. The carbohydrates content was analysed based on developed methods that quantify certain compounds of sugars found in the coffee beverage. In addition, the results were compared with the previous outcomes of the sensory evaluation of EC carried out by sensory panellists. The research experiments were based on two separate phases of carbohydrates, initial and after dialyses phases, in which examining in particular the content of high molecular weight (HMW) compounds. The correlation between galactomannans and arabinogalactans on two cultivars showed that they are both dependent on the amount of ground coffee: for instance, arabinogalactans can be increased by lowering the amount of R&G coffee in Arabica. In the case of the Robusta cultivar, the galactomannans are higher in the samples at 4mm of the perforated disc with 12g, whereas arabinogalactans are higher at 7mm of the perforated disc with 14g. This study shows that, according to the different solubility of arabinogalactans and galactomannans, they are optimally extracted at certain different conditions, whether using different combinations of supplementary devices, or

different separated cultivars, with consequently different results in terms of foam conditions (foamability or foam stability of EC).

8.5 Reuse of spent coffee from EC residue (study 5)

This final study contributes to develop methods of exploiting the waste deriving from the food production and transformation industries, such as spent coffee grounds. This waste that commonly ends up in the dustbin could be collected to produce an extract with added values. In this case study, by considering the promising results obtained in terms of phenol content compounds, the total antioxidant capacity, we supposed to use spent coffee extract as a source of active and attractive ingredient in cosmetic formulations for aged skin. Further studies will be devoted to include the coffee spent ground extract in cosmetic formulations.

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