UNIVERSITÀ CATTOLICA DEL SACRO CUORE

Sede di Piacenza

Dottorato di ricerca per il Sistema Agro-alimentare

Ph.D. in Agro-Food System

Cycle XXXV

S.S.D. AGR13, AGR15

Multifactorial traceability and characterization of Specialty and High-quality coffee

Coordinator:

Ch.mo Prof. Paolo Ajmone Marsan

Candidate:

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Matriculation n: 4915197

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Coffee is more than a cup,
coffee is all of us dreaming behind that cup.

Ai viaggi, ai luoghi e ai momenti
che non mi lasciano mai smettere di sognare

Summary

1. Introduction

1.1. SPECIALTY COFFEE AND HIGH-QUALITY COFFEE: AN OVERVIEW

Specialty, premium, fine…yes, surely all of these are adjectives describing something more than a regular raw material, as in the words, something "above the average" for one or more measurable features characterizing all the products from the same class.

This statement is, however, so general and it could be declined for thousands of craft products from the most differentiated chains, from dresses to technology, from jewels to foodstuff but no one of them could be compared to the highest quality lot of a so fascinating and complex supply and value chain as the coffee one is.

Now, to start the journey that will drive us from the tropical region where coffee plants grow to taste of the cups of coffee consumed all over the world, let's start defining what specialty coffee, premium coffee and fine coffee mean and to outline which are the main procedures to evaluate the quality of a coffee lot.

It is necessary to do a step backward, to the organisms that are the two cornerstones in the pretty young but already rich story of coffee quality protection: the Specialty Coffee Association (SCA) and the Coffee Quality Institute (CQI).

Historically divided by continent in which coffee community settled, Specialty coffee association story started about forty years ago in America, where a branch of coffee people decided at the end of a conference to organize themselves in an Association aimed to set standards for the commercialization of coffee, called from few years "specialty". Later on, little sister Europe, also discovered that something really close to what was experienced in America was also occurring in the old continent, that's why, at the very end of XX century, Specialty coffee Association of Europe was born.

The changings in society settlement and an everyday more connected world in which exchange of goods, information and trends are every moment faster, easier and interconnected, they also direct reflected in the relationship established among SCA chapters growing in every continent. That's the way now, all the people involved in the specialty coffee sector feel themselves part of a unique and worldwide diffused association that embraces all the different stake and shareholders of the coffee market, the Specialty Coffee Association.

Together with the SCA which was more involved in trade of coffee lots, in 1996 it was born the Coffee Quality Institute (CQI), aimed to be the scientific and research branch of the "Specialty world".

The CQI these days is playing as an organism involved in the education of coffee producers and all the people involved in the certification and assurance of quality. The main goal is to teach a common language to all the players of coffee value chain that allows to communicate among the coffee community all over the world and to share information about market needs, agricultural and processing innovation, continuous changes in desired flavors and willingness for voluntary certification that drives coffee consumers' choice.

Whit its social function for the community, the CQI also plays a fundamental role in scientific research field. Thanks to its different programs they are a solid bridge between in-origin academic institutions, researchers, private companies, and coffee producers. This way it is possible to perform high-end research on field, promptly apply innovative solutions for coffee production and selection, and share high quality knowledge out of the academic institutions straight to the coffee farmers that, at the very end, can get novel strategies to face new challenges like climate change, increased demand for high quality coffee, reduction of water consumption and soil conservation and prevention of biotic contamination.

Now that we know who the organisms are, given the rules and language of the game, it's time to understand how operatively coffee can be evaluated to get the Arabica Specialty Coffee or Fine Robusta designation.

Among the above-mentioned programs by the CQI, there are 2 courses intended for people involved in the quality control of coffee that gives the title of Arabica and Robusta Q-Grader respectively. To get the certification of Q-Grader, students are asked to demonstrate a complete understanding of the key points of the coffee supply chain and to be up to date on the new trends in coffee industry. On top of that the focus of the Q-Grade certification course is to build a consensus on the way to describe – in terms of intensity and quality – coffee flavors, base tastes, mouthfeel and aftertaste: to achieve this, a solid scientific strategy is at the base of the 18 to 20 exams (depending on the specie subject of the course) people are expected to pass.

The main skills tested are:

- green coffee and roasted coffee grading. In these tests, students are asked to check 350g of green coffee and 100g of roasted on to evaluate frankness from defects that don't allow them to grade the coffee lot as Specialty or Fine. Defects are codified for both coffee specie, for each of the two stages in which grading is required, and graders are asked to recognize defected beans, count them, properly convert in number of defects and then finally state if the coffee is eligible to be Specialty or not;

- the capability to discriminate coffees from the same area and same post harvesting process, this is tested using triangulation test in a red lighted environment to push trainees to base their decision on olfactory and taste perception, removing the bias usually provided by the expectations generated from the visual perception;

- the ability to recognize base fragrances present in coffee, being these both positive or negative features for a cup, smelling them directly to standard solutions known as "le nez du café", a powerful tool to build a consistent matching between perception and semantic label used by q-graders to describe cup profiles and – of course – defects. When Robasta coffee is considered, together with "le nez du café" students have also

the consider fragrances includes in "le nez du vin" because of the particular flavors characterizing this specie;

- identification and discrimination power of the different types of acidity, one of the base tastes characterizing high quality coffee together with bitterness sweetness and– only in the case of Canephora coffee - saltiness. The type of acidity considered are those expressed by the most common organic acids characterizing the two coffee specie: acetic acid, malic acid, phosphoric acid, and citric acid for Arabica, which, in case of the Q-Robasta Course are accompanied by lactic acid and quinic acid too;

- tastes intensity recognition. This is a three steps test in which, part by part, are evaluated the ability to recognize different intensities of the base tastes characterizing coffee, discriminate between tastes at different intensities and, in step three, the skill to correctly identify the intensity of three different taste stimuli when blended in a single cup;

- identification of roasting defects. This is a crucial skill for a Q-Grader because, not conform roasting leads to a misevaluation of the coffee lot so, in this exam students are cupping 4 roasting defects, namely under-roasted, over-roasted, baked, and underdeveloped, together with an on-standard coffee and identify correctly and describe each cup issues.

- cupping skill. These exams (4 in Arabica and 5 in Robusta) are divided by origin of coffee and post harvesting processes. They are the most important part of the Q course because they summarize all the technical skills evaluated before and used then to evaluate coffee quality. Every exam requires to cup 6 coffees following the cupping protocol by SCA and to fill properly the cupping form, at the end students are evaluated in terms of calibration with the panel, discrimination power and ability to detect defects and not specialty coffee.

Once a candidate has passed all the exams he gets the title of Q-grader, and he is allowed to grade coffee lots to give them scores. Every three years, to assure

that the community is always calibrated and graders all over the world have uniform scoring system, the certified people are asked to be attend a calibration course in which 4 cupping exams are made on a single day and, at the end of that, the certification is reissued for additional three years.

Q-graders are this way ready to grade coffee but, to do this the protocol is crucial: it is easy to understand that without a common protocol no one around the world would perceive in the same way the same lot of coffee and no agreement on scores and sensory profile would be achievable.

As said before, the protocol is made by SCA and starts with green coffee grading. In this phase 350g of coffee is inspected to identify if defective beans are contained and, if so, to identify their severeness. The severity is evaluated considering the impact that a defective bean, when present, can have on the final cup profile and it is expressed by the number of beans needed to count a defect. That's why we have primary defects that are not allowed in specialty coffee and ask for a lower number of defected seeds to count a defect, and secondary defects that are allowed in specialty coffee for a maximum of 5 out of 350g because they are less compromising in terms of sensory impact and -for that – they have a higher number of beans to defect conversion coefficient. As an example, a full black bean could strongly impact on the sensory profile of a cup, giving dirty, moldy, sour, and phenolic notes, so it is needed just one bean counts as one primary defect and – therefore – a coffee sample containing 1 full black bean is not specialty. Conversely, three partial black beans count for 1 secondary defect so up to 15 partial (<50% of the surface of the beans) blacks are allowed. Additionally, the color and the odor of the bean mass are evaluated to check if they are in the color scale standard and frank from off-odors (Kosalos et al., 2013)

After green, also roasted beans are visually evaluated to ensure that no quackers are present in 100g of coffee. This type of beans does not achieve the color of the roasted mass, remaining pale brown, due to a lack of nutrients that allows to easy detection in the coffee batch.

Once roasted, coffee is weighted and ground cup by cup to limit the spread of a punctiform defect through all 5 cups required to evaluate the sensory profile, the uniformity, and the quality of a coffee lot. The fragrance of the coffee, as it is after grinding and before infusing it with water, is evaluated and then scored once compared with the aroma of the infused one.

After the infusion time, set in the protocol from SCA as all the other requirements for the evaluation of coffee, the cups are tasted individually at 3 different temperatures to evaluate flavors, acidity, body, aftertaste balance, and overall impression for coffee. The 5 cups are needed to assure that if a cup is defected the evaluation of coffee is guaranteed by the other four and the grader just need to subtract the number of points related to the defected cup.

If, at the end of all the evaluation stages, coffee scores 80 points or more, it is awarded the title of specialty coffee.

To conclude this brief overview, it must be underlined that to get a specialty or high-quality coffee it is not only mandatory to select coffee once ready to be sold as green, but it is a long journey that starts into the fields when the farmers chose the more suitable variety of coffee to plant in each slope of their parcel. Following, the meticulous labor of pickers – in some cases also assisted by machinery –, selectively harvesting only the fruits at the right ripening stage for the post-harvesting process the owner planned (or sometimes is able) to do. Additionally, all the cleaning and selection stages of the coffee fruit before processing, now a day, are year by year more assisted by high-tech expensive machinery, and to conclude the know-how of every single farmer that can find the right balance between innovation and tradition to keep the flavor of their lands while facing the challenges of climate change, scarcity of resources and increased demands. All these are vital parts of the production of quality, all of them are people who pay the extra value they give and, at the end, all of them are "who" the works presented in the following pages are addressed to.

1.2. AIM AND SCOPE

In the context of the valorization of the specialty and high-quality coffee supply chain, to provide with consistent basis not for only the economic value of coffee on the market in terms of absence of defects and alignment with quality standards, but also to prove the importance of all the stages of the complex journey from origins to cup on the total food quality of this products , the works here presented are focusing the attention on that small share of coffee market to identify the extra values that Specialty coffee can express, dealing with the intrinsic and extrinsic values deriving from the different countries of origin, the processes and the extraction methods.

Going through the different chapters, a multiplicity of factors dealing with quality and traceability of coffee are investigated.

Chapter 2 *(accepted by Journal of the Science of Food and Agriculture on February 4th,2023 – Manuscript number : JSFA-22-3497.R1; "Specialty and high-quality coffee: discrimination through elemental characterisation via ICP/OES, ICP/MS, and ICP/MS-MS of origin, species, and variety" by Fontanella, Mariachiara; Vezzulli, Fosca; Lambri, Milena; Beone, Gian Maria)*, dealing with the characterization of the elemental composition of green and roasted coffee lots, together with an overview on the evaluation of that related to silver skin – the main roasting by-products – is aimed to provide with a solid ground on which built a composition-based traceability system of Specialty coffee, from green to roasted and, potentially to the final coffee beverage.

The focus on the by-product is also innovative and look forwarding since the reduction of waste on one hand and on the other the continuous research of food additives able to supplement the intake of key nutrients such as minerals and phenolic compounds, are the challenges to face in a fast-growing world and part of the goals settled by Agenda 2030.

Thanks to the results of this analytic approach, together with the statistical treatment of data, it is expected to extend what is already provided for commercial green and roasted separately in a vertical model that links green to roasted coffee and – the two – to the origin and to the variety of coffee from which the beans originated. Lastly, because of the large and vary number of

samples included in the analysis, it also improves the already wide knowledge on mineral composition of coffee and its discriminant power with a robust characterization performed with standard analytical conditions and a wide-ranging discriminant model.

Chapter 3. *(Published on Foods 2023, 12, 489. https://doi.org/10.3390/ foods12030489. "Volatile Compounds in Green and Roasted Arabica Specialty Coffee: Discrimination of Origins, Post-Harvesting Processes, and Roasting Level" Vezzulli, F.; Lambri, M.; Bertuzzi, T.)* as a coherent consequence of the previous, using the same statistical approach on a different dataset, focuses on the most complex part of the coffee experience, namely the volatile compounds perceived as aroma, fragrance, flavors and concurring in the aftertaste.

In this piece of work, the head-space analysis via SPME-GC/MS was used to get chromatograms from the different Specialty Arabica coffee lots both as green ground coffee and roasted ground. The identification of the compounds and the relative quantification were crucial to draw the unique profile of each lot and identify similarities and differences provided by origin, post-harvesting processes, and roasting levels.

From a preliminary study of the available literature was detected that just a few works were dealing with a high number of high quality and traceable samples well representing the variability available on the market in terms of combination of origins and post-harvesting processes and, additionally, a lack of work including – for the same samples – data from green coffee and coffee roasted at different roasting levels.

Additionally, the flavor of coffee is, on the consumers' side, strongly linked with origins where the different varieties of Arabica coffee are grown, to post-harvesting processes applied to get the green coffee and, to the roasting profile applied too.

For those reasons, this work aimed to identify the molecules composing the volatile fraction of coffee and to assess if and at which intensity, those are modified in terms of relative concentration by the origin, post-harvesting processes and, for the roasted coffee, by the roasting levels to prove and sustain

what empirically experienced and proved from other authors, with different experimental designs, on the complexity and variability of coffee aroma.

For chapter 4 (*Published on. Int. J. Food Sci. Technol. 2022, 1–9, doi:10.1111/ijfs.15900. "Acrylamide: Impact of Precursors Concentration, Origin, Post-Harvesting Process and Roasting Level in High-Quality Arabica and Robusta Coffee" Vezzulli, F.; Triachini, S.; Mulazzi, A.; Lambri, M.; Bertuzzi, T.)* the attention moved to the safety side of Specialty coffee.

In the last decade several works pointed out that coffee as many of the foodstuff submitted for their preparation to thermal treatments like frying, baking, and roasting are supposed to develop different process contaminants in relation to their composition. Since coffee, as potato and bakery products, is rich in reducing sugar and amino acids that during roasting play as substrates for Maillard reactions, it was under a magnifying glass for its potential contamination from acrylamide.

Considering that the concentration of the above-mentioned contaminant was shown to be also related with the occurrence of seeds rich in asparagine, an investigation on the causes provided with this scenario was carried out and the presence of defective and immature beans in the lot was identified as source of asparagine. Because of that, as Specialty coffee is – by definition – selected and frank from high number of defective seeds, it was interesting to investigate if this higher quality of the raw materials matches whit a lower occurrence of the contaminant after roasting but also with a lower level of asparagine.

From several works it was also demonstrated that acrylamide occurrence depends not only on the composition of the beans but also to on the roasting profile, in that respect it was also investigated if nevertheless, starting from a green coffee with a lower risk of acrylamide development, any process condition could lead to a high level of contamination.

From previous prelaminar research (shown in Chapter 5.), it was expected that Arabica Specialty Coffee thanks to their particular selection method could represent a valid solution to prevent exceeding the benchmark from EFSA on acrylamide in roasted coffee even when light roasted.

Moving from green to roasted and finally to coffee beverage, in respect of the specialty coffee they were not often consumed after espresso extraction since the historically larger consumers of this beverage were not keen to the high acidity and floral/fruity flavors characterizing high quality arabica coffees. In the last years, a wider range of origins and post harvesting processes, together with more suitable roasting profiles available on the market tried to merge the Italian espresso and these lots of coffee into a balanced and well-loved beverage.

In chapter 5. *(Published on Int. J. Food Sci. Technol. 2021, doi:10.1111/ijfs.15380 "Sensory Profile of Italian Espresso Brewed Arabica Specialty Coffee under Three Roasting Profiles with Chemical and Safety Insight on Roasted Beans." Vezzulli, F.; Bertuzzi, T.; Rastelli, S.; Mulazzi, A.; Lambri, M.)* the above-mentioned problem was investigated. From previous literature, not much was available about the sensory profile of espresso coffee obtained from single origin Arabica coffee nor much about roasting profile optimization to exalt in espresso the flavors detected in Specialty coffee with official tasting methods.

Starting from that, a study was conducted on a smaller number of samples than the previous because it was conduct not on a laboratory scale but by roasting coffee on industrial machineries broadly diffused in small roastery of specialty coffee.

After roasting, to provide with characterization of roasting level not based only on color, some chemical parameters were evaluated together with a safety assessment on the amount of acrylamide produced during roasting and residual in coffee samples. Once proved the safety, coffee was submitted to sensory analysis to identify the best roasting profile to exalt a specialty coffee but also to match the expectation of traditional Italian espresso consumers to give a scientific-based tool to spread the market of Specialty coffees also in areas not accustomed to filter coffee.

To conclude, starting from the peculiarities already mentioned about extraction methods, this topic was investigated to identify molecular markers able to justify the differences among beverages and to identify the magnitude of those, net of coffee variability.

At state of the art, not many works approaching the extraction of coffee focused only on the extraction methods but more on the combination of extraction and other parameters such as – among the other – temperature, time of contact, and brew ratio.

In Chapter 6. *(Published on Foods 2022, 11, doi:10.3390/foods11060807.* *"Metabolomics Combined with Sensory Analysis Reveals the Impact of Different Extraction Methods on Coffee Beverages from Coffea Arabica and Coffea Canephora Var. Robusta." Vezzulli, F.; Rocchetti, G.; Lambri, M.; Lucini, L.)* to investigate the topic of extraction method, 2 standard samples of Arabica and Robusta coffee widely used in Italian espresso blends were prepared with 4 extraction systems, and then submitted to metabolomics and sensory analysis.

The combination of the two systems was chosen to confirm that the differences identified by the metabolomic approach were also perceivable from the human senses and that the hierarchical distances showed by the statistical analysis of the former data set are identifiable when analyzing the sensory analysis outcomes.

2. Specialty and high-quality coffee: discrimination through elemental characterization via ICP/OES, ICP/MS, and ICP/MS-MS of origin, species, and variety

2.1. ABSTRACT

2.1.1.BACKGROUND

This study aimed to establish the elemental profiling and origin combined with the genetic asset of coffee samples collected from major coffee-producing countries. A total of 76 samples were analyzed for 41 elements using inductively coupled plasma (ICP)-optical emission spectroscopy (OES), ICP-mass spectrometry (MS), and ICP-triple quadrupole (MS/MS). The mineral composition of the silver skin detachment during the roasting process was also evaluated to verify the loss of minerals during roasting, differences in composition with beans, and between species.

Application of linear discriminant analysis provided models with an accuracy of 93.3% for continents, 97.8% for countries of cultivation, and 100% for species. Discrimination between Arabica, Canephora coffee, and Eugenoides, and different varieties of Arabica species were identified in both models with Ca, Ba, Cd, Rb, and Sr as significant discriminant elements. Rb, Sr, S, and Tm were significant discriminant elements in both models for geographical distinction at different scales. Most of the elements had significantly higher values in silver skin than those in roasted coffee at different magnitudes, with exceptions of P and Rb.

In summary, the determination of mineral elements, processed by multivariate statistical analysis, was demonstrated to be discriminant for different coffee species. Linear discriminant analysis of the elemental analysis of samples from the seven major producing countries provided a reliable prediction model. Elemental analysis of major and minor elements is relatively easy and can be used together with other traceability systems and sensory evaluations to authenticate the origin of roasted coffee, different species, and varieties.

2.2. INTRODUCTION

Coffee is the accumulation of roasted beans from the green seeds of drupes produced by a bush belonging to the family *Rubiaceae* and genus *Coffea* [1]. The two main species cultivated in the tropical area between the two tropics were *Coffea arabica* and *Coffea canephora*[2].

The genetic differences between the two species and several related varieties ascribable to them are reflected in tree behaviour when housed at diverse latitudes, longitudes, altitudes, and soils. This is one of the main reasons why the origin of coffee lots is one of the main features that influence the cup sensory profile, together with the roasting process and non-defective nature.

As for other crops and food products whose origin and varieties are linked to different levels of quality and price [3-5], coffee is of major importance to assure the downstream players of the supply chain on the traceability of lots and to guarantee to final consumers that the financial outlay paid for a specific cup of coffee is directly proportional to the intrinsic quality in terms of the sensory profile and origin of the raw material.

In addition, it is necessary to identify a reliable method to guarantee that the origin and variety declared on roasted coffee packages conform to the true ones to prevent fraud. Considering the role of roasting on the physical features of green coffee and its impact on molecular composition, it is challenging to identify systems that track the origin using these features [6-8].

As provided by many authors, the elemental composition of coffee beans, being roasted or green, is helpful to discriminate the quality of that raw material, identifying if the seed is defective or non-defective and whether it is already roasted. In addition [9], it can differentiate between different regions of cultivation when considering Arabica coffee from a specific country and, in the same context, seeds of other subspecies [10-12].

In addition, elemental characterization conducted via different analytical approaches has already been applied to trace the origin of coffee; however, to the best of our knowledge, there are only a few studies [13,14] based on a comprehensive approach to traceability, linking green coffee to the origin and

roasted to the related green, considering a vast range of elements, such as macro, micro, and rare earth elements, in samples of specialty or premium specialty coffee.

Therefore, this study evaluated the elemental composition of different lots of coffee via inductively coupled plasma (ICP)-optical emission spectroscopy (OES), ICP-mass spectrometry (MS), and ICP-triple quadrupole (MS/MS) to first verify whether the elemental profile of coffee is stable once green coffee is roasted, considering the losses caused by the detachment of silver skin during the roasting process. In addition, this study investigated whether the differences in geographical origin, combined with the genetic assets of coffee trees cultivated in different countries, provide differences in the elemental composition of the seeds.

2.3. MATERIALS AND METHODS

2.3.1. Coffee samples

From 75 samples of coffee from species Arabica, Canephora, and Eugenoides (Table S1), 1 kg was drawn as a representative sample from GrainPro bags of 60 kg each containing Arabica coffees recognised as 'Specialty' or 'Premium' coffee according to the Specialty Coffee Association. These coffees scored at cupping 80/100 or more, and beans were free from primary defects (only a small number of unripe or broken beans were allowed). The selected Canephora samples were obtained from the higher quality standards for each country (Supremo NV, 2021) involved in the sample list. Coffee samples also represent the main varieties cultivated worldwide, considering the variability available in the coffee market. Finally, all post-harvesting processes applied to the coffee chain were included in our sample set.

Green coffee samples (500 g) were frozen and milled using a cyclone hammer mill (1 mm sieve, Pulverisette, Fritsch GmbH, Idar-Oberstein, Germany) and then homogenised. Thereafter, 300 g of the sample was collected and stored at -20 °C until analysis.

2.3.2. Roasting

The samples were roasted using an IKAWA Model V2-PRO (IKAWA Ltd., UK, 2018). This equipment is a convective roaster that applies airflow into the roasting chamber that agitates and roasts simultaneously. The silver skin was removed using a cyclone system and collected in a jar. Each roasting batch weighed 50 g (± 0.5 g). The roasting profile applied was settled as follows: the chamber was preheated at 174–175 °C before the coffee inlet, and the roasting ended at 215 °C for 6.46 minutes. Once roasted, all samples were accurately weighted and, both for roasted coffee and silver skin, stored at -20 °C. At the time of analysis, the frozen seeds were ground using a Moulinex blender (Model AR110830).

The relative stability of the elemental composition between green and roasted coffee was verified by analyzing the silver skin detached during roasting and calculating the mass balance dry-based.

2.3.3. Roasted coffee analysis

In this study, 0.5 g of milled roasted coffee was mineralized in a Teflon tube with 5 mL of ultrapure HNO_3 65% (Carlo Erba (Milan, Italy) and 1 mL of H_2O_2 30% (Carlo Erba (Milan, Italy) using a microwave system (Mars 5 Express, CEM) at 800 W, following two temperature steps: one at 140 °C for 20 min hold time and the next one at 200 °C for 20 min hold time. After cooling, the mixtures were added to a final volume of 50 mL of ultrapure water (18.2 MΩ cm, ELGA PURELAB flex, Veolia Water Solutions and Technologies, Ontario, Canada) in polypropylene tubes (DigiTUBES, SCP Science, Champlain, NY, USA). The extracts were filtered using a 0.45 µm Teflon filter (DigiFILTER, SCP Science, Champlain, NY, USA).

The digests were diluted with distilled water, and the microelements were analyzed using an inductively coupled plasma-mass spectrometer (ICP-MS 7850 Agilent Technologies, Santa Clara, CA, USA). The operating conditions are listed in Table S2. The macroelements were determined using an inductively coupled plasma-optical emission spectrometer (ICP-OES 5100 Agilent Technologies, Santa Clara, CA, USA), and the operating conditions are listed in

Table S3. The analysis of rare earth elements (REE) was performed by inductively coupled plasma-mass spectrometer triple quadrupole (ICP-MS/MS 8900 Agilent Technologies, Santa Clara, CA, USA) (Table S4).

The method detection limit (MDL) for coffee treatments was calculated as three times the standard deviation of six analytical blanks prepared and diluted in the same manner as the samples [15].

Analytical quality control was periodically carried out in triplicate with two certified reference materials: tea (NCS DC 73351) and rice flour (NIST 1568a).

2.3.4. *Statistical analysis*

Statistical analysis of the elemental composition was carried out using the IBM SPSS statistics package (ver. 27, Inc., Chicago, IL, USA) and Microsoft Excel. The homogeneity of variance was checked. A t-test was conducted to evaluate whether significant differences were present between Robusta and Arabica coffee compositions. One-way analysis of variance and discriminant analysis were applied to assess significant differences in elemental composition among continents and countries of origin. Tukey's post hoc test was applied. A t-test and PCA were performed on the silver skin composition data to discriminate between coffee species.

2.4. RESULTS AND DISCUSSION

2.4.1. *Validation of quality control procedure*

The concentrations of the elements determined in tea (NCS DC 73351) and rice flour (NIST 1568a) are listed in Table 1. The actual experimental values of these contents were in line with the specified concentrations in the certified reference materials, with recoveries ranging between 80% and 110% and relative standard deviations below 18%. The detection limits for this method are listed in Table 1.

Table 1. Validation parameters for the determinations of macro, microelements, and REE in roasted coffee: method detection limits (MDL), values of certified reference materials (NCS DC 73351 and NIST1568a), recovery on certified matrices, and relative standard deviation (RSD).

	MDL (mg kg^{-1}) ICP-OES	BCR NCS DC 73351 (mg kg^{-1})	Recovery (%)	RSD (%)	BCR NIST 1568a (mg kg^{-1})	Recovery (%)	RSD (%)
Ba	0.05	58	97	0.9			
Ca	7.53	4300	88	1.1	118	94	0.9
Fe	0.69	264	81	6.4	7	84	6.4
K	34.4	16600	88	1.6	1280	96	3.0
Mg	0.35	1700	85	2.6	560	85	3.0
Na	0.23						
P	0.56	2840	96	0.3	1530	94	1.7
S	2.00	2450	97	0.4	1200	89	1.3
Zn	2.96	26	95	1.8	19	92	1.1
	ICP-MS						
Li	0.014						
Be	0.0051						
Al	0.25				4.40	88	15
V	0.0054						
Cr	0.013	0.80	80	8			
Mn	0.017	1240	100	10	20	88	8
Co	0.0070	0.180	99	9			
Ni	0.0095	4.60	99	10			
Cu	0.44	17.30	99	7	2.40	88	14
As	0.0060	0.280	93	11	0.290	95	14
Se	0.011				0.38	88	18
Rb	0.036	7.4	97	4	6.14	95	5
Sr	0.020	15.2	95	8			
Mo	0.013	0.038	104	12	1.46	91	10
Ag	0.13						
Cd	0.0054	0.057	107	6	0.022	102	5
Sb	0.0058	0.056	80	7			
Pb	0.098	4.40	94	10			
	ICP-						
	(µg kg^{-1})						
La	2.40	600	96	9.1			
Ce	2.55	1000	91	9.2			
Pr	2.12						
Nd	3.12						
Sm	3.99	85	96	8.5			
Eu	3.93	18	110	12			
Gd	3.71						
Tb	3.25						
Dy	3.94						
Ho	2.79						
Er	2.66						
Tm	2.23						
Yb	3.82	44	86	7.1			
Lu	2.46						

2.4.2.Distribution of elements

The elements analysed in this study were classified as macro, micro, and trace based on their concentration levels, as previously reported in the literature [11,16-26].

Brazilian and Ethiopian are the most investigated roasted coffee, both as an elementary composition and for geographical discrimination (Table S4a). However, roasted coffees, ground, or beans from Costa Rica, Panama, and India have been less studied or differentiated (Table S4b). Therefore, it can be inferred that the basic composition of these coffees is unknown and that there is no information on some fundamental elements, such as P, S, and Na.

The magnitude distributions of essential mineral concentrations, including rubidium, in roasted coffee divided by nation, are presented in Table 2.

Table 2. Distribution of most concentrated elements in roasted coffee from different nations.

Nation	Samples	Element distribution
Brazil	N = 9	K > Mg > P > S > Ca > Fe > Mn > Rb
Colombia	N = 8	K > Mg > P > S > Ca > Mn > Fe > Rb
Costa Rica	N= 3	K > Mg > P > S > Ca > Rb > Fe > Mn
Ethiopia	N = 13	K > P > Mg > S > Ca > Rb > Fe > Mn
India	N = 5	K > P > Mg > S > Ca > Fe > Na > Rb
Indonesia	N= 6	K > P > Mg > S > Ca > Rb > Fe > Mn
Panama	N= 5	K > Mg > P > S > Ca > Fe > Rb > Mn

The same distribution was described by other authors in roasted coffee from the Brazilian region, except for Bitter et al. (2020) [16], who published a different order of magnitude between Mn and Fe. Debastiani et al. (2014) [20] (2019)[18] (2021) [20,21] found a lower concentration of sulphur in all articles (Table S4a).

In the Colombian database, only Cloete et al. (2019) [24] reported a different distribution of macro elements, with higher values of Ca and Rb and lower values of P (Table S4b). The same author wrote about the lower distribution of P in the Ethiopian group. However, Feleke et al. (2018) [11] wrote about three times higher Ca concentration and an extremely high value of iron (Table S4a).

The macroelement with the highest concentration was potassium, followed by magnesium or phosphorus, with some differences from different origins. Central and southern American coffee show higher concentrations of

magnesium than phosphorus, and the opposite behavior is shown by samples from Africa and Asia.

The elements in Tables 3, 4, and 5 are reported as the concentrations of macroelements, microelements, and rare earth elements, as already defined by Habte et al. (2016) [10].

Through the determination of the method detection limit, elements that were near or below the limit of detection in the sample were suppressed from multivariate analysis, like lithium and beryllium and different rare earth elements, such as samarium, europium, gadolinium, terbium, holmium, ytterbium, and lutetium.

 No significant differences depending on the origin were detected by Tukey's post hoc test (0.05) for the concentrations of barium, sodium, lithium, beryllium, copper, arsenic, selenium, molybdenum, silver, and antimony, and different rare earth elements, such as samarium, gadolinium, terbium, dysprosium, holmium, erbium, thulium, ytterbium, and lutetium.

Table 3. Means ± standard deviation (SD) of the macroelements contents in roasted coffee from different nations. Different letters indicate significant differences in element content (ANOVA, Tukey's test, $p < 0.05$) between nations.

CONTINENT	NATION		Ba mg kg^{-1}		Ca mg kg^{-1}		Fe mg kg^{-1}		K mg kg^{-1}		Mg mg kg^{-1}		Na mg kg^{-1}		P mg kg^{-1}		S mg kg^{-1}		Zn mg kg^{-1}	
AMERICA	BRASIL	MEAN	3.32	a	1075	b	29.6	ab	19548	b	1922	c	13.8	a	1609	ab	1524	c	5.64	ab
	n = 9	SD	1.60		161		2.8		1251		55		23.6		173		67		0.61	
		MAX	7.08		1467		34.4		21391		1992		67.0		2019		1609		6.92	
		MIN	2.14		913		26.0		18053		1829		1.1		1438		1409		4.86	
	COLOMBIA	MEAN	7.27	a	990	ab	27.1	ab	17077	a	1769	ab	5.8	a	1754	abc	1411	b	6.39	b
	n = 8	SD	2.07		208		2.9		1229		117		4.0		109		62		1.51	
		MAX	9.57		1463		30.4		17961		1938		12.9		1885		1494		9.60	
		MIN	3.06		777		24.1		14503		1586		1.4		1542		1311		5.10	
	PANAMA	MEAN	6.55	a	909	a	25.5	a	18329	ab	1780	ab	7.8	a	1557	a	1401	ab	5.28	ab
	n = 5	SD	2.46		246		2.6		785		117		8.4		144		39		1.00	
		MAX	9.03		1319		28.4		19246		1943		21.9		1720		1464		6.58	
		MIN	3.70		683		21.7		17373		1660		0.9		1405		1364		4.46	
	COSTA RICA	MEAN	4.71	a	1023	ab	35.4	ab	16891	a	1882	bc	5.3	a	1625	ab	1420	b	4.90	ab
	n = 3	SD	1.89		145		13.8		579		53		5.5		145		64		0.77	
		MAX	6.43		1170		51.3		17560		1941		11.7		1719		1460		5.57	
		MIN	2.68		880		26.6		16554		1840		2.0		1457		1347		4.06	
AFRICA	ETHIOPIA	MEAN	3.75	a	884	ab	23.4	a	17227	a	1720	ab	8.9	a	1780	bc	1313	a	4.62	a
	n = 13	SD	0.83		66		2.9		429		48		6.0		98		49		0.36	
		MAX	5.67		1000		28.8		17727		1820		21.3		1959		1391		5.41	
		MIN	2.16		786		19.5		16473		1628		2.7		1647		1235		4.13	
ASIA	INDIA	MEAN	3.59	a	1142	b	35.9	b	19288	b	1683	a	28.0	a	1869	c	1471	bc	6.08	ab
	n = 5	SD	0.71		69		13.5		422		163		12.4		202		15		0.88	
		MAX	4.52		1208		59.4		19715		1909		48.2		2092		1492		7.62	
		MIN	2.76		1030		24.9		18580		1545		17.9		1625		1451		5.51	
	INDONESIA	MEAN	4.21	a	1072	ab	30.2	ab	18499	ab	1701	a	9.6	a	1758	abc	1405	ab	5.71	ab
	n = 6	SD	2.47		66		5.1		840		89		4.5		120		36		0.58	
		MAX	8.56		1176		37.9		19695		1794		15.0		1912		1455		6.59	
		MIN	1.15		994		22.8		17125		1553		3.6		1656		1351		5.04	

Table 4a. Means ± standard deviation (SD) of the microelements contents in roasted coffee from different nations. Different letters indicate significant differences in element content (ANOVA. Tukey's test. $p < 0.05$) between nations. *excluded by ANOVA

CONTINENT	NATION		Li* (µg kg⁻¹)		Be* (µg kg⁻¹)		Al (µg kg⁻¹)		V (µg kg⁻¹)		Cr (µg kg⁻¹)		Mn (µg kg⁻¹)		Co (µg kg⁻¹)		Ni (µg kg⁻¹)		Cu (µg kg⁻¹)	
AMERICA	BRASIL	MEAN	<14		<5		3153	abc	<5.4	a	78	a	27594	abc	180	a	276	a	13671	a
	n = 9	SD					1575				148		8076		113		102		1715	
		MAX	18		5		5126		7.8		470		44504		397		383		15798	
		MIN	<14		<5		<250		<5.4		<13		14851		35		123		10167	
	COLOMBIA	MEAN	13		<5		2097	ab	5.7	a	86	a	32773	bc	143	a	424	a	12954	a
	n = 8	SD	10				1578		3.9		85		9439		129		185		2093	
		MAX	26		14		4314		9.8		209		49021		368		702		15117	
		MIN	<14		<5		<250		<5.4		<13		22014		17		57		9132	
	PANAMA	MEAN	<14		<5		2719	a	<5.4	a	90	a	16615	ab	48	a	400	a	13903	a
	n = 5	SD					4474				123		1821		10		202		875	
		MAX	11		<5		7884		<5.4		273		18795		58		716		14538	
		MIN	<14		<5		<250		<5.4		<13		14892		31		171		12366	
	COSTA RICA	MEAN	<14		<5		8822	c	37.2	b	1475	b	32241	c	92	a	840	a	14367	a
	n = 3	SD					6591		3.5		2381		3893		8		925		868	
		MAX	17		<5		13257		39.7		4224		36155		98		1899		15196	
		MIN	<14		<5		1248		<5.4		78		28369		83		188		13465	
AFRICA	ETHIOPIA	MEAN	<14		<5		2991	abc	6.8	a	35	a	14012	a	35	a	305	a	12600	a
	n = 13	SD					2484		3.9		36		1008		12		251		987	
		MAX	22		10		7588		9.5		116		15451		51		1078		14673	
		MIN	<14		<5		<250		<5.4		<13		12650		14		104		10471	
ASIA	INDIA	MEAN	<14		<5		6049	bc	54.0	ab	73	a	20040	abc	311	b	2379	**b**	13998	a
	n = 5	SD					6164				38		9099		37		813		1234	
		MAX	18		5		16589		54.0		127		33062		340		3295		15520	
		MIN	<14		<5		1817		<5.4		31		13092		250		1531		12256	
	INDONESIA	MEAN	14		<5		6180	abc	<5.4	a	161	a	21790	abc	59	a	232	a	12798	a
	n = 6	SD	1				2881				250		11078		61		124		2824	
		MAX	14		<5		9552		11.6		668		37735		178		401		15873	
		MIN	<14		<5		1944		<5.4		16		11864		16		114		8207	

Table 4b. Means ± standard deviation (SD) of the microelements contents in roasted coffee from different nations. Different letters indicate significant differences in element content (ANOVA, Tukey's test, $p < 0.05$) between nations.

CONTINENT	NATION		As $\mu g\ kg^{-1}$		Se $\mu g\ kg^{-1}$		Rb $\mu g\ kg^{-1}$		Sr $\mu g\ kg^{-1}$		Mo $\mu g\ kg^{-1}$		Ag $\mu g\ kg^{-1}$		Cd $\mu g\ kg^{-1}$		Sb $\mu g\ kg^{-1}$		Pb $\mu g\ kg^{-1}$	
AMERICA	BRASIL	MEAN	<6.0	a	63	a	26264	a	5435	ab	73	a	<134	a	<5.4	ab	6.6	a	<98	a
	n = 9	SD			138		13029		1987		72						8.0			
		MAX	<6.0		375		44037		8732		248		283.5		6.7		20.4		461	
		MIN	<6.0		<11		9856		3252		<13		<134		<5.4		<5.8		<98	
	COLOMBIA	MEAN	<6.0	a	17	a	24005	a	8559	bc	102	a	<134	a	8.5	ab	13.7	a	<98	ab
	n = 8	SD			8.9		8480		1490		114				7.0		9.6			
		MAX	9.0		25		34114		11088		306		<134		22.9		20.5		367	
		MIN	<6.0		<11		11452		6471		<13		<134		<5.4		<5.8		<98	
	PANAMA	MEAN	<6.0	a	<11	a	20283	a	12889	c	106	a	<134	a	<5.4	a	8.5	a	<98	a
	n = 5	SD					2453		3354		88						0.0			
		MAX	<6.0		14.1		22502		16014		236		<134		<5.4		8.5		133	
		MIN	<6.0		<11		16167		9054		28		<134		<5.4		<5.8		<98	
	COSTA RICA	MEAN	15.9	a	<11	a	38578	a	9389	bc	316	a	<134	a	7.5	b	12.7	a	129	b
	n = 3	SD	21.3				7644		2897		426				6.9		0.0		202	
		MAX	31.0		<11		47232		12498		618		<134		15.4		12.7		362	
		MIN	<6.0		<11		32749		6765		<13		<134		<5.4		<5.8		<98	
AFRICA	ETHIOPIA	MEAN	20.1	a	101	a	29024		3882	a	106	a	1068	a	<5.4	a	5.5	a	<98	ab
	n = 13	SD	42.0		69		8153		505		79		1763				3.1			
		MAX	105.9		213		46932		4998		263		3104		<5.4		8.3		196	
		MIN	<6.0		<11		19716		3024		<13		<134		<5.4		<5.8		<98	
ASIA	INDIA	MEAN	<6.0	a	34	a	23996	a	3831	a	51	a	<134	a	7.0	b	6.4	a	<98	ab
	n = 5	SD			7		4694		560		19				3.5		3.1			
		MAX	<6.0		41		29325		4470		79		<134		9.9		8.9		105	
		MIN	<6.0		25		16557		3300		<13		<134		<5.4		<5.8		<98	
	INDONESIA	MEAN	<6.0	a	21	a	71253	b	6799	ab	102	a	<134	a	<5.4	ab	<5.8	a	<98	ab
	n = 6	SD			20		22927		2722		91									
		MAX	<6.0		58		88558		11825		223		<134		9.0		<5.8		160.5	
		MIN	<6.0		<11		28446		3725		<13		<134		<5.4		<5.8		<98	

Table 5a. Means ± standard deviation (SD) of the REE contents in roasted coffee from different nations. Different letters indicate significant differences in element content (ANOVA. Tukey's test. $p < 0.05$) between nations. *excluded by ANOVA

CONTINENT	NATION		La µg kg^{-1}		Ce µg kg^{-1}		Pr µg kg^{-1}		Nd µg kg^{-1}		Sm* µg kg^{-1}		Eu* µg kg^{-1}		Gd* µg kg^{-1}	
AMERICA	BRASIL	MEAN	11.1	ab	11.7	ab	<2.12	ab	5.1	a	<3.99		<3.93		<3.71	
	n = 9	SD	7.4		6.7				3.9							
		MAX	23.1		23.2		2.74		11.9		<3.99		<3.93		<3.71	
		MIN	<2.40		<2.55		<2.12		<3.12		<3.99		<3.93		<3.71	
	COLOMBIA	MEAN	<2.40	a	<2.55	a	<2.12	ab	<3.12	a	<3.99		<3.93		<3.71	
	n = 8	SD														
		MAX	4.9		4.0		<2.12		<3.12		<3.99		<3.93		<3.71	
		MIN	<2.40		<2.55		<2.12		<3.12		<3.99		<3.93		<3.71	
	PANAMA	MEAN	10.0	ab	28.1	ab	4.85	ab	9.6	ab	<3.99		<3.93		<3.71	
	n = 5	SD	14.3		32.4		5.82		16.3							
		MAX	34.8		64.2		8.96		38.2		9.26		<3.93		7.57	
		MIN	<2.40		<2.55		<2.12		<3.12		<3.99		<3.93		<3.71	
	COSTA RICA	MEAN	3.1	a	3.7	a	<2.12	ab	<3.12	a	<3.99		<3.93		<3.71	
	n = 3	SD	2.0		2.8											
		MAX	5.3		6.8		<2.12		3.5		<3.99		<3.93		<3.71	
		MIN	<2.40		<2.55		<2.12		<3.12		<3.99		<3.93		<3.71	
AFRICA	ETHIOPIA	MEAN	4.5	a	6.8	ab	<2.12	ab	3.4	a	<3.99		<3.93		<3.71	
	n = 13	SD	4.1		8.2				3.9							
		MAX	15.2		28.5		2.71		13.2		<3.99		<3.93		<3.71	
		MIN	<2.40		<2.55		<2.12		<3.12		<3.99		<3.93		<3.71	
ASIA	INDIA	MEAN	22.0	b	27.7	b	5.17	b	18.1	b	<3.99		<3.93		<3.71	
	n = 5	SD	35.7		48.6		7.31		32.8							
		MAX	85.9		114.5		13.61		76.8		8.8		4.3		6.9	
		MIN	4.5		3.1		<2.12		<3.12		<3.99		<3.93		<3.71	
	INDONESIA	MEAN	4.4	a	5.5	ab	<2.12	ab	<3.12	a	<3.99		<3.93		<3.71	
	n = 6	SD	2.0		2.1											
		MAX	7.1		9.4		<2.12		3.9		<3.99		<3.93		<3.71	
		MIN	<2.40		4.2		<2.12		<3.12		<3.99		<3.93		<3.71	

Table 5b. Means ± standard deviation (SD) of the REE contents in roasted coffee from different nations. Different letters indicate significant differences in element content (ANOVA. Tukey's test. $p < 0.05$) between nations. *excluded by ANOVA

CONTINENT	NATION		Tb*	Dy*	Ho*	Er		Tm*	Yb*	Lu*
			µg kg⁻¹	µg kg⁻¹	µg kg⁻¹	µg kg⁻¹		µg kg⁻¹	µg kg⁻¹	µg kg⁻¹
AMERICA	BRASIL	MEAN	<3.25	<3.94	<2.79	<2.66	a	<2.23	<3.82	<2.46
	n = 9	SD								
		MAX	<3.25	<3.94	<2.79	<2.66		<2.23	<3.82	<2.46
		MIN	<3.25	<3.94	<2.79	<2.66		<2.23	<3.82	<2.46
	COLOMBIA	MEAN	<3.25	<3.94	<2.79	<2.66	a	<2.23	<3.82	<2.46
	n = 8	SD								
		MAX	<3.25	<3.94	<2.79	<2.66		<2.23	<3.82	<2.46
		MIN	<3.25	<3.94	<2.79	<2.66		<2.23	<3.82	<2.46
	PANAMA	MEAN	<3.25	7.4	<2.79	4.0	a	<2.23	<3.82	<2.46
	n = 5	SD								
		MAX	<3.25	7.4	<2.79	4.0		<2.23	<3.82	<2.46
		MIN	<3.25	<3.94	<2.79	<2.66		<2.23	<3.82	<2.46
	COSTA RICA	MEAN	<3.25	<3.94	<2.79	<2.66	a	<2.23	<3.82	<2.46
	n = 3	SD								
		MAX	<3.25	<3.94	<2.79	<2.66		<2.23	<3.82	<2.46
		MIN	<3.25	<3.94	<2.79	<2.66		<2.23	<3.82	<2.46
AFRICA	ETIOPIA	MEAN	<3.25	<3.94	<2.79	<2.66	a	<2.23	<3.82	<2.46
	n = 13	SD								
		MAX	<3.25	<3.94	<2.79	<2.66		<2.23	<3.82	<2.46
		MIN	<3.25	<3.94	<2.79	<2.66		<2.23	<3.82	<2.46
ASIA	INDIA	MEAN	<3.25	<3.94	<2.79	<2.66	a	<2.23	<3.82	<2.46
	n = 5	SD								
		MAX	<3.25	9.9	<2.79	3.4		<2.23	<3.82	<2.46
		MIN	<3.25	<3.94	<2.79	<2.66		<2.23	<3.82	<2.46
	INDONESIA	MEAN	<3.25	<3.94	<2.79	<2.66	a	<2.23	<3.82	<2.46
	n = 6	SD								
		MAX	<3.25	<3.94	<2.79	<2.66		<2.23	<3.82	<2.46
		MIN	<3.25	<3.94	<2.79	<2.66		<2.23	<3.82	<2.46

There are non-essential elements that have no functional effects on the body. Lead (Pb), cadmium (Cd), and arsenic (As) are of particular concern because of their adverse health effects. To avoid toxic effects, the EU Commission has set maximum permitted levels for Pb, Cd, and As, in a number of foods in its Regulation No. 1881/2006. The maximum levels for arsenic, cadmium and lead are 0.1–0.25 mg kg^{-1}, 0.05–1 mg kg^{-1}, and 0.02–1.5 mg kg^{-1}, respectively. The reported concentrations of heavy metals were below the maximum levels set for other foodstuffs or food supplements as set in Commission Regulation (EC) No 1881/2006 [27] and food supplements as set in Regulation (EU) No 488/2014 [28], except for a few exceptions in the Ethiopian batch for As and Pb, with higher values in the South American group.

Calcium was higher in Brazilian and Indian coffee than that in Panama, which, in contrast, peaked in the concentration of iron. Potassium was significantly higher in Brazilian and Indian coffee samples than that in Colombian, Costa Rican, and Ethiopian samples. Magnesium was higher in American (especially Brazilian) coffee than that in Asian coffee, whereas the opposite was true for phosphorus. African coffee was the least concentrated in sulphur, zinc, and cadmium, together with Panama. Costa Rican coffee had an average higher concentration of aluminium, vanadium, chromium, manganese, and strontium, together with Panamanian and Colombian coffee. Indian coffee peaked at concentrations of Co and Ni, similar to Indonesian coffee, for rubidium. Brazilian and Panamanian coffee had lower concentrations of Pb, and Ethiopian coffee had lower concentrations of Mn.

The Al, Ba, Ca, Cr, Cu, Fe, K, Mg, Mn, Ni, Rb, and Sr concentrations found in our sample of Ethiopian coffee are consistent with those reported by Worku et al. (2019) [12].

Regarding rare-earth elements, Indian coffee peaked for lanthanum, cerium, praseodymium, neodymium, europium, and dysprosium. No significant differences were found in the concentrations of the other rare earth elements; this also depends on the fact that the concentrations were generally as low as the MDL for all elements.

2.4.3.Discrimination of species, variety, continent, and county of origin

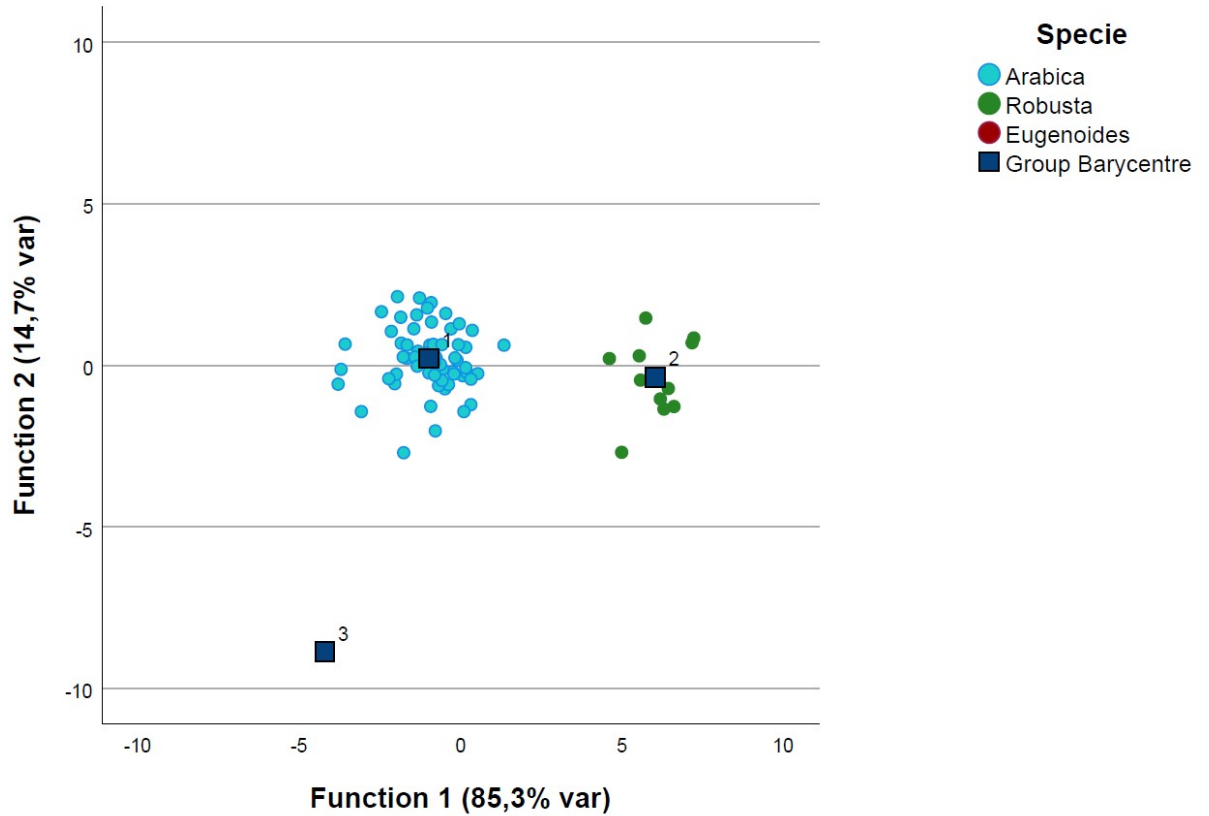The result of the discriminant analysis carried out among the species is shown in Figure. 1.

Figure 1. Graphical outcome of discriminant analysis of Coffea specie.

As provided by many authors [29-33] Arabica and Robusta coffee seeds profoundly differ in terms of macromolecular concentration, such as proteins, sugars, trigonelline, chlorogenic acids, fatty acids, and alkaloids. In addition, a deep genetic difference, namely diploid genome for Robusta and tetraploid for Arabica, is used to identify the two species and their presence in blends [34]; that feature also reflects in the characteristic physiology of the Coffea species and a different elemental composition of the seeds [7]. Using canonical discriminant analysis on elemental composition, it is possible to discriminate between Arabica and Canephora coffee and the Eugenoides. This ancestor species concurred in the speciation process with Canephora coffee to create Arabica coffee [35].

The three subgroups were clustered via functions based on the concentrations of Ba, Ca, K, Mg, P, Zn, Mn, Co, Rb, Sr, Cd, and Pb. Almost 100.0% of the 75 original grouped cases were classified correctly by the two discriminant functions, explaining the 85.3% and the 14.7% of the variance, respectively.

Applying leave-one-out validation to verify the power of the discriminant model, we reached 97.3% of cases classified correctly.

Since only one sample of Eugenoides coffee beans was collected at state of art, it is not possible to conclude that Eugenoides species had a significantly different composition from Arabica and Canephora. However, compared to previous studies, we can confirm that the discrimination is possible due to the molecular compositions [6,36].

Using a t-test ($p < 0.05$ – Cohen's D > 2), it was also possible to state that Robusta coffee had significantly higher concentrations of La, Ce, Nd, P, S, Co, and Ni than Arabica, and that, in contrast, Arabica had higher concentrations of Ba, K, and Mg than Robusta.

The differences in the elemental composition of soils from different continents have widely been reported, and this, together with the characteristic deposition of the element in coffee beans, allowed, via canonical discriminant analysis, to get an efficient clustering (93.3%) of samples based on the continent of origin (Figure. 2).
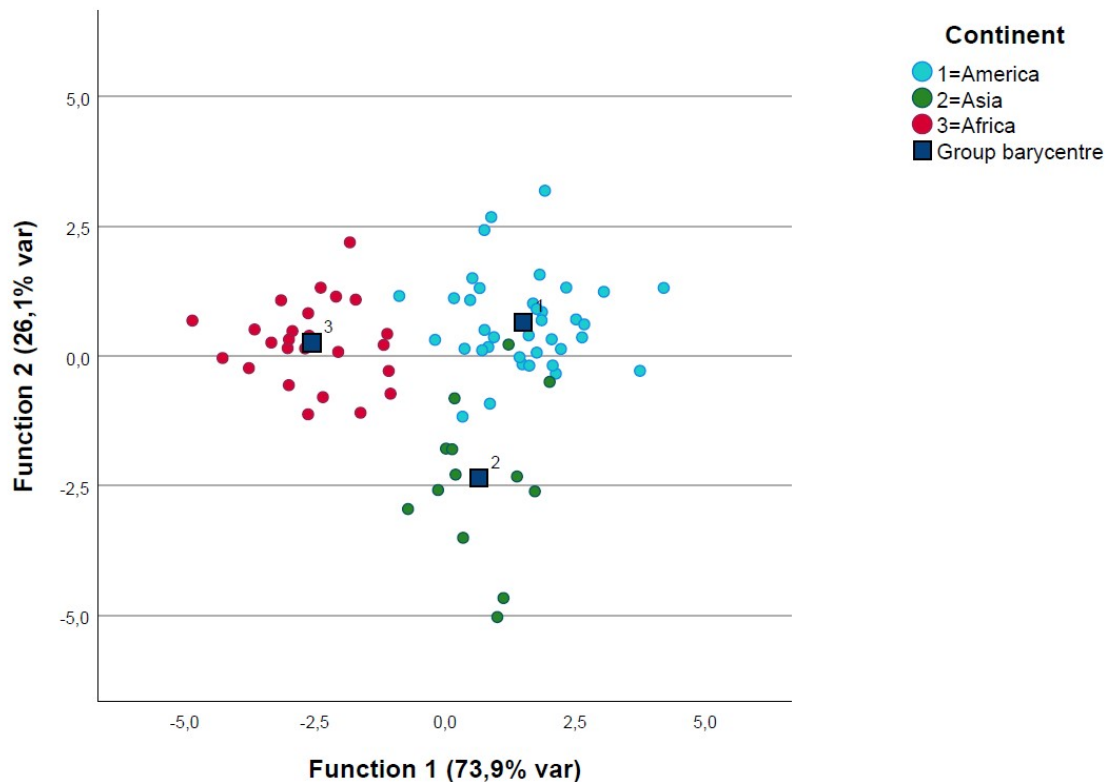
Figure 2. Graphical outcome of discriminant analysis of continent of origin.

The discriminant model identified Ce, Mg, Nd, Rb, S, Se, Sr, Tm, and Zn as the significant discriminant elements. The first two discriminant functions explained the 73.9% and the 26.1% of the variance, respectively. Leave-one-out validation was applied to verify the power of the discriminant model, which showed that 90.7% of cases were classified correctly.

As already provided by Liu et al. (2014) [37], Sr and Rb are powerful elements in the discrimination of macroareas of coffee origin.

A higher level of detail was reached only by selecting samples from a country of origin represented in the sample set by a few samples of five or more coffee lots.

Figure. 3 shows the graphical result of the canonical discriminant analysis conducted to investigate whether common trends in the elemental composition of coffees from the same country of origin but different farms of area are present and if these can provide clustering of them.
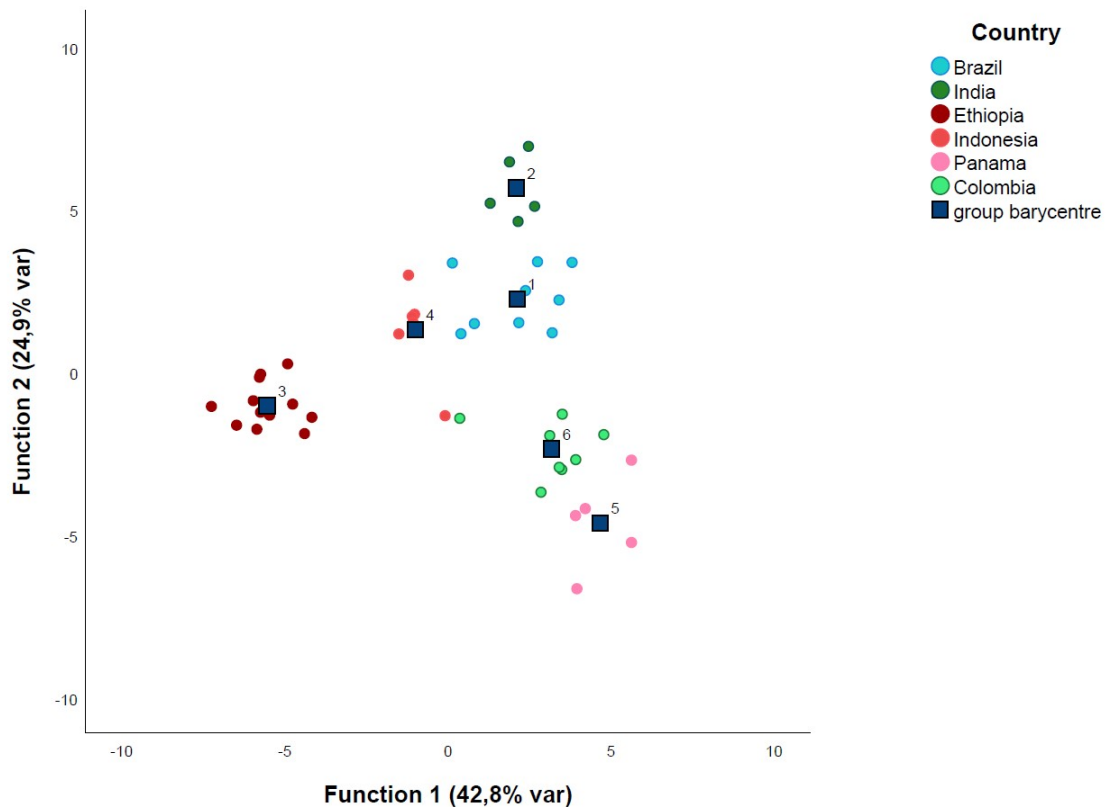
Figure 3. Graphical outcome of discriminant analysis of country of origin.

As shown, Ethiopian coffee is the most unique in the set due to the presence of indigenous varieties in Ethiopia, many of which are not listed in the taxonomic records responsible for the expression of an incomparable product of variety × terroir. Statistically, no misclassifications were detected during the leave-one-out validation of the model for this origin. The same was true for Indian coffee, even if samples representing the origin were from Arabica, Robusta, and Panamanian species.

Misclassification occurred during the validation between Brazilian and Indonesian coffees, and Colombian coffee was identified as Panamanian. All that considered, the two discriminant functions explaining the 42.8% and the 24.9% of the total variance had correctly classified 97.8% of the 46 original grouped cases. In addition, 93.5% of the cases were correctly classified using leave-one-out validation. In this context, discrimination was built on the Tm, Ca, Fe, K, P, S, Mn, Ni, Rb, and Sr concentrations.

Four identified elements, namely Ca, Mn, Rb, and Sr, have already been identified as discriminants for the different growing regions of Ethiopian coffee by Worku et al. (2019) [12].

By analyzing the elemental composition data, considering only monovarietal lots of coffee, canonical discriminant functions could classify samples by *C. arabica* varieties (Figure. 4), even if they were from different countries. The varieties used in our study were bourbon, caturra, geisha, and heirloom. For the heirloom variety, the only one represented by Ethiopian samples because it is a group of indigenous genotypes, it is possible to state that the phenotype and terroir were discriminants. Indeed, Sr and Rb were strongly discriminated for this cluster. Bourbon samples were from Burundi, Colombia, Rwanda, and El Salvador; Caturra was from Panama, Bolivia, and Nicaragua; and Geisha was from Panama, Colombia, Costa Rica, and Guatemala.

The discriminant elements leading to the setting of the two functions explaining 73.8% and 23.8% of the total variance were Ba, Ca, Cd, Lu, Mg, Rb, and Sr.

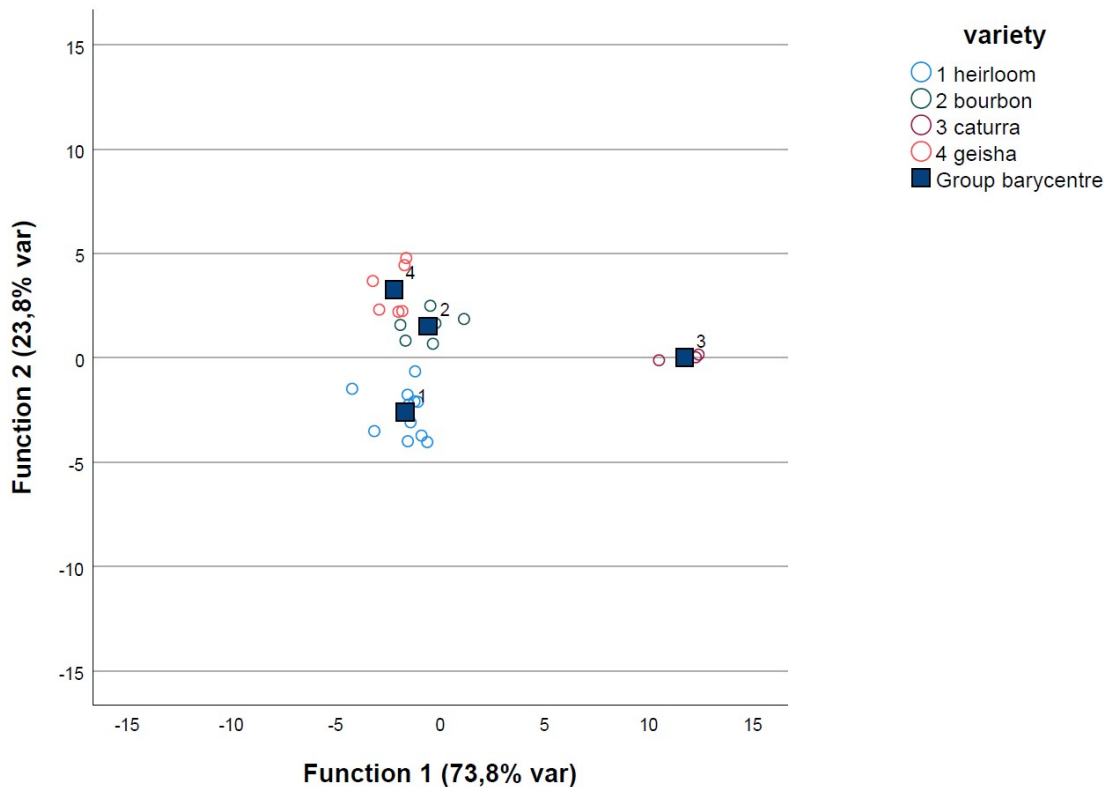**Figure 4.** Graphical outcome of discriminant analysis of C. arabica varieties.

2.4.4. Silver skin

Since the concentration of macro-and micro-elements slightly changed from green to roasted coffee (dry-based measurements), the silver skin composition was analyzed to verify the loss of minerals during roasting and whether differences in the composition of this layer were present when compared with

roasted beans and between coffee species. The elemental composition of this by-product confirmed the stability of the elements considered in our study during the thermal process. Differences between green and roasted beans are explained by the detachment of silver skin (0.5–1% of green coffee weight) (data not shown).

We verified the correlation between coffee seed composition and silver skin using Pearson's test. Concentrations of Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, S, and Sr were directly correlated ($p < 0.05$) in the two matrices; no correlation was found for Ca and Zn, which seemed to be influenced by parameters independent of the genetic deposition of elements in the different tissues of the fruit and seed. Most elements had higher values in silver skin than in roasted coffee. Li, V, and some REE, such as La, Ce, Pr, Nd, Sm, and Gd, had much higher values, even higher than 20 times those of the roasted samples. Ba, Ca, Na, Be, Cr, and Sr had concentrations 10 and 20 times greater than those in the roasted samples. In contrast, P and Rb were more concentrated in roasted coffee than in silver skin; even with only silver skin from Robusta coffee, these differences were reduced.

From our quantification, the most concentrated elements were K > Ca > Mg > S > P > Fe > Al > Na > Ba > Sr > Cu > Mn > Rb > Zn in all silver skins analyzed. This mineral distribution changes only if the Arabica variety is considered: Ca > K > Mg > S > P > Fe > Na > Ba > Al > Sr > Mn > Cu > Zn > Rb. The following trend was observed in the Robusta silver skin: K > Ca > Mg > S > P > Fe > Al > Na > Cu > Sr > Ba > Rb > Mn > Zn. Using t-test and PCA, we also identified different compositions of the silver skin. Robusta coffee silver skin showed significantly ($p < 0.05$) higher concentrations of aluminium, iron, potassium, magnesium, and sulphur when compared to Arabica coffee, which, in contrast, had higher concentrations of Ba and Mn. Nzekoue et al. (2022) [38] published similar distribution but with a lower concentration of P and particularly of S. In addition, Gottstein et al. 2021 [39] reported a similar order of magnitude of the analysed elements, except for Fe, Al, Mn, and Cu, with concentrations two to five times higher than the values of our study (Table S5). The same author described the distribution inside silver skin from the Robusta variety with a

recorded value of Na approximately three times greater, Al and Rb at three or two times greater than the concentrations found in our study (Table S6).

Considering the above-mentioned elements, it was possible to build two principal components to explain 87.02% of the variance via PCA (61.97% and 25.05%, respectively). Figure 5 shows a graphical representation of the PCA and its sample distribution.
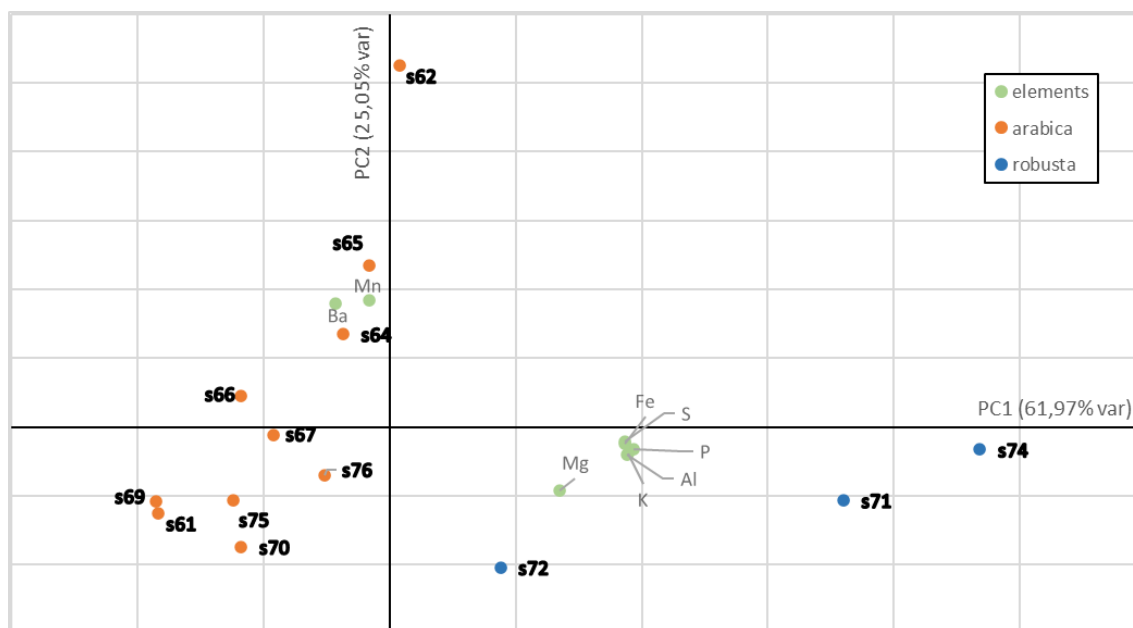

Figure 5. Graphical distribution of silver-skin samples, based on Coffee specie, via PCA (87,02% cumulate explained variance).

Because of the novelty of the topic and interest in the reuse of this by-product, to the best of our knowledge, it is not possible to compare the results presented here with similar work in the literature.

2.5. CONCLUSION

The elemental composition profiles of the coffee samples collected from the main producing countries, representing the variability in terms of origin and variety present in the market, were assessed for the first time. Macro, micro, and trace element analyses were performed with an adequate number of samples to represent the variability of coffee world production. For each roasted coffee sample, potassium had the highest concentration, and manganese or rubidium had the lowest concentration levels. The contents of

trace toxic elements (Pb, Cd, and As) were below the maximum levels set for other foodstuffs or food supplements as set in the Commission Regulations.

Linear discriminant analysis of the elemental analysis of samples from different continents provided a reliable prediction model with 93.3% accuracy and 90.7% prediction ability. The model constructed, based on the elemental compositions divided by countries, was found to be effective in classifying the coffee samples into their respective production zones with 93.5% prediction ability, even if the Panamian group influenced misclassification. In both models, Rb, Sr, S, and Tm are significant discriminant elements for geographical distinction at different scales.

Using canonical discriminant analysis on elemental composition, it is possible to discriminate between Arabica and Canephora coffee and tentatively also the Eugenoides and different varieties of Arabica species identified in both Ca, Ba, Cd, Rb, and Sr as significant discriminant elements.

The chemical analysis and statistics of data from silver skin have helped to determine the distinctive characteristics of coffee species and between this by-product and roasted coffee.

Elemental analysis of major and minor elements is relatively easy and can be used together with the existing paper-based traceability system and sensory evaluation to reliably authenticate the origin of roasted coffee, different species, and varieties. Further studies are ongoing to verify the potential of our model for more accurate discrimination (varieties and regions of origin) and to develop the knowledge of silver skin composition and possible applications.

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2.7. SUPPORTING INFORMATION INDEX

Table S1. Samples list

ID	Nation	Cod continent	Region	Farm	Specie	Variety	Process	Altitude (masl)
1	Brazil	1	Mantiqueira de minas	Cocarive cooperative	Arabica	Yellow catuai	Natural	1200
2	Brazil	1	Cerrado	---	Arabica	---	Natural	---
3	Brazil	1	---	---	Arabica	---	Natural	---
4	Brazil	1	Minas geiras	Salitre	Arabica	Red catuai	Pulped natural	---
5	Brazil	1	Alta mogiana franca	---	Arabica	---	Natural	---
6	Brazil	1	Mantiqueira de minas	Cocarive cooperative	Arabica	---	Natural	---
7	Brazil	1	---	---	Arabica	---	---	---
8	Peru	1	---	---	Arabica	---	---	---
9	India	2	---	---	Arabica	Kent	Washed	---
10	India	2	---	---	Robusta	---	Washed	---
11	Haiti	1	---	---	Arabica	---	Natural	---
12	Santo domingo	1	Juncalito mountains	Finca nunez	Arabica	Typica. caturra	Washed	---
13	Ethiopia	3	Yirgacheffe	Worka's cooperatives	Arabica	Heirloom	Washed	2000 - 2200
14	Ethiopia	3	Yirgacheffe . beriti village. gedeb	Halo beriti	Arabica	Heirloom	Natural	1870 – 1900
15	Ethiopia	3	Sidamo	---	Arabica	---	Washed	---
16	Ethiopia	3	Yirgacheffe	Konga	Arabica	Heirloom	Natural	---
17	Ethiopia	3	Yirgacheffe	---	Arabica	Heirloom	Natural	---
18	Ethiopia	3	Gedeb. yirgacheffe	Chelbesa farm	Arabica	Wolisho and dega	Washed	1950 – 2200
19	Ethiopia	3	Gedeb. yirgacheffe	Halu fafate. worka	Arabica	Heirloom	Washed	2050-2200
20	Ethiopia	3	Guji. oromia	Uraga washing station	Arabica	Heirloom	Washed	2250 - 2300
21	Indonesia - Bali	2	Singaraya dan tabanan	Putu mulyati	Arabica	Linie s	Washed	2000
22	Costa Rica	1	Brunca	Finca organica hamacas	Arabica	Sarchimor and iapar	Honey	1100

23	Kenya	3	Nyeri	Mahiga farm	Arabica	Sl-28. sl-34	Washed	1700 – 1900
24	Kenya	3	Kurugoya. kirinyaga	Mutira cooperative	Arabica	Sl-28. sl-34. ruiru 11. batian. k7	Washed	1700
25	Indonesia	2	Aceh. north sumatra	Cooperative	Arabica	Sigararutang. jember s795. ateng	Giling basah	1400-1500
26	Burundi	3	Bugendana. gitega	Gaterama. agahore coffee	Arabica	Bourbon mbrizi	Washed	1600 – 1850
27	Congo	3	Mususa. butembo. north kivu	Coopade	Arabica	Blue mountain. katwai. rumangabo	Washed	1900
28	Honduras	1	Celaque. copán	Francis arturo romero	Arabica	Lempira	Natural	1200
29	Panama	1	Volcan	Carmen estate	Arabica	Caturra	Washed	1800-2100
30	Panama	1	Boquete. palmira	David pech	Arabica	Pacamara	Honey	1250 – 1400
31	Colombia	1	Tolima	Arrango family	Arabica	Pink bourbon	Honey	1800 – 1900
32	Colombia	1	Quindìo	Finca insula	Arabica	Castillo	Washed	1650
33	Indonesia	2	Sumatra	---	Arabica	---	---	---
35	Colombia	1	Tolima	Bahamon. bejarano. jaramil	Arabica	Colombia	Natural	1700 – 1875
36	Uganda	3	Mount elgon	Smallholders	Arabica	Sl14. sl34	Washed	1200-1600
37	Indonesia	2	Toba lake	---	Arabica	Various	Giling basah	1600
38	Colombia	1	Cali. valle del cauca	Inmaculada	Eugenoides	---	Natural	1900
39	Honduras	1	---	---	Arabica	---	Washed	---
40	Uganda	3	---	---	Robusta	Nganda. erecta	Natural	---
41	India	2	---	---	Robusta	---	Natural	---
42	Indonesia	2	Flores island	---	Robusta	---	Natural	---
43	Panama	1	Santa clara. volcàn	Finca hartmann	Arabica	Geisha	Honey	1400-2000
44	Panama	1	Santa clara. volcàn	Finca hartmann	Arabica	Geisha	Anaerobic	1400-2000
45	Costa Rica	1	Tarrazu	Andrey carranza	Arabica	Catuai. caturra	Anaerobic	1400-1700
46	Ethiopia	3	Bensa district. sidama region	Bombe washing station	Arabica	Heirloom	Washed	2000-2400
47	Colombia	1	Cali. valle del cauca	Inmaculada	Arabica	Geisha	Natural	1750
48	Colombia	1	China alta. tolima	Benedicto puentes gonzález	Arabica	Geisha	Washed	1760

49	Costa Rica	1	Brunca	Finca hamacas	Arabica	Geisha	Honey	1500
50	Indonesia	2	Java	---	Robusta	Mixed	West indische bereiding	---
51	Colombia	1	---	---	Arabica	Mixed	Washed	---
52	Bolivia	1	Samaipata	Jair gonzales	Arabica	Caturra	Washed	1750
53	Guatemala	1	Huehuetenango	---	Arabica	Mixed	Washed	---
54	Burundi	3	Kayanza province. north burundi	Mpanga washing station	Arabica	Red bourbon	Washed	1950
55	Rwanda	3	Western. lake kivu. kibaya village	Intango washing station	Arabica	Red bourbon	Pulped natural	1560
56	Ethiopia	3	Jimma	Cooperative	Arabica	Heirloom	Washed	1950-2050
57	El Salvador	1	Santa ana volcano	Finca el cipres	Arabica	Bourbon	Natural	1700
58	Brazil	1	Espirito santo	---	Robusta	Conillon	Natural	---
59	Vietnam	2	---	---	Robusta	Mixed	Wet polished	---
60	Guatemala	1	Sacatapéquez. antigua	Various	Arabica	Bourbon. caturra. catuai	Washed	1550-1900
61	Ethiopia	3	Guji. oromia	Jiratamo	Arabica	Heirloom	Washed	2100
62	Colombia	1	Antioquia	Ubeimar flórez henao	Arabica	Castillo	Washed	2037
63	India	2	Malabar	---	Arabica	Malabar spiga	Monsooned	---
64	Rwanda	3	Gitega hills	Bernard uwitije	Arabica	Red bourbon	Washed	1600
65	Honduras	1	Santiago de puringla. montecillos. la paz	Finca oropendula	Arabica	Catuai. bourbon. lempira	Anaerobic honey	1500
66	Nicaragua	1	Dipilito. nueva segovia	Ener elias rodas	Arabica	Caturra	Washed	1200-1300
67	Panama	1	Boquete. chiriquí	Cafetalera fernández	Arabica	Typica	Washed	1300-1600
68	Guatemala	3	San pedro yepocapa. chimaltenango	Proyectos del pacifico	Arabica	Gesha	Washed	1500
69	Ethiopia	3	Gedeb district	Cooperative	Arabica	Heirloom	Washed	1900-2200
70	Ethiopia	3	Oromia	Hambella farm	Arabica	Heirloom	Honey	2100-2300
71	Congo	3	---	---	Robusta	---	Natural	---
72	India	2	---	---	Robusta	---	Washed	---

73	Vietnam	2	---	---	Robusta	---	Hulled	---
74	Uganda	3	---	---	Robusta	---	Natural	---
75	Uganda	3	Upper bukyabo	Various farmers	Arabica	Sl14. Sl28. nyasaland	Washed	1800-2200
76	Brazil	1	Três pontas. state of minas gerais	Fazenda zaroca	Arabica	Mundo novo. catuai. yellow bourbon	Natural	1100

Table S2. ICP-MS operating conditions.

ICP-MS parameters	Setting
RF power	1550 W
RF Matching	1.80 V
Carrier gas flow rate (Argon)	1.00 L min^{-1}
Dilution Mode	ON
Dilution Gas (Argon)	0.20 L min^{-1}
Sampling depth	8.0 mm
S/C temp	2 °C
Collision Gas	He
Collision gas flow rate	4.0 mL min^{-1} (10 mL min^{-1}†)
Isotope monitored	^{7}Li, ^{9}Be, ^{27}Al, ^{51}V, ^{52}Cr, Mn, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{75}As, ^{78}Se†, ^{85}Rb, ^{88}Sr, ^{95}Mo, ^{107}Ag, ^{111}Cd, ^{121}Sb, ^{208}Pb.
Internal standards	^{6}Li, ^{45}Sc, ^{72}Ge, ^{89}Y, ^{115}In, ^{159}Tb.
Integration Time/Mass	0.6 sec

Table S3. ICP-OES operating conditions.

Instrument parameters	Setting
Read time	5 s
Replicates	3
Sample uptake delay	35 s
Fast pump	Yes
Stabilization time	40 s
Pump speed	12 rpm
RF power	1450 W
Aux flow rate	1.00 L min^{-1}
Plasma flow rate	12.0 L min^{-1}
Nebulizer flow rate	0.70 L min^{-1}
Elements	Ba (455.406 nm)†, Ca (393.375 nm), Fe (259.940 nm)†, K (766.491 nm)†, Mg (279.553)†, Na (588.995 nm), P (213.618 nm), S (181.972 nm), Zn (206.200 nm).
Viewing modes	Axial. radial (†)
Viewing height	8 mm
Background correction	fitted

Table S4. ICP-MS/MS operating conditions.

ICP-MS parameters	Setting
RF power	1550 W
RF Matching	1.80 V
Carrier gas flow rate (Argon)	1.00 L min^{-1}
Dilution Mode	ON
Dilution Gas (Argon)	0.20 L min^{-1}
Sampling depth	8.0 mm
S/C temp	2 °C
Gas mode	O_2
Cell gas flow	25 %
m/z Q1	^{139}La, ^{140}Ce, ^{141}Pr, ^{146}Nd, ^{147}Sm, ^{153}Eu, ^{157}Gd, ^{159}Tb, ^{163}Dy, ^{165}Ho, ^{166}Er, ^{169}Tm, ^{172}Yb, ^{175}Lu.
m/z Q2	^{155}La, ^{156}Ce, ^{157}Pr, ^{162}Nd, ^{163}Sm, ^{169}Eu, ^{173}Gd, ^{175}Tb, ^{179}Dy, ^{181}Ho, ^{182}Er, ^{185}Tm, ^{188}Yb, ^{191}Lu.
Internal standards Q1	^{89}Y, ^{103}Rh.
Internal standards Q2	^{105}Y, ^{103}Rh.
Integration Time/Mass	0.6 sec

Table S4a. Tables of comparison between data on roasted coffee from literature and this study, divided by country of origin.

BRAZIL		This study			Bitter et al. 2020	Kalschne et al. 2020		Debastiani et al. 2019		Carter et al (2016)	Debastiani et al. 2014		Debastiani et al. 2021		Albals et al. (2021)		Sabrina et al. (2017)	
		mean		SD	mean value	mean value	RSD%	mean	SD	mean	mean	SD	mean	SD	mean	SD	mean	SD
		n=9			n=5	n=21		n=144		n=1	n=6		n=102		n = 6		n=50	
Macro Elements																		
Ba	mg kg^{-1}	3.32	±	1.60	3.14					2.6					7.0	1.7		
Ca	mg kg^{-1}	1075	±	161	1000	1134	21	1437	303	1118	1365	299	1441	276	1484	987		
Fe	mg kg^{-1}	30	±	3	26	109.5	26	68	23	39.0	65	21	60.7	17.3	186	39		
K	mg kg^{-1}	19548	±	1251	21710	22383	11	22451	3436	19447	21279	1702	21258	1498				
Mg	mg kg^{-1}	1922	±	55	2014	2289	8	2092	323	2174	1713	119	1776	167	4899	459		
Na	mg kg^{-1}	13.8	±	23.6														
P	mg kg^{-1}	1609	±	173				1761	303	1719	1480	106	1475	128				
S	mg kg^{-1}	1524	±	67				1313	180		1253	102	1261	111				
Zn	mg kg^{-1}	5.6	±	0.6	5.69	8.3	16	8.7	2.5	6.6	8.4	2.1	8.65	2.55	7	10	6.62	2.26
Micro Elements																		
Li	mg kg^{-1}	<0.014			1.48													
Be	mg kg^{-1}	<0.005			0.0004													
Al	mg kg^{-1}	3.15	±	1.57	4.96			91	26	2.6			83.5	25.6	115	83		
V	mg kg^{-1}	<0.0054			0.008										0.9	0.9		
Cr	mg kg^{-1}	0.078	±	0.148	0.013										2.0	0.7	0.34	0.3
Mn	mg kg^{-1}	27.6	±	8.1	33.6	28.1	16	32	8	34.0	33	5	31.8	5.1	79	9	19.44	4.32
Co	mg kg^{-1}	0.180	±	0.113	0.21										0.7	0.1		
Ni	mg kg^{-1}	0.28	±	0.10	0.36					0.4					0.7	0.2	0.7	0.38
Cu	mg kg^{-1}	13.7	±	1.7	16.1	18.3	15	18.5	4.6	17.0	19	4	18.6	3.5	27	7	10.38	2.52
As	mg kg^{-1}	<0.0056			0.04													
Se	mg kg^{-1}	0.063	±	0.138	0.05													
Rb	mg kg^{-1}	26.3	±	13.0	26.7			48	20		45	22	41.7	14.7				

Element	Units	Value	±	Uncert.															
Sr	mg kg^{-1}	5.4	±	2.0	4.0					3.7	13	6			6.2	1.2		
Mo	mg kg^{-1}	0.07	±	0.07	0.087													
Ag	mg kg^{-1}	<0.134																
Cd	mg kg^{-1}	<0.0054			0.0058										0.1	0.1		
Sb	mg kg^{-1}	0.0066	±	0.008	0.0007													
Pb	mg kg^{-1}	<0.098			0.0103										1.2	0.8	0.75	0.33
Rare Earth Elements																		
La	mg kg^{-1}	1.11E-02	±	7.41E-03	5.71E-03													
Ce	mg kg^{-1}	1.17E-02	±	6.67E-03	7.04E-03													
Pr	mg kg^{-1}	<2.12E-03			7.30E-04													
Nd	mg kg^{-1}	5.14E-03	±	3.94E-03	2.54E-03													
Sm	mg kg^{-1}	<3.99E-03			5.57E-04													
Eu	mg kg^{-1}	<3.93E-03			7.47E-04													
Gd	mg kg^{-1}	<3.71E-03			4.76E-04													
Tb	mg kg^{-1}	<3.25E-03			6.83E-05													
Dy	mg kg^{-1}	<3.94E-03			3.66E-04													
Ho	mg kg^{-1}	<2.79E-03			7.95E-05													
Er	mg kg^{-1}	<2.66E-03			2.44E-04													
Tm	mg kg^{-1}	<2.23E-03																	
Yb	mg kg^{-1}	<3.82E-03			2.90E-04													
Lu	mg kg^{-1}	<2.46E-03			5.59E-05													

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		This study			Bitter et al . 2020	Carter et al (2016)		Cloete et al. 2019		Gure et al. (2018)			
ETHIOPIA		Mean		Sd	Mean conc.	Mean	Sd	Mean	Sd	Mean (min)	Sd	Mean (max)	Sd
		N=13			N= 3	N=1		N=13 (tutti)			Da inserire		
Macroelements													
Ba	mg kg⁻¹	3.75	±	0.83	4.04	3.9							
Ca	mg kg⁻¹	884	±	66	952	967		1205	447	790		1530	60
Fe	mg kg⁻¹	23.4	±	2.9	21.0	26.0		25	7	37.3		47.5	5.5
K	mg kg⁻¹	17227	±	429	20314	18130		17315	2610	14300		19400	970
Mg	mg kg⁻¹	1720	±	48	1895	1957				1670		1890	20
Na	mg kg⁻¹	8.9	±	6.0									
P	mg kg⁻¹	1780	±	98		1912		995	643				
S	mg kg⁻¹	1313	±	49				1936	594				
Zn	mg kg⁻¹	4.62	±	0.36	4.33	4.8		4	2	6		30.4	0.5
Microelements													
Li	mg kg⁻¹	<0.014			1.32								
Be	mg kg⁻¹	<0.005			2.38e-04								
Al	mg kg⁻¹	2.991	±	2.484	1.84	3.6							
V	mg kg⁻¹	0.0068	±	0.0039	0.010								
Cr	mg kg⁻¹	0.035	±	0.036	0.022					0.43		0.56	0.01
Mn	mg kg⁻¹	14.0	±	1.0	13.9	17.0		16	6	15		20	1
Co	mg kg⁻¹	0.035	±	0.012	0.037					5.8		19.3	0.5
Ni	mg kg⁻¹	0.305	±	0.251	0.208	0.3				1		2	0.01
Cu	mg kg⁻¹	12.6	±	1.0	13.2	13.0		10	2	13		27.6	3
As	mg kg⁻¹	0.020	±	0.042	0.037								
Se	mg kg⁻¹	0.101	±	0.069	0.066								
Rb	mg kg⁻¹	29.02	±	8.15	23.3			51	23				
Sr	mg kg⁻¹	3.88	±	0.51	3.67	5.0		3	2				

Mo	mg kg⁻¹	0.106	±	0.079	0.13									
Ag	mg kg⁻¹	1.068	±	1.763										
Cd	mg kg⁻¹	<0.0054			4.06e-03					<0.01		<0.01	
Sb	mg kg⁻¹	0.0055	±	0.0031	5.91e-04								
Pb	mg kg⁻¹	<0.098			5.80e-03					<0.05		0.07	0.01
Rare Earth Elements														
La	mg kg⁻¹	4.50e-03	±	4.12e-03	1.93e-03								
Ce	mg kg⁻¹	6.77e-03	±	8.21e-03	2.50e-03								
Pr	mg kg⁻¹	<2.12E-03			3.50e-04								
Nd	mg kg⁻¹	3.42e-03	±	3.86e-03	1.41e-03								
Sm	mg kg⁻¹	<3.99E-03			3.98e-04								
Eu	mg kg⁻¹	<3.93E-03			9.31e-04								
Gd	mg kg⁻¹	<3.71E-03			3.68e-04								
Tb	mg kg⁻¹	<3.25E-03			5.17e-05								
Dy	mg kg⁻¹	<3.94E-03			2.91e-04								
Ho	mg kg⁻¹	<2.79E-03			6.20e-05								
Er	mg kg⁻¹	<2.66E-03			1.64e-04								
Tm	mg kg⁻¹	<2.23E-03											
Yb	mg kg⁻¹	<3.82E-03			2.21e-04								
Lu	mg kg⁻¹	<2.46E-03			4.33e-05								

Ethiopia	Tesfay et al. (2015)				Albals et al. (2021)		Feleke et al. (2019)			
	Mean (min)	Sd	Mean (max)	Sd	Mean	Sd	Mean (min)	Sd	Mean (man)	Sd
					N=1		N=3		N=3	
Macroelements										
Ba					15.9					
Ca	931	17	1009	18	1210		2360.4	159.4	2510.03	58.4
Fe					320		125.2	5.1	4149.7	118
K	18563	477	19610	343			15738.4	539.9	17862.01	114
Mg	1943	45	2030	98	5247					
Na	446	73	484	85						
P							1224.4	34.71	1686.3	43.9
S							1135.5	51.9	1292.4	933
Zn	14	0.3	18	0.3	11.2		2.87	0.6	7.2	1.3
Microelements										
Li										
Be										
Al					111					
V					2.88					
Cr					1.54					
Mn	19	0.2	23	0.5	43.8		24.36	2.16	35.6	1
Co					0.32					
Ni					0.89					
Cu	9	0.3	13	0.2	28		12.89	0.81	16.66	1.06
As										
Se							0.14	0.03	2.8	4.26
Rb							20.4	3.11	57.48	3.43

Sr					9.0		3.78	3.52	9.03	1.5
Mo										
Ag										
Cd	N.d		N.d		0.45					
Sb										
Pb	N.d		N.d		3.0					
Ree										
La										
Ce										
Pr										
Nd										
Sm										
Eu										
Gd										
Tb										
Dy										
Ho										
Er										
Tm										
Yb										
Lu										

Table S4b. Tables of comparison between data on roasted coffee from literature and this study, divided by country of origin.

COLOMBIA		*This study*			*Bitter et al . 2020*	*Carter et al (2016)*		*Cloete et al. 2019*	
		mean		SD	mean conc.	mean	SD	mean	SD
			n=8		n=4	n=3		n=13	
Macro Elements									
Ba	mg kg⁻¹	7.3	±	2.1	5.8	6.1	1.7		
Ca	mg kg⁻¹	990	±	208	976	1200	121	1686	758
Fe	mg kg⁻¹	27.1	±	2.9	24.6	32	2	27	7
K	mg kg⁻¹	17077	±	1229	18692	17265	159	16543	3632
Mg	mg kg⁻¹	1769	±	117	1914	2008	54		
Na	mg kg⁻¹	5.8	±	4.0					
P	mg kg⁻¹	1754	±	109		1866	17	766	521
S	mg kg⁻¹	1411	±	62				1328	397
Zn	mg kg⁻¹	6.4	±	1.5	6.02	7.3	0.7	6	3
Micro Elements									
Li	mg kg⁻¹	0.013	±	0.010	1.29				
Be	mg kg⁻¹	<0.005			4.43E-04				
Al	mg kg⁻¹	2.10	±	1.58	3.38	2.73	0.61		
V	mg kg⁻¹	0.0057	±	0.0039	0.007				
Cr	mg kg⁻¹	0.086	±	0.085	0.049				
Mn	mg kg⁻¹	32.8	±	9.4	30.5	38	14	32	17
Co	mg kg⁻¹	0.14	±	0.13	0.15				
Ni	mg kg⁻¹	0.42	±	0.18	0.30	0.29	0.02		
Cu	mg kg⁻¹	12.95	±	2.09	14.5	14.7	0.6	11	5
As	mg kg⁻¹	<0.006			0.031				
Se	mg kg⁻¹	0.017	±	0.0089	0.037				
Rb	mg kg⁻¹	24	±	8	23.4			63	26

Sr	mg kg^{-1}	8.6	±	1.5	10.2	6.5	2.4	19	17
Mo	mg kg^{-1}	0.10	±	0.11	0.083				
Ag	mg kg^{-1}	<0.134							
Cd	mg kg^{-1}	0.0085	±	0.007	0.01				
Sb	mg kg^{-1}	0.014	±	0.0096	8.86E-04				
Pb	mg kg^{-1}	<0.098			0.041				
Rare Earth Elements									
La	mg kg^{-1}	<2.40E-03			2.09E-03				
Ce	mg kg^{-1}	<2.55E-03			1.95E-03				
Pr	mg kg^{-1}	<2.12E-03			3.02E-04				
Nd	mg kg^{-1}	<3.12E-03			1.13E-03				
Sm	mg kg^{-1}	<3.99E-03			3.87E-04				
Eu	mg kg^{-1}	<3.93E-03			1.24E-03				
Gd	mg kg^{-1}	<3.71E-03			3.22E-04				
Tb	mg kg^{-1}	<3.25E-03			4.76E-05				
Dy	mg kg^{-1}	<3.94E-03			2.61E-04				
Ho	mg kg^{-1}	<2.79E-03			6.17E-05				
Er	mg kg^{-1}	<2.66E-03			1.85E-04				
Tm	mg kg^{-1}	<2.23E-03							
Yb	mg kg^{-1}	<3.82E-03			2.54E-04				
Lu	mg kg^{-1}	<2.46E-03			5.59E-05				

COSTA RICA		This study			Bitter et al . 2020
		mean		SD	mean conc.
		n=3			n= 3
MacroElements					
Ba	mg kg-1	4.7	±	1.9	4.6
Ca	mg kg-1	1023	±	145	998
Fe	mg kg-1	35.4	±	13.8	24.2
K	mg kg-1	16891	±	579	20300
Mg	mg kg-1	1882	±	53	2029
Na	mg kg-1	5.3	±	5.5	
P	mg kg-1	1625	±	145	
S	mg kg-1	1420	±	64	
Zn	mg kg-1	4.09	±	0.77	5.77
MicroElements					
Li	mg kg-1	<0.014			1.53
Be	mg kg-1	<0.005			2.57E-04
Al	mg kg-1	8.82	±	6.59	1.71
V	mg kg-1	0.037	±	0.003	0.004
Cr	mg kg-1	1.475	±	2.38	0.020
Mn	mg kg-1	32.2	±	3.89	28.6
Co	mg kg-1	0.092	±	0.008	0.19
Ni	mg kg-1	0.840	±	0.93	0.37
Cu	mg kg-1	14.367	±	0.87	14.1
As	mg kg-1	0.016	±	0.02	0.034
Se	mg kg-1	<0.011	±	0.00	0.032
Rb	mg kg-1	38.6	±	7.64	15.8
Sr	mg kg-1	9.39	±	2.90	11.0
Mo	mg kg-1	0.316	±	0.43	0.044
Ag	mg kg-1	<0.134			
Cd	mg kg-1	0.0075	±	0.0069	0.007
Sb	mg kg-1	0.013	±	0.00	0.002
Pb	mg kg-1	0.129	±	0.20	0.014
Rare Earth Elements					
La	mg kg-1	3.12E-03	±	1.95E-03	2.09E-03
Ce	mg kg-1	3.74E-03	±	2.78E-03	1.20E-03
Pr	mg kg-1	<2.12E-03			2.42E-04
Nd	mg kg-1	<3.12E-03			8.96E-04
Sm	mg kg-1	<3.99E-03			3.78E-04
Eu	mg kg-1	<3.93E-03			1.02E-03
Gd	mg kg-1	<3.71E-03			2.86E-04
Tb	mg kg-1	<3.25E-03			4.22E-05
Dy	mg kg-1	<3.94E-03			2.44E-04
Ho	mg kg-1	<2.79E-03			5.40E-05
Er	mg kg-1	<2.66E-03			1.58E-04
Tm	mg kg-1	<2.23E-03			
Yb	mg kg-1	<3.82E-03			2.22E-04
Lu	mg kg-1	<2.46E-03			4.83E-05

PANAMA		This study			Bitter et al . 2020
		Mean		SD	Mean conc.
		N=5			N=2
Macroelements					
Ba	mg kg⁻¹	6.55	±	2	6.87
Ca	mg kg⁻¹	909	±	246	950
Fe	mg kg⁻¹	25.5	±	2.6	25.9
K	mg kg⁻¹	18329	±	785	17521
Mg	mg kg⁻¹	1780	±	117	1776
Na	mg kg⁻¹	7.8	±	8.4	
P	mg kg⁻¹	1557	±	144	
S	mg kg⁻¹	1401	±	39	
Zn	mg kg⁻¹	5.28	±	1.00	5.95
Microelements					
Li	mg kg⁻¹	<0.014			1.82
Be	mg kg⁻¹	<0.005			1.57E-04
Al	mg kg⁻¹	2.72	±	4.5	1.19
V	mg kg⁻¹	<0.0054			4.59E-03
Cr	mg kg⁻¹	0.090	±	0.12	5.68E-03
Mn	mg kg⁻¹	16.6	±	1.82	17.7
Co	mg kg⁻¹	0.048	±	0.01	0.04
Ni	mg kg⁻¹	0.400	±	0.20	0.31
Cu	mg kg⁻¹	13.9	±	0.87	14.5
As	mg kg⁻¹	<0.006			0.03
Se	mg kg⁻¹	<0.011			0.04
Rb	mg kg⁻¹	20.3	±	2.5	16.6
Sr	mg kg⁻¹	12.9	±	3.4	16.5
Mo	mg kg⁻¹	0.106	±	0.088	0.17
Ag	mg kg⁻¹	<134			
Cd	mg kg⁻¹	<0.0054			4.37E-03
Sb	mg kg⁻¹	0.008	±	0.00	7.55E-04
Pb	mg kg⁻¹	<0.098			7.64E-03
REE					
La	mg kg⁻¹	1.00E-02	±	1.43E-02	8.76E-04
Ce	mg kg⁻¹	2.81E-02	±	3.24E-02	7.23E-04
Pr	mg kg⁻¹	4.85E-03	±	5.82E-03	1.07E-04
Nd	mg kg⁻¹	9.64E-03	±	1.63E-02	3.97E-04
Sm	mg kg⁻¹	<3.99E-03			1.98E-04
Eu	mg kg⁻¹	<3.93E-03			1.24E-03
Gd	mg kg⁻¹	<3.71E-03			1.73E-04
Tb	mg kg⁻¹	<3.25E-03			2.29E-05
Dy	mg kg⁻¹	7.37E-03	±	0.00E+00	1.21E-04
Ho	mg kg⁻¹	<2.79E-03			2.65E-05
Er	mg kg⁻¹	3.98E-03	±	0.00E+00	9.73E-05
Tm	mg kg⁻¹	<2.23E-03			
Yb	mg kg⁻¹	<3.82E-03			1.60E-04
Lu	mg kg⁻¹	<2.46E-03			4.70E-05

Table S5. Mean, standard deviation, minimum and maximum values of macro elements in silver skin from Arabica variety, compared with the values in the literature.

| Elements | unit | this study | | | | Gottstein et al. (2021) | Nzekoue et al. (2022) |
| | | From Ethiopia, Colombia, Rwanda, Honduras, Panama, Uganda, Brasil, Nicaragua (n=10) | | | | from Brazil, India, Mexico, El Salvador (n=3) | n=3 |
		mean	DS	MIN	MAX	values	mean ± DS
Ba	mg kg^{-1}	103	58	46	246	~130	66.75 ± 12.4
Ca	mg kg^{-1}	10641	1754	8095	12853	>10000	10800 ± 690
Fe	mg kg^{-1}	185	38	132	257	~1000	238 ± 11
K	mg kg^{-1}	7883	4219	2628	15541	~10000	9720 ± 460
Mg	mg kg^{-1}	2598	495	2071	3517	>2000	2570 ± 180
Na	mg kg^{-1}	173	101	16	350	200	110 ± 10
P	mg kg^{-1}	344	97	209	518		124 ± 8
S	mg kg^{-1}	2199	160	1944	2438		51.9 ± 1.3
Zn	mg kg^{-1}	17	5	11	28	25	31.9 ± 5.3

| Elements | unit | this study | | | | Gottstein et al. (2021) | Nzekoue et al. (2022) |
| | | From Ethiopia. Colombia. Rwanda, Honduras, Panama, Uganda, Brasil, Nicaragua (n=10) | | | | from Brazil. India, Mexico, El Salvador (n=3) | n=3 |
		mean	DS	MIN	MAX	values	mean ± DS
Li	mg kg^{-1}	0.34	0.13	0.25	0.70		
Be	mg kg^{-1}	0.06	0.02	0.03	0.11		≤0.01
Al	mg kg^{-1}	101.6	32.4	53.4	174.2	215	89.0 ± 6.5
V	mg kg^{-1}	0.14	0.04	0.06	0.19	0.45	0.2 ± 0.06
Cr	mg kg^{-1}	1.24	1.44	0.55	5.30	4	0.23 ± 0.02
Mn	mg kg^{-1}	57.1	36.7	17.1	123.1	145	46.7 ± 2.8
Co	mg kg^{-1}	0.30	0.10	0.20	0.46	0.6	0.2 ± 0.06
Ni	mg kg^{-1}	0.8	1.1	0.3	4.1	1.9	0.5 ± 0.01
Cu	mg kg^{-1}	44.2	14.0	25.7	71.5	98	37.9 ± 9.7
As	mg kg^{-1}	0.1	0.1	0.01	0.20		0.1 ± 0.04
Se	mg kg^{-1}	0.35	0.22	0.06	0.65		0.1 ± 0.03
Rb	mg kg^{-1}	11.6	12.4	3.0	44.4	10	
Sr	mg kg^{-1}	84	33	4.41	158	68	
Mo	mg kg^{-1}	0.50	0.37	0.11	1.04	0.26	0.2 ± 0.04
Ag	mg kg^{-1}	0.22	0.13	0.10	0.49	n.d	0.03 ± 0.006
Cd	mg kg^{-1}	0.19	0.28	0.00	0.78	0.075	0.07 ± 0.01
Sb	mg kg^{-1}	0.02	0.01	0.01	0.05		0.05 ± 0.009
Pb	mg kg^{-1}	1.18	1.66	0.01	4.95	0.75	0.3 ± 0.005

Elements	unit	this study				Gottstein et al. (2021)
		From Ethiopia, Colombia, Rwanda, Honduras. Panama. Uganda, Brasil, Nicaragua (n=10)				from Brazil. India, Mexico, El Salvador (n=3)
		Mean	DS	MIN	MAX	values
La	mg kg^{-1}	0.136	0.057	0.049	0.241	0.15
Ce	mg kg^{-1}	0.181	0.098	0.057	0.379	0.21
Pr	mg kg^{-1}	0.020	0.011	0.007	0.044	n.d
Nd	mg kg^{-1}	0.083	0.045	0.031	0.178	0.11
Sm	mg kg^{-1}	0.030	0.016	0.015	0.056	
Eu	mg kg^{-1}	0.006	0.004	0.002	0.014	
Gd	mg kg^{-1}	0.013	0.007	0.006	0.026	
Tb	mg kg^{-1}	<3.25E-3		<3.25E-3	<3.25E-3	
Dy	mg kg^{-1}	0.008	0.005	0.002	0.019	
Ho	mg kg^{-1}	0.001	0.001	<MDL	0.002	
Er	mg kg^{-1}	0.004	0.003	0.001	0.011	
Tm	mg kg^{-1}	<2.23E-3		<2.23E-3	<2.23E-3	
Yb	mg kg^{-1}	0.002	0.002	<3.82E-3	0.005	
Lu	mg kg^{-1}	<2.46E-3		<2.46E-3	<2.46E-3	

Table S6. Mean, standard deviation minimum and maximum values of macro elements in silver skin from Robusta variety, compared with the values in the literature.

Elements	unit	this study				Gottstein et al. (2021)
		From India, Congo, Uganda (n=3)				from India (n=3)
		mean	DS	MIN	MAX	values
Ba	mg kg^{-1}	47	20	26	67	73
Ca	mg kg^{-1}	8097	1430	7030	9722	>10000
Fe	mg kg^{-1}	471	170	275	578	>600
K	mg kg^{-1}	27236	6920	19802	33490	~20000
Mg	mg kg^{-1}	4048	405	3595	4375	>4000
Na	mg kg^{-1}	135	73	80	218	360
P	mg kg^{-1}	1212	544	585	1556	
S	mg kg^{-1}	3022	181	2863	3219	
Zn	mg kg^{-1}	20	1	18	21	33

Elements	unit	this study				Gottstein et al. (2021)
		From India, Congo, Uganda (n=3)				from India (n=3)
		mean	DS	MIN	MAX	values
Li	mg kg^{-1}	0.336	0.064	0.264	0.387	
Be	mg kg^{-1}	0.058	0.016	0.041	0.072	
Al	mg kg^{-1}	441	242	175	651	155
V	mg kg^{-1}	0.714	0.361	0.322	1.033	0.3
Cr	mg kg^{-1}	2.3	2.0	1.0	4.6	2.9
Mn	mg kg^{-1}	26	6	20	32	43
Co	mg kg^{-1}	0.78	0.36	0.39	1.10	0.95
Ni	mg kg^{-1}	3.5	1.8	2.0	5.5	2.3
Cu	mg kg^{-1}	105	25	77	126	185
As	mg kg^{-1}	0.07	0.02	0.04	0.08	
Se	mg kg^{-1}	0.18	0.20	0.07	0.41	
Rb	mg kg^{-1}	41	9	31	48	18
Sr	mg kg^{-1}	62	21	39	77	38
Mo	mg kg^{-1}	0.52	0.43	0.09	0.95	0.21
Ag	mg kg^{-1}	0.19	0.12	0.07	0.30	0.065
Cd	mg kg^{-1}	0.11	0.06	0.05	0.17	0.1
Sb	mg kg^{-1}	0.02	0.01	0.01	0.03	
Pb	mg kg^{-1}	0.55	0.37	0.17	0.90	0.65

Elements	unit	this study				Gottstein et al. (2021)
		From India, Congo, Uganda (n=3)				from India (n=3)
		mean	DS	MIN	MAX	values
La	mg kg^{-1}	0.589	0.570	0.078	1.204	0.15
Ce	mg kg^{-1}	1.326	1.268	0.113	2.642	0.18
Pr	mg kg^{-1}	0.109	0.105	0.012	0.220	n.d
Nd	mg kg^{-1}	0.389	0.355	0.049	0.758	0.1
Sm	mg kg^{-1}	0.082	0.058	0.018	0.133	
Eu	mg kg^{-1}	0.015	0.013	0.002	0.028	
Gd	mg kg^{-1}	0.052	0.046	0.009	0.101	
Tb	mg kg^{-1}	0.006	0.006	0.002	0.011	
Dy	mg kg^{-1}	0.035	0.034	0.006	0.073	
Ho	mg kg^{-1}	0.005	0.006	0.000	0.012	
Er	mg kg^{-1}	0.017	0.017	0.003	0.036	
Tm	mg kg^{-1}	0.002		<2.23E-3	0.002	
Yb	mg kg^{-1}	0.010	0.015	0.000	0.028	
Lu	mg kg^{-1}	<2.46E-3		<2.46E-3	<2.46E-3	

3. Volatile compounds in green and roasted Arabica Specialty coffee: discrimination of origins, post harvesting processes and roasting level

3.1. ABSTRACT

The aroma of coffee is a complex mixture of more than 1000 compounds. The volatile compounds in green and roasted coffee were analyzed to detect several features related to quality, roasting level, origins, and the presence of specific defects. With respect to specialty coffee, the flavor profile and peculiarities of the aforementioned characteristics are even more relevant knowing the expectations of consumers to find, in a cup of coffee, unicity bestowed by its origin and post-harvesting processes. In this work, which dealt with 46 lots of specialty Arabica coffee, we used HS-SPME/GC–MS to detect the volatile compounds in green coffees together with those in the same coffees roasted at three different levels to identify whether differences in headspace composition were ascribable to the origin, the post-harvesting processes, and the roasting pro-files. The main results are related to the discriminant power of the volatile compounds in green coffee, which are impacted by the origins more than the post-harvesting processes. Compounds such as linalool and 2,3-butanediol were more concentrated in natural coffees, while hexanal was more concentrated in washed varieties ($p < 0,05$). In roasted coffees, the differences in composition were due to roasting levels, countries of origin, and the post-harvesting processes, in descending order of significance.

3.2. INTRODUCTION

As with many of the best-loved foodstuffs, coffee and coffee beverages are chosen and identified first by their unique aroma, which plays a crucial role as a marker of coffee quality and characteristics, both for green and roasted coffee [1]. A number of chemical compounds, especially volatile compounds, are involved in the flavor profile of roasted coffee. More than 1000 volatile compounds (mainly produced by Maillard reactions) of several chemical classes have been identified in roasted coffee, but only a small percentage (about 5%) play a relevant role in coffee aroma [2]. Consumers are increasingly attracted to single-origin coffees rather than blends thanks to the specific flavor profile these

coffees can offer [3]. The exponential increase in demand for one such highly traceable coffee, known as Arabica Specialty coffee, with its certified ori-gins, varieties, and post-harvesting processes [4], has played a crucial role in the inves-tigation into the impacts these parameters have on the volatile profile of both green and roasted beans [5].

The composition of the volatile fraction in coffee is influenced by many factors, such as the variety, the agro-ecological zone of cultivation (climate, soil, altitude, etc.), the post-harvesting processes (e.g., fermentation, washing, and drying), and the roast-ing and brewing parameters (temperature, pressure, etc.). The characterization of the volatile fraction has helped to identify defects [6–8], the different roasting levels [9], the origins of [10,11] raw or roasted coffee, and the influence of altitude and the climatic conditions of farming [12,13] on raw and roasted coffee. Some studies have also evalu-ated the influence of the post-harvesting processes on the volatile compounds found in coffee [14,15].

Headspace solid-phase micro-extraction (HS-SPME) coupled with gas chroma-tography–mass spectrometry (GC–MS) is often used in volatile fraction characterization [16,17]. Moreover, composition data can be processed to evaluate the possible rela-tionships between volatile composition and specific factors of coffee, such as origin, post-harvesting processes, and roasting levels [18].

In this context, we carried out HS-SPME/GC–MS analysis of 46 specialty Arabica coffees from Asia, Africa, Central, and South America, including green samples and samples roasted at three different levels. Volatile composition results were then pro-cessed to evaluate whether they were relevant in the discrimination of coffee origin, the post-harvesting processes used to remove the pericarp of the fruit from the beans (natural, honey, and washed methods, also known as dry, semi-dry, and wet methods), and the roasting levels. Finally, the correlations among volatile compounds occurring in green and roasted coffee were studied.

3.3. MATERIALS AND METHODS

3.3.1. Sampling

In all, 46 samples of Arabica green coffee of the 2020–2021 crop, originating from Brazil, Burundi, Colombia, Costa Rica, Democratic Republic of Congo, Dominican Re-public, El Salvador, Ethiopia, Guatemala, Haiti, Honduras, India, Indonesia, Kenya, Peru, Republic of Panama, Rwanda, and Uganda were shipped in 60 kg GrainPro bags, sampled by a local supplier, and delivered to our laboratories. All Arabica coffees were graded as "Specialty" or "Premium" coffees according to the protocol established by the Specialty Coffee Association. Specifically, the samples were required to have a cupping score of 80 points or more and needed to be free of primary defects (sour beans, foreign matter, or insect/fungus damage). Only a small number of unripe or broken seeds were allowed. Coffee samples represented 30 of the different varieties of Arabica cultivated in the countries of origin, increasing the variability and representativeness of the sample sets. In the post-harvest processes, 17 samples were subjected to the dry process, 4 to the semi-dry process, and 25 to the wet process [19].

A total of 500 g of green coffee was frozen, milled using a cyclone hammer mill (1 mm sieve, Pulverisette, Fritsch GmbH, Idar-Oberstein, Germany), and homogenized. Then, an aliquot of 2 g was stored at −20 °C until the time of analysis.

After 3 different roasting processes (see Section 2.2) of each Arabica green coffee sample, 3 different samples of roasted coffee were obtained for a total of 138 samples of roasted coffee.

3.3.2. Roasting

The sample roaster IKAWA Model V2-PRO was used to roast all the coffee samples as described by Vezzulli et al. [16]. Each roasting batch was 50 g (+/−0.5 g). Three roasting profiles, referred to in this work as light, medium, and dark, reached roasting levels comparable to the industrial levels between the first and second crack [19]. The chamber was preheated to 174–175 °C before the coffee was dropped in. Light roasting was achieved at 205 °C in 5.46 min, medium roasting at 210 °C in 6.16 min, and dark roasting at 214 °C in 6.46 min.

After being discharged from the roasting chamber, samples were cooled and then ground using a Moulinex blender (Model AR110830). The heating of the beans was minimized during the milling. Immediately after this, 2 g of ground coffee was placed in a 15 mL vial, closed by a crimp cap with a Teflon-lined septum (Supelco, Bellefonte, PA, USA), and frozen until analysis.

3.3.3. Volatile Compounds Analysis

The volatile compounds in the coffee samples were detected using an HS-SPME/GC–MS system (Thermo-Fisher Scientific, San Jose, CA, USA) according to previous studies [11,18,20]. After being defrosted at 5 °C and stabilized at 20 °C, each sample was incubated at 50 °C under agitation for 10 min. Then, the headspace was sampled (30 min) using an SPME fiber coated with DVB/CAR/DMS (75 µm) (Supelco, Bellefonte, PA, USA), pre-conditioned according to manufacturer recommendations, at 50 °C for 30 min under continuous agitation. Afterward, the fiber was thermally de-sorbed at 220 °C for 3 min in splitless mode. The volatile compounds were analyzed using a TraceGQ Ultra coupled with an ISQ single quadrupole mass spectrometry (Thermo-Fisher Scientific, San Jose, CA, USA). The volatile compounds were separated on a capillary column Rtx-5MS, 30 m x 0.25 mm i.d., 0.25 µm film thickness (Restek Corporation, Bellefonte, PA, USA). Helium, the carrier gas, was maintained at a con-stant flow rate of 1.0 mL/min. The oven temperature was set at 40 °C for 3 min. Then, the temperature was increased to 180 °C at 12 °C/min and held for 5 min. Finally, the tem-perature was increased to 240 °C at the rate of 40 °C/min up and held for 5 mins. The transfer line temperature was set at 250 °C and the MS source at 250 °C. Mass spectra were acquired in the electron impact mode at 70 eV, using an m/z range of 50–650. When reference compounds were not available, compounds were identified after comparing the mass spectra with the NIST database. The linear retention indices (LRIs) of the volatiles were compared with data from the literature. ThermoQuest Xcalibur 1.2 software was used to obtain the data. The results were expressed as the relative per-centage of each compound's peak area to the sum of the identified GC–MS peak area. Each analysis was carried out in duplicate. The absence of

contaminants was verified by the injection of a blank sample every three injections.

3.3.4. Statistical Analysis

Statistical analysis of the volatile compounds was conducted using the IBM SPSS statistics software (ver. 27, Inc., Chicago, IL, USA). The homogeneity of variance was checked. A t-test was applied to discriminate between the set of data from Ethiopian and American coffees and between washed and natural samples. One-way ANOVA with a Waller–Duncan post-hoc test and discriminant analysis was applied to evaluate the significant differences among coffees belonging to the three Arabica subgroups (natural, honey, and wet), among coffees from different continents and countries of origin, and among coffees roasted at different levels. Data from the volatile composition obtained via the headspace analysis of the green and roasted coffee samples, expressed in terms of area under the chromatogram, were statistically treated via discriminant canonic analysis to detect whether the volatile profile allowed one to discriminate among coffees from different countries of origin, coffees that had undergone different post-harvesting processes, and coffees roasted to different levels.

3.4. RESULTS

3.4.1. Green coffee volatile compounds

In our study, HS-SPME/GC–MS analysis helped detect 51 compounds, which were divided into 12 chemical groups (Table 1). The hydrocarbon group was the most nu-merous (n = 13). However, this group was difficult to identify due to the similar mass spectrum of some isomers and the lack of standards. For this reason, only the number of carbon atoms was reported. The composition of the volatile fraction showed different profiles among the samples. In each coffee sample, 23 to 40 different compounds were detected; 8 compounds were detected in not more than 5 samples, whereas 35 were detected in more than 20 samples. No relevant differences were found between the average number of compounds detected in dry and wet coffees (35 vs. 34).

Considering the markers of defective beans, benzaldehyde was detected mainly in dry coffee (12 of 13 samples), whereas 2-methylpyrazine was detected in 14 samples (5 dry and 9 wet coffees).

Table 1. List of compounds and related Linear Retention Index (LIR) detected in the green coffee headspace, listed by chemical class

Alcohols	LRI	Linear Alkanes	LRI
Ethanol	565	Trimethyl-octane	904
3-Methylbutanol	742	n-Decan	1000
2,3-Butanediol	804	2,6-Dimethyloctane	1016
2-Heptanol	873	Tetramethyl-octane	1068
Octanol	1007	3-Methylnonane	1064
Phenylethyl alcohol	1126	Tetramethyl-heptane	1026
Acids	**LRI**	Undecane	1100
Acetic acid	595	3-Methylundecane	1176
3-Metyl butanoic acid (isovaleric acid)	856	Dodecane	1200
2-Methyl butanoic acid	863	3-Methyl-tridecane	1374
3-Methyl-but-2-en-oic acid	884	Tetradecane	1400
Pentanoic acid (valeric acid)	894	Pentadecane	1500
3-Methyl-pentanoic acid	903	**Terpenes**	**LRI**
Esters	**LRI**	β-Pinene	898
Methyl ester isovaleric acid	809	Farnesane (2,6,10-trimethyl-dodecane)	1241
2-Methyl-ethyl ester butanoic acid	840	α-Pinene	930
3-Methyl-ethylestere butanoic acid (ethyl ester isovaleric acid)	843	Carene	1010
Propanoic acid methyl hexyl ester	1058	D-limonene	1023
Aldehydes	**LRI**	α-Linalool	1104
Hexanal	798	**Pyrazines e pyridines**	**LRI**
Benzaldehyde	907	Methylpyrazine	832
Nonanal	1110	3-Methoxypyrazine-2-isobutyle	1181
Ketones	**LRI**	**Furans**	**LRI**
1-Methoxy-2-propanone	582	2-Pentylfuran	985
Cyclopentanone-2-sec-butyl	1221	Furfuryl-alcohol	831
6,10,14-Trimethyl-2-pentadecanone	1752	**Contaminants**	**LRI**
Diphenyl-propane	1654	Ethylbenzene	848
Alkaloids	**LRI**	Tributyl-phosphate	1602
Caffeine	1772	Phthalic acid dibutyl-ester	1764
		Diethyl phthalate	1558

3.4.2. Roasted coffee volatile compounds

After samples were roasted at three different levels, the volatile compositions of the obtained samples were again analyzed via headspace analysis, and the values were expressed in terms of area under the chromatogram.

Origin and roasting conditions significantly influence flavor formation and aroma quality. It is well known that roasting can modify, modulate, and

generate the final aroma of coffee, generating more than 100 compounds [20,27–33].

A total of 70 compounds were identified and, after validation, 56 were included in the analysis, where they were divided into nine chemical groups (Table 2). Furan was the most numerous group (n = 19). Almost all the compounds were detected in all the samples. However, the abundance of 10 compounds was discriminant for each of the three roasting levels (pyridine; furfural; furfuryl alcohol acetate; 2,4-dimethyl-1,3-cyclopentanedione; 1-methyl-2-acetonirtrile-pyrrole; maltol; 1,2,-furanyl-methyl-1-pyrrole; furan-5-methyl-2,2-methylendi; furfu-ryl-3-methyl-butanoato; and 2,2-oxydimethylen-difurane), their concentration increas-ing with the roasting level, as already reported by Moon et al. [34].

Only one compound (3,5-dietil-2-metil pyrazine; floral odor) decreased with an increasing roasting level.

Table 2. List of compounds and related Linear Retention Index (LIR) detected in the roasted coffee headspace, listed by chemical class.

Furans and Derivatives	LRI	Pyrazole	LRI
Dihydro 2-methyl-3-furanone	820	Pyrazole-3,4-pyrimidine	1227
Furfural	842	**Pyridines**	**LRI**
Furfuryl alcohol	831	Pyridine	776
Furfuryl formate	890	1-Acetyl-1,4-dihydropyridine	990
3-Furanone-2,5-dimethyl-2-((hydroxy-1-acetyl) ethyl)	930	**Pyrazines**	**LRI**
5-Methylfurfural	942	Methylpyrazine	832
Furfuryl acetate	955	4,6-Dimethylpyrimidine	906
1-Propanone-2-furanyl	968	2-Ethyl-6-methylpyrazine	959
2,2-Bifuran	1023	2-Ethyl-5-methylpyrazine	962
Alanine N ethyl furfuryl ester	1072	2-Methyl-6-vinyl pyrazine	982
Furan, 2,2′-methylenebis	1117	2-Acetyl pyrazine	998
4-Hydroxy-2,5-dimethyl-3(2H)-furanone	1128	3-Ethyl-2,5-dimethyl pyrazine	1112
5-Methyl-2,2-dimethylene furan	1217	1-(6-Methyl-2-pyrazinyl) ethenone	1163
Furfuryl methyl disulfide	1258	2-Acetyl-3-methyl pyrazine	1155
Furfuryl-3-methylbutanoate	1262	5-Methyl-6,7-dihydro-5H-cyclopenta pyrazine	1181
3-Phenylfuran	1272	3,5-Diethyl-2-methylpyrazine	1197
6-(5-Methyl-furan-2-yl)-hexan-2-one	1291	2-Methyl-5(1-propenyl) pyrazine	1242
2,2′-Difurylmethane	1130	3,5-Dimethyl-2-acetyl pyrazine	1265
Furfuryl methylamine	1401	6-Methyl-2-isoamyl pyrazine	1294
Ketones	**LRI**	**Acids**	**LRI**
2,3-Pentanedione	590	Isovaleric acid	856
3-Methyl-1,2-cyclopentanedione	1013	2-Methylene-4-hydroxy butyric acid	1028
2,4-Dimethyl-1,3-cyclopentanedione	1050	**Pyrroles**	**LRI**
Methyl acetyl acetone	1143	2-Formyl-1-methylpyrrole	965
3-Ethyl-2-hydroxy-cyclopentene-1-one	1135	Pyrrole-2-carboxyaldehyde	1007
Terpenes	**LRI**	Pyrrole-2-acetonitrile-1-methyl	1150

		Alcohols	**LRI**
Linalool	1104		
Damascenone (2,6,6-trimethylcyclohexa-1,3-diene)	1379	Maltol	1157
4-Ethyl-2-methoxy phenol	1320		
2-Methoxy-4-vinyl phenol	1353		

3.5. DISCUSSION

3.5.1. *Green Coffee Origin and Discrimination and Characterization Based on the Post-Harvesting Processes*

Volatile compounds in green coffee have been widely studied for detecting defective elements and the influence of altitude or climatic conditions. Specifically, the presence of defective beans, such as black, sour, immature, or moldy beans deriving from inappropriate agricultural, harvesting, and post-harvesting practices, impacts the volatile compounds profile of a coffee lot. Cantergiani et al. [6] identified some compounds causing a moldy/earthy flavor in coffee and suggested their presence as being influenced by post-harvest drying. Toci et al. [7] identified 2-methylpyrazine and 2-furylmethanol acetate in black immature beans as well as benzaldehyde and 2,3,5,6-tetramethylpyrazine as markers of defective beans in general. Bertrand et al. [21] reported that butan-1,3-diol and butan-2,3-diol are correlated with acidity, a reduction in aroma quality, and an increase in earthy and green flavors. Moreover, they assumed that high temperatures induce higher levels of these compounds.

Figure 1 provides the results of the discrimination of green coffee samples by country of origin. Only countries represented by more than five samples in the sample set were considered (n = 29). The discriminant compounds included in the model are ethanol; 1-methoxy-2-propanone; isovaleric acid methyl ester; hexanal; 2,3-butanediol; methylpyrazine; butanoic acid 2-methylethyl ester; isovaleric acid ethyl ester; xylene; isovaleric acid; butanoic acid 2-methyl; pinene; alpha-pinene; methyl nonane; benzal-dehyde; pentanoic acid 3-methyl; 2-pentylfuran; n-decan; octanol; carene; and 2,6-dimethyloctane. The two-function model was able to correctly group 100% of the samples. However, unfortunately only a small number (<15%) of the samples were correctly identified by the leave-one-out validation. This can be justified by the fact that the aroma profile of a coffee increases in complexity and intensity once it is

roasted since the precursors, and some fixed compounds, undergo Maillard and browning reactions originating new volatile molecules.
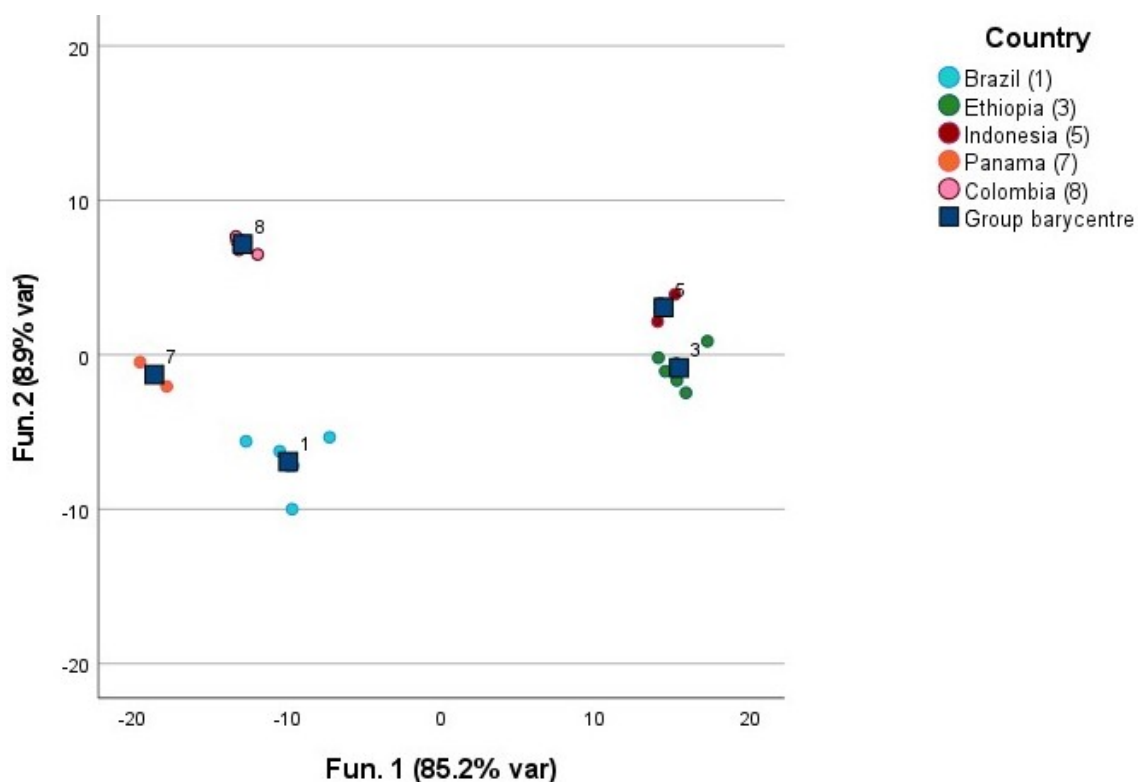
Figure 1. discrimination of country of origin of green coffee samples

A t-test was conducted to identify differences in the composition of the volatile fractions in American and African coffee samples. In this respect, American samples were richer in methyl ester isovaleric acid, tetramethyl octane, and 3-methoxypyrazine-2-isobutyl, while the African samples were more concentrated in 2-methyl butanoic acid, pinene, and D-limonene ($p < 0.05$).

Additionally, an ANOVA combined with a Waller–Duncan post hoc test showed that Asian coffees were the richest in 3-methylbutanol, 2-pentylfurane, and 3-hydroxybutanoic acid 2,2,4-trimethylpentil ester. Conversely, African coffees were characterized by pinene and D-limonene and the American coffees showed to be the least concentrated in butanoic acid.

To improve the reliability of the discrimination, the variables in the model were reduced by selecting samples only from one continent at a time. Figure 2 provides the results obtained when discriminating samples from three American countries: Brazil, Colombia, and Panama. The discriminant compounds were, in this case, ethanol, methyl nonane, n-decan, D-limonene, and tributyl phosphate. All the samples (100%) were properly clustered, and

contrary to the earlier result, 95.8% of the samples were also correctly associated after leave-one-out validation. Guyot et al. [22] found that coffee from higher elevations in Guatemala exhibit higher beverage quality, successively confirmed in several Central American countries [12,23,24]. Tsegay et al. [25] identified volatile compounds in Ethiopian coffee samples and found only weak correlations with the altitude of the cultivation area.
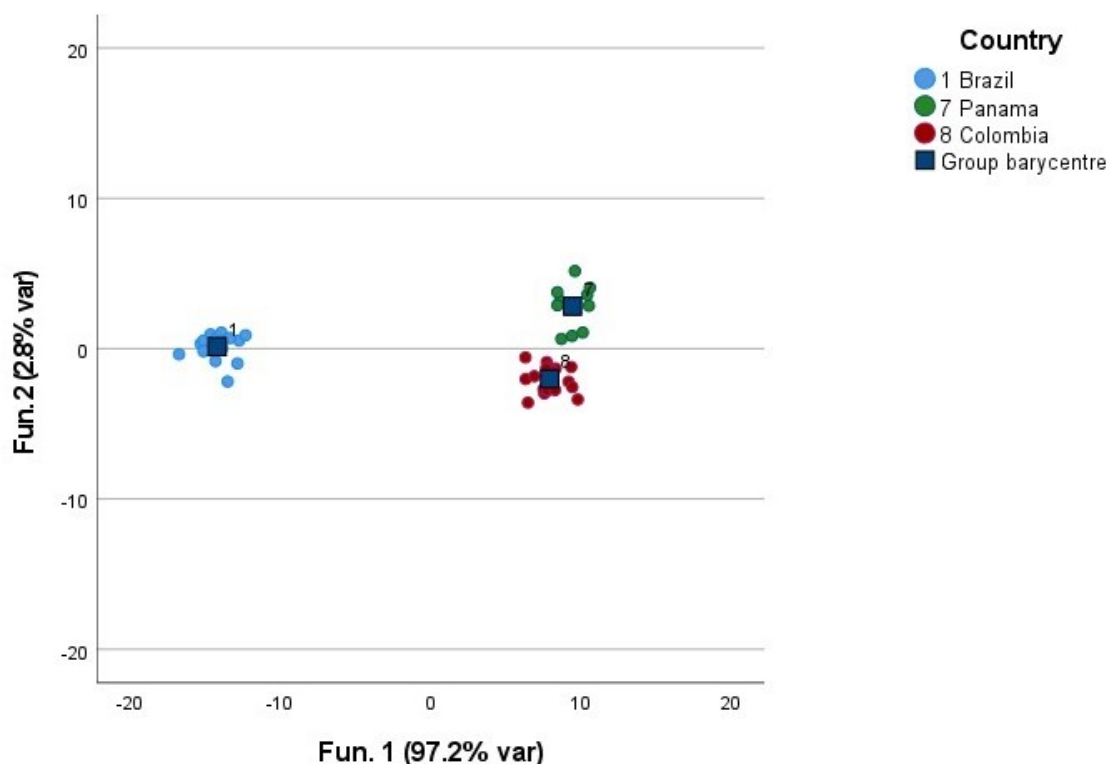
Figure 2. discrimination of Brazilian, Colombian, and Panamanian green coffee in American subcluster

Interestingly, reducing the variability to only a post-harvesting process, the continent of origin was also discriminated considering only washed processed samples. The model, built on the concentrations of ethanol; butanoic acid 2-methyl; 2,6-dimethyloctane; D-limonene; phenylethyl alcohol; 3-methoxypyrazine-2-isobutyl; and 3-hydroxybutanoic acid 2,2,4-trimethylpentil ester, was able to properly cluster 88.6% of the samples and 77.1% of them were also validated via the leave-one-out test.

Dealing with the post-harvesting processes, discriminant canonic analysis was performed to classify the samples obtained via natural and washed processes. Honey, pulped, and anaerobic processes were not included in the model due to their variability and the small number of samples available. Figure 3 provides

the clustering obtained by modeling the concentration of 2,3-butanediol; pinene; and octanol, which turned out to be the discriminant molecules for the post-harvesting processes. In the model, 77.8% of the samples were correctly clustered and the leave-one-out validation also provided the same discriminant power.

As per Gonzalez-Rios et al. [14], the volatile fraction of green coffee beans is primarily given by the alcohols, acids, esters, and aldehydes which are mainly formed during the fermentation stage of the post-harvesting process. Acids and aldehydes may also be formed during drying. They also reported that the fermentation stage increases the volatile compound fraction, particularly if fermentation was carried out in water.
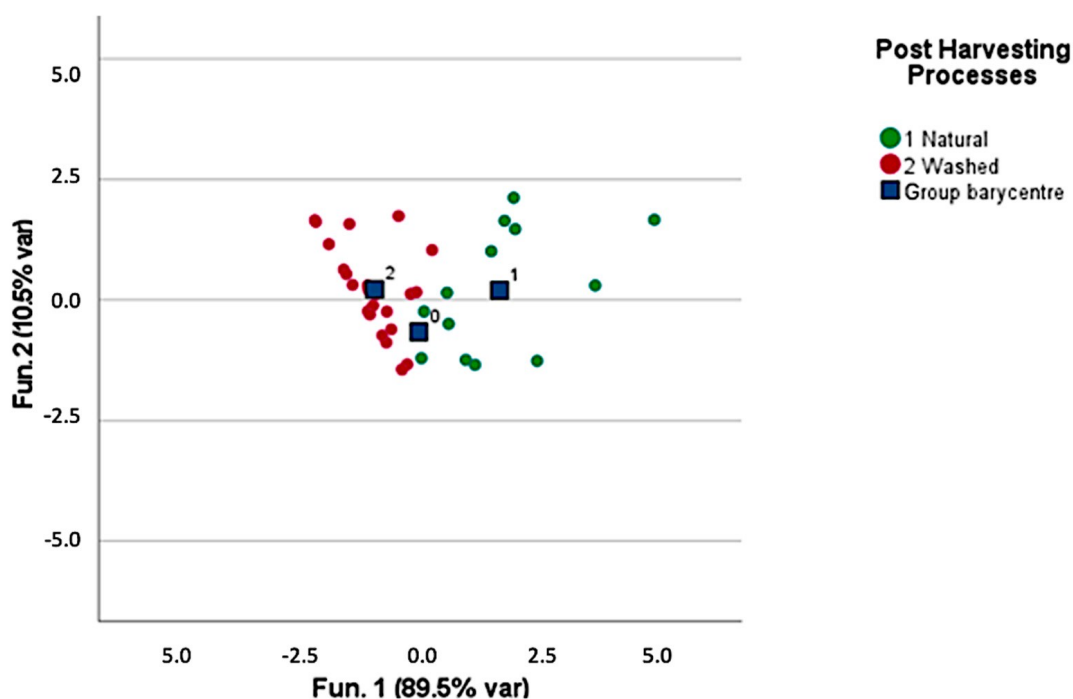
Figure 3. discrimination between washed and natural post-harvesting processed green coffee samples.

T-test was conducted to identify differences in the composition of the volatile fraction from American and African coffee samples: in this respect American resulted richer in methyl ester isovaleric acid, tetramethyl octane, and 3-methoxypyrazine-2-isobutyl while African more concentrated in 2-methyl butanoic acid, Pinene, and D-limonene ($p < 0,05$).

ANOVA combined with Waller-Duncan's post hoc test additionally showed that Asian coffee are the richest in 3-methylbutanol, 2-pentylfurane, and 3-hydroxybutanoic acid 2,2,4-trimethylpentil ester, conversely African are for pinene and D-limonene and American showed to be the least concentrated in butanoic acid class.

T-test was also performed to compare washed and natural processed coffee: washed coffee appeared to be less concentrated in acetic acid, 2,3-butanediol, 3-methylpentanoic acid, octanol, furfuryl alcohol, alpha-linalool, 3-methoxypyrazine-2-isobutyl and higher in hexanal, 3-methylundecane, and undecane. The results are the same for both the whole set of samples and for the test within a single origin subset. The outcomes can be justified considering the solubility of the different chemical classes that alternatively can be lost or not during the washing process. 2,3-butanediol and hexanal, as reported by Bertrand et al. [18], showed high correlation earthy at-tribute and with acidity and bitterness respectively. The fact can be supported by the study of Marin et al. [23] that reported hexanal can be produced by oxidative degradation of unsaturated fatty acids during storage and, in wet coffees, it can be formed during the long stage of washing. The simple analysis and quantification of these two compounds allow to verify which post-harvesting process was carried out.

Finally, results showed that wet process probably reduced the compounds belonging to butanoic class (methyl butanoate, 3-methyl butanoic acid, 2-methyl butanoic acid, 3-methyl-but-2-en-oic acid); considering samples coming from the same Origin, as Ethiopian coffees, the reduction in butanoic compounds for wet samples was more than 50% (-57.2%). Our results confirmed the recent findings of Elhalis et al. [15] which evaluated the effect of wet fermentation on flavor volatiles: in this study, significantly higher level of hexanal and a lower level of butanoic acid in wet green coffee was found.

3.5.2.Discrimination and Characterization of the Origin, Post-Harvesting Processes,
and Roasting Level of Roasted Coffee

Data were statistically treated via discriminant canonic analysis to detect whether the volatile profile allowed one to discriminate among different countries and continents of origin, roasting levels, and post-harvesting processes.

Figure 4 provides a general overview of the distribution of the coffee samples after three different roasting processes in clusters homogeneous for the continent of origin of the green coffee. It can be stated that, independently of the roasting level, coffee kept a certain degree of unicity deriving from its area of origin. The discriminant functions were built on the concentrations of discriminant volatile molecules, such as pyridine; methyl pyrazine; furfuryl alcohol; isovaleric acid; furfuryl formate; furfuryl alcohol acetate; 2-methyl-6-ethyl pyrazine; benzene acetaldehyde; 2,4-dimethyl-1,3-cyclopentanedione; 3-ethyl-2,5-dimethyl pyrazine; fu-ran-2,2-methylenbis; 3-furanone-4-hydroxy-2,5-dimethyl; 3-methyl-2,4-pentanedione; pyrazole-3,4-pyrimidine; 2-acetyl-3,5-dimethyl pyrazine; 3-phenylfurane; 5,6-methylfuran-2-yl-hexan-2-one; and ethyl-pentamethyl-benzene. Discriminant canonic analysis was able to correctly classify 98.6% of the samples, and 95.8% of the samples were also validated via the leave-one-out test. Our results are in line with those already provided by different authors about pyridine as a discriminant molecule for identifying the continent of origin of coffee, together with its impact on the coffee sensory profile delivering roasted and nutty aromas [10,11,20,35]
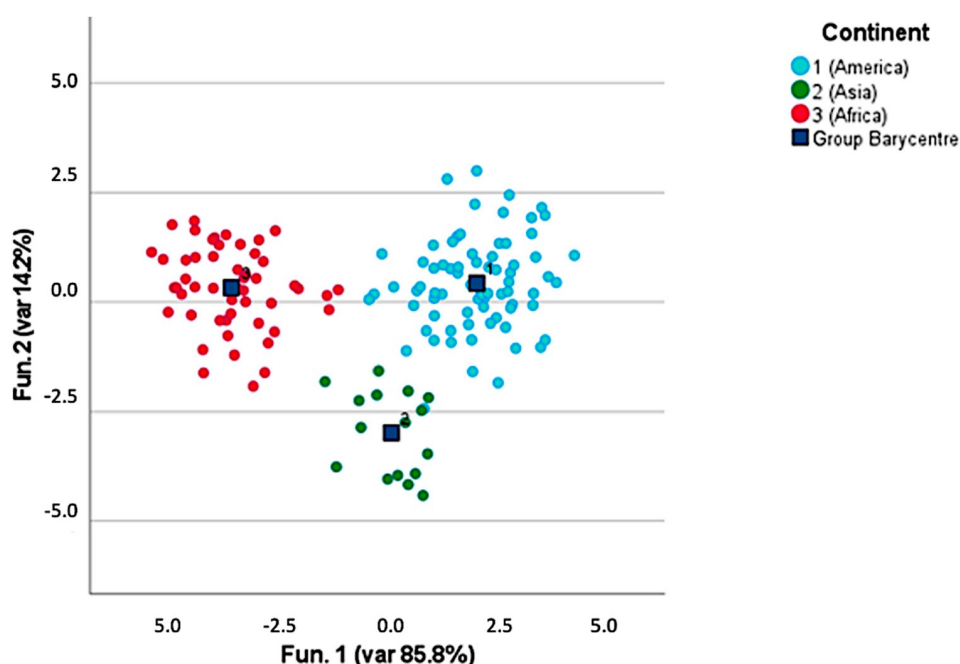
Figure 4. discrimination among continents of origin of roasted coffee samples.

The discrimination of the roasting levels of none of the samples was satisfactory in terms of the classification of samples (<90% of correct association). Therefore, discrimination of subclusters homogeneous for the continent of origin and the post-harvesting process was conducted.

Figures 5 and 6 provide the graphical results of the canonic discriminant analyses conducted on African coffees (Figure 5), and washed samples (Figure 6).

The former discrimination showed that furfuryl alcohol; 5-methyl-2-furfuraldehyde; furfuryl alcohol acetate; and 3,5-diethyl-2-methyl pyrazine were the discriminant molecules for the roasting level among samples of the same origin. The latter identified 1-furfuryl-etanone; 2,4-dinethyl-1,3-cyclopentanedione; 2,2-methylenbisfurane; 3-furanone-4-hydroxy-2,5-dimethyl; 3-methyl-2,4-pentanedione; 3,5-diethyl-2-methyl pyrazine; 1,2-furanylmethyl-1-pirrol; and damascenone. Both models correctly clustered 90% of the samples and were also confirmed by the leave-one-out validation.

It was difficult to identify post-harvesting processes on the basis of the volatile profile of coffee. To enhance the discriminant power, the analysis was conducted considering only one roasting profile at a time, and the best discrimination was obtained on light-roasted coffee, explained by the fact that

the lighter the roast, the more preserved the acidic and volatile profile deriving from the fermentation/drying phases. Irrespective of any improvements, only 79.2% of the samples were classified in the proper post-harvesting process group, and 68.8% of them were validated. These poor results must also be considered in light of the small number of discriminant molecules, such as methylpyrazine; 3,5-diethyl-2-methylpirazyne; and 2,2-oxydimethylen-difurane.
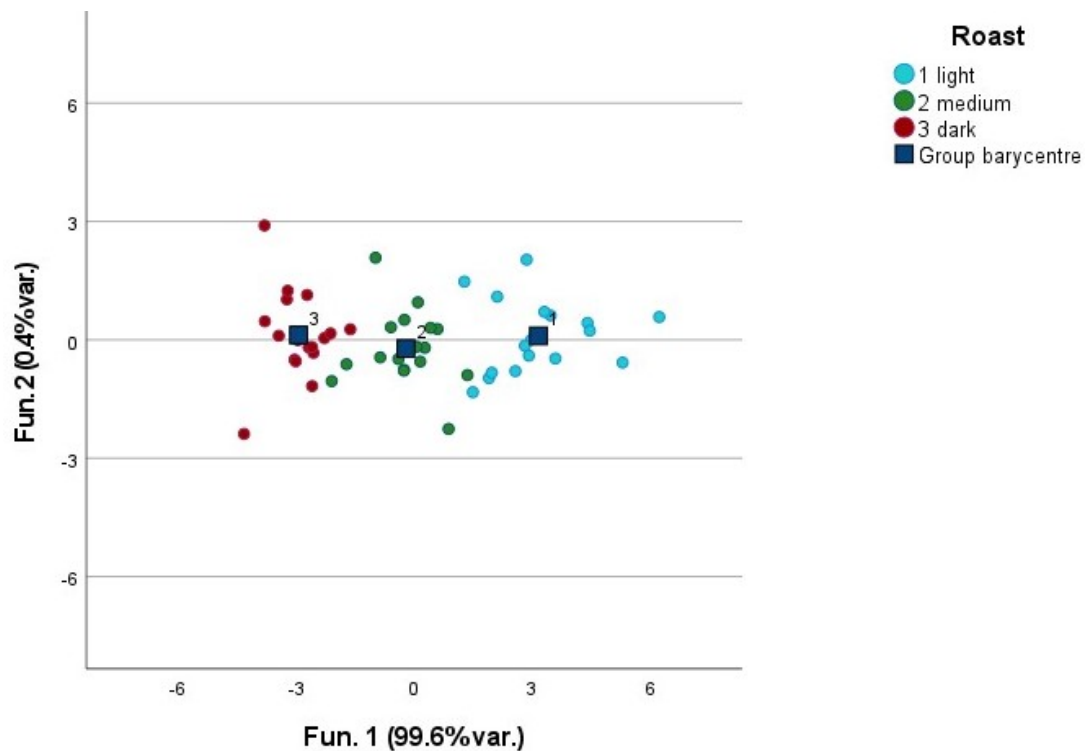
Figure 5. discrimination among roasting levels in African coffee subcluster.
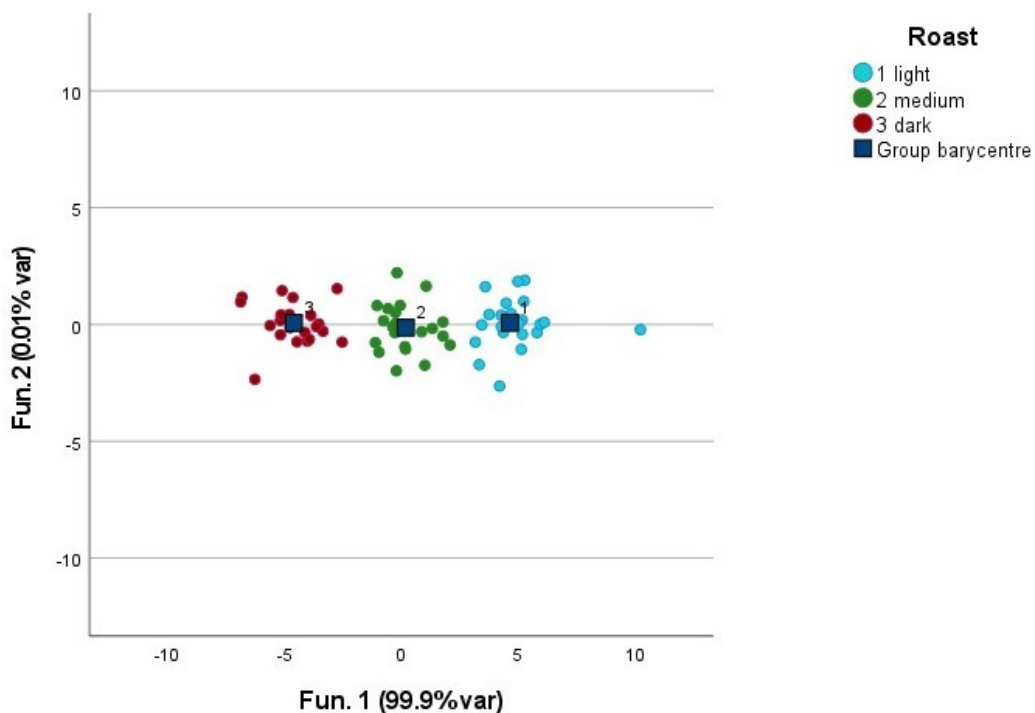
Figure 6. discrimination among roasting levels in washed coffee subcluster.

As the same issues in post-harvesting process discrimination were outlined by Caporaso et al. [18], and tentatively connected with variability provided by origins, a discriminant model was built considering only African (Figure 7) coffees at different roasting levels, and 96.1% of the samples were correctly classified with 96.1% confirmed after cross-validation.

A t-test ($p < 0.05$) was conducted to identify any significant differences in the composition of the volatile profiles of natural and washed coffee. Methylpyrazine; 2,3-pentanedione (and its linear and cyclic methylate and dimethyl derivates); furfuryl formate; 5-methyl-2-furfuraldehyde; 2 formyl-1-methyl pyrrole; linalool; maltol; and other compounds ascribable to the classes of furans and pyrazine were identified as being significantly different in the two sets of samples.
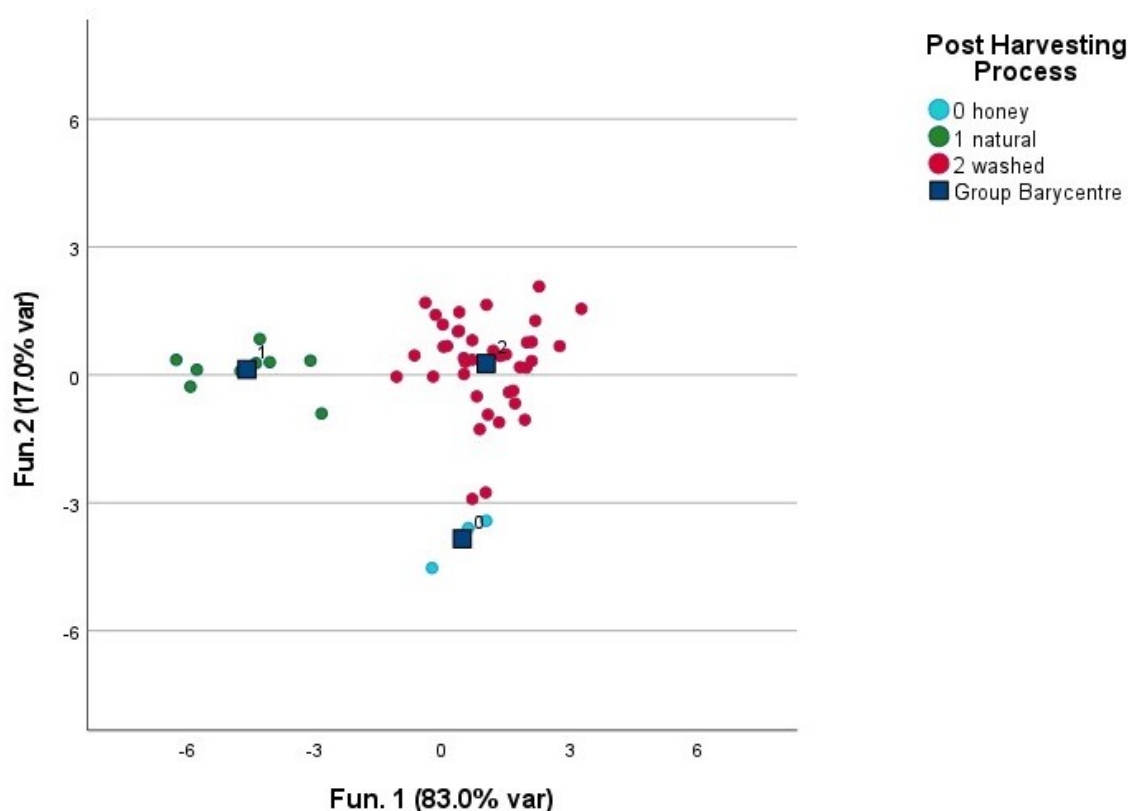
Figure 7. discrimination among post-harvesting processes in African coffee subcluster.

In contrast, the countries of origin were best identified when considering only the dark-roasted samples, potentially due to the intense thermal treatments that push the reaction involving fixed compounds in the beans—the source of coffee's complex aroma profile, the reduction in the number variables, and the variability provided by the post-harvesting processes, may allow features of origins to show their effect in terms of the volatile profile (Figure 8). In this case, 100.0% of the samples were consistently classified and 85.7% of them were confirmed via leave-one-out validation. That is a satisfying result when compared with the outcomes obtained from green coffee (<15%). These outcomes confirm the prevalence of roasting already stated by Zakidou et al. [11] and are also in line with those reported by Caporaso et al. [18], even if, in the present work, the number of samples was increased (n = 30).

The discriminant molecules were 4,6-dimethylpyrimidine; 5-methyl-2-furfuraldhyde; 1-methyl-2-formylpyrrole; 2-furfurylpropan-1-one; benzene-acetaldehyde; 2,5-dimethyl-4-hydroxyfuran-1-one; and 2-methoxy-vinylphenol.
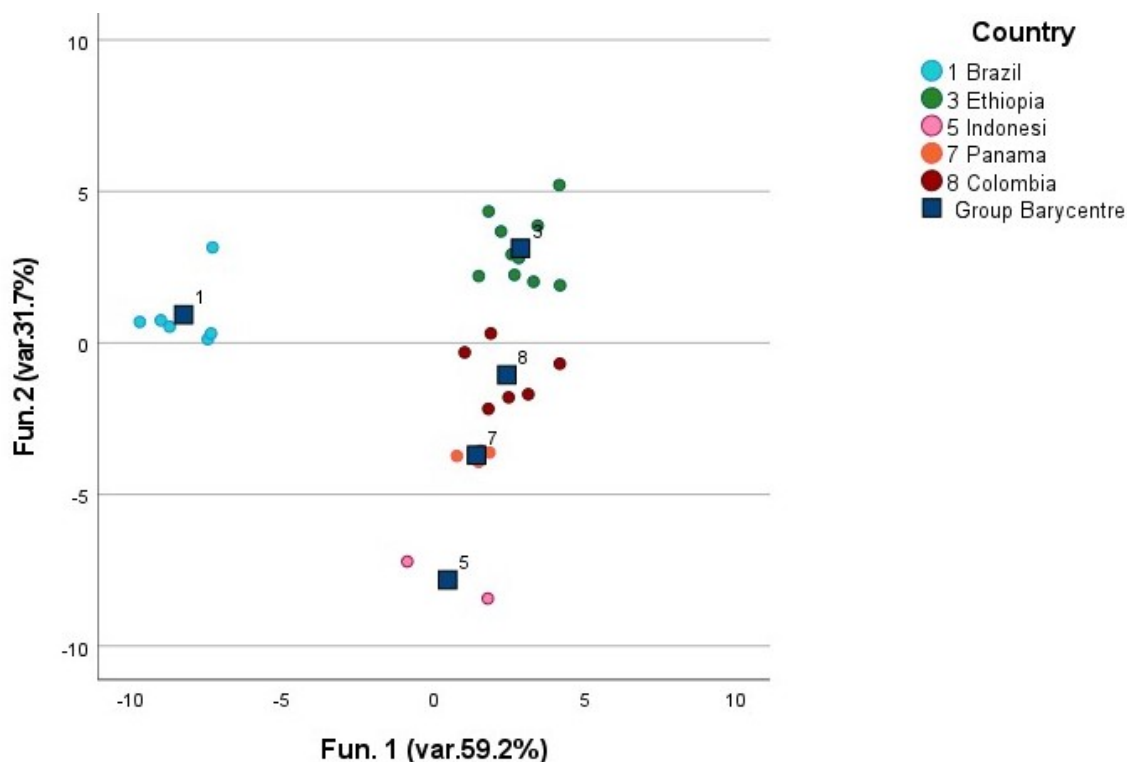
Figure 8. discrimination among countries of origin in dark roasted coffee subcluster.

Lastly, in the results obtained, for both country and post-harvesting processes, discriminations are in line with those already assessed for green coffee, confirming that the volatile profile is more suitable for origin rather than post-harvesting processes discriminant models. However, it is useful to identify discriminant compound markers of natural and washed processed coffee in both green and roasted samples, as observed in the t-test results.

3.6. CONCLUSIONS

To conclude, this work provided a deep overview of the volatile composition of Specialty Arabica coffee, both as green and roasted coffee.

The findings support the generally accepted peculiarities of Specialty coffees that link the aromatic composition to the origin, the post-harvesting process, and the roasting level.

Even if the roasting profiles have a strong impact on the volatile composition of coffee, features deriving from the origin and post-harvesting processes are preserved in roasted coffee because they act as precursors in specific

biochemical pathways, potentially helping discriminate among them via the headspace analysis.

It is confirmed that the origin has a stronger impact on the volatile profile than the post-harvesting process. Even when coffee of a single origin is kept for analysis, it is possible to identify the post-harvesting processes.

Further studies are needed to identify the correlation between the volatile profiles of green and roasted coffee and to potentially predict the effect, in terms of the aroma composition, of roasting on a defined green coffee lot.

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4. Acrylamide: impact of precursors concentration, origin, post-harvesting process and roasting level in high quality Arabica and Robusta coffee

4.1. ABSTRACT

Origin of coffee, precursors concentration, post-harvesting processes, and commercial roasting degrees were evaluated for their impact on acrylamide content in roasted coffee. 47 Specialty Arabica and 7 high quality Robusta green coffee samples were analyzed to determine sugars, asparagine and Aw. Acrylamide was quantified on light, medium and dark roasted samples. In green coffee, glucose and fructose content resulted lower after wet and honey processes, no remarkable differences were found for sucrose and asparagine. In all samples, the content of asparagine was generally lower than what provided in previous works. Acrylamide concentration never exceeded the limit of 400 µg kg^{-1} in Arabica samples and it does once in Robusta, it peaked between light and medium roasting, and it was higher in Robusta. Moreover, it was lower in honey coffee than in others. Acrylamide correlated with asparagine in Robusta, while with monosaccharides and Aw in dry and honey Arabica. Coffee Origin impacted on precursors and acrylamide.

4.2. INTRODUCTION

Coffee is one of the most popular beverages in the world thanks to its sensory profile, the beneficial impact on human health of some bioactive components, and the effect played by caffeine on psychophysical performances. The roasting process, which is the most important and impactful unit operation capable for the development of antioxidant and bioactive compounds, as well as many chemicals, physical and sensory characteristics of the final cup, is also the major responsible for the creation of hazardous compounds contaminating roasted coffee as furans and acrylamide (AA) [1]. As regards AA, EFSA reported that this contaminant could increase the risk of developing cancer in all age groups [2]. Following the EFSA opinion, the European Commission published the Regulation (EU) 2017/2158, establishing mitigation measures and benchmark levels for the reduction of the AA presence; the benchmark level for coffee was fixed at 400 µg kg^{-1} [3]. AA is mainly formed during heat processing (T>120°C,

optimal range 170-190°C) and it results prevalently by specific pathways of Maillard reactions, particularly from the reaction of reducing sugars with asparagine forming N-glycosylasparagine, an adduct that leads to more stable decarboxylated Schiff base. The Schiff base may directly decompose into AA and an imine or, after hydrolysis, it forms carbonyl compounds and 3-aminopropionamide; this last compound may also originate AA after the elimination of an ammonia group [4]. Sucrose, the main sugar in green coffee, can also contribute to AA formation, since in the early stages of roasting, it decomposes to reducing monosaccharides. Other minor pathways of formation, starting from 5-hydroxymethylfurfural (a substance generated during roasting), acrolein and acrylic acid (deriving from fats and amino acids, respectively) together with ammonia and asparagine, can contribute to the final acrylamide content in coffee [5].

Through all the coffee chain, from the different stages of selection and post harvesting processes till the roasting, the final AA level can be decreased by specific good manufacture practices (GMP) that can provide with a reduction of precursors level. After harvest and selection of coffee fruits, three types of post-harvesting process (dry, wet and honey) can be alternatively applied [6], with the common aim of extracting green beans from coffee fruits. Briefly, the dry process is characterized by the direct drying of the entire cherries under sunlight or in mechanical dryers. After a cleaning step to remove leaves, stones, hydraulic separation of overripe and unripe drupes from properly ripe, cleaned fruits are spread on patios or African beds (tables made of mesh), to allow irradiation by sun and air to circulate all around the fruits. After a variable period of 10-20 days, a hulling machinery will remove the dry skin and the parchment before green coffee sorting and shipping. Opposite, wet process starts, after fruits sorting by flotation, with the pulping of coffee fruits by mechanical pulper that removes skins and the majority of the mesocarp. As for natural process, unripe drupes are processed separately because of the higher energy needed to them be squeezed. After a fermentation period, the mucilage layer covering the parchment is soluble so that it can be easily washed. Lastly, green beans in parchment are dried under sunlight or in mechanical driers then

hulled and sorted to be sold. As well known, the wet process reduces the concentration of reducing sugars in green beans [7]. Finally, the honey (semi-dry) process is an intermediate treatment between two already mentioned: in this case, cherries pass through the pulper that leaves on seeds in parchment a variable quantity of mucilage, depending on farmer goals. This time the polysaccharidic layer is not fermented as in wet process but dried and removed in a dehuller with parchment.

All that considered, the objective of this work was to investigate if, besides the roasting level, the precursors concentration, specie, variety, geographical origin of green coffee and the applied post-harvesting process could influence the AA formation. To improve the consistency of sampling and samples information, together with a certified quality of green coffee, Specialty Arabica and high-quality Robusta coffees, coming from the main producer countries, were collected by a local roaster. The samples were analyzed for determination of the AA precursors and, after roasting at three different levels, for AA quantification. Then, all the results were processed to evaluate if the data on green coffee features showed correlations with AA levels in the roasted products. Then, it was also investigated if AA mitigation in roasted coffee might be obtain by appropriate selection of green coffee lots.

4.3. MATERIALS AND METHODS

4.3.1. Sampling

A total of 54 green coffee samples, both Arabica (47 samples from Brazil, Burundi, Colombia, Costa Rica, Democratic Republic of Congo, Dominican Republic, El Salvador, Ethiopia, Guatemala, Haiti, Honduras, India, Indonesia, Kenya, Peru, Republic of Panama, Rwanda, and Uganda) and Robusta (7 samples from Brazil, India, Indonesia, Uganda and Vietnam) were drawn (1 kg) from GrainPro bags of 60 kg each (S1, Supplementary Materials). All Arabica coffees were recognised as "Specialty" or "Premium" coffee according to the protocol established by the Specialty Coffee Association. To obtain this certification, they must have at least a cupping score of 80/100 and beans must be almost free of defects (no sour beans, foreign matter, or insect/fungus

damage and only a small number of unripe or broken beans are allowed). Robusta coffee samples were selected among the coffee reaching the higher quality standard for each country [8] considered in the study. Coffee samples were from 35 different varieties, representing the wide diversity of coffee on the market. Regarding the post-harvest processes, 15 Arabica and 4 Robusta samples were subjected to dry, 6 Arabica to honey and 26 Arabica and 3 Robusta to wet process.

Frozen green coffee samples (500 g) were milled using a cyclone hammer mill (1 mm sieve, Pulverisette, Fritsch GmbH, Idar-Oberstein, Germany) and homogenized. After milling and homogenization, an aliquot (300 g) of the sample was taken and stored at -20°C until the time of analysis.

4.3.2. Roasting

The sample roaster IKAWA Model V2-PRO (IKAWA Ltd., UK, 2018) was used to roast the coffee samples. In this equipment, a fan draw air into a heating element and then into the roaster so that beans are agitated and roasted evenly. Chaff is removed by a cyclone system and fell into a collection jar, to separate it from the seeds. Each roasting batch was of 50 g (± 0,5g). Three roasting profiles, namely "light" (I roast), "medium" (II roast) and "dark" (III roast), were applied reaching commercial roasting levels [1], No adjustment was applied to roasting profile based on the differences of specie and post-harvesting processes to reduce the variability deriving from the treatment. Chamber was preheated at 174-175°C prior to coffee inlet, "light" roasting ended at 205°C in 5.46 minutes, "medium" at 210°C in 6.16 minutes and the "dark" one at 215°C in 6.46 minutes. Moving from I roast to the II roast and finally to III roast level, there was every time an increase of 5°C and 30 seconds between one and the other. The temperatures were measured using the probe present in the roasting chamber. After roasting, all samples were stored at -20°C. Before the analyses, frozen seeds were ground using the Moulinex blender (Model AR110830), paying attention not to heat the beans during the milling. The I roast was replicated keeping the same roasting conditions, to confirm the repeatability of the process.

4.3.3. Moisture and Aw determination

Moisture was determined by gravimetric method after evaporation at 105°C for 24 hours. Water activity (Aw) was measured using AquaLab Pre (Meter Food, Pullman, WA, USA).

4.3.4. Reagents and standards

The solvents and chemicals used for the extraction and clean-up were ACS grade or equivalent (Carlo Erba, Milan, Italy); deionized water was purified through a Milli-Q treatment system (Millipore, Bedford, MA, USA). Solvents and formic acid used for LC-MS/MS analysis were HPLC grade (Merck, Darmstadt, Germany). Deuterium labelled d3-acrylamide (AA-d3; internal standard) standard solution, AA, fructose, glucose, sucrose and asparagine were obtained from Sigma-Aldrich (St. Louis, MO, USA). AA stock and working standard solutions were prepared as reported in our previous work [9]. Sugars standard solutions were prepared at concentration from 0.2 to 4 mg l^{-1}; asparagine standard solutions from 8 to 160 µg kg^{-1}. All the solutions were stored at –20 °C when not in use.

4.3.5. Low molecular sugar determination

Sugars were extracted from 0.5 g of ground green coffee into a centrifuge tube with 50 ml of 80% (v/v) ethanol for 10 min in an ultrasonic bath at 80°C, according to Bertuzzi et al. [10]; after centrifugation (3500 g, 5 min), the extract was diluted (0.5 + 9.5 v/v) using H_2O:CH_3CN = 25 + 75 (v/v). Quantification was performed by LC-MS/MS. The LC-MS/MS system consisted of a LC 1.4 Surveyor pump (Thermo Fisher Scientific, San Jose, CA, USA), a PAL 1.3.1 sampling system (CTC Analytics AG, Zwingen, Switzerland) and a Quantum Discovery Max triple quadrupole mass spectrometer; the system was controlled by an Excalibur 1.4 software (Thermo Fisher Scientific). Chromatographic separation was obtained using an Xbridge BEH Amide column (2.5 µm particle size, 100 × 2.1 mm i.d., Waters Corporation, MA, USA) and a gradient elution 8 mM ammonium formate - CH_3CN. The linear gradient was from 25% to 55%

ammonium formate within 5 min, isocratic for 6 min and conditioning of the column for 7 min. The flow rate was 0.2 ml min^{-1}. For fructose and glucose, the ionization was performed in negative mode (ESI interface), considering the $[M^+HCOO]^-$ ion (225 m/z), while for sucrose in positive mode, considering the $[M^+Na]^+$ ion (365 m/z). The fragment ions were 90, 113 and 179 m/z for fructose, 90, 119 and 179 m/z for glucose, 185 and 203 m/z for sucrose. For all sugars, the limit of detection (LOD) and of quantification (LOQ) were 100 and 300 mg kg^{-1}, respectively.

4.3.6. Free asparagine determination

Asparagine was quantified by LC-MS/MS (Thermo Fisher Scientific) as reported by Bertuzzi et al. [10] briefly, after extraction from 2 g of green coffee using 50 ml 0.01 M formic acid for 40 min and dilution (1+9 v/v) with $H_2O:CH_3CN$ = 90:10 (v/v), asparagine was separated using a X-Select HSS T3 column (2.5 μm particle size,150×2.1 mm i.d., Waters Corporation) and a gradient elution H_2O-CH_3CN (both acidified with 0.2% formic acid; pH = 2.6). The gradient program was 100% acidified H_2O for 2.5 min.; linear gradient to 15% acidified CH_3CN within 0.5 min, then isocratic for 1 min; conditioning of the column 7 min. The flow rate was 0.2 ml min^{-1}. The ionization was performed in positive mode (ESI interface) and the fragment ions were 116, 87 and 74 m/z. The LOD and the LOQ were 0.5 and 1.5 mg kg^{-1}, respectively.

4.3.7.. Acrylamide determination

Acrylamide quantification was performed following the method by Bertuzzi et. al [9]. Briefly, an aliquot of sample (2 g) was weighed into a centrifuge vial, 20 ml of Milli-Q water, 5 ml of hexane, 1 ml of Carrez I and 1 ml of Carrez II solutions were added and the mixture was agitated using a rotary-shaking stirrer for 45 minutes. After centrifugation (4500 g for 10 minutes), 10 g of $MgSO_4$, 1 g of NaCl and 10 ml of CH_3CN were added to 5 ml of aqueous phase. The vial was shaken for 5 minutes, centrifuged again at 4500 g for 10 minutes and 3 ml of the organic phase were transferred in a vial together with 150 mg of basic Al_2O_3, hand shaken and centrifuged for 3 minutes. Finally, an aliquot of

the organic phase (1 ml) was purified on the column OASIS HLB column (60 mg, Waters Corporation, Bedford, MA, USA) previously conditioned with 3 ml of H_2O and 3 ml of CH_3CN; AA was collected into a vial, adding 1 ml of CH_3CN to complete the elution. The liquid was almost completely evaporated using a gentle stream of nitrogen and the residue was immediately re-dissolved in 1 ml of CH_3CN: formic acid 0.2% (v/v) aqueous solution 10:90 (v/v). An aliquot of 100 µl of a deuterium-labelled AA (AA-d_3) internal standard solution (1 mg l^{-1}) was added to 900 µl of the extract; then, 20 µl were injected into the LC-MS/MS (Thermo-Fisher Scientific) in positive mode. Chromatographic analysis was performed using a X-Select HSS T3 column (2.5 µm particle size, 150 × 2.1 mm i.d., Waters Corporation) and a gradient elution H_2O-CH_3CN (both acidified with 0.2% formic acid). The gradient program was 100% acidified H_2O for 3 min.; linear gradient to 15% acidified CH_3CN within 5 min, then isocratic for 2 min; conditioning of the column for 7 min. The fragment ions were: 55 and 44 m/z for AA, 58 m/z for AA-d_3. The LOD and the LOQ were 5 and 15 µg kg^{-1}, respectively. All the results were corrected for the recovery (83.2%). The trueness of the method was evaluated by AA analysis in a FAPAS coffee reference material with reference value of 638 µg kg^{-1} (expanded uncertainty U = 109 µg kg^{-1}). The average concentration of three replicates, corrected for the recovery, was 658 µg kg^{-1} with a standard deviation of 6.3%. To confirm these findings, 30% of samples for each roasting level was analyzed in duplicate.

4.3.8. Statistical analysis

Statistical analysis of AA precursors and AA concentration data was carried out using package IBM SPSS statistics (ver. 27, Inc., Chicago, IL, USA). Homogeneity of variance was checked. T-test was conducted to evaluate if significant differences was present between wet and dry Robusta coffee and discrimination between set of data from Ethiopian and Southern American coffees. One-way ANOVA and discriminant analysis were applied to evaluate significant differences between the three Arabica subgroups (dry, honey and wet) and Robusta. The Waller Duncan post-hoc test was applied in

homoscedastic sets (AA concentration in Arabica Coffee) while Games-Howell in heteroscedastic (AA precursors in Arabica Coffee). The relationship between AA precursors in green coffee and AA was evaluated with Excel linear regression model.

4.4. RESULTS AND DISCUSSION

4.4.1. AA precursors in green coffee

In Supplementary materials (S2 and S3) moisture, Aw and the AA precursors (Sucrose, Fructose Glucose and Asparagine) concentrations in green coffee are shown. Aw and moisture showed no significant differences depending on the origin, specie and post harvesting process. On the contrary and as expected from what provided by several works [11], the concentration of sugars and asparagine in Specialty Arabica and high-quality commercial Robusta coffees revealed some important differences.

As regards Arabica coffee, glucose and fructose levels are strongly affected by the post-harvest processing, confirming, and substantiating the findings of Knopp et al. [7], Kleinwächter & Selmar [12] and Amalia et al. [13]. Although the initial level of the two monosaccharides in coffee fruits was not known, Waller Duncan's test provided with significant differences between their concentration in dry and the two other processes (Table 1). In detail, glucose and fructose content in dry processed beans (15 samples) is higher (p<0.01) than honey (6 samples) and wet one (26 samples). These findings also support what provided by Tarzia et el. [14] in the respect of the amount of extractable saccharides in coffee obtained by different post-harvesting processes: natural process is characterized by an higher time of contact between coffee seeds and whole pulps – in comparison with honey and wet process – and by the absence of fermentation that result in the scenario mentioned above. Even if a larger number of data are required to provide with consistent results, with a preliminary analysis no differences was observed between dry (n=4) and wet (n=3) Robusta coffee samples.

Table 1: Average sucrose (g kg^{-1}), glucose (mg kg^{-1}), fructose (mg kg^{-1}) and asparagine (mg kg^{-1}) content in dry, honey, wet Arabica and Robusta green coffee.

	Dry Arabica	Honey Arabica	Wet Arabica	Robusta
Sucrose (g kg^{-1})	65.1 ± 4.6[A]	71.6 ± 4.3 [B]	70.8± 7.7 [B]	32.6 ± 2.0
Glucose (mg kg^{-1})	1760.8 ± 636.2[B]	707.9± 563.7[A]	515.9 ± 270.6[A]	941 ± 291.1
Fructose (mg kg^{-1})	3472.2 ± 1437.4[B]	814.6 ± 755.6[A]	560.9 ± 408.8[A]	1093 ± 305.0
Asparagine (mg kg^{-1})	231.0 ± 35.0[a]	242.0 ± 55.5[a]	246.4 ± 61.9[a]	365.6 ± 99.1

[A,B,a,b] Letters in superscript provides with significant differences from Games-Howell (uppercase) and Waller Duncan's (lowercase) test among Arabica coffees on the same line.

In respect of sucrose, even if it is not a direct AA precursor being a no reducing sugar, many studies demonstrated it has a role in the AA formation. In particular, Oosterveld et al. (2003) evaluated the carbohydrate composition of extracts obtained from roasted coffee beans and proved that most of the sucrose was converted into sugar degradation products, even at mild roasting conditions. Stadler et al. [16] found that AA could be formed by the pyrolysis of asparagine and other amino acids with an equimolar amount of fructose, galactose, lactose or sucrose, giving comparable yields in terms of AA. As reported in previous works, sucrose in Arabica coffee is not affected by the post-harvesting processing [17];[12]; [7]. Our data on Arabica coffee confirmed these findings; the slightly lower average content found for dry processed beans is probably attributable to sample variability rather than post-harvesting process, together with level of cherry ripening, required to be higher for fruits to be submitted to natural process, that is directly related with the degree of sugar depolymerization. As already known, sucrose was markedly lower in Robusta coffee.

Asparagine levels was not affected by the post-harvest processing in Arabica and Robusta coffee. Robusta showed higher asparagine levels, confirming what already provided in several works [18]; [19]; [20]. From our data, the level of asparagine in the green coffee samples was always below 400 and 500 µg kg^{-1} in Arabica and Robusta, respectively, and the mean content was often lower than those provided by other studies. Several authors proved that unripe and defective beans had normally a higher asparagine content [21,22], then, the low level of asparagine detected in our samples can be a confirm of the high quality of Arabica "Specialty" and high-quality Robusta coffees. Indeed, the

certification is released only whether defective beans are not present or at a very low level (<10 beans/300g green coffee).

Finally, discriminant analysis was conducted considering all AA precursors concentrations: besides a discrimination between Robusta and Arabica samples, these last were subclustered in dry group and wet and honey one (Figure 1).
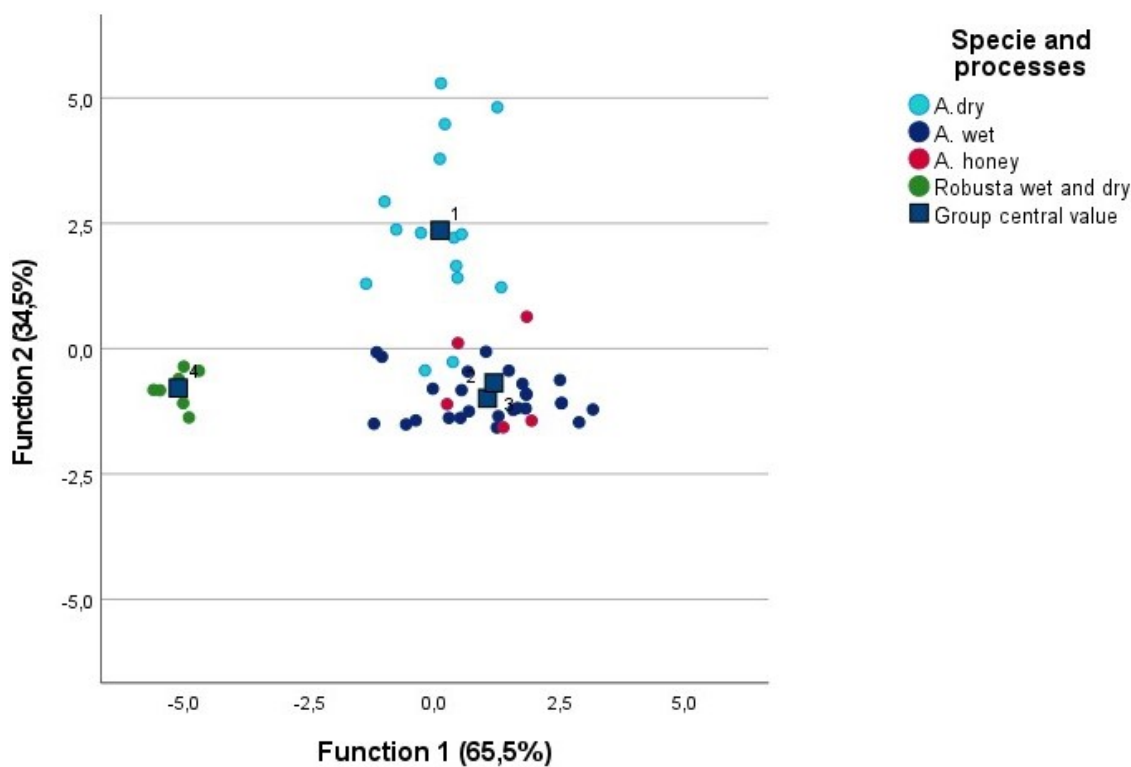
Figure 1. Discriminant analysis based on species and post-harvesting processes.

4.4.2.AA content and correlations with its precursors

The concentration of AA was analyzed after coffee was submitted to three commercial roasting profiles (I, II and III roast). AA levels of I roast and its replicate were similar; then, no replicates were performed for II and III roast. Regarding AA analysis, no remarkable difference was found between the replicates. AA concentration levels for each sample at different roasting degree are reported in Supplementary materials 4 and 5.

Results confirmed that AA, during roasting, reached a maximum and then degraded (Table 2), as already demonstrated by many authors [23]. In our work, at light roasting degree, about half of dry and wet Arabica and all Robusta samples reached the maximum contamination. The other half of dry

and wet Arabica showed higher levels at the medium roasting degree, as most of the honey Arabica samples did. The III roast (dark) caused a decrease of AA concentration for all the samples, independently from Origin and processes, confirming the pathway of AA decomposition increasing the roasting level [18,22]

Table 2: Average acrylamide content (μg kg^{-1}) in dry, honey, wet Arabica and Robusta coffee at three different roasting levels.

	Dry Arabica	Honey Arabica	Wet Arabica	Robusta
AA I roast (μg kg^{-1})	212.1 ± 70.6[b]	148.2± 48.0[a]	193.3 ± 56.6[a,b]	300.4 ± 97.4
AA I roast-replicate (μg kg^{-1})	212.8 ± 87.6[b]	130.3 ± 53.5[a]	196.0 ± 81.5[a,b]	309.5 ± 188.8
AA II roast (μg kg^{-1})	197.9 ± 45.6[.b]	159.0 ± 34.8[a]	199.2 ± 38.2[.b]	229.9 ± 68.4
AA III roast (μg kg^{-1})	150.7 ± 32.8[b]	104.2 ± 28.3[a]	134.3 ± 40.4[a,b]	213.5 ± 78.9

[a,b] Letters in superscript provides with significant differences from Waller Duncan's test among Arabica coffees on the same line.

Usually, to a light roasting degree corresponds a higher AA level because the process ends when the pathway of formation is favored in the respect to degradation one. However, this is not a general trend, because roasting curve parameters are not standardized, and coffee behave is different due to the species, the chemical composition, and the post-harvest processing. Independently from the roasting degree, honey Arabica always presented the lowest mean AA levels: after I and III roast, AA level of honey Arabica was significantly ($p<0.05$) lower than that of dry Arabica and after II roast it was lower ($p<0.05$) than both other two processes. This fact can be explained considering the processing flow that characterize honey coffee: together with the removal of exocarp also the outer layers of the mesocarp are mechanically removed before drying [11], resulting in a lower reducing sugar concentration in green beans once compared with dry process. Additionally, to get depulped coffee with a consistent amount of mucilage left on the parchment, drupes must be all homogeneously ripe, that results in a very low probability to have underripe seeds rich in Asparagine [21] – more suitable for wet one. Even if the statistics was not strictly due to high difference in samples abundance (47 vs 7), Robusta was always the most contaminated, confirming previous data from literature (Table 2).

In general, AA concentration found in our samples never exceeded the benchmark level of 400 µg kg^{-1} fixed by Commission Regulation [3] in Arabica samples and it does once in Robusta (average value 471.5 µg kg^{-1}). AA levels were lower than those presented by the EFSA Scientific opinion on AA in food of 2015 (the mean level, independent from the roasting degree, is 185 µg kg^{-1} vs 249 µg kg^{-1}). Additionally, more recent scientific works [9,23–25], provided with AA higher than ours, except for the study by Lachenmeier et al. [24] which reported comparable data. However, the number of samples involved in this study was less representative (only 2 samples).

In our whole sample set, no correlation was found between AA and its precursors in green coffee. However, a linear correlation was found for dry Arabica coffees between AA level at I roast and the sum of glucose and fructose by Aw value in green coffee (r = 0.7579; n=15, Figure 2), following the equation Acrylamide = 0,0434[(fructose+glucose)*Aw]+ 82,27. Just mentioned correlation was also verified including honey Arabica coffees in the model (r = 0.7812, n=21) and, despite the small number of samples available (6), it persists in honey subcluster processed at II roast (r=0,9027; n=6, Figure 3) thanks to the AA increase from I to II roast. Even never reported before in coffee, Aw value improved the significance of our correlation; its impact on AA formation can be justified by its role as an enhancer of Maillard reaction rate when ranging values between 0.6 and 0.8 [26]. No significant improvement in correlation (r=0,730;) was found including asparagine concentration in our equation, proving that low levels of this amino acid did not affect AA formation.
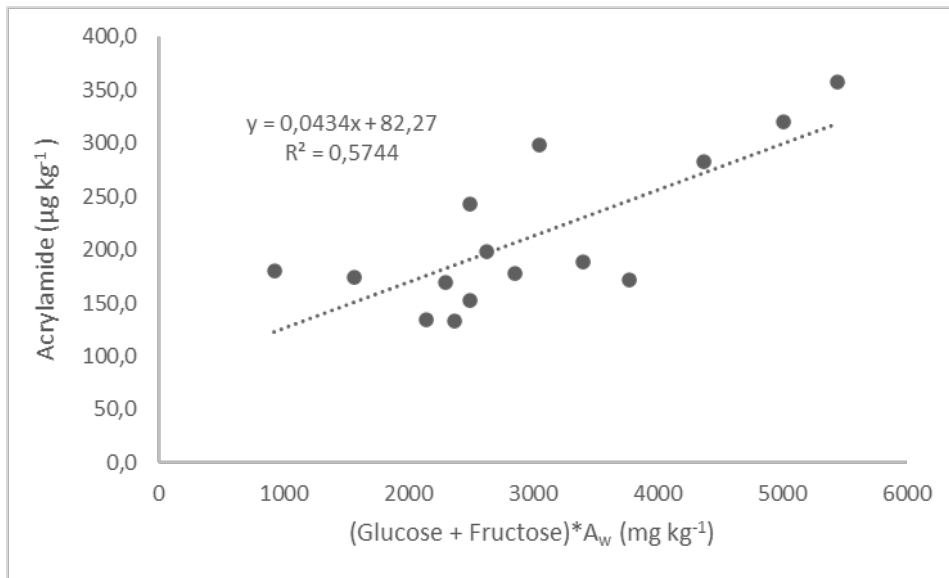
Figure 2. Relationship between (Glucose + Fructose)*Aw (mg kg⁻¹) in Arabica dry green coffees and AA level of I roast (μg kg⁻¹).
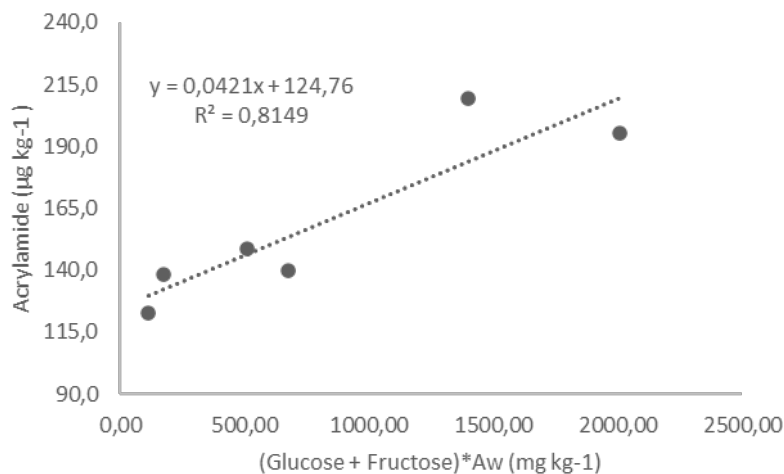
Figure 3. Relationship between (Glucose + Fructose)*Aw (mg kg⁻¹) in Arabica honey green coffees and AA level of II roast (μg kg⁻¹).

On the contrary, in Robusta coffees asparagine correlated with AA values at light roast (r = 0.8269), while no correlation was found with sugars concentration.

As expected, no correlations were identified for III roast, confirming the hypothesis of Lantz et al. [19] which reported that correlations between green coffee precursors and AA formed in the early stages of roasting process are obscured by its reduction during the following stages. The same authors reported a correlation between asparagine and AA (r= 0.7485) in 20 commercial coffee samples (15 Arabica and 5 Robusta), while glucose levels in the green

coffees did not show correlation with AA. Similarly, Bagdonaite et al. [18] reported that an increased content of asparagine resulted in a higher AA level. All that considered, it is possible to conclude that the method of production of Specialty Arabica and high-quality Robusta coffees, including selective picking and appropriate sorting of the drupes, permitting to process only ripe and not defective drupes, resulted a global low AA level after roasting.

4.4.3.Impact of Origin on AA formation and its precursors

From our data (Table 3), significant higher levels of fructose ($p<0.05$), glucose and sucrose ($p<0.1$) are verifiable in samples of dry coffee from Ethiopia when compared with dry samples from Southern America (Brazil and Colombia). In particular, coffees from Ethiopia, very likely due to the high level of selection of the green beans and the higher average altitude of their plantation, are characterized by high levels of low molecular sugars, according to Worku et al. [27], about 1.5 times higher than Brazilian and Colombian ones. Consequently, the AA concentration reached by Ethiopian dry coffees is significantly ($p<0.05$) higher than the level quantified in southern Americans, both in light and medium roasted samples.

Table 3. Sucrose (g kg^{-1}), glucose (mg kg^{-1}), fructose (mg kg^{-1}) asparagine (mg kg^{-1}) and acrylamide content (µg kg^{-1}) in Ethiopian and South American dry Arabica green and roasted coffee.

	Ethiopian dry	South American dry
Sucrose (g kg^{-1})*	68.0 ± 3.7	61.0 ± 3.8
Glucose (mg kg^{-1})*	2413.8 ± 417.2	1663.1 ± 863.4
Fructose (mg kg^{-1})**	5583.0 ± 357.1	3346.2 ± 1082.2
Asparagine (mg kg^{-1})	223.6 ± 16.4	228.2 ± 45.7
AA I roast average (µg kg^{-1})**	325.0 ± 37.0	180.4 ± 27.7
AA II roast (µg kg^{-1})**	262.2 ±33.3	170.6 ± 23.5
AA III roast (µg kg^{-1})	160.6 ± 30.6	163.6± 23.3

(*T-test significance $p<0.10$; **T- test significance $p<0.05$)

4.5. CONCLUSIONS

Outcomes from this work suggest that the certification as Specialty Arabica coffees allows at getting not only a coffee with a superior sensorial quality, but also a safer product giving rise to less AA. On a total of 47 Arabica coffee roasted at three different levels, AA never exceeded the benchmark limit of 400

µg kg⁻¹ fixed by EFSA; only 1 Robusta coffee of high quality showed a slightly higher concentration (at I roast). As regards the post-harvest process, it was confirmed that wet and honey treatments reduced the content of glucose and fructose, AA precursors; the coffee samples subjected to honey process showed a slightly lower average AA content after each roasting level. During the roasting, maximum AA level was reached at I or II roast, depending on the species, the chemical composition, and the post-harvest processing. Considering the species Arabica and Robusta, higher AA levels were found in the samples belonging to Robusta, due to a higher asparagine content in green beans. Different correlations were found among AA precursors and AA content, depending on the post-harvesting process. Finally, coffee origin can also impact on AA content.

Through our findings, AA mitigation in roasted coffee might be obtain by an appropriate selection of green coffee lots, depending on easily available information.

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S1: Moisture (% w/), Water activity (Aw), sucrose (% w/w), glucose (mg/kg), fructose (mg/kg) and asparagine (mg/kg) content in Arabica green coffee.

Process	Sample	Crop	Moisture %	Aw	Sucrose (%)	Glucose (mg/kg)	Fructose (mg/kg)	Asparagine (mg/kg)
DRY	Brasile	2020	9.04	0.499	5.97	1461	3539	280.9
	Brasile	2020	9.81	0.526	5.83	1406	4027	200.2
	Brasile	2020	10.69	0.571	6.52	1911	4690	163.4
	Brasile	2019	10.13	0.542	6.68	915	3333	212.5
	Brasile	2019	9.82	0.553	6.28	1063	3451	178.7
	Haiti	2014	9.60	0.605	6.78	1665	3382	217.5
	Etiopia	2019/2020	10.76	0.587	7.23	1938	5501	242.3
	Etiopia	2019/2020	11.03	0.627	6.58	2717	5274	216.7
	Etiopia	2019/2020	12.40	0.635	6.54	2587	5974	211.8
	Honduras	---	10.70	0.564	6.71	1351	2851	259.3
	Colombia	2019/2020	9.30	0.619	5.59	2847	2651	245.5
	Panama	2019/2020	6.46	0.502	6.66	1908	1220	266.5
	Costa Rica	2019	7.71	0.462	6.72	1990	2646	235.0
	Colombia	2019/2020	8.44	0.512	6.31	698	1108	277.0
	El Salvador	2018	9.52	0.598	7.26	1957	2434	258.1
WET	India	2019	11.76	0.617	6.84	566	1040	170.5
	Santo Domingo	2018	10.61	0.56	7.35	454	1007	193.7
	Etiopia	2019/2020	11.27	0.661	6.85	511	362	232.6
	Etiopia	2019	11.75	0.612	7.21	352	245	224.4
	Etiopia	2019/2020	10.32	0.529	5.68	267	260	275.8
	Etiopia	2019/2020	9.96	0.502	7.56	564	579	247.3
	Etiopia	2019/2020	10.15	0.547	7.99	541	392	236.2
	Indonesia - Bali	2019	10.22	0.516	7.98	834	789	277.0
	Kenya	2019/2020	9.81	0.551	7.55	575	345	206.8
	Kenya	2019/2020	11.04	0.59	8.00	527	398	227.8
	Indonesia	---	12.75	0.657	7.07	1103	1356	385.8
	Burundi	2020	11.15	0.599	6.41	400	788	153.5
	Congo	2020	11.93	0.631	6.76	504	731	151.7
	Panama	2019/2020	10.80	0.531	5.72	1175	1473	179.2
	Colombia	2019/2020	10.32	0.584	7.56	475	594	316.9
	Uganda	---	9.53	0.606	6.19	328	269	217.8
	Indonesia	2019/2020	9.98	0.651	6.07	392	213	235.8
	Honduras	2019	9.05	0.572	6.60	272	273	335.3
	Ethiopia	2019/2020	8.40	0.496	7.19	212	54	320.5
	Colombia	2020	9.16	0.555	7.45	353	363	328.4
	Colombia	2019	8.10	0.473	6.75	406	259	277.7
	Bolivia	2019	8.55	0.507	7.52	612	768	319.5
	Guatemala	2019	9.66	0.616	7.40	416	335	249.3
	Burundi	2019	9.06	0.548	8.21	149	48	230.5
	Ethiopia	2019	9.58	0.586	8.38	289	250	154.1
HONEY	Costa Rica	2019/2020	10.84	0.571	7.58	1613	1903	232,6
	Panama	2019/2020	8.48	0.464	6.58	588	510	218,6
	Colombia	2019/2020	9.34	0.527	6.72	1116	1536	195,4
	Panama	2019/2020	8.00	0.502	7.19	585	759	296,1
	CostaRica	2019/2020	9.06	0.539	7.62	190	130	323,1
	Rwanda	2019	8.88	0.551	7.27	155	49	186,4

S2: Moisture (% w/), Water activity (Aw), sucrose (% w/w), glucose (mg/kg), fructose (mg/kg) and asparagine (mg/kg) content in Robusta green coffee. (Samples are clustered by post harvesting processes)

Process	Sample	Crop	Moisture %	Aw	Sucrose (%)	Glucose (mg/kg)	Fructose (mg/kg)	Asparagine (mg/kg)
DRY	Uganda	2019/2020	7.29	0.566	3.03	1132	1079	446.2
	India	2019/2020	9.78	0.611	3.27	893	1250	454.4
	Indonesia	2019/2020	8.26	0.553	3.39	481	582	397.6
	Indonesia	2019/2020	10.14	0.689	2.95	881	1093	233.7
WET	Indonesia	2020	9.36	0.48	3.33	760	1454	270.6
	Brasile	2020	9.17	0.594	3.32	1045	832	469.7
	Vietnam	2019	7.55	0.505	3.52	1401	1363	286.9

S3: Acrylamide content (μg/kg) in Arabica coffee roasted at different roasting levels (Samples are clustered by post harvesting processes)

Process	Sample	Crop	AA I roast (μg/kg)	AA I roast replicate (μg/kg)	AA II roast (μg/kg)	AA III roast (μg/kg)
DRY	Brasile	2020	152.1	154.7	145.2	126.4
	Brasile	2020	177.9	152.2	168.7	159.6
	Brasile	2020	172.0	163.4	139.2	134.3
	Brasile	2019	168.8	161.8	192.7	212.0
	Brasile	2019	242.7	245.5	202.2	194.9
	Haiti	2014	298.5	332.1	214.6	131.3
	Etiopia	2019/2020	283.1	257.1	225.3	163.0
	Etiopia	2019/2020	319.8	350.5	271.6	187.2
	Etiopia	2019/2020	357.2	397.5	289.8	140.7
	Honduras	---	133.3	89.8	225.4	99.3
	Colombia	2019/2020	188.9	196.2	150.6	172.7
	Panama	2019/2020	173.8	179.0	215.9	140.1
	Costa Rica	2019	134.0	125.7	137.3	97.5
	Colombia	2019/2020	180.8	184.5	187.3	136.7
	El Salvador	2018	198.9	201.5	202.5	164.8
WET	India	2019	287.5	398.5	199.5	168.8
	Santo Domingo	2018	181.0	175.5	134.8	103.5
	Etiopia	2019/2020	277.4	328.0	243.3	231.4
	Etiopia	2019	232.0	290.6	204.9	144.2
	Etiopia	2019/2020	275.5	271.6	269.4	161.9
	Etiopia	2019/2020	281.3	329.0	231.8	179.2
	Etiopia	2019/2020	219.2	235.8	166.1	98.2
	Indonesia	2019	267.5	260.7	173.2	62.5
	Kenya	2019/2020	207.1	195.9	233.9	92.7
	Kenya	2019/2020	138.6	141.6	253.0	105.3
	Indonesia	2019	202.2	202.2	212.2	188.6
	Burundi	2020	91.0	67.8	203.0	109.4
	Congo	2020	116.0	121.1	244.8	104.2
	Panama	2019/2020	73.6	60.9	227.3	148.2
	Colombia	2019/2020	150.1	154.2	200.9	102.1
	Uganda	2020	198.3	168.4	206.2	124.5
	Indonesia	2019/2020	161.9	152.1	184.2	186.0
	Honduras	2019	180.1	165.7	229.3	132.7
	Ethiopia	2019/2020	178.5	186.4	194.8	140.6
	Colombia	2020	245.2	229.3	177.2	111.0
	Colombia	2019	168.3	149.5	155.2	102.0
	Bolivia	2019	175.3	181.5	188.9	209.0
	Guatemala	2019	180.2	153.0	135.4	119.3
	Burundi	2019	180.5	171.9	130.8	110.2
	Ethiopia	2019	171.4	150.1	153.1	147.6
HONEY	Costa Rica	2019/2020	191.8	194.3	195.4	139.2
	Panama	2019/2020	74.3	56.7	148.6	60.2
	Colombia	2019/2020	153.2	102.5	209.1	89.2
	Panama	2019/2020	186.7	175.2	139.7	116.2
	Costa Rica	2019/2020	177.3	158.0	138.5	124.4
	Rwanda	2019	105.7	94.9	122.7	96.0

S4: Acrylamide content (μg/kg) in Robusta coffee roasted at different roasting levels (Samples are clustered by post harvesting processes)

Process	Sample	Crop	AA I roast (μg/kg)	AA I roast replicate (μg/kg)	AA II roast (μg/kg)	AA III roast (μg/kg)
DRY	Uganda	2019/2020	289.7	306.2	243.1	254.4
	India	2019/2020	387.0	411.7	344.6	359.8
	Indonesia	2019/2020	319.9	351.9	206.5	215.8
	Indonesia	2019/2020	268.0	249.0	239.3	124.2
WET	Indonesia	2020	214.8	193.3	153.8	150.2
	Brasile	2020	453.7	489.3	272.9	223.0
	Vietnam	2019	169.6	161.0	149.4	167.0

5. Sensory profile of Italian Espresso brewed Arabica Specialty Coffee under three roasting profiles with chemical and safety insight on roasted beans.

5.1. ABSTRACT

Specialty Coffee (SC) has been showing an increasing interest from the consumers which appreciate its traceability and the peculiar flavours from each single Origin. Additionally, the processes to which coffee fruits underwent to get green coffee characterize the beans in terms of macromolecules acting as substrates during the roasting. This work evaluates via sensory analysed eight SC, roasted at light, medium, and dark level, submitted to Italian espresso extraction, to assess how different roasting levels exalt the expected cup profile obtained by the suppliers via cupping in origin countries. Finally, roasted beans were characterized for physicochemical features (pH, titratable acidity, caffeine, melanoidins, polyphenols and acrylamide). Sensory analysis demonstrated that the intermediate roasting level and espresso extraction matches better attributes from in-Origin cupping. Melanoidins (mmol/g coffee d.b.) was able to discriminate among roasting levels (light 0,12±0,01; medium 0,13±0,003; dark 0,14±0,01; α=0,05). Acrylamide analyses assured the compliance with the food safety standard (light 301,9±37,2 ppb; medium 126,1±19ppb; dark 107,9±22,5ppb). Physicochemical features were able to cluster samples from different Origin within the same roasting level (α=0,05). Results shown correlations (α=0,01) between sensory analysis and physicochemical values: direct for caffeine and astringency, reverse for perceived acidity in relation to astringency, roasted, dried fruits and nuts notes.

5.2. INTRODUCTION

Particularly in the last two decades, coffee market permitted consumers to appreciate the connection between origin, processes and taste of different single-origin coffee [1,2] and the certified quality [3] of those products started to be mostly required [4]. From the selected species of the genus Coffea, the variety and the terroir of the harvesting country [5,6], going through the post-

harvest processing to obtain coffee beans [7], up to the different roasting systems and sensory profiles of a cup of coffee, it can straightforwardly be understood the complexity and the huge number of variables involved to obtain high quality coffee [8–13];

In this panorama, Specialty Coffee (SC) has been started to become a new positive trend in the world coffee market, with helpful reflections on producers' life-conditions and improvement of productions quality [14] . SC as defined by Coffee Quality Institute and Specialty Coffee Association are traceable and well identified lots of coffee, evaluated firstly as green and roasted beans to ensure the absence of primary defects, quakers and up to five secondary defects, then submitted as roasted and ground coffee to a standard extraction and lastly cupped to be scored in terms of sensory quality. People in charge of this quality control procedure are certified experts known as Arabic Q-Graders [15,16]. Besides the availability of more efficient industrial plants and the parallel increased potentiality of data recording and process monitoring, a big improvement in cropping, selection, processing, roasting and extraction processes has been done leading to a quick development in SC sector. Many studies on these topics indeed are aimed to evaluate quality indicators and to assess the authenticity of raw material [11,17–21].

Further, detailed studies and scientifical approaches about industrial coffee roasting in order to exalt the most green's features [22] and to evaluate changings in given and perceived quality under different extraction systems are missing [23–25]. Such a knowledge may also be spent on an industrial scale along with the definition of a set of routine analytical methods scientifically based suitable to monitor the roasting profiles and to detect the main quality markers of the roasted powder and of the final coffee brew.

Indeed, during post-harvesting and roasting processes, enzymatic modifications, fermentations, caramelization, non-enzymatic browning, thermal degradations and pyrolysis, along with the Maillard reactions [7] are all responsible for the final cup profile [3,22]. Some of these reactions are directly involved in beneficial compounds metabolic pathways like polyphenols [26], antioxidant compound attributable to melanoidins group [27] and caffeine, of

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which daily intake coffee is one of the main sources. On the other hand, also hazardous low molecular weight compounds deriving from specific pathways of Maillard reactions and thermal degradation are produced during roasting, namely acrylamide [28,29]. In this respect, the analysis of acrylamide, caffeine, titratable acidity, melanoidins, polyphenols should be used as chemical quality control tool to support and complement sensory analysis of brewed coffee in the evaluation of perceived quality levels of beverage related to changings in the roasting profiles.

All that considered, this work deals with eight samples of Arabica SC that, after being roasted at 3 different levels, were assessed for the consistency between the experimental sensory profile obtained from sensory analysis of espresso extraction and the expected profile got from technical sheets of green coffee to evaluate which roasting level better allow the perception of the expected sensory attributes. Roasted beans have been then physicochemical analyzed following methods feasible by small-medium roasting company to get measurable quality markers.

5.3. MATERIALS AND METHODS

5.3.1. Coffee samples

The analysis had been conducted on 8 different micro-lots of green Arabica SC from 5 different producing countries from 2018/2019 crop as detailed in Table 1. Four micro-lots belonged to washed coffees, the other four were naturals including semi-washed/honey processes according to the CQI Protocol for cupping. These 8 micro-lots have been roasted as detailed below with a Giesen W6A drum roaster applying three roasting profiles (light-medium-dark) (Supplementary material 1) giving rise to 24 independent samples.

Table 1. Characterization of green coffee samples by origin, variety, altitude, post harvesting processes, and physical parameters.

Sample	Origin/Name	Process	Region	Farm	Altitude (m)	Variety	Screen	Density (g/L)	Moisture (%)
1	Kenia AB	Washed sun-dried	Nyeri Country	Ibutiti	1700	N/A*	16/18	866	9.7

2	Brazil FB	Natural	Minas Gerais	Tabuoes Boa vista	1140 – 1150	Bourbon, Mundo Novo	16/18	834	8.7
3	Guatemala G	Washed	San Marcos	El Platillo	1260	Geisha	18/19	800	10.8
4	El Salvador N	Natural Honey	Cordillera Apaneca Ilamatepec	San Juan Bosco	1400 – 1600	Pacas Bourbon	16/18	846	10.7
5	Dom. Republic	Natural	Barahona	Toral	1000	Caturra, Typica	N/A	828	9.5
6	Brazil SW	Pulped natural	Chiapada Diamantina	Sitio Santana II	1380	Red & yellow Catuai	16/18	853	10.7
7	Guatemala B/C/C	Washed	Los Humitos Amatitlan	Los Humitos	1400 – 1600	Bourbon Caturra Catuai	16/18	834	10.4
8	El Salvador W	Washed	Cordillera Apaneca Ilamatepec	Santa Gregoria	1350 – 1500	Pacas 80% Catimor 20%	16/18	820	10.4

*N/A not available

5.3.1.1. *Producers' description of coffee samples sensory profile*

Overall and score by features obtained from in-Origin cupping [15] and reported in technical sheets of the SCs are listed in Table 2.

Hereby, one by one, a brief qualitative description of every sample:

- Brazil semi washed: Light aroma with cherry, sugar cane, tangerine, black currant and vanilla notes; high sweetness. Cherry, sugar cane, tangerine, black currant, vanilla and dried fruit in aftertaste.

- Brazil Full Bloom: medium to high aroma with chocolate and roasted nuts notes, silky and syrupy body; milk chocolate in aftertaste.

- Dominican Republic AA: intense aroma with caramel, red and yellow fruits in aftertaste; sweet. Juicy and light body. Apricot, caramel and sugar cane in aftertaste

- El Salvador natural: pineapple, lemon, apricot, cherry, red apple and dark chocolate in both aroma and aftertaste. Nuts in aftertaste.

- El Salvador: citrus, dried fruits, peach, sugar, apple and nuts in aroma. Citrus, grapefruit, peach, sugar, nuts and chocolate in aftertaste.

- Kenia AB: intense aroma with floral, peach, banana, orange, tea and chocolate notes; juicy body. Floral, sugar cane, banana and orange notes in aftertaste.

- Guatemala: orange, lemon, yellow fruit, cherry, caramel and milk chocolate notes both in aroma and aftertaste.

- Guatemala Geisha: jasmine, apricot, tangerine, notes both in aroma and aftertaste.

Table 2. Overall and scores by classes given during in-Origin cupping and reported in technical sheets of each lot.

	Guatemala Geisha	Guat emala	Kenia AB	El Salvador	El Salvador natural	Dominican Republic AA	Brazil Full Bloom	Brazil semi washed
Aroma	8.25	8	8	8	8.5	8	8.5	7.75
Taste	8.25	8	8	8.25	8.5	8	9	7.75
Acidity	8.25	8	8	8.25	8.25	8	8.5	7.5
Aftertaste	7.75	8	8	7.75	8.25	7.75	8.5	7.5
Body	8.25	8	8	7.75	8.25	7.75	8.5	7.75
Balance	8.25	8	8	8	8.25	7.75	9	7.5
Uniformity	10	10	10	10	10	10	10	10
Sweetness	10	10	10	10	10	10	10	10
Clean cup	10	10	10	10	10	10	10	10
Overall	8	8.25	8	8	8.25	7.75	8.5	7.5
TOT	**87**	**86.25**	**86**	**86**	**88.25**	**85**	**90.5**	**83.25**

5.3.2. Roasting process

Roasting process had been conducted on a 6 kg professional drum roasting machine (Giesen coffee roasters W6A), with traditional conductive/convective heat exchange system led by a 0-100% modulable burner fed by LPG from domestic distribution. Roasting process parameters were in continuum recorded on Cropster Roasting Intelligence software. The air flow during all roasts was stably adjusted (drum inner pressure at 103 Pa). Drum speed was set at 49 Hz. Setpoint of the exhausted air was at 235°C. Coffee was dropped in the preheated drum at 180°C ±1°C, with burner off before the charge of raw beans. At turning point of temperature plot, the burner was turned on to manage a proper roasting profile.

The roasting process was performed to obtain three different roasting levels for each micro-lot (Supplementary material 1), modulating the development time ratio on total roasting time. Samples were taken after 5 minutes of cooling from the mass of roasted coffee for the three roasting levels (Table 3).

Table 3. Time/temperature parameters for the three roasting levels. Additional samples at 3′ and 5′ are obtained only from light roasting to check acrylamide behaviour. In brackets are reported the numerical code used to identify roasting levels.

Sample	Origin/Name	light roasted (0)				medium roasted (1)				dark roasted (2)				3' roasted		5' roasted	
		yellow (min)	first crack (min)	time (min)	end T (°C)	yellow (min)	first crack (min)	time (min)	end T (°C)	yellow (min)	first crack (min)	time (min)	end T (°C)	time (min)	T (°C)	time (min)	T (°C)
1	Kenia AB	3:34	5:43	07:01	198.1	4:01	5:14	07:11	203.3	3:58	5:29	07:36	207.2	03:00	136.7	05:00	170.1
2	Brazil FB	3:35	4:52	07:08	196.9	3:45	5:41	07:07	197.3	3:20	5:15	07:32	207.1	03:00	137.3	05:00	172.3
3	Guatemala G	N/A*	5:45	06:49	195.7	N/A	6:02	07:14	201.2	N/A	6:08	07:50	206.9	03:00	140.4	05:00	173.5
4	El Salvador N	N/A	6:11	07:07	194.9	4:05	6:04	07:40	199	3:54	5:58	08:01	205.3	03:00	134.6	05:00	169.4
5	Dom. Republic	3:59	5:58	07:25	194.9	3:48	5:37	07:25	200	3:56	5:39	07:39	204.1	03:00	130.5	05:00	164.3
6	Brazil SW	4:13	6:27	05:54	191.3	N/A	6:05	06:59	196.3	4:23	5:59	07:00	203.7	03:00	139.7	05:00	179.7
7	Guatemala B/C/C	3:53	6:01	06:52	187.9	4:01	6:06	06:40	192.9	4:01	6:00	07:08	198.1	03:00	128.2	05:00	165.4
8	El Salvador W	3:55	5:51	06:44	194.7	3:44	6:02	07:17	200.3	3:56	5:59	07:39	204.3	03:00	137.9	05:00	170.9

*N/A not available.

Table 4. Physical characterization of samples after roasting. Roasting was performed on a 2.5kg batch of green coffee. Inlet temperature was constant between 180°C and 181°C.

Sample	Process	Roasting Level	Final density (g/L)	Moisture (%)	Final weight (g)	Roasting time (min)	Final T (°C)	Weight loss (%)
Kenia AB	Washed	light	502	1.7	2200	06:52	187.9	12.0
Kenia AB	Washed	medium	483	1.5	2167	06:40	192.9	13.3
Kenia AB	Washed	dark	436	1.5	2142	07:08	198.1	14.3
Brazil FB	Natural	light	434	1.6	2191	05:54	191.3	12.4
Brazil FB	Natural	medium	476	1.5	2170	06:59	196.3	13.2
Brazil FB	Natural	dark	410	1.4	2143	07:00	203.7	14.3
Guatemala G	Washed	light	385	1.7	2168	06:49	195.7	13.3
Guatemala G	Washed	medium	397	1.5	2118	07:14	201.2	15.3
Guatemala G	Washed	dark	416	1.4	2090	07:50	206.9	16.4
El Salvador N	Natural	light	408	1.6	2153	07:01	198.1	13.9
El Salvador N	Natural	medium	416	1.5	2116	07:11	203.3	15.4
El Salvador N	Natural	dark	439	1.5	2093	07:36	207.2	16.3
Dominican Republic	Natural	light	429	1.6	2177	07:08	196.9	12.9
Dominican Republic	Natural	medium	423	1.6	2150	07:07	197.3	14.0
Dominican Republic	Natural	dark	380	1.4	2115	07:32	207.1	15.4
Brazil SW	Pulped natural	light	442	1.5	2156	07:25	194.9	13.8
Brazil SW	Pulped natural	medium	442	1.5	2125	07:25	200.0	15.0
Brazil SW	Pulped natural	dark	416	1.5	2112	07:39	204.1	15.5
Guatemala B/C/C	Washed	light	469	1.7	2171	07:07	194.9	13.2
Guatemala B/C/C	Washed	medium	457	1.6	2136	07:40	199.0	14.6
Guatemala B/C/C	Washed	dark	429	1.4	2102	08:01	205.3	15.9
El Salvador W	Washed	light	436	1.7	2168	06:44	194.7	13.3
El Salvador W	Washed	medium	442	1.5	2127	07:17	200.3	14.9
El Salvador W	Washed	dark	403	1.5	2105	07:39	204.3	15.8

Moreover, 15g of each coffee were taken from the drum of the roasting machine by sampler probe at minute 3:00 and at minute 5:00 during the light roast of every green coffee. Samples were immediately tested for weight, moisture, and density with Sinar BeanPro 6070 (Table 4), stored in triple layers (PET+PETmet+PE) barrier bags with unidirectional valve, then welded.

5.3.3. Italian espresso extraction

A traditional Italian espresso coffee was prepared with professional espresso machine (Sanremo Café Racer) using softened water from a Brita Purity C150 (30% bypass) to obtain acceptable total and carbonate hardness according to SCA water control chart.

Coffee was ground on Marlkonig E65s, set to obtain the proper percolation (30 ml in 25''). Extraction was made at 91°C, with 6'' at 1 bar of prewetting, to get a final brew ratio of 1:2 (g/g). Coffee was professionally ground and extracted by AST (Authorized SCA Trainer) [30] for Barista & Brewing module.

5.3.4. Sensory analysis

Within 2 weeks from the roasting date, coffee samples were submitted to sensory analysis. The 24 samples were split into 3 groups of 8 samples each, homogeneous for roasting level. Tasting was performed from lighter to darker roasted group to prevent draft effect given by bitter compounds.

A Panel of 6 coffee tasters (3 of them certified Q-Arabica Graders, 2 expert panelists from I.i.a.c. (Istituto Italiano Assaggiatori Caffè) and a food technologist with a second level master in sensory analysis) was asked to fill in M34 Trialcard Plus form (Supplementary material 2) by "Centro Studi Assaggiatori – Italian tasters". The validation and replicability power of the panel were evaluated via analytical replicate. Panel calibration was made by checking the results obtained from the evaluation of a reference 100% arabica coffee not included in samples list and considering the median as panel central value. Data was recorded with ADS System by Horizon Design and Centro Studi Assaggiatori Brescia.

5.3.5. Acrylamide and caffeine analysis

For caffeine determination, 2.25 g of coffee were extracted with 50 ml of distilled water for 30 minutes, in a thermostatic bath. Extract was cooled at room temperature, paper filtered and consequently micro-filtered using syringe filters (0.45 μm), diluted 100 times and transferred in vial for the chromatographic determination. Separation was performed using a reverse phase C-8 Select B column and isocratic elution with CH_3CN: acidified water (2% acetic acid) = 10:90. UV detector was set at 275 nm [31]. Quantification was made with external calibration.

Acrylamide was tested at 5 points of the roasting process. All the samples have been ground and were extracted according to Bertuzzi et al., 2017. A Quechers separation, clean-up of the extract on Al_2O_3 and HLB 60 cc (60 mg) column (Oasis Waters) was performed. Separation and quantification by LC-MS/MS were performed using a X-Select HSS T3 2.5 μm column, gradient elution with acidified water and acetonitrile. Ionization was performed by ESI and the cation (72 m/z) was fragmented by Argon collision then detected and quantified as ion fragments (55 and 44 m/z). Quantification was made with internal standard d3-acrylamide from Sigma-Aldrich (St. Louis, MO, USA).

5.3.6. pH, acidity, total phenolics, melanoidin analysis of roasted SCs

For pH and acidity analysis, extract described for caffeine, after filtration, was split in 2 aliquots of 15 ml each and then directly tested. Automatic combined system formed by a pH-meter and an automatic titrator (CRISON MICRO TT 2050, Carpi, Modena, Italy) was calibrated with standard solutions, then used for samples analysis. Potentiometric titration was performed with NaOH 0.1N. Samples were analyzed in duplicate, and results were expressed as percentage of equivalent chlorogenic acid on roasted ground coffee mass.

For melanoidins quantification, 10 g of ground coffee was suspended with 100 ml of distilled water, covered with a watch glass to prevent solvent loss during extraction (30 minutes, in a thermostatic bath at 100°C). Samples were cooled at room temperature then vacuum micro-filtered using 1.2 μm paper membrane filters on a glass filter. 0.5 ml of the extract were diluted in 10

ml of distilled water and put into poly-carbonate cuvette for UV-Visible spectrometry (1 cm optical path). Absorbance at λ=420 nm was read in duplicate on a Shimadzu UV-1601 spectrophotometer (Shimadzu Europe, Duisburg, Germany) [33]. Before tests, the instrument was double zeroed using distilled water as blank. Melanoidins were expressed as mmol/g coffee dry base (d.b.) using molar extinction coefficient of 0.97±0.07 L mmol^{-1} cm^{-1} at 420 nm as reported by Martins & van Boekel, (2003).

Total phenolics were analyzed according to Singleton & Rossi, (1965). 100 µL of the same filtrate used for melanoidin was added of 0.5 ml of Folin-Ciocalteau reactive, 5 ml of a water solution at 20% of Na_2CO_3, then made up to volume in a 50 ml graduated flask, mixed and stored in a dark place at room temperature for 30 min. Blank was prepared in the same way. After storing, samples were put into poly-carbonate cuvette for UV-Visible spectrometry (1 cm optical path). Absorbance at λ=700 nm was read in duplicate on a Shimadzu UV-1601 spectrophotometer (Shimadzu Europe, Duisburg, Germany). Before tests, the instrument was double zeroed using the blank. Concentration of total phenolics was calculated with external calibration and reported as percentage of grams of chlorogenic acid equivalents on roasted coffee (w/w).

5.3.7. Statistical Analysis

All the data were subjected to Microsoft Excel 2003 and multivariate analysis of variance (ANOVA). Correlations between process, chemical and sensory parameters were performed by Pearson's test. Factorial analysis with PCA elaboration was applied to highlight relationships within variables and between variables and samples, which were clustered as for origin, process and roasting level. Statistical elaboration was carried out by IBM SPSS Statistics 27 (IBM Corporation, New York, USA).

5.4. RESULTS AND DISCUSSION

Regarding the roasting process, all the 8 samples followed the expected trends in terms of decreasing of density, moisture and weight when passing from a

light, to a medium, to a dark roasting and compared with green beans. This confirms the loss of water and organic matter, together with the increase in volume.

5.4.1. Sensory profile of SC espresso and its correlations with parameters from roasted SC

Spider-graphs reported in Figure 1 outlined the overall vision on how Specialty Coffee is perceived by consumers in the south European countries [36] where there is rooted belief about coffee consumption and on the descriptors that must be founded in espresso coffee [37]. Going through different Origins, samples of SCs here studied were really appreciated for the presence of remarkable aromas and the extremely low level of the defective ones [38]. This confirmed both the overall high quality of the raw beans and the suitable roasting applied. The sensory profile provided a comparison among the perceptions scored for the three roasting modalities which matched with data from technical sheets and literature [39].

The Kenyan coffee was the only one representing washed coffee from Africa in the set, it was expected to be differently characterized than other samples. Roasting modulation enhanced the peculiar characteristics of that coffee, scored 86 by the producer (Table 2). The region is well known for the high quality of its coffees, finesse of their cup and the impressive acidity, complex and bright, fruity and berry notes, all harmonized by good sweetness. Aftertaste and aroma were characterised by orange, peach, banana and floral notes, juicy body, tea and cocoa fragrance and can sugar-like sweetness. As well shown by the spider graph (Figure 1), acidity was better perceived in medium roasted cup because in the lighter it was a little masked by an astringent mouthfeel given by chlorogenic acids. The intense floral and fruity notes have always been recognized as well as the strong and pleasant olfactive and retro-olfactive components.

The two Brazilian samples were from different producing regions with altitude and climate (Table 1) providing the best condition of harvesting for different varieties. The beans peculiar characteristics properly exalted the low

and elegant acidity, heavy round body, chocolaty sweetness and nutty flavours as outlined from spider graph in Figure 1. Natural coffees from Minas Gerais, scored 86 points at Q-Grading (Table 2) and were characterized by high and balanced intensity of all the descriptors, with a stress on the excellent velvety body; aroma was described as medium to intense with remarkable chocolate and roasted nuts notes, aftertaste and flavours are of milk chocolate. Panellists were more aligned with these traits for the medium and dark roasted coffees than for light roasted sample which was also discriminated for the pleasant and intense acidity and lower body than the other two (Figure 1). The second Brazilian sample, from one of the Nordics producing region called Chiapada Diamantina, was described as well balanced but with weaker intensity and complexity if compared with the previous one (Table 2). Panellists properly discriminated the differences in quality and intensity between the two coffees, showing a decrease in floral and fruity notes as the roasting degree increased, even the second sample was more attractive in all the roasting version. This last point was powerful to demonstrate how the traditional consume of less complex coffees, usually blended with different Robusta percentages, remarkably influences the acceptance of espressos characterized by unusual taste and aromas [36] .

Guatemala is known to produce a wide assortment of different coffee, from light, sweet and floral, to heavy, fruity chocolatey full cups [39]. The two samples from Guatemala were both wet processed but from harvesting regions (Table 1) far one to the other and used to plant specific distinct varieties of Arabica coffee. Guatemala coffee from San Marcos is a Geisha, washed processed, scored 87 (Table 2) at Q-Grading, reported as excellent for body, balance, taste and aroma, and a little less impressive for aftertaste lasting and complexity. The main descriptors for these characteristics are jasmine, apricot, tangerine, and aromatic herbs. Panellists evaluated this coffee as described by producer, but the different roasts discriminated cups for the intensity of roast taste, intensity of floral and fruity notes, body and acidity (Figure 1). Medium roasted coffee gave the more complex cup, thanks to a proper development of all the aromatic and volatile compounds in the beans that, conversely, in lighter

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and in darker roasting level were, on the one hand, at the precursor stage or, on the other, degraded. Opposite to what provided by the producer, the intensity for the retro-olfactive perception ranged medium to high through the roasting levels, with a maximum for the medium roasted. The second sample was from volcano Amatitlan area. This washed micro lot was different thanks to the special mix of three varieties harvested, cropped and processed all together from the origin. At Q-Grading, this coffee has been scored 86.25 (Table 2), characterized by notes, both for aroma and taste, of citrus, yellow fruits, cherry, caramel, and milk chocolate. From our results (Figure 1), this sample seemed not to meet expectation: panel founded low intense notes of the descriptors mentioned and didn't score as high as supposed also for the balance. As already said for the Brazil Semi-washed sample, the less complexity was considered more attractive in all the roasting version, if compared with the Geisha variety.

The peculiarity of the two samples from El Salvador is that they came from the same region and were harvested and processed by the same producer in two different farms following once washed and once natural process (Table 1). These two micro lots were composed respectively by two varieties but the most abundant is Pacas. Those were one of the best examples of what central America coffee is [39]: as "milds", the generic name for these coffees says, they are elegant, sweet, clean, and balanced with complex malic, lactic, and citric acidity that also impacts on body and tactile sensation. The first sample considered is the natural one, it was scored 88.25 by the Q-graders (Table 2) and the cup should be characterized by aromas of pineapple, apricot, cherry, lemon, and dark chocolate, all founded also in taste and aftertaste accompanied by nut notes. As provided by panel (Figure 1), the roasting level that, in quantity and quality, matched better the expected profile of coffees was the medium one. In this case light roasted coffee seemed to be considered as a little underdeveloped or backed due to lack of good acidity, balance and retro olfactive good notes. The discrimination between roasting levels has been properly done by the intensity of roasted notes. The second sample i.e., the washed one, was characterized by more citrus and light fruits notes, sugary sweetness, and some nutty and dry fruit hint.
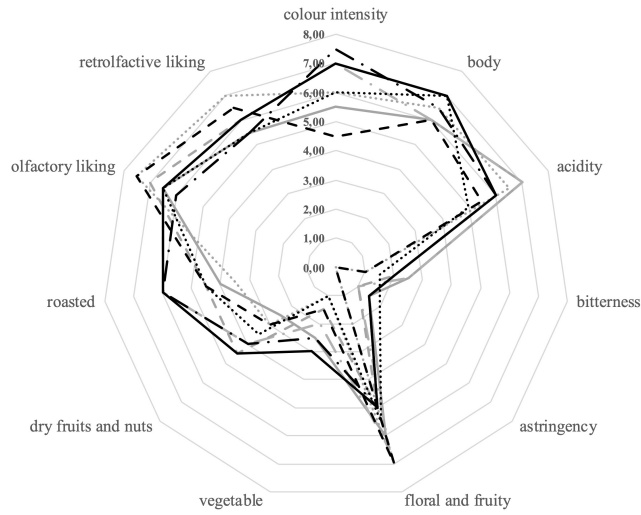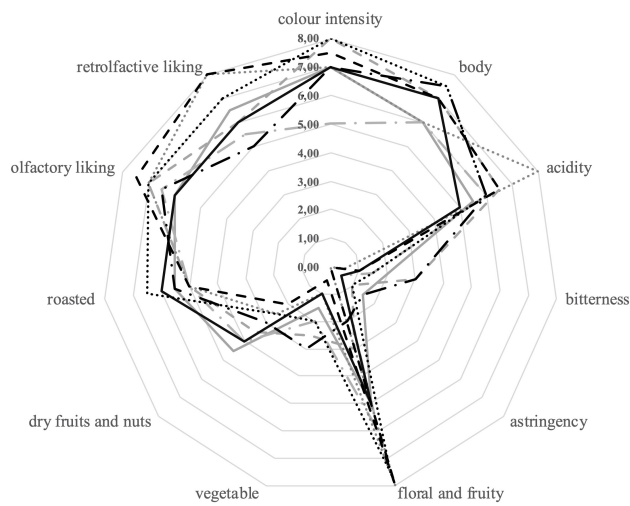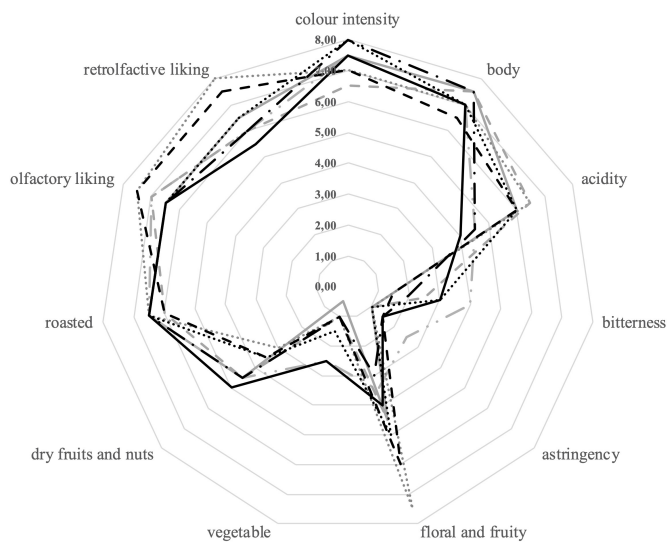
Figure 1. Spider graphs from sensory analysis of espresso brewed SCs clustered by roasting level.

This lot scored 86 points (Table 2) at cupping, the higher marks were given to taste and acidity as expected for washed coffee yet. Body and aftertaste should not be impressive but however good and pleasant. As the radar graph shows (Figure 1), panellists agreed with all the notes provided for this coffee when light and medium roasts were analysed. Body increased its intensity only in the dark roasted cup when also all the other descriptors connected to darker coffees appear; on behalf all the positive floral, fruity, and nutty notes were masked by the roasted taste and aroma. As provided for Brazilian and Guatemalan coffee this loss of complexity exalted the average appreciation of the sample.

Lastly, considering Dominican sample, from many descriptions available about coffees from this origin and especially from Barahona island, this micro-lot was expected to have typical attributes characterizing coffee from islands [39]. The lot was scored 85 points at Q-Grading (Table 2), mild and clean cup, full of different aromas, with good balanced acidity. From the producer it was described as intense in positive odours of yellow and red fruits, caramel, juicy clean and delicate at taste with apricots, caramel, and cane sugar aftertaste. By the panellists (Figure 1), there was generally a good discrimination of the three roasting levels by increased perception of roasted taste, bitterness, spicy notes, body, odours intensity and darkness of "crema" colour from light to dark roast; at the counter part a decrease of astringency, acidity and vegetable notes is observed. Light and medium roasted references exalted the most expected characteristics   provided   for   this   micro-lot.

Table 5. Chemical characterization of roasted beans. Waller-Duncan test (α=0,05) was conducted within roasting level to discriminate samples (lower case) and among averages to discriminate roasting levels (upper case).

ANALYSIS	ROASTING / SAMPLE	light	medium	dark
Caffeine (% w/w d.b.)	Kenia AB	1.14±0.02 bc	1.02±0.02 c	1.25±0.03 e
	Brazil FB	1.12±0.02 b	1.21±0.02 e	1.12±0.02 c
	Guatemala G	1.00±0.02 a	0.92±0.02 a	0.81±0.02 a
	El Salvador N	1.49±0.03 f	1.10±0.02 d	1.22±0.02 de
	Dom. Republic	1.39±0.03 e	1.26±0.03 e	1.16±0.02 cd
	Brazil SW	1.16±0.02 bc	1.00±0.02 bc	1.26±0.03 e
	Guatemala B/C/C	1.22±0.02 cd	0.94±0.02 ab	1.59±0.03 f
	El Salvador W	1.25±0.02 d	1.13±0.02 d	1.03±0.02 b
	AVERAGE	1.22±0.16 A	1.20±0.32 A	1.18±0.21 A
pH	Kenia AB	5.14±0.05 a	5.28±0.26 a	5.30±0.24 a
	Brazil FB	5.36±0.05 ab	5.57±0.28 a	5.90±0.11 b
	Guatemala G	5.52±0.22 bc	5.65±0.04 a	5.75±0.04 b
	El Salvador N	5.71±0.09 c	5.70±0.03 a	5.84±0.11 b
	Dom. Republic	5.51±0.01 bc	5.51±0.01 a	5.76±0.06 b
	Brazil SW	5.54±0.08 bc	5.48±0.23 a	5.88±0.07 b
	Guatemala B/C/C	5.44±0.06 abc	5.69±0.21 a	5.66±0.07 b
	El Salvador W	5.52±0.24 bc	5.65±0.16 a	5.82±0.08 b
	AVERAGE	5.47±0.18 A	5.57±0.19 A	5.76±0.21 B
Acidity (g chlorogenic eq/kg coffee d.b.)	Kenia AB	53.9±0.7 d	47.2±3.5 b	46.9±3.4 c
	Brazil FB	40.9±0.4 bc	34.6±3.4 a	28.7±0.5 a
	Guatemala G	42.6±0.9 bc	36.7±0.5 a	33.2±0.6 ab
	El Salvador N	39.6±0.7 b	37.1±0.8 a	32.8±2.5 ab
	Dom. Republic	31.1±1.9 a	39.5±0.9 ab	38.3±0.4 b
	Brazil SW	40.6±1.0 bc	36.4±4.2 a	30.5±0.6 a
	Guatemala B/C/C	42.8±0.5 bc	35.7±1.9 a	37.3±1.0 b
	El Salvador W	44.4±1.7 c	42.2±1.3 ab	36.5±0.7 b
	AVERAGE	42.0±6.17 B	38.7±4.8 AB	33.6±10.5 A
Acrylamide (ppb)	Kenia AB	318.4±22.3 ab	108.3±7.6 a	69.2±4.8 a
	Brazil FB	294.0±20.6 ab	113.4±7.9 ab	119.5±8.4 cd
	Guatemala G	372.1±26.1 b	100.3±7.0 a	101.6±7.1 bc
	El Salvador N	263.9±18.5 a	142.8±10.0 bc	113.5±7.9 bc
	Dom. Republic	297.8±20.8 ab	130.7±9.2 abc	113.8±7.9 bc
	Brazil SW	294.5±20.6 ab	113.4±7.9 ab	89.3±6.3 ab
	Guatemala B/C/C	253.1±17.7 a	146.2±10.2 c	146.0±10.2 d
	El Salvador W	324.5±22.7 ab	154.0±10.8 c	110.2±7.7 bc
	AVERAGE	301.9±37.2 B	126.1±19.9 A	107.9±22.5 A
Melanoidins (mmol/g coffee d.b.)	Kenia AB	0.12±0.01 e	0.13±0.01 d	0.14±0.01 d
	Brazil FB	0.13±0.01 c	0.12±0.01 bc	0.14±0.01 c
	Guatemala G	0.11±0.01 b	0.13±0.01 c	0.13±0.01 a
	El Salvador N	0.12±0.01 d	0.13±0.01 e	0.14±0.01 b
	Dom. Republic	0.11±0.01 a	0.11±0.01 a	0.13±0.01 a
	Brazil SW	0.12±0.01 d	0.13±0.01 e	0.14±0.01 c
	Guatemala B/C/C	0.11±0.01 a	0.12±0.01 b	0.14±0.01 e
	El Salvador W	0.12±0.01 c	0.14±0.01 f	0.14±0.01 c
	AVERAGE	0.12±0.01 A	0.13±0.003 B	0.14±0.01 C
Polyphenols (% w/w d.b.)	Kenia AB	4.99±0.01 c	5.55±0.04 f	1.54±0.03 a
	Brazil FB	5.17±0.03 d	5.41±0.02 e	4.63±0.01 e
	Guatemala G	6.22±0.04 e	4.95±0.01 d	5.40±0.01 g
	El Salvador N	6.21±0.01 e	2.55±0.01 a	2.48±0.01 b
	Dom. Republic	4.40±0.16 b	4.18±0.01 b	4.67±0.02 f
	Brazil SW	5.05±0.01 cd	5.59±0.01 f	5.49±0.01 h
	Guatemala B/C/C	3.16±0.01 a	4.86±0.01 c	4.53±0.01 d
	El Salvador W	5.02±0.01 c	5.79±0.01 g	4.41±0.01 c
	AVERAGE	5.03±0.95 B	4.86±1.03 AB	4.15±1.35 A

The bell pattern of acrylamide concentration during roasting (Figure 2) was obtained by data from quantitative analysis on samples of the 8 coffee lots taken from roaster at 5 different moments of the process (Table 3). After 5-minute of roasting in a range of temperature between 164.3°C and 173.5°C, six out of the eight coffees showed acrylamide concentration above 400 µg kg^{-1} (i.e., the limit stated by (EU, 2017)). Then, acrylamide rapidly decreases due to the prevalence of breakdown pathways, as already evidenced by different authors [41,42]. Despite SC is usually light roasted, such a behavior did not origin safety issues for the final consumers, in fact by data (Table 5) relative to acrylamide concentration for the 3 roasting levels it is higher in the light, lower in medium and dark one but always under 400 µg kg^{-1}. Additionally, a reduction of the human intake at the consumption stage is guaranteed from every extraction method [43]

Figure 2. Acrylamide trend during roasting.
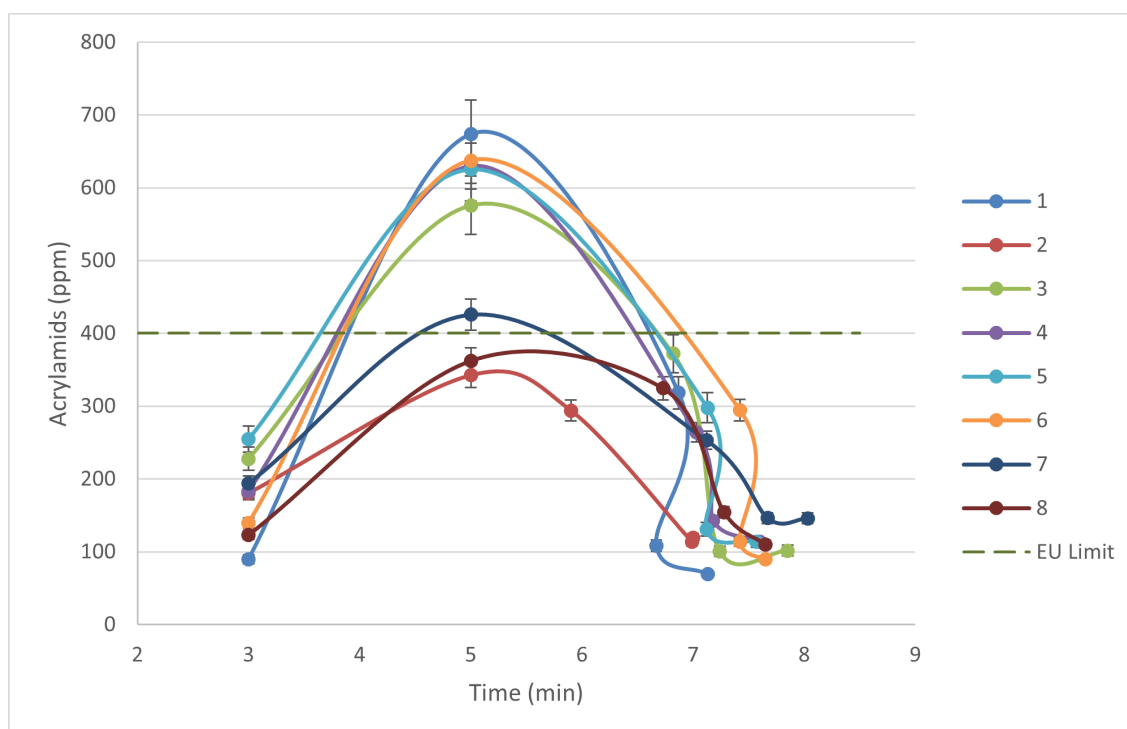
As expected, no significant changings in concentration of caffeine were recorded (Table 5). This might be justified by the loss of water and organic

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matter and the sublimation of a fraction of caffeine during roasting [7]; reversely, concentration defers among different coffee lots due to variety and origin variability [44].

5.4.3. pH, acidity, total phenolics, and melanoidins of roasted SCs

Regarding pH, all the values were around 5.58±0.22 (Table 5) aligned with the expected average pH value for brewed coffee that ranges from 5.2 to 5.8 [45]. Results from the different roasting levels (Table 5) demonstrated how the pH followed the same trend: lower in light and medium roasted than in dark coffees with no significant differences between washed and natural coffees. In the subset of natural coffees, the weaker difference in pH between the two milder roasting processes may reflect acidic fraction modification of these samples due to their distinctive fermentation pathway and possible occurrence of off-fermentations [46].

Further, titratable acidity could properly discriminate light roasted from darks. As already been provided by Ginz et al., (2000) this could be due to the progressive rising in the degradation rate of the organic acids during the early stage of roasting. Thanks to the different thermolability of these acids, pH could gradually increase from light to dark roasts (Table 5), when roasting is performed by applying the same profile. As a consequence, titratable acidity showed to be inversely related to pH (Table 5) confirming the progressive breakdown and evaporation of organic acids when the amount of thermal energy increased moving from light to dark roast.

The concentration in phenolic compounds of roasted coffees has been expressed (Table 5) as a percentage weight by weight of equivalent chlorogenic acid, responsible for the majority of the antioxidant power of a coffee extract [48]. In this respect, samples followed the trend suggested by Schouten et al., (2020) that is: coffees got the highest antioxidant power which is likely given by both the ratio between high and low molecular weight phenolics and the relative extent of degraded or newformed antioxidant molecules. Actually, data from these authors and results from this work (Table 5) confirm the average decrease in phenolic concentration going from light to darker roasting level.

As like as reported by Martins & van Boekel, (2003), melanoidins gradually increased in each sample when roasting led to darker color (Table 5) as higher temperatures and longer times promote final stages of Maillard and caramelization reactions [27]. Melanoidin concentration was capable to discriminate among the roasting levels with a same trend for washed and natural samples. In particular, a proportional increase in concentration was shown when darker roasts are achieved (Table 5).

5.4.4. Correlations between sensory profile of espresso SC and chemical data of roasted beans

From data obtained with Pearson test, some noticeable interdependences were available between chemical and sensory perceptions. Acrylamide, that was more abundant in light roasted coffees (Figure 1 and Table 5) showed a positive correlation ($p < 0.05$) with the perception of astringency which characterize the light roasted SCs for the larger preservation of chlorogenic compounds, and with floral/fruity notes also widely reported in milder thermal treatments [49]. Caffeine, as described by Poole & Tordoff, (2017), can serve as a bitter compound but also gives tactile perceptions: this was true for samples where a superior caffein content, occurred along with bigger body, higher bitterness, and astringency perceptions ($p < 0.05$).The low pH, as an estimator for the acidity of the brewed coffee [51], was correlated ($p < 0.05$) with the perceivable acidity, the floral/fruity notes, the hedonic level, the vegetable notes and with the lack in balance and palatability. This last consideration led to confirm the well-known lower acceptability of light roasted single Origin coffees by espresso coffee consumers due to the low pH [51]. Additionally, the perceived acidity, that is generally higher where astringency and roasted notes of dried fruits and nuts are low [3], show an inverse correlation ($p < 0.05$) with the olfactory acceptability of the samples.

Finally, also physical parameters (Table 1 and 3) were correlated with some sensory perceptions: in particular, the green moisture and the green density impacted on the cup profile modulating the visual, the olfactory and the tactile properties of the coffee brew. In detail, the green density was positively

correlated ($p<0.05$) with the vegetable and bitter notes and negatively ($p<0.05$) with positive retro-olfactory perceptions. In high density green coffee lots, thanks to the higher mass per unit of volume leading to a lower heat transfer rate, the bean development during roasting is limited, especially when the roasting degree is light leading to vegetable notes in cup; conversely, if dark roasts are performed, more bitter compounds are produced as a result of the abundance of vegetal structures undergoing to pyrolytic reactions [52].

5.5. CONCLUSION

In conclusion, the 8 lots of Arabica SC proved to be comparable in sensory attributes, both quantitative and qualitative, with the cup profile given after official in-Origin cupping session when prepared via Italian espresso extraction and tasted by a trained panel. Medium roasting level appeared to be the most suitable to exalt coffee sensory attributes under espresso preparation. Physicochemical analyses were capable to outline similarities and dissimilarities in the final products obtained from the 3 different roasting processes and, potentially, to obtain reliable parameters to assess the conformity of roasting final products with a given quality standard. Further studies may be carried out to identify possible correlations between physicochemical traits of green and roasted beans, and cup profile.

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5.7. ADDITIONAL INFORMATION

Supplementary material 1. Roasting profiles

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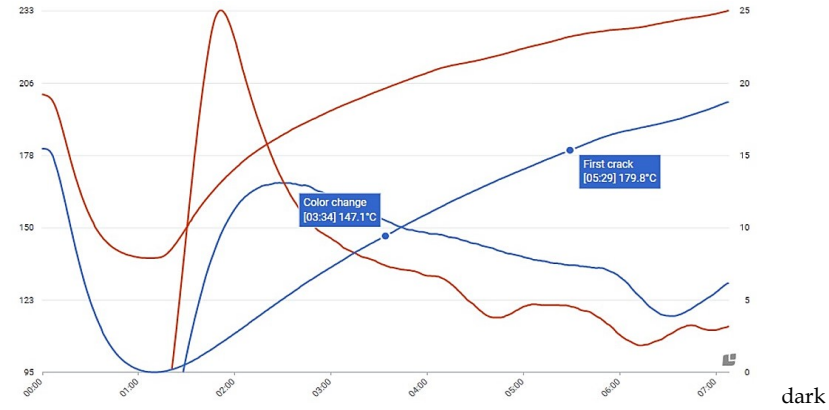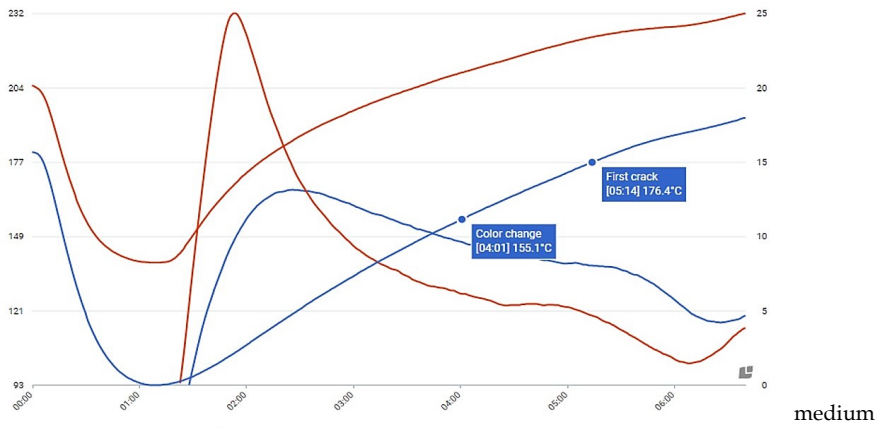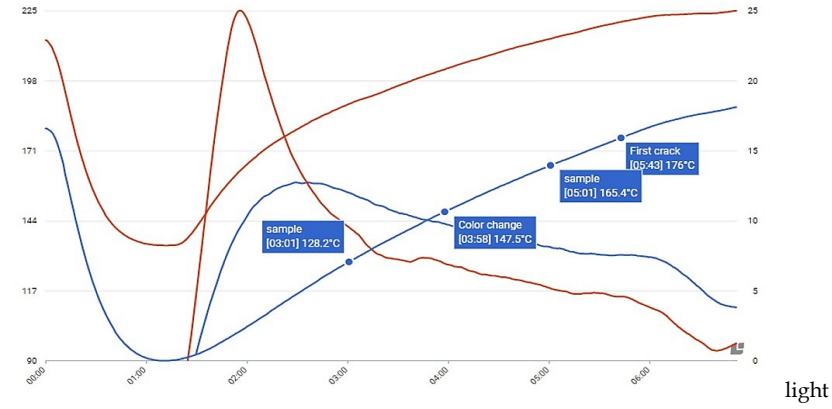
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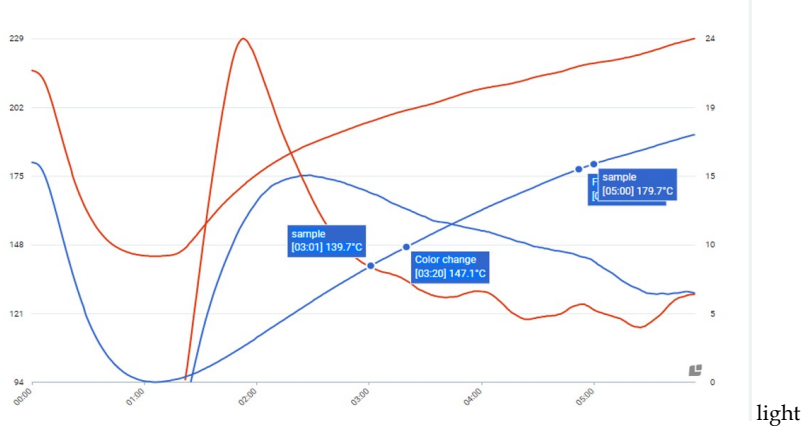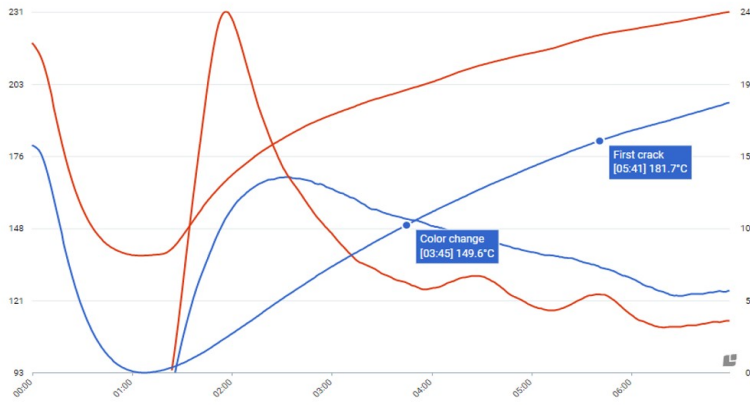
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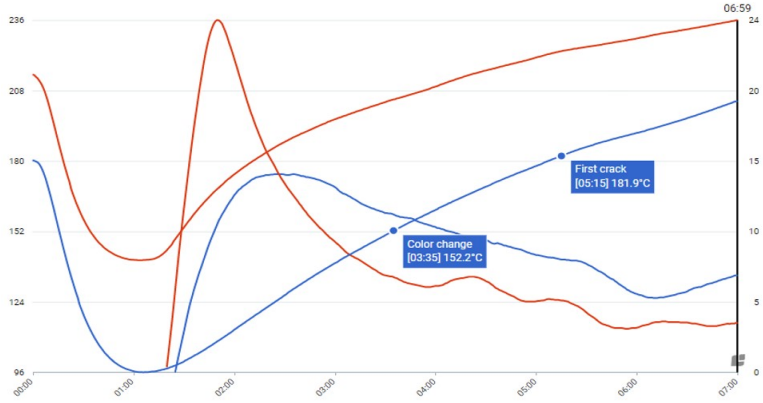
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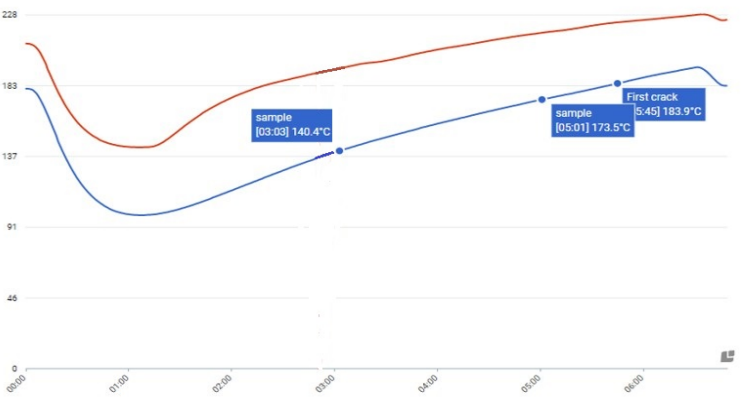
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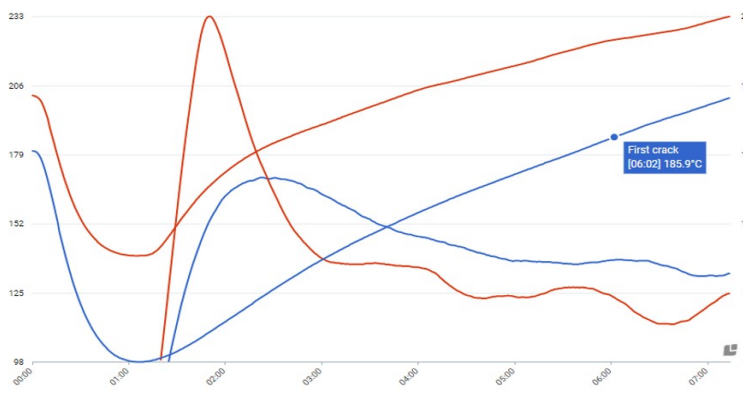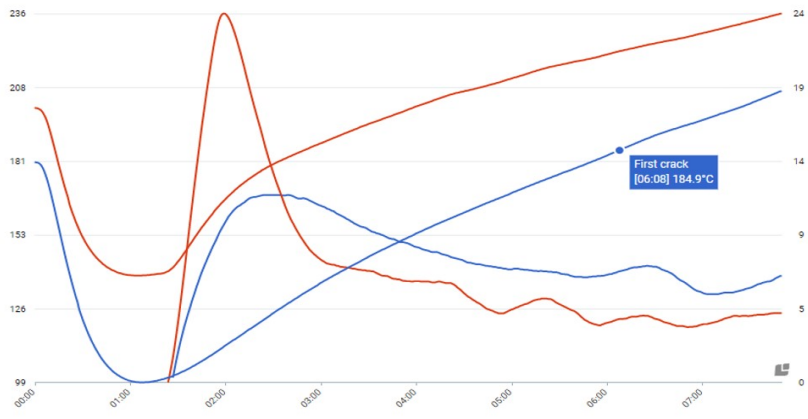
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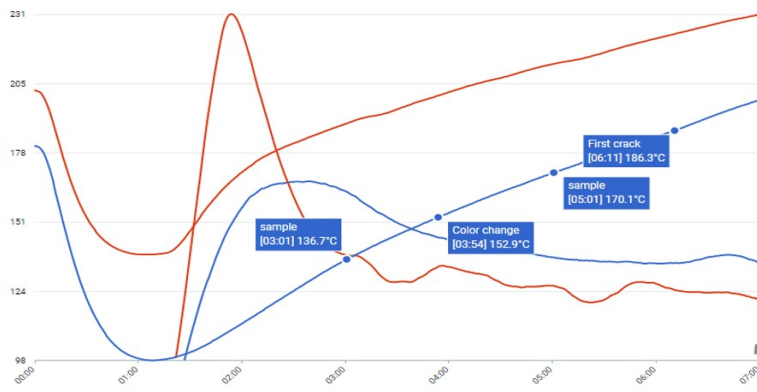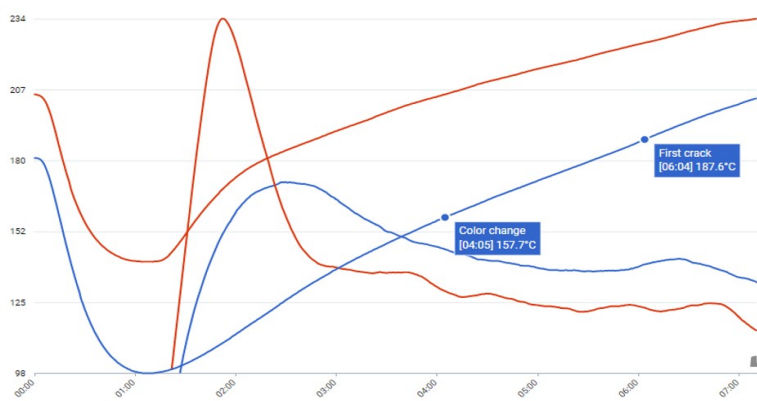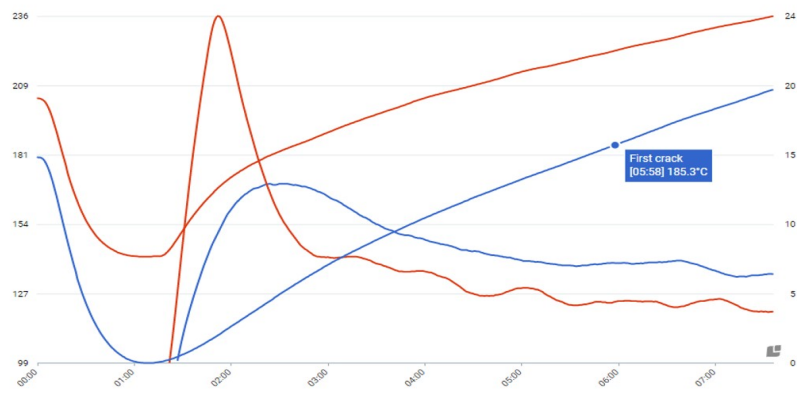
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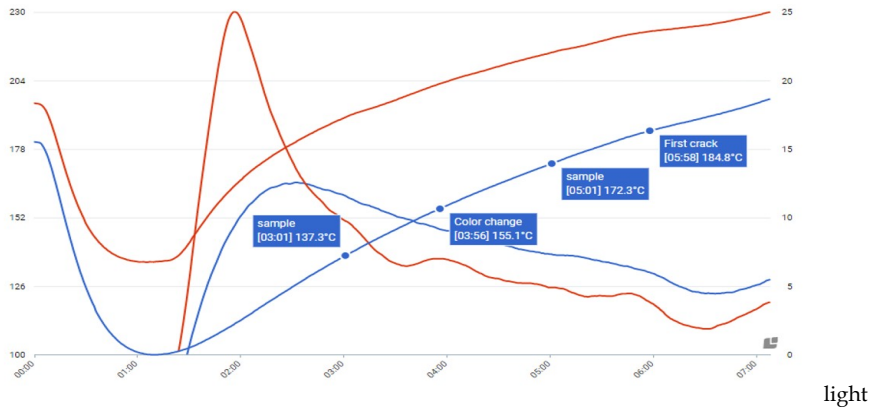
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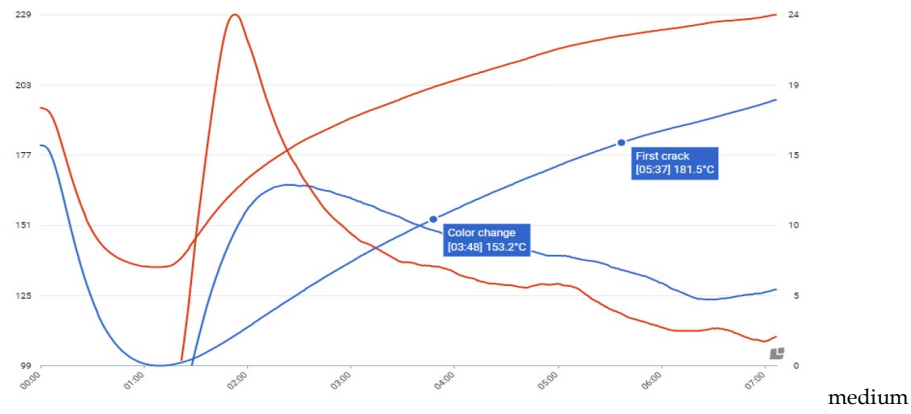
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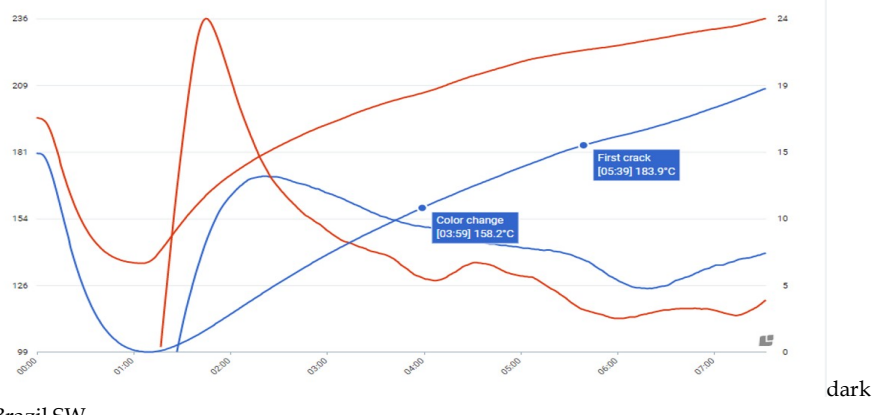
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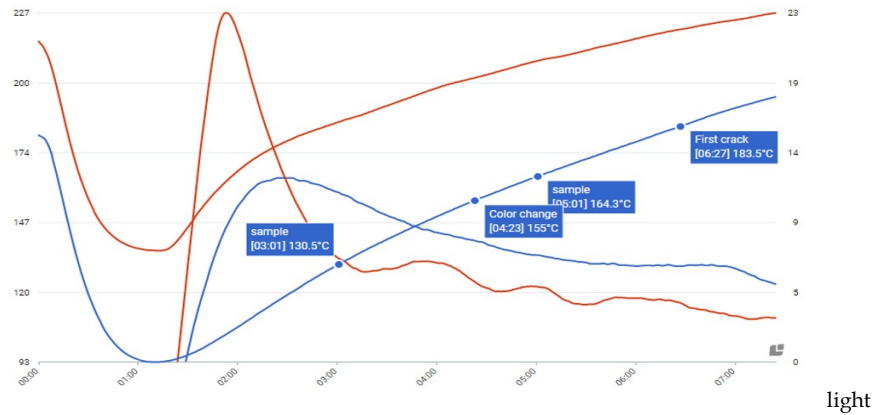
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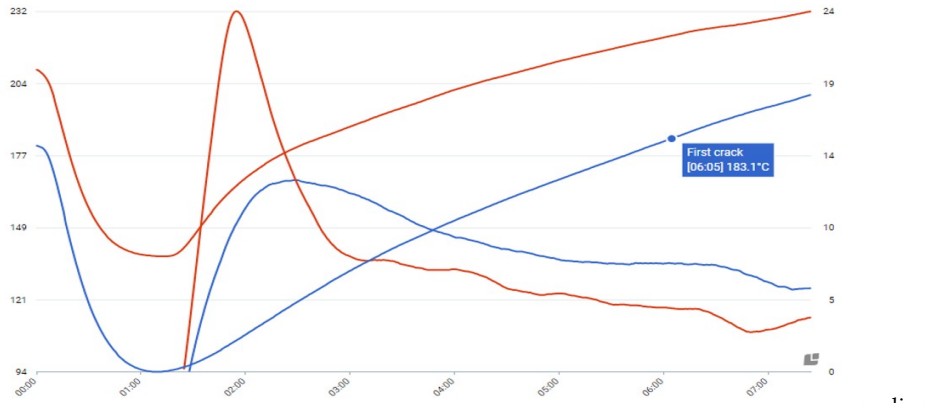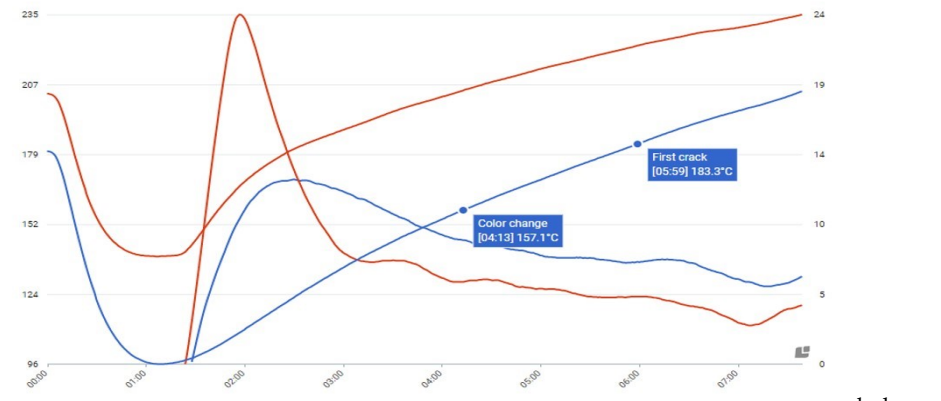
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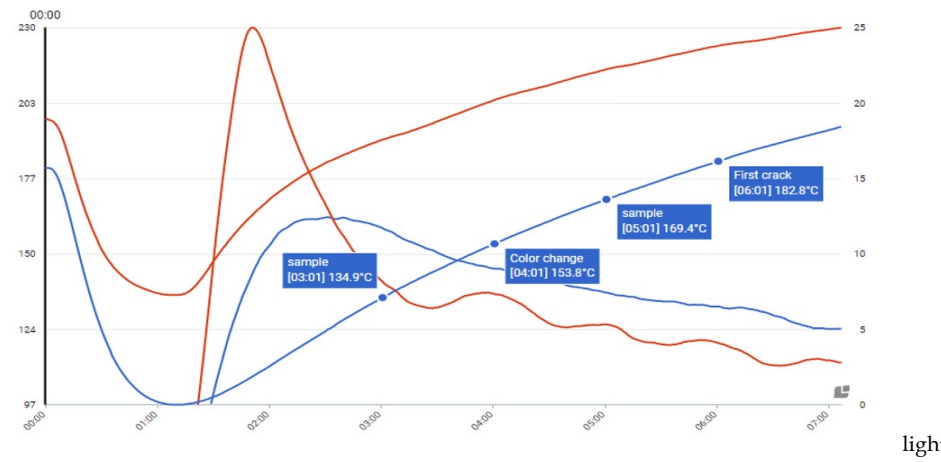
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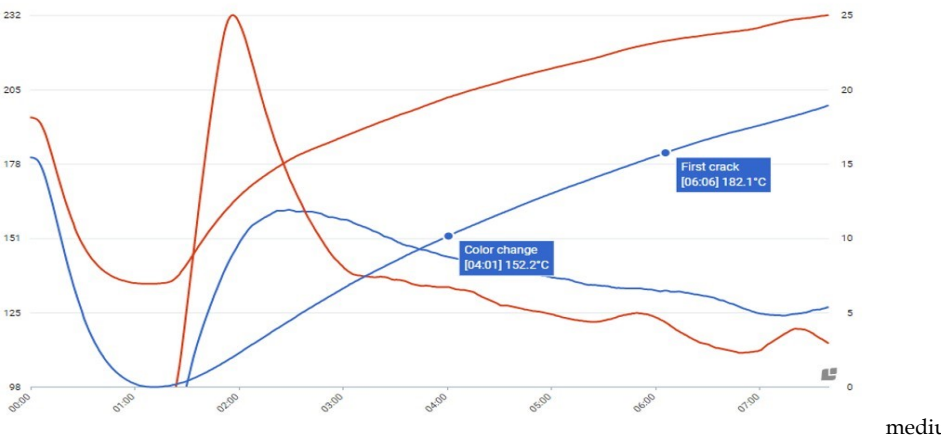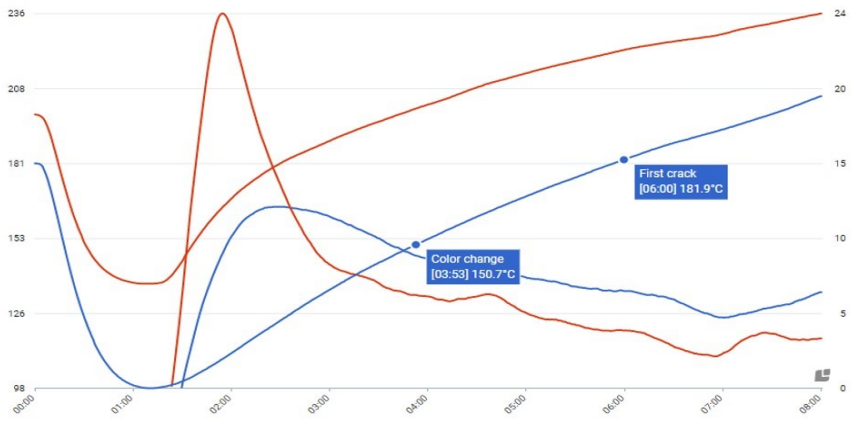
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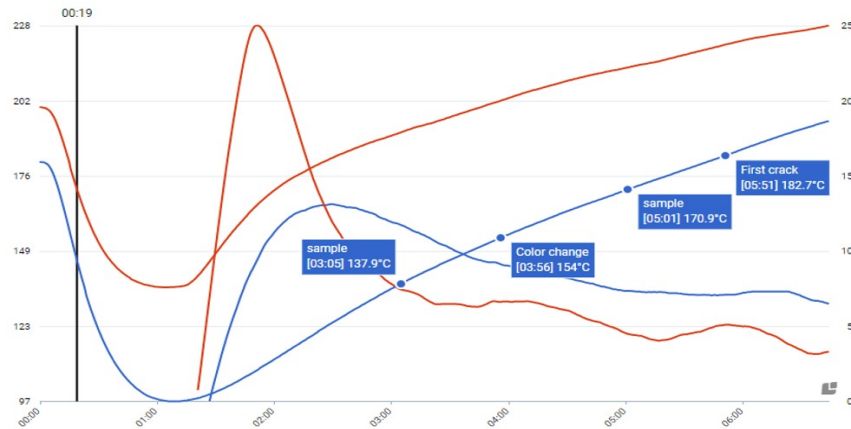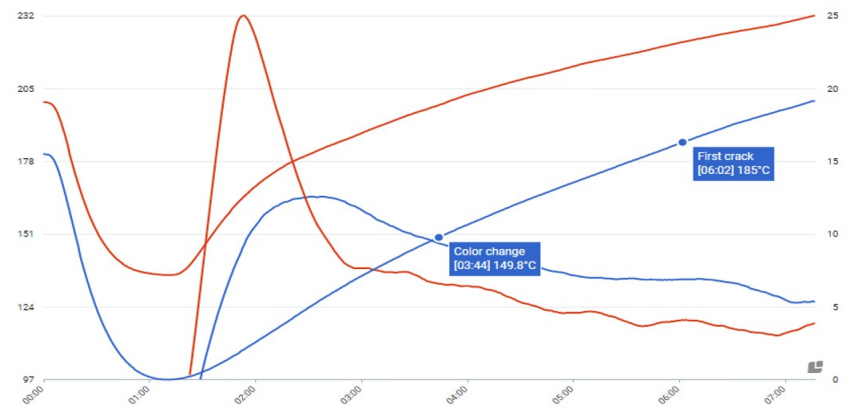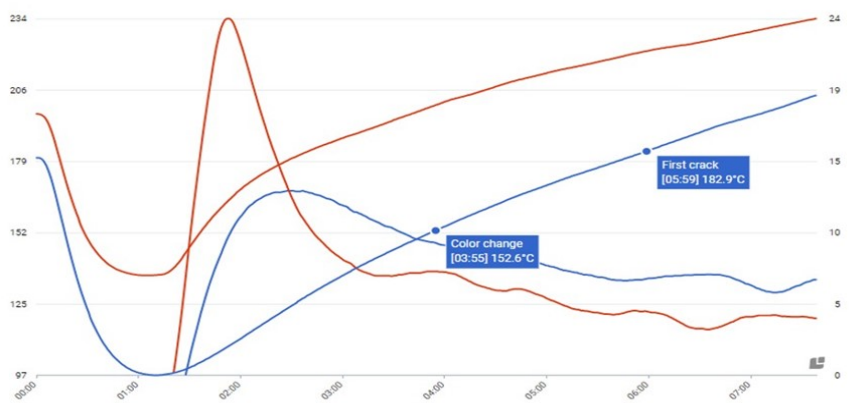
Supplementary material 2. TrialCard Plus Form

Prodotto	Data	Giudice		Tria

Codice giudice

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A B C D E F G H I L M N O P Q R S T U V Z

Codice serie   **Codice campione**

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0 1 2 3 4 5 6 7 8 9

Sezione descrittori codificati		0 1 2 3 4 5 6 7 8 9	*Sezione descri*
Percezione visiva	Intensità colore	O O O O O O O O O O	
	Tessitura	O O O O O O O O O O	
	■ ATTRAENZA	O O O O O O O O O O	
Percezione olfattiva	Intensità olfattiva	O O O O O O O O O O	
	■ PIACEVOLEZZA OLF.	O O O O O O O O O O	
Percezione tattile/gustativa	Corpo	O O O O O O O O O O	
	Acido	O O O O O O O O O O	
	Amaro	O O O O O O O O O O	
	Astringenza	O O O O O O O O O O	
	■ EQUILIBRIO	O O O O O O O O O O	
Percezione retrolfattiva	Fiori e frutta fresca	O O O O O O O O O O	
	Vegetale	O O O O O O O O O O	
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6. Metabolomics combined with sensory analysis reveals the impact of different extraction methods on coffee beverages from Coffea arabica and Coffea canephora var. robusta

6.1. ABSTRACT

An untargeted metabolomics approach combined with sensory analysis was used to depict the impact of different traditional Italian extraction methods (i.e., Espresso, Neapolitan, Moka) along with Filter, on Coffea arabica and Coffea canephora var. robusta beverages. To this aim, polyphenols, Maillard reaction products, and coffee metabolites were screened by high resolution mass spectrometry and elaborated through both unsupervised and supervised multivariate statistical approaches. Multivariate statistics showed a distinctive chemical profile for Espresso preparation, while Moka and Neapolitan were very similar. The orthogonal projection to latent structures discriminant analysis allowed identifying 86 compounds showing a high VIP (Variable importance in projection coefficient) discrimination score (i.e., > 0.8). The 2,5-dimethyl-3-(methyldithio)-furan was marker for the Filter preparation while 1,2-disinapoylgentiobiose characterized both Filter and Neapolitan extractions. Caffeine (known to be a bitter compound) was highly up accumulated in Filter vs. Espresso, although at sensory profile bitterness was more perceived in Espresso. Vegetal aroma carried by pyrazines, pyridines, and phenolic acids were markers of Espresso, with Robusta showing higher values than Arabica. Notwithstanding, our findings showed that the extraction process played a hierarchically higher role in driving the chemical composition of the beverages and when compared to coffee species.

6.2. INTRODUCTION

Two Coffea species, namely Coffea arabica and Coffea canephora var. robusta, are the most cultivated worldwide and dominate in terms of market volume [1]. These species are deeply different genetically (polyploid for the former, diploid for the latter) [2], require different pedoclimatic conditions [3], have different biochemical ripening processes, and undergo different post-harvesting

processes [4–7]. Therefore, chemical profiles and flavour precursors characterizing the green beans reflect the previously cited diversity [8,9]. Also, the transformations occurring during roasting and extraction steps can lead to exclusive metabolites, such as aromatic compounds and bioactive molecules, providing a cup profile to the beverage that testifies all those characters [10,11]. Together with the coffee powder used, the extraction method is recognized to strongly impact the sensory profile of coffee beverages [12]. Among others, the traditional Italian extraction methods, namely Moka, Neapolitan pot, and Espresso, are well-known to give consumers a unique and recognizable aromatic and gustative perception [13,14]. In this context, the market availability of automatic filter coffee domestic machines has contributed enhancing the consumption of less intense and longer coffee, even in a historically espresso-consumer population [15]. Reversely, an increasing interest in the rediscovery of home extraction systems stressed the need for accurate studies on technical aspects [16] to exalt and differentiate the final cup from Moka Neapolitan pots [17].

In the last years, several analytical strategies have been implemented for the quality and integrity of foods, including coffee and coffee beverages, such as isotope ratio mass spectrometry (IRMS), liquid chromatography coupled with mass spectrometry (LC-MS), gas chromatography coupled with mass spectrometry (GC-MS), near infrared spectroscopy (NIRS), and nuclear magnetic resonance spectroscopy (NMR) [18]. In this regard, liquid chromatography quadrupole time-of-flight mass spectrometry approach has allowed discriminating coffee brewed by different extraction methods [19]. Similarly, high-resolution mass spectrometry techniques have been efficiently applied for evaluating coffee quality and the potential correlations with the sensory attributes [13]. Interestingly, several studies on C. canephora have been carried out in the last years by using a metabolomics [20,21]. In this regard, this species is considered to have a lower cup quality compared to C. arabica. Accordingly, some authors were able to identify potential markers for the early selection of C. canephora plants with desirable cup quality traits [21]. Therefore, metabolomic approaches demonstrated a solid potential to investigate several

aspects related to coffee quality, including processing conditions (e.g., roasting, grinding, and brewing methods), authentication, traceability, the correlation with sensory quality, and the quality improvements of selected cultivars. However, to the best of our knowledge, there is still a lack in the scientific literature about the link between comprehensive metabolomic phytochemical profiles of coffee and sensory traits, related to different extraction methods. Therefore, this study aimed to explore the potential correlations existing between metabolomic profiles of C. arabica and C. canephora var. robusta beverages obtained through four traditional extraction methods (Moka, Neapolitan pot, Espresso, and Filter) and their sensory profile. This information is relevant to unravel the effect of extraction method and coffee species combinations, in terms of both sensory and chemical profiles. This piece of information can complement the more consolidated knowledge already available on other quality-related aspects such as planted cultivar and edaphic conditions, processing and roasting, as well as shelf life and packaging, in a "one-quality" perspective.

6.3. MATERIALS AND METHODS

6.3.1. Coffee samples

Two roasted coffee samples were supplied from a local industrial roaster (Musetti, Piacenza, Italy) with the same roasting process. The two commercial blends were constituted either by 100% C. Arabica natural processed from Brazil or 100% C. Canephora var. robusta natural processed from India. Coffee samples were ground with La Cimbali ELECTIVE (Gruppo Cimbali S.p.A., Binasco, Milan, Italy) grinder-doser to reach the proper granulometry for each extraction.

6.3.2. Extraction methods

Moka extraction was performed using the Bialetti "Moka Express" as provided by [22], applying an adjusted brew ratio of 76 g/L for both Arabica and Robusta samples, for both sensory and chemical analysis. Filter coffee was prepared using a commercial drip coffee maker Ariete Vintage, setting a "strong coffee"

modality, and using a brew ratio of 50 g/L. Neapolitan coffee was prepared using an aluminum traditional Neapolitan pot (Ilsa, Turin) following the procedure described by [17] using a brew ratio of 72 g/L.

Traditional Italian espresso coffee was prepared with the professional Espresso machine Cimbali M100 (Gruppo Cimbali S.p.A., Binasco, Milan, Italy) using water softened from a Brita Purity C150 (30% bypass) to obtain acceptable total and carbonate hardness, according to the SCA water control chart [23]. The extraction was made at 92 °C, with 6 sec of pre-infusion at a ratio between coffee powder and beverage of 1:2 (w/w). About 16 g of coffee were packed in a double shot coffee basket for both Arabica and Robusta samples.

6.3.3. Sensory analysis

The sensory evaluation was performed by a single panel of 6 trained panelists in two different sessions, the former for espresso and filter coffees, the latter for Moka and Neapolitan pot extractions, both carried out in laboratory "SensoryLab", compliant with UNI ISO 8589 standards, at Università Cattolica del Sacro Cuore (Piacenza – Italy). The validation and replicability were tested by the presence of a replicate sample per session. Medians of the scores given to each descriptor from a single panelist to the repeated samples should not differ of more than +/-1 point to consider the panelist repeatable. Panel calibration was made by delivering to panelists the median score reached by an extra sample tasted before starting each analysis. The attributes graded by the judges after description of their definition, aligned to those reported by [24], are reported in M34 Trialcard Plus form by "Centro Studi Assaggiatori – Italian tasters", used during each session. Panelists were asked to evaluate each attribute on a scale from "0" meaning "absence of attribute" to "9" meaning "net and very intense perception of the attribute" for qualitative descriptors and "0" meaning "unperceivable" to "9" meaning "extremely intense" for those quantitative. Data was collected with ADS System by Horizon Design and Centro Studi Assaggiatori Brescia, to be statistically elaborated.

6.3.4. Extraction of metabolites from coffee samples

For the metabolomics analysis, a total of 44 samples was analyzed, when considering Espresso (20 replicates), Neapolitan (8 replicates), Moka (8 replicates), and Filter (8 replicates) preparations. In this regard, 1 mL of each coffee beverage (as resulting from Moka, Neapolitan pot, Espresso, and Filter preparations) were extracted in 5 mL of 70% aqueous methanol (LC-MS grade, VWR, Milan, Italy) acidified with 0.1% formic acid. Regarding the starting ground coffee samples of C. arabica and C. canephora var. Robusta, four replicates (1 g) of each sample were extracted using an Ultra-Turrax homogenizer (IKA T25, Staufen., Germany) using the same extraction solution. The extracts were then centrifuged (Eppendorf 5810R, Hamburg, Germany) at $10000 \times g$ for 10 min at 4 °C and filtered using 0.22 μm cellulose syringe filters into amber vials.

6.3.5. Untargeted metabolomic profiling through UHPLC-QTOF mass spectrometry

The untargeted metabolomic profile of the different coffee extracts was investigated through an UHPLC-QTOF-mass spectrometry. To this aim, a 1290 liquid chromatograph was coupled with a G6550 mass spectrometer detector via a Dual Electrospray Jet Stream ionization system (from Agilent Technologies, Santa Clara, CA, USA) under previously optimized instrumental conditions [13]. The instrument worked in Full-SCAN mode, acquiring positive ions in the range of 100–1200 m/z. Samples were acquired in "extended dynamic range" mode with a nominal resolution of 40,000 FWHM. The injection volume was 6 μL, while the sequence injection was randomized. Also, Quality Control samples (QC) were injected in the UHPLC-QTOF and consisted of a pooled aliquot of each extract. In this regard, QCs were injected at the beginning of the sequence and every 10-sample injection and analyzed in data-dependent MS/MS mode using 10 precursors per cycle (1 Hz, 50–1200 m/z, positive polarity, active exclusion after 2 spectra), with typical collision energies of 10, 20, and 40 eV. The raw mass features were aligned and deconvoluted using the Agilent Profinder B.06 software. In this regard, the find-by-formula algorithm was used to annotate molecular features (MFs) following mass and

retention time alignment. The detailed information regarding the post-acquisition process is accurately described elsewhere [13]. Three databases were combined for the identification process, namely the FoodDB (https://foodb.ca/- using the list of compounds already reported in coffee), Phenol-Explorer 3.6 (http://phenol-explorer.eu/- to profile polyphenols), and a custom database on Maillard reaction products. Based on our process, each compound was identified according to a Level 2 of confidence (putative annotation based on high mass accuracy, exploiting the isotopic profile of each mass feature) as reported by COSMOS Metabolomics Standards Initiative [25]. Besides, the level of confidence in annotation was increased by using the spectral information reported in the QC. These latter were elaborated using the software MS-DIAL (version 4.70) for a further identification and/or confirmation step [26]and compared against the publicly available MS/MS experimental spectra available in the same software (e.g., Mass Bank of North America) and MS-Finder in-silico fragmentation from compounds in Lipid Maps, FoodDB, and PlantCyc [26].

6.3.6. Multivariate statistical analysis

6.3.6.1.   Metabolomic data

The raw data obtained following metabolomics were aligned and normalized using the Agilent Mass Profiler Professional B.12.06 software, according to the workflow reported in a previous work [13]. Then, two different multivariate statistical approaches were used to elaborate the raw data, namely an unsupervised hierarchical cluster analysis (both HCA - distance measure: Euclidean; clustering algorithm: Ward's, and PCA - principal component analysis) and a supervised orthogonal partial least squares discriminant data analysis (OPLS-DA). In particular, the supervised model was created considering as class discrimination the "extraction process". Besides, each OPLS-DA model was inspected for outliers, cross-validated (CV-ANOVA), and evaluated for potential overfitting (permutation testing with 200 random permutations). The model parameters (goodness of fit: R2Y and goodness of prediction: Q2Y) were also inspected to evaluate the overall goodness of the

prediction model. The variables importance in projection (VIP) was finally used to select those compounds having the highest discrimination potential (VIP score > 0.8) and potentially related to the sensorial profile [13,27].

6.3.6.2. Sensory data

All the data were collected with Microsoft Excel 2007 and elaborated by radar graphs. Discriminant power of the extraction method was assessed via one-way analysis of variance (ANOVA). Factorial analysis with principal component analysis (PCA) elaboration was applied to highlight relationships within variables and between variables and samples, which were clustered as for specie and extraction method. Statistical elaboration was carried out by IBM SPSS Statistics 27 (IBM Corporation, New York, USA).

6.4. RESULTS AND DISCUSSION

6.4.1. Untargeted profiling by UHPLC-QTOF-mass spectrometry

In this work, the untargeted metabolomics approach based on UHPLC-QTOF-mass spectrometry resulted in the putative identification of 228 compounds annotated according to a Level 2 of confidence [13,25]. Besides, the analysis of QC samples allowed to confirm the structural identity of 94 compounds, such as caffeoylcholine, caffeine, phloroglucinol, and trigonelline, among the others. A comprehensive list reported the relative abundance of each compound annotated, together with its composite MS and MS/MS spectra, can be found in the supplementary material (Table S1).

As the first step, we used a Volcano plot analysis combining ANOVA (p < 0.05) and Fold-Change analysis (FC cut-off > 1.2) to check the chemical differences between the raw ground coffee samples under investigation (i.e., C. arabica and C. canephora var. Robusta) before to run the different extraction processes. The output obtained is reported in Table S1. As can be observed, the volcano plot for the comparison C. arabica vs. C. canephora var. robusta showed 92 significant compounds (including the isomeric structures), with 72 compounds significantly up accumulated for the C. arabica ground coffee, thus revealing a broader and complex phytochemical profile when compared to C. canephora var. robusta.

Regarding specific and typical compounds, according to [28], caffeine was strongly up accumulated in C. canephora var. robusta (Fold Change value = 10.81; p-value = 0.044), followed by Na-p-Hydroxy-coumaroyl-tryptophan (Fold Change value = 3.10; p-value = 1.7×10^{-6}), (R)-2-Hydroxy-4,7-dimethoxy-2H-1,4-benzoxazin-3(4H)-one 2-glucoside (also known as HDMBOA-Glc) (Fold Change value = 2.01; p-value = 8.5×10^{-6}) and coffeasterene (Fold Change value = 1.95; p-value = 1.2×10^{-5}). Overall, the amino acid conjugates of hydroxycinnamic acids (e.g., Na-p-Hydroxy-coumaroyl-tryptophan) have been previously reported as potential marker compounds to discriminate among coffee cultivars [29]. In this regard, [30] showed that p-coumaroyl-N-tryptophan was a characteristic marker compound of the C. canephora species, thus confirming our findings. Also, the compound coffeasterene belongs to the class of organic compounds known as stigmastanes and derivatives. These are sterol lipids with a structure based on the stigmastane skeleton, which consists of a cholestane moiety bearing an ethyl group at the carbon atom C24; however, little information is available in the literature concerning its ability as related to cultivar discrimination. Finally, HDMBOA-Glc has been reported as a marker of biological interest when considering defense mechanisms of the plant [31]; therefore, our findings suggested a potential up-accumulation of this metabolite in C. canephora var. robusta as a response to terroir-related factors, such as pedoclimatic conditions, together with agronomic and post-harvest practices [32].

Regarding the significant marker compounds of C. arabica, those showing the highest variations were p-HPEA-AC (Fold Change value = 2.13; p-value = 3.8×10^{-6}), 5-Methylquinoxaline (Fold Change value = 1.88; p-value = 1.4×10^{-4}) and isomeric forms of cyclopentanedione (Fold Change value = 1.83; p-value = 5.1×10^{-5}). The compound p-HPEA-AC belongs to the class of organic compounds known as tyrosols and derivatives. These antioxidant compounds are minor phenolic compounds in the coffee plant, although their presence has been previously documented [13,33]. Besides, 5-Methylquinoxaline belongs to the chemical class of quinoxalines; these compounds contain a quinoxaline moiety, a bicyclic heterocycle made up of a benzene ring fused to a pyrazine ring.

According to literature, 5-Methylquinoxaline contributes to the coffee flavor development, being associated with sensorial descriptors, such as burnt, roasted, nutty, and roasted corn [34]. Finally, cyclopentanedione derivatives (such as 3,5-dimethyl-1,2-cyclopentanedione), also known as benzyl-related compounds, belong to the class of organic compounds known as cyclic ketones, usually described as sweet, maple, sugar, caramel, and coffee tasting compounds, and then considered potential biomarkers for the consumption of coffee and coffee products [35].

6.4.2. Multivariate statistical discrimination of the different extraction methods

In the next part of this work, untargeted metabolomics based on UHPLC-QTOF mass spectrometry was used to explore the major differences imposed on the chemical profile by the four different extraction methods under investigation, thus accounting for the variability imposed specifically by each processing method. As can be observed from the unsupervised hierarchical cluster analysis heat map (Figure 1), the Espresso preparation was characterized by the most distinctive chemical profile, being included in a separate cluster. Interestingly, Filter, Moka, and Neapolitan preparations were included in another cluster, with Moka and Neapolitan providing a more similar profile, being included in the same sub-cluster.

Figure 1. Unsupervised hierarchical cluster analysis (HCA) based on fold-change heat map (similarity: Euclidean; linkage rule: ward) for the different coffee samples included in the different extraction category (i.e., Espresso, Filter, Moka, and Neapolitan).
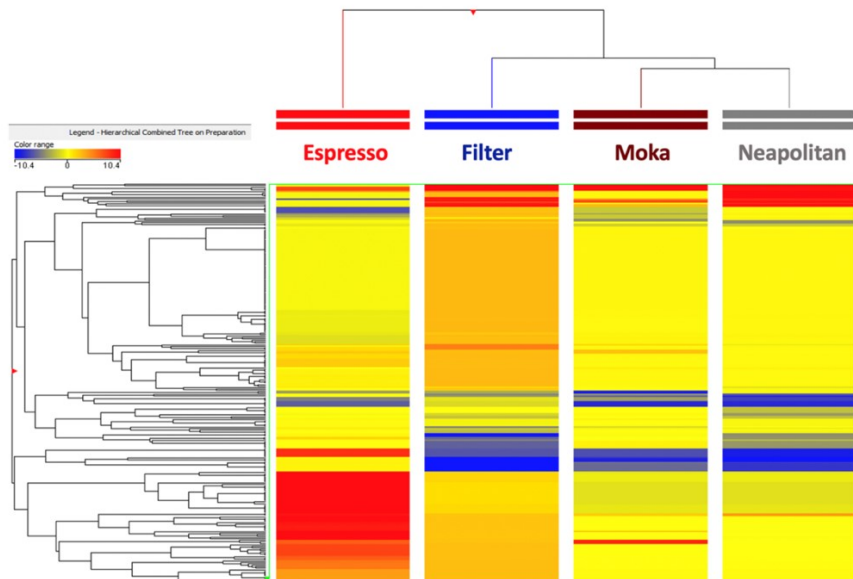
Besides, a PCA score plot was inspected to assess the dispersion of each sample according to the measured chemical profile. As clearly reported in Figure 2, the 2 main principal components (PC1 and PC2) were found to explain a total of 77.8% of the variability among each group, thus revealing a clear ability of the statistical model to dis-criminate the different extraction methods. Also, a high variability between the Espresso samples was observed, mainly driven by the different cultivars considered (i.e., Coffea arabica and Coffea canephora var. robusta).

Figure 2. Principal Component Analysis (PCA) score plot for the different coffee samples included in the different extraction category (i.e., Espresso, Filter, Moka, and Neapolitan).
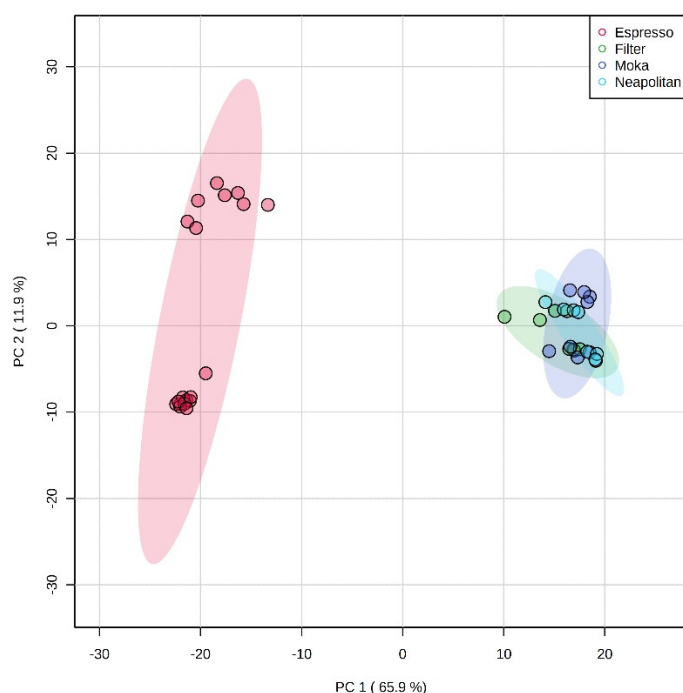
Thereafter, to better investigate the compounds or classes of compounds explaining most of the variability observed, a following supervised multivariate statistical approach, namely OPLS-DA, was used. The OPLS-DA score plot is reported in Figure 3. The goodness model parameters were highly significant, being correlation R^2Y (cum) = 0.772, R^2X = 0.762, and Q2Y prediction ability = 0.616. Also, the prediction model was cross-validated using a Cross Validation-ANOVA (p-value = 2.15 x 10^{-14}) and both strong outliers and overfitting could be excluded (Table S1). Besides, Figure 3 indicates that the orthogonal components were effective in separating the Espresso vs. the other extraction methods, while the chemical distance between coffee samples included in the Filter, Moka, and Neapolitan groups was smaller.

Figure 3. Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) score plot for the different coffee samples included in the different extraction category (i.e., Espresso, Filter, Moka, and Neapolitan).
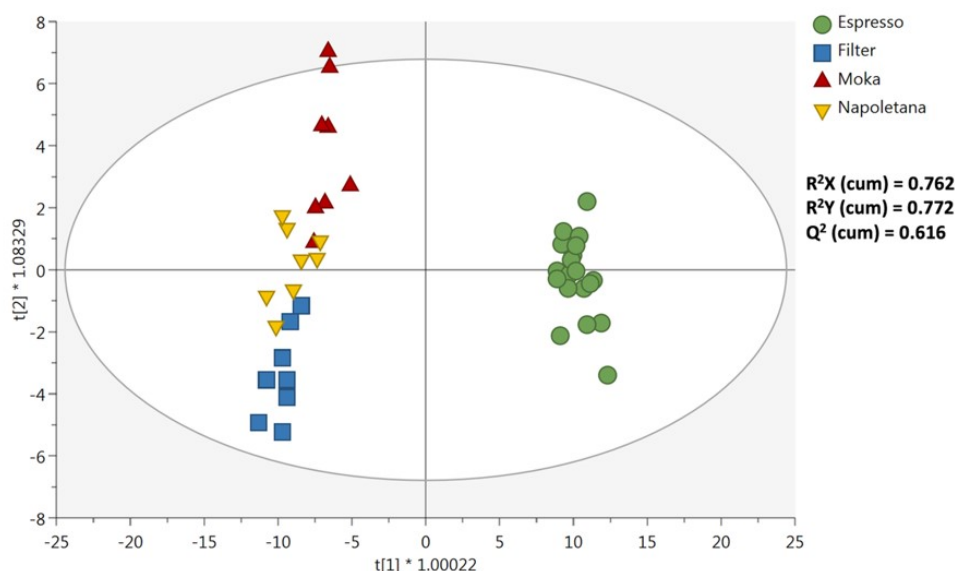
After that, the identification of the most important variables in the orthogonal projection was carried out through the VIP method. This latter ranked compounds as a function of their ability to determine the OPLS-DA score plot observed in Figure 3. These discriminant compounds are reported in Table 1, together with their VIP scores (cut-off > 0.8) and Log2 Fold-Change values (resulting from Fold-Change analysis with cut-off = 1.2 and having a p value < 0.05). The Espresso category was used as reference in Fold-Change analysis. Overall, we classified 86 discriminant compounds (excluding the potential isomeric structures), showing large differences between the different coffee samples extracted with the four extraction methods. Among the discriminant compounds, we found a large abundance of polyphenols (42%), followed by amino acids analogues, pyrazines, pyridines, and aryl-alkyl-ketones. Overall, two compounds were characterized by the highest VIP scores, namely 2,5-dimethyl-3-(methyldithio)-furan (VIP score = 1.72) and 1,2-disinapoylgentiobiose (VIP score = 1.48), belonging to furan derivatives and phenolic acids classes, respectively. Interestingly, these latter were highly discriminant for the Filter preparation, as can be observed by checking the LogFC variations reported in Table 1.

Table 1. Classified VIP discriminant compounds (VIP score > 0.8) following the OPLS-DA supervised statistics and considering the comparisons of Filter, Moka, and Neapolitan vs the Espresso extraction system, according to the Log2 Fold-Change (FC) variations.

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Class	Discriminant compounds (OPLS-DA)	VIP score	LogFC Filter vs Espresso	LogFC Moka vs Espresso	LogFC Neapolitan vs Espresso
Alkaloids	Caffeine	1.01	3.44	-0.12	-2.48
	Calystegine A6	0.99	0.74	-0.99	-1.96
Alkyl-phenylketones	3',4'-Dihydroxyacetophenone	1.26	3.78	1.62	1.47
	1-Phenyl-1-propanone	1.10	-16.45	-18.91	-18.98
Amino acids, peptides and analogues	L-Homoserine	1.07	1.66	-0.25	-0.50
	N-(carboxymethyl)lysine	1.04	-3.50	-1.20	-1.61
	N6-formyl lysine	1.03	0.27	0.34	-0.03
	N-(carboxyethyl)lysine	0.91	-13.47	-15.97	-15.99
	N6-Acetyl lysine	0.89	-4.13	-6.44	-6.65
	N-Caffeoyltryptophan	0.81	4.74	2.54	2.34
Aryl-compounds	1-Methyl-2-carboxaldehyde pyrrole/2-Acetylpyrrole	1.37	3.25	1.29	1.20
	1-(2-Furanyl)-1-butanone	1.37	0.10	0.53	0.20
	2-Acetyl-6-methylpyridine/2-Acetyl-5-methylpyridine	1.09	-0.53	-0.22	-0.52
	4-Acetyl-3-methylpyridine/4-Acetyl-2-methylpyridine	1.09	-0.52	-0.21	-0.52
	Ethyl 2-furanyl diketone	0.90	3.89	1.58	1.49
	1-(5-Methyl-2-furanyl)-1,2-propanedione	0.82	3.87	1.56	1.47
Azoles	5-Ethyl-2-methyloxazole/5-Ethyl-4-methyloxazole/4-Ethyl-2-methyloxazole/2-Ethyl-5-methyloxazole/2-Ethyl-4-methyloxazole	1.07	-15.21	-17.68	-17.73
	4-Ethyl-2,5-dimethyloxazole/5-Methyl-2-propyloxazole/5-Ethyl-2,4-dimethyloxazole	0.97	0.18	-0.01	-0.29
	4,5-Dimethyl-2-propyloxazole	0.88	2.06	-0.46	-0.93
Flavonoids	Narirutin 4'-O-glucoside	1.08	-4.06	2.30	-6.58
	Neodiosmin/Diosmin	1.07	3.51	0.97	1.02
	Neohesperidin/Hesperidin Quercetin 3-O-galactoside 7-O-rhamnoside/Kaempferol 3-O-sophoroside/Quercetin 3-O-rutinoside/Quercetin 3-O-rhamnosyl-galactoside/Kaempferol 3,7-O-diglucoside	1.03	3.54	1.01	1.03
	Pigment A/Peonidin 3-O-rutinoside/Peonidin 3-O-(6''-p-coumaroyl-glucoside)	1.03	3.52	0.99	1.01
	Delphinidin 3-O-rutinoside/Cyanidin 3,5-O-diglucoside/Cyanidin 3-O-sophoroside	1.03	3.62	1.06	1.08
	Cyanidin 3-O-glucosyl-rutinoside	0.96	17.55	12.95	15.08
	(+)-Catechin/(-)-Epicatechin	0.96	19.18	-1.36	-1.36
	Nepetin/Isorhamnetin/Rhamnetin	0.91	4.11	5.53	5.89
Furans	Dihydroactinidiolide	1.15	-6.14	-2.05	-0.21

	2,5-Dimethyl-3-(methyldithio)furan	1.72	3.27	-2.17	-4.47
	(R)-Roemerine	1.06	-14.13	-8.48	-14.04
	4-[(2-Furanylmethyl)thio]-2-pentanone	0.95	-11.56	-14.05	-14.06
	2-Ethyl-4,5-dimethyloxazole	0.94	0.15	-0.03	-0.31
Other phenolics	Tyrosol/4-Ethyl-1,2-benzenediol/3-Ethyl-1,2-benzenediol/4-Ethylcatechol	1.37	0.10	0.53	0.20
	4-Hydroxyphenylacetic acid	1.17	3.89	1.74	1.55
	Sinapaldehyde	1.07	-11.90	-0.47	-4.80
	Hydroxytyrosol	0.99	2.62	0.57	0.38
	p-HPEA-AC	0.99	2.34	0.24	-4.35
	threo-Syringoylglycerol/erythro-Syringoylglycerol	0.86	2.73	0.48	0.21
	Epirosmanol/Rosmanol	0.85	-4.83	-7.10	-4.08
	Umbelliferone/4-Hydroxycoumarin	0.85	9.67	4.94	6.94
	3,4-Dihydroxyphenylacetic acid	0.83	0.72	0.45	0.41
	p-HPEA-EA/Ligstroside-aglycone	0.81	4.72	2.53	2.32
	Vanillin	0.80	3.87	1.56	1.47
	Dimethylmatairesinol	1.34	17.81	0.63	13.29
	Leonuriside A	1.07	2.04	-0.28	2.20
Pyrazines	2-Acetyl-3,6-dimethylpyrazine/2-Acetyl-3,5-dimethylpyrazine	1.11	-0.46	-0.02	-0.52
	Ethylpyrazine/2-Ethylpyrazine/2,5-Dimethylpyrazine/2,6-Dimethylpyrazine/Dimethylpyrazine/2,3-Dimethylpyrazine	1.08	-15.30	-17.61	-17.96
	2-Isopropyl-6-methoxypyrazine/2-Isopropyl-5-methoxypyrazine	1.01	-5.01	-0.38	-0.76
	2-Methyl-3-(2-methylpropyl)pyrazine	0.85	-2.83	-5.32	-5.35
	2,5-Diethyl-3-methylpyrazine	0.84	-2.76	-5.26	-5.29
	2,3-Diethyl-5-methylpyrazine	0.84	-2.81	-5.31	-5.33
	3,5-Diethyl-2-methylpyrazine	0.83	-2.79	-5.29	-5.31
Pyridines	6-Acetyl-2,3,4,5-tetrahydropyridine	0.98	0.20	-0.06	-0.29
	3-Ethyl-pyridine	0.89	0.35	0.12	-0.15
	2-Ethyl-5-methylpyridine	0.88	2.08	-0.45	-0.92
Pyrroles	N-furfurylpyrrole/1-Furfurylpyrrole	0.89	-14.44	-16.93	-16.96
	1-(2-Furanylmethyl)-1H-pyrrole	0.82	-14.45	-16.93	-16.97
	2-Acetyl-1-pyrroline	1.07	-15.21	-17.68	-17.73
Stilbenes	Pinosylvin	1.15	7.24	0.94	4.90
	4-Vinylsyringol	1.10	-14.94	-17.44	-17.46
	Pterostilbene	0.94	-14.20	-16.69	-16.72
Phenolic acids	1,2-Disinapoylgentiobiose	1.48	9.30	0.63	9.01
	Gallic aldehyde/2,4-Dihydroxybenzoic acid/Protocatechuic acid/3,5-Dihydroxybenzoic acid/2,6-Dihydroxybenzoic acid/2,3-Dihydroxybenzoic acid/Gentisic acid	1.11	-14.46	-16.95	-16.98
	5-Caffeoylquinic acid/3-Caffeoylquinic acid/Cryptochlorogenic acid/4-	1.07	-8.44	-0.33	-8.44

	Caffeoylquinic acid/1-O-Caffeoylquinic acid/trans-Neochlorogenic acid				
	p-Coumaric acid ethyl ester	1.07	-4.10	2.16	-6.62
	1-Sinapoyl-2-feruloylgentiobiose	1.07	-4.06	2.30	-6.59
	Caffeic acid ethyl ester	1.07	-11.90	-0.47	-4.80
	p-Coumaroyl tartaric acid	0.95	-8.69	-11.19	-11.21
	m-Coumaric acid/o-Coumaric acid	0.90	-15.13	-17.44	-17.65
	Caffeic acid/trans-Caffeic acid	0.85	9.67	4.94	6.94
	Vanillic acid	0.83	0.72	0.45	0.41
	4,5-Dicaffeoylquinic acid/3,4-Dicaffeoylquinic acid/3,5-Di-O-caffeoylquinic acid/3,5-Dicaffeoylquinic acid/4,5-Di-O-caffeoylquinic acid	0.80	0.67	-1.83	-1.85
Other compounds	Floribundine	1.07	-13.84	-16.34	-16.37
	2-Methylbenzaldehyde/4-Methylbenzaldehyde/3-Methylbenzaldehyde/Phenylacetaldehyde/4-Vinylphenol	1.05	-14.37	-6.53	-8.65
	3,5-Dimethyl-1,2-cyclopentanedione/3-Ethyl-1,2-cyclopentanedione/3,4-Dimethyl-1,2-cyclopentanedione/3-Methyl-1,2-cyclohexanedione	1.10	-13.81	-15.96	-17.06
	Damascenone	0.92	-2.38	-0.05	-0.32
	(R)-2-Hydroxy-4,7-dimethoxy-2H-1,4-benzoxazin-3(4H)-one 2-glucoside	0.90	2.90	0.29	0.15
	Rubrofusarin 6-[glucosyl-(1-3)-glucosyl-(1-6)-glucoside]	0.96	16.79	12.16	16.38
	b-D-Glucuronopyranosyl-(1-3)-a-D-galacturonopyranosyl-(1-2)-L-rhamnose	0.80	0.67	-1.83	-1.85
	5-Methylquinoxaline	1.09	-14.22	-16.72	-16.74
	O-Methylcorypalline	1.06	2.30	-0.05	-0.34
	3-Mercapto-3-methyl-1-butanol/4-(Methylthio)-1-butanol	1.04	-14.31	-6.46	-8.59

Ubiquitously present in thermally processed foods, furans exposure studies revealed that coffee contributes most significantly to an adult's dietary exposure. This aspect might be of concern, considering that the International Agency for Research on Cancer classified furan as type 2B (i.e., possibly carcinogenic to humans). Besides, coffee is one of the only foods known where 2-methylfuran levels consistently exceed those of furan. However, as [36] reported, methyl-furans appear to be metabolized, at least in part, in a similar manner to furan, thus resulting in highly reactive intermediates with similar

toxicity. Regarding their presence in coffee beverages, initially absent in green coffee beans, furan derivatives are generated upon roasting from the thermal degradation of endogenous components. As reported by [37], methyl-furan forms are generated from the condensation of carbohydrate moieties arising from the Maillard reaction, while the origins of 3-methyl-, 2,5-dimethyl-, and 2,3-dimethyl-furan derivatives have yet to be fully established [38]. Besides, 1,2-disinapoylgentiobiose is a phenolic acid that belongs to the sub-class of hydroxycinnamic acids. Coffee is known to be a rich source of polyphenols, especially hydroxycinnamic acids such as different isomers of caffeoylquinic acid [39]. In our experimental conditions, this compound was highly abundant in Filter (LogFC = 9.30) and Neapolitan (LogFC = 9.01) extractions when compared with Espresso (Table 1). On the other hand, we found that the Espresso category was the best in providing the highest recovery of chlorogenic acid isomers (Table 1), with Filter and Neapolitan category characterized by a strong down-accumulation for these compounds (on average: -8.44; Table 1). Looking at other typical compounds, we found that Filter was the best extraction system for the recovery of caffeine (VIP score = 1.01; LogFC vs. Espresso = 3.44), while the group of pyrazines mainly characterized the Espresso preparation with the families of 2-acetyl-dimethyl-pyrazines showing the highest discrimination potential (VIP score = 1.11). According to data from the literature, pyrazines and furans are the major compounds in terms of concentration and the main classes contributing to coffee characteristic aroma through their impact on flavor, imparting earthy, musty woody, and papery notes. In previous work, [40] identified 12 pyrazines in different brands of capsule-brewed Espresso samples, with a significant abundance of 2-ethylpyrazine, 2-ethyl-6-methylpyrazine, and 2-ethyl-3,5-dimethylpyrazine, that have also been indicated as potent key odorants. In our untargeted experimental conditions, we detected several isomeric forms of different pyrazine-derivatives belonging to ethyl-, acetyl-, diethyl-, and dimethyl-derivatives (Table 1). Therefore, the distribution of pyrazines demonstrated that Espresso preparation was the best in enhancing the potential development of typical coffee aroma.

Looking at some recent works about coffee brewing, [41] evaluated the distribution of α-dicarbonyl compounds (α-DCs) and 4-methylimidazole in 72 Espresso coffee made with different roasting and brewing conditions, demonstrating that a cold brewing method provides the maximum concentration of these potentially hazardous compounds when largest coffee bean particles were used. Besides, the level of α-DCs was higher in C. arabica than in C. robusta, while C. robusta showed higher levels of 4-MI when compared with C. arabica. In our experimental conditions (UHPLC-QTOF-MS), we did not evaluate the presence of these Maillard reaction/caramelization-related intermediates considering that the untargeted full Scan acquisition ranged from 100 up to 1200 m/z. However, as showed in Table 1, among the discriminant compounds we listed several isomeric forms of methylated and dimethylated oxazoles. Interestingly, the formation of the different heterocyclic volatile compounds in coffee represents a complex interplay involving several chemical reactions, such as the so-called Strecker degradation, in which the dicarbonyl reagent undergoes transamination, thus leading to an α-aminocarbonyl. The α-aminocarbonyls are not only the precursors of pyrazines but can also lead to pyrrole derivatives (some of them included among the best discriminant marker compounds; Table 1), as well as imidazole and oxazole derivatives (for these latter, a shared and parallel formation mechanism has been previously proposed by [42]). Our findings revealed that both pyrrole and oxazole derivatives were marker compounds of the Espresso preparation, thus confirming once again the most complex chemical profile as potentially related to its typical aroma.

6.4.3. Sensory analysis

All the panelists properly performed, in terms of repeatability, so the raw data from the 6 panelists are validated and provided in Table 2 as the average of the panel score for each sensory descriptor for each sample under investigation. Interestingly, the sensory evaluation was more relevant in discriminating the different extraction methods than the coffee species.

Table 2. Average score values of sensory descriptors for different coffee beverage corresponding to four extraction systems applied on Arabica and Robusta roasted coffee. The description of each descriptor was explained to panelist according to [24].

Sensory descriptors [24]	Arabica Moka	Arabica Neapolitan pot	Robusta Moka	Robusta Neapolitan pot	Arabica Espresso	Robusta Espresso	Arabica Filter	Robusta Filter
Colour intensity	7.0	6.7	6.9	6.1	7.0	7.3	7.0	7.2
Aroma intensity	5.4	4.1	5.6	4.0	6.7	6.2	4.8	5.2
Body	4.7	4.1	4.9	3.4	6.0	5.8	2.3	2.7
Acidity	3.1	1.4	1.9	2.1	3.8	3.3	1.8	2.2
Bitter	3.4	3.1	3.3	2.9	4.0	4.7	1.2	3.5
Astringency	2.1	2.3	1.7	1.4	2.0	2.8	1.0	1.3
Honey	2.0	1.6	1.0	1.4	0.7	0.3	0.5	0.5
Floral & fruity	2.0	1.3	1.3	1.6	1.3	0.7	1.5	1.0
Dry vegetal	2.3	2.7	3.9	2.4	2.2	3.8	2.0	1.8
Vegetal	2.7	3.1	3.9	2.9	2.3	3.1	2.0	1.7
Stone fruit	2.9	2.3	2.9	2.2	3.2	3.3	2.7	1.8
Nuts and dry fruits	3.9	3.3	4.0	3.1	2.7	3.3	2.8	2.2
Cereals	2.9	2.6	3.9	3.1	3.5	3.4	3.7	2.5
Caramel	1.9	2.0	2.4	1.6	2.7	3.0	1.3	1.5
Cocoa	2.3	2.4	2.3	2.4	1.5	2.7	0.7	1.2
Pastry	2.6	2.0	1.0	1.8	2.5	1.6	1.5	1.0
Roasted	3.9	3.6	4.9	4.0	4.3	4.6	3.5	3.2
Burnt	1.7	1.0	1.9	1.1	0.0	0.8	0.5	0.7
Positive aromas	4.4	3.7	3.9	3.5	4.3	4.6	3.8	3.5
Aroma persistence	4.4	3.7	4.1	2.9	4.2	5.6	3.2	2.3

The analysis of variance (ANOVA) provided the classification of samples by significantly variant descriptors, namely color intensity, aroma intensity, body, acidity, vegetal, stone fruit, nuts and dry fruits, caramel, cocoa, burnt, positive aromas, and aroma persistence. Espresso samples were highlighted as the most intense (p < 0.05) in terms of aroma, acidity, body and aroma persistence, the best characterized in terms of caramel and stone fruits notes, the most persistent for aroma and the weakest for burnt notes.

On the other hand, Neapolitan pot resulted as the weakest in colour and aroma intensity, acidity, stone fruits notes but the majorly characterized for cocoa notes. For what concern moka and filter coffee, they hardly never peaked in any category apart from burnt notes, that were higher in Moka. Body, vegetal, nuts, and dried fruits, caramel, cocoa and aroma persistence resulted the weakest for intensity in filter coffee. Additionally, Figure 3 provides the sample distribution in two dimensions rotate space drove from PCA on average data obtained by

sensory analysis of different coffee beverages corresponding to four extraction systems applied on Arabica and Robusta roasted coffee.

PCA graph provides with the different sensory perceptions (bitter taste, body, aroma persistence, burnt, caramel, honey, roasted, stone fruits and global positive aroma) that – via factorial analysis – proved to be reliable to discriminate between samples. In particular, it shows the reciprocal distribution of samples, and, from our data, they fall, paired by extraction method, in the four different quadrants of the plane outlining once more the huge impact that different extraction methods have to the final sensory profile of coffee. To conclude, the two components (PC1 and PC2) are together able to explain the 79.9% of the variance, outlining once more the reliability of sensory attributes in extraction methods discrimination.

Figure 3. Unsupervised PCA score plot resulted from the average data obtained by sensory analysis of different coffee beverage corresponding to four extraction systems applied on Arabica and Robusta roasted coffee.
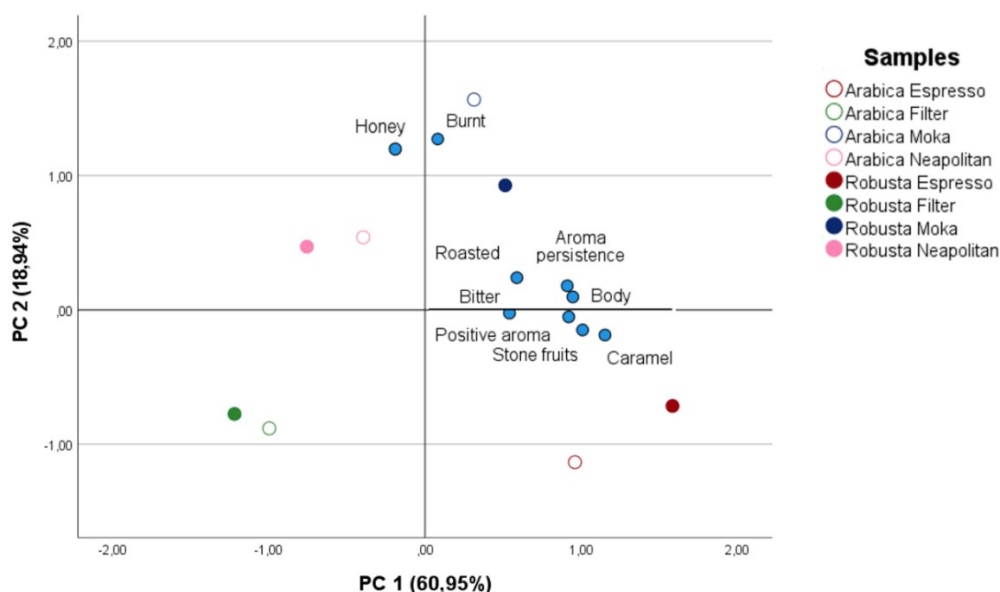
Additionally, by inspecting the distribution in Figure 4 (A, B, C, D), it was possible to define the most discriminant descriptors of different extraction. In this regard, the Moka brewing (Fig. 4A) was found to exalt body, roasted, and caramel aromas for Robusta, but it was relevant for the extraction of the positive odorants and honey notes characterizing moka coffee obtained from

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Arabica while increasing their persistency. What stated also reflected the high relative abundance of 2-acetylpyrrole (caramel, bread and beaked) and 4,5-dimethyl-2-propyloxazole (roasted) in Arabica that justify the cereal and pastry taste and the higher presence in Robusta of 3-ethylpyridine (grassy) and 6-acetyl-2,3,4,5-tetrahydropyridine (creamy, bread crust) (Table 1).

Figure 4. Sensory profiles of different coffee beverage corresponding to four ex-traction systems applied on Arabica and Robusta roasted coffee.
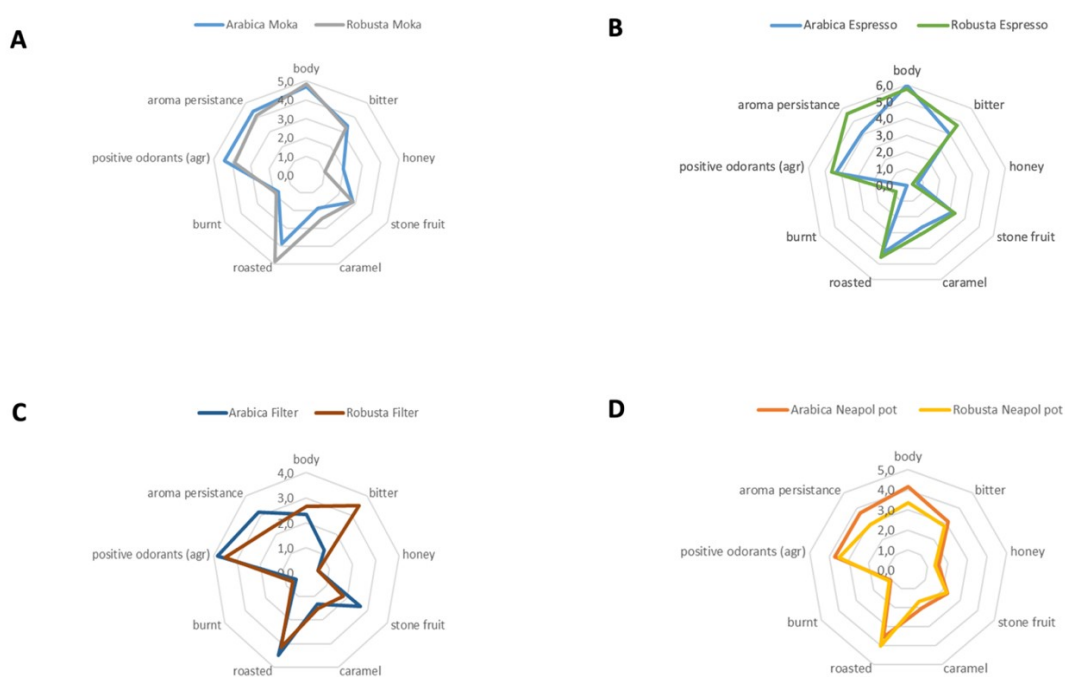
When considering the Espresso extraction (Fig. 4B), Arabica overcame Robusta thanks to the higher body, the richness in positive aroma and fragrances and the pastry notes; reversely, Robusta showed higher bitterness and aroma persistence, together with aromas of caramel, roasted and stone fruits. As confirmed by the shape of the spider graphs, the PCA and the cluster analysis of metabolites Espresso extraction were closer to the other in terms of perceptions and composition than the other extractions prepared with the same roasted coffee.

Regarding filter coffee prepared with an automatic home dripper (Fig. 4C), it was characterized by constant descriptors reported for both Arabica and Robusta excepted for the bitterness that was peculiar only for the drip-coffee

obtained with Robusta beans. Two samples differed for the predominance of roasted, stone fruits notes and a more intense olfactory perception always in Arabica coffee; reversely, Robusta was found to provide more caramelized notes – in terms of quantity – and higher body.

Finally, the Neapolitan pot (Fig. 4D) remarkably impacted the profile of the beverage. In fact, as highlighted from the slight distances of samples both in the PCA graph and on the spider chart, the two species were lower in scores for all the descriptors if compared with other extraction methods and were mutually close in terms of sensory profile. The only notable differences revealed by the panelists were body and aroma persistence, majorly perceived in Arabica sample, roasted and brunt notes higher in Robusta coffee.

6.5. CONCLUSIONS

The combination of UHPLC-QTOF untargeted metabolomics and sensory analysis al-lowed depicting the impact of different Italian traditional extraction methods, namely Espresso, Neapolitan, Moka, and Filter coffee. The chemical and sensory profile of coffee beverage was evaluated using both Coffea arabica and Coffea canephora var. robusta. Interestingly, the ability of our approach to discriminate coffee beverages, prepared with different methods, was hierarchically higher than the coffee species considered. The combination of sensory analysis and metabolomics allowed to build distinctive profiles characterizing brewed coffees, thus outlining mutual differences and similarities. Further ad-hoc studies, based on more targeted approaches, are advisable to better evaluate the degree of correlation between sensory perceptions and chemical markers as a function of the extraction technique considered.

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6.7. ADDITIONAL INFORMATION

Moderated T-Test [Arabica] Vs [Robusta] P <= 0.05 FC >=1.2
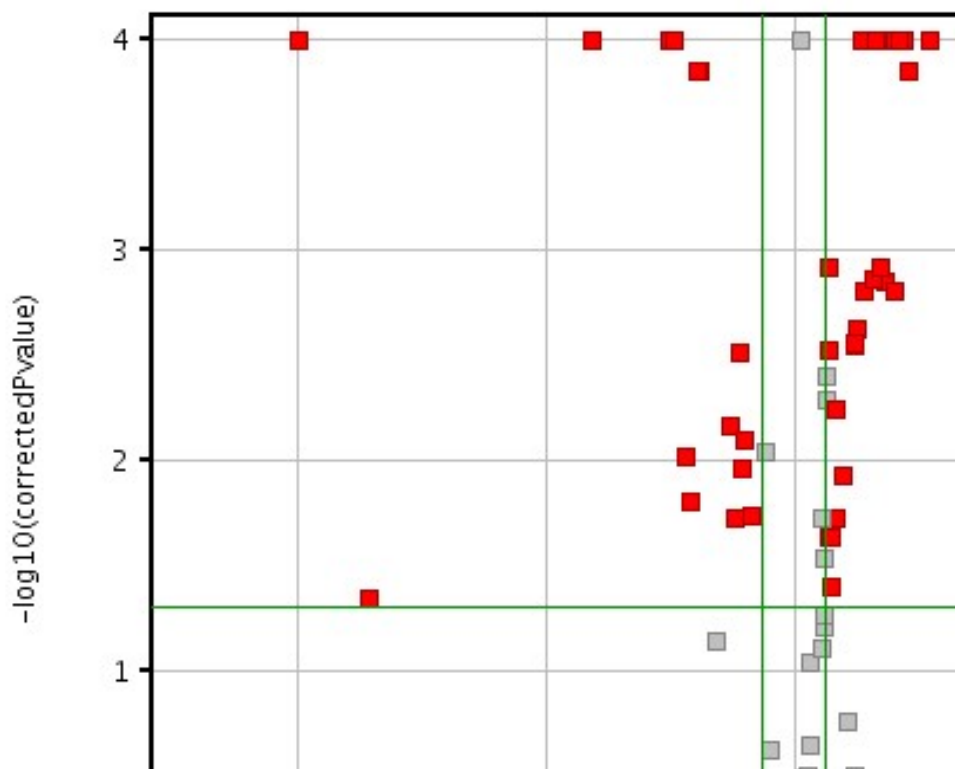
Compound	p-value (Corr)	Regulation	Fold Change
2-Methyl-5-vinylpyrazine	0.018284678	down	-1.2686316
(1-Methylethenyl)pyrazine	0.018284678	down	-1.2686316
1-Propenylpyrazine	0.018284678	down	-1.2686316
2-Isopropyl-5-methylpyrazine	0.007880719	down	-1.3243846
2-Methyl-3-(2-methylpropyl)pyrazine	0.010927588	down	-1.3454139
3,5-Diethyl-2-methylpyrazine	0.003014799	down	-1.3687001
2,3-Diethyl-5-methylpyrazine	0.003014799	down	-1.3687001
Hydroxytyrosol	0.018663792	down	-1.4037865
Isoferulic acid	0.018663792	down	-1.403838
Ferulic acid	0.018663792	down	-1.403838
2,5-Diethyl-3-methylpyrazine	0.006875375	down	-1.4355471
threo-Syringoylglycerol	0.000140036	down	-1.6982014
erythro-Syringoylglycerol	0.000140036	down	-1.6982014
(R)-Roemerine	0.000140036	down	-1.726407
Tetramethylpyrazine	0.015689315	down	-1.784934
3-Ethyl-2,5-dimethylpyrazine	0.009390499	down	-1.8473926
Coffeasterene	0.000012391	down	-1.9568543
(R)-2-Hydroxy-4,7-dimethoxy-2H-1,4-benzoxazin-3(4H)-one 2-glucoside	0.000008515	down	-2.0184622
Na-p-Hydroxycoumaroyltryptophan	0.000001730	down	-3.1094658
Caffeine	0.044851754	down	-10.811226

Compound	p-value (Corr)	Regulation	Fold Change
p-HPEA-AC	0.000003814	up	2.137861
5-Methylquinoxaline	0.000140036	up	1.8854108
3,5-Dimethyl-1,2-cyclopentanedione	0.000051390	up	1.8357165
3-Ethyl-1,2-cyclopentanedione	0.000051390	up	1.8357165
3,4-Dimethyl-1,2-cyclopentanedione	0.000051390	up	1.8357165
3-Methyl-1,2-cyclohexanedione	0.000051390	up	1.8357165
Nepetin	0.000000000	up	1.7972527
Isorhamnetin	0.000000000	up	1.7972527
Rhamnetin	0.000000000	up	1.7972527
3-Mercapto-3-methyl-1-butanol	0.001559218	up	1.7479894
2-Methylbenzaldehyde	0.001559218	up	1.7479894
4-(Methylthio)-1-butanol	0.001559218	up	1.7479894
4-Methylbenzaldehyde	0.001559218	up	1.7479894
3-Methylbenzaldehyde	0.001559218	up	1.7479894
Phenylacetaldehyde	0.001559218	up	1.7479894
4-Vinylphenol	0.001559218	up	1.7479894
2-Ethyl-5-methylpyridine	0.000000000	up	1.7395109
Vanillic acid	0.001418573	up	1.6618196
3,4-Dihydroxyphenylacetic acid	0.001418573	up	1.6618196
3-Ethylpyridine	0.001201384	up	1.6119117
3-Ethyl-pyridine	0.001201384	up	1.6119117
Ethyl acetoacetate	0.000000000	up	1.5700772
L-Homoserine	0.001359416	up	1.5503743
2-[(Methylthio)methyl]-2-butenal	0.000000000	up	1.5216334
Sinapaldehyde	0.001559218	up	1.4737191
Caffeic acid ethyl ester	0.001559218	up	1.4737191
3-Mercapto-3-methylbutyl formate	0.000000000	up	1.4590257
4-Ethyl-2-methyloxazole	0.002359660	up	1.417234
2-Ethyl-5-methyloxazole	0.002359660	up	1.417234
2-Ethyl-4-methyloxazole	0.002359660	up	1.417234
2-Acetyl-1-pyrroline	0.002719308	up	1.4025335
5-Ethyl-2-methyloxazole	0.002819824	up	1.3983815
5-Ethyl-4-methyloxazole	0.002819824	up	1.3983815
Dihydroactinidiolide	0.011709164	up	1.3011712
2,5-Diethylthiazole	0.005617291	up	1.2584088
4-Ethyl-2,5-dimethylthiazole	0.005617291	up	1.2584088
2-Isobutylthiazole	0.005617291	up	1.2584088
2,4-Diethylthiazole	0.005617291	up	1.2584088
5-Ethyl-2,4-dimethylthiazole	0.005617291	up	1.2584088
2-Ethyl-4,5-dimethylthiazole	0.005617291	up	1.2584088
2-Isopropyl-4-methylthiazole	0.005617291	up	1.2584088
4-Vinylsyringol	0.018663792	up	1.2570382

2,5-Diethylpyrazine	0.022928998	up	1.2248167
1-Phenyl-1-propanone	0.039181694	up	1.2184347
Calystegine A6	0.022907278	up	1.2167718
Neodiosmin	0.002967672	up	1.2090663
Diosmin	0.002967672	up	1.2090663
(+)-Catechin	0.001201384	up	1.2048963
Quercetin 3-O-xylosyl-rutinoside	0.001201384	up	1.2048963
Glyoxal lysine dimer	0.001201384	up	1.2048963
trans-Chlorogenic acid	0.001201384	up	1.2048963
Rubrofusarin 6-[glucosyl-(1-3)-glucosyl-(1-6)-glucoside]	0.001201384	up	1.2048963
4,5-Dicaffeoylquinic acid	0.001201384	up	1.2048963
Bisdemethoxycurcumin	0.001201384	up	1.2048963
Pterostilbene	0.001201384	up	1.2048963
3,4-Dicaffeoylquinic acid	0.001201384	up	1.2048963
3,5-Di-O-caffeoylquinic acid	0.001201384	up	1.2048963
Narirutin 4'-O-glucoside	0.001201384	up	1.2048963
Cyanidin 3-O-glucosyl-rutinoside	0.001201384	up	1.2048963
3,5-Dicaffeoylquinic acid	0.001201384	up	1.2048963
(-)-Epicatechin	0.001201384	up	1.2048963
4-Demethylsimmondsin 2'-(E)-ferulate	0.001201384	up	1.2048963
Quercetin 3-O-acetyl-rhamnoside	0.001201384	up	1.2048963
4,5-Di-O-caffeoylquinic acid	0.001201384	up	1.2048963
Cyanidin 3-O-xylosyl-rutinoside	0.001201384	up	1.2048963
Kaempferol 3-O-acetyl-glucoside	0.001201384	up	1.2048963
3-Demethylsimmondsin 2'-(Z)-ferulate	0.001201384	up	1.2048963
Cyanidin 3-O-sambubioside 5-O-glucoside	0.001201384	up	1.2048963
1,2-Disinapoylgentiobiose	0.001201384	up	1.2048963
1-Sinapoyl-2-feruloylgentiobiose	0.001201384	up	1.2048963
Dimethylmatairesinol	0.001201384	up	1.2048963
b-D-Glucuronopyranosyl-(1-3)-a-D-galacturonopyranosyl-(1-2)-L-rhamnose	0.001201384	up	1.2048963
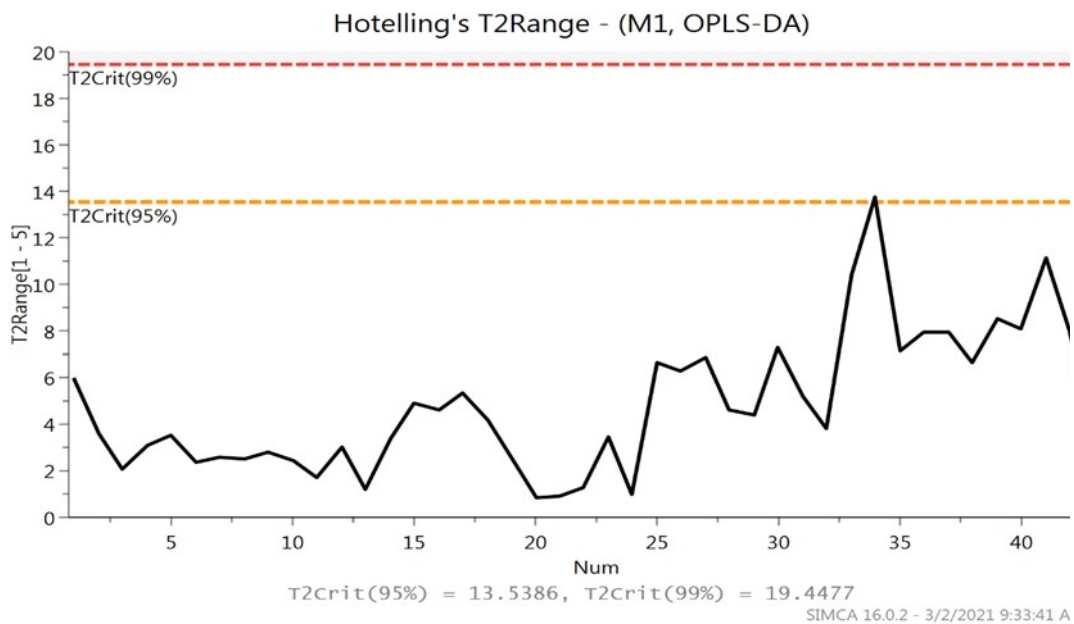
OPLSDA validation – Hotelling's T2Range
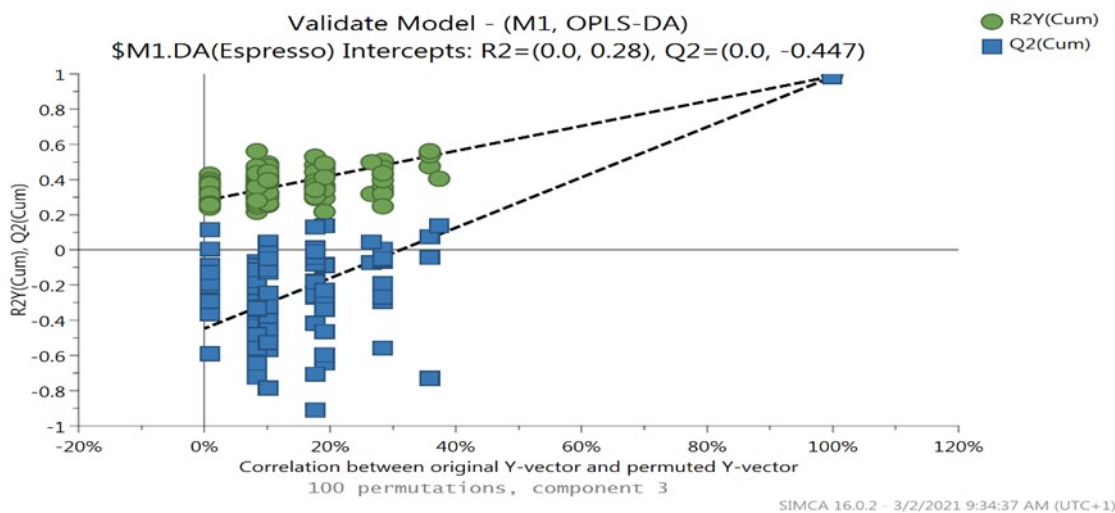
OPLSDA validation – goodness of fitness and prediction

M1	SS	DF	MS	F	p-value	SD
Total corr.	129	129	1			1
Regression	88.836	30	2.961	7.298	2.15×10^{-14}	1.720
Residual	40.164	99	0.405			0.636

OPLSDA validation – model structure

7. Conclusion and further perspectives

To conclude, the presented works, dealing with the world of high-quality and specialty coffee at different levels of the supply and value chain outlined the main features able to distinguish this segment in a huge market as coffee one is, characterizing the targeted sector in terms of intrinsic as extrinsic values as identified by the Specialty Coffee Association in the last-released definition provided for Specialty Coffee.

The fil rouge linking the different studies was a vast sample set of coffee well representing the variability that can be found in the real market, crop after crop, preserving anyway a common denominator that is their link with the origin. This indissoluble connection between coffee beans and the plantations was demonstrated at two different levels thanks to the results obtained via the analysis of the elemental and volatile profile, leading to the results that both inorganic and organic composition of coffee beans depends on the place where they were cultivated and these characteristics are detectable both in green and roasted coffee.

The elemental composition of coffee beans was also useful to demonstrate that the variety of Arabica coffee, an attribute that in the specialty coffee sector is starting to be also communicated to the final consumer, it has an important role in determining the final composition of the beans – net of the origin – and, parallelly, it can be discriminated thank to elemental analysis.

What provided for variety by elemental analysis was true for post-harvesting processes and roasting levels considering the volatile compounds. In this respect, post-harvesting processes were not as impactive as origin in green coffee and less modulative for the volatile profile than the roasting level. Once combined, these results can provide with a robust traceability and discrimination model capable of linking coffee to its origins when already roasted and potentially detecting frauds and adulteration of coffee from different origins and post-harvesting processes.

To valorize the quality expressed by Specialty Coffee, assessing the higher level of safety in terms of reducing the occurrence of process contaminants in these

lots was of crucial importance. In this respect, the results obtained were able to show a correlation between precursors and acrylamide concentration not already provided in literature and, additionally to demonstrate that the absence of defective seeds in Arabia coffee, and potentially also in Robusta, leads to a lower level of acrylamide when compared with data available from the survey conducted by EFSA. Also, origin, not only the post-harvesting processes, seemed to impact the concentration of acrylamide precursors giving even more importance to the traceability of coffee lots.

In respect of Specialty coffee roasting and extraction, the metabolomics approach allowed to prove the unique profile given to coffee beverages by the extraction method capable of drawing, from the same coffee, different shades. Additionally, it demonstrated the unicity of Italian espresso extraction method when compared with percolation and low-pressure extraction system, justifying the focus placed on this beverage.

Regarding the Italian market, proud consumer and inventor of Italian espresso machine, the work here presented put an important base on which built and sustain the trend undertaken by the specialty coffee roasters aimed to profile roasting intended for every different extraction method, preserving, nonetheless, the sensory profile characterizing Specialty coffees of different origins.

Further works are now needed to go in depth into the different areas here exported.

To maximize the sustainability of coffee sector, silver-skin must be investigated and potentially upcycled in high added value food products, together with all the by-products deriving from coffee fruits processing.

The development of a predictive model of the volatile compounds deriving from well-characterized green coffee after roasting would be of strategic interest for developing roasting profiles that suit the expectation of coffee consumers and exalts the potentiality of every lot of coffee.

Also, the coffee beverage needs to be investigated the most, characterizing it for the elemental composition to find some stable element able to link the cup of coffee back to its origin.

Moreover, the impact of water composition – a main ingredient of a cup of coffee– on the sensory and chemical profile of the beverage would be investigated to bring the best from coffee in the cups.

Lastly, a lot of work is to be done in the producing country, trying to minimize the losses caused by spoilage and contamination but also encouraging producers to develop more sustainable post-harvesting processes and preservative cultural methods able to give the same outstanding results in terms of cup profile obtained in the last decade.

8. Acknowledgement