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Importance of Espresso Coffee Machine Parameters on the Extraction of Chlorogenic Acids in a Certified Italian Espresso by Using SPE-HPLC-DAD

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Abstract

Chlorogenic acids (CGA) are a group of phenolic acid derivatives, which are commonly found in coffee at concentrations reaching 0.1-0.2%. A method based on high performance liquid chromatography-diode array detector (HPLC-DAD) is proposed for the simultaneous determination of three chlorogenic acids, i.e. 3-*O*-caffeoylquinic acid (3-CQA), 5-*O*-caffeoylquinic acid (5-CQA) and 3,5-di-O-caffeoylquinic acid (3,5-diCQA), in time portions of espresso coffee. Two different espresso coffee machines working with different pressure and temperature curves, and two different blends (i.e. Arabica and Robusta) were used. The method presents good linearities (correlation coefficient greater than 0.99) and recoveries (in the range 67-99%) for the 3 chlorogenic acids. The concentration of total CGAs in a cup of Certified Italian Espresso ranged from 1522.5 to 2223.4 mg kg^{-1} and CGA isomer contents were, in decreasing order, 5-CQA > 3-CQA > 3,5-diCQA.

The concentration of total chlorogenic acids was higher in Espresso coffee (EC) prepared with Aurelia machine rather than with Leva; Arabica blend possessed higher level ot total chlorogenic acids than Robusta samples.

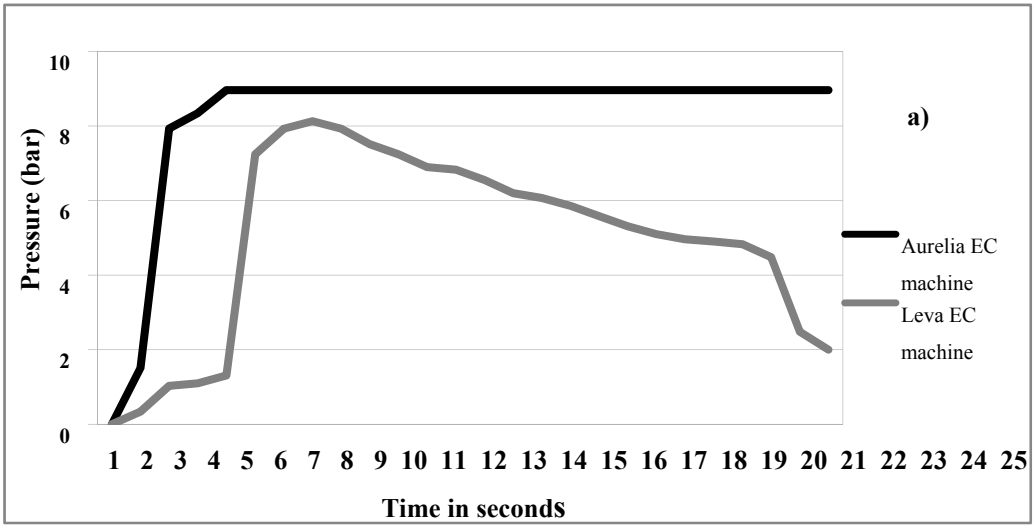
Keywords: coffee, certified Italian espresso, chlorogenic acids, antioxidant, SPE-HPLC-DAD, espresso coffee machines

1. Introduction

Brew coffee is a very popular beverage in the world (Parliament & Stahl, 1995). Consumers appreciate this drink for its enjoyable aroma, appreciable directly through the nose. Espresso coffee (EC) has specific aroma characteristics, mainly due to the presence of foam, which traps the volatilized aromas and doses their emission into the atmosphere (Blumberg, Frank, & Hofmann, 2010; Illy & Viani, 1995; Maeztu et al., 2001a). EC preparation is affected by factors related to the quality of coffee and water, and to settings of the machine. Water is brought to the desired pressure (normally about 9 atmosphere) and then passed through a heat exchanger, which gives to the water the set temperature (normally between 91°C and 96°C). Then, water is sprayed over the coffee and the extraction of the espresso components begins (Caprioli et al., 2012; Odello & Odello, 2006). The Certified Italian Espresso is the drink-in-a-cup conforming to the strict production specifications issued by the Italian Espresso National Institute and approved by a third-party Body operating in conformity with ISO standard 45011, and it is safeguarded and promoted through a product certification (certificate of product conformity Csqa n. 214-24 September 1999, DTP 008 Ed.1). To prepare a Certified Italian Espresso the following technical conditions are reported in literature, though only those would not be sufficient to completely fulfill quality requirements: needed portion of ground coffee of 7 g ± 0.5, temperature of water coming out from the unit of 90°C ± 2°C, coffee temperature in the cup of 67°C ± 3°C, water pressure of 9 bar ± 1, percolation time of 25 seconds ± 2.5, viscosity at 45°C > 1.5 mPas, total fat > 2 mg/ml, caffeine < 100 mg/cup, volume in the cup (inclusive of foam) 25 ml ± 2.5 (Odello & Odello, 2006). Chlorogenic acid is an ester formed between caffeic acid and quinic acid, and it is a phenolic compounds present in coffee that possess important biological effects since some previous findings reported that these phenolic compounds can acts as antioxidant, antitumor, antimutagenic and anticarcinogenic agents (Cetto & Wiedenfeld, 2000; Jiang et al., 2000; Moseira, Spitzer, Schapoval, & Schenkel, 2001). Caffeoylquinic acid derivatives, like 3-*O*-caffeoylquinic acid (3-CQA),

5-*O*-caffeoylquinic acid (5-CQA) and 3,5-di-O-caffeoylquinic acid (3,5-diCQA), are natural pohenolic compounds that have been isolated from a variety of traditional medicine plants and present a broad spectrum of pharmacological properties, including antioxidant, hepatoprotectant, antibacterial, antihistaminic and other biological effects (Basnet, Matsushige, Hase, Kadota, & Namba, 1996; Kwon, Jung, & Shin, 2000). Recently, it has been demonstrated that caffeoylquinic acid derivatives possess also neuroprotective effects (Hur, Soh, & Kim, 2001; Soh, Kim, Sohn, Lee, & Kim, 2003). More, it is reported that 3,5-diCQA exhibited neuroprotective properties against neuronal cell death that can be applied in the development of brain protection, as well as in the treatment of neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease and ischemia (Kim, Park, Jeon, Kwon, & Chun, 2005). Several authors have suggested a relationship between the composition of the CGA fraction and the quality of the beverage; addition of dicaffeoylquinic acids was negative for coffee flavor, whereas addition of mono caffeoylquinic acids brought about positive results (Ohiokpehai, Brumen, & Clifford, 1982). Tressl (1977) reported a more direct link between CGA fraction and beverage quality, through the influence on beverage aroma of organoleptically significant CGA degradation products such as caffeic acid, quinic acid and others. Clifford (Clifford & Ohiokpehai, 1983) and Naish (Naish, Clifford, & Birch, 1993) investigated the astringency of dicaffeoylquinic acid (DCQA) in depth; they reported a response for 5CQA similar to that of tannic acid and grape seed tannin, which traditionally are associated to astringency. Ohiokpehai (Ohiokpehai, Brumen, & Clifford, 1982) reported that the caffeoylquinic acid CQA/DCQA molar ratio on green coffee beans may influence the pleasantness of obtained beverage. Moreover, Nagel (Nagel, Herrick, & Graber, 1987) demonstrated that CQA does not show the bitter taste when its acid character is masked. There have been many reports on the presence of CGA in green coffee beans (Clifford, 1979; Van der Stegen & Van Duijin, 1980) or in brew coffee (Fujioka & Shibamoto, 2008) but not in ECs. For example, the content of CGA in various green coffee beans (21 species) from Cameroon and Congo ranged from 0.8% to 11.9% on a dry matter basis (Campa, Doulbeau, Dussert, Hamon, & Noirot, 2005), while it was ~5-15 mg g^{-1} in brew coffee (Fujioka & Shibamoto, 2008). The CGA content in brewed coffee is influenced by the kind of coffee beans used, because Arabica beans contain less CGA than Robusta ones (Ky et al., 2001). Most commercial brands of coffee are, however, made up of both Arabica and Robusta beans. The roasting method might also play an important role in the CGA content of the final coffee product. In fact, in the only article available on the CGA concentration in espresso coffee, it is reported that the CGA content is higher in Arabica rather than in Robusta EC samples (Ludwig et al., 2012). Various analytical methods have been proposed for the quantification of chlorogenic acids in coffee: HPLC (High-performance liquid chromatography) is the most common method, coupled with UV detector (Bicchi, Binello, Pellegrino, & Vanni, 1995; Fujioka & Shibamoto, 2008; Ky, Noirot, & Hamon, 1997; Maeztu et al., 2001) or with a mass spectrometer (Blumberg, Frank, & Hofmann, 2010). Other procedures, such as the simultaneous determination of total CGA and caffeine in coffee by high performance gel filtration (HPGF) chromatography has also been reported (De Maria, Trugo, & Moreira, 1995).

To the best of our knowledge, to date, no quantification of chlorogenic acids in espresso coffees wasperformed by comparing two EC machines working on different principles with the combination of two different blends. In fact, the aim of our work was to quantify the three most concentrated chlorogenic acids, i.e. 3-*O*-caffeoylquinic acid (3-CQA), 5-*O*-caffeoylquinic acid (5-CQA) and 3,5-di-O-caffeoylquinic acid (3,5-diCQA), in time portions of espresso coffees, comparing two different espresso machines, working with different pressure and temperature curves, and two different blends, Arabica and Robusta. The kinetic extraction of the Aurelia Competizione (A) EC machine was compared with that of the Leva Victoria Arduino (B). In this case, the settings of machine A were 92°C and 9 bar while espresso machine B is unsettable (Figure 1).



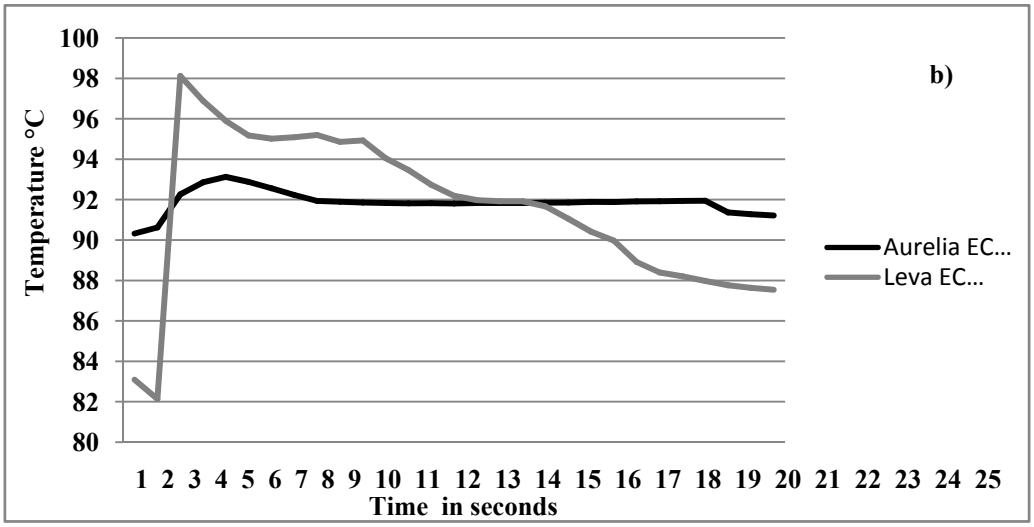


Figure 1. Curves of pressure (a) and temperature (b) on the coffee filter exhibited by Aurelia Competizione and Leva "Victoria Arduino" EC machines

2. Methods

2.1 Materials and Standards

The analytical standards of 5-*O*-caffeoylquinic acid (5-CQA), 3-*O*-caffeoylquinic acid (3-CQA), and 3,5-di-O-caffeoylquinic acid (3,5-diCQA) were purchased from Sigma-Aldrich (Milano, Italy).

Individual stock solutions were prepared by dissolving 100 mg of each compound in 100 ml of methanol (HPLC-grade, 99.9%; Sigma-Aldrich, Milano, Italy) and stored in glass-stoppered bottles at 4°C. Standard working solutions, at various concentrations, were daily prepared by appropriate dilution of aliquots of the stock solutions in methanol.

HPLC-grade formic acid was supplied by Merck (Darmstadt, Germany). Deionized water (> 8 MΩ cm^{-1} resistivity) was obtained from the Milli-Q SP Reagent Water System (Millipore, Bedford, MA). All the solvents and solutions were filtered through a 0.45 µm PTFE filter from Supelco (Bellefonte, PA, USA) before use. Cartridges SPE Strata-X 6 ml, 200 mg, were purchased from Supelco (Bellefonte, PA, USA).

2.2 Coffee Types and Espresso Machines

2 Kind of coffee, Arabica (pure *Coffea Arabica* from Colombia) and Robusta (95:5 blend of *Coffea canephora* and *Coffea Arabica*) and two EC machines, the Aurelia Competizione and the Leva Victoria Arduino working

with peculiar and different curves concerning pressure and temperature (Figure 1), were a gift from a local company (Nuova Simonelli, Belforte del Chienti, Italy). The variance in settings derives from the production method of the two EC machines. Aurelia is equipped with an electric pump and a heat exchanger, while in Leva pressure is given by a spring and water is brought to the desired temperature by passing it through a boiler.

2.3 Certified Italian Espresso Sample Preparation

Powdered coffee was made using a coffee grinder set in such a way that the obtained EC had a volume of 25 ml in 25 seconds of extraction in the coffee machine. Toasted coffee beans were ground immediately before each preparation; 7.5 g of the obtained coffee powder was used for each EC. After in-depth studies performed from our research group on the quality of EC, 9 bar of water pressure and 92°C were chosen as settings for the EC machine A for preparation and analysis of 7 time portions of the EC sample (0-10, 11-15, 16-20, 21-25, 26-30, 31-35, 36-40 sec.), with the aim at comparing these results with those from the same analysis performed using EC B. The conditions of EC "B" (T and P) are unsettable: pressure and temperature change as reported in Figure 1.

2.4 Sample Purification: Solid Phase Extraction (SPE)

The solid phase extraction was carried out using SPE Strata-X extraction cartridge (200 mg, 6 ml). The SPE cartridge was conditioned with methanol (3 ml) followed by water (6 ml). A volume of 0.5 ml of espresso coffee diluted with 2 ml of water was loaded onto each cartridge using a vacuum pump and collected. The elution was performed using methanol (7.5 ml). The eluent was evaporated under vacuum (60 mbar) at 30°C by a Büchi apparatus (Büchi R200, Labortechnik, Flawil, Switzerland); 10 ml of methanol were added to the residue, the solution filtered (0.45 μm nylon membrane) and transferred to a glass vial before injection.

2.5 LC/DAD Analysis

The separation was achieved on an analytical column Polar-RP 80Å (150 x 4.6 mm I.D., 4 μm) from Phenomenex (Chesire, U.K.). The mobile phase for LC/DAD analysis was water (A) and methanol (B), both containing 0.1% of formic acid at a flow rate of 1 ml min^{-1}. The solvent composition varied from 0-5.5 min: 25% B (v/v); 5.5-8 min: 50% B (v/v); 8-13.5 min: 50% B (v/v); 13.5-18 min: 25% B (v/v). The injection volume was 5 μl.

LC/DAD experiments were performed using a Hewlett Packard (Palo Alto, CA, USA) HP-1090 Series II, made of an autosampler and a binary solvent pump, equipped with a diode-array detector (DAD). LC/DAD analysis were performed monitoring two different wavelengths: 325 nm for 5-*O*-caffeoylquinic acid (5-CQA) and 330 nm for 3-*O*-caffeoylquinic acid (3-CQA), and 3,5-di-O-caffeoylquinic acid (3,5-diCQA).

2.6 Statistical Analysis

Analysis of variance (ANOVA) was performed using the SPSS software package, Chicago, IL, USA) for Windows. Values of $p < 0.05$ were considered as statistically significant.

3. Results and Discussion

3.1 Pressure and Temperature Curves From the Two EC Machines

Considering the pressure curve (Figure 1a), values increased up to a maximum of 9 and 8 bar for EC machines Aurelia and Leva, respectively. After that, the pressure curve in machine Aurelia remained unchanged, due to being equipped with an electric pump, while in machine Leva the pressure decreased until it reached 2 bar after 26 s.

Considering the temperature curve (Figure 1b), in the first 3 seconds of extraction the values increase up to a maximum of 93°C for Aurelia and 98°C for Leva. Due to the differences in construction, for Aurelia, the temperature value remains fairly constant, while for Leva the temperature drops about 10 degrees during extraction (Figure 1).

3.2 Comparison of Chlorogenic Acids Content in Coffee Samples Using Two EC Machines

Different trends of concentration have been obtained for ECs prepared by using two different blends, i.e. Arabica and Robusta (Figure 2).

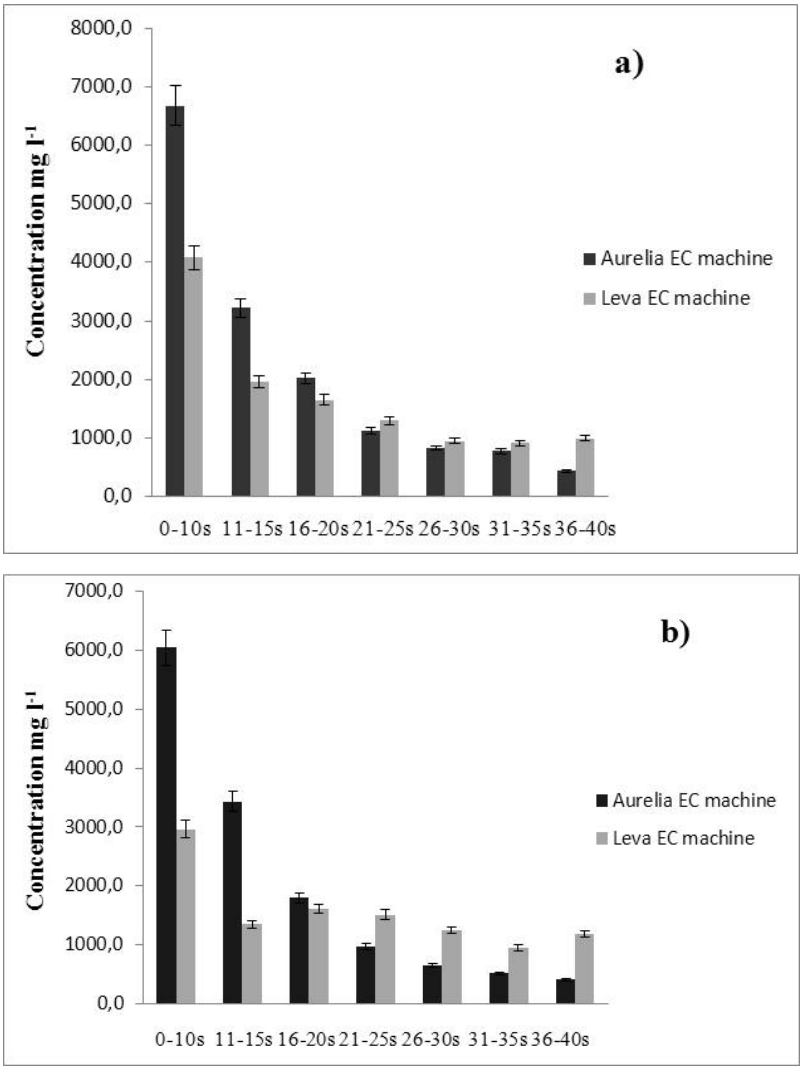


Figure 2. Trend of concentration (mg l^{-1}) of chlorogenic acids in time portions of ECs in Arabica (a) and Robusta (b) with the two EC machines (n = 3 RSD% < 5.7)

The concentrations of the three chlorogenic acids in each time portions is constantly decreasing. By using Leva EC machine, the chlorogenic acids concentration is greatly higher in Arabica rather than in Robusta in the first three portions, meanwhile in the other fractions an opposite trend is observed.

Similar trends of concentration have been obtained for ECs prepared by using two different EC machines, i.e. Aurelia and Leva, with a fixed blend (Figure 2). By comparing the trends of chlorogenic acids concentration obtained with Robusta blend, the chlorogenic acid concentration is greatly higher in samples obtained with Aurelia rather than with Leva in the first three fractions. These concentration values displayed large differences, especially in the first two fractions (0-10 sec. and 11-15 sec.). In fact, we found a concentration of chlorogenic acids of 6041.1 and 3431.7 mg l^{-1} in Aurelia, and of 2958.5 and 1349.7 mg l^{-1} Leva EC samples, respectively, showing great differences and high relative Aurelia/Leva ratio (2.042 and 2.543). Also by comparing the trends of chlorogenic acids concentration obtained with Arabica, the total concentration is higher in samples obtained using Aurelia rather than Leva in the first three fractions. In detail, the concentration of chlorogenic acid in the first two fractions (0-10 sec. and 11-15 sec.) is of 6684.7 and 3217.7 mg l^{-1} in Aurelia and of 4083.5 and 1966.3 mg l^{-1} in Leva EC samples, showing a relative ratio of 1.637 and 1.636, respectively.

As can be seen in Figures 2a and 2b, the extraction of chlorogenic acid, both in terms of concentration and of content (*data not show*) is higher in Aurelia than in Leva EC machines in the first three fraction (i.e. 0-10 s, 11-15 s, and 16-20 s).

In Figure 3 it is reported an overlapping of an HPLC-DAD chromatograms referred to a Robusta coffee sample (0-10 s of extraction time) prepared with Aurelia (gray) and Leva (black) in which it is clearly evident the most effective extraction of chlorogenic acid with Aurelia EC machine.

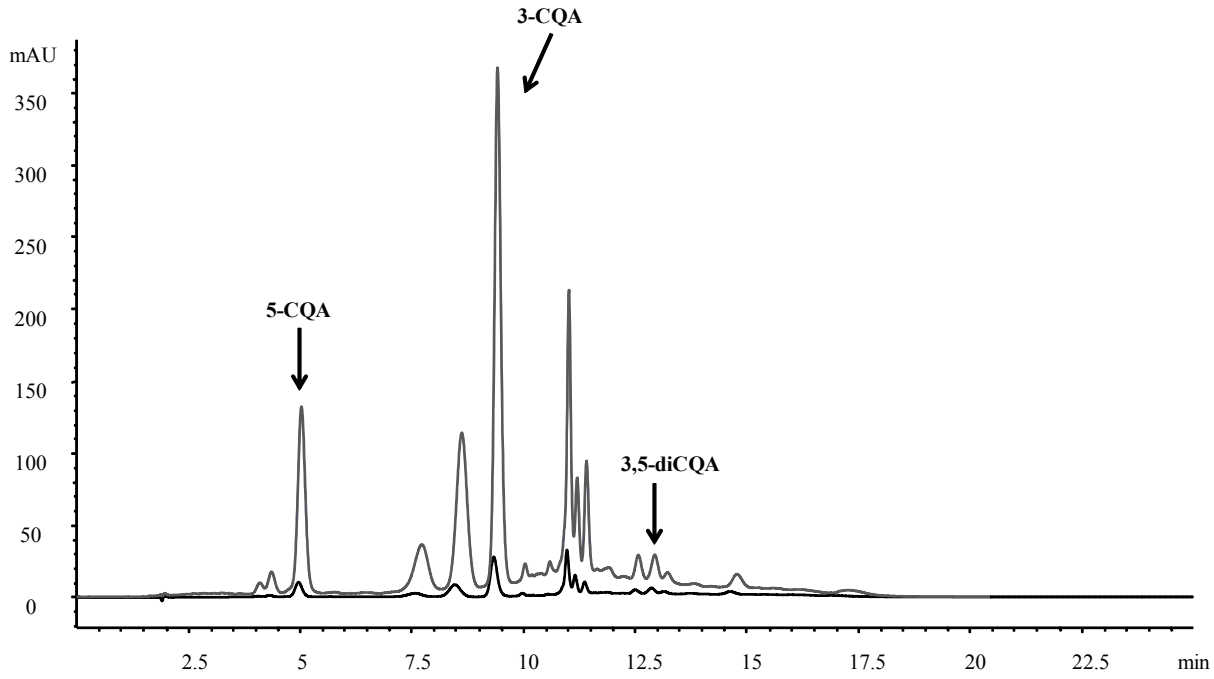


Figure 3. Overlapping of an HPLC-DAD chromatograms referred to a Robusta coffee sample (0-10s of extraction time) prepared with Aurelia (gray) and Leva (black)

These results can be explained by considering the temperature, higher in Leva respect to Aurelia up to 15 seconds (Figure 1). A lower temperature value seems to be more suitable to extract chlorogenic acids. As clearly visible in Figure 2, chlorogenic acids extraction is more efficient in the first fractions. In detail, for both Arabica and Robusta, the extraction of CQAs in the first fractions is more efficient for Aurelia than Leva. Because of this, concentration is: a) higher in coffees made with Aurelia in the first 2 fractions, b) very similar between the coffees made with the two EC machines in the central fractions, c) higher in EC made with Leva in the last fractions.

As shown in Figure 1, the water pressure in Aurelia (9 bar) is always higher than that in Leva EC machine. This value of 9 bar is reported to be the best extraction pressure condition for 5-CQA (Andueza et al., 2002). Probably, in Leva EC machine, because of the previous considerations, the pressure parameter seems to be not highly influent for chlorogenic acid extraction.

In our work, we have screened chlorogenic acid content in a long time of extraction for EC (40 sec, including a total of 7 fractions), in such a way to investigate all the type of preparation of EC worldwide.

By considering that chlorogenic acids play a beneficial role for human health, due to their antioxidant action, the concentration and the content of the three compounds in a cup of espresso (25 ml, i.e. the volume of a Certified Italian Espressso) have been considered (Table 1).

Table 1. Concentration (mg l^{-1}) and content (mg) of each chlorogenic acids in a cup of espresso coffee (i.e. 25 ml) (n = 3 RSD% < 5.0).

EC machine	Blend	3-CQA (mg l^{-1})	3-CQA (mg)	5-CQA (mg l^{-1})	5-CQA (mg)	3,5-diCQA (mg l^{-1})	3,5-diCQA (mg)	Total concentration in a cup of coffee	Total content in a cup of coffee
Leva	Arabica	422.8	10.6	1220.2	30.5	90.7	2.3	1733.7	43.3
	Robusta	394.9	9.9	986.1	24.7	141.5	3.5	1522.5	38.1
Aurelia	Arabica	555.8	13.9	1559.9	39.0	107.8	2.7	2223.4	55.6
	Robusta	529.3	13.2	1416.1	35.4	177.1	4.4	2122.5	53.1

RSD: Relative Standard Deviation; CQA: CaffeoylQuinic Acid.

CGA isomer concentration were in decreasing order 5-CQA > 3-CQA > 3,5-diCQA, with a concentration ranging from 986.1 to 1559.9 mg l^{-1}, 394.9 to 555.8 mg l^{-1}, and 90.7 to 177.1 mg l^{-1}, respectively. The amount (mg) in a cup of EC ranged from 24.7 to 39 mg, 9.9 to 13.9 mg and 2.3 to 4.4 mg for 5-CQA, CQA and 3,5-diCQA, respectively.

The most concentrated CGA was 5-CQA and was found in Arabica coffee prepared with Aurelia EC machine with a higher concentration of 1559.9 mg l^{-1}, in agreement with previous findings (Ludwig et al., 2012). The highest level of 3-CQA was also detected in Arabica coffee sample prepared with Aurelia EC machine (555.8 mg l^{-1}). On the contrary, the highest concentration of 3,5-di-CQA was found in Robusta coffee sample prepared with Aurelia EC machine (177.1 mg l^{-1}).

The total concentrations of the three compounds in a cup of coffee (25 ml) ranged from 1522.5 to 2223.4 mg l^{-1} and the total amount ranged from 38.1 to 55.6 mg (Table 1). Coffee samples made with Arabica blend and Aurelia EC machine displayed the highest concentration and content of total chlorogenic acids; however, samples obtained by using Robusta with the same EC machine (Aurelia) showed also the highest total concentration (2122.5 mg l^{-1}) and highest total content (53.1 mg). On the contrary, coffees made with Leva EC machine displayed a lower concentration and a lower content of total chlorogenic acid compounds, with a concentration of 1733.7 and 1522.5 mg l^{-1} and a content of 43.3 and 38.1 mg, for Arabica and Robusta, respectively.

3.3 Method Validation

The method was validated by determining linearity, recovery at three fortification levels, repeatability and with-in reproducibility, limits of detection (LODs) and limits of quantification (LOQs) (instead of CCs alpha and CCs beta).

Calibration curves of the analyzed compounds were constructed injecting 5 µl of standard solutions at seven different concentrations, i.e. 1, 2.5, 5, 7.5, 10, 25 and 50 mg l^{-1} in HPLC/DAD. Five replicates for each concentration were performed and the relative standard deviations (RSDs) ranged from 1.10 to 1.48 % for run-to-run precision, and from 2.25 to 4.33 % for day-to day precision. All the calibration curves of the analyzed compounds showed a correlation coefficient greater than 0.99.

In the HPLC/DAD analysis of espresso coffee samples, the recoveries, obtained by spiking the beverage solution with a final concentration of 10, 20 and 50 mg l^{-1} with a standard mixture of the three chlorogenic acids, were in the range 67-99% for all analyzed compounds (Table 2).

Table 2. Percent recovery and repeatability of the method evaluated by HPLC-DAD on ECs at three fortification levels (n = 5)

Compounds	Spiked Concentration in matrix (mg l^{-1})	Recovery %	RSD%	LOD (mg l^{-1})	LOQ (mg l^{-1})
5-CQA	**200**	89	3.94		
	400	87	3.71	0.08	0.25
	1000	99	0.73		
3-CQA	**200**	88	4.93		
	400	67	4.04	0.1	0.3
	1000	80	1.46		
3,5-diCQA	**200**	97	1.3		
	400	90	0.79	0.1	0.3
	1000	99	0.15		

RSD: Relative Standard Deviation;

LOD: Limits Of Detection;

LOQ: Limits Of Quantification.

The repeatability of the method was calculated on fortified samples at 10, 20 and 50 mg l^{-1} (n = 8), giving RSD% that were in a range 1.3-3.94%, 0.79-3.25 % and 0.15-1.46%, respectively.

The Limits Of Detection (LOD) and the Limits Of Quantification (LOQ) of the three chlorogenic acids, expressed in mg kg^{-1}, calculated in the matrix, were estimated on the basis of 3:1 and 10:1 S/Ns. LODs and LOQs of chlorogenic acids were in the range 0.08-0.1 and 0.25-0.3 mg l^{-1}, respectively.

Retention time stability was utilized to demonstrate the specificity of the method. Reproducibility of the chromatographic retention time for each compound in coffee samples was examined five times over a 5-day period (n = 25). The retention times using this method were stable, with a percent RSD value ≤ 1.38%.

4. Discussion

From the results obtained, it is clear that similar trends for the concentration of CGA have been obtained for different coffee blends made with the same espresso machine, even if the total concentration of the three compounds is slightly higher in Arabica than in Robusta especially in the first three fractions. Regarding the peculiarity of the two different EC machines, the total concentration and content of the three chlorogenic acids is higher with Aurelia EC machine rather than with Leva, very likely due to differences in extraction temperature; pressure seems to be not very influent on this process. The selection of EC machine utilized in preparing coffee seems to influence the extraction of chlorogenic compounds more than the selection of blends. This data concerning the comparison of two different EC machine are, to the best of our knowledge, the first available in literature. The total concentration of the three chlorogenic acids in a cup of coffee (i.e. 25 ml as reported above) ranged from 1522.5 to 2223.4 mg l^{-1} meanwhile the total amount ranged from 38.1 to 55.6 mg. These data are of great interest considering that the consumption of coffee is a great source of antioxidants for many populations that worldwide daily consumption of coffee is currently increasing and considering the importance of these compounds for their health benefits in the prevention of many illnesses, especially related to central nervous system.

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References

Andueza, S., Maetzu., L., Dean, B., De Pena, M. P., Bello, J., & Cid, C. (2002). Influence of water pressure on the final quality of Arabica espresso coffee. Application of multivariate analysis. *Journal of Agricultural and Food Chemistry, 50*, 7426-7431. http://dx.doi.org/10.1021/jf0206623

Basnet, P., Matsushige, K., Hase, K., Kadota, S., & Namba, T. (1996). Four di-O-caffeoyl quinic acid derivatives from propolis. Potent hepatoprotective activity in experimental liver injury models. *Biological and Pharmaceutical Bulletin, 19*, 1479-1484. http://dx.doi.org/10.1248/bpb.19.1479

Bicchi, C. P., Binello, A. E., Pellegrino, G. M., & Vanni, A. C. (1995). Characterization of Green and Roasted Coffees through the Chlorogenic Acid Fraction by HPLC-UV and Principal Component Analysis. *Journal of Agricultural and Food Chemistry, 43*, 1549-1555. http://dx.doi.org/10.1021/jf00054a025

Blumberg, S., Frank, O., & Hofmann, T. (2010). Quantitative Studies on the Influence of the Bean Roasting Parameters and Hot Water Percolation on the Concentrations of Bitter Compounds in Coffee Brew. *Journal of Agricultural and Food Chemistry, 58*(6), 3720-3728. http://dx.doi.org/10.1021/jf9044606

Campa, C., Doulbeau, S., Dussert, S., Hamon, S., & Noirot, M. (2005). Qualitative relationship between caffeine and chlorogenic acid contents among wild Coffea species. *Food Chemistry, 93*, 135-139. http://dx.doi.org/10.1016/j.foodchem.2004.10.015

Caprioli, G., Cortese, M., Cristalli, G., Maggi, F., Odello, L., Ricciutelli, M., ... Vittori, S. (2012). Optimization of espresso machine parameters through the analysis of coffee odorants by HS-SPME-GC/MS. *Food Chemistry, 135*, 1127-1123. http://dx.doi.org/10.1016/j.foodchem.2012.06.024

Cetto, A. A., & Wiedenfeld, H. (2001). Hypoglycemic effect of *Cecropia obtusifolia* on streptozotocin diabetic rats. *Journal of Ethnopharmacology, 78*, 145-149. http://dx.doi.org/10.1016/S0378-8741(01)00335-X

Clifford, M. N. (1979). Chlorogenic acids – their complex nature and routine determination in coffee beans. *Food Chemistry, 4*, 63-71. http://dx.doi.org/10.1016/0308-8146(79)90031-1

Clifford, M. N., & Ohiokpehai, O. (1983). Coffee astringency. *Analytical Proceedings, 20*, 83-86. http://dx.doi.org/10.1039/ap9832000083

De Maria, C. A. B., Trugo, L. C., & Moreira, R. F. A. (1995). Simultaneous determination of total chlorogenic acid, trigonelline and caffeine in green coffee samples by high performance gel filtration chromatography. *Food Chemistry, 52*, 447-449. http://dx.doi.org/10.1016/0308-8146(95)93298-6

Fujioka, K., & Shibamoto, T. (2008). Chlorogenic acid and caffeine contents in various commercial brewed coffees, *Food Chemistry, 106*, 217-221. http://dx.doi.org/10.1016/j.foodchem.2007.05.091

Hur, J. Y., Soh, Y., & Kim, B. H. (2001). Neuroprotective and neurotrophic effects of quinic acids from Aster scaber in PC12 cells. *Biological and Pharmaceutical Buletin, 24*, 921-924. http://dx.doi.org/10.1248/bpb.24921

Illy, A., & Viani, R. (1995). Espresso Coffee: The Chemistry of Quality (pp. 24-28). In A. Illy, R. Viani (Eds.). London: Academic Press Limited.

Jiang, Y., Kusama, K., Satoh, K., Takayama, F., Watanabe, S., & Sakagami, H. (2000). Induction of cytotoxicity by chlorogenic acid in human oral tumor cell lines. *Phytomedicine, 7*, 483-491. http://dx.doi.org/10.1016/S0944-7113(00)80034-3

Kim, S. S., Park, R. Y., Jeon, H. J., Kwon, Y. S., & Chun, W. (2005). Neuroprotective Effects of 3,5-Dicaffeoylquinic Acid on Hydrogen Peroxide-induced Cell Death in SH-SY5Y Cells. *Phytotherapy Research, 19*, 243-245. http://dx.doi.org/10.1002/ptr.1652

Kwon, H. C., Jung, C. M., & Shin, C. G. (2000). A new caffeoyl quinic acid from Aster scaber and its inhibitory activity against human immunodeficiency virus-1 (HIV-1) integrase. *Chemical and Pharmaceutical Bulletin, 48*, 1796-1798. http://dx.doi.org/10.1248/cpb.48.1796

Ky, C. L., Louarn, J., Dussert, S., Guyot, B., Hamon, S., & Noriot, M. (2001). Caffeine, trigonelline, chlorogenic acids and sucrose diversity in wild Coffea arabica L. and C. canephora P. accessions. *Food Chemistry, 75*, 223-230. http://dx.doi.org/10.1016/S0308-8146(01)00204-7

Ky, C. L., Noirot, M., & Hamon, S. (1997). Comparison of Five Purification Methods for Chlorogenic Acids in Green Coffee Beans (*Coffea* sp.). *Journal of Agricultural and Food Chemistry, 45*, 786-790. http://dx.doi.org/10.1021/jf9605254

Lua, H. T., Jiang, Y., & Chen, F. (2004). Application of preparative high-speed counter-current chromatography for separation of chlorogenic acid from *Flos Lonicerae*. *Journal of Chromatography A, 1026*, 185-190. http://dx.doi.org/10.1016/j.chroma.2003.11.004

Ludwig, I. A., Sanchez, L., Caemmerer, B., Kroh, L. W., Paz De Pena, M., & Cid, C. (2012). Extraction of coffee antioxidants: impact of brewing time and method. *Food Interntional Research, 48*, 57-64. http://dx.doi.org/10.1016/j.foodres.2012.02.023

Maeztu, L., Andueza, S., Ibanez, C., Paz de Pena, M., Bello, J., & Cid, C. (2001). Multivariate Methods for Characterization and Classification of Espresso Coffees from Different Botanical Varieties and Types of Roast by Foam, Taste, and Mouthfeel. *Journal of Agricultural and Food Chemistry, 49*, 4743-4747. http://dx.doi.org/10.1021/jf0103141

Maeztu, L., Sanz, C., Andueza, S., Paz, M. P. D., Bello, J., & Cid, C. (2001). Characterization of espresso coffee aroma by static headspace GC-MS and sensory flavor profile. *Journal of Agricultural and Food Chemistry, 49*(11), 5437-5444. http://dx.doi.org/10.1021/jf0107959

Moseira, A. S., Spitzer, V., Schapoval, E. E. S., & Schenkel, E. P. (2000). Antiinflammatory activity of extracts and fractions from the leaves of *Gochnatia polymorpha. Phytotherapy Research, 14*, 638-640. http://dx.doi.org/10.1002/1099-1573(200012)14:8%3C638::AID-PTR681%3E3.0.CO;2-Q

Nagel, C. W., Herrick, I. W., & Graber, W. R. (1987). Is chlorogenic acid bitter? *Journal of the. Science of Food and Agriculture, 52*, 213. http://dx.doi.org/10.1111/j.1365-2621.1987.tb14007.x

Naish, M., Clifford, M. N., & Birch, G. G. (1993). Sensory astringency of 5-0-caffeoylquinic acid, tannic acid and grape-seed tannin by a time-intensity procedure. *Journal of the. Science of Food and Agriculture, 61*, 57-64. http://dx.doi.org/10.1002/jsfa.2740610110

Odello, L., & Odello, C. (2006). *Espresso Italiano Tasting*. Centro Studi Assaggiatori. Artigianelli Spa, Brescia, Italy.

Ohiokpehai, O., Brumen, G., & Clifford, M. N. (1982). The chlorogenic acids content of some peculiar green coffee beans and the implications for beverage quality. *Proceedings of the 10th International Scientific Colloquium on Coffee, Salvador, San Salvador* (pp. 177-185). Paris: ASIC.

Parliment, T. H., & Stahl, H. D. (1995). What makes that coffee smell so good? *Chemtech*, *25*, 38-47.

Soh, Y., Kim, J. A., Sohn, N. W., Lee, K. R., & Kim, S. Y. (2003). Protective effects of quinic acid derivatives on tetrahydropapaverolineinduced cell death in C6 glioma cells. *Biological and Pharmaceutical Bulletin, 26*, 803-807. http://dx.doi.org/10.1248/bpb.26.803

Tressl, L. R. (1977). Gas chromatographic mass spectrometric examination of phenols and diphenols in roasted coffees of various sorts. *Proceedings of the 8th International Scientific Colloquium on Coffee, Abidjan, Ivory Coast* (pp. 117-120). Paris: ASIC..

Van der Stegen, G. H. D., & Van Duijn, J. (1980). *Analysis of chlorogenic acids in coffee*. Colloque Scientifique International sur le Cafe, *9*, 107-112.