



Original Research Article

HPTLC fingerprint profile analysis of coffee polyphenols during different roast trials

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ABSTRACT

Coffee beans are a rich source of bioactive phytochemicals such as chlorogenic acids (CGA). During the roasting process, the CGA content changes dramatically. An effective HPTLC method was developed which allows a fast characterization of the eight main CGA present in green and roasted coffee beans and their distribution during roasting trials. The method was optimized with regard to three caffeoylquinic acids (CQA), two feruloylquinic acids (FQA) and three dicaffeoylquinic acids (di-CQA).

The best separation for a fingerprint consisting of eight phenolic compounds as markers was achieved on silica gel 60 F₂₅₄ HPTLC plates with a solvent mixture of diethyl ether, formic acid, acetic acid, water, acetophenone and heptane (30:3:9:50:30:10) (v/v/v/v/v/v). Staining with natural product reagent A enabled visualization and quantitative evaluation. Principal component analysis can help to visualize the evolution of the chlorogenic acids throughout the process also with the aim of controlling the roasting degree.

1. Introduction

Drinking coffee is a great socio-cultural event, which evokes a sense of belonging. A pleasant working atmosphere is associated with its living coffee culture. Coffee beverages are not only consumed for their stimulating effect but also for their attractive flavor properties like aroma and taste. Due to the high number of food conscious consumers, there is an increasing interest in fully transparency of origin, roasting parameters and brewing methods of coffee. Consequently, information about eco-physiological parameters like species, origin, variety, harvest time, and processing, become more and more important and have a significant impact on the selection of raw material. One of the most important factors for the characteristic taste are the roasting conditions, which lead to the main structural, physical and chemical changes of coffee.

One type of main compounds found in coffee beans is the large group of chlorogenic acids (CGA), which are naturally occurring in plant and plant-based beverages. CGA play an important role as therapeutics with pharmacological effects such as antioxidants, free radical scavenger and a central nervous system stimulator (Naveed et al. 2018). Coffee products are among the foods with a high proportion of these available phenolic acid compounds. CGA are bioactive components, which are

mainly composed of quinic acid and at least one group of caffeic, ferulic, or coumaric acid. Compounds of this class include nine isomers, which are the predominant polyphenols present in green or processed coffee, whereby quinic acid builds isomers in positions 3, 4, and 5 to form caffeoylquinic, dicaffeoylquinic, and feruloylquinic acids. In detail, in coffee beans three CGA groups consist of caffeoylquinic acids (3-, 4-, and 5-CQAs), di-caffeoylquinic acid (3,4-, 3,5-, and 4,5-di-CQAs), and feruloylquinic acid (3-, 4-, and 5-FQAs) with 83%, 11%, and 6%, in *Coffea arabica* varieties and 73%, 16%, and 11% in *Coffea canephora* varieties (Narita and Inouye, 2015). According to Monteiro and Farah (2012), the distribution of CGA classes is similar in all cultivars and crops. That means, a crop-to-crop variation for one cultivar may be as great as from cultivar to cultivar.

Coffee is not only consumed for its stimulating effect, but also for its pleasant bitter and sour taste. To differentiate between bitter tasting compounds and their impact on taste, Chen (1979) reported that caffeine contributed to approximately 30% and trigonelline to a maximum of 1% to the overall bitterness of coffee beverage. CGA and their accumulation in green coffee beans also contribute to coffee brew bitterness (Campa et al., 2005). Phenolic compounds like CGA can decompose upon roasting and form caffeic acid and quinic acid, which,

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primarily, confer to bitterness and astringency in coffee brew (Aree, 2019). More extensive studies done by Frank et al. (2006) identified two classes of bitter taste stimulants: chlorogenic acid lactone and hydroxylated phenylindanes, whereby both of the substances are derived from *O*-caffeoylquinic acids. Roasting can break phenolic acids down to form hydroxyhydroquinone or can epimerize and dehydrate the acids to give various chlorogenic acid lactones that provide a 'pleasant, coffee-like bitter taste quality'. This is why a precise investigation of the time when decomposition reactions take place is necessary for understanding the sensory properties of coffee brew.

The varying composition of CGA isomers is also responsible for the significant biological properties such as antioxidant activity (Preedy, 2015) and antimicrobial properties (Bajko et al., 2016). Different *in vitro* and *in vivo* methods are being used for antioxidant evaluation purpose (Ciarrelli et al., 2019), whereby it is difficult to compare one method with each. Also, antioxidant activity *in vivo* can not be led back on a single antioxidant test *in vitro*. For the present study it was important to emphasize that CGA is not only linked to sensory and taste characteristic but also to health property. Antioxidant and radical scavenging properties of CGAs are well known (Niseteo et al., 2012), but they present also other interesting biological effects (Upadhyay & Mohan Rao, 2013), such as a protective effect against cardiovascular diseases, and antihypertensive, anti-inflammatory, anti-viral, anti-fungal, immunoprotective, anti-cancer (Palmioli et al., 2017) actions. Roasting has probably the highest impact on bioactive compounds including antioxidants. A study by Opitz et al. (2017) states a general decrease of antioxidants towards slower and darker roasts, which cause heat degradation of active compounds. Bioactive compounds and their behavior are dependent on the speed, temperature and the airflow conditions of the roast (Kwak et al., 2017). Most of the published studies in the field of coffee roasting are focusing on the individual phenolic compounds between green and final roasted coffee beans.

The present study aimed to display the detailed course of the individual CGA in one-minute steps to the desired roasting degree. Four roast trials at different speeds have been described in an attempt to understand the formation and destruction of CGA isomers.

Another major aim of the present study was to demonstrate the suitability of High-Performance Thin-Layer Chromatography (HPTLC) for reliable and accurate routine analysis, not only of single compounds of interest but also of profiles of CGA isomers. This is the first time that HPTLC was used to monitor the complete coffee roast process. The results obtained with the proposed new HPTLC method were compared with results from liquid chromatography / mass spectrometry (LC-DAD/MS) and complemented by color characteristics from a roast degree analyzer.

2. Material and methods

2.1. Reagents

Chlorogenic acids (CGA) including 3-*O*-caffeoylquinic acid (3-CQA, neochlorogenic acid), 4-*O*-caffeoylquinic acid (4-CQA, cryptochlorogenic acid), 5-*O*-caffeoylquinic acid (5-CQA, chlorogenic acid), 3,4-di-*O*-caffeoylquinic acid (3,4-di-CQA), 3,5-di-*O*-caffeoylquinic acid (3,5-di-CQA), 4,5-di-*O*-caffeoylquinic acid (4,5-di-CQA), 4-*O*-feruoylquinic acid (4-FQA), 5-*O*-feruoylquinic acid (5-FQA), and caffeic acid were purchased from Sigma Aldrich Chemie GmbH (Buchs, Switzerland).

For plate impregnation, a 2.5 % aqueous potassium dihydrogenphosphate solution was used (Sigma). Derivatization reagents like 2-aminoethyl diphenylborinate (natural product reagent A, NPA), were purchased from Sigma.

The solvents acetic acid, acetophenone, diethyl ether, formic acid, and heptane were of HPLC grade and obtained from Roth AG (Arlesheim, Switzerland). HPTLC silica gel plates 60 F₂₅₄ were purchased from Merck Schweiz AG (Zug, Switzerland).

2.2. Coffee roasting and color analysis

In the present study, all coffee roasts were performed on a single batch of *Coffea arabica* L. from Columbia called Excelso Huila Supremo, a special sweet and citric sort. All samples were roasted in a 1 kg batch roaster using the FZ-94 Lab Roaster (Kaffeemanufaktur Riesen AG, Triesen, Principality of Liechtenstein). The roasting process was varied to produce a broad range of roasted coffees. Green coffee beans were roasted in four profiles: fast (10 min), fast-medium (12 min), medium-slow (14 min) and slow (21 min) (see continuous line in Fig. 1). Other roasting parameters like temperature or airflow condition were kept constant.

A large number of trial roasts were performed aiming at obtaining a similar color for all roasted coffee beans (see dashed line in Fig. 1). Samples were taken every other minute. The roast degree was analyzed with a reflection measurement system Colorette 3b (Probat-Werke von Gimborn Maschinenfabrik GmbH, Emmerich am Rhein, Germany). Previous studies have shown a high reproducibility of the roasting conditions and the trial roasts (data not shown) using the FZ-94 Lab Roaster.

2.3. Sample preparation

For evaluating the CGA composition of the coffee beans, samples were prepared according to Pedan et al. (2018) with small modifications: 20 g of the green, partially roasted and fully roasted coffee beans were frozen in liquid nitrogen before grinding to obtain a fine powder, defatting with *n*-hexane and subsequently freeze-drying. 1.0 g of the fine powder was weighed into a 50 mL centrifuge vessel and extracted three times with 3.0 mL water at 60 °C using a heating thermomixer (MHR 23, Huberlab AG, Aesch, Switzerland). For quantitative statement, the final volume was set to 9.0 mL. This procedure results in a maximum recovery for phenolic compounds (data not shown). To avoid overloading the HPTLC plate, samples were diluted 1:5 with water.

2.4. Single phenolic compounds – standard solutions

For HPTLC analysis individual standard stock solutions of 3-CQA, 4-CQA, 5-CQA, 3,4-di-CQA, 3,5-di-CQA, 4,5-di-CQA, 4-FQA and 5-FQA were prepared at 1 mg/mL in methanol. A working standard solution mixture at 0.1 mg/mL was obtained by a dilution with methanol. All solutions were stored in the dark at –20 °C. For determination of the limits of detection (LOD) and quantitation (LOQ), the working solution

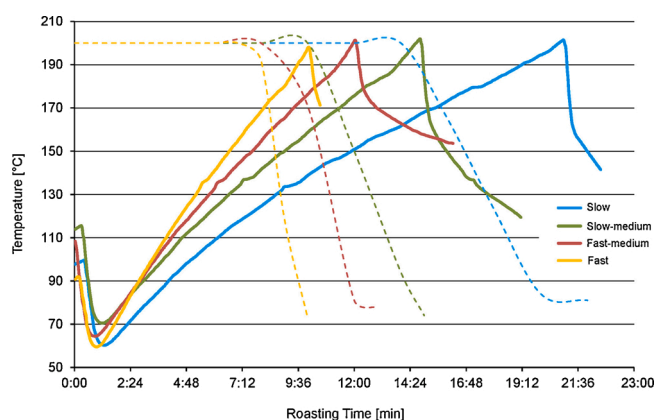


Fig. 1. Time-temperature profiles of the four different roast trials that were conducted with *Coffea arabica* beans. Coffee beans were roasted in different roast profiles from fast, fast-medium, slow-medium to slow, as it is visible with increasing roasting time illustrated as solid lines. The colored dashed lines symbolize the color gradient of the respective roasting curve measured using colorette. For the present study samples were taken after every other minute ($n = 10$ for each roast profile).

mixture was applied onto the plate to yield 100 ng to 1500 ng per zone. For quantitative analysis, linear regression was used ($R^2 > 0.99$ and $n = 2$).

For a comparative LC-DAD/MS analysis the same working solution mixture was used in a calibration range from 0.001 to 0.4 g/L ($R^2 > 0.99$ and $n = 2$).

2.5. HPTLC analysis

2.5.1. Sample application

The sample application followed Pedan et al. (2017): aqueous coffee extracts (2.0–5.0 μL) were sprayed onto 20 x 10 cm HPTLC glass plates as 8 mm bands, 8 mm from the lower edge of the plate and a distance between tracks of 11.4 mm (15 tracks per plate) with a 25 μL syringe (dosage speed 100 nL/s) using the Automatic TLC Sampler 4 (ATS 4, CAMAG, Muttenz, Switzerland). To minimize carryover effects, the syringe was rinsed twice with pure ethanol and twice with the upcoming sample.

2.5.2. Chromatography

For impregnation, the HPTLC plate was immersed into a 2.5% aqueous potassium dihydrogenphosphate solution (Immersion Device III, CAMAG) at a speed of 5 mm/s and a dwell time of 0 sec. Prior to use, the plate was dried for 20 minutes in a drying oven at 120 °C. This impregnation makes a subsequent detection of alkaloids such as caffeine and trigonelline impossible.

As developing solvent, 10 mL heptane, 50 mL deionised water, 30 mL acetophenone, 30 mL diethyl ether, 9 mL acetic acid and 3 mL formic acid were combined in a 250 mL glass bottle and well shaken. After 15 minutes, the upper organic phase was removed and kept in the dark until use.

The activity of the plate was adjusted at 33% r.H. with a saturated magnesium chloride solution. Development was performed in an unsaturated 20 x 10 cm twin trough chamber (ADC 2, CAMAG) without saturation pad. The plate was pre-preconditioned in the chamber for 5 min with mobile phase vapor before plate development and dried automatically for 5 min after plate development.

2.5.3. Plate evaluation

Scanning densitometry was used for qualitative and quantitative analysis of the target compounds. Spectra of the corresponding zones were recorded in the absorbance-reflectance mode from $\lambda = 200$ –700 nm, using the TLC Scanner 4 with *visionCATS* 2.5 software (CAMAG). The densitometric documentation for CGA measured using a mercury lamp (366 < /400 nm). Quantification was performed prior to derivatization via peak area using a linear calibration function of respective compounds. The limit of detection (LOD) and limit of quantification (LOQ) were calculated as signal-to-noise ratio of 3 and 10, respectively. Images of chromatograms were captured under white light, as well as under UV 254 and 366 nm with a TLC Visualizer 2 (CAMAG).

2.5.4. Post-chromatographic derivatization of coffee polyphenols

The developed HPTLC plate was heated at 100 °C for 3 min on a TLC plate heater (CAMAG). Natural product reagent A (NPA) was prepared by dissolving 1 g NPA in 100 mL methanol. The derivatization was carried out using a TLC Derivatizer (CAMAG) with 3 mL NPA reagent, spraying level 3, green nozzle. After derivatization, the plate was dried for 5 min at room temperature. Using NPA as derivatization reagent enables a HPTLC visualization of flavonoids and plant acids (Reich and Schibli, 2007).

2.6. LC-DAD/MS analysis

For comparison reason crude coffee extract was additionally analyzed by RP-LC-DAD-ESI/MS. Evaluation was done using LC-DAD, whereby ESI/MS was applied for identification reason only. All

discussed results are based on LC-DAD measurements. LC-DAD/MS analysis was performed on an Agilent 1200 series liquid chromatography and single quadrupole mass spectrometer with electrospray ionization interface (LC/MS 6120, G6100 series, Agilent Technologies AG, Waldbronn, Germany). The column temperature was set at 40 °C. The crude coffee extracts were analyzed using a gradient mixture of water:formic acid (99.9:0.1, v:v) (solvent A) and acetonitrile:water:formic acid (94.9:5:0.1, v:v:v) (solvent B). A 3.0 x 150 mm Eclipse XDB-C18 (3.5 μm) column (Agilent Technologies AG, Waldbronn, Germany) was used. The separation was affected using a linear gradient with a flowrate of 0.2 mL/min as follows: 2 % B at 0–0.2 min, 2–12 % B at 0.2–8 min, 12–15 % B at 8–18 min, 15–22 % B at 18–22 min, 22–24 % B at 22–27 min, 24–40% B at 27–33 min, 40–99 % B at 33–36 min, and 99 % B at 36–40 min. The re-equilibration time was 7 min and the injection volume was 1 μL .

For ESI/MS analysis, the positive capillary voltage was set at 4,000 V and the negative at 3,000 V. The drying gas temperature was 330 °C and the drying gas flow 11 mL/min. The samples were analyzed using a full scan from 100–2,000 m/z in positive ionization mode.

Retention times, UV-spectra, and characteristic fragmentation patterns were obtained using standard substances for identification of chemical components. LC-DAD/MS analysis was performed by monitoring different wavelengths. All of the CGA were detected at the maximum wavelength at $\lambda_{\text{max}} = 320$ nm.

2.7. Statistical analysis

All experimental data were statistically processed using the statistics software R (Version 3.6.2, R Core Team, 2019). Principal component analysis (PCA) was carried out using the R package FactoMineR (Le et al., 2008) in order to identify underlying structures and to characterize the temporal development. In addition, Pearson correlation was carried out to compare the results of the analytical methods HPTLC and LC-DAD/MS.

3. Results and discussion

3.1. Color analysis

Using a fast and non-destructive tool is essential to ensure high quality of a final coffee product. A first evaluation of roasting parameters was performed by color measurement. Although there is a lack of an universal standard method to classify green as well as roasted coffee beans, many coffee roasters are using Colorette as a spectrometric method for quality evaluation. In the present study, the color of the roasted coffee beans between the roast trials did not show any differences (Fig. 1). Within each profile, coffee beans were roasted to the same roasting degree. It can be described as follows: starting with a colorette value of 200 and ending up with a value of 81 for the slow roast trial, 73 for the slow-medium, 74 for the fast-medium and 78 for the fast roast. The presented roasted coffee beans belong to a medium brown roast with a slightly shiny surface (Jansen, 2006).

3.2. Quantification of chlorogenic acids

The proposed HPTLC method was found to be a fast screening tool for a comprehensive study of CGA isomers and their changes during large roast trials. The HPTLC fingerprint, which can be influenced by genotype, origin, postharvest techniques, or the above-mentioned roast profiles, can be evaluated and quantified easily (Pedan et al., 2018). However, the quantification of all compounds is not necessarily relevant for quality control. In the following, the applicability of this method was tested with a large set of different samples of varying roasting degrees. However, all coffee roasts were performed on a single batch of *Coffea arabica* beans, whereby there can be tremendous variation between varieties, origin, harvest etc. and the ability of compounds to react by

Table 1

5-CQA content determined by HPTLC. Results are expressed in milligrams per gram of the non-fat dry matter (w/w). Data has been calculated as mean \pm SD (n = 3). *Compound marked by an asterisk were determined by RP-LC-DAD detection at 320 nm. The bold entries refer to further explanations in the text. Pearson correlation r was applied to compare both methods.

Minutes [min]	Slow [mg/g]	Slow-medium [mg/g]	Fast-medium [mg/g]	Fast [mg/g]
green beans	29.1 \pm 3.2	29.1 \pm 3.2	29.1 \pm 3.2	29.1 \pm 3.2
	23.2 \pm 1.6 *	23.2 \pm 1.6 *	23.2 \pm 1.6 *	23.2 \pm 1.6 *
	27.5 \pm 6.2	27.3 \pm 4.6	26.8 \pm 4.3	28.1 \pm 3.1
2	29.6 \pm 5.3 *	33.2 \pm 3.4 *	30.9 \pm 0.9 *	38.1 \pm 1.0 *
	33.6 \pm 2.9	29.9 \pm 3.5	34.7 \pm 4.1	43.3 \pm 1.2
4	39.9 \pm 2.3 *	38.0 \pm 5.9 *	43.4 \pm 2.0 *	44.9 \pm 1.1 *
	45.1 \pm 0.7	33.6 \pm 0.3	36.3 \pm 2.5	42.5 \pm 2.6
6	43.0 \pm 2.7 *	44.6 \pm 0.4 *	46.0 \pm 1.3 *	45.9 \pm 1.7 *
	49.1 \pm 1.3	31.8 \pm 2.3	28.8 \pm 2.6	28.0 \pm 4.1
8	42.9 \pm 2.4 *	44.9 \pm 0.5 *	40.2 \pm 1.7 *	27.9 \pm 0.4 *
	34.5 \pm 2.7	24.1 \pm 0.4	19.6 \pm 1.0	1.3 \pm 0.4
10	46.2 \pm 1.1 *	36.5 \pm 0.2 *	23.0 \pm 0.4 *	4.0 \pm 0.8 *
	29.1 \pm 3.3	19.6 \pm 2.1	2.7 \pm 0.1	-
12	42.4 \pm 1.5 *	23.7 \pm 0.4 *	6.3 \pm 1.9 *	-
	18.4 \pm 2.7	7.1 \pm 1.4	-	-
14	36.7 \pm 1.5 *	8.5 \pm 1.4 *	-	-
	16.6 \pm 0.8	2.8 \pm 0.3	-	-
16	25.9 \pm 0.1 *	5.3 \pm 1.8 *	-	-
	5.1 \pm 0.4	-	-	-
18	16.1 \pm 0.2 *	-	-	-
	2.6 \pm 0.3	-	-	-
20	7.3 \pm 0.2 *	-	-	-
	1.6 \pm 0.2	-	-	-
22	4.6 \pm 3.2 *	-	-	-
Total Σ	312	205	178	172
r Correlation	0.87	0.96	0.98	0.94

high temperature. In this study, CGA were quantified using external calibration analysis. For each analyte, the peak area was plotted against the concentration of the stock solution (Table 1).

The chromatographic results are presented as images (Figs. 2 and 3). The identification of CGA was based on R_F values and color of zones obtained for standards and coffee extract. The HPTLC chromatograms indicate similarities and differences in the composition of the identified compounds during the roasting process. Blue and green zones dominate the pattern for coffee. Here, 3-CQA, 4-CQA and 5-CQA show light-blue fluorescence zones, whereby 4-FQA and 5-FQA show deep-blue fluorescence zones and the three dicaffeoylquinic acids like 3,4-di-CQA, 3,5-di-CQA and 4,5-di-CQA show light-green fluorescence zones.

Chemometric evaluation of *Coffea arabica* and *Coffea canephora* germplasm using HPTLC could identify deep-blue fluorescence zones in ascending order of the R_F as 3-CQA, 5-CQA, 4,5-di-CQA, 3,4-di-CQA, and 3,5-di-CQA (Lebot et al., 2020). The mentioned study determined three bluish fluorescence zones which remained unidentified but assumed to be minor chlorogenic acids. They eluted at the top of the HPTLC plate with high R_F .

3.2.1. Caffeoylquinic acids (CQAs)

CQA are in the presumption to contribute to the overall coffees' bitterness (Campa et al., 2005). In addition, the presence of CQA may have a role concerning the biological activity of coffee brew due to their antioxidant activity. In particular 3-CQA > 4-CQA > 5-CQA showed the highest correlations with antioxidant activity assays like FRAP (ferric reducing antioxidant power) ($r > 0.88$) (Fujioka and Shibamoto, 2008; Moreira et al., 2005).

In the present study, three major caffeoylquinic acids, present in green and roasted coffee beans, were determined by HPTLC (Supplementary Material). The major zones for CQA were detected for 3-CQA at $R_F = 0.12$, for 5-CQA at $R_F = 0.15$ and for 4-CQA at $R_F = 0.18$ as shown in Fig. 2 (a, b, c). The present study observed the highest content in all roast trials for 5-CQA > 3-CQA > 4-CQA. An initial raise of all the CQA was observed in at 8 - 10 min of roast. In detail, the concentration of 5-CQA increased subsequently by 70% within the slow roast from 29.1-

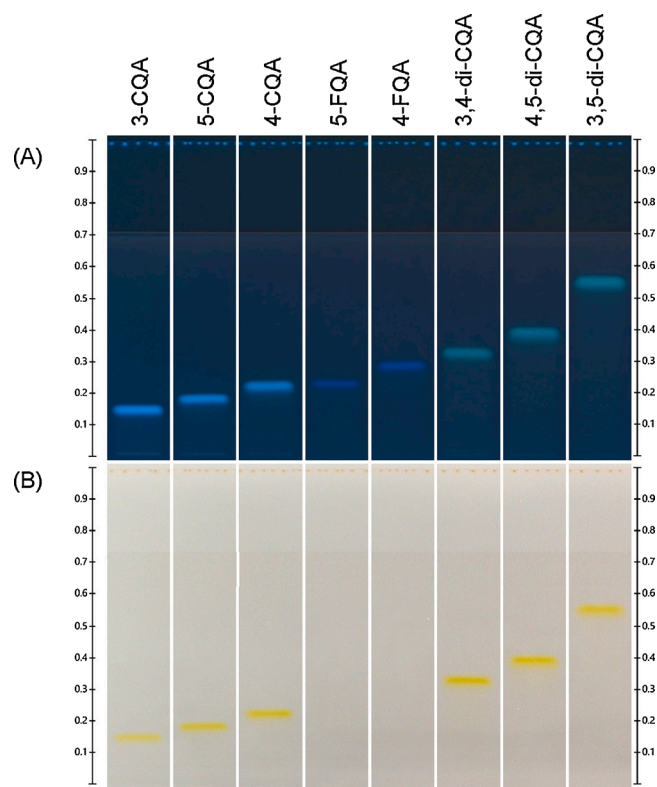


Fig. 2. HPTLC fingerprint of individual CGA. The major bands for CGA were detected for 3-CQA at $R_F = 0.12$, for 5-CQA at $R_F = 0.15$, for 4-CQA at $R_F = 0.18$, for 5-FQA at $R_F = 0.21$, 4-FQA at $R_F = 0.25$, for 3,4-di-CQA at $R_F = 0.27$, for 4,5-di-CQA at $R_F = 0.34$ and for 3,5-di-CQA at $R_F = 0.49$ and for caffeic acid at $R_F = 0.80$. (A) HPTLC plate with UV 366 nm, derivatization with NPA reagent. (B) HPTLC plate under white light, derivatization with NPA reagent.

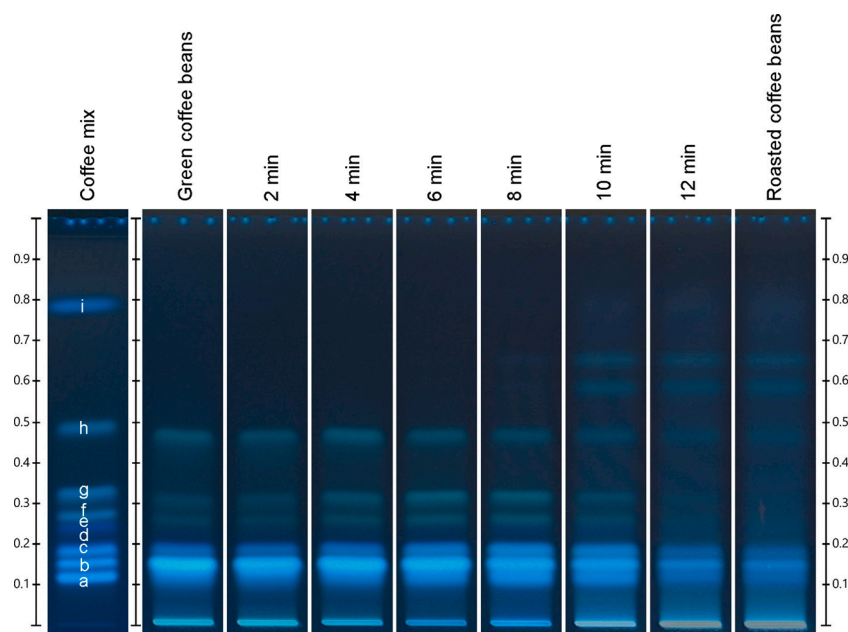


Fig. 3. Comparative HPTLC fingerprints of the whole roasting process. Left track is the coffee mixtures for CGA with the separated compounds (a) 3-CQA, (b) 5-CQA, (c) 4-CQA, (d) 5-FQA, (e) 4-FQA, (f) 3,4-di-CQA, (g) 4,5-di-CQA, and (h) 3,5-di-CQA illuminated with UV 366 nm, derivatization with NPA reagent.

49.7 mg/g at 0-8 min and decreased from 34.5-1.6 mg/g at 10-22 min (Supplementary Material). Whereas, the levels of 3-CQA and 4-CQA increased by three to four times of their original value. The reduction rate of the total CQA was calculated according to Moon et al. (2009). At the end of the roast, the reduction rate for all roast trials were > 95%. The results of the reduction rate are also in accordance to Trugo and Macrae (1984). Here they mentioned a degradation of seven CGA during roasting with a loss of about 60% when mild roasting conditions were used and almost 100% after severe roasting.

The present study was able to determine the following distribution of CGA compounds in green coffee beans with: 5-CQA (75%), 4-CQA (1.4%), 3-CQA (~7%), 5-FQA (~7%), 4-FQA (<1%), 3,4-di-CQA (1.7%), 3,5-di-CQA (3.5%) and 4,5-di-CQA (3%).

Studies done by Farah et al. (2005) determined a CQA content with about 80% of the total CGA found in green *Coffea arabica* beans as the most abundant phenolic class. In detail, 5-CQA was evaluated as the major CQA in green coffee beans with 62%, followed by the total di-CQA content with 15% and a total level of FQA represented by 5.2%. They also described a loss of CGA during the roasting process, whereby high temperatures cause a break of the C-C bond of CGA, resulting in isomerization and degradation. Also conceivable are other chemical transformations like dehydration of the quinic acid moiety and the formation of a lactone ring (Scholz and Maier, 1990). Longer periods of roast or higher temperatures results in a loss of total CGA.

The latest studies done by Farah and Lima (2019) provide a more detailed distribution about the composition CGA in coffee brew, in order of abundance is with: 5-CQA (41 - 48%), 4-CQA (20 - 25%), 3-CQA (17 - 20%), 5-FQA (4 - 8%), 4-FQA (2 - 5%), 3-FQA (1 - 4%), 3,4-di-CQA (1 - 2.5%), 3,5-di-CQA (1 - 1.5%), 4,5-di-CQA (~1%), others (< 1%).

The results of the present study are in contrast to the work done by Wei and Tanokura (2015) who show a continuous decline from time point 0 - 9 minutes of 5-CQA from 120 integral value to almost 0, as well as 3-CQA and 4-CQA from 40 integral value to almost 0. As mentioned in that cited study, the integral value is described as a signal due to caffeine, which was set to a constant of 100.

However, analysis done by Moon et al. (2009) postulated in green coffee beans, a decreasing order of CQA isomers with CQAs > di-CQAs > FQAs. They also mention that the content of 5-CQA was highest, with Ethiopian beans with 50.7 ± 1.6 mg/g to Panamanian with 40.2 ± 0.7

mg/g. Among CQA found in coffee beans, 5-CQA has also been reported as the highest content (Fujioka, Shibamoto 2008; Perrone et al., 2008). In the study done by Moon et al. (2009), percentages of 5-CQA in total CQA of green coffee beans were 73.4% for Ethiopian, 65.7% for Nicaraguan, 46.5% for Panamanian, and 65.5% in Sumatran.

Work done by Moon et al. (2009) mentions no significant differences in the content of total CQA among commercially available roasted coffee beans. However, they found high differences in total CQA in green coffee beans starting with green coffee beans from Colombia (41.6 ± 3.3 mg/g) > Mexico > Guatemala > Papua > Ethiopia > Nicaragua > Sumatra (34.4 ± 1.5 mg/g).

A study by Opitz et al. (2017) determined a continuous degradation of low molecular weight compounds starting already with mild roast conditions and a reduction by half for slow and dark roasts. The same study examined chlorogenic acids and their differences in roasted coffee beans coming from different roast trials. A recent study by Gutiérrez Ortiz et al. (2019) analyzed the roasting behavior of 3-, 4-, and 5-CQA during a period of up to 45 min. They observed a continuous decrease of 5-CQA from about 110 - 15 mg/g and a simultaneous increase of 3-, and 4-CQA from 18 - 30 mg/g at 0 - 8 min with a subsequent degradation to 15 mg/g at 8 - 45 min.

Although the concentration of CQA in green beans is higher than in roasted beans, it should be emphasized that chemical compounds do not disappear but can transform into each other. In detail, a decrease of both, quinic and caffeic acid, can be observed due to degradation of the chlorogenic acid (Preedy, 2015). Vignoli et al. (2014) mention a partial degradation of phenolic compounds during the roasting process with an accompanying formation of melanoidins and a maintenance or even increase of the antioxidant activity. They observed an increase of the antioxidant values with increasing temperature and an optimum at around 200 °C. This change might affect the biological activity of the coffee.

It should be kept in mind, that although the roasting process degrades CGA, it can form potent antioxidants like the high molecular, brown-colored melanoids, thus play an important role in the preparation of high-antioxidant low-acid coffee beverage (Kamiyama et al., 2015). The same study stated that the degree of antioxidant activity of brewed coffee is inversely proportional to the total CGA content. 70 % of 5-CQA degrades upon hydrolysis into other antioxidants like caffeic acid and

4-ethylcatechol (Fujioka and Shibamoto, 2008).

3.2.2. Feruloylquinic acid (FQA)

FQA is formed by the esterification of quinic acid and the trans-cinnamic acid derivativ ferulic acid, whereby three isomers occur as 3-, 4-, and 5-FQA. FQA were visualized after derivatization with NPA at UV 366 nm as shown in Fig. 2 (d, e) with a R_F value for 5-FQA at $R_F = 0.21$ and 4-FQA at $R_F = 0.25$. However, no yellow zones were visible under white light after derivatization with NPA for 4-FQA or 5-FQA, as it is the case for other CGA.

The ratio of the FQA isomers in green coffee beans were as followed: 5-FQA > 4-FQA with 3.2 mg/g of 5-FQA in green beans and 1.9 mg/g of 4-FQA. During the roasting process, individual FQA undergo the same changes as the CQA, with an initial increase at the beginning of the process and a drop after a certain temperature with around 160 °C. During the slow roast the content of 4-FQA almost triples to 0.62 mg/g after 12 minutes, which represents the largest change in measured CGA. However, also other roast trials observed similar alterations.

Quantification of FQA in green *Coffea arabica* beans (n = 54) showed a content of 0.45 ± 0.1 mg/g for 4-FQA and 3.06 ± 0.5 mg/g for 5-FQA (Badmos et al., 2019)

According to Preedy (2015) a total FQA content was observed for green coffee beans with 1.9 - 14.3 mg/g whereby the content decreases

during the roasting process to 0.6 - 3.4 mg/g. This result was consistent with the findings of the present study.

The antioxidant activity of CGA is well documented in various studies. Significant correlations with antioxidant activity assays like FRAP were also found for total FQA with 5-FQA > 4-FQA ($r > 0.76$) and total di-CQA with 3,4-di-CQA > 3,5-di-CQA > 4,5-di-CQA ($r > 0.80$) (Moreira et al., 2005). That study could identify the contribution of specific components to the iron-reducing activity in coffee beverages measured by FRAP. However, different *in vitro*, *in vivo* and *ex vivo* methods need to be tested to determine the contribution of specific components to the antioxidant capacity of coffee.

To sum up, many studies have shown that it is possible to separate the isomers of FQAs by HPLC. However, Jeon et al. (2017) reported chromatographic problems in the determination of FQA, where it seems to be impossible to separate 4-FQA and 5-FQA by HPLC. Nevertheless, the present study could distinguish between 4-FQA and 5-FQA using HPTLC. Although the differences of the R_F values between 4-CQA with $R_F = 0.18$ and 5-FQA with $R_F = 0.21$ are not sufficient, a better separation could be performed by immersion the HPTLC plate with NPA reagent and a further UV measurement under white light. The spots for the three CQA get visible by showing yellow zones with a sharp contour, whereby both FQA zones diminish under white light and a previous derivatization with NPA reagent.

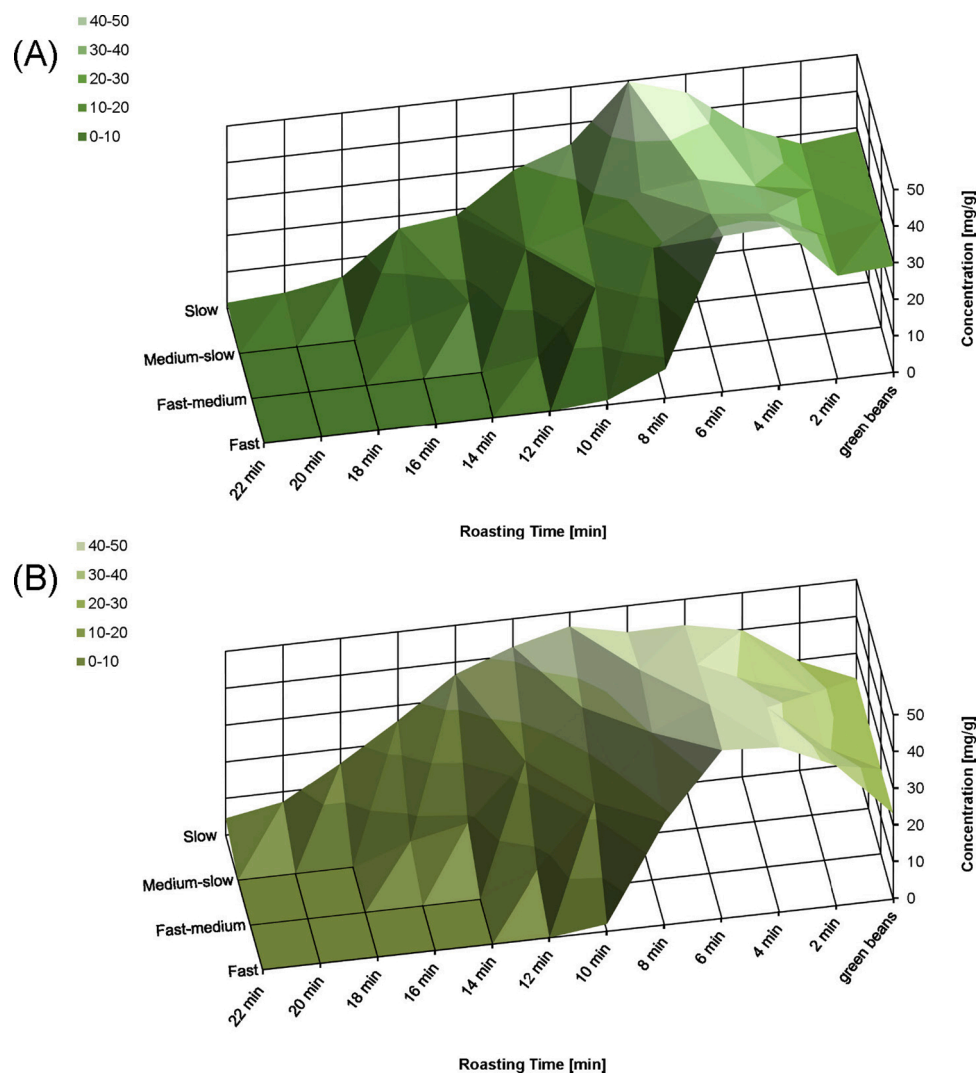


Fig. 4. (a) 5-CQA profile of the coffee roast trial depending on the four different stages of the roast process determined by HPTLC. (b) 5-CQA profile of the coffee roast trial depending on the four different stages of the roast process determined by RP-LC-DAD.

3.2.3. Dicafeoylquinic acids (di-CQAs)

The HPTLC fingerprint for dicafeoylquinic acids (di-CQAs) is shown in Figs. 2 (f, g, h) with R_F values for 3,4-di-CQA at $R_F = 0.27$, for 4,5-di-CQA at $R_F = 0.34$ and for 3,5-di-CQA at $R_F = 0.49$. The present study observed the highest content in all roast trials for 4,5-di-CQA > 3,5-di-CQA > 3,4-di-CQA. During the slow roast 4,5-di-CQA increases from an initial content of 1.7 mg/g in green coffee beans to 2.7 mg/g from 0-8 min and decreased to 0.04 mg/g in the final product. With a reduction rate of > 97%, 4,5-di-CQA undergoes the greatest change during roasting. Same behavior was observed for 3,5-di-CQA with an initial content of 1.8 mg/g in green coffee beans and a moderate increase to 1.9 mg/g during 0-6 min with a subsequent descent to 0.7 mg/g. Here, the reduction rate for 3,5-di-CQA was about 60%.

The study done by Moon et al. (2009) exhibited for 3,4-di-CQA a content of 0.60 mg/g for Ethiopian and 0.94 mg/g for Papuan green coffee beans. The same study conducted for 3,5-di-CQA a content of 1.14 mg/g for Sumatran and up to 2.35 mg/g for Colombian coffee beans. The content for 4,5-di-CQA was determined with 0.90 mg/g for Nicaraguan and 1.43 mg/g for Colombian coffee beans.

Other researchers detected a content of 0.1 - 0.2 mg/g for 3,4-di-CQA, 0.2 - 0.6 mg/g for 3,5-di-CQA and 0.2 - 0.4 mg/g for 4,5-di-CQA in raw *Coffea arabica* beans (Debry, 1994).

To underline the significance of di-CGA Liang et al. (2016) mentioned that they might contribute to different taste qualities, such as bitter/metallic taste found in certain coffees. With respect to the taste profile of coffee, particularly Robusta coffees, or blends of coffees that contain a proportion of Robusta beans hence higher amounts of di-CGA.

In a cultured gastric epithelial model, multiple CGA isomers showed intact transfer across the gastric barrier at an acidic apical pH, with di-CQA having a relatively higher permeability coefficient compared to CQA (Farrell et al., 2011).

In addition, the two major species can be differentiated by their CGA content, whereby each single compound such as 3-CQA, 5-CQA, 3,4-di-CQA, 3,5-di-CQA, 4,5-di-CQA presented higher values in *Coffea canephora* (Lebot et al., 2020).

3.3. Method comparison

The CGA content were determined and compared by HPTLC

densitometry and LC-DAD/MS. The quantitative results of both analytical methods did not show any statistical differences. As a matter of fact, the correlation between both methods was notably high, as revealed by Pearson correlation with $r > 0.87$ for 5-CQA during the slow roast e.g. (Supplementary Material). However, with respect to the total di-CQA content, HPTLC determined a content of 3.75 mg/g which is twice as high as measured using LC-DAD/MS with 1.67 mg/g for green beans. For LC-DAD/MS the total di-CQA can be further subdivided into 0.4 mg/g for 3,4-di-CQA, 0.84 mg/g for 3,5-di-CQA and 0.42 mg/g for 4,5-di-CQA.

Both methods could be equally used to determine valuable compounds present in green or roasted coffee beans, respectively their changes during roasting process. As shown in Fig. 4, the course of 5-CQA did not differ over the entire roasting process, whether measured by HPTLC or LC-DAD/MS. Therefore, HPTLC densitometry and LC-DAD/MS were found to be equal and could be used for routine analysis of CGA in coffee. It can be highlighted that HPTLC has specific advantages, like simultaneously running of several samples and standards on one plate causing better analytical accuracy as compared to LC-DAD/MS, whereby one sample after the other can be analyzed. HPTLC provides a good separation and enables a visual inspection of compounds of interest. However, both methods are not in competition with each other. HPTLC can be considered as a complementary or even alternative analytical tool to HPLC.

3.4. Principal component analysis

To better understand the interaction between roasting process and chemical composition data, the present research used PCA as a multivariate data tool (Fig. 5). The PCA was applied using a matrix of the four coffee roasts fast, fast-medium, medium-slow and slow with the individual amounts of the nine phenolic compounds as variables. The first two principal components explained 78.2% of the total variance with $PC1 = 64.1\%$ and $PC2 = 14.1\%$. Each point in the score plot represents the coffee bean from one of the roast to time combinations, with f_0 representing the fast roast at 0 minutes, f_2 representing the fast roast at 2 minutes. The coffee roasts tended to group according to roasting time. The roast degree can be projected as a trajectory moving according to the roasting process.

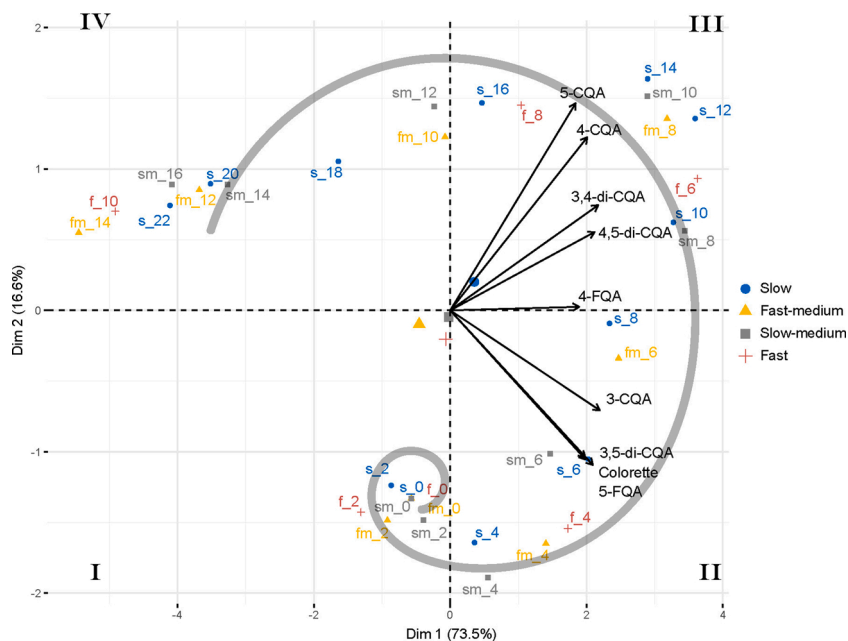


Fig. 5. The PCA chart shows the relationships between roast and time for the four coffee roast trials. The initial letter marks the roast profile with s = slow roast, sm = slow-medium roast, fm = fast-medium roast and f = fast roast, whereby the listed number marks the roast time in minutes.

The first quadrant (see label I in Fig. 5) characterizes the samples within the beginning of the roast process with f_0, f_2, fm_0, fm_2, sm_0, sm_2, f_0 and f_2. None of the phenolic compounds are loaded in the first quadrant. The second quadrant is characterized by the samples f_4, fm_4, fm_6, sm_4, sm_6, s_4 and s_6, which represent coffee samples at temperatures about 110 °C. Here, the negatively loaded 3-CQA, 3,5-di-CQA and 5-FQA and the color value evaluated by colorette are plotted with each other. This observation indicates a high correlation with the phenolic compounds and the color value mentioned. The third quadrant is characterized by 5-CQA, 4-CQA, and 3,4-di-CQA, 4,5-di-CQA and 4-FQA. Here, higher temperatures were applied to the coffee beans about 140 °C. The fourth quadrant is characterized by the samples f_10, fm_10, fm_12, fm_14, sm_12, sm_14, sm_16, s_18, s_20, s_22, whereby all these samples were processed at about 190 °C. No phenolic compounds are loaded in the fourth quadrant.

The individual ingredients of each roast create a trajectory in the two-dimensional space that starts in the first quadrant and moves across the second, third and fourth quadrants. This is shown as a grey line in Fig. 5. Within the trajectory, the beans started from an initially light roast by low temperatures towards high roasts with high temperatures.

A study done by Wieland et al. (2012) observed the same dynamic behavior in a 3D PCA space for volatile organic compounds during the roasting process. They stated that a time-resolved analysis provides a detailed picture of the evolution of the roasting process and allows establishing a real-time process control tool that ensures highest consistency of the roast degree.

In addition, Fig. 5 provides the centroid as the mean for each variable for the four different roast trials, which are very close together. This is in accordance with the primary interest of the present study to develop different roasting profiles that produce a similar coffee quality.

Research done by Velásquez et al. (2019) about volatile and sensory characterization of roast coffees used also statistical analysis to distinguish between different stages of maturity. Based on PCA, sensory or volatile differences existed among fully immature coffee beans and all other maturity stages (yellow-green to overripe).

4. Conclusion

The results of the present study emphasize the roasting process as an important manufacturing step with its high impact on the CGA content. However, high level of CGA are also associated with a low-quality coffee beverage (Farah et al., 2006). To retain the balance between health beneficial phenolic compounds and sensory attributes, optimization of roasting condition specific to the coffee variety are required. For these reasons, analytical tracking of phenolic components during the processing is important.

The present study underlines the usefulness of HPTLC as a reliable tool to assess quality and quantity parameters of the coffee roasting process. The established method is a comprehensive tool for compound analysis in laboratories with high sample throughput. The fingerprints obtained are useful for quality control of coffee beans, the influence of roasting parameters of bioactive compounds and can help to estimate traceability.

CRedit authorship contribution statement

Vasilisa Pedan: Supervision, Data curation, Writing - original draft. **Elisa Stamm:** Visualization, Investigation. **Tiën Do:** Conceptualization, Methodology, Software. **Mirjam Holinger:** Software, Validation. **Eike Reich:** Conceptualization, Writing - review & editing.

Declaration of Competing Interest

There is no conflict of interest. The manuscript was approved for publication by all authors.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jfca.2020.103610>.

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