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Flow Injection Analysis for the Direct Quantification of the Total Polyphenol Content in Coffee Brews

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Abstract

A flow-injection analysis (FIA) procedure with colorimetric detection was developed, to determine the total polyphenol content (TPP) in coffee brews with no need for sample work-up. A comparison of three different officially accepted colorimetric methods was performed and the Folin-Ciocalteu reaction was found to be the most suitable assay with respect to sensitivity, selectivity, lack of matrix effects and cost. Furthermore, the FIA setup adopted here was characterized by its simple experimental design, relatively inexpensive equipment and for providing results that are excellent in view of rapidity, accuracy and precision. FIA key parameters were optimised for sensitivity, linearity, working range and matrix effects, and calibrated against gallic, caffeic, ferulic and tannic acid equivalents. The method was successfully tested against false-positive adsorptions from non-polyphenol components. The method was applied first to brews from two different *C. Arabica (Coffea arabica L.)* and *C. Robusta (Coffea canephora var. robusta L)* coffees (light roast). *Robusta* showed 25% higher TPP content compared to *Arabica*. Secondly, coffees roasted to three different roast degrees, each along three different time-temperature roasting profiles, were compared: High-temperature with short roasting times showed higher TPP content in the brew than low-temperature with longer roasting times, an observation that indicates the potential for optimising the roasting process to increase polyphenol content.

Keywords Coffee, Polyphenols**,** Antioxidants, Flow Injection Analysis (FIA), Folin-Ciocalteu method (FC), Folin-Denis method (FD), 4-Aminoantipyrine method (4-AAP), Roasting, *Arabica*, *Robusta*

1. Introduction

Epidemiological studies are increasingly revealing the positive benefits of coffee consumption for our health and wellbeing. Indeed, there is mounting evidence that a moderate consumption of 3 to 5 cups of coffee per day has protective effects against the development of certain neurological disorders, such as Parkinson's diseases [1], inflammatory and cardiovascular diseases [2], type 2 diabetes [3], liver cirrhosis [4], and various types of cancers [5]. The question that arises here is whether these benefits can be linked to specific ingredients in the coffee brew. A serving of coffee may contain up to four times as much antioxidant activity as a serving of green tea [6], and coffee is the major source of antioxidants in many peoples diet [7]. Polyphenols, the major group of antioxidants in coffee, have been reported to exert a variety of biological actions, such as free radical scavenging, metal chelation, modulation of enzymatic activity and, more recently, effects on signal transduction, activation of transcription factors and gene expression. In coffee, the most abundant polyphenols are chlorogenic acids (CQAs). There are several factors that affect the variability of polyphenol content in coffee. E.g. in green coffee, the CQA content is higher in *C. Robusta* than in *C. Arabica* [8]. Also, processes such as roasting [9] and brewing affect the final CQA content in the brew to varying degrees.

Linking these observations, there is reasonable evidence to suggest that the reported health benefits of coffee are a consequence of its exceptionally high polyphenol content. This has tentatively been traced back to compound-classes such as chlorogenic acids [10], which are most abundant in the green bean and degrade during roasting, and to Maillard reaction products (melanoidins), which are formed during roasting [11]. Yet, the metabolic pathways responsible for the benefits, stability and bioavailability still need to be researched further [12].

Several analytical procedures with different detection systems were developed to detect identify and quantify phenolic compounds, including: liquid chromatography [13], capillary electrophoresis [14], voltammetry [15], amperometric analysis [16], batch spectrophotometry [17] and flow injection analysis with colorimetric detection [18-20]. These methods can be divided in two classes: specific/targeted methods and global/untargeted methods.

Specific methods - detailed chemical characterisation: In order to identify and quantify individual polyphenols, chromatographic methods using separation, detection, identification and quantification can be applied. Yet, the chemical diversity of phenolic compounds makes such a detailed analytical approach challenging and some polyphenols may be missed by such a targeted approach. Hence, chromatographic procedures will only be implemented if the exact chemical nature of the polyphenols is relevant.

Global methods - Total Polyphenol Content (TPP): Alternatively, if one is interested in the total polyphenol content, global and untargeted methods are more appropriate, both from the perspectives of time and cost. This is particularly valid if they can be applied directly to the brew, eliminating sample work-up. Several colorimetric methods such as the Folin-Ciocalteu [21], the Folin-Denis [22] and the 4-aminoantipyrine methods [23] have been used to determine the presence of polyphenols in various food products. The FC method has been adopted as an official procedure for determining total phenolic levels in wine; the *Office International de la Vigne et du Vin (OIV)* [24].

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Similarly, the FD assay is one of the most widely used procedures for quantification of total phenolics in plant materials, fruits and beverages. The 4-AAP method is mainly used for the determination of the "phenol index" in water and wastewater (Environmental Protection Agency Method Method 9066) and was recently used for the determination of polyphenols in wines and beer [20, 25].

Colorimetric methods are calibrated against a standard polyphenol [26], gallic acid (GA) often being used as a universal reference standard due to its good solubility, high stability and low price.

The aim of the presented project was to develop a simple and robust method for the determination of the TPP content in coffee brews, without the need of a workup procedure. More specifically: **(i)** test a series of official colorimetric method and calibration equivalents, in order to select and optimize the most suited system for measuring the total polyphenol content (TPP) directly from the coffee brew; **(ii)** convert the experimental steps performed in the course of a typical colorimetric assay from a manual batch procedure to automated operations; and **(iii)** demonstrate the potential of the optimized method on two applications in the field of coffee (different coffee varieties and impact of processing).

To meet the quest for speed, improved reproducibility and higher automation a Flow Injection Analysis (FIA) setup was adopted and optimized for these investigations. The key parameters of the FIA system, the selectivity and sensitivity to polyphenols, were optimized and implemented for the quantification of the total polyphenol (TPP) content in different coffee brews. Selectivity is mainly related to the absence of false positive responses. Sensitivity is mainly affected by two kinetic processes that occur simultaneously - the *physical* process of sample dispersion and the *chemical* process resulting from reactions between the analyte and reagent [27] – and related to the spectroscopic absorption cross section of the reaction product at the adopted wavelength.

2. Material and Methods

2.1 FIA Setup

A 4 channel Ismatec® IPC (High Precision Multichannel Dispenser) peristaltic pump, with a low pulsation pump-head, fitted with 8 rollers and operated with color-coded Ismatec® pump tubes (ENE 10 tubes i.d. 0.95 mm, colour code white/black) was used to propel the solutions. A second peristaltic pump (4 channels Ismatec® MS-4 Reglo) was used for continuous dilution of coffee samples. A 6 port KNAUER rotary valve with Rheodyne sample loop (20, 50, 100 and 200 µL) was used as the injector and flow lines were made of PTFE tubings (i.d. 0.8 mm). The spectrophotometric readings were made by means of a single-beam UV/VIS Perkin Elmer Lambda 1 spectrophotometer. A set of three Hellma® flow-through quartz cells (resistant to coffee acids and high temperatures) cells were used as the detection system. The optical path-length of each cell was 10 mm and the chamber volume varied between 8, 30 and 390 µL. The photometer was interfaced with a PC through an IOtech (Personal Daq/3000™) Multifunction Data Acquisition Module. The software used was DASYLab® (Data Acquisition System Laboratory). A schematic representation of the FIA unit is shown in Figure 1.

Based on the system outlined in Figure 1, three different reaction systems were tested: the Folin-Ciocalteu (FC), the Folin-Denis (FD) and 4-Aminoantipyrine (4-AAP). Optimisation and selection of the most suitable method was based on the following criteria: the methods shall allow quantifying of the extract without sample work-up, at the highest possible sensitivity and with the least amount of interference (false-positive responses).

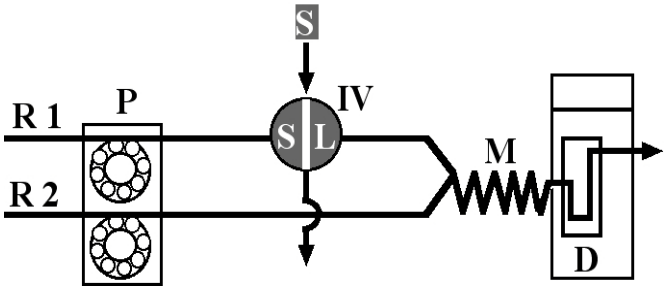

Figure 1. Schematic diagram of the Flow Injection Analysis (FIA) system. (P) Peristaltic pump, (R1) (R2) Reagents, (S) Sample, (SL) Sample Loop, (IV) Injection Valve, (M) Mixing Coil, (D) Detector; flow-through cell in photometer, (DAQ) Data Acquisition

2.2 Folin-Ciocalteu (FC) and Folin-Denis (FD) procedures

The active reagent used in both methods consists of a yellow acidic solution containing complex polymeric ions formed from phosphomolybdic and phosphotungstic acids. The sample is first injected into a carrier stream (Folin-Ciocalteu's Phenol Reagent or Folin- Denis' Reagent) and then merged with a second reagent stream, comprised of an alkaline NaOH solution. The coloured product of the reaction has a broad absorbance peak and may be measured at any setting between λ = 700-800 nm. Referring to Figure 1, R1 is the Folin-Ciocalteu or Folin-Denis and R2 the NaOH solution.

2.3 4-Aminoantipyrine (4-AAP) procedure

The sample is first injected into a carrier stream (4-AAP) and then merged with a second reagent stream, comprised of a buffered potassium hexacyanoferrate (III) solution. The coloured product of the reaction is measured at λ = 520 nm. Referring to Figure 1, R1 is the 4-AAP solution and R2 the buffered potassium hexacyanoferrate(III) solution.

2.4 Reagents and solutions

The Folin-Ciocalteu's Phenol Reagent (2 M), Folin-Denis' Reagent, 4-aminoantipyrine, potassium ferricyanide, sodium hydroxide, Patent Blue V, gallic acid monohydrate, tannic acid, caffeic acid, ferulic acid and all other chemicals were purchased from Sigma Aldrich and were of analytical grade. Furthermore, the following solutions were prepared:

Folin-Ciocalteu reagent working solution (0.2 M) was prepared by tenfold dilution of the commercial Folin-Ciocalteu's Phenol reagent solution (Sigma Nr. 47641). *Folin-Denis* reagent working solution was prepared by fourfold dilution of the commercial Folin-Denis' reagent solution (Sigma Nr. 47641). *Alkaline solution (FC):* 10 g/L NaOH. *4-Aminoantipyrine* (4-AAP) aqueous solution: 0.65 g/L. *Buffered potassium ferricyanide* (4-AAP) solution was prepared by dissolving 2.0 g potassium ferricyanide, 3.1 g boric acid, and 3.75 g potassium chloride in 800 mL water. The pH was adjusted to 10.3 with 1 N sodium hydroxide and diluted to 1 L. *Gallic- caffeic-, ferulic-, and tannic acid standard solutions*: Standard solutions were prepared daily by appropriate dilution of a stock solution

(1.0 g /L). Calibration standards for the respective working concentration ranges were prepared as dilutions of the respective stock solutions. *Dye (Patent Blue V)* solution (2 mg/L) was used to evaluate the dispersion.

2.5 *C. Arabica* and *C. Robusta* coffee brews (application 1): The two different *C. Arabica* and *C. Robusta* coffees for TPP content measurement were prepared as follows. Coffees were roasted in a 100 g capacity BRZ Probat sample roaster at Strauss Commodities AG in Zug/CH to a very light (roast degree ≈ 124 on a Colorette 3b from *Probat*) and to a light roast degree (Colorette ≈ 109). Approximately 15 g of roasted beans were ground in a Ditting Espresso grinder KED 640 (Setting: 8). Exactly 12 g of ground coffee were placed in a French Press from Bodum (cylindrical glass urn fitted with a metallic filter mesh on a plunger). 190 mL of hot (90 °C) and softened water (Britta Purity 600 Quell ST, 4 ° carbonate hardness) was added to the grounds in the bottom of the urn, the coffee was left to sit for two minutes (extraction). Then the plunger was pressed down, separating the grounds from the liquid coffee extract. After two additional minutes, the coffee extract was poured out to stop the extraction process. The freshly brewed coffee was poured into a 100 mL bottle and cooled in a water-bath tempered to 25 °C. The coffee was then diluted to fit the working range and used for FIA analysis. All coffee brews were measured with 5 repetitions, for each repetition the whole sample preparation procedure was repeated with different roasted coffee beans from the same roasting batch. The average and the relative Standard Deviations (*RDS* %) for TPP content were around 1.6 % p = 95% for all coffees, expressed as Gallic Acid Equivalents (**GAE**).

2.6 Profile roasting (application 2): 15.6 kg green *Arabica* coffee from Guatemala were roasted by Bühler AG in Uzwil/CH on a 15-20 kg hot-air Petroncini *TT 15/20* roaster to a light, medium and dark roast degree, respectively. The roast degree was measured by a Minolta Chroma Meter (DP-301/CR-300) and expressed in "Colorette" via the formula: Colorette = f (Minolta) = 17.62x - 619.37 (R^2 = 0.992); x is the reading on the Minolta scale. To vary the roasting time at a fixed roast degree, the hot air inlet temperature (HAIT) was varied between low, medium and high, so as to achieve for each roast degree, roasting times of approx. 1200 s, 870 s and 570 s, respectively. Each profile was roasted in five repetitions. After roasting, the beans were discharged from the roaster, air-cooled to approximately room temperature, packaged under an inert atmosphere in 1 kg bags and stored at room temperature until needed for the measurement. For each determination of the TPP content in the brew, a 1 kg bag of roasted coffee was opened. Exactly 48 g of freshly ground coffee beans (Ditting KED 640, Degree 9.0) were placed in a French-Press (Bodum) and extracted with 800 g of softened water at 90 °C. From this point on, exactly the same protocol was followed for the sample preparation as for the Arabica and Robusta samples in the first application.

3. Results and Discussion

In a typical FIA method, a large range of often interrelated experimental parameters can be optimized, and there is no universal protocol to convert a batch procedure to a FIA method. In theory the application of a sophisticated multivariate method could be the method of choice, but in practice a knowledge based "one-factor-at-a-time" approach is usually the most practical solution and was applied here.

The various optimisation, validation and calibration steps involved in the development of the FIA method are described next. This includes, firstly, the optimisation of the FIA manifold design. Aspects like dispersion, sensitivity, linearity and expected matrix effects were investigated. Since the physical parameters of the experimental setup are identical for all three methods (FC, FD, 4-AAP), the optimisation of the main parameters is achieved based on the FC method using gallic acid (GA) as a reference standard. Throughout the following optimisation process, the concentration of the standard GA solution, the experimental temperature and the detection wavelength were fixed as follows:

Table 1.
Optimisation of the FIA manifold design

		Fixed Parameter			
Investigated Parameter	**Range**	**Flow Rate (mL/min)**	**Injection Volume (µL)**	**Flow-through Cell Chamber Volume (µL)**	**Reaction Coil Length (cm)**
Flow-Rate	**0.5-4 mL/min**		50	30	50
Flow-through Cell Chamber Volume	**8-390 µL**	2	100		50
Reaction Coil Length	**50-200 cm**	2	100	30	

Concentration of GA standard solution = 60 mg/L; Temperature = 22 °C – 23 °C; Wavelength λ = 765 nm.

The most significant experimental parameters for achieving maximum sensitivity that affect the dispersion are the flow-rate, the injection volume, the volume of the flow-through cell chamber and the length of the reaction coil. In order to find the optimal conditions affecting the zone dispersion and chemical reaction of each one of these four settings a set of optimization trials was carried out, applying the parameters summarised in Table 1.

3.1 Optimized Parameters

Based on the results obtained from the optimisation experiments, the experimental conditions for the FIA setup were chosen as follows: (**i**) flow rate: *2 mL/min*; (**ii**) sample volume introduced to the system: *100 µL*; (**iii**) flow-cell chamber volume: *30 µL*;

(**iv**) reaction coil length: *100 cm*. Using these conditions, the sample throughput was approximately 110 samples per hour.

3.2 Experimental Dispersion Coefficient

The experimental dispersion was measured using a Patent Blue V (2 mg/L) solution (detection at λ 632 nm) [27]. No chemical reaction is involved in these experiments and only the physical process is taken into consideration. The dispersion coefficient of the optimised FIA system was found to be 4.9, +/- 1% $_{(n\ 10,\ p\ 95\%)}$ *RSD*. This value can be considered satisfactory according to the widely accepted classification proposed by Růžička [27] and indicates that the mixing between the sample solution and the carrier stream (reagent) is adequate.

3.3 Comparison of Colorimetric Determination Methods

Based on the optimised FIA experimental conditions, the **FC**, **FD** and **4-AAP** methods were compared. Gallic (**GA**)-, caffeic (CA)-, ferulic (**FA**)-, and tannic-acid (**TA**) were used as total polyphenol equivalent chemical standards (**STD**).

Table 2. Linear working ranges and equations for the calibration curves

Method	STD	Working range (mg /L)	Equation	R^2
FC	**GA**	**0.5-80**	**y=0.0116x - 0.0118**	**0.999**
	CA	**0.5-70**	**y=0.0124x - 0.0020**	**0.998**
	FA	**0.5-70**	**y=0.0104x + 0.0092**	**0.998**
	TA	**0.5-70**	**y=0.0105x - 0.0191**	**0.999**
FD	**GA**	**0.5-80**	**y= 0.0115x - 0.015**	**0.998**
	CA	**0.5-70**	**y= 0.0100x + 0.008**	**0.998**
	FA	**0.5-70**	**y= 0.0103x + 0.019**	**0.992**
	TA	**0.5-90**	**y = 0.0089x - 0.004**	**0.999**
4-AAP	**GA**	**5-600**	**y= 0.0004x + 0.0042**	**0.999**
	CA	**5-400**	**y= 0.0009x - 0.0003**	**0.998**
	FA	**0.5-140**	**y= 0.0054x - 0.0111**	**0.997**
	TA	**5-600**	**y = 0.0005x + 0.0102**	**0.996**

As outlined above, these FIA optimised conditions were based on the FC method and the GA standard. For the following comparison between the three colorimetric methods (FC, FD, 4-AAP), these optimised settings were applied throughout the experiments. The only difference was the detection wavelength: for both the FC and FD methods, the detection wavelength was λ 765 nm, while 520 nm was used for the 4-AAP procedure.

3.3.1 Folin methods

It has been stated that the Folin-Ciocalteu reagent is interchangeable with the Folin-Denis formulation [21]. Yet, in the context of this specific study of direct analysis of coffee brews, and without prior work-up of the extract, it appeared important to revisit this question, and check whether both the FC and FD methods are indeed equivalent. Calibration curves, determined with the FC and FD method showed linearity in the range of 0.5 - 80 mg equivalent chemical/L, as summarized in Table 2 (The standard solutions were analysed in triplicate).

3.3.2 4-AAP method

Calibration curves (range 0.5 - 60 mg equivalent chemical /L) determined with the 4-AAP method showed an approximately tenfold lower sensitivity compared to the Folin methods (Tab. 1) For the application and experimental setup suggested in this work, the FC method was found to be on par with the FD method, as shown in Table 1. In terms of standardisation, both Folin procedures are equivalent. Yet, in economic terms, the cost of the reagent per measurement for the FC method is lower. The precision was assessed by percentage of relative standard deviation (% *RSD*) of repeated measurements at two concentrations of the GA standard solutions. At the concentrations of 10 mg GA/L and 60 mg GA/L, the % $RSD_{(n=10,\ p=95\%)}$ was 1.8 and 1.5 respectively. Based on the above discussion, the FC method was selected as being the most appropriate one.

3.4 Interferences

To validate that the method does indeed measure the TPP in the brew, three types of tests were conducted.

3.4.1 Absorption without Addition of Reagents

A potential interference in UV-visible spectrophotometry is the absorption of non-polyphenol compounds in the brew at the probing wavelength that may obscure the detection of the PPT signal in the sample, in particular if no sample work-up is performed. In order to evaluate whether such interference is indeed occurring, tests were run with an espresso brew at two different signal wavelengths; λ 730 and 765 nm, and at various dilutions of the extract, without the addition of reagents.

Table 3. Absorbance of diluted espresso coffee

Dilution	λ 730 nm	λ 765 nm
10 x	0.048	0.035
20 x	0.030	0.020
40 x	0.022	0.009

As shown in Table 3, at λ 765 nm, a 40-fold dilution of the sample is sufficient for the intrinsic absorbance of the coffee brews to be negligible, relative to the TPP signal (below 1%) in the same brew, and therefore no correction measurements of background absorbance were needed. Hence, subsequent measurements were performed with at least a 40-fold dilution.

3.4.2 Matrix interference

Interference with the colorimetric reaction from compounds, present in coffee beverages (matrix) was evaluated by determining the recovery rate of samples spiked with a known concentration of analyte. The recoveries were determined by adding low, medium, and high levels of gallic acid to diluted coffee samples. The procedure was as follows: *1)* 1 mL gallic acid standard solution was added to 100 mL coffee sample → absorbance response = (AR_1). *2)* 1 mL water was added to 100 mL coffee sample → absorbance response = (AR_2). 3) 1 mL gallic acid standard solution was added to 100 mL water → absorbance response = (AR_3).

The added standard solution leads to a concentration increase of 10, 20 and 40 mg GA/L with recovery rates of 102.6±2.1 %, 102.0±1.2 % and 103.2±1.8 % respectively.

The recovery rates were calculated according to Eq. 1.

$$RR(\%) = \frac{(AR_1 - AR_2)}{AR_3} 100\% \quad \text{(Eq. 1)}$$

The measured recovery rates reflect no (or negligible) influence of the sample matrix on the adopted determination method.

3.4.3 Possible interactions from non-polyphenols

The colour contribution of selected non-polyphenolic coffee compounds in the Folin-Ciocalteu reaction was evaluated by measuring solutions of such compounds in coffee at a concentration of 50 mg/L. The results revealed no interaction from citric acid, acetic acid, quinic acid, sucrose, trigonelline-hydrochlorid or caffeine.

To achieve satisfactory results, it is recommended that calibration is performed at least once a day. The calibration run is followed by analytical runs, then, after a fixed interval of measurements, calibration is repeated.

3.5 Applications

Two applications of the optimised and validated method will be discussed. *First*, the TPP content in two *coffea arabica* (*Arabica*) and two *coffea canephora* (*Robusta*) coffees were evaluated. *Secondly*, Guatemala *Arabica* coffee beans were roasted using three different time-temperature roasting profiles (fast, medium and slow roasting), and the TPP content of the brews were determined for each.

3.5.1 Application 1 - Robusta and Arabica

The TPP content of four coffee brews, prepared from two Arabica and two Robusta green coffee varieties were measured (see Figure 2).

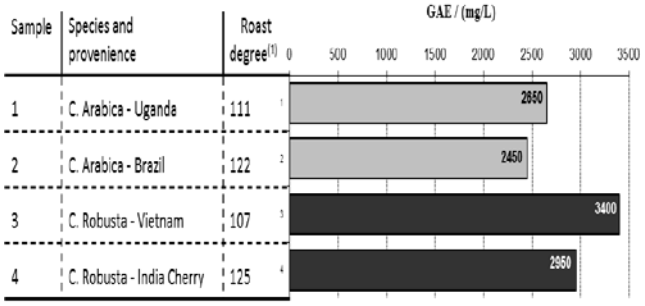

Figure 2. Sample characteristics and TPP content in different coffee brews. The values correspond to the average of 5 repetitions, with an RSD of 1.6 % $_{(p=95\%)}$ for all coffees.
(1)The roast degree was measured using a Probat Colorette 3b

Referring to published work [6, 9, 28], the polyphenols and the concomitant high antioxidant activity in green coffee is mainly attributed to CQA. The total CQA content in green *Robusta* coffee is as high as 8-10%, while *Arabica* has approximately 6% CQA. Although the CQA content of the beans decreases during roasting [9], the antioxidant activity is reported to initially increase up to a medium roast degree. Medium roasts were reported to produce coffee with maximum in vitro oxygen scavenging and chain breaking activities [29]. This difference was observed despite a 20% and 45% decrease in the CQA content of light and medium roast coffees respectively, implying that other compounds make important contributions to the total antioxidant activity of roasted coffee.

Melanoidins are brown polymers, rich in polyphenol functionalities, formed by the Maillard reaction during the roasting of coffee beans, and account for up to 25% of the dry matter in medium to dark roasted coffee. It has been shown that coffee melanoidins have significant in vitro antioxidant activity. Furthermore, there is evidence that a fraction of the CQAs that "disappear" during roasting are integrated into the melanoidins [30, 31], hence, the two most significant polyphenol compound families in roasted coffee are the CQAs and the melanoidins. While green coffee is very rich on chlorogenic acids, they gradually decrease during roasting and are barely detected in dark roasted coffee [32]. In contrast melanoidins are gradually formed [31] and both together make up the bulk of the antioxidant capacity of roasted coffee, which are chemically most probably related to polyphenols.

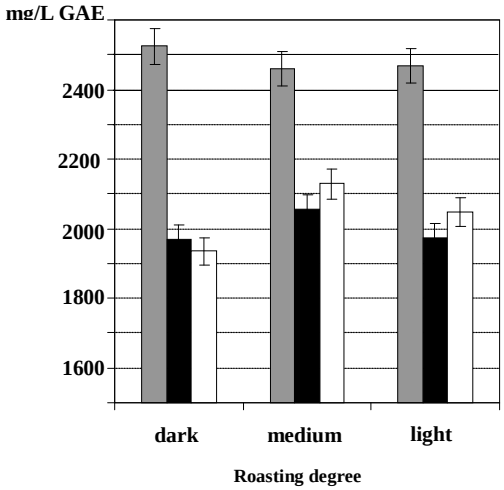

Figure 3. *Arabica* coffee from Guatemala was roasted to three different roast degrees, each one along three different time-temperature profiles. Mean value from 5 roastings, at a confidence level of 95%.
□ hot air inlet temperature (HAIT) high (≅10 min), □ HAIT medium (≅15 min), ■ HAIT low (≅20 min)

The results in Figure 2 are in line with the literature. The TPP content is approx. 25% higher in the brews prepared from the two *Robusta* coffees (average from two different provenances) compared to those prepared from the two *Arabica* coffees [33]. Also the fact that the TPP increases slightly from a very light roast (Colorette ≅ 124) to a light roast (Colorette ≅ 110) is consistent with the observed increase in antioxidant activity during roasting (up to a medium roast).

3.5.2 Application 2 - Profile Roasting

In a second series of experiments, the TPP content was measured in coffee brews from coffees roasted to different roast degrees and with different time-temperature profiles. Roasting parameters are shown in Table 4, while the measured TTP contents for the various roasting profiles are shown in Figure 3. The most interesting observation is the higher TTP content, by approximately 25 %, for the fast roasted coffee, irrespective of the roast degree, and relative to both the medium and slow roasted coffee. It appears that a fast roast of approx. 10 minutes or respectively with a high roasting temperature results in a higher TPP content compared to either a medium (15 min) or a slow roast (20 min), under the conditions and for the coffee variety used in this study.

At a confidence level of 95%, the TPP content between a low and a medium HAIT cannot be differentiated. Further studies are on-going to examine the process conditions and the chemical processes responsible for this surprising observation.

Table 4. Relevant parameters for each roast profile *Arabica* coffee from Guatemala was roasted to three different roast degrees, each one along three different time-temperature profiles. Mean value from 5 roastings, at a confidence level of 95%. (Roast degree **M**=Minolta, **C**=Colorette)

HAIT	Roast degree	Roast time/s (±2%)	Final Temp./°C (±1%)	Roast degree M (±1%)	Roast degree C 3b (±1%)
low	light	1130	184.6	41.38	109.9
low	medium	1199	192.2	40.28	90.4
low	dark	1258	198.9	39.54	77.5
medium	light	831	185.7	41.40	110.3
medium	medium	869	192.7	40.31	91.1
medium	dark	907	198.3	39.56	77.8
high	light	546	185.9	41.36	109.5
high	medium	572	191.4	40.31	91.1
high	dark	593	195.4	39.56	77.9

To conclude, a semi-automated flow injection analysis system with colorimetric detection was optimised and validated, for the quantification of the total polyphenol (TPP) content directly in the coffee extract. It is based on the Folin-Ciocalteu (FC) method and expresses the TPP content in terms of gallic acid equivalents (GAE). The method was found to be superior to alternative methods such as the Folin-Denis (FD) and the 4-aminoantipyrine (4-AAP). The advantages of the FC procedure are: high sensitivity, no need for colorimetric correction of the background colour of the coffee brews, negligible matrix influence and availability of the FC-reagent from commercial sources. In particular it can be applied directly on the brew. The developed analytical method is applicable over a wide working range from 0.5 – 80 mg GA/L, has high throughput of up to 110 samples/hr and a high precision of *RSD* of < 2% $_{n=10,\ p=95\%}$. Some additional advantages of the optimized FIA method are: Miniaturization (from milli- to microliters) with corresponding improvement in reagent economy and reduction of generated waste; furthermore, a fully automated sequential analysis of coffee compounds - e.g. TPP, melanoidins, caffeine - in a continuous process monitoring setup (on-line measurements) would be an additional features that can be implemented**.** The method was applied to two different coffee systems. First, the brews from different coffee varieties were compared. It was shown that a light roasted *Robusta* has approx. 25% higher TPP content than *Arabica*. Secondly, an *Arabica* coffee was roasted using different time-temperature profiles and to varying roast degrees and it was demonstrated that a fast roasted coffee (10 min), respectively one roasted at higher roast temperature, had an approximately 25 % higher TTP content as compared to slower roasted coffees (or a coffee roasted at a lower roast temperature), irrespective of the roast degree.

4. Acknowledgment

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