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Research paper

Review on coffee quality markers

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Coffee is a very popular hot drink, highly appreciated all over the world. The objective of this paper is to review the analysis of coffee quality markers in coffee, mainly by HPLC-DAD. HPLC - DAD a rapid validated method for the simultaneous determination of six quality markers (caffeine, trigonelline, nicotinic acid, *N*-methyl-pyridinium ion, 5-monocaffeoylquinic acid (5-CQA) and 5-hydroxymethyl furfur in coffee. The method showed good linear correlation, precision, sensitivity, and recovery for all compounds. HPLC method is simultaneous, rapid, and valid allow us to realize the use of industries in expanding biodiversity is cultivated worldwide. In addition, the HPLC-DAD method could be applied to green, roasted coffee and espresso coffee.

Keywords: Caffeine, Coffee, Quality, Green, HPLC, Roasting

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INTRODUCTION

Coffee is a very popular hot drink, highly appreciated all over the world. It ranks second after petroleum in international trade to earn foreign exchange in many agriculture-oriented countries. The two most important species of coffee, *Coffea arabica* and *Coffea canephora* (Robusta), differ significantly in price, quality and consumer preference [1]. Arabica coffee is more appreciated due to its fine taste, aroma and strong body. It is green to pale green in color and has an oval shape in contrast in robusta that is round and brown in color [2]. Arabica coffee is more acidic, with a more intense aroma and a richer body than Robusta, which is instead more bitter and characterized by a typical earthy and woody flavor [1].

Ethiopia is the single known center of origin and genetic diversity for Arabica coffee (*Coffea arabica L.*).The endowment of Ethiopia with diverse coffee types and agro-ecology allowed the production of high-quality coffee to the world market. Coffee grows widely in variable environments in Ethiopia has a variety of characteristics sought in the international market [3].

The quality of coffee can be defined as its organoleptic cup-quality, physical appearances and inherent chemical constituents of a green bean produced. Coffee quality is of critical importance to the coffee industry. Quality coffee is a product that has desirable characteristics such as clean raw and roasted appearance, attractive aroma, and good cup taste. Coffee quality encompasses beans flavor in fragrance, aroma, flavor, sweetness, acidity or overall taste felt by the consumer after drink as well as physical characteristics such as length, width, thickness or weights, shape and color of coffee beans [3-4].

Coffee quality is typically evaluated by professional cuppers trained to assess subjective contributors to flavor and aroma. Coffee quality in terms of cuppers can bring biasness and far from acceptance because it is different from person to person. It is better to get a common objective evaluation. Quality may also be monitored via a more objective evaluation, particularly through the content of compounds known as “coffee taste

descriptors" namely, caffeine, trigonelline, nicotinic acid, *N*-methyl pyridinium ion, 5-monocaffeoylquinic acid (5-CQA), and 5-hydroxymethyl furfural.

Compounds trigonelline, nicotinic acid, *N*-methylpyridinium ion are also key markers in the roasting process. Coffee quality markers green and roasted coffee was not determined in related to varies coffee diversity and geographic location in Southwest Ethiopia. The objective of this paper is to review the analysis of coffee quality markers; mainly by HPLC-DAD in coffee.

Coffee Quality

The quality of coffee can be defined as its organoleptic cup-quality, physical appearances and inherent chemical constituents of a green bean produced [6,3]. Coffee quality is of critical importance to the coffee industry. Production and supply of coffee with excellent quality appear more crucial than ever before for coffee exporting countries. Quality coffee is a product that has desirable characteristics such as clean raw and roasted appearance, attractive aroma, and good cup taste. Coffee quality encompasses beans flavor in fragrance, aroma, flavor, sweetness, acidity or overall taste felt by the consumer after drink as well as physical characteristics such as length, width, thickness or weights, shape and color of coffee beans [3].

The quality of coffee is strongly influenced by environmental factors. Altitude, daily temperature fluctuations, amount and distribution of rainfall and the physical and chemical characteristics of the soil are very important factors. The production of good quality coffee beans in specific areas characterized by their climatic conditions clearly showed that climate is one of the important factors in determining the quality of coffee beverages. According to genetic origins greatly influenced coffee quality [4].

Moisture content

The moisture content of the coffee beans is an important attribute and indicator of quality. The high moisture content of the beans is a loose sensorial defect. If coffee beans are too wet (above12.5 % moisture), can mold easily during storage [4]. In addition, if the beans are too dry (below8 % moisture) they lose flavor. The moisture content can influence the way coffee roasts and the loss of weight during roasting. Green coffee with low moisture content tends to roast faster than those with high moisture content [8].

Chemical attributes

The chemistry of coffee quality is highly complex with a wide range of compounds that change during fruit development. A few key components, such as caffeine, trigonelline, lipids, sucrose and chlorogenic acids (CGAs), are regarded as significant in influencing coffee quality. These components either stay stable and act as flavor attributes reaching the coffee brew or are degraded during roasting accounting for flavor precursors **[6, 9]**.

Green coffee chemical composition

Non-volatile compounds in green coffee

The non-volatile fraction of green coffee is composed primarily of water, carbohydrates and fiber, proteins and free amino acids, lipids, minerals, organic acids, chlorogenic acids, trigonelline, and caffeine (Table.1). Of these compounds found in green coffee, chlorogenic acids, caffeine, trigonelline, soluble fiber, and diterpenes from the lipid fraction are most likely to be bioactive, and they may also be important contributors to the beverage flavor after roasting.

Table.1. Nonvolatile compounds in green C. Arabica and C. Canephora.

Component	Concentration[a] (g/100 g)	
	Coffea arabica	*Coffea canephora*
Carbohydrates/fiber		
Sucrose	6.0–9.0	0.9–4.0
Reducing sugars	0.1	0.4
Polysaccharides	34–44	48–55
Lignin	3.0	3.0
Pectin	2.0	2.0
Nitrogenous compounds		
Protein/peptides	10.0–11.0	11.0–15.0
Free amino acids	0.5	0.8–1.0
Caffeine	0.9–1.3	1.5–2.5
Trigonelline	0.6–2.0	0.6–0.7
Lipids		
Coffee oil (triglycerides with unsaponifiables, sterols/tocopherols)	15–17.0	7.0–10.0
Diterpenes (free and esterified)	0.5–1.2	0.2–0.8
Minerals	3.0–4.2	4.4–4.5
Acids and esters		
Chlorogenic acids	4.1–7.9	6.1–11.3
Aliphatic acids	1.0	1.0
Quinic acid	0.4	0.4

Caffeine

Caffeine (1, 3, 7-trimethylxanthine), an alkaloid of the methyl xanthine family is a naturally occurring substance found in the leaves, seeds or fruits of over 63 plants species worldwide. The most commonly known sources of caffeine are coffee, cocoa beans, cola nuts and tea leaves [8]. Caffeine is one of the most important bitterness attributes contributing to coffee quality. When caffeine is consumed moderately by humans, increased energy availability, alertness, and concentration decreased fatigue and boosted physical performance have been reported, however, too much caffeine may result in undesired effects such as cardiovascular disease, depression, and even addiction. Nowadays, caffeine is the world's most famous behaviorally active drug and is consumed primarily from coffee [6, 9].

Chlorogenic acids

Coffee has one of the highest concentrations of CGA of all plant constituents [11]. Chlorogenic acids (CGAs) are a group of phenolic compounds that show multiple attributes. During roasting, a large percentage of the CGAs degrade to form caffeic acid, lactones, and other phenol derivatives through Maillard and Strecker's reactions, which result in increased bitterness, astringency and aroma [6].

Chlorogenic acids play a great role in the formation of pigments, taste, and flavor of coffee beans, which determine the quality and acceptance of the beverages. They contribute to the final acidity of the beverages and the formation of lactones and other phenol derivatives responsible for flavor and aroma [10]. CGAs are thermally unstable and in Arabica coffee the loss of CGAs after light roasting and after very dark roasting of beans corresponds to 60.9% and 96.5% respectively while in Robusta this loss corresponds to 59.7% to almost 98% respectively. Although most CGAs are lost by roasting, a sharp increase in total antioxidant activity was reported in the coffee beverage which suggested that the breakdown products of CGAs are antioxidants. For the coffee plant itself, CGAs are significant plant metabolites that are associated with the protection of plant cells against stress, for example, oxidative stress, UV irradiation and pathogen infection [6, 7].

The total CGA content of green coffee beans varies according to species, degree of maturation and less importantly agricultural practices, climate, and soil. In general, the percentage of CGA for regular green coffee beans on dry matter basis varies from 4 to 8.4% for Arabica and 7 to 14.4% for Robusta with some hybrids presenting intermediate levels [6, 8].

In addition to caffeine and trigonelline, chlorogenic acids are major chemical components of coffee beans, whose content is closely related to the quality of coffee beverages [12].

Trigonelline

Trigonelline is an alkaloid biologically derived from enzymatic methylation of nicotinic acid. It contributes to the bitterness of the brew and is a precursor for the formation of different classes of volatile compounds during roasting such as pyrroles and pyridines, some of which according to Flament may confer an “objection - able flavor.” Regarding potential bioactivity, trigonelline has inhibited the invasiveness of cancer cells *in vitro*. In addition, this compound has been able to regenerate dendrites and axons in animal models, suggesting that it may improve memory. More recently it has been considered a novel phytoestrogen. Trigonelline demethylation during coffee roasting produces nicotinic acid, a B-complex vitamin also known as niacin [11, 12].

Coffee roasting

In newer fluid bed roasters, the seeds are in contact with hot air/gases. Fluid bed roasters are preferred for industrial use because they are faster, allow better control of air temperature and speed inside the roasting chamber, and produce a more homogeneous color than other roasters [12]. The aroma of green coffee seeds is quite different from what we imagine when we hear the word coffee. It is only through roasting that the seeds gain the characteristic aroma and flavor of coffee [12,13]. The high roasting temperatures cause a series of physical and chemical changes in the seeds. The specific roasting conditions strongly influence these changes and consequently affect the bioactivity and flavor of the beverage [12].

The temperatures used to roast the seeds depend on the roaster type, but the maximum temperatures used in industrial fluid bed roasters generally vary from 210°C to 240°C. In the initial phase of roasting, free water evaporates. When the seed temperature reaches 130°C, sucrose caramelizes, and the seeds begin to brown and swell. Chemical changes in this initial phase are relatively small compared to those that occur at the end of the roasting process. At temperatures higher than 160°C, a series of exothermic and endothermic reactions take place; the seeds become light brown, their volume increases considerably, and aroma formation begins [12].

The chemical reactions responsible for the aroma and flavor of roasted coffee are triggered at approximately 190◦C. During the Maillard and Strecker reactions, which involve carbohydrates (reducing sugar), proteins, and other classes of compounds, low- and high-molecular-weight compounds such as melanoidins are simultaneously degraded and produced. During this process, light brown seeds can become almost black [12].

Changes in coffee chemical composition during roasting

Nonvolatile components in roasted coffee

The seed composition dramatically changes during roasting as a consequence of pyrolysis, caramelization, and Maillard reactions. Some compounds are destroyed and others are formed, including bioactive compounds and substances of high and medium volatility, which are important for the aroma and flavor of the brew [14]. The final composition of roasted coffee varies according to the raw material, roasting degree, and other roasting variables such as roaster type and the time, temperature, and air-flow speed in the roasting chamber. The moisture content of roasted coffee (1.5%–5%) is much lower than that of green coffee and varies depending on the roasting degree [15].

A portion of the coffee protein is degraded, and free amino acids and peptides are consumed by Strecker reactions. Some of the amino acids react with reducing sugars to form (via Maillard reaction) low-molecular-weight compounds and melanoidins that incorporate into their structures other components, such as chlorogenic acids, galactomannans, and arabinogalactan-proteins. Melanoidin polymers, which exhibit variable composition and molecular mass, are responsible for the brown color of roasted coffee and approximately 25% of its dry matter [15, 16].

Different studies suggest that melanoidins are partially responsible for the antioxidant, antibacterial, and metal-chelating properties of coffee beverages and therefore may be considered bioactive compounds [16, 17].

Caffeine is of major importance with respect to the physiological properties of coffee, also in determining the strength, body & bitterness of brewed coffee. During roasting, there is no significant loss in terms of caffeine. Caffeine presents significant stability, both in terms of lack of mobility within the coffee plant. While roasting temperatures generally exceed caffeine’s sublimation point (178°C). Caffeine losses in roasting are insignificant, probably due to pressure build-up within the bean & a poor rate of vapor diffusion through the bean’s outer layers [12].

Table.2. Nonvolatile compounds in roasting C. Arabica and C. Canephora

Compounds	Concentration[a] (g/100 g)	
	Coffea arabica	*Coffea canephora*
Carbohydrates/fiber		
Sucrose	4.2-tr	1.6-tr
Reducing sugars	0.3	0.3
Polysaccharides (arabinogalactan, mannan, and glucan)	31–33	37
Lignin	3.0	3.0
Pectins	2.0	2.0
Nitrogenous compounds		
Protein	7.5–10	7.5–10
Free amino acids	ND	ND
Caffeine	1.1–1.3	2.4–2.5
Trigonelline	1.2–0.2	0.7–0.3
Nicotinic acid	0.016–0.026	0.014–0.025
Lipids		
Coffee oil (triglycerides with unsaponifiables)	17.0	11.0
Diterpene esters	0.9	0.2
Minerals	4.5	4.7
Acids and esters		
Chlorogenic acids	1.9–2.5	3.3–3.8
Aliphatic acids	1.6	1.6
Quinic acid	0.8	1.0
Melanoidins	25	25

Antioxidant chlorogenic acids combine phenolic acids with quinic acid in various combinations. Up to half of the CGAs in green coffee beans degrade during high-temperature roasting, which causes a host of chemical reactions. Some of the CGAs hydrolyze to form free phenolic acids or dehydrate to bitter-tasting chlorogenic acid lactones. Others are involved in Maillard browning reactions to give a wide range of compounds including brown colored and very bitter tasting antioxidant polymers called melanoidins [16].

Chlorogenic acid contents in commercial roasted coffees may vary from 0.5–6 g/100 g, dry weight, depending on the type of processing, blend, roasting degree, roasting method, and analytical conditions. Chlorogenic acid lactones contribute considerably to the bitterness of the coffee beverage, an important aspect of quality. These lactones have also received attention because of their potential effects on brain function independent of the pharmacologic effects of caffeine. However, their relatively weak *in vitro* affinity to opioid receptors suggests that acute pharmacologic effects are unlikely with normal coffee consumption [12].

Roasting degrades trigonelline, producing a variety of compounds including nicotinic acid (3%) and volatile compounds such as pyrrols (3%), pyridines (46%), pyrazines, and methyl nicotinate. Nicotinic acid, also called niacin, vitamin B3, or vitamin PP is formed via trigonelline demethylation. In humans, nicotinic acid participates as a coenzyme in various metabolic processes, and its deficiency causes pellagra, a disease characterized by skin lesions. Although niacin production increases as roasting progress, a 100-mL cup of a medium-roast coffee can supply approximately 20% of the daily dietary reference intake recommendation. Fast roasting tends to produce coffees with higher trigonelline content than slow roasting [17].

Trigonelline

Trigonelline (0.6-2%) is a pyridine derivative present in green coffee beans, known to contribute indirectly to the formation of desirable & undesirable aroma compounds during roasting. Trigonelline content correlated with good cup quality (17, 18). It contributes to bitterness & a part of it is converted to nicotinic acid or niacin, a soluble water vitamin B through demethylation during coffee roasting. Trigonelline comprises about 2% of the dry weight of green coffee but does not survive to roast, breaking into nicotinic acid, pyridine and other volatile compounds [18].

Trigonelline is another alkaloid in coffee beans besides caffeine which on contrary to caffeine, largely disappears. Trigonelline degrades into N-methylpyridinium (NMP) or nicotinic acid (3). Its esters depending on whether a decarboxylation or demethylation of trigonelline occurs [12, 18].

Method development

A rapid and validated HPLC-diode array detector method for the simultaneous quantitation of caffeine, trigonelline, nicotinic acid, *N*-methyl pyridinium ion, 5-caffeoylquinic acid, and 5-hydroxymethyl furfural that is applicable to three coffee matrixes: green, roasted, and instant. Nicotinic acid, *N*-methyl pyridinium ion, and

trigonelline are well studied nutritional biomarkers present in coffee, and they are indicators of thermal decomposition during roasting. The method showed good linear correlation (r^2 > 0.9985), precision (less than 3.9%), sensitivity (LOD = 0.023–0.237 µg/mL; LOQ = 0.069–0.711 µg/mL), and recovery (84–102%) for all compounds. RP-HPLC coupled to a diode array detector (DAD) or to a mass spectrometer (LC/MS) is the methodology normally applied to measure nonvolatile compounds such as caffeine, trigonelline, nicotinic acid, *N*-methylpyridinium ion, 5-caffeoylquinic acid, and 5-hydroxymethyl furfural. However, none of the HPLC methods available to date permits the simultaneous quantitation of these six analytes.

There are reports on the quantitation of caffeine, trigonelline, nicotinic acid together with 5-caffeoylquinic acid or 5-hydroxymethyl furfural, but no RP-HPLC method has been reported in the evaluation of *N*-methylpyridinium ion in the presence of trigonelline, nicotinic acid. The highly hydrophilic character of trigonelline, and its thermal degradation products, nicotinic acid, *N*-methylpyridinium ion, has rendered the simultaneous determination of these three important coffee roasting markers a difficult task [27].

Recently, nonconventional HPLC methods, which include the use of two columns of different stationary phases connected in series or hydrophilic interaction liquid chromatography coupled to MS, have been implemented in order to improve the resolution between caffeine, trigonelline and caffeine, and, *N*-methylpyridinium ion. The herein RP-HPLC-DAD method allowed baseline separation and quantitation of quality markers caffeine, trigonelline, nicotinic acid, *N*-methylpyridinium ion, 5-caffeoylquinic acid, and 5-hydroxymethyl furfural in 30 min. The identities of caffeine, trigonelline, nicotinic acid, *N*-methylpyridinium ion, 5-caffeoylquinic acid, and 5-hydroxymethyl furfural in coffee were confirmed by LC/MS/MS [27].

The determination of organic acids in brewed coffee by using solid-phase extraction in UV/HPLC was described by Rodrigues, [26]. A solid-phase extraction (SPE) method was adapted to perform brewed coffee sample clean-up for seven organic acids (acetic, citric, formic, malic, chlorogenic acid, quinic and caffeine) determination by reversed-phase UV high-performance liquid chromatography (RP/UV-HPLC). 20 brewed coffee samples from the two types of coffee (robusta vs. arabica) were tested. Brewed coffee samples were prepared according to [ISO 6668. Green coffee—preparation of samples for use in the sensory analysis] and the results were compared to sensory evaluation obtained from a panel of coffee tasters. Roasting conditions also seem to affect final acidity in brewed coffee for both coffee types analyzed. All Chemicals and reagents used, chromatographic conditions, sample preparation for Solid-phase extraction Organic acids were described in the published article in detail [26].

A simple method for the simultaneous determination of caffeine and chlorogenic acid content in green coffee was reported (28). The method was based on the use of UV/Vis absorption. It is relevant that the quantification of both caffeine and chlorogenic acid factors without their preliminary chemical separation despite their spectral overlap in the range 250–350 nm. [28]. The estimate of concentration values was in agreement with the one obtained by High-Performance Liquid Chromatography quantification. The method use of UV/Vis spectrophotometer is easy, fast, and cheap device, today available in most laboratories and allows us to realize routine controls during the coffee production. In addition, it could be applied to roasted coffee and espresso coffee [28].

Chemicals and apparatus

All standard compounds were purchased from Sigma-Aldrich (St. Louis, MO). *N*-methyl-pyridinium iodide and 6 were synthesized and fully characterized by NMR spectrometry and electrospray ionization (ESI)/MS analysis following the available literature procedures. Methanol (MeOH; HPLC grade) was purchased from J.T. Baker (Griesheim, Germany), and the water for HPLC-DAD and LC/MS analyses was MilliQ purified (EMD Millipore Corp., Billerica, MA). Green specialty coffee (*Coffea arabica*) was obtained from Jaen-Cajamarca, Peru. The coffee was roasted by professionals from CENFROCAFE (Cooperativa de Servicios Multiples, Peru). The instant coffee sample was purchased from a commercial supermarket in the United States. Coffee samples were frozen under nitrogen and kept at –78°C until use [27].

Instrumentation

A closed vessel microwave system Mono-wave 300 (Anton Paar GmbH, Graz, Austria), with a maximum control pressure of 435 psi and a maximum control temperature of 300°C was used for the green coffee microwave-assisted extraction (MAE). HPLC analysis was performed using a Model 1200 series DAD equipped instrument with a binary pump unit, UV-Vis detector, degasser, column oven, and an auto-sampler and controlled by Chem - station software LC3D (Agilent Technologies, Inc. Santa Clara, CA).

The LC/ESI-MS experiments were carried out on an Esquire 6000 composed of an ion-trap mass spectrometer controlled by Compass 1.3 for Esquire / HCT software (Bruker Daltonik GmbH, Bremen/Germany) [27].

HPLC-DAD and LC/MS Conditions

The chromatographic separation was performed on a phenyl-hexyl Luna column (250 × 4.6 mm, 5 µm; Phenomenex, Torrance, CA). The mobile phase was a mixture of two solvents: A (0.3% aqueous formic buffer, pH 2.4) and B (MeOH). The elution gradient was 0–10% B in 5 min, 10–25% B in 8 min, 25% B for 3 min, 25–35% B in 6 min, and 35–40% B in 8 min (total elution time = 30 min). The flow rate was 1 mL/min, and the temperature of the column was set at 30°C. Compounds nicotinic acid and *N*-methylpyridinium ion were monitored at 260 nm; caffeine, trigonelline and 5-hydroxymethyl furfural at 270 nm; and 5-monocaffeoylquinic acid (5-CQA) at 330 nm. All samples were filtered through a 45 µm syringe filter (Millipore, Darmstadt, Germany) prior to analysis. Each sample was injected (10 µL) in duplicate [27].

The same elution program was used for the LC/MS analysis. The ESI-MS operating conditions for the positive ionization mode were drying gas (N_2) flow, 12 L/min; nebulizer pressure, 65 psi; gas drying temperature, 350°C; capillary voltage, –4000 V; scan mode, *m/z* 50–500; and injection volume, 10 µL. For the negative ionization mode, the voltage was set to 3800 V. The identity of compounds caffeine, trigonelline, nicotinic acid, *N*-methylpyridinium ion, 5-monocaffeoylquinic acid (5-CQA), and 5-hydroxymethyl furfural in coffee extracts was confirmed by co-injection with authentic standards and by comparison against their mass and UV spectra.

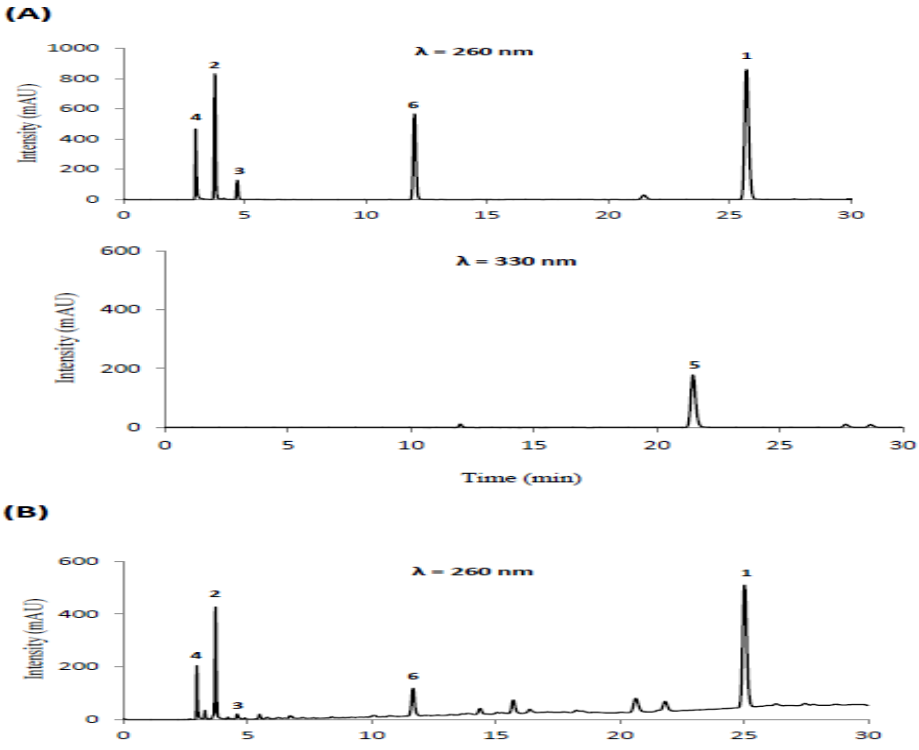

Figure 1: HPLC-DAD chromatograms at 260 nm and 330 nm of (A) standard mixture of quality 6 markers (B) representative [27].

Table 3. Calibration curves of analytes 1–6 and sensitivity of the HPLC-DAD method

Compound	Concn range, µg/mL	Linear regression		LOD, µg/mL	LOQ, µg/mL
		y = mx + b[a]	r^{2} [b]		
N-methylpyridinium ion (**4**)	27.1–216.8	2524.3x – 30.668	0.9992	0.237	0.711
Trigonelline (**2**)	50.30–402	2048.0x – 1.813	0.9988	0.105	0.315
Nicotinic acid (**3**)	1.19–15	2623.6x – 1.211	0.9990	0.064	0.192
5-(Hydroxymethyl)furfural (**6**)	4.23–70.50	6442.3x – 3.594	0.9999	0.023	0.069
5-Caffeoylquinic acid (**5**)	30.15–603	10322.4x – 58.050	0.9985	0.046	0.170
Caffeine (**1**)	90.27–722	5625.5x + 34.786	0.9998	0.023	0.078

[a] y = Peak area; x = concentration in µg/mL; m = slope; b = intercept.

[b] Correlation coefficient.

Sample Preparation and Recovery Studies

Roasted coffee beans were ground in a Kitchen Aid (St. Joseph, MI) Artisan Burr Grinder set at grade 6.5. Ground material (1.65 g) was extracted with water at 93°C (30 mL) for 4 min, vortexed using an MS 1 Vortexer shaker (IKA Works, Inc., Wilmington, NC), and allowed to stand for 6 more minutes without agitation in a 50 mL closed plastic centrifuge tube (Nalgene Oak Ridge, Thermo Scientific Inc., Waltham, MA).

The extract was centrifuged (12 000 × *g*) for 10 min at 10°C. The supernatant (25–26 mL) was lyophilized using a LABCONCO Freeze Dry System/FreeZone 4.5 (Kansas City, MO) to yield 274 mg dry mass. For quantitative analysis, 12 mg lyophilized material was diluted with MilliQ water to a final volume of 1 mL. Five independent extractions were performed (n = 5). Green coffee beans were ground to a fine powder using an electric grinder (Sigma-Aldrich, Model Z278181). The ground material (200 mg) was suspended in water (20 mL) and placed in the microwave oven programmed at 120°C/3 min/ 200 psi [27].

Table 4. the precision of retention time (tr) and peak area of analytes 1–6 in the HPLC-DAD method

Compound[a]	t_r, min	Intraday precision (CV, % n = 5)		Interday precision (CV, % n = 5)	
		t_r	Peak area	t_r	Peak area
N-methylpyridinium ion (**4**)	2.911	0.766	0.217	1.202	3.284
Trigonelline (**2**)	3.718	0.226	1.025	0.661	2.589
Nicotinic acid (**3**)	4.624	0.136	0.334	1.369	3.870
5-(Hydroxymethyl)furfural (**6**)	12.117	0.051	0.198	0.255	1.730
5-Caffeoylquinic acid (**5**)	20.866	0.325	0.385	1.622	1.390
Caffeine (**1**)	25.236	0.261	0.202	1.651	1.678

[a] Concentration range of analytes 0.034 to 1.860 mM.

The total runtime under this setting was 10 min. The sample was then cooled and centrifuged under the same conditions described above. The supernatant recovered (approximately 18 mL) was brought to a final volume of 20 mL with MilliQ water. The extraction process was repeated five times (n = 5). Instant coffee was used as purchased. For quantitative analysis, a sample (12 mg) of the material was diluted with MilliQ water to a final volume of 1 mL. Five independent evaluations of a representative instant coffee sample were performed (n = 5) [27].

Recovery of the complete analytical extraction protocol for roasted and green coffees was evaluated by enriching roasted coffee (1.65 g) with 20, 10, and 5 mg standard compounds caffeine, trigonelline and 5-monocaffeoylquinic acid (5-CQA) and 1, 0.5, and 0.25 mg compounds nicotinic acid, *N*-methylpyridinium ion, and 5-hydroxymethyl furfural and green coffee (0.2 g) with 5, 2.5, and 0.5 mg standard compounds caffeine, trigonelline and 10, 5, and 1 mg compound 5-monocaffeoylquinic acid (5-CQA). The recovery was expressed as the percentage of the total amount recovered. The process was replicated three times ($n = 3$) [27].

Recent development method for analysis of two coffee quality markers in Simultaneous Determination of Caffeine and Chlorogenic Acids in Green Coffee by UV/Vis Spectroscopy, other than the HPLC-DAD method.

Validation Validated HPLC-Diode Array Detector Method

The HPLC method was validated according to the International Conference on Harmonization requirements. The external calibration curves were prepared with solutions containing standard compounds within the following ranges: 90–722 µg/mL for caffeine, 50–402 µg/mL for trigonelline, 1.9–15 µg/mL for nicotinic acid, 27–217 µg/mL for *N*-methylpyridinium ion, 30–603 µg/mL for 5-monocaffeoylquinic acid (5-CQA), and 4.2–70.5 µg/mL for 5-hydroxymethyl furfural. Linearity was checked by a regression analysis of five different concentrations for each compound [27].

Each concentration level was prepared a minimum of three times, and each solution was evaluated by HPLC in duplicate. Intraday and interday precision were confirmed by performing injections on the same day ($n = 5$) and over 5 different days, respectively, at a given concentration for each compound. LOD and LOQ values were obtained from highly diluted solutions based on S/N of 3 and 10, respectively. Accuracy of the method was evaluated through a recovery study using standard compounds caffeine trigonelline, nicotinic acid, *N*-methyl pyridinium ion, 5-monocaffeoylquinic acid (5-CQA), and 5-hydroxymethyl furfural at three concentration levels. Samples were prepared according to the previously described procedure [27].

Table 5. Recovery of the HPLC-DAD method evaluated using roasted and green coffee

		Recovery, %[a]				Recovery, %[a]		
Sample	Spike, mg	Caffeine (**1**)	Trigonelline (**2**)	CQAs[b]	Spike, mg	Nicotinic acid (**3**)	NMP (**4**)	5-HMF (**6**)
Roasted coffee	20	83.9 ± 3.2	90.5 ± 4.9	95.9 ± 5.3	1	95.4 ± 3.5	91.9 ± 0.5	98.8 ± 2.8
	10	90.9 ± 2.5	94.0 ± 4.6	100.4 ± 4.5	0.5	99.0 ± 3.4	98.5 ± 2.6	101.9 ± 2.2
	5	95.7 ± 0.5	85.3 ± 1.1	95.1 ± 0.6	0.25	92.2 ± 2.0	101.8 ± 0.8	99.1 ± 2.1
		Caffeine (**1**)	Trigonelline (**2**)			CQAs[b]		
Green coffee	5.0	96.2 ± 0.8	98.5 ± 0.3		10	100.6 ± 0.9		
	2.5	97.6 ± 0.6	98.9 ± 1.4		5.0	101.7 ± 2.2		
	0.5	98.3 ± 2.5	97.9 ± 0.6		1.0	100.8 ± 2.9		

[a] Values are the average of three determinations ± SD.

[b] Value corresponds to the sum of 3-, 4-, and 5-CQA isomers.

RESULTS AND DISCUSSIONS

Chromatographic Analysis

The simultaneous separation of structurally alike, highly polar, aromatic heterocycles trigonelline, nicotinic acid and *N*-methylpyridinium ion in the presence of less polar compounds, such as caffeine, 5-monocaffeoylquinic acid (5-CQA) and 5-hydroxymethyl furfural was achieved exploiting the demonstrated aromatic selectivity (through π-π bonding interactions) of the phenyl- hexyl - Luna column. Compound trigonelline resolved almost 1 min apart [retention time

difference (Δtr) = 0.9 min] from nicotinic acid (Figure 8A), a result that at the moment represents the best separation achieved between these two compounds using RP chromatography. The more polar *N*-methylpyridinium ion was also cleanly resolved (Δtr = 0.8 min) from trigonelline and nicotinic acid [27].

The results corresponded well with those achieved in a recent LC/MS method using NP chromatography. The resolution between the remaining compounds 5-hydroxymethyl furfural, 5-monocaffeoylquinic acid (5-CQA), and caffeine, compared with other reported RP-HPLC methods in which longer chromatographic runs or the use of a mixture of three solvents as the eluting system were required [27].

Table 6. Content of compounds caffeine, trigonelline, nicotinic acid, *N* - methylpyridinium ion, 5-monocaffeoylquinic acid (5-CQA) and 5-hydroxymethyl furfural expressed in mg/g dry weight present in roasted, green, and instant coffee samples.

Coffee extract (n = 5)	Caffeine (**1**)	Trigonelline (**2**)	Nicotinic acid (**3**)	NMP (**4**)	CQAs[b] (**5**)	5-HMF (**6**)
Roasted	7.79 ± 0.09	4.63 ± 0.07	0.125 ± 0.002	0.290 ± 0.004	12.37 ± 0.19	0.293 ± 0.005
CV, %[c]	1.2	1.5	1.6	1.3	1.5	1.7
Green	10.33 ± 0.20	6.88 ± 0.10	ND[d]	ND	55.10 ± 0.92	ND
CV, %	1.9	1.4	—	—	1.7	—
Instant	52.44 ± 0.87	20.02 ± 0.20	1.014 ± 0.015	3.75 ± 0.05	63.02 ± 0.81	0.828 ± 0.014
CV, %	1.7	1.0	1.5	1.4	1.3	1.7

[a] Each value is expressed as the average of five independent extractions ± SD.

[b] Value corresponds to the sum of 3-, 4-, and 5-CQA isomers.

[c] Percentage coefficient of variation.

[d] ND = Not detected.

The presence of compounds caffeine, trigonelline, nicotinic acid, *N* - methylpyridinium ion, 5-monocaffeoylquinic acid (5-CQA) and 5-hydroxymethyl furfural in the aqueous roasted coffee extract (Figure 8 B) was confirmed through comparison of their tr values, UV profiles, and LC/MS/MS data against those of pure compounds. The molecular ions $[M+H]^+$ at *m/z* 94.1, 124.4, 127.4, 138.4 and 195.2 confirmed identities of *N*-methyl pyridinium ion, nicotinic acid, 5-hydroxymethyl furfural, trigonelline, and caffeine , respectively, whereas $[M - H]^-$ at *m/z* 353.6 confirmed 5-monocaffeoylquinic acid (5-CQA) as well as the 3- and 4-CQA isomers. The isomers were differentiated by the characteristic fragmentation patterns in their MS/MS spectra [27-28].

Validation of the HPLC-DAD Method

The regression parameters obtained for the six different calibration curves indicated a good linear correlation in the concentration range established for each compound (Table 3). Appropriate sensitivity was achieved with LOD values in the range of 0.023–0.237 µg/mL and LOQ values of 0.069–0.711 µg/mL. Reproducibility, in terms of tr and peak area, was verified by intraday and interday repetitive analysis (n = 5) with satisfactory results, displaying a CV below 2.6% in all cases except for nicotinic acid and *N*-methylpyridinium ion , which reached 3.3 and 3.9%, respectively, for peak area and interday precision (Table 6). Good recoveries were achieved at the three concentration levels addressed for each of the six compounds (Table 5) [27].

Quantitation of Compounds Six Quality Markers in Coffee Extracts

Coffee samples were extracted by two established different procedures, varying with the type of coffee. For roasted samples, the ratio between coffee mass and water was the same, as were water temperature and extraction time, like those used in the preparation of specialty coffee for quality assessment. For green coffee, a recently validated MAE protocol was used. Having confirmed the identity of the six quality markers, the content of compounds 1–6 in each type of coffee extract was calculated using the corresponding standard calibration curves. The results attained, shown in Table 6, agreed with those reported in the literature for roasted, green and instant coffees. Total chlorogenic acid content is reported here as the sum of 5-monocaffeoylquinic acid (5-CQA), 4-CQA, and 3-CQA (27).

The isomers were quantitated using the calibration curve available for 5-monocaffeoylquinic acid (5-CQA) since they exhibit the same coefficient of absorptivity at λ = 330 nm. This correction is needed to account for the isomerization of 5-monocaffeoylquinic acid (5-CQA) upon heating.

As expected, the results showed that caffeine, trigonelline, and chlorogenic acid contents are higher in green coffee than in roasted coffee. Also, compounds nicotinic acid and *N*- methylpyridinium ion, and 5-hydroxymethyl furfural were found only in roasted coffee samples. The CV percentage values were lower than 2% in all cases (Table 6) [27].

CONCLUSIONS

In the conclusion, a specific procedure for the determination and quantification of six quality markers (caffeine, trigonelline, nicotinic acid, *N*-methyl-pyridinium ion, 5-monocaffeoylquinic acid (5-CQA) and 5-hydroxymethyl furfur in coffee was reviewed.

An enhanced validated HPLC-DAD method, that allows a rapid and short time within half an hour and simultaneous quantitation of six important quality markers of coffee. The resolution attained between compounds trigonelline and nicotinic acid, and between trigonelline and *N*-methyl pyridinium ion, is the best reported to date in this type of chromatography. RP-HPLC-DAD method such as the one implemented here, based on readily available and robust instrumentation (HPLC-DAD), is needed for quick, accurate, and precise quality assessment.

Moreover, HPLC-DAD methods also applied on roasted, espresso or instant coffee; and considering the increasing interest of coffee industries in expanding biodiversity in cultivated coffee worldwide.

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