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ORIGINAL ARTICLE

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Simultaneous Determination of Acrylamide, Caffeic Acid, and Caffeine in Commercial Coffee Capsules Using a Rapid and Validated HPLC-DAD Approach

Wassila Belkhiri-Beder [1,2] **Lamia Taouzinet** [3] ✉ **Zahra Toutou** [4,5] **Khodir Madani** [2,3] **Sofiane Fatmi** [1,4]

1 Université de Bejaia, Faculty of Technology, Department of Process Engineering, 06000 Bejaia, Algeria. wassila.beder@univ-bejaia.dz / sofiane.fatmi@univ-bejaia.dz
2 Université de Bejaia, Faculté des Sciences de la Nature et de la Vie, Laboratoire de Biomathématique, Biophysique, Biochimie et Scientométrie, 06000, Bejaia, Algeria. khodir.madani@univ-bejaia.dz
3 Centre de Recherche en Technologies Agro-Alimentaires, Route de Targa Ouzemmour, Campus, Universitaire, Bejaia 06000, Algeria. lamia.taouzinet@crtaa.univ-bejaia.dz
4 Université de Bejaia, Faculty of Natural and Life Sciences, Associated Laboratory in Marine Ecosystems and Aquaculture, 06000 Bejaia, Algeria. zahra.toutou@univ-bejaia.dz
5 Université de Bejaia, Faculty of Technology, LMPA, 06000, Bejaia, Algeria.

ABSTRACT

Background: Coffee capsules are increasingly consumed worldwide and represent a complex source of bioactive compounds, notably caffeine (stimulant), caffeic acid (antioxidant), and acrylamide (toxicant).

Aims: The objectives of this investigation were twofold: (i) assess total phenolic content (TPC), antioxidant capacity, pH, and browning index, across seven commercial capsule products (comprising six Algerian brands widely consumed locally and one European comparator with undisclosed blend/roast); and (ii) to develop and validate an HPLC – Diode Array Detection (DAD) procedure for the simultaneous determination of caffeine, caffeic acid, and acrylamide , serving as joint indicators of product quality and safety.

Methods: The TPC was determined employing the Folin–Ciocalteu method, while antioxidant activity was assessed by the DPPH radical scavenging assay. The simultaneous quantification of caffeine, caffeic acid, and acrylamide were performed by a validated HPLC – DAD procedure in accordance with International Council for Harmonization (ICH) guidelines.

Results: The resulting coffee brews exhibited pH values ranging from 5.64 to 6.25 and browning indices between 0.280 and 0.482, reflecting roasting differences. Despite a modest TPC (0.9 to 2 mg GAE/g), the antioxidant activity was high, (up to 95.3% inhibition DPPH). Caffeine was consistently detected (0.05 – 0.33 mg/25 mL), confirming its stimulant role. Caffeic acid was present in lower concentrations (0.001 – 0.14 mg/25 mL), contributed to the antioxidant potential. In contrast, acrylamide was detected in all analyzed samples ranging from 4.75 to 28.15 µg/25 mL). On a dry coffee basis, these concentrations correspond to 458 – 4023 µg/kg. A majority of the capsules yielded levels that exceed the European Commission's benchmark of 400 µg/kg for roasted coffee, thereby underscoring consumer-relevant exposure level and raising toxicological concern.

Conclusions: Coffee capsule brews demonstrated notable antioxidant capacity despite modest TPC, with caffeine ubiquitous and caffeic acid detectable. The presence of acrylamide was confirmed in all samples at levels that warrant monitoring. The validated HPLC–DAD workflow enables the simultaneous determination of these markers and offers a robust basis for integrated quality control and risk assessment in encapsulated coffee products.

Keywords: Coffee capsule; Antioxidant activity; High-Performance Liquid Chromatography (HPLC); Caffeic acid; Caffeine; Acrylamide.

Article Information

✉ **Corresponding author:** Lamia Taouzinet
E-mail: lamia.taouzinet@crtaa.dz
Tel. (+213) 656 62 14 13

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1 INTRODUCTION

Coffee constitutes one of the most widely consumed beverages worldwide, valued for both its sensory qualities and physiological effects (McLellan *et al.*, 2016; Nehlig, 2016). Two species dominate global production—*Coffea arabica* (*Arabica*) and *Coffea canephora* (*Robusta*) that differ in chemical composition, sensory profile, cultivation ecology, and market positioning. *Arabica* typically exhibits a sweeter, milder flavor with greater perceived acidity, whereas *Robusta*

is more bitter and harsher; these attributes reflect species-specific chemotypes, including higher caffeine generally observed in *Robusta* and species-dependent profiles of chlorogenic acids (CGAs) and related phenolics (Dias & Benassi, 2015). Differences in precursors particularly free asparagine and reducing sugars that drive Maillard chemistry further shape the potential formation of acrylamide during roasting (Schouten *et al.*, 2021). Collectively, these contrasts directly influence expected in-cup levels of caffeine, CGAs (precursors to caffeic acid and related compounds), and acrylamide and provide a rationale for species- and blend-aware analytics.

Within commercial frameworks, the utilization of *Arabica*/*Robusta* blends has become increasingly prevalent—especially within the single-serve capsule segment—as a strategic means of optimizing cost-efficiency alongside organoleptic profiles. Compositional screening via ^{1}H-NMR spectroscopy has consistently identified *Robusta*-specific markers across diverse pod and capsule formats, underscoring real-world variability in species composition and, by extension, in sensory attributes and bioactive potential (Monakhova *et al.*, 2015; Speer & Kölling-Speer, 2006). This industry prevalence and its implications on chemical constitution serve as the empirical justification for our sample selection, while simultaneously elucidating the heterogeneity observed in capsule brews.

From a contemporary analytical perspective, three analyte classes are particularly informative. Caffeine is a well-established central nervous system stimulant associated with increased Alertness and aspects of cognitive performance; its concentration varies by species (typically higher in *Robusta*) and processing, and extraction is further modulated by brewing parameters (Farah, 2012; McLellan *et al.*, 2016; Nehlig, 2016). Caffeic acid and the broader CGA profile are major contributors to coffee's antioxidant capacity; roasting transforms CGAs (e.g., formation of caffeic acid and CGA-lactones), and brewing conditions modulate in-cup levels, with reported links to mitigation of oxidative stress and markers relevant to health-promoting effects such as neutralization of reactive oxygen species, reduction of oxidative stress, and potential lowering of the risk of chronic diseases including cardiovascular disorders, cancer, and neurodegenerative diseases including Alzheimer's and Parkinson's diseases (Cämmerer & Kroh, 2006; Caporaso *et al.*, 2014; Santana-Gálvez *et al.*, 2017; Tajik *et al.*, 2017; Vignoli *et al.*, 2014). By contrast, acrylamide is an undesirable process contaminant formed predominantly early in roasting via the Maillard reaction between asparagine and reducing sugars; its levels depend on species, roast degree, and the balance between formation and degradation. Acrylamide is classified as a probable human carcinogen (IARC, 1994), and risk-management benchmarks in the European Union are 400 µg kg^{-1} for roasted coffee and 850 µg kg^{-1} for instant coffee (European Commission, 2017; EFSA, 2015).

Accordingly, we pursued three aims: (i) to characterize in-cup quality attributes of brews from seven commercial capsule products six Algerian brands widely consumed locally and one European comparator by measuring pH, browning index, total phenolic content (Folin–Ciocalteu), and antioxidant activity (DPPH); (ii) to develop and validate, in accordance with ICH Q2(R2)/Q14 (ICH, 2023a, 2023b), a rapid and robust HPLC–DAD method for the simultaneous quantification of caffeine, caffeic acid, and acrylamide in capsule brews; and (iii) to apply this workflow across brands and Arabica/*Robusta* blends to map compositional variability and to contextualize acrylamide levels with respect to current EU benchmark values. To the best of our knowledge, this represents one of the first validated methodologies to integrate these disparate chemical markers within a single workflow for capsule-based matrices, providing actionable evidence for quality control and consumer safety.

2 Methods

2.1 Chemicals

Folin-Ciocalteu, sodium carbonate, 2,2-diphenyl-1-picrylhydrazy (DPPH), caffeine, caffeic acid, and acrylamide were purchased from Sigma Aldrich. HPLC-grade methanol was purchased from BIOCHEM Chemopharma.

The coffee capsules specimens investigated in this study were purchased from various supermarkets in Bejaia, Algeria. The sample set comprised six Algerian brands (identified as C1, C3, C4, C5, C6, C7) and one leading European brand (C2) serving as a reference comparator. Based on the available manufacturer labeling, samples C1, C2, and C3 were identified as *Arabica* and *Robusta* blends; however, the precise botanical proportions for these, as well as the full compositions of the remaining four brands, were not disclosed.

Furthermore, the specific degree of roasting (e.g., light, medium, or dark) was not specified by the producers. Physical examination of the coffee grounds within the capsules revealed a fine-to-medium particle size distribution, estimated within the range of 250 – 350 μm. All samples were stored in their original hermetically sealed packaging at room temperature in a dry environment until analysis to ensure matrix integrity.

2.2 Coffee Beverage Preparation

The extraction of coffee beverages was carried out through a domestic capsule-based pressurized system operating at a nominal pressure of approximately19 bar. Each specimen was extracted according to the standard "long coffee" setting, yielding a final volume of 25 mL. To eliminate analytical

variability arising from mineral content, deionized water was employed for all extraction cycles. The resulting infusions were collected immediately upon elution and stabilized for subsequent physicochemical characterization.

2.2.1 Determination of pH and Melanoidin Content

The pH of the different coffee beverages was measured employing a calibrated pH meter. For the assessment of brown compounds, 100 µL of each coffee beverage was diluted with 4 mL of ultrapure water. The concentration of brown compounds was then quantified by measuring the absorbance of the sample at 420 nm, following established protocols (Bekedam *et al.*, 2006; Vignoli *et al.*, 2011)

2.2.2 Quantification of Total Phenolic Content

The concentration of phenolic compounds and flavonoids serves as a critical indicator for antioxidant capacity and a preliminary screening metric for functional food matrices. The TPC was quantified via the Folin–Ciocalteu colorimetric assay (Singleton *et al.*, 1999; Vignoli *et al.*, 2011). Briefly, 300 µL of the coffee sample was reacted with 1.5 mL of Folin–Ciocalteu reagent (diluted 1:10, v/v). Following a 2-minute stabilization period, 1 mL of sodium carbonate solution (7.5%) was added to the mixture. The mixture was incubated under dark condition, and the absorbance was measured at 765 nm. Results were expressed as milligram equivalents of caffeic acid per milliliter of coffee beverage, using a calibration curve (y = 0.162x – 0.009) and expressed as milligrams of caffeic acid equivalents per milliliter of beverage (mg CAE/mL).

2.2.3 Anti-Free Radical Effect (DPPH)

The antioxidant capacity was further evaluated using the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). In its radical form, DPPH exhibits a deep purple coloration with a maximum absorbance at 517 nm. Upon reduction by hydrogen-donating antioxidant species, the radical is quenched, resulting in a stoichiometric decolorization of the solution. The intensity of discoloration is inversely proportional to the ability of antioxidants present in the medium to donate protons (Sánchez-Moreno *et al.*, 1998). The scavenging activity was calculated according to the following equation:

$$\text{DPPH+ (AH)n} \longrightarrow \text{DPPH-H + (A)n}$$

(AH)n / compound capable of yielding a hydrogen to the DPPH radical (violet) to reduce it to diphenyl picrylhydrazine (Yellow).

To determine the antioxidant capacity, a 100 μL aliquot of each coffee beverage was reacted with 2.9 mL of a methanolic DPPH solution. The absorbance was then measured at 515 nm as described by López-Galilea *et al.* (2006). The scavenging capacity was expressed as the percentage of DPPH inhibition, calculated according to the following equation:

$$\%\ Inhibition\ of\ DPPH\ radical = \frac{Abs\ control - Abs\ sample}{Abs\ control}\ x\ 100$$

Where *Abs control* denotes the absorbance of the radical solution without the sample, and *Abs sample* represents the absorbance of the analyzed coffee infusion.

2.3 HPLC Analysis

Instrumentation and chromatographic conditions

Quantitative of acrylamide, caffeic acid, and caffeine was performed utilizing an HPLC system coupled with a UV-Vis detector set at 210 nm. The HPLC-UV system (UltiMate 3000 RS-Variable Wavelength Detector) included an LC 1650 auto-injector, a vacuum degasser unit, a temperature controlled well-plate autosampler, a column thermostat, a quaternary pump, and a photodiode array detector.

Isocratic separation was carried out employing a C18 column (150 × 4.6 mm, 5 µm particle size, 80 Å pore size; Thermo, Bellefonte, PA, USA). The mobile phase comprised a mixture of methanol and ultrapure water (40:60, v/v), delivered at a flow rate of 1.5 mL/min. The injection volume was fixed at 20 μL, and the column temperature was maintained at 36 ± 0.5 °C to ensure retention time stability.

Box 1. Chromatographic condition of HPLC analysis
Column: A reverse-phase C-18 (150 x 4.6 mm, 5 µm)
Mobile phase: Methanol: Water (40:60, v/v)
Flow rate: 1.5 ml/min
Detector: UV detector, 210 nm
Injection volume: 20 μL
Temperature: 36 °C

Preparation of Standards and Samples

Standard solutions of acrylamide, caffeic acid, and caffeine were prepared in water at concentrations ranging from 0.08 to 0.12 mg/mL for acrylamide and caffeine, and 0.04 to 0.06 mg/mL for caffeic acid. These solutions were utilized to evaluate linearity, accuracy, and precision. Specificity was assessed by preparing individual solutions of acrylamide, caffeic acid, and caffeine at their target concentrations. For the analysis of commercial matrices, various capsule-based coffee brands were diluted in ultrapure water to achieve concentrations within the validated linear range.

Analytical Method Validation

Method validation was conducted in accordance with International Council for Harmonization (ICH) guidelines (2005) and established analytical protocols:

- **Specificity:** System specificity was evaluated by analyzing solutions containing acrylamide, caffeic acid, caffeine, methanol, the mobile phase, and a mixed solution containing all analytes. Chromatograms were carefully inspected to detect any potential co-elution of the analytes with interfering peaks.
- **Linearity and Range:** Calibration curves were constructed over the concentration ranges of 0.02–0.12 mg/mL for acrylamide and caffeine, and 0.01–0.07 mg/mL for caffeic acid.
- **Accuracy**: Accuracy was evaluated through recovery experiments performed at multiple fortification levels in triplicate.
- **Precision:** System repeatability (intra-day) was determined via five independent injections of certified reference standards within a single 24-hour period. Intermediate precision (inter-day) was assessed over three consecutive days. Results were expressed as the relative standard deviation (RSD, %).
- **Sensitivity:** The Limits of Detection (LOD) and Limit of Quantification (LOQ) were calculated based on the signal-to-noise ratio (*S/N*) and the standard deviation of the response relative to the slope of the calibration curve (ICH, 2005; Shrivastava & Gupta, 2011; Taouzinet *et al.*, 2021; Taverniers *et al.*, 2004; Toutou *et al.*, 2022).

2.4 Statistical Analysis

Data are expressed as mean ± standard deviation (SD) of triplicate measurements. Statistical significance was evaluated employing one-way analysis of variance (ANOVA). Post-hoc mean comparisons were performed through Tukey's Honestly Significant Difference (HSD) test. Statistical significance was defined at a threshold of p-value < 0.05.

3 Results

3.1 pH and Browning Index

The pH values of the Algerian coffee capsule beverages ranged from 5.36 to 5.73, whereas the European brand (C2) exhibited a pH of 5.46. The absorbance index of brown compounds, measured at 420 nm varied between 0.280 and 0.482 across the analyzed samples (Table 1).

3.2 Total Phenolic Content (TPC)

Statistical evaluation revealed a significant variation in TPC among the seven capsule coffee varieties (ANOVA, p < 0.05). Tukey's post-hoc analysis indicated that sample C6 possessed the highest phenolic concentration (6.47 ± 0.28 mg EAC/cup), which was significantly greater than other analyzed infusions. Conversely, the lowest content was observed in C3 (2.29 ± 0.02 mg EAC/cup), while the European brand C2 occupied an intermediate position (3.36 ± 0.26 mg EAC/cup) (Figure 1).

Table 1. pH and Browning Index (absorbance at 420 nm) of Coffee Capsule Brews

Samples	pH (mean ± SD)	Browning Index (abs. at 420nm) (mean ± SD)
C1	5.73 ± 0.01^{e}	0.307 ± 0.002 ^{c}
C2	5.46± 0.01^{b}	0.238 ± 0.001^{a}
C3	5.64± 0.02^{d}	0.280 ± 0.001^{b}
C4	5.54± 0.01^{c}	0.370 ± 0.002^{e}
C5	5.64± 0.02^{d}	0.482 ± 0.002^{f}
C6	5.36 ± 0.01^{a}	0.479 ± 0.001^{f}
C7	5.62 ± 0.01^{d}	0.364 ± 0.001^{d}

Note: Abs. Absorbance

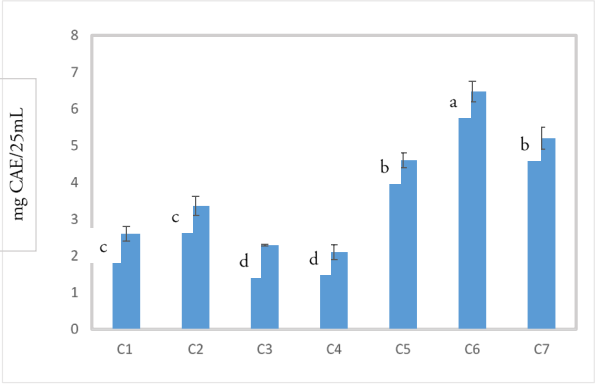

Figure 1. TPC Content of Coffee Capsule Beverages (mg EAC/25 mL)

Error bars represent standard deviation (SD) of triplicate determinations

3.3 Radical Scavenging Activity (DPPH)

The antioxidant activity of the coffee beverages was quantified via the DPPH radical scavenging assay. One-way ANOVA followed by Tukey's test (p < 0.05) revealed significant differences in scavenging efficacy across the sample set (Figure 2). The most robust antioxidant activities were recorded for the Algerian brands C5 and C3, yielding inhibition rates of 95.32% ± 5.37 and 89.56% ± 1.62, respectively. The European brand (C2) demonstrated a radical scavenging activity of 80.10% ± 4.33, ranking fourth overall.

Table 2. Calibration Curve Data for Acrylamide, Caffeic Acid, and Caffeine

Regression parameters	Acrylamide	Caffeic acid	Caffeine
Regression coefficient (R^2)	0.999	0.999	0.996
Linear equation	y = 3.68 x - 28.59	y =1.14 x + 2.18	y = 0.49 x + 1.5
Slope	3.68	1.14	0.49
Concentration range (mg/ml)	0.02 – 0.12	0.01 – 0.07	0.02 – 0.12
Number of points	8	8	8

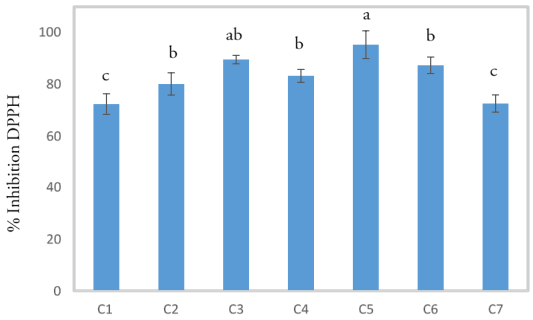

Figure 2. Anti-Radical Activity of Coffee Capsule Beverages
Error bars represent standard deviation (SD) of triplicate determinations.

3.4 Analytical Method Validation

3.4.1 Specificity and Chromatographic Profiles

Chromatograms of the individual standards of caffeic acid, caffeine and acrylamide are illustrated in Figure 3. Under the optimized chromatographic conditions, the retention times (t_R) were determined to be 2.50 min, for caffeic acid, 3.15 min for caffeine, and 5.00 min for acrylamide. The sharp, symmetrical peaks and baseline separation confirm the high specificity of the method for these analytes in coffee matrices.

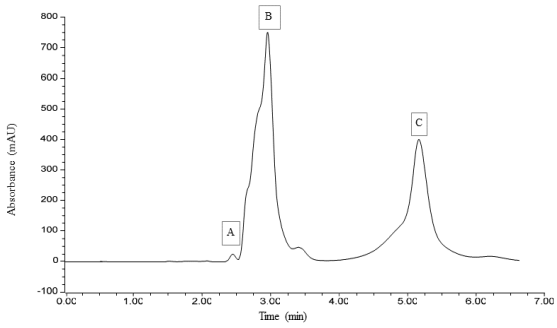

Figure 3. Chromatograms of Standard Solution
(A) Caffeic acid; (B) Caffeine; (C) Acrylamide

3.4.2 Linearity and Regression Analysis

The analytical method exhibited excellent linearity over the tested concentration ranges (0.02 – 0.12 mg/mL for acrylamide and caffeine; 0.01 – 0.07 mg/mL for caffeic acid). The regression equations and their corresponding correlation coefficients (R^2) are summarized in Table 2:

- Acrylamide: y = 3.68*x* - 28.59 (R^2 > 0.995)
- Caffeic acid: y = 1.14*x* + 2.18 (R^2 > 0.995)
- Caffeine: y = 0.46x + 1.5 (R^2 > 0.995)

3.4.3 Accuracy, Precision, and Sensitivity (LOD/LOQ)

Both intra-day and inter-day accuracy and precision parameters remained within the predefined acceptance criteria (Table 3), confirming the reliability of the method. The sensitivity of the assay was characterized by the following Limits of Detection (LOD) and Quantification (LOQ):

- **Acrylamide:** LOD = 1.192 x 10^{-5} mg/mL; LOQ = 3.97 x 10^{-5} mg/mL
- **Caffeic acid:** LOD = 9.59 x 10^{-5} mg/mL; LOQ = 3.19 x 10^{-5} mg/mL
- **Caffeine:** LOD = 9.52 x 10^{-5} mg/mL; LOQ = 3.20 x 10^{-4} mg/mL

The detection limits for acrylamide, caffeic acid, and caffeine were 1.192 * 10-5, 9.59 * 10-6, and 9.52 * 10-5 mg/mL, respectively, while the quantitation limits were 3.97 * 10-5, 3.19 * 10-5, and 0.00032 mg/mL, respectively.

3.4.4 Quantitative HPLC Analysis of Coffee Samples

The validated HPLC method was applied to the quantification of target analytes in various capsule coffee beverages. The resulting concentrations for caffeine, caffeic acid, and acrylamide are detailed in Table 4, providing a comparative chemical profile of the Algerian and European samples.

Table 3. Validation Results of the Analytical Method

Sample	Added standard (mg/mL)	Linearity (R2)	Accuracy (%recovery)	Precision (%RSD)		LD (mg/mL)	LQ (mg/mL)
				Intra-day	Inter-day		
Acrylamide	0.08	0.999	99.35	0.19	0.66	$1.192*10^{-5}$	$3.97*10^{-5}$
	0.09		100.44				
	0.1		100.41				
	0.11		100.07				
	0.12		99.70				
Caffeic acid	0.04	0.998	99.80	0.27	0.79	$9.59*10^{-6}$	$3.19*10^{-5}$
	0.045		100.32				
	0.05		100.33				
	0.055		99.19				
	0.06		100.36				
Caffeine	0.08	0.996	100.81	0.79	1.79	$9.52*10^{-5}$	0.00032
	0.09		99.98				
	0.1		99.00				
	0.11		99.58				
	0.12		100.72				

Table 4. Quantification of Caffeine, Caffeic Acid, and Acrylamide in Different Coffee Beverages

	Caffeic acid (mg/25mL)	Caffeine (mg/25mL)	Acrylamide (µg/25mL)
C1	0.001	0.11	26.15
C2	0.037	0.10	4.75
C3	0.033	0.09	2.98
C4	0.13	0.21	10.58
C5	0.07	0.24	12.15
C6	0.11	0.33	18.09
C7	0.14	005	25.49

4 Discussion

4.1 pH and Brown Compounds

The pH of coffee is a fundamental determinant of beverage quality, directly modulating flavor perception, chemical stability, and consumer acceptability. Reported values typically oscillate between 5.0 to 6.0 (Clarke & Vitzthum, 2001; Ginz *et al.*, 2000) the results obtained in this study (5.36–5.73) remain within this interval, confirming the typical acidic profile of capsule coffees. Minor fluctuations in pH may reflect differences in botanical composition, since *Arabica* tends to exhibit slightly higher pH than *Robusta* or *Arabica–Robusta* blends (Clarke & Vitzthum, 2001). Furthermore, a lower pH has also been associated with increased acidity perception and pH dependent stability of bioactive compounds. For instance, chlorogenic acids undergo accelerated degradation at higher pH, thereby linking pH to both sensory and functional properties of the beverage (Narita & Inouye, 2013; Yeager *et al.*, 2021).

The analysis of brown compounds (Table 1) provides further insights into the roasting kinetics of the samples. These compounds—predominantly melanoidins synthesized via the Maillard reaction—contribute not only to the typical color and mouthfeel of coffee but also to antioxidant capacity of the beverage, the latter attributed to the incorporated phenolic residues into their polymeric structures (Bekedam *et al.*, 2008; Echavarría *et al.*, 2012). Spectrophotometric absorbance at 420 nm is a robust indicator of browning intensity (Vignoli *et al.*, 2011). In the current study, the minimum absorbance was recorded for the European branded coffee C2, which is consistent with a lighter roasting degree and lower melanoidin levels (Bekedam *et al.*, 2008; Vignoli *et al.*, 2011). Conversely, the higher absorbance values observed in Algerian brands suggest more intense roasting and possibly a greater proportion of *Robusta*, which is consistent with their fuller body and higher bitterness (Farah *et al.*, 2005). Given that melanoidin levels increase during roasting while native phenolics decrease, the balance between botanical composition and roast degree ultimately shapes the antioxidant potential of the final product (Del Castillo *et al.*, 2002; Moreira *et al.*, 2019).

4.2 Total Phenolic Content (TPC)

The observed variability in TPC among the capsule coffees may be attributed to several factors, including species (*Arabica* vs. *Robusta*), roast degree, capsule geometry, and brewing conditions (Bartel *et al.*, 2015; Farah *et al.*, 2005; Ludwig *et al.*, 2012). A significant determinant is the contribution of melanoidins (measured at 420 nm), which reflects roasting intensity. The relatively higher TPC values in

certain Algerian capsules coincided with stronger absorbance at 420 nm, whereas the European brand C2, characterized by lower absorbance than most Algerian brands, displayed comparatively lower TPC values, suggesting a milder roasting profile (Bekedam *et al.*, 2008; Moreira *et al.*, 2019; Vignoli *et al.*, 2011). It is noteworthy that C1, C2, and C3 are classified as blends, which influence their phenolic composition, while the varietal origin of the other capsules remained not specified.

Beyond global phenolic measurements, the specific contribution of individual isomers must be emphasized. Coffee is rich in chlorogenic acids (CGAs)—notably 5 caffeoylquinic acid (5 CQA), 3 CQA and 4 CQA—which constitute the dominant phenolics in green beans (Clifford *et al.*, 2017). During roasting, partial hydrolysis and other reactions yield caffeic acid and related derivatives; caffeic acid is particularly reactive in the Folin–Ciocalteu assay and therefore significantly influences TPC values (Moon *et al.*, 2009; Singleton *et al.*, 1999). These compounds contribute substantially to antioxidant activity, although CGA concentrations generally decrease with increasing roasting intensity (Beder-Belkhiri *et al.*, 2018; Ludwig *et al.*, 2012; Moon *et al.*, 2009; Perrone *et al.*, 2012). The TPC values reported here are consistent with those observed for *Arabica* and *Arabica–Robusta* blends across different roasting degrees in prior literature (Moreira *et al.*, 2019; Vignoli *et al.*, 2011). In synthesis, the interplay between varietal composition—including the blending strategies employed in certain capsules (C1, C2, C3)—and roasting-derived metabolites such as caffeic acid and melanoidins emerge as decisive factors shaping the phenolic content and potential bioactivity of capsule coffee beverages.

4.3 Radical Scavenging Activity (DPPH)

The maximum radical scavenging activity was identified in Algerian brands C5 and C3, whereas the European brand C2 demonstrated comparatively lower efficacy. As all samples were brewed under standardized conditions, these disparities are primarily attributable to roasting intensity and blend composition (Del Castillo *et al.*, 2002; Hecimovic *et al.*, 2011; Vignoli *et al.*, 2011).

The DPPH values are consistent with the synergistic contribution of phenolic derivatives and melanoidins. CGAs and their hydrolysis products (e.g., caffeic acid) are potent radical scavengers, while melanoidins—formed during the Maillard reaction—add complementary antioxidant activity via their polymeric structures (Clifford *et al.*, 2017; Echavarría *et al.*, 2012; Moon *et al.*, 2009; Perrone *et al.*, 2012). As roasting proceeds, CGAs generally decline, but newly formed melanoidins partly compensate—so the net DPPH response reflects a balance between loss of native phenolics and formation of melanoidins (Del Castillo *et al.*, 2002; Perrone *et al.*, 2012). Regarding trends reported in the literature, large scale analyses and comparative studies indicate that lighter roasts often exhibit higher antioxidant activity by DPPH/FRAP than darker roasts, whereas *Robusta* rich coffees tend to display higher activity than *Arabica* at a given roast degree (Hecimovic *et al.*, 2011; Melliyanti *et al.*, 2023; Vignoli *et al.*, 2011). Methodology also matters: DPPH outcomes can vary with assay parameters (e.g., DPPH concentration, reaction time), so cross study comparisons require caution (Mishra *et al.*, 2012). Overall, our pattern (C5/C3 > C2) is consistent with more intense roasting and/or higher *Robusta* proportion in the Algerian capsules, whereas C2 appears milder roasted and Arabica leaning—yielding lower DPPH.

4.4 HPLC Method Validation and Quantification

The developed HPLC method was successfully validated for the simultaneous quantification of acrylamide, caffeic acid, and caffeine in accordance with ICH guidelines (ICH, 2005; U.S. Food and Drug Administration, 2021; Lopes *et al.*, 2022).

Chromatograms of individual compound solutions confirmed the absence of additional peaks at the retention times of these active molecules. Furthermore, no peak interferences were observed in the coffee samples. The results detailed in Table 2 confirm the suitability of this HPLC method for the precise quantitative determination of acrylamide, caffeic acid, and caffeine.

Caffeic Acid and Caffeine

The composition of coffee capsules—specifically blend formulation and roasting intensity—exerts a major influence on the concentrations of caffeic acid, caffeine, and acrylamide. Roasting progressively degrades chlorogenic acids (CGAs), thereby lowering caffeic acid content (Clifford *et al.*, 2017; Vignoli *et al.*, 2014; Monteiro & Farah, 2012). In the current study, measured caffeic acid concentrations were slightly lower than those generally reported in the literature. This outcome can be attributed not only to the darker roasting applied to Algerian capsules, which promotes phenolic degradation, but also to methodological factors. These include the reduced extraction volume (25 mL per capsule versus a standard cup of 50 – 100 mL), potential differences in extraction efficiency among capsule machines, and the intrinsic quality of the beans (Eiermann *et al.*, 2020; Ludwig *et al.*, 2012).

Algerian capsules, characterized by higher melanoidin formation and lower phenolic levels, appear to undergo more intense roasting, frequently associated with *Coffea canephora* (*Robusta*)-rich blends. Consequently, they contained lower caffeic acid than the European capsule, which displayed a more balanced profile of melanoidins and phenolics,

suggesting a moderate roast and an *Arabica*-dominant blend (Moon *et al.*, 2009; Moreira *et al.*, 2012).

Caffeine levels, unlike caffeic acid, remain largely unaffected by roasting due to the relative thermal stability of the molecule (Crozier *et al.*, 2012). Instead, concentrations are primarily dictated by species composition, with *Coffea arabica* generally containing less caffeine than *C. canephora* (Ludwig *et al.*, 2014). The lower caffeine concentrations observed in the European capsule are consistent with *Arabica* dominance, whereas the higher levels found in Algerian capsules likely reflect the inclusion of *Robusta* in the blends. Additionally, the relatively reduced extraction volume (25 mL) may partly explain why absolute caffeine levels were lower than those typically reported for conventional brewed coffee (Ludwig *et al.*, 2012; Ludwig *et al.*, 2014). These compositional differences contribute not only to the stimulant properties and bitterness of the beverages but may also influence functional properties such as antioxidant activity.

Acrylamide Formation and Mitigation

Acrylamide formation is influenced by both species and roasting degree. *Robusta* possesses higher concentrations of free asparagine—the primary precursor of acrylamide in the Maillard reaction, accounting for the higher levels generally observed in the Algerian capsules (Capuano & Fogliano, 2011; Friedman, 2003; Vezzulli *et al.*, 2022). However, acrylamide is thermally labile, and its concentration decreases during the advanced stages of browning (Strocchi *et al.*, 2022). In the present study, capsules with the highest browning indices, such as C3, exhibited markedly reduced acrylamide levels, whereas those with lower browning indices, like C1 and C7, retained significantly higher amounts. Interestingly, the European capsule C2 also exhibited relatively low acrylamide content despite a moderate browning index. This can be explained by the predominance of Arabica or Arabica-rich blends in its composition, since Arabica generally contains lower free asparagine than *Robusta*, reducing the potential for acrylamide formation (Vezzulli *et al.*, 2022). Overall, a negative association was observed between melanoidin formation and acrylamide content, supporting the hypothesis that advanced stages of the Maillard reaction can lead to acrylamide depletion via secondary reactions (Pastoriza *et al.*, 2012). Importantly, when expressed per kilogram of coffee powder (assuming 6.5 g per Algerian capsule and 5.5 g for the European capsule for normalization), acrylamide levels ranged from ≈458 µg/kg (C3) reaching approximately 4023 µg/kg with C1. Most tested capsules exceeded the European Commission's benchmark level of 400 µg/kg for roasted coffee—an indicator for mitigation rather than a legal maximum—with only the lowest (C3) approaching this value (European Commission, 2017).

5 Conclusions

In conclusion, this study successfully developed and validated a robust HPLC-DAD method for the simultaneous quantification of caffeine, caffeic acid, and acrylamide in commercial coffee. The methodology demonstrated high specificity, linearity, and accuracy, with the limits of detection (LOD) and quantification (LOQ) confirming its sensitivity for identifying trace levels of these analytes.

Analysis of seven coffee capsule samples revealed notable variability in antioxidant activity, total phenolic content, and concentrations of caffeine, caffeic acid, and acrylamide. Notably, the Algerian brand C5 exhibited the highest antioxidant activity, whereas sample C6 contained the highest caffeine concentration. Acrylamide—a compound of toxicological concern—was detected in all analyzed samples, with some levels exceeding the European Union benchmark value established for instant coffee, underscoring the importance of continuous monitoring.

Beyond its analytical contribution, this study provides practical insights for the coffee industry, highlighting how species composition and roasting profiles directly influence both the functional and safety attributes of capsule beverages. For regulatory authorities, the findings emphasize the requirement for more stringent surveillance of acrylamide in both emerging and established markets. From a consumer perspective, the observed variability among brands reinforces the importance of transparent information to guide healthier and safer dietary choices. Overall, this validated method serves as a valuable tool for quality control programs, supporting both industry innovation and the protection of public health.

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