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Acrylamide and 4-methylimidazole in robusta amazônico and conilon capixaba coffees: A comparative analysis of their levels and antioxidant capacity in high-quality Brazilian *Canephora*☆

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ABSTRACT

Coffee is one of the most widely consumed beverages worldwide, and concerns regarding heat-formed contaminants such as acrylamide and 4-methylimidazole (4-MEI) have increased. Data on these compounds in specialty *Coffea canephora* remain limited, despite the growing relevance of Conilon and Robusta production in Brazil. This study evaluated specialty Conilon Capixaba (Espírito Santo) and Robusta Amazônico (Rondônia) coffees. Acrylamide and 4-MEI were quantified by LC-MS/MS and GC–MS, respectively, along with selected chemical precursors, total polyphenols, and antioxidant activity (ORAC). A total of 30 roasted and 11 green coffee samples were analyzed. Acrylamide levels ranged from 94.32 to 343.06 μg/kg, consistent with values reported in the literature and below the European Union benchmark of 400 μg/kg for roasted coffee. In contrast, 4-MEI concentrations were low (<LOD–106.16 μg/kg), remaining below those typically reported for non-specialty Canephora and Arabica coffees. Green coffees presented an average polyphenol content of 53.38 mg GAE/g, decreasing to 37.94 mg GAE/g after roasting due to thermal degradation. Antioxidant activity (ORAC) varied considerably among green samples, ranging from 105.55 to 189.11 μmol Trolox/g (average: 144.88 μmol/g). Statistical analyses indicated a positive correlation between reducing sugars and acrylamide formation, as well as an association between polyphenol content in green coffee and 4-MEI. Multivariate analysis revealed marked chemical variability even among samples from the same region, underscoring the heterogeneity of Brazilian specialty Canephora coffees. Overall, the results confirm that specialty Canephora coffees exhibit low levels of heat-induced contaminants while maintaining significant bioactive potential, supporting their valorization within the Brazilian specialty coffee sector.

1. Introduction

Brazil is currently the second-largest global producer of *Coffea canephora*, with increasing recognition of the quality of its cultivars, which have traditionally been destined for the instant coffee industry. In recent decades, significant advancements in cultivation practices, selective harvesting, post-harvest processing, and sensory evaluation protocols have driven the emergence of so-called specialty coffees of the *C. canephora* species, elevating their status in both national and international markets (Baqueta et al., 2023). In this context, varieties such as Robusta Amazônico cultivated by indigenous farmers, originating from the Amazon region of Rondônia state, and Conilon Capixaba, produced mainly in Espírito Santo state, stand out for their superior sensory profiles and hold Geographical Indication (GI) certifications. These certifications reflect not only regional typicity but also the adoption of sustainable and socially inclusive practices throughout the production

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chain (Baqueta et al., 2023; Baqueta et al., 2025).

Alongside the growing appreciation for sensory quality, concerns have emerged regarding chemical food safety, particularly with respect to the formation of contaminants during coffee roasting (da Costa et al., 2023). Acrylamide, for example, is a well-studied process contaminant, primarily formed through the Maillard reaction, which results from the interaction between reducing sugars (such as glucose and fructose) and the amino acid asparagine at temperatures higher than 120 °C (Vezzulli et al., 2022). This compound has genotoxic properties and is classified by the International Agency for Research on Cancer (IARC) as probably carcinogenic to humans (Group 2 A) (International Agency for Research on Cancer (IARC), 1994). No tolerable intake levels of acrylamide could be established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (World Health Organization, 2011) and the European Food Safety Authority (EFSA (European Food Safety Authority), 2015). Its presence in food has prompted various risk management initiatives, including the establishment of a benchmark level of 400 μg/kg for roasted coffee by the European Union (Commission Regulation, 2017).

Another compound of concern formed during coffee roasting is 4-methylimidazole (4-MEI), a nitrogen-containing heterocyclic compound mainly generated through caramelization and Maillard reaction involving ammonia and reducing sugars. The IARC classifies 4-MEI as possibly carcinogenic to humans (Group 2B) (Internacional Agency for Research on Cancer (IARC), 2025). The United States National Toxicology Program (NTP) identified 4-MEI as a carcinogenic substance, with the ability to induce pulmonary neoplasia (including alveolar/bronchiolar adenomas and carcinomas) in mouse after exposure at high doses (170 mg of 4-MEI per kg body weight) (National Toxicology Program, 2007). In response, the Office of Environmental Health Hazard Assessment (OEHHA) has established a No Significant Risk Level (NSRL) of 0.05 mg/day body weight for 4-MEI (OEHHA, 2011). While maximum concentrations of 200 mg/kg and 250 mg/kg for Class III and Class IV caramel colorants are set in some regions (EC., 2012), there is currently no specific legislation regulating the presence of 4-MEI in coffee products. To date, no consolidated scientific evidence has confirmed the direct influence of bioactive compounds, such as chlorogenic acids or other phenolics present in green coffee, on the formation of 4-MEI during roasting (da Costa et al., 2023; Farah & Donangelo, 2006).

Despite worldwide advancements in monitoring acrylamide and 4-MEI in commercial coffee samples, a significant gap persists in the scientific literature regarding their occurrence in Brazilian coffees, including specialty *C. canephora* coffees, particularly those certified by GI (Alves et al., 2010; Bertuzzi et al., 2020; Vezzulli et al., 2022; da Costa et al., 2023; Baqueta, Diniz, et al., 2024; Baqueta, Marini, et al., 2024). Given the increasing penetration of these coffees into niche markets, the generation of data on process contaminants, including acrylamide and 4-MEI, is essential to ensure food safety (Baqueta et al., 2025; da Costa et al., 2023).

In this context, the present study aimed to evaluate the levels of acrylamide and 4-MEI in roasted samples from two Brazilian *Coffea canephora* varieties certified with GI (Robusta Amazônico and Conilon Capixaba). For the Conilon Capixaba variety, potential precursors and antioxidant capacity were also assessed. Additionally, green beans were analyzed for the main precursors of acrylamide and 4-MEI — asparagine, glucose, and fructose — to investigate possible correlations with contaminant levels after roasting.

2. Materials and methods

2.1. Samples

The origin, botanical variety, post-harvest method, and overall quality score of *Coffea canephora* samples previously provided by Embrapa Rondônia (Porto Velho, Brazil), are presented in Table 1. The samples were collected from the Brazilian States of Espírito Santo and

Table 1
Coffee samples information.

Sample ID	State	City/Origin	Botanical variety	Post-harvest	Overall quality
ES 94	Espírito Santo	Nova Venécia	Conilon Capixaba	Natural	75.00
ES 225	Espírito Santo	Nova Venécia	Conilon Capixaba	Natural	83.00
ES 278	Espírito Santo	Nova Venécia	Conilon Capixaba	Natural	79.00
ES 326	Espírito Santo	Nova Venécia	Conilon Capixaba	Natural	77.50
ES 495	Espírito Santo	Nova Venécia	Conilon Capixaba	Natural	77.10
ES 746	Espírito Santo	Nova Venécia	Conilon Capixaba	Natural	74.60
ES 763	Espírito Santo	Nova Venécia	Conilon Capixaba	Natural	74.00
ES 909	Espírito Santo	Nova Venécia	Conilon Capixaba	Natural	80.50
ES 397	Espírito Santo	Nova Venécia	Conilon Capixaba	Natural	74.50
ES 250	Espírito Santo	Nova Venécia	Conilon Capixaba	Natural	80.50
ES 661	Espírito Santo	Nova Venécia	Conilon Capixaba	Natural	74.00
ES 795	Espírito Santo	Nova Venécia	Conilon Capixaba	Natural	75.00
ES 927	Espírito Santo	Nova Venécia	Conilon Capixaba	Natural	83.00
ES 963	Espírito Santo	Muniz freire	Conilon Capixaba	Natural	79.00
ES 990	Espírito Santo	Nova Venécia	Conilon Capixaba	Natural	77.50
RO IND 59	Rondônia	Terra Indígena Sete de Setembro	Robusta Amazônico	SIAF	85.50
RO IND 30	Rondônia	Terra Indígena Sete de Setembro	Robusta Amazônico	SIAF	85.50
RO IND 56	Rondônia	Terra Indígena Sete de Setembro	Robusta Amazônico	SIAF	85.13
RO IND 14	Rondônia	Terra Indígena Sete de Setembro	Robusta Amazônico	SIAF	87.50
RO IND 28	Rondônia	Terra Indígena Sete de Setembro	Robusta Amazônico	SIAF	84.13
RO IND 44	Rondônia	Terra Indígena Rio Branco	Robusta Amazônico	SIAF	83.81
RO IND 09	Rondônia	Terra Indígena Sete de Setembro	Robusta Amazônico	SIAF	83.21
RO IND 63	Rondônia	Terra Indígena Sete de Setembro	Robusta Amazônico	SIAF	80.63
RO IND 52	Rondônia	Terra Indígena Sete de Setembro	Robusta Amazônico	SIAF	86.88
RO IND 38	Rondônia	Terra Indígena Sete de Setembro	Robusta Amazônico	SIAF	83.25
RO IND 31	Rondônia	Terra Indígena Sete de Setembro	Robusta Amazônico	SIAF	88.94
RO IND 46	Rondônia	Terra Indígena Rio Branco	Robusta Amazônico	SIAF	82.75
RO IND 62	Rondônia	Terra Indígena Sete de Setembro	Robusta Amazônico	SIAF	84.31
RO NON IND 1	Rondônia	Novo Horizonte do Oeste	Robusta Amazônico	SIAF	88.65
RO NON IND 2	Rondônia	Cacoal	Robusta Amazônico	SIAF	88.80

SIAF: self-induced anaerobic fermentation.

Rondônia. From Espírito Santo, 15 samples of the Conilon variety were collected, primarily from Nova Venécia municipality; these coffees were processed using the dry (natural) method. From Rondônia, 15 samples of the Robusta Amazônico variety cultivated by indigenous, were collected from Indigenous Lands (Sete de Setembro and Rio Branco) and municipalities of Novo Horizonte do Oeste and Cacoal; these coffees were processed using self-induced anaerobic fermentation (SIAF), by the farmers themselves.

All coffee samples were roasted according to the Specialty Coffee Association standard protocol (Specialty Coffee Association, 2015), using a professional sample roaster, model Lab Roaster (Carmomaq®, Brazil), equipped with two roasting chambers and a capacity of 160 g per batch. Roasting time ranged from 9 to 10 min, aiming for a medium roast level. The degree of roast was standardized using the Agtron color scale, with values between 55 and 65, corresponding to a medium roast classification according to Specialty Coffee Association standard protocol (SCA) parameters.

In addition, 11 green coffee samples of *Coffea canephora*, botanical variety Conilon Capixaba (Sample ID: ES 94, ES 225, ES 250, ES 326, ES 495, ES 746, ES 763, ES 909, ES 927, ES 963, and ES 990) were ground by cryogenic grinding using liquid nitrogen and subsequently processed in a household coffee grinder (Cadence, model MR35). Particle size standardization was performed using a 20 mesh/Tyler sieve (0.85 mm). After grinding, the coffee samples were stored in hermetically sealed containers at −20 °C.

2.2. Chemicals and reagents

Folin–Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA), sodium carbonate (Na_2CO_3, ≥99 %, Sigma-Aldrich, St. Louis, MO, USA), dipotassium hydrogen phosphate (K_2HPO_4, ≥99 %, Sigma-Aldrich, St. Louis, MO, USA), and monopotassium dihydrogen phosphate (KH_2PO_4, ≥99 %, Sigma-Aldrich, St. Louis, MO, USA) were used for buffer preparation. Sodium fluorescein (≥95 %, Sigma-Aldrich, St. Louis, MO, USA) was employed as a fluorescent probe. 2,2′-Azobis(2-methylpropionamidine) dihydrochloride (AAPH, ≥97 %, Sigma-Aldrich, St. Louis, MO, USA) was used as a radical generator and hydrochloric acid (HCl, ≥37 %, analytical grade, Synth, Diadema, SP, Brazil) was used for pH adjustment and sample treatment. Potassium hexacyanoferrate (II) trihydrate (≥99 %, Sigma-Aldrich, St. Louis, MO, USA) and zinc sulfate heptahydrate (≥99 %, Sigma-Aldrich, St. Louis, MO, USA) were also used. Organic solvents of HPLC grade included acetone (≥99.8 %), glacial acetic acid (≥99.7 %), acetonitrile (≥99.9 %), ethyl acetate (≥99.8 %), and dichloromethane (≥99.8 %), all purchased from Merck (Darmstadt, Germany). Ultrapure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA).

Gallic acid (3,4,5-trihydroxybenzoic acid, $C_7H_6O_5$, ≥98 %, Sigma-Aldrich, St. Louis, MO, USA) was used as the phenolic standard and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, ≥97 %, Sigma-Aldrich, St. Louis, MO, USA) as the antioxidant standard. Acrylamide analytical standard (≥99 %) and isotopically labeled internal standard 2,3,3-d_3-acrylamide (≥98 %) were obtained from Sigma-Aldrich (St. Louis, MO, USA). The standard of 4-methylimidazole (≥99 %) was acquired from Sigma (West Chester, PA, USA), while ethylimidazole (2-EI, ≥98 %) was obtained from Aldrich (Steinheim, Germany) as an internal standard. Bis(2-ethylhexyl) phosphate (BEHPA, ≥98 %) was purchased from Aldrich (Steinheim, Germany) and isobutyl chloroformate (IBCF, ≥99 %) from Sigma (West Chester, PA, USA). Isooctane and acetonitrile, both LiChrosolv grade, were obtained from Merck (Darmstadt, Germany). Pyridine (over molecular sieve, ≥99.8 %), acetic acid (≥99.7 %), chloroform (≥90 %), and isobutanol (≥99.8 %) were acquired from Fluka (Neu-Ulm, Germany).

Isolute Multimode® cartridges (300 mg, 3 mL; Biotage, Uppsala, Sweden) and PVDF membrane filters (13 mm diameter, 0.22 μm pore size; Analitica, São Paulo, SP, Brazil) were used for sample preparation and filtration. The phosphate buffer (0.2 M; pH 6) was prepared using potassium dihydrogen phosphate and dipotassium phosphate (≥99 %, Sigma, West Chester, PA, USA). Ultra-high purity helium for GC–MS and nitrogen for solvent evaporation were obtained from Gasin (Maia, Portugal). All other reagents were of analytical grade and purchased from various suppliers.

2.3. Chemical characterization of green and roasted coffee samples

Fig. 1 illustrates the experimental design and the chemical analyses performed on two distinct groups of coffee samples: green coffee and roasted coffee. The green coffee group consisted of 11 samples of the Conilon variety from Espírito Santo (Sample ID: ES 94, ES 225, ES 250, ES 326, ES 495, ES 746, ES 763, ES 909, ES 927, ES 963, and ES 990). These samples were analyzed for precursor compounds (reducing sugars and asparagine), total phenolic content, antioxidant capacity using the Oxygen Radical Absorbance Capacity (ORAC) method, and moisture content. The roasted coffee group was divided into two subgroups: (I) 11 samples of the Conilon variety from Espírito Santo analyzed for total phenolics and (II) 30 samples (15 of the Conilon variety from Espírito Santo and 15 of the Robusta variety from Rondônia) analyzed for acrylamide and 4-MEI. In the following sections, we detail the methodologies used for each chemical analysis.

2.3.1. Precursors analysis (reducing sugars and asparagine)

D-glucose, D-fructose, and L-asparagine were quantified in green coffee samples from the Conilon Capixaba variety using enzymatic methods with Megazyme kits (Chicago, IL, USA) adapted for coffee matrices. Absorbance was measured in 96-well microplates at 340 nm (BMG LABTECH, FLUOstar Omega, Germany) and concentrations were calculated from standardized spreadsheets. L-asparagine was extracted from 2 g of ground coffee with 25 mL of 0.1 N HCl, sonicated for 20 min, centrifuged (4500 rpm, 20 min), and microfiltered (0.22 μm) following Abbood et al. (2009)Abbood (2009, adapted). D-glucose and D-fructose were extracted from 0.5 g of coffee using 50 mL of deionized water at 80 °C for 10 min, followed by centrifugation (10,000 × *g*, 5 min) and filtration (0.22 μm), according to Santos et al. (2018, adapted).

2.3.2. Total phenolics (TP)

Total polyphenols were quantified in 11 green and 11 roasted Conilon Capixaba coffee samples using the Folin–Ciocalteu method (Singleton et al., 1999). Polyphenols were extracted from 1 g of sample in 20 mL of acetone:water (50:50), homogenized, and centrifuged. Absorbance was measured at 760 nm using a FLUOstar Omega microplate reader (BMG Labtech, Germany).

2.3.3. Determination of antioxidant capacity by oxygen radical absorbance capacity (ORAC)

The antioxidant capacity was determined using the ORAC (Oxygen Radical Absorbance Capacity) method, based on the procedures described by Cao et al. (1993) and Ou et al. (2001). Fluorescence decay was monitored over time to calculate the area under the curve (S) for blanks, Trolox standards, and samples. The mean blank value was subtracted from standard and sample values to correct background fluorescence. A calibration curve was constructed from corrected Trolox values against known concentrations, allowing calculation of antioxidant capacity via the regression equation. Results were expressed as micromoles of Trolox equivalents per gram of sample (μmol TE/g), adjusted for dilution factors.

2.3.4. Acrylamide determination

The modified method of Arisseto et al. (2008) was applied for acrylamide extraction from coffee. One gram of sample was spiked with 25 μL acrylamide-*d3* (10,000 μg/mL), extracted with 10 mL ultrapure water at 60 °C, vortexed, and treated with Carrez I (15 % potassium hexacyanoferrate (II)) and Carrez II (30 % zinc sulfate), followed by defatting with hexane. Liquid–liquid extraction with ethyl acetate/NaCl

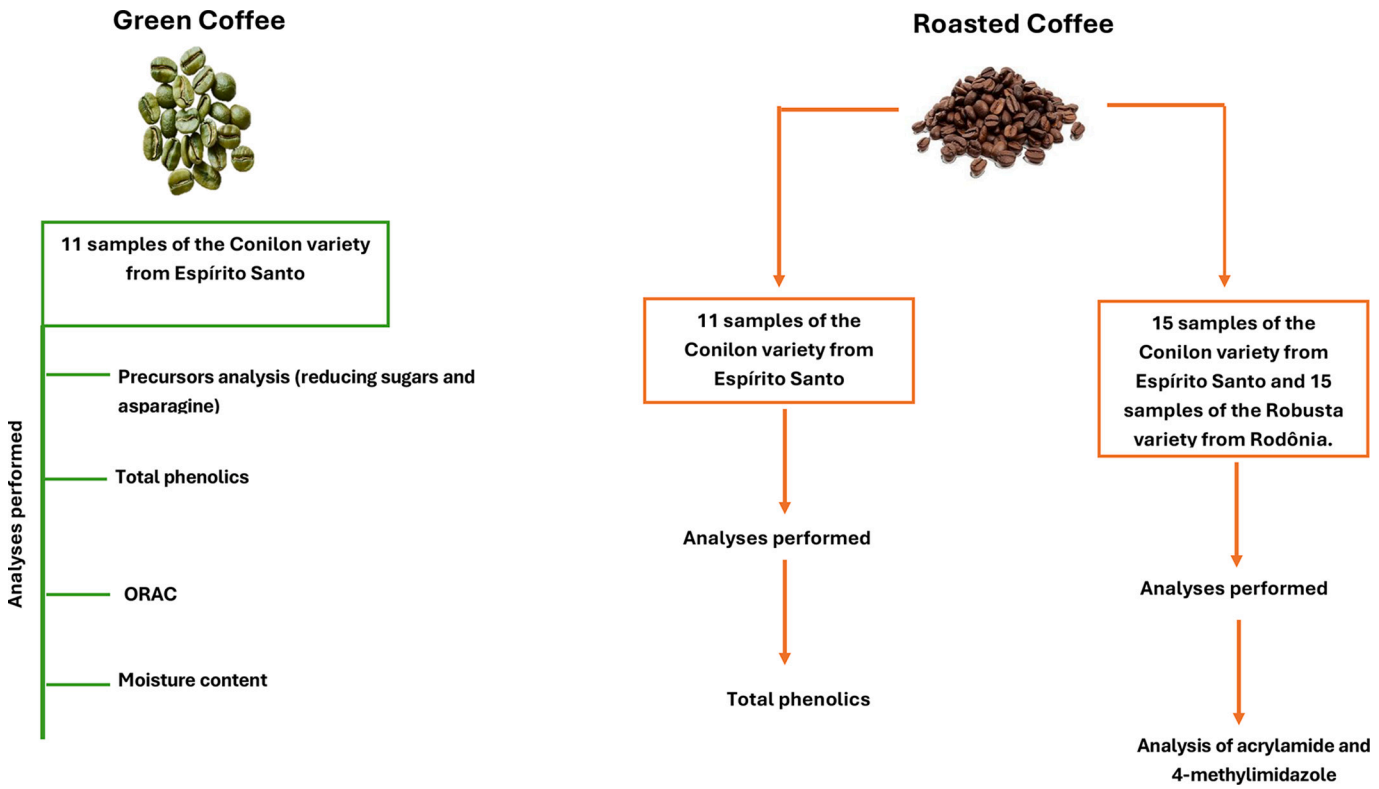

Fig. 1. Flowchart of the chemical analyses performed on green and roasted coffee samples. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

was performed twice; the organic phase was washed with acidified water (0.01 % formic acid), evaporated, and adjusted to 2 mL. Cleanup was performed on Isolute Multimode® SPE cartridges preconditioned with methanol/water. UHPLC–MS/MS models 1290 Infinity and 6460 Triple quadrupole (Agilent Technologies, Santa Clara, CA, USA) was used for analyses. The chromatography was performed using a Poroshell 120 EC-C18 reverse-phase column (2.7 μm, 2.1 mm × 150 mm), binary mobile phase (0.01 % formic acid in water and 0.01 % formic acid in acetonitrile), 30 °C, 0.200 mL/min flow, and 2.0 μL injection. ESI+ (SRM) monitored *m/z* 72.1 → 55.1 (quantifier) and *m/z* 72.1 → 44.0 (qualifier) for acrylamide, and *m/z* 75.0 → 58.0 for the internal standard (IS). The calibration curve ranged from 20 to 1000 μg/kg. All data acquisition was acquired and processed using MassHunter software version 8.00 (Agilent Technologies, Santa Clara, CA, USA).

2.3.5. 4-MEI determination

The methodology was adapted from Cunha et al. (2016) with modifications for 4-MEI analysis in roasted and ground coffee. Duplicate 2.0 g samples were spiked with 100 μL of 2-EI internal standard (10 mg/L) and extracted three times with 4 mL methanol under agitation (10 min each). The combined extract was evaporated under nitrogen, reconstituted in 4 mL phosphate buffer (0.2 M, pH 6), vortexed, centrifuged, and subjected to liquid–liquid extraction with 0.1 M BEHPA in chloroform. The organic phase was transferred to 0.1 M HCl, manually mixed, centrifuged, and 1 mL of aqueous phase derivatized with acetonitrile, isobutanol, pyridine, and isobutyl chloroformate. Isooctane extraction was performed and 1 μL was injected into a GC–MS (Agilent 6890/5975, DB-5 ms column, EI 70 eV, SIM mode aquicsition). Chromatographic conditions followed a splitless injection at 270 °C, helium carrier gas at 1 mL/min, and an oven program from 80 °C to 280 °C. Quantification used *m/z* 182 (quantifier) and m/z 82, 109, 81 (qualifiers) for 4-MEI, and m/z 196 (quantifier) for the internal standard (IS). Data acquisition and control were performed using Agilent ChemStation software.

2.3.6. Moisture content

The moisture content was determined using the gravimetric method, which involves direct drying in an oven, as described by Zenebon et al. (2008). Briefly, 3 g of green coffee sample were placed in previously weighed and desiccated crucibles. These crucibles were then placed in an oven set at 105 °C ± 5 °C until a constant weight was reached.

2.4. Statistical analysis

Analyses for precursors (glucose, fructose, and asparagine), moisture, phenolics and ORAC were carried out in triplicate, while acrylamide and 4-MEI quantifications were conducted in duplicate. Statistical analyses were performed using R software (version 2025.05.1 + 513). Data normality was assessed with the Shapiro-Wilk test ($p > 0.05$ indicating normal distribution). For normally distributed data, one-way analysis of variance (ANOVA) was used to detect significant differences between treatment means ($p < 0.05$), followed by Tukey's test for pairwise comparisons. When normality was not observed, the non-parametric Kruskal-Wallis test was applied to compare group medians.

2.5. Multivariate data analysis

Multivariate analyses were employed to investigate relationships among analytical variables that cannot be captured through univariate approaches. The analysis included data on: i) asparagine, fructose, glucose, and total polyphenols in green coffee; ii) total polyphenols, ORAC, acrylamide, 4-MEI, and overall quality in roasted coffee. The resulting data matrix was processed using MATLAB R2019a (The MathWorks, Natick, MA) coupled with PLS_Toolbox 8.6 (Eigenvector Research Inc.).

Initially, Pearson correlation coefficients were calculated to evaluate the strength and direction of associations between variables. Multiple coefficients were calculated for pairs of variables in the dataset, aiding in the identification of relational patterns between variables. Coefficients

closer to +1.00 indicate strong positive correlations (i.e., as one variable increases, so does the other), while those closer to −1.00 indicate strong negative correlations (i.e., as one variable increases, the other decreases). The closer the value is to ±1.00, the stronger the correlation.

Additionally, principal component analysis (PCA) was performed to evaluate variability among the samples based on the same set of analytical variables, complementing the correlation analysis by identifying the most influential variables contributing to sample differentiation. The data matrix was auto scaled to account for differences in measurement units and to ensure equal weighting of all variables. Principal components (PCs) explaining the highest variance were selected for interpretation. In this analysis, the first four PCs were considered informative. Two biplots were generated to visualize the relationships: one showing the interaction between PC1 and PC2, and another between PC3 and PC4. These biplots represent the samples through scores and the variables through loadings.

3. Results and discussion

3.1. Occurrence of acrylamide and 4-MEI in robusta amazônico and conilon capixaba roasted coffees

The concentrations of acrylamide and 4-MEI are presented in Table 2. Acrylamide levels ranged from 94.32 to 343.06 μg/kg, with a global average of 192.7 μg/kg. All samples presented values above the LOQ (20 μg/kg), reinforcing its ubiquitous formation during coffee roasting, which is promoted by the Maillard reaction between free asparagine and reducing sugars — the main precursors of acrylamide formation in foods (Tareke et al., 2002; Yashwanth et al., 2024).

The Student's *t*-test indicated no statistically significant difference in the mean acrylamide levels between Conilon Capixaba and Robusta Amazônico coffees ($p > 0.05$). Thus, within the scope of this study, differences in the drying methods used for each group were not a determining factor for acrylamide formation. However, although the group means did not differ statistically, individual samples exhibited substantial variability, with some Conilon Capixaba samples showing notably higher concentrations than others. This intra-group variability was confirmed by one-way ANOVA followed by Tukey's test, which revealed significant differences ($p < 0.05$) among specific samples. This demonstrates that, despite the absence of significant differences between the group means, a relevant level of chemical heterogeneity exists within each group. Within the Espírito Santo group, originating from different localities within the state, both the highest mean concentrations (ES 495, ES 746, and ES 278) and the lowest (ES 927 and ES 963) were observed, while in the Robusta Amazônico group, samples RO IND 31 and RO IND 62 showed the lowest values (Table 2). These results suggest that factors such as differences in the biochemical composition of the beans and environmental conditions associated with the growing regions may have played a more relevant role in acrylamide variability (Vezzulli et al., 2022).

In the analyzed samples, the Conilon Capixaba coffee underwent natural drying, while the Rondônia coffee was subjected to induced anaerobic fermentation. The roast degree and conditions were kept similar between the groups, ensuring comparability in acrylamide formation. On the other hand, different post-harvest processes can alter the levels of free asparagine and reducing sugars — critical precursors for

Table 2
Concentrations of acrylamide and 4-methylimidazole (4-MEI) in *Coffea canephora* samples from the Conilon Capixaba (Espírito Santo) and Amazonian Robusta (Rondônia) varieties.

Code	Sample ID	Mean acrylamide concentration (μg/Kg) ± *SD	Min–Max (μg/Kg) Number of replicates = 2	Mean 4-MEI concentration (μg/Kg) ± SD	Value-Z	Min–Max (μg/Kg) Number of replicates = 2
1	ES 397	149.02 ^{m,n,o} ± 10.34	140.95–157.08	106.16 ± 8.91	3,39	93.57–116.42
2	ES 250	162.60 ^{l,m,n} ± 0.29	154.54–170.67	51.46 ± 1.17	-0,64	50.21–53.32
3	ES 661	223.01^{e,f,g} ± 3.64	214.95–231.07	55.65 ± 1.21	0,04	52.91–55.99
4	ES 795	139.13^{o} ± 1.42	131.07–147.20	< LOQ	-1,20	44.72–46.40
5	ES 927	94.32^{p} ± 6.96	86.26–102.39	60.07 ± 0.83	1,32	59.00–60.89
6	ES 963	103.02 ^{p} ± 3.82	94.96–111.08	60.39 ± 1.85	1,42	58.50–62.44
7	ES 990	146.05 ^{n,o} ± 1.13	137.99–154.12	58.29 1.12	0,86	57.12–60.01
8	ES 94	228.04 ^{e,f,g} ± 1.23	219.98–236.10	77.69 ± 1.78	2,98	78.92–79.59
9	ES 225	256.21^{c,d} ± 7.96	248.14–264.27	< LOQ	−1.20	44.54–53.66
10	ES 278	266.49^{c} ± 7.47	258.43–274.55	53.43 ± 5.65	−0,03	47.85–62.44
11	ES 326	255.47^{c,d} ± 4.49	247.41–263.53	62.24 ± 3.42	1,84	56.80–66.27
12	ES 495	343.06^{a} ± 8.68	335.00–351.12	62.94 ± 0.92	2,06	61.63–64.23
13	ES 746	325.23^{a,b} ± 10.82	317.16–333.29	55.04 ± 2.33	0,18	52.42–58.76
14	ES 763	229.35^{e,f,g} ± 6.26	221.28–237.41	60.98 ± 4.26	1,48	56.11–65.58
15	ES 909	217.27 ^{f,g,h} ± 1.08	209.21–225.33	71.10 ± 3.10	2,70	66.64–72.36
16	RO IND 59	319.28^{e} ± 2.70	311.21–327.34	54.4 ± 2.06	0,01	52.65–57.80
17	RO IND 30	136.22^{o} ± 1.58	128.16–144.29	61.15 ± 2.63	1,56	57.59–64.83
18	RO IND 56	331.99^{a,b} ± 2.43	323.93–340.06	61.9 ± 0.55	1,77	61.12–64.83
19	RO IND 14	214.02^{g,h} ± 1.06	205.96–222.08	<LOD	−2,46	<LOD
20	RO IND 28	237.42^{d,e,f} ± 7.89	229.35–245.48	81.24 ± 4.76	3,08	77.09–89.28
21	RO IND 44	197.10^{h,i,j} ± 1.31	189.04–205.17	<LOD	−2,46	<LOD
22	RO IND 63	171.40 ^{k,l,m} ± 3.61	163.34–179.46	<LOD	−2,46	<LOD
23	RO IND 52	174.72 ^{j,k,l} ± 1.27	166.66–182.79	<LOD	−2,46	<LOD
24	RO IND 38	190.38^{i,j,k} ± 2.62	182.31–198.44	<LOD	−2,46	<LOD
25	RO IND 31	173.14 ^{k,l} ± 8.27	165.07–181.20	<LOD	−2,46	<LOD
26	RO IND 46	210.60^{g,h,i} ± 2.00	202.54–218.66	<LOD	−2,46	<LOD
27	RO IND 62	132.39^{o} ± 4.70	124.33–140.46	<LOD	−2,46	<LOD
28	RO NON IND 1	216.50 ^{f,g,h} ± 12.72	208.43–224.56	<LOD	−2,46	<LOD
29	RO NON IND 2	243.29^{d,e} ± 3.97	235.22–251.35	53.26 ± 1.57	0,01	51.56–55.06
30	RO IND 09	214.02 ^{g,h} ± 1.06	205.96–222.08	56.24 ± 3.37	1,56	51.14–60.40

SD: Standard deviation. Values were expressed as mean ± standard deviation. Different letters in the same column (mean acrylamide concentration) indicate statistical differences ($p < 0.05$) between the results, according to Tukey's test. After identifying significant differences for 4-methylimidazole using the non-parametric Kruskal-Wallis test ($p < 0.05$), the Mann-Whitney test revealed a statistically significant difference between the Amazonian Robusta and Capixaba Conilon coffees. The Z value in the 4-methylimidazole concentration data represents the standardized score of the mean (*Z*-score), indicating how far each sample's mean is from the overall mean of the data. The limits of detection (LOD) and quantification (LOQ) for 4-methylimidazole were 20 μg/kg and 50 μg/kg, respectively.

acrylamide formation during roasting. Induced anaerobic fermentation, for instance, has been associated with a greater degradation of these precursors by modifying the chemical profile of the beans, potentially reducing acrylamide formation (Halagarda & Obrok, 2023). Natural drying, in turn, may favor the preservation of these compounds. Therefore, the higher acrylamide concentrations observed in some Conilon Capixaba samples may be related to specific pre-roasting processing characteristics (Akıllıoglu & Gökmen, 2014).

The acrylamide concentrations found in this study are consistent with previously reported levels in roasted Robusta coffee samples (Bagdonaite et al., 2008; Rattanarat et al., 2021). Bertuzzi et al. (2020), for instance, found acrylamide levels in Robusta coffee ranging between 100 and 350 μg/kg. The values obtained in the present study are below the European Union's benchmark level of 400 μg/kg established for roasted coffee (EC., 2012). However, the need for continuous monitoring is evident since acrylamide is considered a genotoxic carcinogen and chronic exposure to low levels may pose long-term health risks.

In contrast to acrylamide, 4-MEI was quantified less frequently. A group predominantly composed of Robusta Amazônico coffees presented concentrations below the limit of detection (LOD, 20 μg/kg). Among the samples with quantifiable levels (above the limit of quantification, LOQ, 50 μg/kg), concentrations ranged from 51.46 to 106.16 μg/kg. The detection of 4-MEI in several samples confirms its formation as a byproduct of the roasting process, particularly at higher roast degrees where Maillard and caramelization reactions are more intense (Hengel & Shibamoto, 2013; da Costa et al., 2023). Its formation is also influenced by the biochemical composition of the beans and post-harvest treatments, which can affect the availability of precursors such as ammonia and reducing sugars (Akbari et al., 2023; Jelena & Yustiantara, 2021).

Non-parametric analysis, performed specifically for 4-MEI due to the non-normal distribution of the data (Kruskal–Wallis, $p < 0.05$, followed by Mann–Whitney), revealed significantly higher concentrations in the Capixaba samples. The data distribution included values well above or below the mean, as indicated by extreme *Z*-scores, suggesting substantial variability among each sample analyzed (Table 2).

Similar to acrylamide, post-harvest processing methods strongly influence the chemical composition of the beans, which directly impact 4-MEI formation during roasting (Halagarda & Obrok, 2023). For instance, Capixaba coffee, naturally dried, showed higher concentrations in some samples, possibly due to better preservation of precursors involved in 4-MEI formation. In contrast, the use of induced anaerobic fermentation in Rondônia coffee may alter the chemical profile of the beans, potentially reducing the formation of 4-MEI (Akıllıoglu & Gökmen, 2014; Halagarda & Obrok, 2023; Jelena & Yustiantara, 2021).

The 4-MEI concentrations detected in this study indicate that, although data on this contaminant in *Coffea canephora* already exist, they remain limited, particularly regarding the commercial varieties Conilon and Robusta. While the presence of 4-MEI has been documented in different coffee types, the available information for roasted *C. canephora* is still scarce, restricting direct comparisons and hindering the understanding of the varietal influence on compound formation.

Among the few studies focused on varietal difference, Casal et al. (2002) reported 4-MEI concentrations of 801 μg/kg (dry weight basis) for Robusta from Ivory Coast and 741 μg/kg for Arabica from Brazil. The levels obtained in the present work for Robusta samples were lower (< LOD to 106.16 - dry weight basis), suggesting that factors such as geographic origin, post-harvest processing, and roasting parameters may significantly affect 4-MEI formation. Studies on other coffee categories also indicate relevant occurrence of 4-MEI. Cunha et al. (2016) found mean 4-MEI concentrations of 223.63 μg/kg in instant coffee and 187.45 μg/kg in decaffeinated coffee, with maximum values of 339.66 μg/kg in instant coffee. Although lower than those reported by Casal et al. (2002) for Robusta, they reinforce the widespread occurrence of 4-MEI across different coffee forms.

3.2. Levels of moisture, D-fructose, D-glucose and L-asparagine in Coffea canephora samples

Table 3 presents the moisture content and mean concentrations ± standard deviations of sugars (D-fructose, D-glucose) and amino acid (L-asparagine) in green coffee beans of *Coffea canephora* (Conilon Capixaba). Differences between groups were evaluated using the Kruskal-Wallis test, with statistical significance set at $p < 0.05$.

Moisture content is a key parameter for ensuring the quality and safety of green coffee beans during storage and transport, and it is also essential for accurately expressing their chemical composition on a dry matter basis. In the samples analyzed, the moisture content ranged from 10.19 % to 12.17 % (Table 3), which falls within the recommended range for preserving the quality of green coffee beans, as reported in the literature (8.0 % to 12.5 %) (Zhu et al., 2021).

The average concentration of asparagine, a key precursor in the formation of acrylamide during the roasting process, was 407.47 mg/kg (dry basis), with levels ranging from 240.04 mg/kg (ES 927) to 590.13 mg/kg (ES 495). This variability reflects the natural differences expected in the chemical composition of green coffee beans, considering that asparagine is highly sensitive to genetic and environmental factors during cultivation and post-harvest processing (Bagdonaite et al., 2008; Murkovic & Derler, 2006). The results obtained are consistent with the findings of Murkovic and Derler (2006), who reported an average of 680 μg/g (equivalent to 680 mg/kg) of asparagine in green Robusta coffee beans, with values ranging from 280 to 960 μg/g across different regions

Table 3
Moisture and mean concentrations of D-Fructose, D-Glucose, and L-Asparagine in green and coffee beans of Conilon Capixaba.

Sample	Moisture content (%) ± SD	L-asparagine (mg/kg, dry basis) ± SD Number of replicates = 3	D-fructose (%, dry basis) ± SD Number of replicates = 3	D-glucose (%, dry basis) ± SD Number of replicates = 3
ES 94	11.66 ± 0.09	390.54 ± 0.03	0.192 ± 0.021	0.133 ± 0.016
ES 225	10.51 ± 2.12	368.74 ± 0.01	0.339 ± 0.063	0.214 ± 0.053
ES 250	10.38 ± 0.08	407.28 ± 0.04	0.171 ± 0.010	0.192 ± 0.001
ES 326	11.55 ± 0.089	435.27 ± 0.03	0.231 ± 0.016	0.166 ± 0.043
ES 495	10.19 ± 0.17	590.13 ± 0.02	0.425 ± 0.013	0.426 ± 0.014
ES 746	11.86 ± 0.56	510.55 ± 0.01	0.318 ± 0.009	0.283 ± 0.013
ES 763	11.28 ± 0.42	479.04 ± 0.04	0.335 ± 0.061	0.216 ± 0.049
ES 909	12.45 ± 1.82	365.51 ± 0.03	0.246 ± 0.030	0.110 ± 0.024
ES 927	10.43 ± 0.05	240.04 ± 0.01	0.222 ± 0.0003	0.175 ± 0.030
ES 963	10.99 ± 0.12	292.10 ± 0.01	0.282 ± 0.017	0.129 ± 0.024
ES 990	12.17 ± 0.30	296.03 ± 0.02	0.239 ± 0.015	0.211 ± 0.016

from the Conilon Capixaba (Espírito Santo) Conilon Capixaba. SD: standard deviation.

of the world. Additionally, Bagdonaite et al. (2008) found even higher levels, ranging from 780 µg/g to 813 µg/g, in Robusta coffees cultivated in India and Vietnam, respectively. Although the average values observed in the present study are slightly lower than those reported in the literature, the maximum concentrations are comparable, highlighting the wide variability of this compound depending on the origin and handling of the green coffee beans.

Regarding reducing sugars, fructose had an average content of 0.277 mg/kg (dry basis), with values ranging from 0.171 mg/kg (ES 250) to 0.425 mg/kg (ES 495), while glucose averaged 0.203 mg/kg, varying between 0.110 mg/kg (ES 909) and 0.426 mg/kg (ES 495). These reducing sugars play a crucial role as precursors in the Maillard reaction during coffee roasting, directly influencing the development of color, aroma, and flavor in the final product. According to Murkovic and Derler (2006), glucose levels in green coffee beans ranged from 0.03 % (0.3 mg/g) to 0.16 % (1.6 mg/g), while fructose levels were found from below the LOQ up to 0.59 % (5.9 mg/g). This wide variation highlights that factors such as soil composition, temperature, altitude, and cultivation conditions significantly influence the sugar profile of coffee beans. Additionally, Kleinwächter et al. (2015) reported that in *Coffea canephora* (Robusta), glucose concentrations ranged from 0.01 % to 0.5 % and fructose concentrations from 0.2 % to 0.55 %. For *Coffea arabica*, glucose levels ranged from 0.01 % to 0.45 %, and fructose levels from 0.02 % to 0.4 %.

3.3. Total polyphenols and oxygen radical absorbance capacity (ORAC)

Table 4 presents the mean concentrations ± standard deviations of total polyphenols and ORAC in of Conilon Capixaba samples. In green coffee beans, both total polyphenols and antioxidant capacity were measured to assess the intrinsic antioxidant potential of the raw material before roasting. In roasted coffee, only total polyphenols were quantified, since roasting induces complex chemical reactions such as the Maillard reaction and caramelization, which can generate non-polyphenolic antioxidant compounds, making it difficult to isolate the specific contribution of polyphenols to the overall antioxidant potential in roasted beans. Differences between groups were evaluated using the Kruskal-Wallis test, with statistical significance set at $p < 0.05$.

The total polyphenol content, responsible for the antioxidant activity of coffee, averaged 53.38 mg GAE/g in green coffee (dry basis), with levels ranging from 45.56 mg GAE/g (ES 963) to 66.70 mg GAE/g (ES 94). As expected, roasting led to a reduction in polyphenol content, with an average of 37.94 mg/g in roasted coffee and values ranging from 24.74 mg GAE/g (ES 94) to 46.21 mg/g (ES 990), reflecting the thermal degradation of phenolic compounds. The antioxidant capacity, measured by the ORAC method (oxygen radical absorbance capacity), varied considerably only among the green coffee samples. The average ORAC value was 144.88 µmol Trolox equivalents/g, with the lowest value observed in sample ES 495 (105.55 µmol/g) and the highest in ES 326 (189.11 µmol/g).

Table 4
Concentrations of total polyphenols and ORAC in green and roasted coffee of Conilon Capixaba.

Total polyphenols (green and ground coffee) (mgGAE/g, dry basis) ± SD Number of replicates = 3	Total polyphenols (roasted and ground coffee) (mgGAE/g, dry basis ± SD Number of replicates = 3	ORAC (green and ground coffee) (µmol Trolox equivalent/g) ± SD Number of replicates = 3
66.70 ± 2.01	24.74 ± 6.07	125.22 ± 9.34
51.02 ± 1.13	36.46 ± 5.39	131.05 ± 15.07
54.78 ± 2.03	34.00 ± 6.57	151.89 ± 21.02
50.40 ± 3.74	38.09 ± 0.23	189.11 ± 17.84
47.05 ± 1.53	36.92 ± 9.44	105.55 ± 12.90
52.08 ± 2.22	40.83 ± 7.24	174.04 ± 21.09
49.53 ± 1.04	36.58 ± 4.30	144.06 ± 10.14
60.26 ± 1.28	39.96 ± 5.54	163.35 ± 22.57
49.79 ± 0.70	40.42 ± 4.47	121.05 ± 8.87
45.56 ± 4.15	43.18 ± 11.24	143.92 ± 16.34
59.61 ± 1.62	46.21 ± 3.77	154.63 ± 19.62

from the Conilon Capixaba (Espírito Santo) Conilon Capixaba. SD: standard deviation; mgGAE/g - milligrams of gallic acid equivalent per gram.

The variation in total polyphenol content between the green and roasted coffee samples analyzed in this study represents a central aspect in evaluating the functional potential of these matrices. The values obtained for green coffee, ranging from 45.56 to 66.70 mg GAE/g (dry basis), were considerably higher than those reported by Alnsour et al. (2022), who found concentrations between 13.82 and 17.25 mg GAE/g in Arabica coffee samples from different origins, including Brazil. This difference can be attributed to multiple factors, including the botanical type of coffee, since the samples analyzed in this study belong to the Robusta and Conilon varieties, which are known to have higher levels of phenolic compounds compared to Arabica. For example, Robusta coffee showed an average concentration of 53.33 mg GAE/g total polyphenols, highlighting its high antioxidant potential. This trend is supported by Tripathi et al. (2025), who observed significantly higher total polyphenol concentrations in Robusta coffees grown in India, reinforcing the role of genetic variety in bioactive composition.

Furthermore, the antioxidant capacity measured by ORAC showed significant values in the green coffee samples, ranging from 105.55 to 189.11 µmol TE/g. The highest values were observed in samples ES 326 (189.11 µmol TE/g), ES 746 (174.04 µmol TE/g), and ES 909 (163.35 µmol TE/g), all associated with intermediate to high levels of total polyphenols. Interestingly, sample ES 963, which had the lowest polyphenol content (45.56 mg GAE/g), still maintained strong antioxidant capacity (143.92 µmol TE/g), suggesting that other compounds present in green coffee besides polyphenols also contribute to this antioxidant activity. These results highlight the complexity of the antioxidant profile of green coffee, which cannot be explained solely by the total polyphenol content but also depends on the specific chemical composition of each sample (Haile & Kang, 2019).

3.4. Variable correlations

Correlation analyses were performed considering 11 green coffee

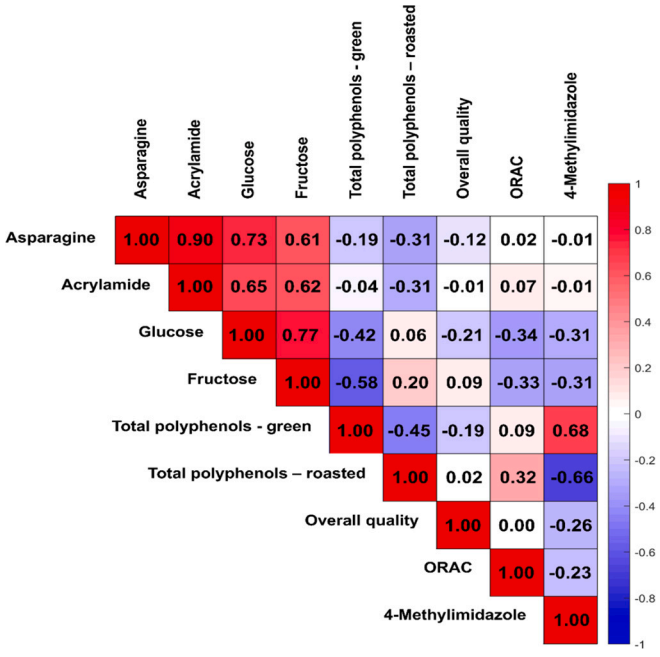

Fig. 2. Pearson correlation coefficients between the variables evaluated for Espírito Santo coffee samples.

samples of Conilon Capixaba (Sample IDs: ES 94, ES 225, ES 250, ES 326, ES 495, ES 746, ES 763, ES 909, ES 927, ES 963, and ES 990). The analyses included fructose, glucose, asparagine, total polyphenols, and ORAC antioxidant capacity in green coffee, as well as total polyphenols in roasted coffee. These biochemical parameters were correlated with the levels of acrylamide and 4-MEI formed during roasting. Additionally, an overall quality assessment was conducted for comparative purposes.

The Pearson correlation coefficients are presented in Fig. 2. Asparagine showed a very strong positive correlation with acrylamide ($\rho = 0.90$), followed by glucose ($\rho = 0.73$) and fructose ($\rho = 0.61$), indicating that higher levels of these precursors in green coffee are associated with increased acrylamide formation. As expected, fructose and glucose also exhibited a strong positive correlation with each other ($\rho = 0.77$), reflecting their shared chemical nature as reducing sugars. Interestingly, fructose showed a significant negative correlation with the total polyphenol content in green coffee ($\rho = -0.58$), suggesting that lower fructose levels are associated with higher polyphenol levels, typically represented by compounds such as chlorogenic acids and caffeic acid, among others. Both fructose ($\rho = 0.62$) and glucose ($\rho = 0.65$) also showed a positive correlation with acrylamide, reinforcing their role as key precursors.

Additionally, total polyphenols in green coffee showed a negative correlation with those in roasted coffee ($\rho = -0.45$), which is consistent with the degradation of these compounds during roasting. A positive correlation was also observed between polyphenols in green coffee and 4-MEI ($\rho = 0.68$), suggesting that the initial phenolic profile may influence the formation of this contaminant during thermal processing. However, this relationship should be interpreted with caution, as the mechanisms linking polyphenols to 4-MEI formation are not yet fully understood and may be affected by factors such as roast intensity, distinct thermal degradation pathways, and the natural variability of the samples. Conversely, total polyphenols in roasted coffee exhibited a negative correlation with 4-MEI ($\rho = -0.66$), which may reflect their degradation or their involvement in reactions contributing to 4-MEI formation during roasting. These findings highlight the complexity of coffee's thermal transformations and indicate that further studies are needed to elucidate the role of phenolic compounds in the formation and degradation of 4-MEI.

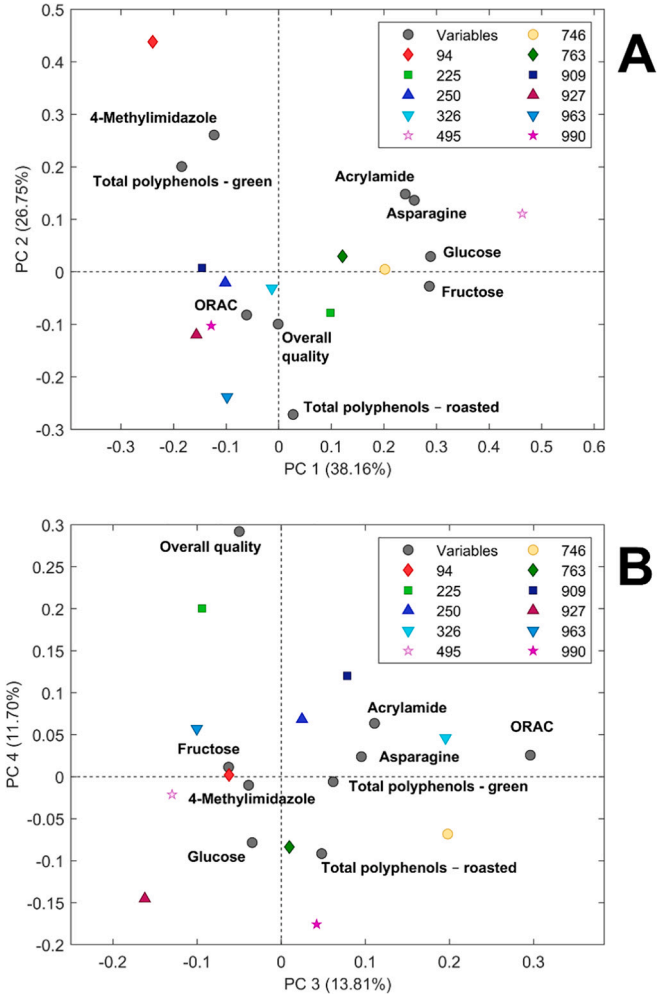

Fig. 3. Principal component analysis of the parameters evaluated in the Conilon Capixaba Espírito Santo samples based on multivariate data acquired. The biplots of PC1 vs. PC2 (A) and PC3 vs. PC4 (B) illustrate the distribution and grouping tendencies among the samples, as well as the relations with the evaluated variables.

Fig. 3 presents the PCA results, which provides a statistical visualization of the interrelationships between variables when the samples are projected onto the plot. In this analysis, only samples from Espírito Santo were considered, as multivariate data were collected exclusively for these samples. Fig. 3A shows the interaction between principal components 1 and 2 (PC1 and PC2), while Fig. 3B displays the interaction between principal components 3 and 4 (PC3 and PC4).

Overall, the samples appear quite distinct from one to another, despite having the same origin. This dispersion may be attributed to factors beyond those studied, such as genetic diversity and differences between producers, since each sample was obtained from a different producer. Although all producers adopted the same natural post-harvest processing method, individual practices and specific characteristics may vary, contributing to the observed variability. Although the variables influence the components differently depending on the direction considered (PC1 or PC2 and PC3 or PC4), it is possible to observe that some variables remain correlated, as previously noted, such as asparagine and acrylamide, as well as 4-MEI and total polyphenols.

The PCA results support findings from the literature regarding the relationships between precursors and contaminants formed during coffee roasting, such as strong correlation between asparagine and acrylamide (Kocadağlı & Gökmen, 2022). In the case of 4-MEI, previous studies indicate that its formation is related to the degradation of phenolic compounds and amino acids during roasting (Alnsour et al., 2022). Considering that the roast degree used in this study was medium, it is likely that 4-MEI concentrations are relatively low, since the formation of this compound tends to increase with darker roasts. Thus, the observed relationship between 4-MEI and total polyphenols may indicate that, in the early stages of roasting, polyphenols are still present and may influence or be associated with the formation of 4-MEI. This suggests that roast intensity directly affects both the amount of 4-MEI formed and the transformation of total polyphenols during the roasting process (Akbari et al., 2023; Hyong et al., 2021).

While classical mathematical and statistical methods are effective in identifying specific and accurate responses of individual properties in experiments involving a single variable, univariate analysis often fails to capture the complexity of interactions among multiple variables. In contrast, multivariate analysis is indispensable for revealing patterns and statistical relationships across several variables simultaneously. This approach enables a more holistic understanding of the interdependencies between properties and experimental conditions, offering more profound insights into the overall data structure that univariate methods may overlook (Héberger, 2008).

4. Conclusion

This study confirmed the occurrence of acrylamide (94.32–343.06 μg/kg) and 4-MEI (<LOD–106.16 μg/kg) in specialty *Coffea canephora* coffees from the Conilon Capixaba and Robusta Amazônico varieties. The variability observed among samples, despite the absence of

statistically significant differences between the two varieties ($p > 0.05$), suggests that contaminant formation is influenced not only by the availability of specific precursors but also by post-harvest processing practices and the intrinsic biochemical composition of the beans. For acrylamide, the levels found were similar to those described in the literature but remained below the 400 μg/kg benchmark level established by the European Union as a monitoring value for roasted coffee. In contrast, 4-MEI concentrations were low, remaining below those commonly reported for non-specialty Canephora and Arabica coffees. The positive correlation between total polyphenols in green coffee and 4-MEI levels further indicates a modulatory role of antioxidant bioactive compounds in the generation of this contaminant during roasting. Moreover, PCA results revealed chemical heterogeneity even within the same producing region, highlighting the complexity of factors that shape quality and safety in specialty coffees. Overall, these findings underscore the importance of an integrated analytical approach that combines contaminant assessment with compositional characterization, providing fundamental insights into the mechanisms underlying the formation of undesirable compounds. At the same time, the results reinforce the potential for promoting specialty Canephora coffees as safe, high-quality products, supporting their valorization and differentiation in both national and international markets.

In practical terms, these findings provide support for understanding how post-harvest steps and roasting conditions influence contaminant formation, enabling more precise adjustments in future studies aimed at mitigating these compounds. For consumers, the results indicate that the evaluated specialty Canephora coffees exhibit reduced levels of the contaminants investigated. From a regulatory perspective, the data expand the scientific basis for monitoring process-related contaminants in coffee, contributing to discussions on risk assessment and potential updates to quality-control guidelines.

CRediT authorship contribution statement

David Silva da Costa: Writing – original draft, Validation, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Camila Akemi Akiyama:** Writing – review & editing, Validation. **Michel Rocha Baqueta:** Writing – review & editing, Visualization, Validation, Data curation. **Enrique Anastácio Alves:** Writing – review & editing, Validation, Resources. **Patricia Aparecida de Campos Braga:** Writing – review & editing, Validation, Investigation. **Juliana Azevedo Lima Pallone:** Writing – review & editing, Validation. **José de Oliveira Fernandes:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition. **Sara Cristina da Silva Cunha:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition. **Adriana Pavesi Arisseto Bragotto:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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