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# Caffeine, trigonelline, chlorogenic acids, melanoidins, and diterpenes contents of *Coffea canephora* coffees produced in the Amazon

Thayna Viencz [a,*], Lucas Bonfanti Acre [a], Rodrigo Barros Rocha [b], Enrique Anastácio Alves [b], André Rostand Ramalho [b], Marta de Toledo Benassi [a]

[a] *Departamento de Ciência e Tecnologia de Alimentos, Universidade Estadual de Londrina (UEL), 86057-970 Londrina, PR, Brazil*
[b] *Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA Rondônia), 76815-800 Porto Velho, RO, Brazil*

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ABSTRACT

This study characterizes the composition profile of *Coffea canephora* coffees of good beverage quality produced in the Brazilian Western Amazon. Samples corresponded to 57 Robusta clones and 10 intervarietal hybrids of Conilon and Robusta (BRS 1216, BRS 2299, BRS 2314, BRS 2336, BRS 2357, BRS 3137, BRS 3193, BRS 3210, BRS 3213, BRS 3220). Roasted coffees were evaluated regarding the contents of caffeine, trigonelline, chlorogenic acids (CGA), melanoidins, and diterpenes (kahweol, cafestol, 16-*O*-methylcafestol). Data were analyzed by ANOVA and Scott-Knott test ($p \leq 0.05$), and Principal Component Analysis. In general, coffees showed high contents of caffeine (1.63–3.57 g 100 $g^{-1}$), trigonelline (0.60–1.15 g 100 $g^{-1}$), CGA (3.93–6.37 g 100 $g^{-1}$), and 16-*O*-methylcafestol (0.082–0.372 g 100 $g^{-1}$). Results showed contents between 10 and 17.9 g 100 $g^{-1}$ for melanoidins, 0.068 and 0.427 g 100 $g^{-1}$ for cafestol, 0.218 and 0.707 g 100 $g^{-1}$ for total diterpenes, and the presence of kahweol in 20% of the samples. The caffeine/ total diterpenes ratio supported identifying *C. canephora* species. Robusta coffees were characterized by the highest content of trigonelline, melanoidins, and 16-*O*-methylcafestol. Hybrid were characterized by the highest content of caffeine, CGA, cafestol, and total diterpenes.

## 1. Introduction

Coffee is a slightly bitter beverage produced with roasted beans (*Coffea* sp.), widely consumed worldwide. In the coffee year 2020–2021, *Coffea arabica* species accounted for 58% of the worldś production, and *Coffea canephora* species, for 42% (International Coffee Organization, 2022).

Perceived for many years as a lower-quality product, *C. canephora* coffee fetched low marketing prices, so growers tried to produce at minimal cost, which resulted in a continually undifferentiated and low-quality commodity, reinforcing the market perception. However, due to the problem of less resistance of *C. arabica* to climate change, interest in *C. canephora* cultivation has been steadily growing, in tandem with increased product quality (Specialty Coffee Association of America, 2020). Currently, *C. canephora* coffees can be recognized as a specialty coffee by the Specialty Coffee Association of America (Lingle and Menon, 2017; Teixeira et al., 2020). Quality evaluation of *C. canephora* beverages is done through the protocol for Fine Robusta Cupping (2010), specific for this species, allowing the identification of coffees with good cup quality (≥ 70 on a 100-point scale) (Uganda Coffee Development Authority, 2010; Lingle & Menon, 2017; Dalazen et al., 2020).

Brazil is the leading producer and exporter of green coffee and the second-world of roasted coffee beverages consumer (Associação Brasileira da Indústria de Café, 2021; Companhia Nacional de Abastecimento, 2022). Vietnam is the firstlargest producer of *C. canephora* followed by Brazil (with 17.7 million bags of 60 kg in 2022); Brazilian production grown more than 50% in the last 20 years, and the increase in *C. canephora* productivity can be attributed to genetic breeding and the introduction of clonal cultivars in the plantations in several coffee regions (Companhia Nacional de Abastecimento, 2013; Ferrão et al., 2020; Companhia Nacional de Abastecimento, 2022; International Coffee Organization, 2022).

Two distinct botanical varieties of *C. canephora* coffee tree, Conilon

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* Corresponding author.

*E-mail addresses:* thayviencz@hotmail.com, thayna.viencz0@uel.br (T. Viencz), lucasbonfanti97@gmail.com (L.B. Acre), rodrigo.rocha@embrapa.br (R.B. Rocha), enrique.alves@embrapa.br (E.A. Alves), andre.rostand@embrapa.br (A.R. Ramalho), martatb@uel.br (M.T. Benassi).

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and Robusta, are commercially cultivated and recognized in the field; in Brazil, there is a predominance in the production of the first (Ferrão et al., 2019a). Conilon plants are smaller, with smaller leaves and fruits, have greater tolerance to water stress and are less resistant to coffee rust (*Hemileia vastatrix*). Robusta plants have greater size and vigor, are less tolerant to water stress, are more resistant to coffee rust and root-knot nematode (*Meloidogyne* spp.) and have greater potential for fine beverage production (Espindula et al., 2019; Ferrão et al., 2019b). Due to the historical introduction of materials carried out in this region, the simultaneous presence of Conilon, Robusta, and plants from hybridization between these varieties is a particular characteristic of the Rondônia state plantations (Silva et al., 2015; Ferrão et al., 2019a). The hybridization process can be natural - when spontaneous crossing occurs between plants in the field - or it can be controlled - when crossings are directed by genetic breeding techniques (Morais et al., 2021) - such as the samples tested in this study.

Initially, *C. canephora* breeding programs focused on productivity, reduction of biennial effect and resistance to pests and diseases, selecting genotypes of the same maturation cycle that together gave rise to the first registered coffee cultivars (Espindula et al., 2019; Teixeira et al., 2020). Studies on the beverage quality have intensified recently, contributing to the valorization of this coffee and better remuneration of the coffee grower (Alves, 2020; Teixeira et al., 2020; Morais et al., 2021). In July 2021, the first worldwide Geographical Indication (Designation of Origin) of *C. canephora* coffee was obtained, known as “Matas de Rondônia”, adding value to the product.

Coffee is a rich source of bioactive compounds such as caffeine, trigonelline, chlorogenic acids, melanoidins, and diterpenes. Thus, regular and moderate beverage consumption benefits the consumeŕs health. It favors the intestinal microbiota, improves cognitive performance, has a hepatoprotective effect, and reduces the incidence of coronary diseases, diabetes *mellitus*, Parkinson’s and Alzheimer’s diseases, and some types of cancer (Ludwig et al., 2014; Hu et al., 2019; Lu et al., 2020; Moeenfard & Alves, 2020; Pereira et al., 2020; Munyendo et al., 2021). In general, *C. canephora* coffees are reported as presenting higher contents of bioactive compounds than *C. arabica* (Dias et al., 2014; Vignoli et al., 2014; Dias & Benassi, 2015; Finotello et al., 2017; Portela et al., 2021); however, data on *C. canephora* composition are still scarce. Most of the works with roasted *C. canephora* evaluated a small number of samples; the exception is the studies focusing on the characterization of diterpenes conducted by Mori et al. (2016) and Francisco et al. (2021) with 30 samples of Conilon and Brazilian natural intervarietal hybrids, respectively, and Finotello et al. (2017), with 39 Robusta coffees originated from Asia and Africa.

This study aimed to characterize the chemical composition profile of *C. canephora* of good beverage quality produced in the Western Amazon region, Robusta and intervarietal hybrid coffees. It also verified the applicability of composition parameters for species identification.

## 2. Materials and methods

### 2.1. Reagents, standards, and equipment

The following reagents, solvents, and materials were used: acetonitrile HPLC grade (Merck, Darmstadt, Germany); acetic acid (purity ≥ 99.8%, Anidrol, Diadema, Brazil); ethanol 96% analytical grade (Êxodo Científica, Hortolândia, Brazil); potassium hydroxide 85% analytical grade (Panreac, Barcelona, Spain); methyl *tert*-butyl ether HPLC grade (Acrós Organics, Morris Plains, USA); 0.22 μm nylon syringe filters and 0.45 μm nylon membranes (Filtrilo, Colombo, Brazil) and 0.40 μm qualitative filter paper.

The water was obtained with an Elga Purelab Option-Q purification and filtration system (Veolia Water Solutions & Technologies, High Wycombe, UK).

The following chromatographic standards were used: caffeine, trigonelline, 5-caffeoylquinic acid (5-CQA), and 16-*O*-methylcafestol (16-OMC) (CAS numbers 58–08–2; 535–83–1; 202650–88–2; 108214–28–4; Sigma Aldrich, Saint Louis, USA), kahweol and cafestol (CAS numbers 6894–43–5; 469–83–0; Axxora, San Diego, USA). Spherisorb ODS-1 column (150 ×4,6 mm, 3 μm; Waters, Milford, USA) was used in the analysis of water-soluble compounds, and Supelcosil LC-18 column (150 ×3 mm, 3 μm; Supelco Park, Bellefonte, USA), in the diterpenes.

Chromatographic analyses were performed in a Waters Acquity ultra-performance liquid chromatograph (Waters, Milford, USA), equipped with an automatic sample injector, quaternary solvent pumping system, column heater/cooler module, and diode array detector, controlled by the Empower 3 program.

### 2.2. Materials

*C. canephora* coffees were harvested in the 2020 crop at the experimental field located at Ouro Preto do Oeste/RO (10°43’55.3"S and 62°15’23.2"W, and 245 m of altitude) in Western Amazon (Brazil). Samples were provided by Embrapa Rondônia (Porto Velho, Rondônia, Brazil). Soil, nutritional, cultural, and phytosanitary management practices were carried out according to the recommendations for coffee cultivation in Rondônia (Marcolan et al., 2009). According to the Köppen classification, the climate in the region is Aw, tropical humid, with a dry season in the winter and a rainy season in the summer, with annual temperatures ranging from 21.2° to 30.3°C, annual rainfall of 1939 mm, and average relative humidity of 81% (Alvares et al., 2013).

To ensure the representativity of each accession, the samples were composed of a blend of fruits from twelve plants (clones) per accession, harvested at a point where each plant has at least 80% of cherry fruits. Coffee fruits were selected (cherry stage) and washed to remove impurities (leaves, stones, sticks, soil) and defects. The fruits were left to dry naturally under a “barge-type” covering (a transparent piece of furniture) until the samples reached 11–12% moisture. After drying, the fruits were peeled, and the coffee beans were sieved (sieve 15 and larger). The green beans were stored in paper packaging until roasting.

The roasting process (around 12 min at 190 ± 10 ºC) was performed with TC-02 Pinhalense roaster (Pinhalense, São Paulo, Brazil) with 2 kg capacity, and it was monitored by color. It was used a SCAA/Agtron Roast Color Classification System, which comprises a set of eight color discs, numbered in 10% increments ranging from “very light” (tile #95) to “very dark” (tile #25). The roasting degree was standardized between Agtron #65 and #55, corresponding to a range between light-medium and medium roasting degrees that is usual for specialty coffees (Morais et al., 2021). After roasting, each sample was separated into two parts of 250 g, packed in laminated packaging, and kept under refrigeration at 8 °C until analyses. One part was used for the classification of cup quality, and the other for the characterization of the composition profile.

The beverage quality classification was carried out in the laboratory of “Sindicato da Indústria do Café ” in São Paulo/SP (Brazil) by six judges/cuppers (R Grader), according to the protocol for Fine Robusta Cupping (Uganda Coffee Development Authority, 2010). The attributes (fragrance/aroma, flavor, aftertaste, salinity/acidity, bitterness/sweetness, mouthfeel, balance, uniform and clean cup, and defects) were evaluated on a scale from 0 to 10, and the final beverage quality was scored by the sum of the scores, resulting in an overall score from 0 to 100 points. The coffees had scores equal to or greater than 70 points (Tables S.2 and S.3); this score indicates, in addition to the good genetic potential, that the harvest and post-harvest procedures were carried out properly and that all the coffees studied had good beverage quality. Data on the beverage attributes cited by the cuppers were also available as Supplementary material (Tables S.2 and S.3).

In total, 67 coffees were evaluated: 57 clones of Robusta variety from the *C. canephora* germplasm bank of Embrapa Rondônia, and 10 intervarietal hybrids of Conilon and Robusta developed by Embrapa and registered in 2019, which are part of the cultivar Robustas Amazônicos (BRS 1216, BRS 2299, BRS 2314, BRS 2336, BRS 2357, BRS 3137, BRS 3193, BRS 3210, BRS 3213 e BRS 3220). More information on hybrid and Robusta coffees are available in Tables S.1 and S.2, respectively.

### 2.3. Characterization of roasted coffees

Roasted beans were ground at fine granulometry with a GVX2 coffee grinder (Krups, Shangai, China). After that it was packed in plastic bags, and kept under refrigeration (8 °C) until analyses. Ground coffees were characterized for color in a Minolta CR-410 colorimeter (Konica Minolta Sensing Inc., Osaka, Japan) with D65 illuminant and diffused illumination and moisture in a MB 45 moisture analyzer (Ohaus, Barueri, Brazil) (analyses in triplicate and duplicate, respectively). The samples had a medium-light roast, with a lightness of 38 ± 2 and a hue of 48 ± 4. Moisture was determined at 105 ºC for 7 min, and an average value of 3.9 ± 0.8 g 100 $g^{-1}$ was observed; the results were used to calculate concentrations on a dry weight basis (db).

#### 2.3.1. Water-soluble compounds analysis

The coffee extract was prepared according to Kalschne et al. (2019). Duplicate extraction was performed, and samples (0.5 g) were extracted with 30 mL of water at 80 °C for 10 min and filtered. The aqueous extract was used in the determination of water-soluble compounds.

The simultaneous determination of caffeine, trigonelline, and chlorogenic acids was carried out according to Kalschne et al. (2019). The coffee extract was diluted with water (10:90 v/v) and filtered. The mobile phase consisted of acetic acid:water (5:95 v/v) (A) and acetonitrile (B), with gradient elution: 0–5 min 5% of B, 6 min 13% of B, and 25 min 13% of B, with a flow rate of 0.5 mL $min^{-1}$ and injection volume of 10 μL. Detection was set at 260 nm for trigonelline, 272 nm for caffeine, and 320 nm for chlorogenic acids. Duplicate analysis was performed. Identification was based on retention times and UV spectra. Quantification was carried out by external standardization using 6-point analytical curves, with triplicate measurements, in the following ranges: 10–60 μg $mL^{-1}$ for caffeine, 1–30 μg $mL^{-1}$ for trigonelline, and 1–60 μg $mL^{-1}$ for 5-CQA. The total chlorogenic acids (CGA) content was estimated by the sum of areas of compounds detected at 320 nm, using 5-CQA as a standard for quantification (Mori et al., 2020). According to the analytical curve, limits of detection (LOD) of 0.059, 0.047, and 0.017 μg $mL^{-1}$ and limits of quantification (LOQ) of 0.178, 0.138, and 0.052 μg $mL^{-1}$ were obtained for caffeine, trigonelline, and 5-CQA, respectively.

Melanoidins were analyzed by diluting 0.6 mL of the aqueous extract with 3.4 mL of water to achieve 2.5 mg of coffee $mL^{-1}$. Duplicate analysis was performed at 420 nm. The melanoidins content was estimated based on the absorptivity value of 1.1289 L $g^{-1}$ $cm^{-1}$ (Mori et al., 2020).

The results of water-soluble compounds were expressed as g 100 $g^{-1}$.

#### 2.3.2. Diterpenes analysis

The determination of kahweol, cafestol, and 16-OMC was carried out according to Dias et al. (2014). Samples (0.2 g) were saponified with 2 mL of potassium hydroxide (2.5 mol $L^{-1}$) in ethanol (96%, v/v) at 80 °C for 1 h, and then added to 2 mL of water distilled. The unsaponifiable fraction was extracted by adding 2 mL of methyl *tert*-butyl ether; the organic phase was collected after shaking and centrifugation (2 min, 3000 RPM, room temperature). This step was repeated three times. For cleaning up, 2 mL of distilled water was added, and the aqueous phase was discarded. The organic extract was collected and evaporated to dryness in a water bath at 70 °C. After resuspension with 4.5 mL mobile phase (45:55 water:acetonitrile), the extract was filtered. Duplicate extraction was performed.

Isocratic elution with water:acetonitrile (45:55 v/v) at a flow rate of 0.7 mL $min^{-1}$ and injection volume of 3 μL were applied in UPLC analysis (Francisco et al., 2021). The detection was set at 230 nm for cafestol and 16-OMC and 290 nm for kahweol. Duplicate analysis was performed. Identification of compounds was based on retention times and UV spectra. Quantification was carried out by external standardization using 6-point analytical curves, with triplicate measurements, in the following ranges: 1–200 μg $mL^{-1}$ for kahweol, 50–300 μg $mL^{-1}$ for cafestol, and 2–400 μg $mL^{-1}$ for 16-OMC. According to the analytical curve, LOD of 0.794, 1.998, and 0.643 μg $mL^{-1}$ and LOQ of 2.406, 6.055, and 1.948 μg $mL^{-1}$ were obtained for kahweol, cafestol, and 16-OMC, respectively. The total diterpenes content was obtained by the sum of contents of kahweol, cafestol, and 16-OMC. The results were expressed as g 100 $g^{-1}$.

### 2.4. Data analysis

In addition to water-soluble compounds and diterpenes, the ratio between cafestol and kahweol contents and the ratio between caffeine and total diterpenes contents were calculated.

Data were analyzed by ANOVA and Scott-Knott test ($p \leq 0.05$) using the software Sisvar (Ferreira, 2014). The composition results (contents of caffeine, trigonelline, 5-CQA, CGA, melanoidins, kahweol, cafestol, 16-OMC, and total diterpenes, and the values of cafestol/kahweol ratio and caffeine/total diterpenes ratio) and the beverage quality scores were compared within each variety (Robusta and intervarietal hybrid coffees). The Scott-Knott test separates treatment means into homogeneous groups, minimizing within-group variation and maximizing between-group variation, and avoiding overlap; thus, the formation of a higher number of groups indicates a greater variability of the parameters studied.

For a more comprehensive exploratory analysis, the Principal Component Analysis (PCA) procedure of the Statistica 7.1 package software (Statsoft Inc., Tulsa, USA) was performed with the total samples. The composition parameters are considered as active variables, and total diterpenes and beverage quality scores, as supplementary variables. The correlation between the variables was verified by the multivariate correlation matrix.

## 3. Results and discussion

There was a significant difference among the samples ($p \leq 0.05$) within each variety (Robusta or hybrid coffees) for all the studied compounds. Overall, there was greater variability (observed by the formation of a higher number of groups for all compounds) among the Robusta coffees compared to that observed for the intervarietal hybrid coffees (Tables 1 and 2); however one should consider the large difference in the number of samples (57 clones and 10 clones, respectively).

Among the studied compounds, there is wide diversity regarding thermal stability. The most stable ones (caffeine and diterpenes) have their contents maintained or eventually increased in the roasted bean due to the loss of other organic compounds in the roasting process; others are easily degraded (trigonelline and CGA) or formed during the process (melanoidins) (Dias et al., 2014; Vignoli et al., 2014; Alves et al., 2020; Moeenfard and Alves, 2020; Munyendo et al., 2021). The variability in the content of compounds in roasted coffees was unrelated to their thermal stability, since caffeine and diterpenes, which are more thermostable, presented CVs from 1.14% to 9.09%, while for CGA, trigonelline and melanoidins CVs between 2.79% and 5.42% were observed (Tables 1 and 2). These results indicate not only that there was a good standardization of the roasting process but also that the variation found could be more associated with the genetics of the clones.

Considering all the coffees analyzed, caffeine was found in the range of 1.63–3.57 g 100 $g^{-1}$, with average values of 2.38 g 100 $g^{-1}$ (CV 1.64%) for Robusta coffees and 2.76 g 100 $g^{-1}$ (CV 1.14%) for hybrid coffees. Overall, the contents (Tables 1 and 2) were above or at the upper end of the caffeine range described in the literature for *C. canephora* coffees.

Brazilian *C. canephora* coffees (without variety identification) with different proportions of defective beans showed caffeine contents between 1.69 and 2.25 g 100 $g^{-1}$ (De Souza and Benassi, 2012; Dias and Benassi, 2015; Kalschne et al., 2019; Reis et al., 2019). Portela et al.

**Table 1**

Contents of caffeine, trigonelline, chlorogenic acids (CGA), melanoidins, kahweol, cafestol e 16-*O*-metilcafestol (16-OMC) (g 100 $g^{-1}$) in *C. canephora* coffees of the Robusta variety.

| Identification | Caffeine[a] | Trigonelline[a] | CGA[a] | Melanoidins[a] | Kahweol[a] | Cafestol[a] | 16-OMC[a] |
|---|---|---|---|---|---|---|---|
| R1 | $2.79^{c} \pm 0.01$ | $0.97^{d} \pm 0.04$ | $4.18^{h} \pm 0.19$ | $16.6^{b} \pm 0.7$ | $0.000^{i} \pm 0.000$* | $0.073^{h} \pm 0.001$ | $0.243^{f} \pm 0.012$ |
| R2 | $2.34^{i} \pm 0.01$ | $1.06^{b} \pm 0.01$ | $4.67^{g} \pm 0.13$ | $14.6^{c} \pm 0.9$ | $0.000^{i} \pm 0.000$* | $0.146^{d} \pm 0.018$ | $0.251^{f} \pm 0.022$ |
| R3 | $2.69^{e} \pm 0.02$ | $1.06^{b} \pm 0.01$ | $4.32^{h} \pm 0.02$ | $16.5^{b} \pm 0.8$ | $0.029^{d} \pm 0.002$ | $0.102^{f} \pm 0.009$ | $0.204^{g} \pm 0.004$ |
| R4 | $2.35^{i} \pm 0.00$ | $0.97^{d} \pm 0.01$ | $4.95^{e} \pm 0.05$ | $15.2^{c} \pm 0.8$ | $0.000^{i} \pm 0.000$* | $0.112^{f} \pm 0.001$ | $0.241^{f} \pm 0.009$ |
| R5 | $2.12^{l} \pm 0.03$ | $0.99^{d} \pm 0.00$ | $4.63^{g} \pm 0.13$ | $12.0^{f} \pm 0.4$ | $0.030^{c} \pm 0.000$ | $0.170^{b} \pm 0.013$ | $0.251^{f} \pm 0.008$ |
| R6 | $2.15^{l} \pm 0.02$ | $0.89^{f} \pm 0.01$ | $4.79^{f} \pm 0.23$ | $17.5^{a} \pm 0.5$ | $0.000^{i} \pm 0.000$* | $0.125^{e} \pm 0.003$ | $0.240^{f} \pm 0.015$ |
| R7 | $2.26^{j} \pm 0.06$ | $1.11^{a} \pm 0.03$ | $5.27^{d} \pm 0.35$ | $17.9^{a} \pm 0.5$ | $0.026^{e} \pm 0.000$ | $0.085^{g} \pm 0.003$ | $0.196^{h} \pm 0.001$ |
| R8 | $1.63^{q} \pm 0.03$ | $0.93^{e} \pm 0.01$ | $3.93^{i} \pm 0.09$ | $14.7^{c} \pm 0.4$ | $0.000^{i} \pm 0.000$* | $0.141^{d} \pm 0.000$ | $0.272^{e} \pm 0.005$ |
| R9 | $2.18^{k} \pm 0.01$ | $0.84^{g} \pm 0.00$ | $4.75^{f} \pm 0.18$ | $17.3^{a} \pm 0.4$ | $0.024^{g} \pm 0.001$ | $0.325^{a} \pm 0.032$ | $0.358^{a} \pm 0.000$ |
| R10 | $2.47^{h} \pm 0.02$ | $0.92^{e} \pm 0.00$ | $4.97^{e} \pm 0.07$ | $16.2^{b} \pm 1.7$ | $0.000^{i} \pm 0.000$* | $0.119^{f} \pm 0.005$ | $0.318^{d} \pm 0.007$ |
| R11 | $2.32^{i} \pm 0.03$ | $0.97^{d} \pm 0.00$ | $5.02^{e} \pm 0.25$ | $12.6^{e} \pm 0.4$ | $0.000^{i} \pm 0.000$* | $0.124^{e} \pm 0.003$ | $0.193^{h} \pm 0.011$ |
| R12 | $2.11^{l} \pm 0.04$ | $0.98^{d} \pm 0.02$ | $4.29^{h} \pm 0.06$ | $11.3^{f} \pm 0.6$ | $0.000^{i} \pm 0.000$* | $0.120^{f} \pm 0.003$ | $0.251^{f} \pm 0.005$ |
| R13 | $2.03^{m} \pm 0.04$ | $0.94^{e} \pm 0.01$ | $4.27^{h} \pm 0.17$ | $16.5^{b} \pm 0.5$ | $0.000^{i} \pm 0.000$* | $0.138^{d} \pm 0.012$ | $0.138^{k} \pm 0.007$ |
| R14 | $2.37^{i} \pm 0.00$ | $1.05^{b} \pm 0.01$ | $5.82^{b} \pm 0.29$ | $17.6^{a} \pm 0.2$ | $0.044^{a} \pm 0.002$ | $0.153^{c} \pm 0.001$ | $0.155^{j} \pm 0.006$ |
| R15 | $2.19^{k} \pm 0.01$ | $0.97^{d} \pm 0.01$ | $4.61^{g} \pm 0.12$ | $12.3^{e} \pm 0.7$ | $0.000^{i} \pm 0.000$* | $0.096^{g} \pm 0.001$ | $0.160^{j} \pm 0.011$ |
| R16 | $2.02^{m} \pm 0.03$ | $0.85^{g} \pm 0.03$ | $3.94^{i} \pm 0.03$ | $14.8^{c} \pm 1.2$ | $0.000^{i} \pm 0.000$* | $0.129^{e} \pm 0.001$ | $0.185^{h} \pm 0.000$ |
| R17 | $2.53^{g} \pm 0.01$ | $0.96^{d} \pm 0.00$ | $4.54^{g} \pm 0.02$ | $16.4^{b} \pm 0.4$ | $0.000^{i} \pm 0.000$* | $0.093^{g} \pm 0.007$ | $0.245^{f} \pm 0.018$ |
| R18 | $2.65^{f} \pm 0.02$ | $0.96^{d} \pm 0.03$ | $5.00^{e} \pm 0.21$ | $15.6^{c} \pm 1.4$ | $0.000^{i} \pm 0.000$* | $0.086^{g} \pm 0.006$ | $0.251^{f} \pm 0.018$ |
| R19 | $1.98^{m} \pm 0.10$ | $1.02^{c} \pm 0.02$ | $4.67^{g} \pm 0.06$ | $11.4^{f} \pm 0.1$ | $0.000^{i} \pm 0.000$* | $0.149^{d} \pm 0.013$ | $0.172^{i} \pm 0.012$ |
| R20 | $2.74^{d} \pm 0.02$ | $0.96^{d} \pm 0.01$ | $5.01^{e} \pm 0.17$ | $16.8^{b} \pm 0.9$ | $0.000^{i} \pm 0.000$* | $0.068^{h} \pm 0.005$ | $0.173^{i} \pm 0.014$ |
| R21 | $2.61^{f} \pm 0.01$ | $0.92^{e} \pm 0.01$ | $4.83^{f} \pm 0.08$ | $15.0^{c} \pm 1.3$ | $0.000^{i} \pm 0.000$* | $0.116^{f} \pm 0.010$ | $0.275^{e} \pm 0.025$ |
| R22 | $1.72^{p} \pm 0.01$ | $0.89^{f} \pm 0.02$ | $4.89^{e} \pm 0.08$ | $13.6^{d} \pm 0.6$ | $0.000^{i} \pm 0.000$* | $0.102^{f} \pm 0.002$ | $0.201^{h} \pm 0.005$ |
| R23 | $2.68^{e} \pm 0.04$ | $0.98^{d} \pm 0.02$ | $4.59^{g} \pm 0.08$ | $15.4^{c} \pm 0.3$ | $0.000^{i} \pm 0.000$* | $0.087^{g} \pm 0.003$ | $0.239^{f} \pm 0.016$ |
| R24 | $2.34^{i} \pm 0.04$ | $0.78^{h} \pm 0.03$ | $4.56^{g} \pm 0.03$ | $15.0^{c} \pm 0.6$ | $0.000^{i} \pm 0.000$* | $0.132^{e} \pm 0.003$ | $0.360^{a} \pm 0.002$ |
| R25 | $2.48^{h} \pm 0.00$ | $0.93^{e} \pm 0.01$ | $4.72^{f} \pm 0.14$ | $13.5^{d} \pm 0.8$ | $0.000^{i} \pm 0.000$* | $0.158^{c} \pm 0.011$ | $0.241^{f} \pm 0.017$ |
| R26 | $2.30^{j} \pm 0.02$ | $1.02^{c} \pm 0.00$ | $5.13^{d} \pm 0.07$ | $14.4^{c} \pm 0.4$ | $0.000^{i} \pm 0.000$* | $0.116^{f} \pm 0.006$ | $0.108^{l} \pm 0.011$ |
| R27 | $2.71^{e} \pm 0.02$ | $1.00^{d} \pm 0.03$ | $5.30^{d} \pm 0.21$ | $12.7^{e} \pm 0.7$ | $0.000^{i} \pm 0.000$* | $0.069^{h} \pm 0.006$ | $0.167^{i} \pm 0.007$ |
| R28 | $2.21^{k} \pm 0.01$ | $1.01^{c} \pm 0.01$ | $4.36^{h} \pm 0.15$ | $14.7^{c} \pm 0.4$ | $0.000^{i} \pm 0.000$* | $0.109^{f} \pm 0.002$ | $0.129^{k} \pm 0.008$ |
| R29 | $2.18^{k} \pm 0.04$ | $0.86^{g} \pm 0.01$ | $4.95^{e} \pm 0.18$ | $13.6^{d} \pm 0.4$ | $0.000^{i} \pm 0.000$* | $0.131^{e} \pm 0.012$ | $0.173^{i} \pm 0.019$ |
| R30 | $2.65^{f} \pm 0.05$ | $0.93^{e} \pm 0.02$ | $4.64^{g} \pm 0.01$ | $14.5^{c} \pm 0.6$ | $0.000^{i} \pm 0.000$* | $0.081^{h} \pm 0.001$ | $0.239^{f} \pm 0.010$ |
| R31 | $2.65^{f} \pm 0.04$ | $0.94^{e} \pm 0.02$ | $4.84^{f} \pm 0.19$ | $12.9^{e} \pm 0.6$ | $0.000^{i} \pm 0.000$* | $0.142^{d} \pm 0.016$ | $0.372^{a} \pm 0.023$ |
| R32 | $1.80^{\circ} \pm 0.02$ | $0.84^{g} \pm 0.00$ | $4.65^{g} \pm 0.04$ | $15.6^{c} \pm 0.8$ | $0.000^{i} \pm 0.000$* | $0.101^{f} \pm 0.006$ | $0.118^{l} \pm 0.004$ |
| R33 | $2.84^{c} \pm 0.08$ | $1.05^{b} \pm 0.16$ | $5.38^{c} \pm 0.05$ | $10.7^{g} \pm 0.7$ | $0.040^{b} \pm 0.002$ | $0.112^{f} \pm 0.005$ | $0.278^{e} \pm 0.005$ |
| R34 | $1.97^{n} \pm 0.01$ | $1.02^{c} \pm 0.04$ | $4.05^{i} \pm 0.01$ | $11.7^{f} \pm 0.8$ | $0.000^{i} \pm 0.000$* | $0.160^{c} \pm 0.003$ | $0.309^{d} \pm 0.014$ |
| R35 | $2.84^{c} \pm 0.07$ | $0.96^{d} \pm 0.03$ | $5.76^{b} \pm 0.05$ | $12.4^{e} \pm 0.7$ | $0.000^{i} \pm 0.000$* | $0.111^{f} \pm 0.000$ | $0.173^{i} \pm 0.009$ |
| R36 | $2.64^{f} \pm 0.02$ | $0.94^{e} \pm 0.01$ | $5.01^{e} \pm 0.16$ | $14.0^{d} \pm 1.2$ | $0.000^{i} \pm 0.000$* | $0.119^{f} \pm 0.001$ | $0.252^{f} \pm 0.010$ |
| R37 | $2.58^{g} \pm 0.01$ | $0.74^{h} \pm 0.01$ | $6.28^{a} \pm 0.08$ | $11.8^{f} \pm 0.7$ | $0.000^{i} \pm 0.000$* | $0.080^{h} \pm 0.008$ | $0.254^{f} \pm 0.000$ |
| R38 | $1.94^{n} \pm 0.02$ | $0.78^{h} \pm 0.02$ | $4.25^{h} \pm 0.16$ | $14.4^{c} \pm 0.3$ | $0.000^{i} \pm 0.000$* | $0.144^{d} \pm 0.009$ | $0.212^{g} \pm 0.025$ |
| R39 | $2.67^{e} \pm 0.14$ | $0.85^{g} \pm 0.04$ | $4.38^{h} \pm 0.05$ | $14.6^{c} \pm 0.3$ | $0.000^{i} \pm 0.000$* | $0.090^{g} \pm 0.006$ | $0.287^{e} \pm 0.010$ |
| R40 | $2.69^{e} \pm 0.06$ | $0.89^{f} \pm 0.05$ | $4.74^{f} \pm 0.13$ | $15.1^{c} \pm 2.0$ | $0.005^{i} \pm 0.000$* | $0.074^{h} \pm 0.005$ | $0.220^{g} \pm 0.021$ |
| R41 | $2.32^{i} \pm 0.06$ | $0.99^{d} \pm 0.02$ | $4.47^{g} \pm 0.11$ | $13.0^{e} \pm 0.5$ | $0.000^{i} \pm 0.000$* | $0.126^{e} \pm 0.004$ | $0.209^{g} \pm 0.026$ |
| R42 | $2.26^{j} \pm 0.04$ | $1.01^{c} \pm 0.04$ | $4.43^{h} \pm 0.03$ | $13.8^{d} \pm 0.4$ | $0.000^{i} \pm 0.000$* | $0.096^{g} \pm 0.003$ | $0.162^{j} \pm 0.018$ |
| R43 | $2.44^{h} \pm 0.04$ | $0.86^{g} \pm 0.03$ | $4.73^{f} \pm 0.23$ | $16.4^{b} \pm 0.3$ | $0.000^{i} \pm 0.000$* | $0.166^{d} \pm 0.015$ | $0.245^{f} \pm 0.026$ |
| R44 | $3.33^{a} \pm 0.02$ | $1.00^{d} \pm 0.02$ | $5.58^{c} \pm 0.20$ | $13.3^{d} \pm 0.5$ | $0.000^{i} \pm 0.000$* | $0.168^{b} \pm 0.019$ | $0.134^{k} \pm 0.001$ |
| R45 | $1.68^{p} \pm 0.02$ | $0.99^{d} \pm 0.02$ | $4.65^{g} \pm 0.06$ | $12.8^{e} \pm 1.1$ | $0.029^{d} \pm 0.001$ | $0.175^{b} \pm 0.014$ | $0.145^{k} \pm 0.006$ |
| R46 | $3.05^{b} \pm 0.08$ | $1.03^{c} \pm 0.05$ | $5.64^{b} \pm 0.29$ | $12.6^{e} \pm 0.3$ | $0.000^{i} \pm 0.000$* | $0.092^{g} \pm 0.003$ | $0.237^{f} \pm 0.011$ |
| R47 | $2.73^{d} \pm 0.06$ | $1.15^{a} \pm 0.02$ | $4.91^{e} \pm 0.42$ | $13.7^{d} \pm 0.4$ | $0.000^{i} \pm 0.000$* | $0.103^{f} \pm 0.000$ | $0.245^{f} \pm 0.005$ |
| R48 | $2.33^{i} \pm 0.03$ | $0.86^{g} \pm 0.01$ | $4.66^{g} \pm 0.04$ | $10.0^{g} \pm 0.2$ | $0.000^{i} \pm 0.000$* | $0.143^{e} \pm 0.010$ | $0.235^{f} \pm 0.027$ |
| R49 | $2.20^{k} \pm 0.02$ | $1.03^{c} \pm 0.02$ | $4.84^{f} \pm 0.28$ | $14.8^{c} \pm 0.3$ | $0.000^{i} \pm 0.000$* | $0.134^{e} \pm 0.000$ | $0.287^{e} \pm 0.002$ |
| R50 | $2.40^{i} \pm 0.00$ | $0.84^{g} \pm 0.00$ | $4.24^{h} \pm 0.20$ | $15.8^{c} \pm 1.0$ | $0.000^{i} \pm 0.000$* | $0.179^{b} \pm 0.020$ | $0.343^{b} \pm 0.013$ |
| R51 | $2.44^{h} \pm 0.09$ | $0.80^{h} \pm 0.05$ | $4.37^{h} \pm 0.15$ | $13.0^{e} \pm 0.2$ | $0.000^{i} \pm 0.000$* | $0.140^{d} \pm 0.011$ | $0.213^{g} \pm 0.013$ |
| R52 | $1.92^{n} \pm 0.07$ | $0.79^{h} \pm 0.05$ | $4.04^{i} \pm 0.21$ | $15.5^{c} \pm 0.5$ | $0.000^{i} \pm 0.000$* | $0.099^{f} \pm 0.007$ | $0.272^{e} \pm 0.012$ |
| R53 | $2.61^{f} \pm 0.08$ | $1.01^{c} \pm 0.04$ | $4.85^{f} \pm 0.36$ | $13.9^{d} \pm 0.7$ | $0.000^{i} \pm 0.000$* | $0.108^{f} \pm 0.008$ | $0.336^{c} \pm 0.031$ |
| R54 | $2.34^{i} \pm 0.02$ | $0.88^{f} \pm 0.01$ | $5.57^{c} \pm 0.12$ | $12.6^{e} \pm 0.3$ | $0.022^{h} \pm 0.001$ | $0.134^{e} \pm 0.007$ | $0.212^{g} \pm 0.017$ |
| R55 | $2.60^{f} \pm 0.04$ | $0.84^{g} \pm 0.00$ | $5.43^{c} \pm 0.17$ | $10.8^{g} \pm 0.4$ | $0.000^{i} \pm 0.000$* | $0.108^{f} \pm 0.007$ | $0.149^{j} \pm 0.013$ |
| R56 | $2.35^{i} \pm 0.05$ | $0.78^{h} \pm 0.03$ | $5.00^{e} \pm 0.24$ | $13.9^{d} \pm 0.9$ | $0.025^{f} \pm 0.001$ | $0.139^{d} \pm 0.005$ | $0.143^{k} \pm 0.000$ |
| R57 | $2.46^{h} \pm 0.00$ | $0.79^{h} \pm 0.00$ | $4.20^{h} \pm 0.09$ | $11.4^{f} \pm 0.5$ | $0.023^{h} \pm 0.001$ | $0.335^{a} \pm 0.023$ | $0.208^{g} \pm 0.022$ |
| **Average value**[b] | 2.38 | 0.94 | 4.78 | 14.2 | 0.005 | 0.127 | 0.226 |
| **CV%** | 1.64 | 3.38 | 3.45 | 5.19 | 9.09 | 8.55 | 5.62 |

[a] Means (duplicate of extraction and analysis, n = 4) ± standard deviation; *zero values correspond to contents below the detection limit (0.794 μg $mL^{-1}$). Means followed by the different letters in the same column showed a significant difference between clones (Scott-Knott, $p \leq 0.05$).

[b] Average value (57 clones) and coefficient of variation (CV%) between clones.

(2021) observed caffeine content of 1.93 g 100 $g^{-1}$ in high quality Brazilian Robusta coffee from Rondônia state. Analyzing Robusta coffees with different roasting degrees, caffeine content from 2.10 to 2.63 g 100 $g^{-1}$ was reported by Klikarová et al. (2022), and from 1.81 to 2.55 g 100 $g^{-1}$ by Heĉimović et al. (2011) for Vietnam and Cherry varieties.

Considering the stability of caffeine to the roasting process, data from green *C. canephora* coffees can also be used for comparison. Alonso-Salces et al. (2009) reported an average caffeine content of 2.66 g 100 $g^{-1}$ for *C. canephora* coffees (without variety identification) from different countries. For Brazilian Conilon, Pinheiro et al. (2019) reported an average caffeine content of 2.45 g 100 $g^{-1}$; for Robusta variety, Klikarová et al. (2022) reported an average content of 2.25 g 100 $g^{-1}$.

Trigonelline content varied from 0.60 to 1.15 g 100 $g^{-1}$, with average values of 0.94 g 100 $g^{-1}$ (CV 3.38%) for Robusta coffees and 0.76 g 100 $g^{-1}$ (CV 3.03%) for hybrid coffees. These contents (Tables 1

**Table 2**

Contents of caffeine, trigonelline, chlorogenic acids (CGA), melanoidins, kahweol, cafestol e 16-*O*-metilcafestol (16-OMC) (g 100 g$^{-1}$) in intervarietal hybrids of *C. canephora* coffee.

| Clones | Caffeine[a] | Trigonelline[a] | CGA[a] | Melanoidins[a] | Kahweol[a] | Cafestol[a] | 16-OMC[a] |
|---|---|---|---|---|---|---|---|
| BRS 1216 | $2.86^{c} \pm 0.02$ | $0.65^{d} \pm 0.01$ | $5.19^{c} \pm 0.18$ | $11.7^{d} \pm 0.6$ | $0.000^{c} \pm 0.000$* | $0.262^{b} \pm 0.018$ | $0.149^{d} \pm 0.011$ |
| BRS 2299 | $2.29^{g} \pm 0.05$ | $0.74^{c} \pm 0.01$ | $4.58^{d} \pm 0.09$ | $12.8^{c} \pm 0.8$ | $0.000^{c} \pm 0.000$* | $0.123^{e} \pm 0.012$ | $0.110^{e} \pm 0.001$ |
| BRS 2314 | $3.09^{b} \pm 0.05$ | $0.71^{c} \pm 0.00$ | $6.37^{a} \pm 0.17$ | $11.9^{d} \pm 0.4$ | $0.000^{c} \pm 0.000$* | $0.427^{a} \pm 0.011$ | $0.249^{b} \pm 0.021$ |
| BRS 2336 | $3.57^{a} \pm 0.01$ | $0.81^{b} \pm 0.02$ | $5.32^{b} \pm 0.22$ | $12.7^{c} \pm 0.3$ | $0.000^{c} \pm 0.000$* | $0.222^{c} \pm 0.016$ | $0.286^{a} \pm 0.015$ |
| BRS 2357 | $2.52^{e} \pm 0.00$ | $0.60^{e} \pm 0.01$ | $4.26^{e} \pm 0.11$ | $16.3^{a} \pm 1.4$ | $0.000^{c} \pm 0.000$* | $0.256^{b} \pm 0.006$ | $0.120^{e} \pm 0.002$ |
| BRS 3137 | $2.46^{f} \pm 0.05$ | $0.82^{b} \pm 0.04$ | $5.16^{c} \pm 0.07$ | $11.3^{e} \pm 0.2$ | $0.031^{a} \pm 0.001$ | $0.180^{d} \pm 0.004$ | $0.279^{a} \pm 0.001$ |
| BRS 3193 | $2.72^{d} \pm 0.05$ | $0.85^{a} \pm 0.00$ | $5.48^{b} \pm 0.24$ | $10.2^{f} \pm 0.8$ | $0.000^{c} \pm 0.000$* | $0.184^{d} \pm 0.018$ | $0.188^{c} \pm 0.002$ |
| BRS 3210 | $2.88^{c} \pm 0.04$ | $0.84^{a} \pm 0.00$ | $5.37^{b} \pm 0.17$ | $11.7^{d} \pm 0.7$ | $0.000^{c} \pm 0.000$* | $0.177^{d} \pm 0.011$ | $0.157^{d} \pm 0.003$ |
| BRS 3213 | $2.68^{d} \pm 0.04$ | $0.72^{c} \pm 0.00$ | $5.14^{c} \pm 0.22$ | $14.0^{b} \pm 0.5$ | $0.000^{c} \pm 0.000$* | $0.225^{c} \pm 0.020$ | $0.105^{e} \pm 0.011$ |
| BRS 3220 | $2.55^{e} \pm 0.01$ | $0.85^{a} \pm 0.02$ | $5.55^{b} \pm 0.04$ | $14.5^{b} \pm 0.4$ | $0.023^{b} \pm 0.001$ | $0.137^{e} \pm 0.014$ | $0.082^{f} \pm 0.007$ |
| **Average value**[b] | 2.76 | 0.76 | 5.24 | 12.7 | 0.005 | 0.219 | 0.173 |
| **CV%** | 1.14 | 3.03 | 2.79 | 5.42 | 9.14 | 5.36 | 5.01 |

[a] Means (duplicate of extraction and analysis, n = 4) ± standard deviation; *zero values correspond to contents below the detection limit (0.794 μg mL$^{-1}$). Means followed by the different letters in the same column showed a significant difference between clones (Scott-Knott, $p \leq 0.05$).

[b] Average value (10 clones) and coefficient of variation (CV%) between clones.

and 2) are generally higher than the range reported in the literature for *C. canephora* coffees; this difference may also be related to the medium-light roasting degree of the beans, with less degradation of the compound.

For *C. canephora* coffees (without variety specification) with differences in the roasting degrees and the proportion of defective beans, the trigonelline contents in the literature vary widely: from 0.07 to 0.68 g 100 g$^{-1}$ (De Souza and Benassi, 2012; Dias and Benassi, 2015; Kalschne et al., 2019). For Brazilian Conilon beverages from Espírito Santo state, Mori et al. (2020) observed trigonelline contents from 206 to 413 μg mL$^{-1}$, which correspond to around 0.14–0.58 g 100 g$^{-1}$ of roasted coffee.

Total CGA contents ranged from 3.93 to 6.37 g 100 g$^{-1}$, with average values of 4.78 g 100 g$^{-1}$ (CV 3.45%) for Robusta coffees and 5.24 g 100 g$^{-1}$ (CV 2.79%) for hybrid coffees. Overall, the CGA contents (Tables 1 and 2) are also above or in the upper part of the range in the literature for *C. canephora* coffees; similarly to trigonelline, the medium-light roasting degree could partially justify this behavior. There is little information regarding the total CGA content in *C. canephora* coffees. For medium-roasted coffees with the presence of defective beans, values from 2 to 2.32 g of CGA 100 g$^{-1}$ were described (Kalschne et al., 2019; Reis et al., 2019). Portela et al. (2021) reported a content of 5.75 g of CGA 100 g$^{-1}$ in high quality Brazilian Robusta coffee from Rondônia state.

Some articles report the content of 5-CQA, the main isomer of the CGA group. In this study, we observed 5-CQA content ranging from 1.25 to 2.43 g 100 g$^{-1}$ (Tables S.2 and S.3); these values are in the upper range of that reported for *C. canephora* coffees with different roasting degrees (0.21–2 g 100 g$^{-1}$) (De Souza and Benassi, 2012; Dias and Benassi, 2015; Klikarová et al., 2022). The 5-CQA contents corresponded, on average, to 33% and 36% of the total CGA, for Robusta and hybrid coffees, respectively. These values were similar to that reported by Perrone et al. (2012) for *C. canephora* coffees with different roasting degrees (31–39%), and by Mori et al. (2020), for Conilon variety (31–40%).

Melanoidins ranged from 10 to 17.9 g 100 g$^{-1}$ with an estimated average value of 14.2 g 100 g$^{-1}$ (CV 5.19%) for Robusta coffees and 12.7 g 100 g$^{-1}$ (CV 5.42%) for hybrid coffees (Tables 1 and 2). Contents from 7.7 to 25 g of melanoidins 100 g$^{-1}$ were described for roasted coffee, with contents increasing with increasing roasting degree (Rufián-Henares and Pastoriza, 2015; Alves et al., 2020). Portela et al. (2021) reported 12.1 g of melanoidins 100 g$^{-1}$ in Brazilian Robusta coffee from the Rondônia state, which also had a low-intensity roasting process (L* of 36.9).

Total diterpenes contents varied in a wide range (0.218–0.707 g 100 g$^{-1}$), with an average value of 0.358 g 100 g$^{-1}$ (CV 4.62%) for Robusta coffees and 0.397 g 100 g$^{-1}$ (CV 3.85%) for hybrid coffees. Overall, total diterpenes contents (Tables S.2 and S.3) are close to those reported in the literature for Robusta coffees from Asia and Africa by Finotello et al. (2017) (0.318–0.461 g 100 g$^{-1}$) and slightly higher than that described by Mori et al. (2016) for Conilon variety (0.191–0.415 g 100 g$^{-1}$). In a previous work of our research group with natural Brazilian intervarietal hybrid coffees produced in six growing sites in Rondônia state, Francisco et al. (2021) reported total diterpenes content (from 0.192 to 0.742 g 100 g$^{-1}$) similar to those observed in this study for the hybrid coffees obtained by breeding (Table S.3).

In a study with *C. arabica* coffees, Zanin et al. (2020) reported the caffeine/total diterpenes ratio as a possible indicator of the coffee species. A significant difference ($p \leq 0.05$) was observed among samples within each variety (Robusta or hybrid coffees), and the caffeine/total diterpenes ratio ranged from 3.1 to 11.5, with average values of 7.0 and 7.5 for Robusta and hybrid coffees, respectively (Tables S.2 and S.3). These results – with similar behavior for Robusta and hybrid coffees (Fig. 1) - confirm the applicability of the ratio for characterization of species and support the proposal of Zanin et al. (2020), who recommended the caffeine/total diterpenes ratio above 2.50 as indicative of the *C. canephora*.

Kahweol contents varied widely, from absence (below the LOD) to 0.044 g 100 g$^{-1}$. The compound was detected in 19.3% of Robusta coffees and 20% of hybrid coffees, with a similar average value in both varieties (0.005 g 100 g$^{-1}$) (Tables 1 and 2). The literature described from absence (Campanha et al., 2010; De Souza and Benassi, 2012; Dias et al., 2014) to contents of 0.016 g of kahweol 100 g$^{-1}$ for Brazilian *C. canephora* coffees (without variety identification) (Kalschne et al., 2019). Finotello et al. (2017) reported the presence of kahweol in 28% of the 39 commercial Robusta coffees from different countries in Asia and Africa, with levels up to 0.020 g 100 g$^{-1}$. Mori et al. (2016) found kahweol in 30% of samples (15 Conilon genotypes produced in two growing sites), with contents of up to 0.014 g 100 g$^{-1}$. In a quite different behavior, Francisco et al. (2021) observed kahweol presence in 77% of samples (30 natural intervarietal hybrid coffees) and maximum content of 0.041 g 100 g$^{-1}$. Despite both originating from Conilon and Robusta varieties, the naturally hybridized coffees described by Francisco et al. (2021) seem to tend to a higher frequency of kahweol presence compared to the hybrid coffees obtained by breeding studied in this work and the original varieties (Conilon and Robusta). However, further studies are needed to confirm this observation since the hybrid coffees obtained by breeding were evaluated in only one growing site.

The large number of samples in this study (67 genotypes) allows us to confirm the high diversity reported in the literature regarding the presence and content of kahweol in the coffees of the *C. canephora* species.

Cafestol values varied from 0.068 to 0.427 g 100 g$^{-1}$, with an average content of 0.127 g 100 g$^{-1}$ (CV 8.55%) for Robusta coffees and

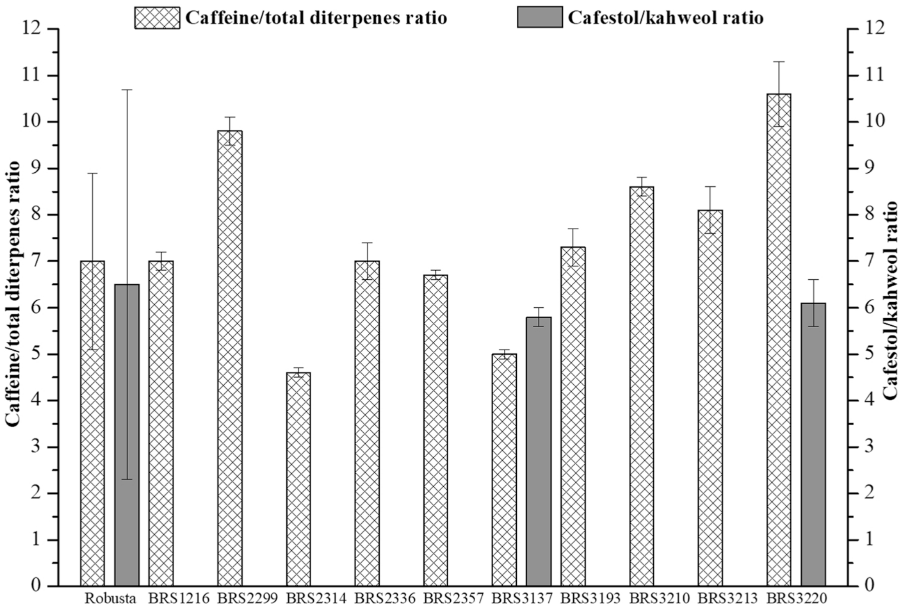

**Fig. 1.** Caffeine/total diterpenes ratio and cafestol/kahweol ratio for *C. canephora* coffees. For Robusta variety, bars indicate the standard deviation (SD) for caffeine/total diterpenes ratio (n = 57 clones) and cafestol/kahweol ratio (n = 10 clones). For intervarietal hybrid coffees (BRS), bars indicate the SD for caffeine/total diterpenes ratio and cafestol/kahweol ratio (n = 4, duplicates of extraction and analysis).

0.219 g 100 $g^{-1}$ (CV 5.36%) for hybrid coffees. Cafestol contents (Tables 1 and 2) are within the range described for *C. canephora* considering coffees without variety identification (0.163–0.491 g 100 g-1) (Campanha et al., 2010; Sridevi et al., 2011; De Souza and Benassi, 2012; Dias et al., 2014; Kalschne et al., 2019), from Robusta variety (0.129–0.272 g 100 $g^{-1}$) (Finotello et al., 2017), from Conilon variety (0.152–0.360 g 100 $g^{-1}$) (Mori et al., 2016), and natural intervarietal hybrid coffees (0.096–0.457 g 100 g-1) (Francisco et al., 2021).

For *C. arabica* coffees, the literature reported that the cafestol/kahweol ratio could be related to beverage quality: higher values indicated better quality (Novaes et al., 2015; Barbosa et al., 2019). The cafestol/kahweol ratio ranged from 2.8 to 14.7, with an average value of 6.5 for Robusta coffees and 5.9 for hybrid coffees. Considering the absence of kahweol in 80% of the studied coffees, the calculation of the ratio can be done for a few samples only, but a significant difference ($p \leq 0.05$) was observed within the Robusta variety (Tables S.2 and S.3). Interestingly, Robusta coffees also showed greater variability in beverage quality evaluated by sensory quality scores (Tables S.2 and S.3). Overall, the cafestol/kahweol ratio was similar between Robusta and hybrid coffees (Fig. 1), an expected result since only coffees with good cup quality were studied; however, the limitations in calculation possibility hinders the use of this parameter for studies with *C. canephora* coffees.

The 16-OMC contents ranged from 0.082 to 0.372 g 100 $g^{-1}$, with the average value of 0.226 g 100 $g^{-1}$ (CV 5.62%) for Robusta coffees and 0.173 g 100 $g^{-1}$ (CV 5.01%) for hybrid coffees (Tables 1 and 2). In general, lower values have been reported in the literature for the varieties Conilon (from 0.026 to 0.132 g 100 $g^{-1}$) (Mori et al., 2016) and Robusta (from 0.120 to 0.223 g 100 $g^{-1}$) (Finotello et al., 2017), and *C. canephora* coffees without variety identification (from 0.144 to 0.184 g 100 $g^{-1}$) (Schievano et al., 2014; Kalschne et al., 2019). Only Francisco et al. (2021) reported, for natural intervarietal hybrid coffees, 16-OMC contents similar to those observed in the present study (from 0.075 to 0.433 g 100 $g^{-1}$), suggesting that hybrid coffees of Conilon and Robusta might tend to higher 16-OMC contents.

Results showed that 30% of the hybrid coffees and 53% of the Robusta coffees presented 16-OMC contents (Tables 1 and 2) above the highest value previously described in the literature for *C. canephora* coffees without variety identification and for Conilon or Robusta varieties (0.223 g 100 $g^{-1}$), indicating that the content of the compound in *C. canephora* species could have been underestimated.

Considering the wide range of values found and the high variability within each variety (already reported by Schievano et al. (2014) and Mori et al. (2016) in studies with Robusta and Conilon coffees), the use of 16-OMC as a single parameter to estimate the percentage of *C. canephora* present in blends with *C. arabica* is not adequate. Our results indicated that the parameter caffeine/total diterpenes ratio could be a support tool in the identification of *C. canephora* species.

In summary, good beverage quality *C. canephora* coffees (of the Robusta variety or intervarietal hybrid coffees) produced in the Western Amazon stood out for their high contents of caffeine, trigonelline, CGA, and 16-OMC compared to that described in the literature for the species.

Usually, the predominance of neutral and less full-bodied beverages for the Conilon variety coffees is expected, while the Robusta variety coffees beverages could be differentiated by nuances (Teixeira et al., 2020). This difference in coffee beverage characteristics (associated with differences in composition) can also be expressed in hybrid plants that can present features of both Conilon and Robusta varieties. Regarding the attributes reported by the cuppers for the whole set samples studied, notes of sweet/caramel/vanilla were the most cited, followed by spice and roasted/woody/cereal, showing the effect of Robusta variety on hybrid coffees; for hybrid coffees, herb notes were also reported, while for Robusta were cited fruity, chocolate/cocoa, and nuts/guarana (Tables S.2 and S.3).

The PCA allowed a global evaluation of the Robusta and hybrid clones, considering their chemical composition profile (Fig. 2). The first two principal components (PC 1 and PC 2) accounted for 51% of the data variance.

PC 1 was positively correlated to trigonelline, melanoidins, and 16-OMC and negatively correlated to caffeine, CGA, and cafestol. PC 2 positively correlated to cafestol and negatively correlated to trigonelline, CGA, and kahweol (Fig. 2A). For the active variables, positive correlations were observed between caffeine and CGA (r = 0.547) and between melanoidins and 16-OMC (r = 0.438), and negative correlation between cafestol and trigonelline (r = −0.506) (Table S.4). For the supplementary variables, a positive correlation was observed between total diterpenes and cafestol (r = 0.712); quality scores were not significantly correlated to the composition parameters, probably because the study was done with a group of coffees with good beverage

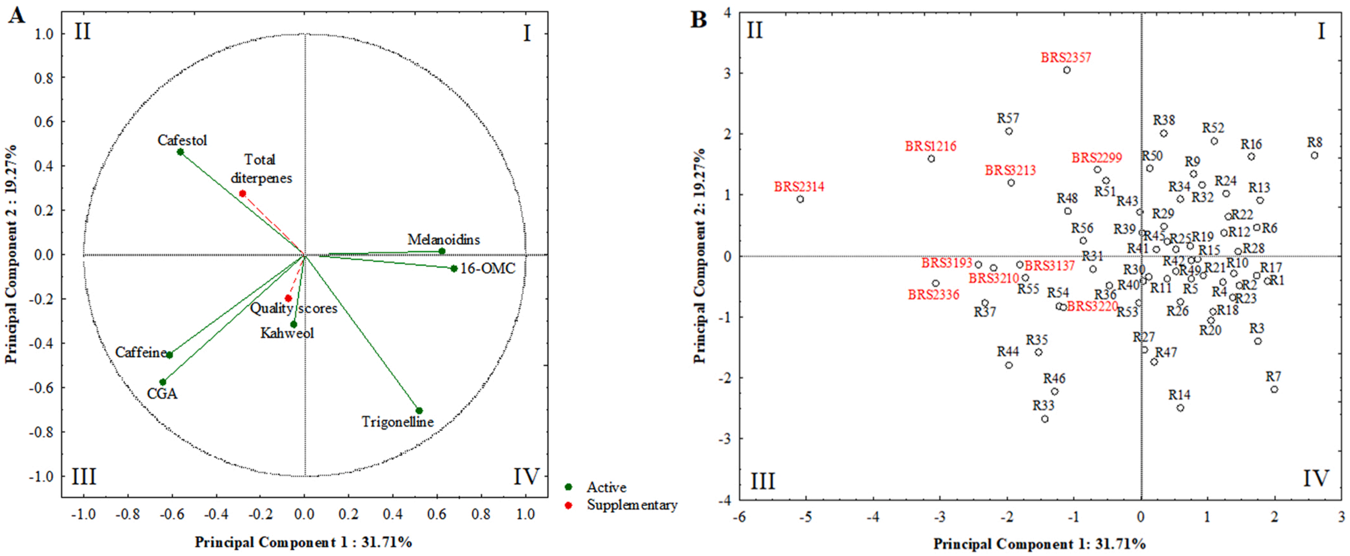

**Fig. 2.** Principal Component Analysis (PCA) considering the chemical composition profile and beverage quality scores of *C. canephora* coffees: A) projection of the variables: caffeine, trigonelline, chlorogenic acids (CGA), melanoidins, kahweol, cafestol, 16-*O*-metilcafestol (16-OMC), total diterpenes and beverage quality scores. B) Sample plot. Identification of Robustas from R1 to R57 in Table S.2; identification of intervarietal hybrid coffees (BRS) in Table S.3.

quality (≥ 70 points) (Table S.4).

Overall the Robusta and hybrid coffees were discriminated mainly by PC 1, with most Robusta coffees located on the right side (quadrants I and IV) and hybrid coffees on the left side of the sample plot (quadrants II and III) (Fig. 2B). The Robusta coffees were mainly characterized by the high contents of trigonelline, melanoidins, and 16-OMC and, the hybrid coffees, by the high contents of caffeine, CGA, cafestol, and total diterpenes (Fig. 2A). According to Souza et al. (2018), the Robusta variety presents beverages with a higher incidence of fruity, exotic, fine, and mild nuances while the Conilon variety presents a higher incidence of neutral flavored beverages, and with fewer stand out sensory attributes. Lemos et al. (2020), relating the composition of green beans of *C. canephora* with quality scores obtained by the protocol of Fine Robusta Cupping, observed that the Conilon genotype with the best beverage quality had the highest trigonelline and caffeine contents and high contents of CGA.

BRS 2314 clone stands out in the sample plot (Fig. 2B) as the most distant from the Robusta group and separated from the other hybrid coffees (quadrant II). BRS 2314 was characterized by higher contents of CGA, cafestol, and total diterpenes (Table 2 and S.3, Fig. 2), which can be associated with the unique genealogy of this hybrid (Emcapa 03 x IAC 640) (Table S.1). Barbosa et al. (2019), studying high quality *C. arabica* coffees originating from coffee quality competitions, correlated higher quality scores with high levels of cafestol. Morais et al. (2021) evaluated the beverage quality of 20 genotypes of C. *canephora* grown in different sites in the Amazon and observed that BRS 2314 differentiated itself sensorially from the other hybrid coffees, showing higher quality scores and beverage characterized by sweet flavor, chocolate aroma with citrus and almond notes, and pleasant acidity.

In the same study, Morais et al. (2021) also highlighted the potential of BRS 1216 and BRS 3220 clones, which were the best performers behind the BRS 2314 clone. BRS 2336, BRS 3193, and BRS 3213 clones show intermediate performance with full-bodied beverages but with more significant variation among growing sites, while BRS 2299, BRS 2357, BRS 3137, and BRS 3210 clones show neutral beverages with few highlighted sensory attributes. The samples dispersion in the first two principal components, considering the composition parameters, also presented BRS 1216 clone with a differentiated behavior from the other hybrid coffees (including BRS 3220 clone), which were located closer to Robusta coffees.

## 4. Conclusions

The *C. canephora* coffees produced in the Western Amazon showed a wide variation in their composition profile, even though they belong to a group of coffees with good beverage quality. The parameter caffeine/total diterpenes ratio was suggested as a support tool in identifying the *C. canephora* species. The studied coffees had high contents of caffeine, trigonelline, CGA, and 16-OMC compared to the literature data for *C. canephora* species. Comparing the varieties, Robusta coffees present higher contents of trigonelline, melanoidins, and 16-OMC, and hybrid coffees were characterized by higher contents of caffeine, CGA, cafestol, and total diterpenes. BRS 2314 clone stood out from the other hybrid coffees for its higher contents of CGA, cafestol, and total diterpenes.

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## CRediT authorship contribution statement

**Thayna Viencz:** Conceptualization, Methodology, Validation, Investigation, Funding acquisition, Formal analysis, Writing – original draft, Visualization. **Lucas Bonfanti Acre:** Methodology, Investigation. **Rodrigo Barros Rocha:** Conceptualization, Formal analysis, Resources, Funding acquisition, Writing – review & editing, Visualization, Supervision. **Enrique Anastácio Alves:** Investigation, Resources, Funding acquisition, Data curation. **André Rostand Ramalho:** Resources, Data curation. **Marta de Toledo Benassi:** Resources, Conceptualization, Formal analysis, Funding acquisition, Visualization, Writing – review & editing, Supervision.

## 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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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2023.105140.

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