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Composition of *Coffea canephora* Varieties from the Western Amazon

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This research aimed to compare the composition profiles of roasted *Coffea canephora* varieties (conilon, robusta, and intervarietal hybrids) grown in the Western Amazon. Ten coffees of each variety were evaluated. No difference in the contents of caffeine (1427 to 3364 mg 100 g^{-1}) and kahweol (absence to 25.7 mg 100 g^{-1}) was observed. Hybrid coffees were discriminated from traditional varieties (conilon and robusta) and stood out for their higher content of trigonelline, chlorogenic acids, and total diterpenes (mean values of 613,3791, and 471 mg 100 g^{-1}, respectively), higher cafestol/kahweol ratio (7.6 to 15.0), and higher frequency of kahweol presence. Traditional varieties only differed in cafestol and 16-*O*-methylcafestol contents. Robusta coffees stood out for their lower cafestol content (116 mg 100 g^{-1}), and conilon for their lower 16-*O*-methylcafestol content (139 mg 100 g^{-1}). Differences between the traditional varieties are smaller than that observed among them and the intervarietal hybrid coffees.

Keywords: chlorogenic acids, caffeine, diterpenes, conilon, robusta, principal component analysis

Introduction

Coffea canephora is one of the most known and commercialized species of the genus *Coffea*. Compared to *Coffea arabica*, which has higher commercial value, *C. canephora* stands out as a more rustic species, with greater resistance to climate stress, less sensitivity to the biannual cycle, and less productivity variation.[1,2]

Brazil, the largest global green coffee grower and exporter, produced 50.92 million 60 kg bags during the 2022 harvest; *C. canephora* accounted for 36% of this total. The country is the second-largest producer of this species, in 2022, the production of *C. canephora* increased by 11.7% in comparison with 2021.[3]

The demand for *C. canephora* coffees is increasing globally due to the expansion of its use, greater competitiveness, and profitability in different sectors of the production chain. Global production has increased progressively in the last 10 years, going from 59 million bags *per* year in 2012 to 69 million in 2017, with an estimate of 74 million for 2022.[4,5] In this way, the global market for *C. canephora* has been consolidating, becoming more attractive and encouraged by the types of beverages demanded, especially by emergent markets.[6]

C. canephora traditional varieties, conilon and robusta, showed different characteristics.[6] Conilon plants have smaller size, early flowering, and higher drought resistance; robusta ones show greater vigor and resistance to diseases and nematodes, with larger fruits of late maturation and beverage with high cup quality.[7,8] Although robusta has advantageous characteristics regarding bean quality and resistance, in Brazil, the cultivation of conilon is predominant since robusta plants need a higher amount of water, increasing the production costs.[9,10] The Western Amazon (Rondônia and Acre states) is the Brazilian coffee region where the two varieties are grown commercially since the frequent and abundant rainfall supplies the water necessary for the robusta plants.[11]

In addition to the favorable climate, the expansion of cultivation in the Amazon region has increased due to genetic breeding research and the introduction of high-yield clonal varieties.[11] Hybridization is an alternative to combine characteristics of the varieties, originating hybrid populations with intermediary phenotypes.[10] The process may occur naturally with spontaneous crossing

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between plants in the field; natural hybrids are among the most cultivated *C. canephora* in the Western Amazon.[7] But the crossings can also be directed by genetic breeding techniques; the controlled hybridization between the conilon and robusta coffees performed by Embrapa Rondônia generated ten clones registered in 2019, the samples here studied.[12]

Comparisons between the conilon and robusta varieties are generally focused on agronomic data, and unlike *C. arabica*, for which the literature offers a large volume of compositional data, there is less information for the *C. canephora* species, especially for roasted coffees. Some data on caffeine, trigonelline, total chlorogenic acids (or the main isomer 5-caffeoylquinic acid), and diterpenes (kahweol, cafestol, and 16-*O*-methylcafestol) contents are reported for roasted coffees from different regions (Asia, Africa, and South America) for robusta[13-16] and conilon varieties.[17] However, in many cases, only the species (*C. canephora*) is specified, and no information on variety is available.[18-23]

In previous works of our research group with *C. canephora* from the Amazon region, Francisco *et al*.[24] reported that natural hybrid coffees stood out for their high contents of diterpenes, and Viencz *et al*.[16] reported that robusta coffees, from the germplasm bank of Embrapa Rondônia, were characterized by a high content of trigonelline and 16-*O*-methylcafestol.

There is no research in the literature with a comprehensive comparison of *Coffea canephora* varieties; thus, considering the potential of the species, there is great interest in characterizing the traditional conilon and robusta varieties and comparing them to the intervarietal hybrids concerning their composition.

Experimental

Reagents, standards, and equipment

For extraction and preparation of the mobile phase, potassium hydroxide (KOH) analytical grade (F. Maia, São Paulo, Brazil), ethanol 96% analytical grade (Êxodo Científica, Hortolândia, Brazil), methyl *tert*-butyl ether HPLC grade (Acrós Organics, Morris Plains, USA), acetic acid P.A. (Sigma-Aldrich, Saint Louis, USA) and acetonitrile HPLC grade (Fisher Scientific, New Jersey, USA) were used. 5-Caffeoylquinic acid (5-CQA), caffeine and trigonelline (Sigma-Aldrich, Saint Louis, USA), kahweol and cafestol (Axxora, San Diego, USA) with 98% purity certified by Alexis Biochemicals (Lausen, USA), and 16-*O*-methylcafestol (16-OMC) (Sigma-Aldrich, Saint Louis, USA) with 98.6% purity were used as standards. The mobile phases and samples were filtered in 0.45 and 0.22 µm membranes (Millipore, Billerica, USA), respectively. The water used to prepare standards and solutions was obtained by Elga Purelab Option-Q purification and filtration system (Veolia Water Technologies, Saint-Maurice, France).

A portable Konica Minolta colorimeter CR 400 (Konica Minolta Sensing Inc., Osaka, Japan) with D65 illuminant was used for color characterization. A gravimetric moisture analyzer MB 45 (Ohaus, Barueri, Brazil) with a halogen lamp and a coffee grinder Krups GVX 2 (Krups, Shanghai, China) were used for moisture analysis and coffee grinding, respectively.

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 photodiode array detector, controlled by the Empower 3 program. A MX-S vortex shaker (Phox Suprimentos Científicos, Colombo, Brazil), laboratory water bath (Marconi Equipamentos para Laboratórios Ltda, Piracicaba, Brazil), and refrigerated laboratory centrifuge 5804 R (Eppendorf, Hamburg, Germany) were also used.

Material

C. canephora coffees were collected in Rondônia (RO) and Acre (AC) states, Brazil, during the 2019 harvest, between April and June. Ten samples of each variety-conilon, robusta, and intervarietal hybrids of conilon and robusta (developed by Embrapa) were provided by Embrapa Rondônia (Porto Velho, RO, Brazil). The coffees (about 450 g of green beans for each sample) came from different locations: Porto Velho (RO), Cruzeiro do Sul (AC), Ouro Preto do Oeste (RO), and Rolim de Moura (RO). Table S1, presented in the Supplementary Information (SI) section, reported information on the local of harvest and clone identification for each coffee; when available, register numbers in the Brazilian Cultivar Register and genealogy were also included. The climate in the regions is type "Aw" by the Köppen classification, defined as tropical humid with a dry winter and rainy summer; more information on the environment characteristics is in Table S2 (SI section).

The fruits were picked manually and selectively to obtain only ripe fruits at the cherry stage. All samples were prepared following the same procedure at Embapa. The coffees 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 at room temperature until roasting.

The beans were roasted in a Rod Bel pilot gas roaster (Rod Bel, São Paulo, Brazil) at around 210 °C, as suggested by Mori *et al.*[17] for conilon coffees. Processing times between 10 and 15 min were used to standardize the roasting degree of the product, considering the differences in size and characteristics of the beans. The roasting process was monitored by weight loss in the range of 15 to 18%, based on Mendes *et al.*[25]

After roasting, coffees were ground using a Burr bench grinder GVX 2 (Krups, Shanghai, China). The ground coffee was classified by manually stirring for 5 min using ASTM sieve stacks No. 20 (0.850 mm mesh opening), No. 40 (0.425 mm mesh opening), and bottom pan; 21% of coffee particles were retained on sieve No. 20; 58% of particles on sieve No. 40 and 21% of particles on the pan indicating a medium granulometry.

Roasted and ground coffees were characterized regarding color (in genuine duplicate with measurements in duplicate) and presented lightness of 32 ± 3 and hue of 26 ± 4, indicating a medium-light roasting degree. Moisture was determined at 105 °C for 7 min (in duplicate), obtaining an average value of 2.4 ± 0.2 g 100 g^{-1}. The results were used to express the contents of constituents on a dry basis (db).

Caffeine, chlorogenic acids, and trigonelline determination

The analysis was performed according to Viencz *et al.*[16] Samples (0.500 g) were extracted in 30 mL of water at 80 °C for 10 min under stirring. After filtering with filter paper, the extracts were diluted with water at a ratio of 5:95 v/v, filtered through a membrane filter directly into vials (1.5 mL), and frozen until analysis.

A Spherisorb ODS-1 column (150 × 4.6 mm, 3 µm) (Waters, Darmstadt, Germany) was used, with a temperature of 26 °C and an injection volume of 5 µL. The samples were eluted in a gradient of 5% acetic acid (A) and acetonitrile (B) with a flow rate of 0.5 mL min^{-1}, in the following conditions: 0 to 5 min: 5% of B; 6 to 25 min: 13% B. Detection was performed at 272 nm for caffeine, 260 nm for trigonelline and 320 nm for chlorogenic acids.

Identification was based on retention times, co-elution with standards, and UV spectra. Quantification was carried out by external standardization, using 6-point analytical curves with triplicate measurements, in the concentration range from 1 to 60 µg mL^{-1} for 5-CQA, 10 to 60 µg mL^{-1} for caffeine, and 1 to 30 µg mL^{-1} for trigonelline. Limits of detection (LOD) of 0.047, 0.059, and 0.017 µg mL^{-1} and quantification (LOQ) of 0.138, 0.178, and 0.052 µg mL^{-1} were obtained for trigonelline, caffeine, and 5-CQA, respectively. The total chlorogenic acids content (CGA) was estimated considering the sum of the compounds detected at 320 nm, using 5-CQA as standard.[16]

The extractions were carried out with genuine duplicates, and duplicate analyses were performed; the results were expressed as mg 100 g^{-1} (db).

Kahweol, cafestol, and 16-OMC determination

The extraction followed the proposed by Dias *et al.*[21] Samples (0.200 g) were saponified in 2.0 mL of 2.5 mol L^{-1} potassium hydroxide in ethanol (96% v/v) at 80 °C for 1 h in a water bath. To extract the unsaponifiable fraction, 2.0 mL of distilled water and 2.0 mL of methyl *tert*-butyl ether were added, followed by agitation and centrifugation (2 min at 3000 rpm at 25 °C) and organic phase collection. This last step of the procedure was repeated three times, totaling 6 mL of solvent. Then, 2.0 mL of distilled water were added for cleaning up, and the organic extract was collected and evaporated in a water bath at 70 °C until drying. The dry extract was resuspended in 4.5 mL of mobile phase (45:55 v/v water:acetonitrile), filtered, added in vials (1.5 mL), and frozen until analysis.

The analysis was performed as described by Viencz *et al.*,[16] with detection at 230 nm for cafestol and 16-OMC and 290 nm for kahweol. A Supelcosil LC-18 column (150 × 3 mm, 3 µm) (Supelco Park, Bellefonte, USA) and a temperature at 26 °C were used. 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 used.

Identification was based on retention times, co-elution with standards, and UV spectra. Quantification was carried out by external standardization, using 6-point analytical curves, with triplicate measurements, in the concentration range of 1 to 200 µg mL^{-1} for kahweol, 50 to 300 µg mL^{-1} for cafestol, and 2 to 400 µg mL^{-1} for 16-OMC. 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 extractions were carried out with genuine duplicates, and duplicate analyses were performed; the results were expressed as mg 100 g^{-1} (db). The total diterpenes content was obtained by the sum of kahweol, cafestol, and 16-OMC contents. The caffeine/total diterpenes ratio and cafestol/kahweol ratio were also calculated.

Statistical analysis

The results were submitted to analysis of variance (one-way ANOVA) and Tukey's test ($p \leq 0.05$), considering the coffee varieties (conilon, robusta, and intervarietal hybrids) as the source of variation. Principal component

analysis (PCA) using the composition parameters (trigonelline, caffeine, CGA, kahweol, cafestol, and 16-OMC) as active variables. All analyses were performed using Statistica 7.1 software.[26]

Results and Discussion

The caffeine contents showed no difference ($p = 0.057$) between conilon and robusta varieties, with an estimated average content of 2402 mg 100 g^{-1}. Higher variability was observed among hybrid coffees (coefficient of variance (CV) of 27%), with values from 1427 to 3364 mg 100 g^{1}, compared to conilon and robusta (CV of 14 and 15%, respectively) (Table 1).

For Brazilian *C. canephora* without botanical variety information, caffeine contents between 1694 and 2100 mg 100 g^{-1} were reported for coffees with differences in the roasting degree and the presence of defective beans.[20,23,27,28] Heĉimović *et al.*[13] reported caffeine contents from 1810 to 2550 mg 100 g^{-1} for robusta variety (Vietnan and Cherry) with light and dark roasting degrees. For Brazilian robusta coffee from Rondônia, Portela *et al.*[15] reported content of 1930 mg 100 g^{-1}, and Viencz *et al.*[16] values ranging from 1630 to 3330 mg 100 g^{-1}. Less data are available for conilon roasted coffee; a wider range of caffeine is described for Brazilian conilon from Espírito Santo state: from 941 to 3200 mg 100 g^{-1}.[29,30]

Considering the thermal stability of caffeine,[31] data on *C. canephora* green coffee can also be helpful.[23] Alonso-Salces *et al.*[32] reported an average caffeine content of 2668 mg 100 g^{-1} for 57 samples of *C. canephora* green coffees from different countries (in America, Africa, Asia, and Oceania) but without identification of varieties. Pinheiro *et al.*[33] described an average caffeine content of 2450 mg 100 g^{-1} for 21 Brazilian conilon green coffees grown in Espírito Santo state. Lemos *et al.*[34] described caffeine content ranges from 2100 to 3400 mg 100 g^{-1} and from 2300 to 2500 mg 100 g^{-1} for Brazilian conilon and robusta coffees, respectively. Thus, the caffeine contents in this study (Table 1) are at the upper end of the range reported for *C. canephora* in the literature.

Conilon and robusta coffees showed no difference in trigonelline content, which varied between 227 and 636 mg 100 g^{-1}. Hybrid coffees stood out for their high trigonelline contents (with an average value of 613 mg 100 g^{-1}), and robusta ones stood out for the high variability within samples (CV of 41%) (Table 2).

The CGA showed similar behavior to trigonelline: no difference was found between conilon and robusta varieties (ranging from 1244 to 2716 mg 100 g^{-1}) and higher contents for hybrid coffees (with an average value of 3791 mg 100 g^{-1}). This class of compounds presented similar variability among varieties (Table 3).

Trigonelline and CGA are compounds that undergo extensive degradation during the roasting process;[23,31] therefore, the comparison with literature data is affected by the differences in roasting degrees and the lack of information on roasted coffee.

A wide range of trigonelline contents (between 70 and 683 mg 100 g^{-1}) was reported for Brazilian roasted coffees with no information on *C. canephora* variety; these samples also presented diversity in roasting degrees and presence of

Table 1. Caffeine contents of *Coffea canephora* botanical varieties: conilon (C), robusta (R), and intervarietal hybrids of conilon and robusta (H)

Caffeine content / (mg 100 g^{-1})					
Botanical varieties					
Genotype[a]	Conilon[b]	Genotype[a]	Robusta[b]	Genotype[a]	Hybrids[b]
C1	2227 ± 2	R1	2646 ± 1	H1	3618 ± 23
C2	1728 ± 103	R2	3301 ± 185	H2	2807 ± 48
C3	1929 ± 141	R3	2432 ± 124	H3	2977 ± 115
C4	2641 ± 151	R4	2534 ± 253	H4	2600 ± 18
C5	1981 ± 46	R5	2743 ± 71	H5	1918 ± 153
C6	1833 ± 46	R6	2092 ± 37	H6	3156 ± 100
C7	1995 ± 141	R7	2695 ± 19	H7	2267 ± 46
C8	2455 ± 44	R8	2472 ± 29	H8	3364 ± 20
C9	1952 ± 15	R9	2331 ± 10	H9	1956 ± 14
C10	2117 ± 46	R10	1879 ± 12	H10	1427 ± 41
Average value[c]	2086 ± 283[A] (14%)		2512 ± 386[A] (15%)		2609 ± 705[A] (27%)

[a]Numbers indicate the sample in each variety; additional information on Table S1 (SI section); [b]means (n = 4, duplicate extraction and analysis) ± standard deviation among extracts; [c]average value (n = 10 clones *per* variety) ± standard deviation and coefficient of variation (CV%) within each variety; means followed by a different capital letter on the same line indicate a significant difference among varieties (Tukey, $p \leq 0.05$).

Table 2. Trigonelline contents of *Coffea canephora* botanical varieties: conilon (C), robusta (R), and intervarietal hybrids of conilon and robusta (H)

Trigonelline content / (mg 100 g^{-1})					
Botanical varieties					
Genotype[a]	Conilon[b]	Genotype[a]	Robusta[b]	Genotype[a]	Hybrids[b]
C1	294 ± 23	R1	636 ± 23	H1	554 ± 16
C2	540 ± 5	R2	329 ± 21	H2	570 ± 12
C3	422 ± 46	R3	360 ± 50	H3	559 ± 22
C4	620 ± 15	R4	260 ± 29	H4	620 ± 29
C5	496 ± 2	R5	263 ± 15	H5	285 ± 52
C6	398 ± 6	R6	632 ± 26	H6	709 ± 13
C7	445 ± 18	R7	314 ± 15	H7	705 ± 20
C8	474 ± 24	R8	253 ± 6	H8	646 ± 11
C9	316 ± 13	R9	338 ± 10	H9	683 ± 4
C10	316 ± 4	R10	227 ± 7	H10	804 ± 85
Average value[c]	437 ± 106[B] (24%)		361 ± 150[B] (41%)		613 ± 140[A] (23%)

[a]Numbers indicate the sample in each variety; additional information on Table S1 (SI section); [b]means (n = 4, duplicate extraction and analysis) ± standard deviation among extracts; [c]average value (n = 10 clones *per* variety) ± standard deviation and coefficient of variation (CV%) within each variety; means followed by a different capital letter on the same line indicate a significant difference among varieties (Tukey, $p \leq 0.05$).

Table 3. Chlorogenic acids contents of *Coffea canephora* botanical varieties: conilon (C), robusta (R) and intervarietal hybrids of conilon and robusta (H)

Chlorogenic acids content / (mg 100 g^{-1})					
Botanical varieties					
Genotype[a]	Conilon[b]	Genotype[a]	Robusta[b]	Genotype[a]	Hybrids[b]
C1	1534 ± 242	R1	2381 ± 62	H1	2855 ± 89
C2	2191 ± 13	R2	2716 ± 304	H2	2339 ± 185
C3	1968 ± 18	R3	2702 ± 176	H3	4441 ± 184
C4	2609 ± 199	R4	1715 ± 117	H4	4466 ± 4
C5	2353 ± 37	R5	1692 ± 67	H5	1997 ± 16
C6	1744 ± 61	R6	2401 ± 6,36	H6	3965 ± 429
C7	1858 ± 104	R7	2060 ± 48	H7	4475 ± 62
C8	1827 ± 111	R8	1660 ± 31	H8	4693 ± 131
C9	1367 ± 69	R9	2488 ± 4	H9	4455 ± 23
C10	1388 ± 13	R10	1244 ± 90	H10	4229 ± 120
Average value[c]	1884 ± 409[B] (22%)		2105 ± 506[B] (24%)		3791 ± 1001[A] (26%)

[a]Numbers indicate the sample in each variety; additional information on Table S1(SI section); [b]means (n = 4, duplicate extraction and analysis) ± standard deviation among extracts; [c]average value (n = 10 clones *per* variety) ± standard deviation and coefficient of variation (CV%) within each variety; means followed by a different capital letter on the same line indicate a significant difference among varieties (Tukey, $p \leq 0.05$).

defective beans.[20,23,27] For Brazilian conilon from Espírito Santo state, values from 145 to 1030 mg 100 g^{-1} were described.[29,30] Viencz *et al.*[16] reported trigonelline contents ranging from 740 to 1150 mg 100 g^{-1}, for medium-light roasted robusta coffees.

CGA contents from 2024 to 2320 mg 100 g^{-1} were observed for Brazilian *C. canephora* roasted coffee without variety specification and with diversity in the presence of defective beans.[27,28] For Brazilian robusta coffee from Rondônia, higher contents were reported by Portela *et al.*[15] (5758 mg 100 g^{-1}) and Viencz *et al.*[16] (4780 mg 100 g^{-1}) for medium-light roasted coffees. Mori *et al.*[30] reported CGA contents between 528 and 942 μg mL^{-1} for the conilon coffee brews, corresponding to the range of 1550 to 2650 mg 100 g^{-1} for roasted coffee.

Few works have described the CGA content in roasted *C. canephora*; some authors[20,23] analyzed only its main isomer. 5-CQA contents between 40 and 518 mg 100 g^{-1} were reported for Brazilian *C. canephora* without variety identification. Higher content of 5-CQA (834 mg 100 g^{-1}) was described for robusta coffee from India,[35] which are comparable to the values obtained by this study (526, 559,

and 1261 mg 100 g^{-1} for conilon, robusta, and hybrids, respectively) (Table S3, SI section).

The literature usually reports 5-CQA as the predominant isomer of the chlorogenic acid class in coffee. 5-CQA corresponds to 38 to 50% of the total CGA in *C. arabica* roasted coffee,[36-38] but lower values (from 31 to 40% of CGA) are described for *C. canephora*.[16,30,39] Our results indicated even smaller percentages, with 5-CQA corresponding, on average, to 28, 27, and 33% of the total CGA for conilon, robusta, and hybrid coffees, respectively (Table S3, SI section).

The contents of trigonelline, CGA, and 5-CQA obtained (Tables 2, 3, and S3) are in the upper part of the range reported in the literature for *C. canephora*. It could be partially due to the use of a mild roasting process but also to the inclusion of hybrid coffees, which stood out for the high content of these compounds.

For the total diterpenes (the sum of kahweol, cafestol, and 16-OMC contents), the hybrid coffees showed a high content (with an average value of 471 mg 100 g^{-1}) and less variability (13%) compared to conilon and robusta coffees (CV of 23 and 38%, respectively, and an average content of 346 mg 100 g^{-1}) (Table S4, SI section). Total diterpenes values described for hybrid coffees (Table S4) also stand out when compared to those found in the literature: from 191 to 415 mg 100 g^{-1} for conilon,[17] from 257 to 707 mg 100 g^{-1} for robusta[14,16] and 192 to 742 mg 100 mg^{-1} for natural intervarietal hybrids.[24]

There was no difference in kahweol contents among the varieties, ranging from absence to 25.7 mg 100 g^{-1}, showing a high variability within each variety (CV between 108 and 211%). However, we highlight that kahweol was more frequently present in hybrid samples (50% of the samples) than in conilon and robusta coffees (20 and 30%, respectively) (Table 4).

Mori *et al.*[17] reported the absence of kahweol in 70% of Brazilian conilon studied (30 coffees, 15 genotypes in 2 growing sites); samples with kahweol showed contents ranging from 3.7 to 14.1 mg 100 g^{-1}. For robusta coffees, Finotello *et al.*[14] reported the presence of kahweol in 28% of samples studied, with contents from 2.5 to 20.0 mg 100 g^{-1}; Viencz *et al.*[16] reported the presence of kahweol in only 19% of the samples studied, with contents up to 44 mg 100 g^{-1}. For Brazilian *C. canephora* without variety identification, both the absence[18,20,21] and the presence of kahweol (16.2 mg 100 g^{-1}) were reported.[27] Natural intervarietal hybrids had a high presence of kahweol (in 77% of the samples), with contents up to 41 mg 100 g^{-1}.[24]

There was no difference in cafestol contents between conilon and hybrid coffees, with values ranging from 106 to 295 mg 100 g^{-1}; the robusta variety stood out for low cafestol content, with an average value of 116 mg 100 g^{-1} (Table 5).

Cafestol contents between 163 and 497 mg 100 g^{-1} were reported for roasted *C. canephora* with no variety identification.[18,19,20,21,27] Cafestol contents from 226 to 264 mg 100 g^{-1} were reported for Brazilian conilon coffee;[17] a higher variation was described for natural intervarietal hybrids: from 96 to 457 mg 100 g^{-1}.[24] For robusta coffees of different origins, a wide range of cafestol contents (between 73 to 335 mg 100 g^{-1}) were reported.[14,16] Thus, robusta coffees here studied also presented cafestol contents at the lower end of the range reported in the literature.

The parameter cafestol/kahweol ratio has already been

Table 4. Kahweol contents of *Coffea canephora* botanical varieties: conilon (C), robusta (R), and intervarietal hybrids of conilon and robusta (H)

Kahweol content / (mg 100 g^{-1})					
Botanical varieties					
Genotype[a]	Conilon[b]	Genotype[a]	Robusta[b]	Genotype[a]	Hybrids[b]
C1	0.0 ± 0.0	R1	0.0 ± 0.0	H1	0.0 ± 0.0
C2	22.2 ± 0.8	R2	0.0 ± 0.0	H2	18.0 ± 0.6
C3	0.0 ± 0.0	R3	0.0 ± 0.0	H3	18.4 ± 0.4
C4	0.0 ± 0.0	R4	0.0 ± 0.0	H4	18.3 ± 0.4
C5	0.0 ± 0.0	R5	0.0 ± 0.0	H5	0.0 ± 0.0
C6	0.0 ± 0.0	R6	17.9 ± 0.8	H6	0.0 ± 0.0
C7	23.1 ± 1.0	R7	0.0 ± 0.0	H7	0.0 ± 0.0
C8	0.0 ± 0.0	R8	23.1 ± 2.7	H8	0.0 ± 0.0
C9	0.0 ± 0.0	R9	17.6 ± 0.4	H9	18.5 ± 0.4
C10	0.0 ± 0.0	R10	0.0 ± 0.0	H10	25.7 ± 0.3
Average value[c]	4.5 ± 9.5[A] (211%)		5.9 ± 9.5[A] (163%)		9.9 ± 10.6[A] (108%)

[a]Numbers indicate the sample in each variety; additional information on Table S1 (SI section); [b]means (n = 4, duplicate extraction and analysis) ± standard deviation among extracts; zero values indicate contents below LOD; [c]average value (n = 10 clones *per* variety) ± standard deviation and coefficient of variation (CV%) within each variety; means followed by a different capital letter on the same line indicate a significant difference among varieties (Tukey, $p \leq 0.05$).

Table 5. Cafestol contents of *Coffea canephora* botanical varieties: conilon (C), robusta (R) and intervarietal hybrids of conilon and robusta (H)

Cafestol content / (mg 100 g^{-1})					
Botanical varieties					
Genotype[a]	Conilon[b]	Genotype[a]	Robusta[b]	Genotype[a]	Hybrids[b]
C1	157 ± 12	R1	78 ± 7	H1	195 ± 19
C2	159 ± 1	R2	165 ± 2	H2	156 ± 6
C3	174 ± 14	R3	141 ± 15	H3	251 ± 27
C4	270 ± 25	R4	79 ± 1	H4	273 ± 19
C5	180 ± 19	R5	172 ± 8	H5	180 ± 1
C6	261 ± 24	R6	87 ± 9	H6	217 ± 15
C7	133 ± 1	R7	86 ± 13	H7	167 ± 17
C8	200 ± 2	R8	136 ± 5	H8	272 ± 13
C9	106 ± 11	R9	96 ± 9	H9	279 ± 41
C10	250 ± 17	R10	119 ± 15	H10	295 ± 36
Average value[c]	189 ± 56^{A} (29%)		116 ± 36^{B} (31%)		228 ± 51^{A} (23%)

[a]Numbers indicate the sample in each variety; additional information on Table S1 (SI section); [b]means (n = 4, duplicate extraction and analysis) ± standard deviation among extracts; [c]average value (n = 10 clones *per* variety) ± standard deviation and coefficient of variation (CV%) within each variety; means followed by a different capital letter on the same line indicate a significant difference among varieties (Tukey, $p \leq 0.05$).

correlated with the quality of the *C. arabica* beverages; Barbosa *et al.*[40] associated the increase in the ratio value with the improvement of the cup quality of coffee brews (66 samples) originating from quality contests. Novaes *et al.*[41] reported that a cafestol/kahweol ratio above 1.20 was related to good quality *C. arabica* (soft beverages). Due to absence of kahweol, it was feasible to calculate this ratio for all coffees; however, a range of values between 4.8 and 7.2 was observed for robusta and conilon, and higher values (7.6 to 15.0) for the hybrid samples, see Figure 1 and Table S5 (SI section). Viencz *et al.*[16] reported values from 3.2 to 14.7 (with a mean of 6.5) for robusta coffees with good cup quality.

16-OMC showed a different behavior from other diterpenes: no difference was observed between robusta and hybrid coffees, with contents between 138 and 294 mg 100 g^{-1}. However, conilon stood out for both high variability (CV of 38%) and lower concentration (average content of 139 mg 100 g^{-1}) of 16-OMC (Table 6).

For roasted coffees, 16-OMC contents between 118 and 372 mg 100 g^{-1} were reported for robustas from different world regions[14,16] and between 26 and 132 mg 100 g^{-1} for

Table 6. 16-*O*-Methylcafestol contents of *Coffea canephora* botanical varieties: conilon (C), robusta (R), and intervarietal hybrids of conilon and robusta (H)

16-*O*-Methylcafestol content / (mg 100 g^{-1})					
Botanical varieties					
Genotype[a]	Conilon[b]	Genotype[a]	Robusta[b]	Genotype[a]	Hybrids[b]
C1	159 ± 5	R1	186 ± 26	H1	200 ± 5
C2	201 ± 24	R2	138 ± 12	H2	200 ± 22
C3	160 ± 5	R3	271 ± 37	H3	270 ± 35
C4	226 ± 6	R4	239 ± 19	H4	281 ± 6
C5	116 ± 14	R5	297 ± 34	H5	231 ± 31
C6	101 ± 4	R6	154 ± 19	H6	251 ± 38
C7	165 ± 22	R7	224 ± 27	H7	294 ± 20
C8	53 ± 5	R8	364 ± 9	H8	219 ± 10
C9	118 ± 1	R9	234 ± 30	H9	186 ± 3
C10	91 ± 3	R10	280 ± 1	H10	180 ± 12
Average value[c]	139 ± 53^{B} (38%)		239 ± 68^{A} (29%)		231 ± 41^{A} (18%)

[a]Numbers indicate the sample in each variety; additional information on Table S1 (SI section); [b]means (n = 4, duplicate extraction and analysis) ± standard deviation among extracts; [c]average value (n = 10 clones *per* variety) ± standard deviation and coefficient of variation (CV%) within each variety; means followed by a different capital letter on the same line indicate a significant difference among varieties (Tukey, $p \leq 0.05$).

conilon from Brazil.[17] Schievano *et al.*[22] and Kalschne *et al.*[27] reported 16-OMC contents from 120 to 185 mg 100 g^{-1} for roasted *C. canephora* without variety identification. Francisco *et al.*[24] reported a 16-OMC content between 75 and 433 mg 100 g^{-1} for natural intervarietal hybrids. 16-OMC values for robusta, conilon, and hybrids (Table 6) remained in the upper range of contents described in the literature.

The literature generally points out the absence of 16-OMC in *C. arabica* coffees,[23] and in recent reports, some authors[42] identified only traces of the compound in this species. This fact, added to the thermal stability of the compound,[43] reinforces the possibility of using it as an indicator of *C. canephora*. The German standard method DIN 10779, initially published in 1999 and revised in 2011, recommends quantifying 16-OMC to evaluate the percentage of *C. canephora* coffee on roasted and ground commercial products.[44] Given the importance of Brazil as a producer and exporter of the species, the data presented here for three varieties may provide information on the presence of 16-OMC, this diterpene, in *C. canephora* coffees.

The wide range observed (ranging from 53 to 297 mg 100 g^{-1}) suggests that data on 16-OMC contents alone might not be sufficient to confidently estimate the percentage of *C. canephora* in blends with *C. arabica*. Schievano *et al.*[22] and Mori *et al.*[17] reported a similar concern, studying robusta and conilon coffees, respectively.

Zanin *et al.*[45] proposed using the parameter caffeine/total diterpenes ratio for *C. arabica* characterization; the authors suggested that values higher than 2.50 may indicate the presence of the *C. canephora* species. Viencz *et al.*[16] described a mean value of 7.0, studying 57 robusta coffees. Our results (with caffeine/total diterpenes values between 2.87 and 10.33, see Figure 1, and Table S6 (SI section)) reinforce the potential of this parameter for *C. canephora* coffees characterization.

PCA was applied to characterize and discriminate the three varieties of *C. canephora* considering the composition profile in a multivariate approach (Figure 2). The main components (CP 1) and (CP 2) accounted for 63% of the variance. CP 1 was positively correlated with the CGA, trigonelline, cafestol, and kahweol parameters, while CP 2 was positively correlated with caffeine and 16-OMC (Figure 2a).

Hybrid coffees were discriminated from the conilon and robusta varieties by CP 1; they were located mainly at the right of the plot in the first and second quadrants (Figure 2), and characterized by high trigonelline, CGA, cafestol, and kahweol contents (Figure 2a).

Conilon and robusta coffees were located mainly in the bottom (third quadrant) and in the upper region of the plot (fourth quadrant), respectively (Figure 2b); these varieties were discriminated by CP 2. Conilon coffees were mainly differentiated by the low caffeine and 16-OMC contents (Figure 2a).

In summary, although the varieties can be discriminated (Figure 2), there was less differentiation in the compositional profile of conilon and robusta than might be expected considering the diversity usually described in the characteristics and cup quality of the beverages of these botanical varieties.[46]

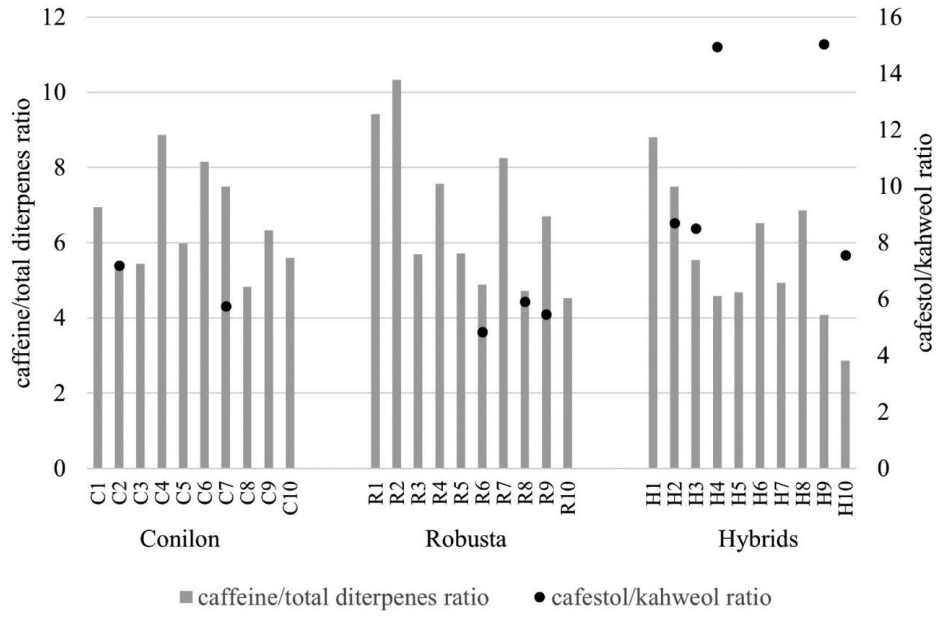

Figure 1. Caffeine/total diterpenes ratio and cafestol/kahweol ratio in *Coffea canephora* varieties: conilon (C), robusta (R), and intervarietal hybrids of conilon and robusta (H). Numbers indicate the sample in each variety; additional information on Table S1 (SI section).

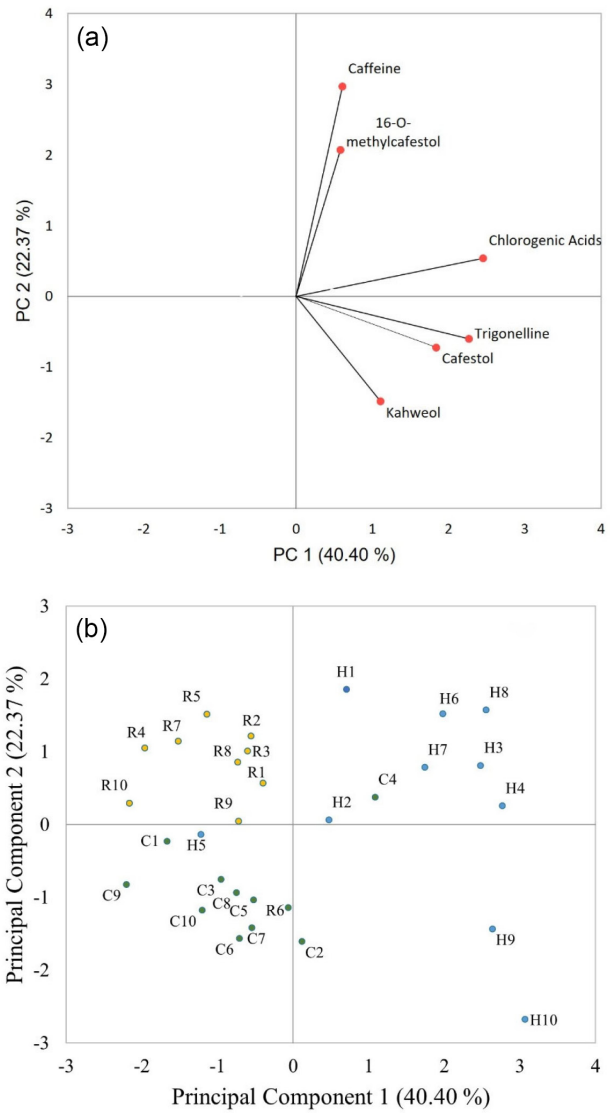

Figure 2. Principal component analysis considering the chemical composition of *Coffea canephora* varieties: (a) projection of the variables: caffeine, trigonelline, chlorogenic acids (CGA), kahweol, cafestol, and 16-*O*-methylcafestol (16-OMC); (b) sample plot. Varieties conilon, robusta, and intervarietal hybrids are represented by letters C, R, and H, respectively, and numbers indicate the sample in each variety, additional information on Table S1 (SI section).

One of the main properties of hybrid individuals is the expression of complementary characteristics of both botanical varieties. However, despite some similarities among hybrid coffees and the traditional varieties (cafestol content did not differ from conilon, 16-OMC content did not differ from robusta, and caffeine content did not differ from both), in general, the hybrid coffees showed a different composition profile, highlighting the higher contents of trigonelline, CGA, and total diterpenes (Tables 1 to 6, Figure 2). This behavior is consistent with observations made in field evaluations, where hybrid coffees have stood out due to their higher productivity and expression of the best characteristics of each of the botanical varieties.[10,12] Therefore, greater diversity in the compositional profile could also be expected.

Conclusions

Caffeine and kahweol were the only compounds with no significant difference comparing traditional varieties (conilon and robusta) and intervarietal hybrids of conilon and robusta. The hybrid coffees stood out for the higher contents of trigonelline, CGA, and total diterpenes, higher incidence of kahweol, and higher values for the cafestol/kahweol ratio (a potential indicator of cup quality) compared to the traditional varieties. Conilon and robusta coffees differ significantly only in cafestol and 16-OMC contents; conilon stood out for its lower 16-OMC content and robusta for its lower cafestol content.

The difference in the compositional profile between the botanical varieties conilon and robusta is smaller than that found among them and the intervarietal hybrid coffees, which exhibited greater diversity in the chemical composition.

Supplementary Information

Supplementary information is available free of charge at http://jbcs.sbq.org.br as a PDF file.

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Author Contributions

Lucas B. Acre was responsible for the conceptualization, methodology, validation, investigation, formal analysis, writing original draft, data curation, visualization; Thayna Viencz for the investigation, writing review and editing, methodology; Julyene Francisco for the investigation, methodology, data curation; Rodrigo Barros Rocha for the writing review and editing, resources, visualization, funding acquisition; Enrique Anastácio Alves for the resources, funding acquisition; Marta T. Benassi for the resources, conceptualization, funding acquisition, visualization, writing review and editing, supervision.

References

1. Companhia Nacional de Abastecimento (CONAB); *Acompanhamento da Safra Brasileira - Café Safra 2020*, http://www.sapc.embrapa.br/arquivos/consorcio/levantamento/conab_safra_2020n1.pdf, accessed in February 2024.

2. Companhia Nacional de Abastecimento (CONAB); *Acompanhamento da Safra Brasileira - Café Safra 2021*, https://www.conab.gov.br/info-agro/safras/cafe/boletim-da-safra-de-cafe/item/download/45166_c217a37038a20bad7beeee5b16525b03, accessed in February 2024.
3. Companhia Nacional de Abastecimento (CONAB); *Acompanhamento da Safra Brasileira - Café Safra 2022*, http://www.consorciopesquisacafe.com.br/images/stories/noticias/2021/2022/dezembro/4_levantamento_safra_conab.pdf, accessed in February 2024.
4. International Coffee Organization (ICO); *Relatório sobre o Mercado de Café, Janeiro 2016*, http://consorciopesquisacafe.com.br/arquivos/consorcio/publicacoes_tecnicas/Relatorio_sobre_o_mercado_de_cafe_Janeiro_2016.pdf, accessed in February 2024.
5. International Coffee Organization (ICO); *Relatório sobre o Mercado de Café, Dezembro 2022*, http://www.consorciopesquisacafe.com.br/images/stories/noticias/2021/2022/dezembro/relatorio_oic_dezembro_2022.pdf, accessed in February 2024.
6. Ferrão, R. G.; Ferrão, M. A. G.; Fonseca, A. F. A.; Volpi, P. S.; Verdin Filho, A. C.; Pacova, B. E. V.; Ferrão, L. F. In *Conilon Coffee*, Ferrão, R. G.; Fonseca, A. F. A.; Ferrão, M. A. G.; Muner, L. H., eds.; Incaper: Vitória, 2019, ch. 6. [Link] accessed in February 2024
7. Rocha, R. B.; Teixeira, A. L.; Ramalho, A. R.; Espindula, M. C.; Lunz, A. M. P.; Souza, F. F.; *Cienc. Rural* **2021**, *51*, e20200713. [Crossref]
8. Dalazen, J. R.; Rocha, R. B.; Pereira, L. L.; Alves, E. A.; Espindula, M. C.; Souza, C. A.; *Coffee Sci.* **2020**, *15*, e151711. [Link] accessed in February 2024
9. *Cafés de Rondônia - O Mundo do Café na Amazônia*, https://www.embrapa.br/rondonia/cafes-de-rondonia, accessed in February 2024.
10. Teixeira, A. L.; Rocha, R. B.; Espindula, M. C.; Ramalho, A. R.; Vieira Júnior, J. R.; Alves, E. A.; Lunz, A. M. P.; Souza, F. F.; Costa, J. N. M.; Fernandes, C. F.; *CBAB, Crop Breed. Appl. Biotechnol.* **2020**, *20*, 1. [Crossref]
11. Souza, F. F.; Ferrão, L. F. V.; Caixeta, E. T.; Sakiyama, N. S.; Pereira, A. A.; Oliveira, A. C. B. In *Café na Amazônia*; Marcolan, A. L.; Espindula, M. C., eds.; Embrapa: Brasília, 2015, ch. 4. [Link] accessed in February 2024
12. Morais, J. A.; Rocha, R. B.; Alves, E. A.; Espindula, M. C.; Teixeira, A. L.; Souza, C. A.; *Acta Sci. Agron*. **2021**, *43*, e52095. [Crossref]
13. Heĉimović, I.; Belščak-Cvitanović, A.; Horžić, D.; Komes, D.; *Food Chem.* **2011**, *129*, 991. [Crossref]
14. Finotello, C.; Forzato, C.; Gasparini, A.; Mammi, S.; Navarini, L.; Schievano, E.; *Food Control* **2017**, *75*, 62. [Crossref]
15. Portela, C. S.; Almeida, I. F.; Mori, A. L. B.; Yamashita, F.; Benassi, M. T.; *LWT -- Food Sci. Technol.* **2021**, *143*, 111090. [Crossref]
16. Viencz, T.; Acre, L. B.; Rocha, R. B.; Alves, E. A.; Ramalho, A. R.; Benassi, M. T.; *J. Food Compos. Anal.* **2023**, *117*, 105140. [Crossref]
17. Mori, A. L. B.; Kalschne, D. L.; Ferrão, M. A. G.; Fonseca, A. F. A.; Ferrão, R. G.; Benassi, M. T.; *J. Food Compos. Anal.* **2016**, *52*, 52. [Crossref]
18. Campanha, F. G.; Dias, R. C. E.; Benassi, M. T.; *Coffee Sci.* **2010**, *5*, 87. [Link] accessed in February 2024
19. Sridevi, V.; Giridhar, P.; Ravishankar, G. A.; *Global J. Med. Res.* **2011**, *11*, 16. [Link] accessed in February 2024
20. de Souza, R. M. N.; Benassi, M. T.; *J. Braz. Chem. Soc.* **2012**, *23*, 1347. [Crossref]
21. Dias, R. C. E.; Faria-Machado, A. F.; Mercadante, A. Z.; Bragagnolo, N.; Benassi, M. T.; *Eur. Food Res. Technol.* **2014**, *239*, 961. [Crossref]
22. Schievano, E.; Finotello, C.; De Angelis, E.; Mammi, S.; Navarini, L.; *J. Agric. Food Chem.* **2014**, *62*, 12309. [Crossref]
23. Dias, R. C. E.; Benassi, M. T.; *Beverages* **2015**, *1*, 127. [Crossref]
24. Francisco, J. S.; Dias, R. C. E.; Alves, E. A.; Rocha, R. B.; Dalazen, J. R.; Mori, A. L. B.; Benassi, M. T.; *Beverages* **2021**, *7*, 77. [Crossref]
25. Mendes, L. C.; Menezes, H. C.; Silva, M. A. A. P.; *Food Qual. Preference* **2001**, *12*, 153. [Crossref]
26. *Statistica*, version 7.1; Statsoft Inc., Tulsa, USA, 2006.
27 Kalschne, D. L.; Viegas, M. C.; de Conti, A. J.; Corso, M. P.; Benassi, M. T.; *LWT -- Food Sci. Technol.* **2019**, *99*, 364. [Crossref]
28. Reis, T. A. D.; de Conti, A. J.; Barrientos, E. A. L.; Mori, A. L. B.; Benassi, M. T.; *Cienc. Agrotecnol.* **2019**, *43*, 1. [Crossref]
29. Agnoletti, B. Z.; Oliveira, E. C. S.; Pinheiro, P. F.; Saraiva, S. H.; *Rev. Virtual Quim.* **2019**, *11*, 785. [Crossref]
30. Mori, A. L. B.; Viegas, M. C.; Ferrão, M. A. G.; Fonseca, A. F. A.; Ferrão, R. G.; Benassi, M. T.; *Br. Food J.* **2020**, *122*, 827. [Crossref]
31. Vignoli, J. A.; Viegas, M. C.; Bassoli, D. G.; Benassi, M. T.; *Food Res. Int.* **2014**, *61*, 279. [Crossref]
32. Alonso-Salces, R. M.; Serra, F.; Reniero, F.; Héberger, K.; *J. Agric. Food Chem.* **2009**, *57*, 4224. [Crossref]
33. Pinheiro, C. A.; Pereira, L. L.; Fioresi, D. B.; Oliveira, D. S.; Osorio, V. M.; Silva, J. A.; Pereira, U. A.; Ferrrão, M. A. G.; Riva-Souza, E. M.; Fonseca, A. F. A.; Pinheiro, P. F.; *Aust. J. Crop Sci.* **2019**, *13*, 2046. [Crossref]
34. Lemos, M. F.; Salustriano, N. A.; Costa, M. M. S.; Lirio, K.; Fonseca, A. F. A.; Pacheco, H. P.; Endringer, D. C.; Fronza, M.; Scherer, R.; *J. Saudi Chem. Soc.* **2022**, *26*, 1014467. [Crossref]
35. Tang, V. C. Y.; Sun, J.; Cornuz, M.; Yu, B.; Lassabliere, B.; *Food Chem*. **2021**, *337*, 128023. [Crossref]

36. Farah, A.; Paulis, T.; Trugo, L. C.; Martin, P. R.; *J. Agric. Food Chem.* **2005**, *53*, 1505. [Crossref]
37. Perrone, D.; Donangelo, C. M.; Farah, A.; *Food Chem.* **2008**, *110*, 1030. [Crossref]
38. Zanin, R. C.; Corso, M. P.; Kitzberger, C. S. G.; Scholz, M. B. S.; Benassi, M. T.; *LWT -- Food Sci. Technol.* **2016**, *74*, 480. [Crossref]
39. Perrone, D.; Farah, A.; Donangelo, C. M.; *J. Agric. Food Chem.* **2012**, *60*, 4265. [Crossref]
40. Barbosa, M. S. G.; Scholz, M. B. S.; Kitzberger, C. S. G.; Benassi, M. T.; *Food Chem.* **2019**, *292*, 275. [Crossref]
41. Novaes, F. J. M.; Oigman, S. S.; Souza, R. O. M. A.; Rezende, C. M.; Aquino Neto, F. R.; *Talanta* **2015**, *139*, 159. [Crossref]
42. Gunning, Y.; Defernez, M.; Watson, A. D.; Beadman, N.; Colquhoun, I. J.; Gall, G. L.; Philo, M.; Garwood, H.; Williamson, D.; Davis, A. P.; Kemsley, E. K.; *Food Chem.* **2018**, *248*, 52 [Crossref]; Guercia, E.; Colomban, S.; Navarini, L.; *J. Mass Spectrom.* **2020**, *55*, e4636 [Crossref]; Portaluri, V.; Thomas, F.; Guyader, S.; Jamin, E.; Bertrand, B.; Remaud, G. S.; Schievano, E.; Mammi, S.; Guercia, E.; Navarini, L.; *Food Chem.* **2020**, *329*, 127129. [Crossref]
43. Speer, K.; Tewis, R.; Montag, A.; *Z. Lebensm.-Unters. Forsch.* **1991**, *192*, 451 [Crossref]; Kemsley, E.; Ruault, S.; Wilson, R. H.; *Food Chem.* **1995**, *54*, 321. [Crossref]
44. Speer, K.; Kölling-Speer, I.; *Braz. J. Plant Physiol.* **2006**, *18*, 201 [Crossref]; *DIN 10779:2011-Analysis of Coffee and Coffee Products: Determination of 16-O-Methyl Cafestol Content of Roasted Coffee - HPLC-Method*, Deutsches Institut Für Normung: Beuth, 2011. [Link] accessed in February 2024
45. Zanin, R. C.; Kitzberger, C. S. G.; Benassi, M. T.; *Braz. Arch. Biol. Technol.* **2020**, *63*, 1. [Crossref]
46. Espindula, M. C.; Teixeira, A. L.; Rocha, R. B.; Ramalho, A. R.; Vieira Júnior, J. R.; Alves, E. A.; Diocleciano, J. M.; Lunz, A. M. P.; Souza, F. F.; Costa, J. N. M.; Fernandes, C. F.; *Novas Cultivares de Cafeeiros Coffea canephora para a Amazônia Ocidental Brasileira - Principais Características*, Comunicado Técnico 413; Embrapa Rondônia: Porto Velho, 2019. [Link] accessed in February 2024; Ferrão, M. A. G.; Ferrão, R. G.; Fonseca, A. F. A.; Filho, A. C. V.; Volpi, P. S. In *Conilon coffee*; Ferrão, R. G.; Fonseca, A. F. A.; Ferrão, M. A. G.; Muner, L. H., eds.; Incaper: Vitória, 2019, ch. 4. [Link] accessed in February 2024

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