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Characterization of Roasted *Coffea arabica* Species by the Relationship Between Caffeine and Diterpenes Contents

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HIGHLIGHTS

- Variations on caffeine and diterpenes contents were superior to previous reported.
- It was described typical profiles of caffeine and diterpenes in roasted *C. arabica.*
- Ratios among caffeine and diterpenes were used for characterization of *C. arabica.*
- A new tool for *C. arabica* discrimination in commercial roasted coffee was proposed.

Abstract: Commercial roasted and ground coffees are usually blends of *Coffea arabica* and *Coffea canephora*. Considering the differences in price and sensory characteristics between these two species, the identification of the presence of each species in commercial blends is of great interest. The aim of this study was to describe typical profiles of caffeine and diterpenes (kahweol and cafestol) contents and the ratios among these compounds to support the characterization of *Coffea* species in roasted coffees. 32 good cup quality Brazilian *C. arabica* coffees (from coffee quality contests) produced using different postharvest treatments were studied. All analysis were performed by HPLC. Higher ranges were observed in diterpene contents – kahweol varied from 1.75 to 10.68 g/kg (coefficient of variation of 510%) and cafestol from 1.76 to 9.66 g/kg (449%) – than caffeine, that varied from 5.1 to 16.2 g/kg (coefficient of variation of 218%). Wide ranges of the kahweol/cafestol ratio (0.63 to 2.77) and the caffeine/kahweol ratio (0.84 to 5.15) were also observed. Hence it was proposed the additional use of a new parameter, the ratio of caffeine/sum of diterpenes (kahweol + cafestol) that presents values from 0.54 to 2.39. The results indicated that the combined use of these parameters could be a potential tool for discriminating *Coffea* species in blends of roasted and ground coffee. It was proposed as potentially indicative of *C. arabica*: values of kahweol/cafestol ratio above 0.50, associated with caffeine/kahweol ratio lower than 5.50 and caffeine/sum of diterpenes ratio lower than 2.50.

Keywords: kahweol; cafestol; caffeine; arabica coffee; roasted coffee.

INTRODUCTION

Coffea arabica L. and *Coffea canephora* L. var. robusta are the two coffee species of commercial importance as they correspond to 62% and 38% of the world coffee production, respectively; Brazil stands out as the largest producer (52%) and exporter (31%) of green *C. arabica* [1]. Brazilian arabica coffee has been recently recognized as a high-quality product, as it is used in blends of the most prestigious commercial brands worldwide [2].

Due to its superior cup quality, *C. arabica* has higher commercial value than *C. canephora*, which is added on blends to give the brew a stronger coffee base [2]. Coffee blends are a good technological alternative since they provide a product with sensory characteristics that fit the consumer's preferences at a lower price. However, the purchaser should be informed about the coffee species in the commercial product as there is an opportunity for fraudulent profit by substituting *C. arabica* with *C. canephora*.

Although green beans are differentiated by physical characteristics such as shape and size, chemical assays become necessary for the discrimination of coffee species after roasting and grinding [3]. Previous reports have described the use of sensory analysis [4], which demands a considerable amount of time, and instrumental methods, which require specialized equipment and well-trained analysts, as ^{1}H NMR spectroscopy [5], mass spectrometry [6,7], molecular methods [8], combined electronic nose and tongue [4,9], and other techniques [3,10].

Usually, the discrimination of coffee species focuses on analytes presented in different concentrations in the two species such as tocopherols, minerals, sugars, fatty acids, chlorogenic acids [11], caffeine, trigonelline, and diterpenes [3,10]. However, several of these compounds are thermolabile, difficulting the discrimination in commercial products, in which the correct intensity of roasting is not provided. In addition, the use of only one class/compound for species identification is a risky approach. As an example, 16-O-methylcafestol, which is consistently described in the literature as exclusive to *C. canephora*, has recently been observed in *C. arabica* [12].

There are substantial advantages associated with using compounds, such as caffeine and diterpenes, that are less affected by the roasting process and that are present in quite different contents in the two coffee species. *C. canephora* has only traces of kahweol (< 0.1 g/kg), 2 to 3 times less cafestol than *C. arabica*, and higher caffeine content (60 to 90%) than *C. arabica* [13-17].

Ratios between the levels of caffeine and diterpenes were also proposed as potential parameters for the identification of the coffee species [15]. However, the ranges that could be characteristic of *C. arabica* are not clearly defined. Diterpenes levels, for example, are highly variable even under standardized conditions for coffee growing and processing [18].

The aim of this research was to establish typical ranges for the contents of kahweol, cafestol, and caffeine in roasted *C. arabica*, as well as to study the ratios kahweol/cafestol and caffeine/kahweol as a tool for species identification. The hypothesis considered in this research is that the relationships between caffeine and diterpenes contents might support the characterization of *C. arabica* in roasted coffees.

MATERIAL AND METHODS

Material

Thirty-two *C. arabica* from the two main producing Brazilian regions, South and Southeast (Table S1), were provided by the Instituto Agronômico of Paraná - IAPAR (Londrina, Brazil). The samples were part of the panel of coffee quality contests (State Contest Cafe Qualidade Parana 2012 and National Contest Cup Excellence 2011) and were produced using different post-harvest processes (natural and pulped).

The coffee beans were roasted (medium roast, weight loss of 17%) in a pilot-type Rod-Bel roaster (São Paulo, Brazil) and ground (Krups GVX208, Shanghai, China) to sieve size 0.84 mm. The samples were characterized by moisture (27 g/kg ± 0.3) using an infrared moisture analyzer (Ohaus-MB45, Parsippany, USA), and color (L*=25.9 ± 2.5) using a Konica Minolta-CR400 colorimeter (Osaka, Japan).

Reagents and standards

The following solvents and standards were used: KOH (Vetec, Rio de Janeiro, Brazil), HPLC-grade methyl tert-butyl ether (Acrós Organics, New Jersey, USA); HPLC-grade acetonitrile (Fisher Scientific, New Hampshire, USA), HPLC-grade acetic acid (Sigma-Aldrich, St. Louis, USA), kahweol and cafestol (Axxora, San Diego, USA), and caffeine (Sigma-Aldrich, St. Louis, USA).

Analysis of caffeine and diterpenes

The extraction and measurements of caffeine and the diterpenes were carried out according to Alves and coauthors [19], and Kitzberger and coauthors [18], respectively. The caffeine extraction was performed using hot water (80°C for 10 min), and injected in a Shimadzu Liquid Chromatograph (Kyoto, Japan) with a UV-Vis detector (SPD-10A). The diterpenes analysis were performed by direct hot saponification with KOH ethanolic, extraction with methyl tert-butyl ether and cleaning up with water and then injected in a liquid chromatography Surveyor Plus (Thermo Scientific, San Jose, USA) diode array detector (Surveyor PDA Plus) with an automatic injector. All three compounds were analyzed using a reversed-phase column Spherisorb ODS 1 (250 mm × 4.6 mm id 5 µm) (Waters, Milford, USA). Caffeine was eluted in a gradient of acetic acid/ultrapure water (5:95 v/v) (A) and acetonitrile (B) as follows: 0 to 5 min, 8% of B; 10 to 35 min, 15% of B and detected at 272 nm. For the diterpenes, an isocratic elution of acetonitrile/water (55:45, v/v) were applied. The detection of cafestol and kahweol was set at 220 and 290 nm, respectively.

Triplicate independent extractions were performed and the quantification was carried out using 6-point calibration curves ($R^2 > 0.99$) with triplicate measurements in the range of 0.5 to 30 g/kg, for caffeine, and 0.5 to 15 g/kg, for kahweol and cafestol.

A completely randomized design was used. The caffeine and diterpenes concentration were analyzed by one-way ANOVA, and Tukey test ($p < 0.05$) using Statistica 10.0 software (StatSoft, Tulsa, USA).

RESULTS

Caffeine, kahweol, and cafestol contents were determined in roasted and ground *C. arabica* and their concentrations are presented in Table 1. Even though it was used coffee from the same species it could observe high variability on these compounds. Caffeine content presented 218% of variability. The diterpenes presented even higher variations, kahweol levels ranged 510% and cafestol ranged 449%, such variability has not been described before.

Table 1. Contents of caffeine, kahweol, cafestol, sum of diterpenes[a] (g/kg) and the ratios kahweol/cafestol, caffeine/kahweol and caffeine/sum of diterpenes of roasted *C. arabica*.

Sample	Caffeine	Kahweol	Cafestol	Sum of diterpenes[a]	Kahweol/ Cafestol (KA/CA)	Caffeine/ Kahweol (CAF/KA)	Caffeine/ Sum of diterpenes (CAF/SUM)
1	$9.67^{cdefg} \pm 0.06$	$4.44^{cd} \pm 0.60$	$2.51^{abc} \pm 0.31$	6.95 ± 1.09	1.77	2.18	1.39
2	$7.82^{ab} \pm 0.06$	$9.34^{no} \pm 0.06$	$5.01^{ghi} \pm 0.04$	14.35 ± 2.25	1.86	0.84	0.54
3	$10.54^{ghijkl} \pm 0.26$	$7.89^{lm} \pm 0.10$	$5.36^{ij} \pm 0.13$	13.25 ± 1.49	1.47	1.34	0.80
4	$10.07^{defghi} \pm 0.12$	$7.41^{kl} \pm 0.14$	$5.74^{ijk} \pm 0.27$	13.15 ± 0.89	1.29	1.36	0.77
5	$10.33^{efghijk} \pm 0.05$	$7.91^{lm} \pm 0.16$	$4.07^{efg} \pm 0.35$	11.98 ± 2.65	1.94	1.31	0.86
6	$10.40^{fghijk} \pm 0.26$	$8.77^{mn} \pm 0.26$	$5.39^{ij} \pm 0.18$	14.16 ± 2.00	1.63	1.19	0.73
7	$10.62^{ghijkl} \pm 0.01$	$10.28^{op} \pm 0.06$	$4.32^{fgh} \pm 0.12$	14.60 ± 1.78	2.38	1.03	0.73
8	$9.87^{cdefgh} \pm 0.04$	$8.77^{mn} \pm 0.34$	$3.17^{cde} \pm 0.26$	11.94 ± 2.37	2.77	1.13	0.83
9	$9.79^{cdefgh} \pm 0.06$	$1.90^{a} \pm 0.22$	$2.90^{bcd} \pm 0.23$	4.81 ± 1.98	0.66	5.15	2.04
10	$9.10^{cd} \pm 0.01$	$6.09^{ghij} \pm 0.17$	$8.30^{rs} \pm 0.08$	14.39 ± 1.81	0.73	1.49	0.63
11	$10.34^{efghij} \pm 0.01$	$5.07^{def} \pm 0.10$	$5.27^{hij} \pm 0.46$	10.34 ± 2.10	0.96	2.04	1.00
12	$6.76^{a} \pm 0.16$	$7.89^{lm} \pm 0.24$	$3.12^{cde} \pm 0.30$	11.01 ± 2.46	2.53	0.86	0.61
13	$11.65^{l} \pm 0.88$	$5.33^{defgh} \pm 0.38$	$6.88^{mnop} \pm 0.52$	12.21 ± 1.03	0.77	2.19	0.95
14	$9.29^{cdef} \pm 0.01$	$6.04^{ghij} \pm 0.09$	$9.66^{t} \pm 0.14$	15.71 ± 1.47	0.63	1.54	0.59
15	$9.36^{cdef} \pm 0.02$	$5.88^{fghij} \pm 0.35$	$7.17^{nopq} \pm 0.17$	13.05 ± 3.05	0.82	1.59	0.72
16	$10.91^{hijkl} \pm 0.01$	$4.04^{hij} \pm 0.12$	$6.10^{efg} \pm 0.30$	10.14 ± 1.44	0.66	2.7	1.08
17	$11.13^{ijkl} \pm 0.52$	$4.80^{de} \pm 0.34$	$6.93^{mnop} \pm 0.36$	11.72 ± 0.80	0.69	2.32	0.95
18	$10.37^{efghijk} \pm 0.09$	$5.71^{efghi} \pm 0.21$	$7.85^{pqr} \pm 0.26$	13.56 ± 1.01	0.73	1.82	0.76
19	$10.21^{defghij} \pm 0.23$	$6.19^{hij} \pm 0.07$	$6.00^{jklm} \pm 0.25$	12.19 ± 2.05	1.03	1.65	0.84
20	$8.87^{bc} \pm 0.02$	$1.75^{a} \pm 0.11$	$1.97^{ab} \pm 0.05$	3.72 ± 2.38	0.89	5.08	2.39
21	$7.82^{ab} \pm 0.02$	$6.79^{jk} \pm 0.90$	$3.68^{def} \pm 0.57$	10.47 ± 1.14	1.85	1.15	0.75

22	9.17^{cde}	±	0.12	3.53^{bc}	±	0.33	3.86^{def}	±	0.42	7.39	±	1.54	0.92	2.6	1.24
23	10.05^{cdefghi}	±	0.09	2.95^{b}	±	0.32	1.76^{a}	±	0.33	4.71	±	1.63	1.68	3.4	2.13
24	10.54^{ghijkl}	±	0.03	7.62^{kl}	±	0.13	4.29^{fg}	±	0.25	11.90	±	2.20	1.78	1.38	0.88
25	11.72^{l}	±	0.34	10.68^{p}	±	0.68	6.79^{lmno}	±	0.49	17.47	±	0.89	1.57	1.1	0.67
26	10.66^{ghijkl}	±	0.19	6.46^{ij}	±	0.08	8.00^{qr}	±	0.26	14.46	±	0.44	0.81	1.65	0.74
27	9.25^{cdef}	±	0.37	7.42^{kl}	±	0.19	7.59^{opqr}	±	0.17	15.01	±	1.04	0.98	1.25	0.62
28	9.67^{cdefg}	±	0.2	6.65^{jk}	±	0.03	6.53^{klmn}	±	0.34	13.19	±	1.48	1.02	1.45	0.73
29	8.91^{bc}	±	0.01	5.11^{defg}	±	0.51	7.16^{nopq}	±	0.77	12.27	±	0.83	0.71	1.74	0.73
30	10.06^{cdefghi}	±	0.03	6.62^{ijk}	±	0.84	8.96st	±	0.77	15.57	±	1.36	0.74	1.52	0.65
31	11.35^{jkl}	±	0.02	6.39^{ij}	±	0.25	5.82^{ijkl}	±	0.22	12.21	±	1.06	1.1	1.78	0.93
32	11.47^{kl}	±	0.03	8.77^{mn}	±	0.58	5.80^{ijk}	±	0.18	14.57	±	2.70	1.51	1.31	0.79

[a] Sum of diterpenes = sum of kahweol and cafestol contents.

Mean of triplicate (± standard deviation) with different superscript letters in the same column indicate significant differences ($p < 0.05$).

The sum of the diterpenes (kahweol + cafestol) contents varied by 370%. In this study, kahweol/cafestol (KA/CA) values ranged from 0.63 to 2.77, and more than 50% of the samples had caffeine/ kahweol (CAF/KA) ratio values below 1.00 (Table 1).

In addition, CAF/KA ranged from 0.84 to 5.15; two samples had CAF/KA values below 1.00 and two samples had CAF/KA above 3.00 (Table 1). Since higher variability was observed on each of the diterpenes levels than for the sum of them, we proposed the use of a new parameter, the ratio caffeine/sum of diterpenes, to contribute to the characterization of *C. arabica* (Table 1).

DISCUSSION

Although it was observed high variation on caffeine concentrations, the results were within the range previously described in the literature (5.1 to 16.2 g/kg) for roasted *C. arabica* of different cultivars, origins and roast degree [14,19,20]. Since all samples are *C. arabica*, the wide range in the caffeine levels (Table 1), is probably due to the variability within *C. arabica* cultivars. In addition, the coffees were produced in different regions and under different edapho-climatic conditions and post-harvest processing, and the expression of the gene for caffeine synthesis depends on the interaction between genotype and the environment [21]. Even though the influence of caffeine on the sensory properties of the brew is well-known [22], it is important to highlight that even with a significant range of caffeine contents, all coffees studied had good cup quality. As a comparison, a medium caffeine content around 20 g/kg was described for samples of roasted *C. canephora* [15].

Regarding the two diterpenes studied and the total diterpene content, expressed as the sum of the kahweol and cafestol levels, they all presented wider ranges (Table 1) than those reported in previous studies [13,14,16,18,23]. As stated before, such great variability in the diterpenes levels could also be related to genetic differences and differences in growing conditions as reported in the study of Kitzberger and coauthors [16] that described significant variations on the contents of kahweol - 79% (1.75-10.68 g/kg) and cafestol 143% (1.76-9.66 g/kg) - on seven cultivars of *C. arabica*, even for coffees harvested in the same edapho-climatic conditions and with a standardized post-harvest process. As a comparison, kahweol content from 0.0 to 0.14 g/kg, and cafestol content from 1.5 to 3.6 g/kg was described for *C. canephora* [24].

To discriminate coffee species in commercial roasted products, it was suggested the use of the ratios between caffeine and diterpenes [15]. The authors pointed out that a kahweol/cafestol ratio (KA/CA) above 1.00 and a caffeine/kahweol ratio (CAF/KA) between 1.00 and 3.00 indicated a *C. arabica* product, on the other hand, the caffeine/kahweol ratio (CAF/KA) above 4.00 was indicative of the presence of *C. canephora*. However, considering the high variation on the compounds content determined in this study, it is necessary to extend the range suggested in literature [15]. For example, the samples 9 and 20 (Table 1), which presented the lowest values of kahweol, have KA/CA values bellow 1.00 and CAF/KA values higher than 4; despite being pure *C. arabica* samples, KA/CA and CAF/KA values extrapolated the ranges previously proposed for *C. arabica* and it could be incorrectly considered as an evidence of the presence of *C. canephora*.

Others authors [16,18] also reported a wide range for KA/CA (0.6 to 3.5) for eleven cultivars of *C. arabica* grown and processed in standardized conditions, highlighting that the genetic diversity within the species could affect this parameter. CAF/KA values (Table 1) also exceeded both the lower and upper limits of the range proposed as being indicative of *C. arabica* (1.00 to 3.00) [15].

Our results demonstrate the interest in these parameters and emphasize the importance of further studies and the evaluation of a large number of samples to obtain ranges that apply to commercial coffees to avoid mistakenly identifying the presence of *C. canephora*. The use of an additional parameter, the caffeine/sum of diterpenes (CAF/SUM) ratio, was proposed. Considering the lower variability in CAF/SUM (from 0.54 to 2.39) compared to CAF/KA (Table 1), this parameter could support the characterization of *C. arabica*.

In summary, besides the new ranges for the parameters KA/CA and CAF/KA established as being indicative of *C. arabica* (KA/CA above 0.50 and CAF/KA lower than 5.50), the use of a new parameter CAF/SUM is proposed (CAF/SUM below 2.50 also indicates *C. arabica*).

CONCLUSION

Significant variations, typical of *C. arabica*, were observed in the kahweol, cafestol and caffeine concentration, highlighting the importance of evaluating a considerable number of samples to provide typical profiles of these compounds in *C. arabica*. The hypothesis that relationships between caffeine and diterpenes content can support the characterization of *C. arabica* species was confirmed. However, considering the wide variabilities in KA/CA and CAF/KA, the additional use of a new parameter (CAF/SUM) was suggested. As a

new assumption, we proposed as potentially indicative of *C. arabica*: values of KA/CA ratio above 0.50, associated with caffeine/kahweol ratio lower than 5.50 and CAF/SUM ratio lower than 2.50.

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Conflicts of Interest: The authors declare no conflict of interest.

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