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Article

Discrimination between Arabica and Robusta Coffees Using Hydrosoluble Compounds: Is the Efficiency of the Parameters Dependent on the Roast Degree?

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Abstract: *Coffea arabica* (arabica) and *Coffea canephora* (robusta) are the most important coffee species. Arabica has higher commercial value and, in general, more favorable sensory characteristics. After roasting, analytical methods are required to differentiate species. Blends with different proportions of arabica/robusta coffees, roasted at three degrees were studied. Color parameters and the levels of chlorogenic (5-CQA) and nicotinic acids, caffeine, and trigonelline were evaluated. Hydrosoluble compounds were analyzed by their efficiency to discriminate coffee species, considering different roast degrees. Caffeine was a good discriminator, regardless of roast degree. The roast degree influenced the efficiency of discrimination of the other hydrosoluble compounds. A model using color parameters and the variables Ratio (5-CQA/caffeine contents ratio) and Sum (sum of nicotinic acid and trigonelline contents) was proposed to the estimation of roasting degree. Considering the use of heat-labile compounds, the discrimination among coffee species should be carried out in two steps: first, the characterization of roasting degree, and subsequently the appropriate parameters are defined for each roasting degree. Thus, the combined use of color parameters and hydrosoluble compounds could be useful to help the differentiation of coffee species in blends of roasted samples.

Keywords: *Coffea arabica*; *Coffea canephor*a; roasted coffee; roasting process; coffee species; differentiation; quality control; hydrosoluble fraction; liquid chromatography; principal components analysis

1. Introduction

Coffee is one of the most important food commodities in the worldwide economy. The genus *Coffea* presents more than 100 species, but commercial trade consists almost entirely of *Coffea arabica* (arabica) and *Coffea canephora* (robusta) [1]. Most commercial roasted and ground coffees are actually blends of the two species. Green beans of both species are distinguished by color, shape, and size. However, after roasting and grounding, analytical methods are required in order to differentiate coffee species, since robusta coffee has lower commercial value and sensory quality. Different types of compositional data evaluated by using several analytical techniques have been applied to characterize samples or achieve discrimination among coffee species [2–14]. However, most studies have focused mainly in green beans or coffees roasted at only one degree [2–4,6,7,9,11].

Several analytes have been proposed as indicators for differentiation between coffee species, such as amino acids [3], metals [7], sucrose [15], and compounds from the lipid fraction, as cafestol, kahweol and especially 16-*O*-methylcafestol [2,16,17].

Some studies were focused on the use of hydrosoluble compounds, mainly trigonelline, caffeine, chlorogenic acid (which the main isomer is 5-caffeoylquinic acid, or 5-CQA [18]), and nicotinic acid [5,17,19]. However, the efficiency of the hydrosoluble compounds for discrimination of coffee species are not consensual. For green beans, discrimination was obtained using levels of caffeine and chlorogenic acids, whereas trigonelline was not considered an efficient parameter [5,6]. The analysis of a series of compounds from hydrosoluble fraction of coffee, including CQAs, enabled the botanical characterization of green coffee beans [20]. Kuhnert *et al.* [21] described the use of isomers of the chlorogenic acids group (3-CQA, 4-CQA and 5-CQA among other analytes) to discriminate arabica and robusta coffees. The authors also proposed the use of a series of chlorogenic acids as unique biomarker for robusta coffee. For roasted coffee, caffeine and trigonelline contents were cited as efficient for discrimination, but not nicotinic acid [5]. On the other hand, for espresso coffee, caffeine, trigonelline and chlorogenic acids levels contributed to classify the arabica and robusta coffee brews [22]. Another difficulty is that differences in the composition are also observed among cultivars of each coffee species. Kitzberger *et al.* [17] reported some variability in the contents of 5-CQA, caffeine, trigonelline, and nicotinic acid for roasted arabica coffee of different cultivars.

The roasting process is carried out under temperatures usually above 200 °C, and the process is typically controlled by the time, weight loss, and color parameters. Roasting defines sensory characteristics and the quality of coffee products, affecting their chemical composition [14,23–25]. At 190 °C, pyrolytic reactions are initiated, which causes oxidation, reduction, hydrolysis, polymerization, decarboxylation, and others processes. As the stability of some hydrosoluble compounds are dependent on the heating conditions [26–29], one probable reason for the discrepancies on the literature regarding the efficiency of these compounds for discrimination of coffee

species is that different roast degrees were used in the studies, with the roast degree not being well characterized or not mentioned.

The detection of the addition of robusta coffee to arabica is interesting for consumers, industries, and regulatory agencies. Brazilian authorities have considered the possibility of mandating the information about composition of blends and roast degree on the packages of roasted and ground commercial coffee [30,31]. Thus, the present research aimed to study the efficiency of caffeine, 5-CQA, trigonelline, and nicotinic acid for discrimination of species in coffee roasted at different degrees. The contribution of each compound was studied on each roast degree in order to verify if these parameters could be widely used for commercial coffee blends (since the roasting degree is not usually informed in the labels). Finally, an additional goal of the research was to describe the efficiency of the combined use of hydrosoluble compounds and color parameters for the characterization of coffee roasting degree.

2. Experimental Section

2.1. Coffee Samples

The mature beans of *Coffea arabica* cv. IAPAR 59 (arabica) and *Coffea canephora* cv. Apoatã (robusta) were supplied by Instituto Agronômico do Paraná (IAPAR). Green beans were harvested at the IAPAR experimental station (Londrina-Paraná-Brazil; latitude 23°08′47″S and altitude 560 m; average annual temperature of 22 °C; average rainfall of 52 mm) [32]. Cherry fruits were manually selected, washed and sun-dried on a patio. The roasting process was carried out in coffee roasting equipment (Rod-Bel, São Paulo, Brazil) at 230 °C using weight loss to control the process: 13% for light, 17% for medium, and 20% for dark roasting. The roasted beans were ground (0.5 mm particle size). Blends of both coffee species were prepared using robusta/arabica coffees ratios of 0:100, 20:80, 30:70, 50:50 and 100:0, at the three roasting degrees. Samples were sealed in plastic bags, and conditioned in a cold chamber (10 °C) until analysis.

2.2. Standards and Solvents

The analytical grade standards of 5-CQA, nicotinic acid, caffeine, and trigonelline were purchased from Sigma (Steinlein, Germany), and the HPLC grade acetonitrile and acetic acid, from J. T. Baker (USA). Water was obtained from a Milli-Q system (Millipore, USA), and mobile phases and extracts of samples were filtered in 0.45 mm membranes (Millipore, USA).

2.3. Color Evaluation

A Minolta CR-10 colorimeter (Konica Minolta Sensing Americas, Inc., NJ, USA) equipped with light source D65 and observation angle of 10° was used. Values of L^* (lightness), a^* (component red-green) and b^* (component yellow-blue) were obtained and the hue angle ($h° = arctan(b^*/a^*)$) was calculated. These analyses were performed in triplicate.

2.4. Determination of Nicotinic Acid, Caffeine, Trigonelline, and 5-CQA

The analyses were carried out according to Alves *et al.* [33]. Samples (0.500 g) were extracted with 30 mL of an acetonitrile:water solution (5:95; v/v) at 80 ℃ for 10 min. Extracts were filtered and properly diluted in the mobile phase before injection. Analysis was carried out in a Shimadzu HPLC (Kyoto, Japan) equipped with two pumps LC-10AD, on line degasser, UV/visible detector SPD-10A, Rheodyne injection valve (20 μL loop) and CBM-101 interface. Detection was performed at 320 nm for 5-CQA and 272 nm for the other compounds of interest. A Spherisorb ODS-1 column (250 mm × 4.6 mm; 5 μm) (Waters, Milford, USA) was employed. A gradient elution of acetic acid/H_2O (5:95 v/v) (A), and acetonitrile (B) were used: 95% of A until 5 min, a linear increase to 87% of A between 5 and 10 min, and 87% of A until the end, 35 min, at a flow rate of 0.7 mL min^{-1}. The identification was based on retention time, UV spectrum and spiking. The method presented good recovery (89% to 104%) and repeatability, and detection limits of 0.01, 0.15, 0.04, and 0.04 mg mL^{-1} were observed for nicotinic acid, trigonelline, 5-CQA and caffeine, respectively [33]. The quantification was carried out by external standardization (six concentrations, in triplicate) and the standard curves demonstrated high correlations ($R^2 \geq 0.99$, for $p \leq 0.001$).

For estimation of the contents in dry base, moisture was determined (107 ℃ for 7 min) using infrared equipment (OHAUS-MB 200, USA).

2.5. Statistical Analysis

Data was evaluated by Principal Components Analysis and Cluster Analysis using Statistica 7.0 (Statistica for Windows-Computer program manual. Version 7.0, Statsoft Inc.: Tulsa, OK, USA, 2005). The hierarchical tree was obtained by unweighted pair-group average as the linkage rule and considering the Euclidian distances as the coefficient of similarity.

Analysis of variance and the Tukey test ($p \leq 0.05$) were applied for group means comparison using a randomized split-plot design. The species represented the main plot and the roasting degree represented the subplot. Data were evaluated using the statistical package SAS (SAS Institute Inc., version 9.1.3, Cary, NC, USA). If a significant *main* × *subplot* interaction ($p \leq 0.05$) was observed, the effect of roast was independently studied for each species. If the interaction was not significant, the comparisons were made with the global means of species on each roast degree and with the global means of roasting on each species.

Models correlating roast degree and species were adjusted by the procedure *multiple linear regression* in Statistica 7.0. The best fit, using forward stepwise and backward stepwise methods, was chosen based on residual analysis and correlation significance.

3. Results and Discussion

3.1. Influence of Roast Degree on the Composition and on Color of Arabica and Robusta Coffees

The roasting process leads to a darker, reddish color. In general, *L** decreased by half, and hue by a quarter, from light to dark roasting considering both coffee species and blends (Table 1). Dias *et al.* [13]

reported a similar behavior: *L** from 55 (2 min of roasting) to 13 (10 min), and hue from 85 (2 min) to 35 (10 min) for arabica and robusta coffees roasted at 230 ℃.

Table 1. Lightness (*L**) * and hue (*h* °) * of arabica (A) and robusta (R) coffees and blends roasted at different degrees.

Values	Species\Roast degree	Light **	Medium **	Dark **
*L**	A 100%	28.0 ^{A,d} ± 0.4	15.9 ^{B,c} ± 0.4	13.0 ^{C,b} ± 0.4
	R 20%	31.0 ^{A,c} ± 0.6	17.9 ^{B,b} ± 0.4	13.4 ^{C,b} ± 0.3
	R 30%	29.3 ^{A,bc} ± 0.5	17.9 ^{B,b} ± 0.4	13.5 ^{C,b} ± 0.2
	R 50%	31.3 ^{A,b} ± 0.7	18.4 ^{B,b} ± 0.3	13.8 ^{C,b} ± 0.4
	R 100%	37.6 ^{A,a} ± 0.3	24.7 ^{B,a} ± 0.4	17.4 ^{C,a} ± 0.4
h °	A 100%	57.4 ^{A,c} ± 0.1	45.1 ^{B,d} ± 0.4	41.6 ^{C,d} ± 1.0
	R 20%	58.0 ^{A,c} ± 0.3	47.8 ^{B,c} ± 0.2	41.2 ^{C,d} ± 0.3
	R 30%	58.6 ^{A,bc} ± 1.2	48.3 ^{B,c} ± 0.3	43.0 ^{C,c} ± 0.6
	R 50%	59.8 ^{A,b} ± 0.2	50.7 ^{B,b} ± 0.3	44.9 ^{C,b} ± 0.2
	R 100%	62.7 ^{A,a} ± 0.2	56.0 ^{B,a} ± 0.2	48.6 ^{C,a} ± 0.2

Notes: Example of sample: R 30% refers to a blend of 30% robusta and 70% arabica. * Means of three repetitions ± standard deviation. ** Different letters: lowercase in the columns and uppercase in the lines indicate significant differences among the means ($p \leq 0.05$).

There is no consensus in literature regarding the relation among the parameters to control the roasting process (time, temperature, and weight loss), the roast degree and color parameters, which makes data comparison difficult. Craig *et al.* [12] proposed a range of *L** for commercial coffees: 23.5 < *L** < 25 (light roasting), 21 < *L** < 23.5 (medium) and 19 < *L** < 21 (dark). Kitzberger *et al.* [17] reported values of 28.7 (*L**) and 40.6 (*h* °) for various arabica coffees at a medium roast degree. For dark roasting, a range from 19 to 37 *L** and from 35 to 55 *h* ° in arabica coffee were reported [12,34–36] and, for robusta, values from 14 to 41 *L** and from 50 to 57 *h* ° were found [36,37].

Values of composition regarding caffeine, trigonelline, 5-CQA and nicotinic acid for roasted coffees (Table 2) are in agreement with literature data [4,19,27,38].

Variability of concentration related to the origin of samples [39], cultivars of the same species [17] and instability to the roasting process [6,25,27,36] must be considered.

In general, there was an inverse correlation between the degree of roasting and the levels of 5-CQA, trigonelline, and nicotinic acid (Table 2).

Levels of 5-CQA ranged from 1.79 g 100 g^{-1} of arabica (light roasting) to 0.09 g 100 g^{-1} of robusta (dark roasting) (Table 2). Studies of 5-CQA degradation during heating are usually focused on arabica coffees [27,36,38] but contents between 0.05 and 3.04 g 100 g^{-1} of arabica and robusta coffees roasted at different degrees were reported [19,26].

For trigonelline, amounts between 0.93 g 100 g^{-1} (light roasting, arabica) and 0.12 g 100 g^{-1} (dark roasting, robusta) were found (Table 2). Values of 0.08 to 0.99 g of trigonelline 100 g^{-1} of samples were described, which is a similar range found here [19].

Table 2. Contents of compounds (g 100 g^{-1}) * for arabica (A) and robusta (R) coffees and blends roasted at different degrees.

Compounds	Species\Roast degree	Light **	Medium **	Dark **
5-CQA	A 100%	1.786 ^{A,c} ± 0.056	0.475 ^{B,a} ± 0.021	0.263 ^{C,a} ± 0.010
	R 20%	1.854 ^{A,bc} ± 0.040	0.499 ^{B,a} ± 0.011	0.249 ^{C,a} ± 0.002
	R 30%	1.871 ^{A,bc} ± 0.003	0.511 ^{B,a} ± 0.016	0.211 ^{C,a} ± 0.002
	R 50%	1.953 ^{A,ab} ± 0.078	0.574 ^{B,a} ± 0.013	0.172 ^{C,b} ± 0.001
	R 100%	2.015 ^{A,a} ± 0.081	0.518 ^{B,a} ± 0.029	0.094 ^{C,b} ± 0.000
Trigonelline	A 100%	0.928 ^{A,a} ± 0.005	0.489 ^{B,a} ± 0.007	0.297 ^{C,a} ± 0.010
	R 20%	0.894 ^{A,b} ± 0.007	0.462 ^{B,a} ± 0.009	0.262 ^{C,b} ± 0.001
	R 30%	0.863 ^{A,b} ± 0.004	0.458 ^{B,a} ± 0.022	0.239 ^{C,bc} ± 0.005
	R 50%	0.865 ^{A,c} ± 0.032	0.456 ^{B,a} ± 0.005	0.206 ^{C,c} ± 0.002
	R 100%	0.683 ^{A,d} ± 0.011	0.380 ^{B,b} ± 0.015	0.119 ^{C,d} ± 0.003
Nicotinic acid	A 100%	0.091 ^{A,a} ± 0.001	0.012 ^{B,a} ± 0.000	0.010 ^{B,a} ± 0.001
	R 20%	0.072 ^{A,b} ± 0.001	0.011 ^{B,a} ± 0.002	0.010 ^{B,a} ± 0.000
	R 30%	0.072 ^{A,b} ± 0.004	0.011 ^{B,a} ± 0.000	0.008 ^{B,a} ± 0.001
	R 50%	0.063 ^{A,c} ± 0.001	0.012 ^{B,a} ± 0.000	0.007 ^{C,a} ± 0.000
	R 100%	0.032 ^{A,d} ± 0.000	0.009 ^{B,a} ± 0.000	0.000 ^{C,b} ± 0.000

Compound	Species\Roast degree	Light	Medium	Dark	Mean values **
Caffeine	A 100%	1.33 ± 0.02	1.35 ± 0.05	1.36 ± 0.06	1.35 ^{d} ± 0.02
	R 20%	1.53 ± 0.05	1.51 ± 0.00	1.52 ± 0.06	1.52 ^{c} ± 0.02
	R 30%	1.62 ± 0.06	1.55 ± 0.07	1.57 ± 0.01	1.58 ^{c} ± 0.04
	R 50%	1.82 ± 0.11	1.79 ± 0.04	1.78 ± 0.06	1.79 ^{b} ± 0.02
	R 100%	2.25 ± 0.13	2.10 ± 0.14	2.20 ± 0.05	2.18 ^{a} ± 0.08
	Mean value **	1.71 ^{A} ± 0.35	1.66 ^{A} ± 0.30	1.69 ^{A} ± 0.32	

Notes: Example of sample: R 30% refers to a blend of 30% robusta and 70% arabica. * Means of two repetitions ± standard deviation. ** Different letters: lowercase in the columns and uppercase in the lines indicate significant differences among the means ($p \leq 0.05$).

Nicotinic acid is produced by trigonelline degradation [19,40], but nicotinic acid is also heat sensitive. Contents from 0.09 mg of nicotinic acid 100 g^{-1} of sample (light roasting, arabica) to the absence (dark roasting, robusta) and high degradation of the compound during roast process (up to 70% from light to medium roasting) was observed (Table 2). Levels of up to 0.017 g of nicotinic acid 100 g^{-1} were cited for arabica roasted coffee [27]; Daglia *et al.* [19] reported a maximum nicotinic acid content for light roast degree coffees, with 11% weight loss.

Caffeine showed stability to the roasting process, and robusta samples presented higher values: contents of 1.35 mg 100 g^{-1} (arabica) and 2.35 mg 100 g^{-1} (robusta) were observed (Table 2). The heat stability of caffeine and its efficiency in the classification of coffee species, as caffeine is present in greater amounts in robusta, are emphasized in the literature [5,6,17,23,36]. Contents from 0.88 to 1.61 g 100 g^{-1} for arabica coffee, and from 1.57 to 2.68 g 100 g^{-1} for robusta were described for different roast degrees [4,19,27]. A value of 1.7 was reported [27] for caffeine level ratio of robusta (from Ivory Coast) and arabica (from Brazil) in different degrees of roasting. An approximate 1.6 ratio was found in the present research.

3.2. Discrimination of the Coffee Species

For an exploratory view of data, principal component analysis (PCA) was applied. The first two components accounted for 98% of the explained variance. Nicotinic acid, trigonelline, 5-CQA and the color parameters hue and lightness were the variables with major relevance for PC 1. The samples located in the right side of the plot presented higher values of these parameters. Caffeine had positive correlation and greater relevance in PC 2. Thus, the caffeine-rich samples were located on the top of plot (Figure 1).

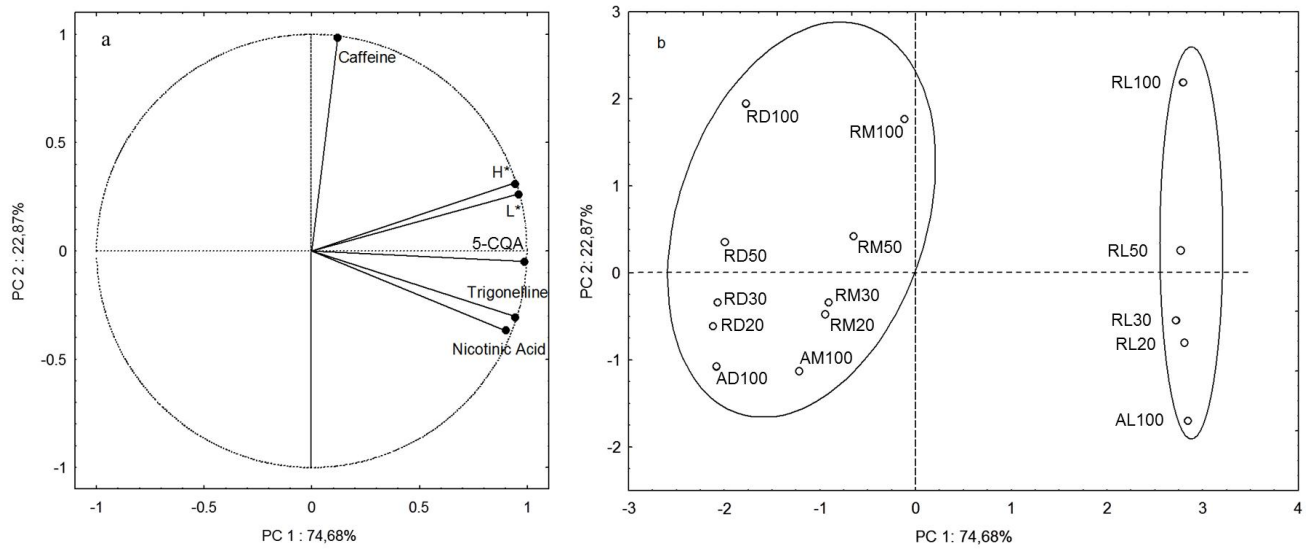

Figure 1. Principal component analysis: variable projection (**a**) and sample plot (**b**). Cluster Analysis defined the groups. Coffee species: arabica (A), robusta (R). Roast degree: light (L), medium (M), dark (D). Example of sample: RD30 refers to a blend of 30% robusta and 70% arabica at the dark roast degree.

PC 1 discriminated samples according to the roast degree, while PC 2 discriminated species of coffee. Two main groups were observed by Cluster Anaysis: (1) light roasted samples; (2) medium and dark roasted samples. PC 2 was able to separate robusta coffees, especially due to their caffeine content, regardless of the roast degree (Figure 1).

To define the relevance for the discrimination of coffee species, each parameter was individually analyzed. It was observed that, in each roast degree, robusta coffees were lighter in color and more yellowish than arabica samples. An interaction among the roast degrees was observed for L^* and $h°$ (Table 1). An increase in roasting reduced L^* and $h°$, and the addition of robusta to arabica coffees led to the opposite behavior. Thus, a blend with higher proportion of robusta and a more intense roast degree could present similar L^* and $h°$ to a blend with lower percentage of robusta at a lighter degree of roasting (Table 1). In conclusion, in a blend of arabica and robusta coffees, color parameters were not efficient to characterize neither species nor roast degree.

For caffeine, no interaction between the main and the second variables was found (Table 2), which indicated that caffeine content only depends on the species, and could be considered a relevant tool for species discrimination, regardless of roast degree.

However, for the heat-labile compounds (trigonelline, nicotinic acid and 5-CQA), an interaction between degree of roasting and coffee species was observed (Table 2).

The amounts of trigonelline and nicotinic acid decreased with increasing of robusta proportion and roast degree. Both compounds were more heat sensitive in the robusta coffee matrix. For trigonelline, a decrease about three times for arabica and above five times for robusta were observed when light and dark roast degree were compared (Table 2). Casal *et al.* [40] also reported that the degradation rate of trigonelline during roasting depended on the coffee species. Comparing arabica and robusta matrices, less difference was observed among trigonelline and nicotic acid levels in medium roasting; thus, both compounds were efficient for discrimination of species in the light and dark roast degrees (Table 2).

Chrologenic acid (5-CQA) was also more degraded in robusta coffee matrix (Table 2), but regarding coffee species discrimination a more complex behavior were observed. A higher level of 5-CQA was observed in the lighter roast degree for robusta coffee (Table 2), which presented the highest content of this compound in green beans [26]. Chlorogenic acid contents were similar for the two coffee species in the medium roast, but in the dark roast, a minor content was found in blends with a greater proportion of robusta (Table 2). Similar behavior was reported [26,36]. Clearly, 5-CQA was more efficient for species discrimination in the dark roasting (Table 2).

Summarizing, the efficiency of trigonelline and nicotinic acid in coffee species discrimination depends on the degree of roasting, but they always presented higher levels in arabica-richer samples. However, higher 5-CQA content could be associated with either a greater proportion of arabica as a greater proportion of robusta, depending on the degree of roasting. It was also verified that it is hard to discriminate coffee species in a medium roast degree (Table 2). In this way, for the use of heat-labile compounds for the discrimination of coffee species, it is necessary to characterize the roast degree of samples beforehand.

3.3. The Use of Combined Parameters

Since the use of only color parameters could not be conclusive to characterize the roast degree in a blend of arabica and robusta coffees, other criteria were considered: 5-CQA/caffeine levels ratio, and the sum of nicotinic acid and trigonelline contents (Sum) [19].

For the Sum parameter, an interaction between species and degree of roast were observed (Table 3) but differences among species were verified into each degree of roasting. Sum values were higher when the roast degree was lighter and the proportion of arabica was higher, varying between 0.12 (robusta 100%, dark roasting) and 1.02 g 100 g^{-1} (arabica 100%, light roasting) (Table 3). Daglia *et al.* [19] reported similar values for arabica and robusta coffees from different geographical origins.

The discrimination achieved for medium roast was better using the Sum parameter (Table 3) than considering trigonelline and nicotinic acid individually (Table 2). Apparently, the Sum parameter could also partially disregard the effect of thermal conversion of trigonelline in nicotinic acid, without losing the ability of origin factors to discriminate species. During the roasting, in addition to the trigonelline/nicotinic acid conversion, thermal degradation of nicotinic acid should be considered. Since both compounds presented stronger degradation rate in the robusta matrix, Sum emphasizes the difference among species, which improves the capacity of discrimination.

Table 3. Sum of nicotinic acid and trigonelline contents (Sum) and 5-CQA/caffeine ratio (Ratio) for arabica (A) and robusta (R) coffees and blends roasted at different degrees *.

	Species\Roast Degree	Light **	Medium **	Dark **
Sum	A 100%	1.02 ^{A,a} ± 0.01	0.50 ^{B,a} ± 0.01	0.31 ^{C,a} ± 0.01
	R 20%	0.97 ^{A,b} ± 0.01	0.47 ^{B,ab} ± 0.01	0.27 ^{C,ab} ± 0.00
	R 30%	0.93 ^{A,b} ± 0.01	0.47 ^{B,b} ± 0.02	0.25 ^{C,bc} ± 0.01
	R 50%	0.87 ^{A,c} ± 0.03	0.47 ^{B,b} ± 0.01	0.21 ^{C,c} ± 0.00
	R 100%	0.72 ^{A,d} ± 0.01	0.39 ^{B,c} ± 0.02	0.12 ^{C,d} ± 0.00
Ratio	A 100%	1.34 ^{A,a} ± 0.02	0.35 ^{B,a} ± 0.00	0.19 ^{C,a} ± 0.00
	R 20%	1.21 ^{A,b} ± 0.01	0.33 ^{B,a} ± 0.01	0.16 ^{C,ab} ± 0.01
	R 30%	1.16 ^{A,c} ± 0.04	0.33 ^{B,a} ± 0.00	0.13 ^{C,bc} ± 0.00
	R 50%	1.08 ^{A,d} ± 0.02	0.32 ^{B,a} ± 0.01	0.10 ^{C,c} ± 0.00
	R 100%	0.90 ^{A,e} ± 0.01	0.25 ^{B,a} ± 0.00	0.04 ^{C,d} ± 0.00

Notes: Example of sample: R 30% refers to a blend of 30% robusta and 70% arabica. * Means (g 100 g^{-1}) of two repetitions ± standard deviation. ** Different letters: lowercase in the columns and uppercase in the lines indicate significant differences among the means ($p \leq 0.05$).

The Ratio parameter also showed interaction between variables (Table 3). In general, the more intense the roast degree, the lower were the Ratio values. A decrease of Ratio was more outstanding in robusta coffee, varying from 0.90 (light roasting) to 0.04 (dark roasting; the lowest overall value) (Table 3). Similar results for samples from distinct geographical origin were reported [19].

Ratio parameter was not efficient for discrimination of the medium roasted samples, but better results at light and dark roast degrees were obtained with Ratio, comparing to the source parameter 5-CQA (Tables 2 and 3). The use of Ratio associated the discrimination of species obtained by caffeine with the differentiation of roast degrees, provided by 5-CQA (Tables 2 and 3). In addition to using a single parameter, Ratio "corrected" the nonlinear behavior of 5-CQA when the roast degrees were compared. In general, Ratio values tended to decrease with increasing of robusta proportion and degree of roasting (Table 3).

Species and degree of roasting were predicted by multiple linear regression with high values of coefficient of determination. A combined use of color data (lightness and hue), Sum, and Ratio was used to propose a model to estimate the roast degree of coffee samples, expressed as weight loss, %WL (Equation (1)). Sum presented the greatest influence on %WL, which made evident the importance in the evaluation of trigonelline and nicotinic acid for roasting characterization of coffee samples (Equation (1) and Table 3):

$$\%WL\ (\pm SD) = 26.81\ (\pm 1.72) - 12.12\ (\pm 1.65)\ Sum + 4.26\ (\pm 1.17)\ Ratio - 0.13\ (\pm 0.05)\ L^* - 0.06\ (\pm 0.06)\ h^\circ \quad (1)$$

Adjusted $R^2 = 0.995$; $N = 15$; $p < 0.001$.

Our results suggested that the discrimination between *C. arabica* and *C. canephora* considering the heat-labile hydrosoluble compounds should be done in two steps. At first, samples are separated by roast degrees (using L^*, h°, Sum and Ratio variables), and subsequently the samples of each roast degree are evaluated for the coffee species discrimination, fitting the equations according to the specific behavior of the variables in each roasting.

This research aimed to contribute by regarding the use of the hydrosoluble compounds that could be used to the discrimination of coffee species. However, to assure the efficiency of the proposed parameters (mainly for Sum and Ratio), additional evaluations with different samples are necessary. Samples with differences in genetics (cultivars), geographical origin, edaphoclimatic conditions, agricultural treatments, and industrial process could be studied.

4. Conclusions

The parameters of color and levels of caffeine, trigonelline, 5-CQA, and nicotinic acid could be used for the discrimination of coffee species, but with different contributions, depending on the degree of roasting. Caffeine was the main responsible for the discrimination of *Coffea arabica* and *Coffea canephora* in blends, regardless of roasting degree. The degree of roast influenced the efficiency of discrimination of the other hydrosoluble compounds. A model using color parameters and the variables Ratio (5-CQA/caffeine contents ratio) and Sum (sum of nicotinic acid and trigonelline contents) was proposed for the estimation of roasting degree. Considering the use of heat-labile compounds, the discrimination among coffee species should be carried out in two steps: first, the characterization of roasting degree, and subsequently the appropriate parameters are defined for each roasting degree.

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Author Contributions

R.D. and M.B. planned and designed the experiments. R.D. performed the experiments in the laboratory. Both analyzed the data and wrote the manuscript. R.D. edited and submitted the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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