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Fingerprint and authenticity roasted coffees by ^{1}H-NMR: the Brazilian coffee case

Aline Theodoro Toci[1] · Marcos Vinícius de Moura Ribeiro[2] · Paulo Roberto Aparecido Bueno de Toledo[2] · Nivaldo Boralle[2] · Helena Redigolo Pezza[2] · Leonardo Pezza[2]

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Abstract With globalization, it has become necessary to adopt policies to regulate the coffee market, addressing problems including the authenticity and traceability of products. It is therefore important to establish methodologies that can help to safeguard the interests of producer countries and add value to products. For this purpose, the use of NMR combined with multivariate statistical procedures can be an attractive option. The aim of this study was to develop a fast and effective technique, using ^{1}H NMR coupled with multivariate statistics, to create a fingerprint of roasted coffees, distinguishing them according to the main Brazilian producer regions. Several compounds suitable for differentiating roasted coffees were identified in the fingerprint. Discriminant analysis revealed good distinction among the samples. The compounds catechol, trigonelline, caffeine, and *n*-methylpyridine were most important for the differentiation. The findings should assist coffee-producing countries in adopting measures to protect their markets and to add value to coffee products.

Keywords Coffee · Authenticity · Traceability · Fingerprint · Discriminant analysis · NMR

✉ Aline Theodoro Toci
aline.toci@unila.edu.br

[1] Federal University of Latin American Integration (UNILA), Av. Tancredo Neves, 6731 – Bloco 6, Foz do Iguaçú, PR 85867-970, Brazil

[2] Chemistry Institute, UNESP-Universidade Estadual Paulista "Júliode Mesquita Filho", Araraquara, SP 14800-060, Brazil

Introduction

Coffee is globally the most widely traded food product, and is especially important to the economies of producing and exporting countries. Brazil is the largest coffee producer and exporter and is the second largest consumer of coffee, second only to the United States [1].

The opening up of markets due to globalization has increased the availability of a wide variety of foods, allowing consumers to experience the flavors and aromas of foods from different regions. Although this offers many commercial opportunities, individual nations endeavor to preserve their market positions by adopting protective economic policies. Some countries have used labeling terminologies such as "typical product", "PGI" (Protected Geographical Indication), and "DOC" (Controlled Denomination of Origin), amongst others, in order to give the products additional value. An important point is that these coffees have particular organoleptic characteristics that are not reproducible in coffees produced in other places. It is only in the last decade that Brazil has begun to implement coffee quality control programs by means of private partnerships. In 2004, the Brazilian Association of Coffee Industries (ABIC), together with the Deliberative Council for Coffee Policy (CDPC), created the "Coffee Quality Program" (PQC) certification, in addition to its existing "Purity Seal". However, such certification is still far from including the entire national market; this private initiative is not supported by many roasting industries, notably those with market leading brands that do not need certification to maintain market positions. A further consideration is that in addition to geographical designations, it is increasingly important to identify fraudulently labeled products that reach national and international markets, for both economic and public health reasons [2]. A difficulty is

that there is a lack of uniformity among coffee-producing countries in terms of the type of classification. In Brazil, the drink classifications are: strictly soft, soft, softish, hard (or hardish), rioy, and rio, in decreasing order of quality. Low quality beverages result from the use of defective seeds with imperfections that mainly occur during harvesting and processing [1, 3, 4]. Gourmet beverages are prepared with seeds classified as strictly soft and soft, obtained from a single region. Meanwhile, the quality of the coffee (or the coffee seeds from which the beverage originates) is greatly influenced by the soil and climate, and therefore also by the geographic origin. In Brazil, the regions that produce the best coffees are in the south of Minas Gerais State and the north of São Paulo State. Given the considerable size of this region, as well as their proximity, the use of the term "geographic origin" seems very broad. Nonetheless, studies have reported the distinction of coffees in terms of the contents of aromatic compounds and minerals, on both the macroscale (country) and the microscale (regions within the same State) [5–8]. It is clear that geographic factors should be taken into account in broadly applicable methodologies developed to evaluate the authenticity of coffees. Nuclear magnetic resonance spectrometry (NMR) coupled with multivariate statistics provides a useful methodology for the determination of adulterants in a variety of food matrices [2, 9, 10]. Consonni et al. [11] differentiated coffees from different continents (Asia, America, and Africa) using ^{1}H NMR spectra. However, as indicated above, the creation of a fingerprint for Brazilian coffees requires a large sample set and correlation of the main factors related to quality, such as origin, seed type, and degree of roasting. To date, to the best of our knowledge, no study has presented a comprehensive and robust methodology considering the various factors that can affect coffee quality [12].

Use of the ^{1}H NMR technique resulted in a large amount of data obtained for the aqueous coffee extract [2]. It was therefore necessary to use multivariate analysis to interpret the results, considering not only the individual variables, but also possible correlations between them. Several multivariate data analysis methods can be used for this purpose. The most widely used is PCA, which aims to reduce the size of the data set while maintaining as much as possible of its variance. Examples of studies of coffee samples that have employed the DA technique include the works of Maeztu and Sanz [13] and Murota [14].

Given this background, the aim of the present work is to create a fingerprint for Brazilian roasted coffees, according to regional provenances, using a ^{1}H NMR technique coupled with multivariate statistics. The findings are expected to assist the country in adopting market protection measures, and to add value to the products in question.

Materials and methods

Samples

Nineteen different arabica coffees (*Coffea arabica*) certified by regional cooperatives in the states of Minas Gerais, Bahia, São Paulo, and Paraná were used. Of these, five samples consisted of defective beans, classified as "Riado/Rio", "Riozona", "Waste", "Varreção" and "Rio soft". The other fourteen samples were good cup quality beans, classified by ABIC as gourmet or soft beverages (Table 1).

Roasting of samples

A portion (100 g) of each sample was roasted for 5 min in a spouted bed roaster (IRoast Gurnee IL USA) using a temperature program of 3 min at 196 °C and 2 min at 235 °C resulting in a light-medium color degree according to the Roast Color Classification System [15]. All samples were ground to pass through a 300 μm sieve. The roasting was performed in duplicate.

Sample extraction

Portions (150 mg) of roasted and ground coffee were vortex-extracted with 1000 μL of deuterated water for 1 min at room temperature. The solutions were then centrifuged at 14,000 rpm for 10 min and final volumes of 750 μL (in 5 mm tubes) were used for the NMR analyses. The extractions were performed in triplicate.

Nuclear magnetic resonance (NMR) analyses

^{1}HNMR spectra were acquired using a 600 MHz Bruker Avance III spectrometer equipped with a PABBO BB/19F-1H/D probe head with gradients, automated tuning, BVT-1000 and BCU-1 accessories for temperature control, and a Sample Express autochanger. Samples were measured at 298 K in manual mode. Automated tuning, matching (ATMA), locking (LOCK), shimming (TOPSHIM), and calibration of 90° pulses (PULSECAL) were performed using standard Bruker routines. Two 1D experiments were performed in sequence:

Experiment 1 The first experiment consisted of a simple single pulse procedure (zg30) with 2 s relaxation delay (RD), 3.6 s acquisition time (AQ), recording of 64 free induction decays (FID) to form a 64k complex data point vector, spectral window of 20.0276 ppm, and detector gain (RG) of 18. After Fourier transformation (without apodization), automatic peak selection was applied to determine the residual water signal at 4.70 ppm. The values at 4.70 ppm were used as the band-selective

Table 1 List of samples (*Coffea arabica*) analyzed

No.	Sample code	State of origin (city-zone)	Beverage quality
1	SP1	São Paulo (North)	Strictly soft
2	SP2	São Paulo (North)	Strictly soft
3	SP3	São Paulo (North)	Strictly soft
4	SP7	São Paulo (North)	Strictly soft
5	SP4	São Paulo (North)	Varreção
6	SP5	São Paulo (North)	Riado/Rio
7	SP6	São Paulo (North)	Waste
8	MG1	Minas Gerais (South)	Gourmet
9	MG2	Minas Gerais (Monte Santo-South)	Gourmet
10	MG3	Minas Gerais (South)	Gourmet
11	MG4	Minas Gerais (Cerrado-South)	Gourmet
12	MG5	Minas Gerais (South)	Riozona
13	MG6	Minas Gerais (South)	Rio soft
14	PR1	Paraná (Tomazina-North)	Soft
15	PR2	Paraná (Ribeirão Claro-North)	Soft
16	PR3	Paraná (Ribeirão Claro-North)	Soft
17	BA1	Bahia (Barra da Choça-South)	Soft
18	BA2	Bahia (Poções-South)	Soft
19	BA3	Bahia (Vitória da Conquista-South)	Soft

saturation field in the next experiment. The phase and baseline were adjusted using TOPSPIN 3.2.5 software (Bruker Biospin).

Experiment 2 The second 1D experiment employed the ZGCPPR suppression pulse sequence, acquisition time (AQ) of 2.76 s, relaxation delay (RD) of 2 s, 128 free induction decays (FID), and water pre-saturation with low power radiofrequency irradiation at 48.05 dB, using the residual water signal at 4.70 ppm. An exponential function with LB = 0.3 was applied before Fourier transformation. The phase and baseline were adjusted with the TOPSPIN 3.2.5 software (Bruker Biospin).

The spectra were aligned for bucket integration of the (trimethylsily)-propionic-2,2,3,3-d4 acid (TSP) signal at 0 ppm, and were reduced by integration in 30 regions (buckets) from 0.15 to 10 ppm (Fig. 1). NMR signals for caffeine and trigonelline, together with caffeic, ferulic, coumaric, quinic, and 3-, 4-, and 5-caffeoylquinic acids (CQA), were confirmed using commercial standards (Sigma-Aldrich). Other signals were assignedaccording to data reported in the literature [11]. Small variation of δ, typically 0.02–0.05 ppm, was observed in the signals for *N*-methylpyridine and chlorogenic acids.

Statistical analysis

A data matrix was constructed by integration of the ^{1}H NMR data signals (region 0.15–10 ppm). Discriminant analysis (DA) was adopted for the statistical modeling. Likewise principal component analysis (PCA) this method is able to reduce data redundancy through the generation of discriminant functions that sort the distribution of each data point within the chosen grouping (classes). In order to overcome the drawback stablished in DA approach, such as biased choices and class dependency, the model was cross-validated by means of rotating coffee samples between the training and validation subsets, in aid of checking the reliability and dependence of the discriminant models to the training datasets. The statistical modeling was performed using the software SPSSv.23.

Results and discussion

Fingerprint of Brazilian roasted coffees

The singular characteristic coffee products from different regions provenances are addressed with different analytical techniques as well as by NMR. Variability of coffees within macroregions (different countries) have frequently been reported in the literature [8, 11, 16–18]. However, the differentiation of coffees from the same country (microregion) is not straightforward. To our knowledge, the present work is the first to describe the microregional differentiation of coffees using ^{1}H NMR spectra.

In the present work, five low quality samples were therefore selected in order to be able to differentiate coffees according to region, irrespective of the presence of

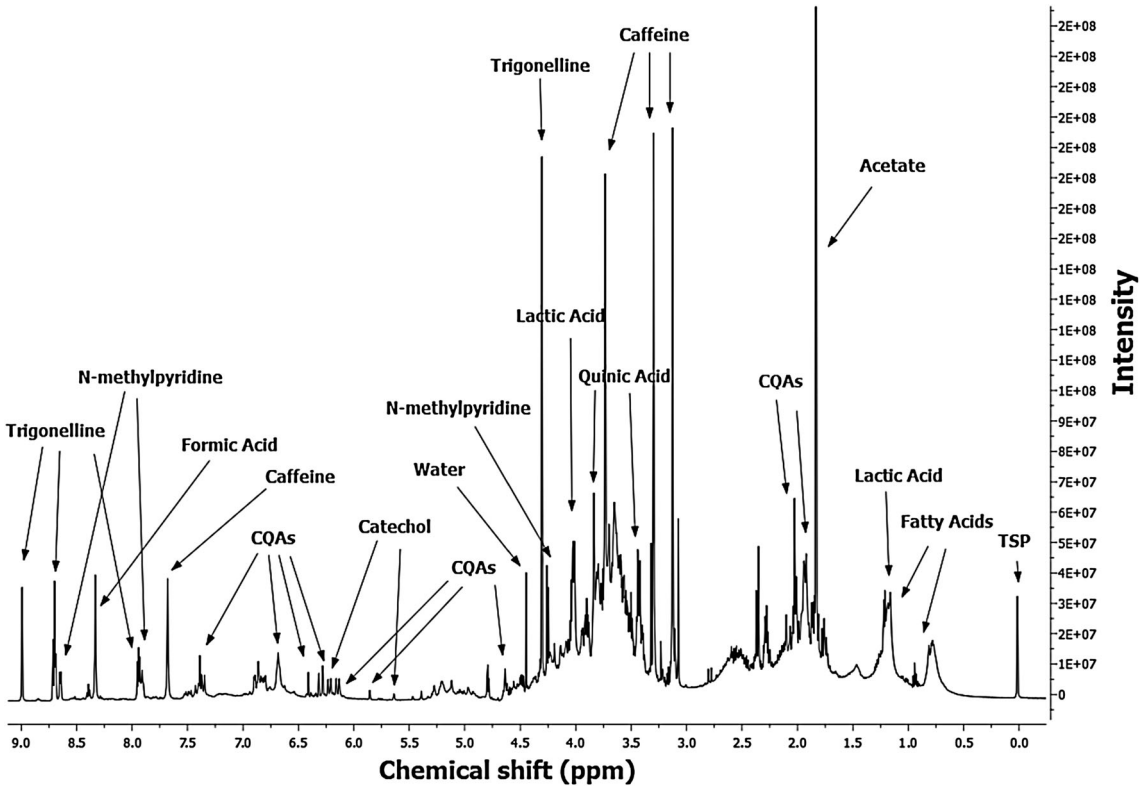

Fig. 1 ^{1}H NMR spectrum fingerprint of Brazilian roasted coffee

defective beans, which are chemically quite distinct [19–21].

Figure 1 shows the ^{1}H NMR spectrum of the solution obtained following deuterium oxide extraction, proposed here as the coffee fingerprint. The spectrum shows a complex resonance pattern due to the numerous compounds present in coffee. Raw coffee has the following composition (on a dry matter basis): 40–50% polysaccharides, with 6–9% sucrose; 15% lipids, of which 75% are related to triglycerides; 9–10% proteins and amino acids; 6–12% chlorogenic acids (caffeoyl–feruloyl-quinic acids—CQAs) and derivatives; 0.6–1.5% alkaloids, mostly caffeine and trigonelline [22, 23]. Roasting is an extremely complex process in which the coffee is exposed to temperatures up to 340 °C. During this procedure, a series of physical and chemical changes occur, involving Maillard and Strecker reactions, hydrolysis, pyrolysis, and other degradations that generate compounds responsible for the color (such as melanoidins) and the aroma. However, these precursor compounds are not degraded completely, remaining part of the roasted coffee composition [24, 25]. The roasting degree applied to the samples (light-medium color) was the degree most widely used for gourmet coffees and emphasizes the aromatic characteristics. The chemical structures of several compounds identified in the coffees are shown in Fig. 2.

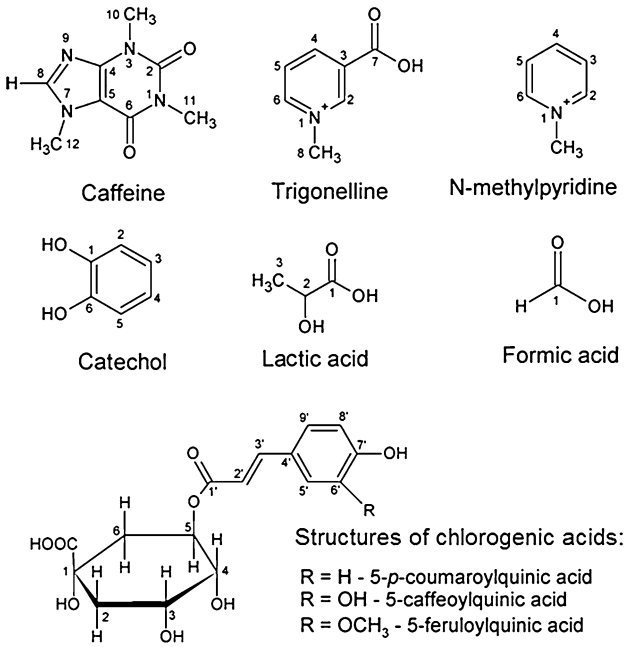

Fig. 2 Structures of some of the compounds identified in the coffees

In the ^{1}H NMR spectra, the water signals were suppressed in order to improve the sensitivity of the other signals, and integrated values were used in the discriminant analysis. Identification of the principal metabolites was by

comparison with reference molecules and chemical shifts reported in the literature [11, 26, 27]. In general, despite the complexity of the samples in terms of the compounds present, the region from 0 to about 3.8 ppm (Fig. 1) was dominated by the resonances of caffeine (*N*-methyl singlets, $\delta = 3.13$, 3.30, and 3.73), acetate ($\delta = 1.80$ and 2.16), lactic acid ($\delta = 1.21$ and 4.03), fatty acids ($\delta = 0.87$ and 1.27), and CQAs ($\delta = 1.80$, 2.16, 3.31, and 3.84). The region between 4.2 and 9.1 ppm (Fig. 1) showed lower complexity, despite the large number of signals, with dominance of trigonelline ($\delta = 4.31$, 7.95, 8.55, 8.68, and 8.99), *n*-methylpyridine ($\delta = 7.92$, 8.40, and 8.65), catechol ($\delta = 5.65$ and 6.22), CQAs ($\delta = 5.12$, 5.88, 6.21, 6.28, 6.41 and 7.40), and (with lower abundance) caffeine ($\delta = 7.68$). The anomeric region from 3.2 to 5.5 ppm included the resonance signals of oligo- and polysaccharides. These signals were highly overlapped, probably due to the large quantities of polysaccharides present. However, it was still possible to discern signals due to caffeine, quinic acid, and lactic acid. The compounds identified in this study are discussed individually below.

Caffeine

The caffeine molecule was clearly identified from its intense signals, notably three *n*-methyl signals at 3.13, 3.30, and 3.73 ppm, as well as the signal at 7.68 ppm due to the aromatic proton (Fig. 1). Caffeine is thermally quite stable [24] and is therefore an excellent quantitative marker for coffees, as shown by Tavares and Ferreira [26] and Wei et al. [27].

Trigonelline and n-methylpyridine

Trigonelline was identified by a signal at 4.31 ppm, together with resonances at 7.95, 8.55, 8.68, and 8.99 ppm (Fig. 1). Trigonelline is strongly degraded during the roasting process and is a precursor of *n*-methylpyridine and niacin (Fig. 2) [24, 28]. The greater the degree of roast, the smaller will be the trigonelline signal and the greater will be the signals corresponding to its products [24]. Due to similarity between molecules, the methyl hydrogen signal (at $\delta = 4.31$) suffered from overlap (Fig. 2). Other *n*-methylpyridine resonances were identified, with signals at 7.92, 8.40, and 8.65 ppm (Fig. 1). Shifts in the aromatic region were due to the presence of the carboxylic grouping in the trigonelline molecule, with reduced shielding of the hydrogens acting to increase the shift. Small differences in the shifts of hydrogens H3, H4, and H1 can be used to determine the degree of roast of the coffee, in any type of sample, since there is a direct relationship with the molecules present. This is a useful finding, because methods employing color-based and weight loss procedures to determine the degree of roast have been shown to be ineffective [16, 29]. For the degree of roast used in the present study, it was observed that the intensity of the trigonelline signal was greater than that of *n*-methylpyridine, corresponding to a medium roast, as expected for gourmet samples.

Considering the trigonelline/*n*-methylpyridine signal ratio ($\delta = 8.99$ and 7.92, respectively), the low quality samples from São Paulo (samples SP05 and SP06) showed higher ratios (0.86 and 1.00), compared to good quality samples (0.65–0.70). This demonstrated that there was less trigonelline degradation in the low quality samples, compared to the good quality samples, despite the fact that both types were roasted for the same time and under the same conditions. These findings were in agreement with a preliminary study showing that for a light-medium degree of roast, the lowest losses of trigonelline occurred for black defective beans (10–12%), while healthy beans showed losses of 26–61% [19]. However, in this study, the low quality samples were composed of a mixture of defective beans. Further work will be required before this parameter can be adopted.

Phenolic compounds

The majority of the phenolic compounds in coffee are known as chlorogenic acids (Fig. 2). This family of compounds is derived from the esterification of quinic acid with cinnamic acid derivatives such as caffeic, ferulic, and *p*-coumaric acids. The main subgroups are isomers of caffeoylquinic (QCA), feruloylquinic (FQA), and dicaffeoylquinic acids (diCQA), together with smaller amounts of *p*-coumaroylquinicacids (*p*-CoQA) [22].

The spectra showed signals in the range 5.18–8.03 ppm that corresponded to unsaturated carbons of caffeoyl derivatives (Fig. 2). Two spin systems were identified in the regions 6.283–7.474 and 6.206–7.420 ppm, and peaks at 1.80, 2.16, 3.31, 3.81, and 4.65 ppm were assigned to protons of the quinic ring.

Catechol was also identified, with a chemical shift at 5.64 ppm attributed to protons of the hydroxyl groups attached to the aromatic ring, and shifts at 5.65 and 6.22 ppm associated with protons bonded directly to the aromatic ring (Fig. 1).

Lipids

The individual lipids present in coffee are degraded to different extents during the roasting process, resulting in greater complexity of chemical composition at higher degrees of roast, due to the degradation of other constituents [24, 30]. Despite the fact that the extraction was performed with deuterium oxide, signals of coffee lipids

Table 2 Non-standardized coefficients of the three canonical discriminant functions for classification of geographical origins

Compounds (V_i)	Function coefficients (β_i)		
	Y_1	Y_2	Y_3
Trigonelline$_1$	-2.18×10^{-08}	5.64×10^{-09}	-1.06×10^{-08}
Trigonelline$_2$	6.12×10^{-09}	-2.81×10^{-08}	-1.27×10^{-08}
N-methylpyridine$_3$	-5.27×10^{-08}	4.62×10^{-09}	7.92×10^{-09}
Formic acid$_4$	-3.63×10^{-08}	2.99×10^{-08}	2.23×10^{-08}
Trigonelline$_5$	2.34×10^{-08}	1.47×10^{-08}	2.62×10^{-08}
Caffeine$_6$	3.82×10^{-08}	-1.19×10^{-08}	-8.58×10^{-10}
N-methylpyridine$_7$	3.50×10^{-10}	-1.44×10^{-08}	-1.38×10^{-08}
CQAs$_8$	-5.70×10^{-09}	8.55×10^{-09}	2.73×10^{-09}
CQAs$_{10}$	-4.97×10^{-08}	-2.78×10^{-08}	3.58×10^{-08}
CQAs$_{11}$	1.37×10^{-07}	8.08×10^{-08}	4.73×10^{-08}
Catechol$_{12}$	-5.36×10^{-10}	-2.32×10^{-07}	-1.90×10^{-07}
Catechol$_{15}$	-3.92×10^{-09}	1.30×10^{-08}	2.26×10^{-08}
Catechol$_{16}$	-2.24×10^{-09}	3.65×10^{-08}	-5.81×10^{-09}
Caffeine$_{25}$	2.98×10^{-09}	-2.55×10^{-09}	1.39×10^{-09}
(Constant)	−12.024	11.995	5.180

were present at 0.87 and 1.27 ppm (Fig. 1). Consonni et al. [11] associated these signals with methyl and methylene protons of fatty acid chains, respectively.

Other compounds

The spectrum also showed the signals of other organic compounds including acetate (at 1.89 and 2.16 ppm) and lactic acid (at 1.21 and 4.03 ppm), as found previously by Tavares and Ferreira [26], Wei et al. [27] and Consonni et al. [11].

Discrimination of Brazilian coffees according to region

The signals of the identified metabolites were divided into 30 spectral regions and each area was used in the discriminant analysis. This approach was adopted because earlier studies have shown that NMR can be used for quantitative analyses [26, 31]. The spectral quality was enhanced by the accumulation of several spectra, as described in Sect. Nuclear magnetic resonance (NMR) analyses. This optimization method enabled all the spectra to be used, which is important in routine analyses.

Using the Discriminant Analyses (DA) technique, linear equations combining the statistically relevant compounds (factors) were generated from the experimental data, as described in Eq. (1).

$$Y = \beta_0 + \beta_1 V_1 + \beta_2 V_2 + \beta_3 V_3 + \cdots + \beta_n V_n \quad (1)$$

where Y is the discriminant function, $\beta_0, \beta_1, \beta_2, \beta_3$, and β_n are the linear discriminant coefficients, and V_1, V_2, V_3, and V_n are the abundance of the compounds that correspond to the β coefficients.

The most important discriminating variables ($p < 0.05$) with their respective p values were: trigonelline$_1$ (0.018), trigonelline$_2$ (0.016), formic acid$_4$ (0.042), trigonelline$_5$ (0.036), caffeine$_6$ (0.039), *n*-methylpyridine$_7$ (0.023), CQAs$_8$ (0.030), catechol$_{15}$ (0.022), catechol$_{16}$ (0.019), and caffeine$_{25}$ (0.014) (Table 2). The application of DA treatment resulted in three discriminant functions (Y). According to the Wilk's lambda test, Y_1 explained 53.8% and Y_2 explained 40.6% of the total variance, totaling 94.4% of the overall variance. Table 2 results show the linear coefficients obtained.

The discrimination of the coffee samples according to their geographical origins is shown in Fig. 3, using only Y_1 and Y_2 (Table 2). For each geographical group, the centroids are plotted using filled symbols, while the data points for each group are plotted according to their position coordinates (unfilled symbols), and are connected with lines to the corresponding group centroid. The areas associated with the different geographic groups are shown using circles.

One of the aspects of the differentiation achieved using DA is the dispersion of data points within a given group. In this respect, attention should be paid to the Minas Gerais category, where the highest relative dispersion between samples was observed, followed by the samples from Paraná and São Paulo. Profiles given by São Paulo and Minas Gerais samples were highly similar, which were only discriminated by the Y_1 function. According to the DA analysis, coffee from these origins appeared to have

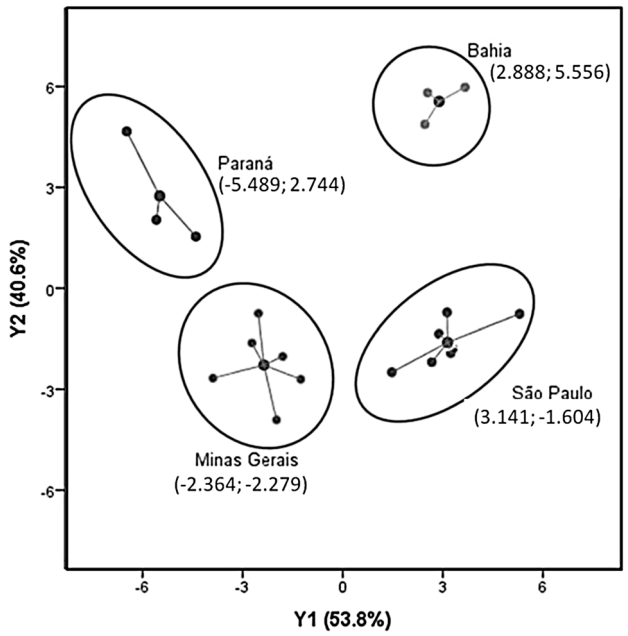

Fig. 3 Plot of the main discriminant functions, with centroid values (Y_1 and Y_2)

intermediate composition, in terms of the discriminating compounds, relative to the other groups.

Comparison of the sample groups indicated that two functions were required to discriminate between the coffees from São Paulo and Bahia, while low dispersion around the centroid was noted for the samples from Bahia, compared to the other groups.

The samples with the greatest squared distances to the group centroids (Mahalanobis distances) were obtained from São Paulo samples SP1 (6.29) and SP2 (3.58), Minas Gerais samples MG3 and MG5 (distances of 3.48 and 8.63, respectively), and Paraná sample PR2 (4.84). A greater distance to the centroid is an indicative of a strong difference in the sample profile probability and possible cross-classification. The MG5 sample was of lower quality (Table 1), providing an explanation for its greater dispersion, because lower quality coffees have different chemical composition. On the other hand, the fact that good and low quality samples from the same region were not differentiated by the model, demonstrated good performance of the method and confirmed the ability of the discriminant functions to classify the samples, following appropriate selection of the compounds used in this procedure.

Table 3 summarizes the performance of the discriminant models regarding the classification of samples from the training database. Accordingly, an entirely correct classification was attained without cross validation (using all coffee samples), which may be attributed to the suitability of the compounds of the database to be used as discriminant factors. Nonetheless, the application of a cross validation exposed some misclassifications involving São Paulo and Bahia samples. On those classes the success of the classifications was reduced to 33.3% (Bahia), and 57.1% (São Paulo) after cross validation. In the case of Minas Gerais and Paraná samples 66.7% of success was attained.

The application of DA in this work aimed at identifying the most relevant compounds, whose concentration variations between coffee samples from different geographic origins are evident enough to be further used to judge/confirm such origins, enabling thus a quality control procedure. In summary, the DA provided good accuracy, enabling chemical differentiation of coffee samples according to their origins. The most suitable compounds for this purpose were trigonelline, *n*-methylpyridine, catechol, and caffeine.

Table 3 Overall classification performance (count and %) of the DA model, with and without cross validation (CV), for four Brazilian states (major coffee producers) differentiation study

Original class	Predicted class (DA model)							
	São Paulo		Bahia		Minas Gerais		Paraná	
	Without CV	With CV	Without CV	With CV	Without CV	With CV	Without CV	With CV
São Paulo	7	4	0	0	0	2	0	1
	(100%)	(57.1%)	(0%)	(0%)	(0%)	(28.6%)	(0%)	(14.3%)
Bahia	0	1	3	1	0	1	0	0
	(0%)	(33.3%)	(100%)	(33.3%)	(0%)	(33.3%)	(0%)	(0%)
Minas Gerais	0	1	0	1	6 (100%)	4	0	0
	(0%)	(16.6%)	(0%)	(16.6%)		(66.7%)	(0%)	(0%)
Paraná	0	0	0	1	0	0	3	2
	(0%)	(0%)	(0%)	(33.3%)	(0%)	(0%)	(100%)	(66.7%)

The proposed ^{1}H NMR fingerprint of roasted coffee provided a simple and effective method for distinguishing products according to the regions of origin, despite the complex resonance patterns due to the numerous compounds present in coffee. The coffees were clearly differentiated, even given the proximity of producer regions such as São Paulo and the south of Minas Gerais and their similar climates and altitudes. The methodology developed offers potential to be used in quality control related to the degree of roast of coffees.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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