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Chemometric discrimination of genetically modified *Coffea arabica* cultivars using spectroscopic and chromatographic fingerprints

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ABSTRACT

Multivariate statistical design and principal component analysis (PCA) applied to RP-HPLC-DAD and FTIR spectroscopic data were performed to investigate the fingerprints of four coffee cultivars, traditional red bourbon and three genetically modified cultivars. The design and response surface results showed that extraction dependence on solvent composition of one of the genetically modified cultivars, IAPAR 59, was very similar to that found for the red bourbon standard. PCA of the FTIR spectra obtained from all the simplex centroid design mixtures indicated that the 1:1 binary ethanol–dichloromethane solution resulted in the best separation of the four cultivars. The IPR 108 cultivar has more intense vibrational bands in the 3200–3600 cm^{-1} and 1100–1600 cm^{-1} regions indicating higher acid and fat levels than those of the other cultivars. The UV absorptions close to 275 nm of the RP-HPLC-DAD spectra are correlated with the strengths of the infrared absorptions between 3400 and 3460 cm^{-1} and can be explained by varying caffeine concentrations in the four cultivars.

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1. Introduction

It is well known that coffee is one of the most popular beverages consumed worldwide. It is one of the firmest pillars of the economy in several Brazilian states, and in the first week of August 2012, the daily revenue with coffee exports reached US $ 21.5 million. The two most important varieties of commercial coffee are *Coffea arabica* and *Coffea canephora* syn. Coffea robusta. Commercial coffee beverage is made from arabica or robusta beans or blends of them, but *C. arabica* is of superior quality. For this reason several papers report methods to discriminate the two species [1–6]. Although there are thousands of papers in the literature about defective and non-defective coffees [7,8] its antioxidant properties [9], the effects of drinking coffee on health [10–12] and the effect of roasting coffee beans [9], among others, few reports on the genetic variability of the Coffea genus can be found. Furthermore no published work on the discrimination of different *C. Arabica* cultivars in terms of genetic variability was encountered. Genetic variability of coffee promotes gains in productivity and desirable agronomical characteristics while also affecting the chemical composition of the product and consequently its attributes and sensory qualities [13].

The Agronomic Institute of Paraná (IAPAR), located in Londrina, Brazil, was established in 1974, and contains a gene bank of *Coffea arabica* with over a thousand hits of this kind. The database also contains a collection of 144 accessions of *C. arabica* collected in Ethiopia, region of the species' origin. This collection has been used as the basis for genetic improvements at IAPAR and resulted in many cultivars with rust resistant genes as well as some with resistance to nematodes [14]. The chemical composition of grain and, consequently, the quality and acceptability of coffee depends on the genetic factors, cropping systems, altitudes, temperatures, water demands, types and levels of fertilization, harvesting times, preparation methods, storage and roasting processes [13].

High performance liquid chromatographic (HPLC) and Fourier transform infrared spectroscopic (FTIR) studies in combination with chemometric techniques have been successfully applied for food quality assessment as well as the detection of food adulteration [15]. Fourier transform infrared spectroscopy is a method that reveals information about the functional groups present in the sample and the relative changes in their amounts.

Recently, fingerprint techniques have become one of the most powerful approaches for the quality control of foods. It describes a variety of analytical methods that can provide the identification and approximate quantification of a group of metabolites associated to specific pathways. Metabolite variations are observed principally by total spectroscopic or chromatographic pattern changes without previous knowledge of the identities of the investigated compounds. Generally, samples with similar spectroscopic or chromatographic fingerprints have similar compositions.

In the last few years, our group has shown that statistical mixture designs permit the development of rigorous but economical

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procedures for demonstrating the effects of solvent changes on the extracted metabolites of plant material [16–21]. Considering the difficulties in chemically differentiating cultivars by conventional means, this research uses a statistical mixture design [22] for four components: (1) ethanol, (2) ethyl acetate, (3) dichloromethane and (4) hexane, to find adequate extraction mixture compositions and experimental conditions for discriminating the traditional cultivar (red Bourbon) from cultivars developed by the Agronomic Institute of Paraná, IAPAR 59, IPR 101 and IPR 108. All these cultivars were produced under the same climatic conditions.

The main objective here was to apply multivariate statistical designs and models associated with liquid chromatographic and spectroscopic data to compare the fingerprints of the four coffee cultivars obtained from different extraction mixtures. As a consequence, method development can be undertaken using experimental conditions capable of differentiating genetically modified cultivars from the traditional cultivar (bourbon) that is of superior quality.

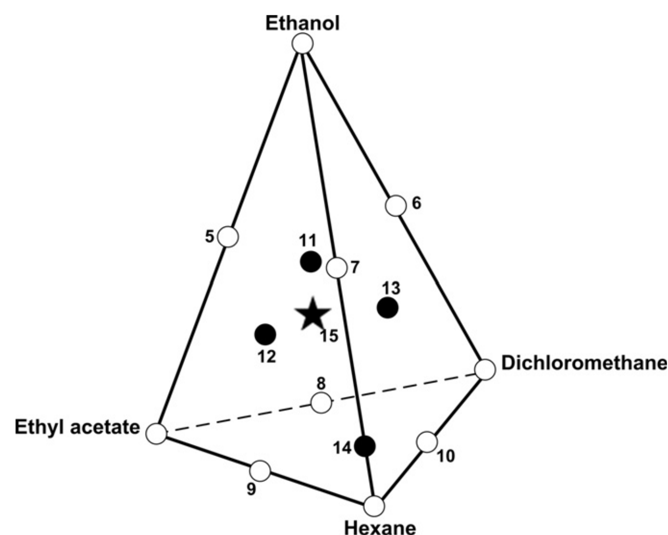

Fig. 1. The simplex centroid mixture design for the ethanol, ethyl acetate, dichloromethane and hexane solvents. Open circles represent pure solvents and binary mixtures, the darkened circles ternary mixtures, and the star a quartenary mixture. Numbers correspond to those in the extract column of Table 1.

2. Materials and methods

2.1. Coffee samples

The green grains of four different cultivars, the traditional red Bourbon cultivar (used as a standard) and cultivars developed by the Instituto Agronômico do Paraná were investigated. IAPAR 59 carries the *Coffea Arabica* genes, Villa Sarchi × Hibrido de Timor (Sarchimor), IPR 101 has Catuaí × Sarchimor (with S_H2, S_H3 rust resistant genes) and IPR 108, the Iapar59 × (Catuaí × Icatu) genes. All samples were kindly provided by the Agronomic Institute of Paraná.

2.2. Reagents

HPLC grade acetonitrile and methanol were purchased from VETEC Química Fina (Rio de Janeiro, Brazil). Mobile phase mixture preparations were made using water prepared with the Millipore Milli-Q purification system. Hexane, dichloromethane, ethyl acetate and ethanol were also purchased from VETEC and were of analytical grade.

2.3. Extract preparation

The grains of green coffees were previously immersed in liquid nitrogen to make them brittle, facilitating their crushing, and then were sieved. The extraction mixtures were chosen according to a Simplex-Centroid Design for four components, amounting to 15 mixtures. Fig. 1 shows the compositions of the experimental extraction mixtures. Each extract was prepared by weighing 10 g and adding 150 mL of one of the solvent mixtures listed in Table 1. These mixtures were placed in an ultrasonic bath (Unique, model Ultracleaner 1400) for 30 min with the bathwater being changed every 15 min to avoid heating. The extracts were filtered through filter paper to separate the solution from the coffee samples. This procedure was repeated two more times. Then an extraction solution of 15 mL was removed and stored in a capped vial under refrigeration for subsequent HPLC analysis. The remainder was evaporated in a rotary evaporator, removing all the solvents still present in the sample, until attaining constant weight. Then the yield of the crude extract was determined and used for chromatographic and spectral analysis.

Table 1
Extract yields (in grams) of the four coffee cultivars for the simplex centroid design mixtures.

Extract	Ethanol	Ethyl acetate	Dichloro methane	hexane	Bourbon	Iapar 59	IPR 101	IPR 108
1	1	0	0	0	1.390	1.505	1.565	1.265
2	0	1	0	0	0.820	0.870	0.920	0.835
3	0	0	1	0	1.050	1.065	1.170	1.070
4	0	0	0	1	0.820	0.850	0.945	0.830
5	0.5	0.5	0	0	1.610	1.490	1.740	1.865
6	0.5	0	0.5	0	2.010	2.170	1.710	1.510
7	0.5	0	0	0.5	1.695	1.830	1.805	1.475
8	0	0.5	0.5	0	0.870	0.915	0.960	0.830
9	0	0.5	0	0.5	0.890	0.975	1.010	0.840
10	0	0	0.5	0.5	1.030	0.940	1.125	1.240
11	0.333	0.333	0.333	0	1.275	1.465	1.650	1.310
12	0.333	0.333	0	0.333	1.255	1.270	1.280	1.185
13	0.333	0	0.333	0.333	1.305	1.290	1.365	1.240
14	0	0.333	0.333	0.333	0.970	0.940	1.090	0.930
15	0.25	0.25	0.25	0.25	1.130	1.145	1.155	1.190

2.4. Analysis by infrared spectroscopy

For Fourier transform infrared analysis (FTIR) 1.5 mg of the crude extract were weighed with 0.3 g of dry solid KBr that was then homogenized in an agate mortar with a few drops of chloroform. The spectra were recorded in the 4000–400 cm^{-1} region, with 4 cm^{-1} resolution and 90 scans, using a Shimadzu FTIR-8300 spectrophotometer. The data analysis was performed using the entire infrared spectra.

2.5. Sample preparation for HPLC analysis

A 20 µL aliquot was pipetted from each 15 mL extraction solution and added to 800 µL of mobile phase. The samples were filtered through 0.22 µm Millex Millipore paper and analyzed immediately. The chromatographic conditions were: Phenomenex C18 column, 2.6 mM Kinetex HILIC 100 A, with dimensions of 150 mm × 4.6 mm, 20 µL injection volume and 1.0 mL min^{-1} mobile phase flow rate. HPLC analysis was conducted on a Finnigan Surveyour 61607 liquid chromatograph equipped with a Finnigan Surveyour PDA Plus diode array detector. Elution was

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