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Validation of near-infrared spectroscopy for the quantification of cafestol and kahweol in green coffee

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ABSTRACT

Near-infrared spectroscopy (NIRS) is among the many tools available to study the biochemical diversity of coffee species. This technique is inexpensive, fast and accurate, and it requires only small amounts of samples. The aim of this study was to use NIRS to estimate the amount of diterpenes (cafestol and kahweol) in green coffee. To construct the prediction model, 126 Ethiopian accessions coffee collection and 44 modern cultivars were analyzed. The total sample set was split into two groups as follows: a group of 130 samples for calibration and a group of 40 samples for the validation step. Reference values of cafestol and kahweol were determined by high performance liquid chromatography (HPLC). Cafestol values ranged from 182.62 g to 1308.62 mg 100 g^{-1}, and kahweol values ranged from 182.69 to 1265.41 mg 100 g^{-1}. To improve the quality of the calibration step, a pretreatment with the second derivative was applied to smooth the raw spectra. The prediction models of cafestol and kahweol were developed using the modified partial least squares regression (mPLS). The performance of these models was evaluated by the ratio of performance deviation (RPD) and R^2 parameters, obtained by the ratio of the NIR prediction data and the corresponding reference data. The prediction models of cafestol (RPD = 2.74; R^2 = 0.89) and kahweol (RPD = 2.2; R^2 = 0.88) confirm the validity of NIRS analysis to determine diterpenes contents in green coffee.

1. Introduction

The interest in coffee as a beverage dates back many centuries and is associated with the stimulating effect of caffeine. However, new compounds in coffee have been identified with beneficial and non-beneficial effects (Cavin et al., 2002; van Cruchten et al., 2010).

Coffee lipids are associated with the aroma and flavor attributes of the coffee beverage. Cafestol, kahweol and 16-*O*-methylcafestol (16-OMC) have been identified in the unsaponifiable lipid fraction of coffee and belong to the class of diterpenes (Speer & Kölling-Speer, 2006). These compounds are found in different species of coffee. Cafestol and kahweol are present in the *Coffea arabica* and *Coffea canephora* species (Speer & Kölling-Speer, 2006), but 16-*O*-methylcafestol is characteristic of the *C. canephora* species (Speer, Tewis, & Montag, 1991).

These compounds have different effects on human health. Diterpenes have positive effects against cancer (Cavin et al., 2002; Roos et al., 1997), induce the degradation of toxic substances and protect against aflatoxin (Cavin et al., 2002), and they also have anti-inflammatory (Kim et al., 2006), antioxidant and hepatoprotective effects (Lee, Choi, & Jeong, 2007). However, negative effects, such as increased blood cholesterol levels, have also been reported to be associated with cafestol (Urgert, Essed, van der Weg, Kosmeijer-Schuil, & Martijn, 1997).

Several studies have demonstrated that such effects are associated with the beverage preparation method. Coffee beverages obtained by filtering contain less cafestol than those prepared without separating the ground coffee. The maximum content of diterpenes is found in French press and espresso coffees, and the minimum content of diterpenes is found in coffee made with filter paper (Roos et al., 1997; Silva, Borges, Santos, & Alves, 2012; Sridevi, Giridhar, & Ravishankar, 2011).

A large majority of commercial coffees are blends of arabica cultivars and canephora clones (Silva et al., 2012; Souza & Benassi, 2012). The control of cafestol content in the brew becomes a complex and difficult process. A plausible solution is to provide cultivars and clones with a diterpene profile that is suitable for human health.

The cafestol and kahweol contents reported in the literature for arabica coffees vary from 100 to 736 mg 100 g^{-1} and from 150 to 700 mg 100 g^{-1}, respectively (Kurzrock & Speer, 2001). In the *C. canephora* species, kahweol values vary from 0 to less than 10 mg 100 g^{-1} (Roos et al., 1997).

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The great variability observed for modern cultivars in the diterpene profile comprises different amounts of cafestol and kahweol, which suggests that the selection of genotypes with higher health benefits is possible. However, current coffee cultivars have a narrow genetic base (Steiger et al., 2002), and it is believed that the wild Ethiopian germplasm available at the Agronomic Institute of Paraná (IAPAR; Londrina, Paraná) may present a higher diversity. A first step to employ the Ethiopian germplasm is to analyze the diterpene profiles of its accessions.

In recent decades, near-infrared spectroscopy (NIRS) has been used as a rapid and reliable method for qualitative and quantitative determinations in various organic agriculture, non-organic agriculture, food industry and pharmaceutical areas (Davrieux et al., 2010; Elfadl, Reinbrecht, & Claupein, 2010; Luypaert, Massart, & Vander Heyden, 2007). This technique has been used to discriminate species and determine several compounds (caffeine, trigonelline, sucrose, lipids and fatty acids) in coffee cultivars. Roasted coffee quality and espresso sensory attributes have been evaluated by NIRS (Esteban-Díez, González-Sáiz, & Pizarro, 2004).

In the area of plant breeding, the NIRS technique has been widely used to measure the oil, protein, total fatty acid and free fatty acid contents in sunflower lines (Biskupek-Korell & Moschner, 2006), and the NIRS technique has also assessed the seed quality of various rapeseed species (Font, Rio-Celestino, & Haro-Bailon, 2006) and identified introgressions between different coffee species (Bertrand, Etienne, Lashermes, Guyot, & Davrieux, 2005).

This indirect method is based on the vibration properties of the chemical bonds within organic molecules and their interactions with infrared radiation (Pasquini, 2003). The diterpenes are pentacyclic alcohols based on the fusion of isoprene units to form the skeleton of 20 kauren carbons. Kahweol differs from cafestol by a double bond between carbons 1 and 2 (Fig. 1) leading to spectra with maximum peak absorptions at different wavelengths: cafestol absorbs at 220 nm, and kahweol absorbs at 290 nm (Kurzrock & Speer, 2001).

Use of the NIRS technique for measuring a chemical compound in a sample requires the development of an accurate and robust prediction model. For this purpose, this indirect method requires a previous analysis of a large number of samples uniformly covering the variability of the compound studied (Elfadl et al., 2010; Plans, Simó, Casañas, & Sabaté, 2012).

The use of fast analytical techniques, such as near-infrared spectroscopy (NIRS), is suggested for exploring the chemical diversity in the Ethiopian coffee collection.

The Agronomic Institute of Paraná (IAPAR) along with other institutions has developed in recent years studies of phenotyping and genotyping of coffee. This task requires the analysis of many samples in large populations of coffee. Considering these analyses are costly and time consuming, the NIRS technique is shown to be suitable to speed studies in search of coffee cultivars with diterpenes content suitable to human health. Thus, the present study aimed to assess the feasibility of NIRS to predict the cafestol and kahweol contents in Ethiopian coffee accessions, traditional and modern coffee cultivars.

2. Materials and methods

2.1. Raw material

The available coffee samples (126) were collected in 2011 from the Ethiopian *C. arabica* collection at the IAPAR Research Centre in Londrina, Paraná, Brazil. The Ethiopian coffee collection is formed by accessions from the *C. arabica* origin center (Ethiopia) that were planted in 1976.

In 2009, thirteen modern cultivars (Iapar 59, IPR 97, IPR 98, IPR 99, IPR 100, IPR 101, IPR 102, IPR 103, IPR 104, IPR 105, IPR 106, IPR107 and IPR 108) and the traditional Catuaí cultivar were collected at the IAPAR Experimental Station in Paraná, Brazil. These same modern and traditional cultivars were also collected in Mandaguari, Paraná, Brazil at the Technologic Park of Agriculture Cooperative (COCARI) in 2009 and 2010. The traditional Bourbon and Icatu cultivars were collected in 2010 at the same experimental field of the COCARI cooperative. Modern cultivars developed by IAPAR are crosses focused for different agronomic traits with greater genetic basis than the current commercial cultivars.

Cherry fruits were manually selected, washed and sun-dried. When moisture contents reached 12.0% to 12.5%, the bark and parchment were removed. The broken beans and defects were removed and stored in a refrigerated room. For chemical determinations and spectra collection, coffee beans were ground in a laboratory disk mill (Perten 3600, Kungens Kurva, Sweden) to a particle size of 0.5 mm. The ground samples were stored in a freezer (−18 °C) and were brought to room temperature before collecting the spectra and performing the chemical analyses.

2.2. Chemical analysis

2.2.1. Chemical reagents

For sample extraction and preparation of the mobile phase and standard the following reagents were used: HPLC grade methyl *tert*-butyl ether (Acrós Organics, New Jersey, USA), analytical grade potassium hydroxide KOH (Quimex, São Paulo, Brazil) and HPLC grade acetonitrile (J.T. Baker, Xalostoc, México), kahweol and cafestol standards (Axxora, San Diego, USA) with a purity of 98% and certified by Alexis Biochemicals).

2.2.2. Cafestol and kahweol determination

The cafestol and kahweol analyses were carried out by the HPLC method proposed by Dias et al. (2010). Samples (0.2 g) were subjected to direct saponification with 2.5 M KOH (2 mL), and the unsaponifiable matter was then extracted with methyl *tert*-butyl ether (t-BME). A clean-up step with water was performed after extraction. HPLC analysis was conducted using a reversed-phase Spherisorb ODS 1 column (250 mm × 4.6 mm; id 5 mm). For the isocratic elution, we injected 25 μL and used a mobile phase of acetonitrile/water (55/45; v/v) with a flow rate of 0.9 mL/min. Detection was performed at 220 and 290 nm for cafestol and kahweol, respectively. An oven temperature of 25 °C was applied for 20 min of the running time. The identification

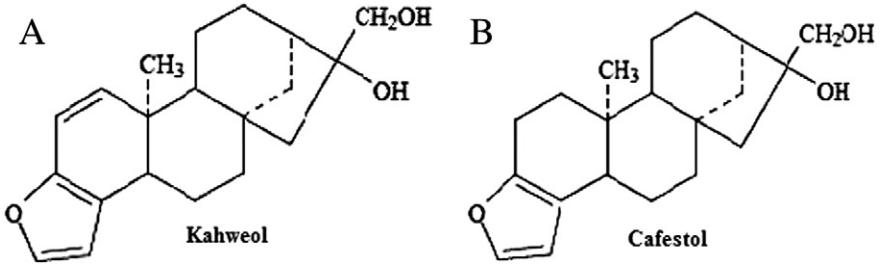

Fig. 1. Structural formulas of cafestol (A) and kahweol (B).

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