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ATR-FTIR FOR CHARACTERIZING AND DIFFERENTIATING DRIED AND GROUND COFFEE CHERRY PULP OF DIFFERENT VARIETIES (Coffea Arabica L.)

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KEYWORDS
Coffee cherry by-products, coffee chemical compounds, infrared spectrum analysis.

ABSTRACT
This study aimed to evaluate the performance of the infrared spectrum in the range of 4000–650 cm⁻¹ for characterizing and differentiating dried and ground coffee cherry pulp of different varieties. The spectral data were subjected to first and second derivative treatments to perform the statistical analyses. Three varieties of coffee pulp were previously characterized for color, water activity, moisture, chlorogenic acids, and caffeine. The results of principal component analysis (PCA) showed that Fourier transform infrared (FTIR) spectroscopy is a viable technique for characterizing and differentiating dried and ground coffee cherry pulp among different varieties, showing the best differentiation with treatment of data from the first derivative, which was mainly associated with the caffeine content and chlorogenic acids. This study is the first investigation of FTIR spectroscopy with attenuated total reflectance for characterizing dried and ground coffee cherry pulp from coffee varieties grown in Colombia.

INTRODUCTION
Coffee is one of the most widely consumed food products globally and one of the most known and traded commodities (Velasquez et al., 2018; Sezer et al., 2018). It is an important crop that provides livelihoods for millions of farmers in 50 countries around the world depend on coffee for their subsistence (Ghosh & Venkatachalapathy, 2015). The coffee cherry is composed of an external red skin attached to the soft yellowish, fibrous, and sweet pulp. This part is commonly referred as “pulp”. This is followed by a translucent, colorless, thin, viscous, and highly hydrated layer of mucilage (Esquivel & Jimenez, 2012). During the typical processing, the pulp is removed mechanically, whereas the mucilage remains attached to the coffee beans during the fermentation process; the mucilage is then removed by washing. In the first processing stages of coffee production, wastes are generated (Selvam et al., 2014) in the form of skin, pulp, and mucilage (Poltronieri & Rossi, 2016); depending on the process used, different amounts of these components are produced. Coffee pulp is the main residue obtained during wet and semi-dry processing; it is essentially composed of sugars, proteins, and minerals. It also contains appreciable amounts of tannins, polyphenols, and caffeine, which are considered toxic in nature (Bonilla-Hermosa et al., 2014). The large volume of coffee produced and processed by the industry results in the generation of a range of waste and by-products, resulting in the contamination of water bodies and lands around the production units; this represents a serious environmental problem for coffee-producing countries (Hikichi et al., 2017).

Colombia is the world’s leading exporter of soft coffee. In the year 2017/18, Colombia produced an average of 14 million 60 kg bags (OIC, 2019), for which 361,200 tons of fresh pulp were generated. Fresh coffee cherries contain over 430 g of coffee cherry pulp per kg and represent 30% of the dry matter of the coffee berry (Heeger et al., 2017). Therefore, alternatives to transform the by-products remaining after harvesting coffee are necessary. These can provide added value to the waste product while offering new renewable materials (Collazo-Bigliardi et al., 2018). Coffee pulp has been used as a food product, for example, in cascara beverages (Heeger et al., 2017), a flour type obtained from dried and ground coffee cherry pulp (Ramirez & Jaramillo, 2013; Gonzalez-Rios et al., 2017), as a mix for cookies (Linxia, 2014; Shuyuan, 2016) and in other applications such as production of bioethanol (Shenoy et al., 2011; Menezes et al., 2013), and cellulose (Selvam et al., 2014).

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It is important to characterize the dried and ground coffee cherry pulp as a raw material for the preparation of different food products. Techniques using Fourier transform infrared (FTIR) spectroscopy have been successfully applied for characterizing a range of agricultural products (Amir et al., 2011). FTIR spectroscopy can also be used non-destructively and quickly to obtain biochemical fingerprints that provide information about the molecular structure and composition of the entire sample (Cebi et al., 2017). However, it has not been implemented as an analysis strategy for dried and ground coffee cherry pulp. The main aims of this study were to characterize the caffeine and chlorogenic acid profile of dried coffee cherry pulp using high-performance liquid chromatography (HPLC) and to evaluate the application of the infrared spectrum with attenuated total reflectance (ATR–FTIR) as an effective tool for obtaining chemical information from the coffee cherry pulp as a postharvest byproduct and its differentiation among different coffee varieties by principal component analysis (PCA).

MATERIAL AND METHODS

Coffee pulp samples

Nine coffee cherry pulp samples (Coffea Arabica L.) of the Castillo, Colombia, and Caturra varieties, from the Huila region of Colombia, were processed at the South Colombian Coffee Research Center (CESURCAFE) of the Universidad Surcolombiana. The ripe cherries were harvested selectively by submerging in water to remove the light or vain fruits (Koskei et al., 2015). The fruits were subsequently depulped using the Gaviota Gv 300 (INGESEC) equipment; subsequently, the coffee pulp samples were dehydrated at 65°C for 24 h in an oven (UF55–Mennert). The dried samples were then ground in a Bunn electric mill (G3 HD BLK, Springfield, Illinois, USA) to obtain a fine particle size between 150 and 250 μm. A generic USB digital microscope (1000x optical zoom) was used to capture images of the dried and ground coffee cherry pulp (Gabriel-Guzmán et al., 2017) as shown in Figure 1.

Moisture content and water activity

The moisture content was measured using an infrared moisture analyzer (OHAUS MB 45; Zanin et al., 2016), with the standard method at 103°C for 10 min. All tests were performed in triplicates. For measuring water activity (aw), 2 to 3 g of dried and ground coffee cherry pulp were placed inside a vapor sorption analyzer (VSA Aqualab Decagon Devices, Inc. Pullman, WA). Before measurement, the aw dewpoint sensor was verified using four saturated aqueous salt solutions 13.41 M LiCl (0.25 ± 0.003 aw), 8.57 M LiCl (0.50 ± 0.003 aw), 6.0 M NaCl (0.76 ± 0.003 aw), and 2.33 M NaCl (0.92 ± 0.003 aw), purchased from the instrument manufacturer (Schmidt & Lee, 2012).

Color

The color parameters of the dried and ground coffee cherry pulp samples were determined using a Konica Minolta colorimeter (CR-410, N.J., USA). A standard white plate was used to calibrate the equipment (Y = 87.0, x = 0.3160, y = 0.3231). The results were expressed according to the CieLab color system (L*: lightness, a*: redness, and b*: greenness) (Homez-Jara et al., 2018).

Aqueous extraction

Sample extraction was conducted in hot water to simulate the conditions of food product preparation. Different particle sizes were separated using sieves of 1.4 mm and 0.71 mm; only the particles retained in the 0.71 mm sieve were used for extraction (Heeger et al., 2017). For aqueous extraction, samples (1.0 g) were added to 20 mL of Milli-Q water for 15 min at 85°C in a water bath (TC-250, Brookfield) and stirred on a magnetic plate at 500 rpm for 10 min. Then, 1.5 mL of the water extracts were centrifuged at 9,000 rpm for 10 min in an Eppendorf Microcentrifuge Heraeus Pico 17 (Thermo Scientific). Extracts were prepared in triplicates. For HPLC analysis, the extracts were filtered using Minisart 0.2 μm nylon filters (Germany).

HPLC-diode array detector analysis of chlorogenic acids (CGAs) and caffeine

HPLC analysis was performed using 1.5 mL of the obtained aqueous extract. Determinations were performed using an Agilent 1260 Infinity II series liquid chromatograph (Agilent Technologies). Santa Clara, CA, USA) with a Poroshell 120-C180 (2.7 μm, 4 mm × 4.6 × 150 mm) column. The sample injection volume was 20 μL. Elution was carried out with a gradient of 100% methanol (eluent A) and water with 0.2% acetic acid (eluent B). Separation started with 80% of A for two min, followed by 2–10 min (A-56%, B-44%), and 10–14 min (A-80%). Detection was performed with a diode-array detector at 280 nm and 324 nm. Chlorogenic acids and caffeine were identified by comparing their retention times and the UV-spectra of the standards, which were prepared in Milli-Q water at concentrations of 100, 200, 300, 400, and 500 mg L−1 for CGAs and 10, 20, 30, 40, and 50 mg L−1 for caffeine.

ATR-FTIR measurements and spectral collection

The spectral measurements were made with an ART-FTIR CARY 630 spectrometer (Agilent, Santa Clara, CA, USA), between the wavelengths 4000–650 (cm−1), with a

FIGURE 1. Fine particles of dried and ground coffee cherry pulp: Castillo (A), Colombia (B), and Caturra (C) varieties.

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resolution of 8 cm\(^{-1}\) and with 20 scanners. The ATR-FTIR measurements were performed in a dry atmosphere at room temperature (20 ± 0.5 °C) (Bahamón et al., 2018; Barrios et al., 2020); approximately 1 g of the dried and ground coffee cherry pulp was placed in the sampling accessory and pressed; the background data was obtained from readings of the accessory without any sample. Once the spectra were obtained, they were exported to the Excel format for analysis. All samples were analyzed in triplicates.

**Statistical analysis**

The results of moisture content, water activity measurements, and HPLC determinations were assessed using analysis of variance (one-way ANOVA) with a confidence level of 95%. Mean comparison analyses were performed to identify statistically significant differences in the parameters evaluated between different categories. Statistical procedures were carried out using StatGraphics Centurion XVI. (Manugistics Inc., Rockville, MD, USA). Processing techniques were applied to the data obtained from the infrared spectra to compensate for any change in the experimental conditions and to improve the results. The pretreatments were first derivative and second derivative, obtained through the Resolutions Pro software (Agilent–USA, 2015). Principal components analysis (PCA) was carried out using these results as well as the raw data, to observe a better differentiation between the different types of dried and ground coffee cherry pulp. Matrices of size 45 × 900 were constructed such that each row corresponded to a sample and each column represented the spectral data at a given wave number. The analysis was carried out using R-statistical software (version 3.6.3, R statistics, St. Louis, MO, USA). Treatments of the first and second derivatives of the spectrum were used because they allowed an increase in e spectral resolution as well as greater differentiation in favor of the fine structures of the spectrum. The increase in resolution allowed resolution of bands that were too close and overlapped in the normal absorption spectrum, and minimized the baseline deviations caused by dispersion effects.

**RESULTS AND DISCUSSION**

Moisture content and water activity are factors that can affect the quality of flours; their increase can accelerate the caking of powdered foods. Thus, when storage moisture increases, the caking rate increases significantly (Carter et al., 2015a). Table 1 shows the statistically significant differences in water activity and moisture content between the dried and ground coffee cherry pulp of different varieties; further, these values were consistent for each variety. The Caturra variety exhibited higher values of water activity and moisture content, continued for Colombia and Castillo, respectively. Duangjaip et al. (2016) reported similar moisture content values for dried coffee pulp powder.

The results in Table 1 can be related to the adsorption isotherms reported in pre-mix powder by Carter et al. (2015a), in wheat flour by Carter et al. (2015b), and in other food products by Schmidt & Lee (2012). In general, the samples were below the critical value of water activity for microorganism proliferation (\(a_w < 0.61\)) reported by Tapia et al. (2008) as well as the maximum value of \(a_w\) for the main multipurpose flours category reported by Schmidt & Fontana (2008).

**TABLE 1. Water activity and moisture content of different varieties of dried and ground coffee cherry pulp.**

| Variety     | Water activity | Moisture content (kg kg\(^{-1}\) d.b.) |
|-------------|----------------|----------------------------------------|
| Castillo    | 0.34±3.46×10\(^{-3}\)\(^{a}\) | 0.061±1.3×10\(^{-4}\)\(^{a}\) |
| Colombia    | 0.356±5.77×10\(^{-4}\)\(^{b}\) | 0.066 ± 1.681×10\(^{-3}\)\(^{b}\) |
| Caturra     | 0.393±6.11×10\(^{-3}\)\(^{c}\) | 0.072±2.652×10\(^{-3}\)\(^{c}\) |

\(^{a}\)Different letters in the same column indicate significant differences (P < 0.05).

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**TABLE 2. Color measurements of different varieties of dried and ground coffee cherry pulp.**

| Variety     | \(L^*\)         | \(a^*\)         | \(b^*\)         |
|-------------|-----------------|-----------------|-----------------|
| Castillo    | 30.02±0.450\(^{a}\) | 7.727±0.262\(^{a}\) | 10.61±0.325\(^{a}\) |
| Colombia    | 29.74±0.115\(^{a}\) | 7.696±0.056\(^{a}\) | 10.70±0.097\(^{a}\) |
| Caturra     | 30.34±0.609\(^{b}\) | 7.452±0.359\(^{a}\) | 10.562±0.433\(^{a}\) |

\(^{a}\)Different letters in the same column indicate significant differences (P < 0.05).

Color is a crucial factor with regard to consumer acceptance; the values in Table 2 show that the obtained \(L^*\) values ranged from 29.7 to 30.3. The highest \(L^*\) value was achieved for the Caturra variety and the lowest values were obtained for the Colombia variety. There were statistically significant differences that can be attributed to the different varieties studied.

**TABLE 3. Content of total chlorogenic acids (CGAs) and caffeine (dry weight) of dried and ground coffee cherry pulp.**

| Variety     | Chlorogenic acids (mg g\(^{-1}\)) | Caffeine (mg g\(^{-1}\)) |
|-------------|----------------------------------|--------------------------|
| Castillo    | 2.141±0.569\(^{a}\) | 5.298±1.189\(^{a}\) |
| Colombia    | 0.722±0.151\(^{b}\) | 7.166±0.794\(^{a}\) |
| Caturra     | 0.506±0.076\(^{b}\) | 7.676±2.225\(^{a}\) |

\(^{a}\)Units in milligrams per gram of product *Different letters in the same column indicate significant differences (P < 0.05).

The highest content of total chlorogenic acids amounting to 2.141 mg g\(^{-1}\) was observed in the Castillo variety; the other varieties showed values between 0.506 to 0.722 mg g\(^{-1}\). The CGAs content in the Castillo variety
presented statistically significant differences compared to the Colombia and Caturra varieties. Heeger et al. (2017) reported similar values of CGA content in dried coffee cherry pulp of the Caturra variety and mentioned high values of CGAs in the Bourbon-Congo variety (~2.15 mg g⁻¹), which can be associated with our results for the Castillo variety. The total caffeine content between the varieties showed no statistically significant differences and showed consistent with those reported by Heeger et al. (2017); however, their different mean values correspond with the absorbance peaks described in Figure 2, which presents the spectra of the dried and ground coffee cherry pulp of the Castillo, Caturra, and Colombia varieties, and their absorbance peaks for each wavelength.

![Image](https://via.placeholder.com/150)

**FIGURE 2.** The mean ATR–FTIR spectra of dried and ground coffee cherry pulp of different Colombian varieties.

The infrared spectrum obtained for each of the samples of dried and ground coffee cherry pulp shown in Figure 2 is similar to the spectrum of roasted coffee reported in literature (Wang et al., 2011; Rivera et al., 2013; Niya & Lim, 2012). Peaks of interest associated with different chemical compounds such as caffeine, carbohydrates, water, and proteins have been identified, which have been reported in different studies on coffee (Ribeiro et al., 2010; Ribeiro et al., 2011; Amir et al., 2011; Reis et al., 2013a; Craig et al., 2014, Barrios et al., 2020). The spectra of the three varieties showed similarity in the number of peaks present but showed differences in absorbance values, wherein the majority of the peaks showed lower absorbance values for the spectrum corresponding to the Castillo variety compared to the spectrum of the other two varieties. Amir et al. (2011) reported peaks for water in the range of 3300–1640 cm⁻¹. In this study, water peaks were observed at a wavelength of 3280 cm⁻¹, and corresponding to Table 1 indicating the values of water activity and moisture content for the three varieties and their statistically significant differences, high values of absorbance were observed for the Caturra, followed by the Colombia and Castillo varieties, respectively.

Reis et al. (2013a) reported that the content of caffeine in coffee husk is similar to that in coffee beans, and that the peaks expressed at the wavelength 2922–2855 cm⁻¹ are likely to be primarily associated with caffeine. Further, caffeine is reported to be detected in the range of 1650–1600 cm⁻¹ in the infrared spectrum (Craig et al., 2014), as shown in Figure 2 (1602 cm⁻¹). The region around 2100 cm⁻¹ is associated with carbohydrates, chlorogenic acids (CGAs), and proteins (Craig et al., 2014). Further, CGAs are a family of esters formed between certain transcinamic acids and chemical acids, and are associated with the presence of absorption peaks in the region of 1450–1000 cm⁻¹ (Lyman et al., 2003). In the present study, this zone presented some absorption peaks (Figure 2), and those with the highest absorbance were associated with the dried and ground coffee cherry pulp of the Colombia and Caturra varieties. According to Ribeiro et al. (2010), wavenumbers ranging from 1700–1600 cm⁻¹ are highly related to chlorogenic acid and caffeine concentration in coffees. In this case, the wavelength of 1603 cm⁻¹ is typical for chlorogenic acid (Capek et al. 2014). As described above, the results of the present study show a higher absorbance value in the peaks for caffeine and CGAs for the samples corresponding to the coffee pulp of the Caturra and Colombia varieties, which agrees with the results shown in Table 3, showing a lower content of these compounds in the Castillo variety; this indicates that it is convenient to implement this technique to obtain rapid results for these types of compounds in dried and ground coffee cherry pulp. Peaks of absorbance were evident at 1237, 1014, and 776 cm⁻¹ and may be associated with saccharose in the range of 1242–1218 cm⁻¹ (Ribeiro et al., 2011), arabinogalactans at 1065–1020 cm⁻¹ (Craig et al., 2018), and carbohydrates at 1500–700 cm⁻¹ wavelength (Reis et al., 2013b). Thus, the dried and ground coffee cherry pulp could be considered as a product rich in chemical compounds. Further, the FTIR technique allows rapid detection of the differences between these compounds across different varieties of coffee.
Determination of spectral variations across the different varieties of dried and ground coffee cherry pulp was determined by means of PCA, which reduced the dimensionality of the IR spectra and facilitated visualization of the data set. Figure 3 shows the PCA biplot from each of the pre-treatments of data used (first derivative and second derivative) as well as the results of the raw data. The best differentiation was found with the processing of data from the first derivative (Figure 3B), which shows a grouping of the samples of dried and ground coffee cherry pulp of the Castillo variety on the left side of CP1, whereas the dried and ground coffee cherry pulp samples of the Caturra variety were grouped on the right side of CP1. Further, samples of dried and ground coffee cherry pulp of the Colombia variety were grouped in the lower part of CP2. The raw data (Fig. 3A) and those treated with the second derivative (Fig. 3C) did not show a clear differentiation between the different varieties of coffee pulp. In both cases, the dried and ground coffee cherry pulp samples of the Caturra and Colombia varieties were overlapped and were separated from the samples of the Castillo variety.

The results show that the infrared spectrum can be used to differentiate between the varieties of dried and ground coffee cherry pulp, by treatment of the first derivative data. They also confirm, in a concrete manner, small differences in the absorbances observed in the spectra described in Figure 2 among the three varieties of dried and ground coffee cherry pulp, which may be related to the data described in Table 2, regarding caffeine and chlorogenic acids. These results show that this technique is a simple but versatile tool for characterizing and differentiating the dried and ground coffee cherry pulp from different varieties, as demonstrated in other studies that have used PCA of infrared spectra as a technique to determine differences between food matrices (Reis et al., 2013b, Craig et al., 2012). Notably, this technique provides coherent information on the differences between the chemical characteristics of different varieties of coffee pulp, which have been confirmed by chemical analyses. Thus, in future, determination of the infra-red spectral information can be considered a factor of relevance for application in the food industry, as it indicates that each coffee variety should be given specific treatment based on the differences found between them.

CONCLUSIONS

Coffee pulp is a waste generated during the processing of coffee, and has high potential as a raw material with multiple uses in the agri-food industry; therefore, it is necessary to establish strategies for its characterization and application. The FTIR spectra showed fingerprints related to the chemical compounds present in the dried and ground coffee cherry pulp as reported in other food products, indicating that the dried and ground coffee cherry pulp is a matrix rich in biomolecules, with great potential in the food industry. These results allowed us to conclude that the FTIR technique facilitates quick identification of the chemical composition of dried and

FIGURE 3. Results of Principal Component Analysis with different data processing: A) Raw data, B) the First derivative and C) the Second derivative.
ground coffee cherry pulp from different varieties, and shows small differences in their absorbance peaks, mainly associated with caffeine and chlorogenic acids. We found that the variety Castillo presented lower contents of the chemical compounds according to the analyzed spectrum. The PCA results of the data obtained from the infrared spectrum showed that it is possible to discriminate the dried and ground coffee cherry pulp according to its variety, by means of the first derivative treatment. Finally, these results indicate that the ATR-FTIR technique provides satisfactory results for characterization and differentiation of coffee varieties, and can be an important tool in the food industry.

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