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ARTICLES

Chemical Discrimination of Arabica and Robusta Coffees by Fourier Transform Raman Spectroscopy

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This article deals with the potential of Fourier transform (FT) Raman spectroscopy in discrimination of botanical species of green and roasted coffees. There are two species of commercial importance: *Coffea arabica* (arabica) and *Coffea canephora* (robusta). It is recognized that they differ in their lipid fraction, especially in the content of the diterpene kahweol, which is present at 0.1−0.3% dry matter basis in arabica beans and only in traces (<0.01%) in robusta. The visual examination of the Raman spectra of the lipid fraction extracted from arabica, robusta and liberica samples shows differences in the mid-wavenumbers region: arabica spectra have two characteristic scattering bands at 1567 and 1478 cm^{-1}. The spectrum of the pure kahweol shows the same bands. Principal component analysis is applied to the spectra and reveals clustering according to the coffee species. The first principal component (PC1) explains 93% of the spectral variation and corresponds to the kahweol concentration. Using the PC1 score plot, two groups of arabica can be distinguished as follows: one group with high kahweol content and another group with low kahweol content. The first group includes samples coming from Kenya and Jamaica; the second group includes samples from Australia. The main difference between these coffees is that those from Kenya and Jamaica are well-known for growing at a high altitude whereas those ones from Australia are grown at a low altitude. To our knowledge, the application of Raman spectroscopy has never been used in coffee analysis.

KEYWORDS: Coffee; Raman spectroscopy; discrimination; botanical species; arabica; robusta; kahweol

INTRODUCTION

Coffee is one of the most popular drinks across the world. Its commercial and social importance is obvious. Coffee is cultivated in more than one hundred different tropical countries, and some 20 million people are engaged in production of 6.7 million tons (*1*). Among more than 100 species in the genus of *Coffea*, only two are of commercial importance: *Coffea arabica* (arabica) and *Coffea canephora* (robusta). Arabica today accounts for some 64% while robusta accounts for about 35% of the world's production; others species with not much commercial value like *Coffea liberica* and *Coffea excelsa* represent only 1%.

The species or varieties, the environmental conditions (soil, rainfall, altitude, etc.), and the methods of processing, especially fermentation, drying, and roasting, influence the quality of coffee, so that the sale prices show wide differences. The arabica coffees have a more pronounced and finer flavor than the robusta coffees; they are considered of better quality; therefore, they are the most expensive ones (*2*). The arabica are, on average, sold at 2−3 times the price of the robusta, and some arabica from different geographical origins are sold at 10 times the price of robusta. The possibility of fraudulence or mislabeling thus arises. A coffee description relates not only to the species or varieties (arabica, robusta, or liberica) but also to the geographical origin and the environmental conditions. By considering the increasing practice of selling coffee based on the varietal and geographical origins, it is necessary to develop methods to discriminate the coffee according to these production parameters.

Strategies utilized to authenticate coffee have traditionally relied on wet chemistry. Some of the chemical data used to characterize the arabica and robusta coffees are the contents of minerals (*3*−*6*), volatile substances (*7*−*9*), chlorogenic acids, and caffeine (*10*−*13*). The analysis of the lipid fraction is also an usual approach, in particular, the determination of fatty acids (*14*, *15*), sterols (*16*, *17*), diterpenes (*18*−*21*), and tocopherols

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(*22*). However, the wet chemistry methods used for this discrimination are all time-consuming and expensive, and the demand for rapid and inexpensive controls is growing (*23*). Fast spectroscopic methods have been studied for this purpose. Infrared spectroscopy has been tested in the botanical authentication of green, roasted coffees (*24*−*27*) and instant coffees (*2*, *28*, *29*). Mass spectrometry has also been tested to identify the geographical origin of some coffees (*30*, *31*). According to the literature overview, Raman spectroscopy has not yet been tested.

Raman spectroscopy is based on the scattering of monochromatic laser radiations in an interaction with the molecular vibrations when these vibrations are accompanied by a change of polarizability in the chemical bonds. Elastic collisions between photons and molecules result in radiations scattered mostly at the incident frequency (Rayleigh scattering); however, concurrent inelastic collisions, resulting from vibrational transitions of chemical bonds, produce a small fraction of scattered radiations with shifted frequencies (Raman scattering). The Raman spectrum of a given compound consists of a unique fingerprint of its molecules and their interactive effects. (*32*) The intensity of scattering is directly proportional to the concentration of the molecules producing the Raman effect (*33*).

MATERIALS AND METHODS

Reagents and Chemicals. All reagents and solvents used were of analytical reagent grade: kahweol (Sigma, Bornem, Belgium), cafestol (ICN Biochemical, Ohio), diethyl ether (Fisher Chemicals, Leicestershire, United Kingdom), and methanol (Merck, Darmstadt, Germany).

Samples Collection. A set of 86 green and 82 roasted coffees (124 arabica, 42 robusta, and two liberica) from 25 different geographic origins was collected for this analysis. The coffee samples were acquired in Belgium from several coffee importers and roasters.

Sample Preparation. Coffee samples were ground to pass through a 0.5 mm sieve (Retsch ZM1 Grinder) and afterward stored in polyethylene flasks until the analysis time. The green coffee beans were ground in liquid nitrogen.

Lipid Extraction. Before extraction, the sample moisture was determined by the weight of water lost during a heating of 12 h at 105 °C. The lipid fraction was extracted from coffee using diethyl ether. Exactly 5 g of ground coffee sample was extracted during 6 h with 110 mL of diethyl ether in a Soxhlet system siphoning six times per hour. Using a vacuum rotary evaporator, the solvent was eliminated and the residue was dried at 105 °C during 5 min and then weighed to obtain the amount of coffee oil.

Standards Preparation. Kahweol and cafestol were dissolved in methanol before the spectra acquisition. The standards spectra were obtained after subtraction of the methanol spectrum from the solution spectrum.

Spectra Acquisition. The Raman measurements were performed on Perkin-Elmer (Boston, MA) NIR FT-Raman Spectrometer 2000R equipped with a Nd:YAG laser emitting at the wavelength of 1064 nm and with an InGaAs detector. The 180° backscattering refractive geometry was used. The spectrometer was managed through the Spectrum software of Perkin-Elmer. The spectral data of coffee oil were obtained at a wavenumber resolution of 4 cm^{-1} at nominal laser power of 500 mW. For each spectrum, 20 scans were accumulated to ensure an acceptable signal-to-noise ratio. The Raman spectra of coffee oil (0.5 mL) were measured in NMR tubes Series 500 from Sigma-Aldrich (Bormen, Belgium). All Raman spectra were collected at room temperature.

Data Treatment. Prior to the visual examination and the statistical analysis, Raman spectra were corrected for baseline variations and normalized to compensate for any change in experimental conditions (variation of the excitation intensity, sample positioning, etc.), resulting in an increased stability of between days measurements (*34*, *35*). The 1700−1400 cm^{-1} range of Raman spectra was used as a descriptor to study a possible discrimination of the coffee samples. The principal component analysis (PCA) was applied for classification as well as for interpretation of the spectral differences in the samples. The PCA involved a mathematical procedure that summarized all of the variations in spectral data into a few new variables called principal components (PC). Each PC was characterized by the spectral variation (loadings) and the corresponding scaling coefficient (scores). When the scores were plotted against the PCs (score plots), it was possible to find similarities and differences among samples; similar samples tended to group together. The PCs loading plots were plots of the "spectra" for the different PCs and can help the interpretation of the relationship between the PCs and the original variables in the spectrum (*36*−*38*).

PC1 accounted for as much of the variation as possible in the data, and each succeeding PC accounted successively for the remaining variation. The chemometric calculations were made by means of the UNSCRAMBLER Software 7.6 (CAMO, Trondheim, Norway).

RESULTS AND DISCUSSION

Moisture and Lipid Content. The moisture contents of green and roasted coffee are 10.0 ± 1.0 and 5.0 ± 1.0%. The lipid content of arabica and robusta samples shows average values of 13.9 ± 1.0 and 9.3 ± 1.0% on a dry matter basis, respectively, for the green coffees. The values for the lipid content in roasted coffees are higher as compared with those in green coffees from which they were derived: 16.8 ± 1.0 and 11.5 ± 1.0% on a dry matter basis, respectively. This is due to the overall dry matter content loss on roasting, comparatively to the loss of lipid matter that is low. This is in agreement with previous reports in the literature (*18*).

Visual Examination of the Spectra. The average Raman spectra of the lipid fraction extracted from the arabica, robusta, and liberica samples are shown in **Figure 1**. There is no large difference between the green and the roasted coffees. The spectra of robusta and liberica display exactly the same profile, while the spectrum of arabica shows additional bands in the mean region of the spectrum. In the high wavenumbers of all of the coffee species, we observe a scattering band at 3008 cm^{-1}, corresponding to the stretching vibrations of ethylenic groups and scattering bands at 2923, 2893, and 2851 cm^{-1} corresponding to symmetric and asymmetric C−H stretchings (*39*, *40*). In the mid-wavenumbers region, we observe a scattering band at 1750 cm^{-1} corresponding to the ester C═O stretching, another band at 1657 cm^{-1} corresponding to the carbon double bond (C═C) stretching, and three bands at 1440, 1302, and 1266 cm^{-1} corresponding to CH_3, CH_2, and CH deformation vibrations (*41*−*45*).

In **Figure 2**, the spectrum of arabica presents two scattering bands at 1567 and 1478 cm^{-1}, which are absent from the robusta and liberica spectra. An unambiguous confirmation of the origin of these specific bands of arabica can be achieved by comparing the coffee spectra with those ones of cafestol and kahweol. These two substances are characteristic diterpenes found in lipid fractions of green and roasted coffee beans. Cafestol and kahweol contents in arabica beans are about 0.3−0.7 and 0.1−0.3% dry matter basis, respectively. Robusta contains less high concentrations of cafestol (∼0.1−0.3%) and only traces of kahweol (<0.01%) (*18*, *46*−*48*).

The spectra of cafestol and kahweol are shown in **Figure 3**. The spectrum of kahweol presents two Raman scattering bands at 1567 and 1478 cm^{-1} while the spectrum of cafestol presents one scattering band at 1500 cm^{-1}. So the characteristic bands of the arabica spectra seem due to the kahweol content. As no scattering bands of kahweol are present in robusta spectra, we can say that the content of kahweol in the analyzed robusta coffees is under the detection limits of Raman spectroscopy.

The molecular structures of cafestol and kahweol are shown in **Figure 4**. These two diterpenes are pentacyclic molecules in

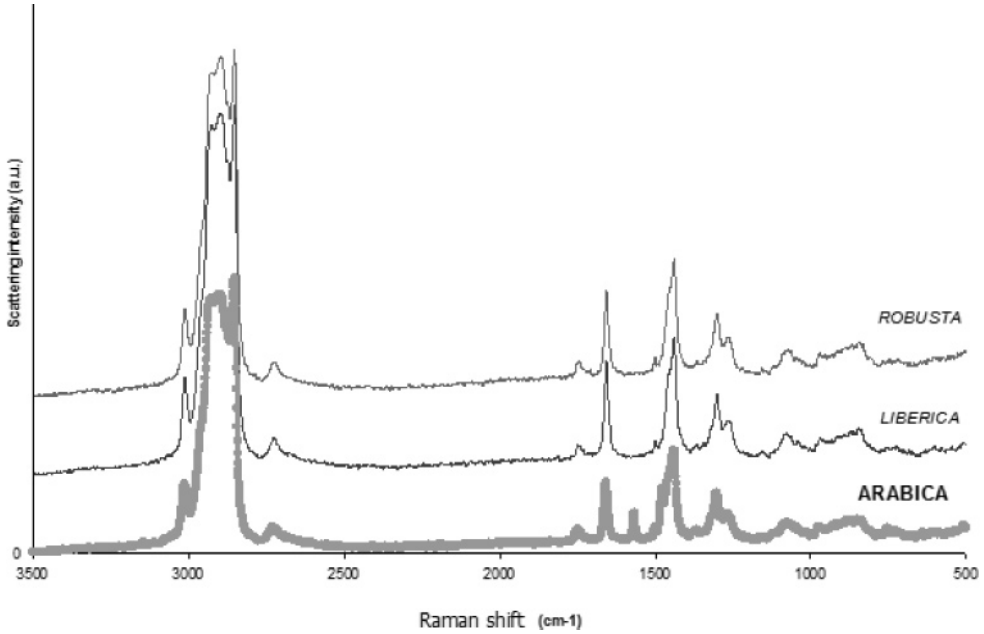

Figure 1. FT-Raman spectra of lipid fractions of arabica, robusta, and liberica coffee species.

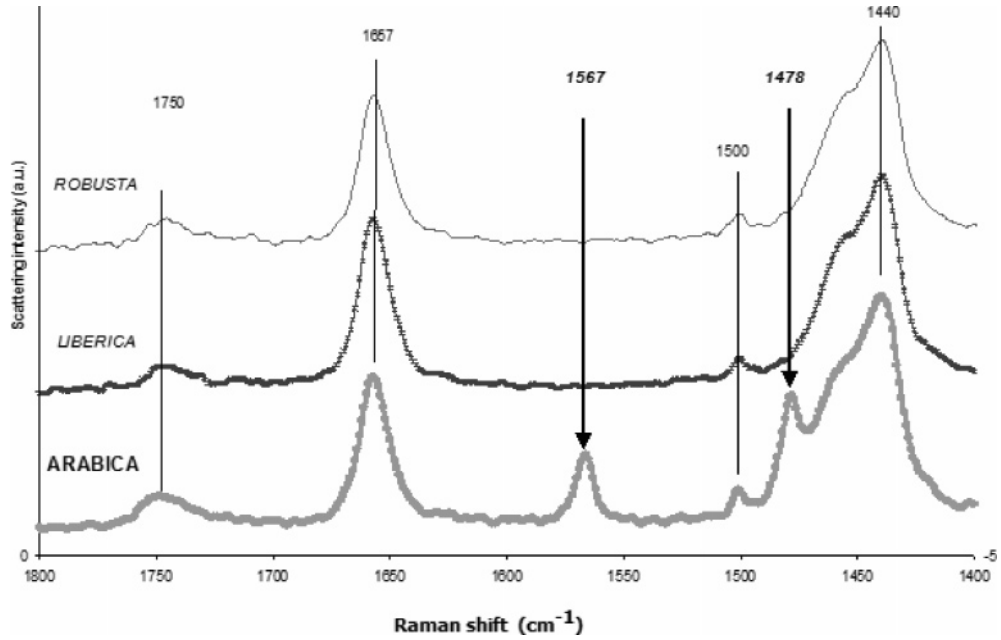

Figure 2. FT-Raman spectra of lipid fractions of coffee species.

which there are three cycles with six carbons (A, B, C), one cycle with five carbons (D), and a furan cycle (E). The difference between the molecular structures of cafestol and kahweol is that the six carbons cycle A is cyclohexene (C_3═C_4) in cafestol while it is 1,3-cyclohexadiene (C_1═C_2, C_3═C_4) in kahweol. The Raman spectra of cyclohexene and cyclohexadiene have been studied by Dilauro et al (*49*, *50*). The C═C stretching wavenumber of cyclohexene is 1657 cm^{-1}, and the C═C stretching of 1,3-cyclohexadiene is 1575 cm^{-1}. This last wavenumber is close to the wavenumber of 1567 cm^{-1} observed in the kahweol spectrum. The wavenumber difference of 8 cm^{-1} in less could be explained by the additional conjugation of the cyclohexadiene double bonds (A) with the double bond (C_{18}═C_{19}) of the furan cycle (E).

The cafestol band at 1500 cm^{-1} is attributed to C═C stretching in the furan cycle (*51*, *52*). The kahweol band at 1478 cm^{-1} can also be associated to the C═C stretching in furan cycle (E), and the decrease of 22 cm^{-1} could be due to the conjugation of the furan double bonds with the double bond C_1═C_2 of cyclohexadiene (A) in kahweol. Such a decrease of wavenumber occurring when there is an increase of conjugated double bonds has already been reported (*45*, *53*).

Statistical Analysis of the Spectra. PCA has been applied with a centered model on the Raman spectra acquired from all of the coffee samples in order to evaluate completely the potential of Raman spectroscopy in coffee discrimination. The 1700−1400 cm^{-1} region of the spectra has been used as a descriptor to exploit the spectral differences between the samples for the best chemical discrimination of the arabica and robusta coffees. The PC1 explains 93%, and the PC2 explains 3% of the total spectral variation of green and roasted coffees. The chemical basis of the discrimination is explored through the interpretation of the PC loadings. We have observed that the PC1 loading corresponds exactly to the spectrum of kahweol

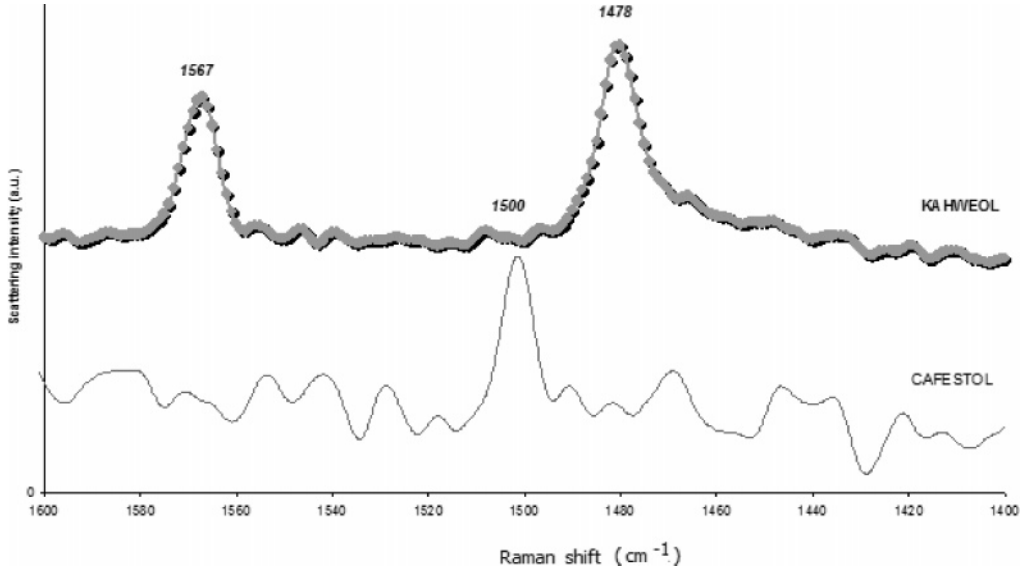

Figure 3. FT-Raman spectra of kahweol and cafestol.

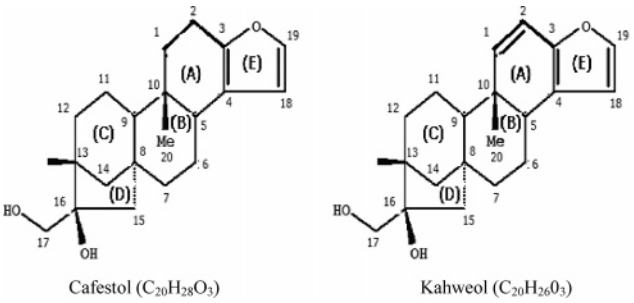

Figure 4. Structural formulas of kahweol and cafestol (*22*).

shown in **Figure 3** and that the second does not correspond to a meaningful spectral profile. The score plot of PC1 in function of PC2 is presented in **Figure 5**. As can be observed, all of the robusta samples are located at the negative side of PC1 score scale and the arabica are located at the positive side of the same scale. So we can conclude that the samples with the highest PC1 score value have the highest content of kahweol. Among them, we have identified the arabica samples coming from Kenya (GA39, GA46, GA49) and Jamaica (GA34). These coffees are well-known for growing at high altitudes from 1500 to 2100 m above sea level (*54*). In contrary, the arabica from Australia (GR42, GR44, GR45) present the lowest PC1 score. These last coffees are normally grown at a low altitude from 15 to 900 m (*55*). De Roos et al. (*48*) studied variations of diterpenes in nine wild African coffee species and found that the kahweol content is related to the geographical distribution of the species; coffee species from West and Central African forests present low concentrations of kahweol, and species originating from East Africa present high concentrations of kahweol (*46*). As the altitude decreases from East of Africa toward the West, the kahweol concentration may be related to the altitude and temperature where coffees are grown (*56*). So, this study seems to indicate the same observation as the PC score plot: arabica coffees coming from high altitude regions such as Kenya and Jamaica present high concentrations of kahweol whereas arabica coffees coming from the low altitude regions such as Australia present the low concentrations of kahweol. Guyot observed a decrease of 0.6 °C in temperature for every increase of 100 m above sea level (*57*). Probably, the temperature is the predominant factor that affects kahweol

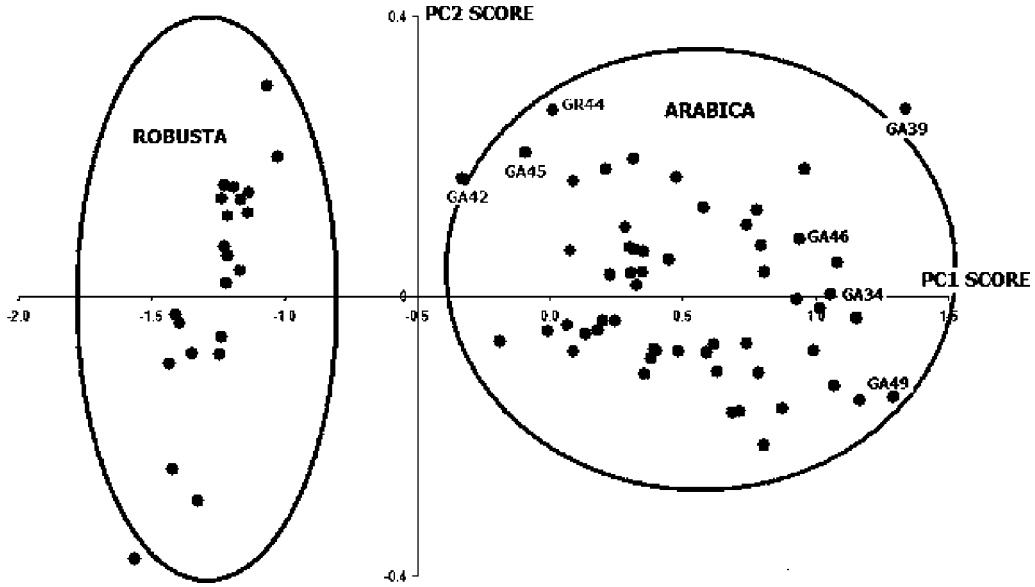

Figure 5. PCA scores plot of the first PCs for green coffees.

metabolism in coffee. However, the kahweol content may be affected by other factors such as coffee cultivars, soil, and postharvest processing. A study of the influence of these factors on arabica coffees in Rwanda has been done and will be published very soon.

In conclusion, according to the results presented here, we can affirm that Raman spectroscopy offers the possibility to distinguish the botanical origin of green and roasted coffees. The analytical performance of the spectroscopic technique is based on the manifestation of kahweol by two specific scattering bands at 1567 and 1478 cm^{-1}. Among arabica samples, it is possible to distinguish by Raman spectroscopy coffees with high contents of kahweol from coffees with low contents.

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