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Discrimination of Green Arabica and Robusta Coffee Beans by Raman Spectroscopy

Anke Keidel,[†] David von Stetten,[†,#] Carla Rodrigues,[§] Cristina Máguas,[§] and Peter Hildebrandt*,[†]

[†]Technische Universität Berlin, Institut für Chemie, Sekr. PC14, Strasse des 17 Juni 135, D-10623 Berlin, Germany, and [§]Stable Isotopes and Instrumental Analysis Facility SIIAF, Faculty of Sciences, Center for Environmental Biology, University of Lisbon, Bloco C2, Campo Grande, 1749-016 Lisboa, Portugal. [#]Present address: European Synchrotron Radiation Facility, 6 rue Jules Horowitz, B.P. 220, F-38043 Grenoble Cedex, France.

This paper presents an approach that may be applied as an accurate and rapid tool for classifying coffee beans on the basis of the specific kahweol content. Using Fourier-transform Raman spectroscopy with 1064 nm excitation it is possible to monitor the characteristic Raman bands of kahweol in green coffee beans without chemical and physical processing of the beans. The procedure was optimized on the basis of 83 and 125 measurements of whole and ground beans, respectively, using coffee samples of two different species, *Coffea arabica* L. and *Coffea canephora* L. (var. Robusta), and different origins (Asia, Africa, and South America). The relative contribution of the kahweol in individual beans can be determined quantitatively by means of a component analysis of the spectra, yielding a spectral kahweol index (σ_{ka}) that is proportional to the relative content of kahweol in a coffee bean. The reproducibility of the spectroscopic measurement and analysis was found to be 3.5%. Individual beans of the same type and origin reveal a scattering of the σ_{ka} values. Nevertheless, an unambiguous distinction between Arabica and Robusta samples is possible on the basis of single-bean measurements as the σ_{ka} values are greater than and less than 10 for Arabica and Robusta coffees, respectively. Measurements of whole and ground beans afforded very similar results, despite the heterogeneous distribution of kahweol within a bean. Unlike conventional analytical techniques, the single-bean sensitivity of the present approach may also allow for a rapid detection of unwanted admixtures of low-value Robusta coffee to high-quality and more expensive Arabica coffee.

KEYWORDS: Arabica coffee; Robusta coffee; kahweol; quality control; Raman spectroscopy

INTRODUCTION

Coffee is a major foodstuff with an annual global consumption of about 7 million tons (*1*). Commercially available coffee roasts consist of two main variants in pure or blended forms: *Coffea arabica* L. and *Coffea canephora* var. *robusta*, commonly referred to as Arabica and Robusta coffees, respectively. Arabica coffees are considered to be of higher quality and of finer taste than Robusta coffees, which is reflected by distinctly higher prices (by >200%) when the cheapest Robusta and the most expensive Arabica coffees are compared (*2*). The price also depends on the geographic origin (*2*). Furthermore, there are substantial variations of the price over time with 10% short-term fluctuations within days and midterm changes by > 100% over years, often related with crop yield variations. In addition, the continuous splitting of large coffee estates, the excessive expansion of new plantations, and the growing number of intermediaries in the marketing chains have resulted in a deterioration of coffee quality and its price. In a situation of overproduction a higher quality coffee is likely to command premium prices. Thus, farmers will have to pay more attention to quality, which may be, in turn, associated with lower yields (*3*), so there is a strong economic interest in safely distinguishing coffee beans of different species (*C. arabica* vs *C. canephora* var. Robusta) and different geographical origins to verify the purity of batches and, specifically, to detect possible admixtures of cheaper (Robusta) to more precious (Arabica) beans.

Currently, the main procedure to distinguish green Arabica and Robusta beans is based on visual inspection of the size, color, and shape of the beans. Not only does this approach depend on the skills and experience of the inspector, but its reliability may also be reduced by natural variations of the appearance of the beans from different species. Among different types of coffee, there are considerable variations in size, shape, and density as a result of both genotype and environmental factors. Furthermore, visual inspection does not allow the safe detection of "contaminations" of Arabica beans by small amounts of Robusta beans. Consequently, developments of more objective methods that can be certified are desirable.

Analytical approaches that are employed for green (*4*−*19*) as well as roasted coffees (*4*, *6*, *8*, *10*, *11*, *16*, *18*, *20*−*26*) may be

*Author to whom correspondence should be addressed (e-mail hildebrandt@chem.tu-berlin.de; phone +493031421419; fax +493031421122).

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grouped in two classes depending on the processing of the coffee. The first class (chemical methods) is based on traditional analytical methods in which coffee beans are mechanically and chemically processed for applying chromatographic techniques to distinguish between the two coffee species on the basis of different compositions of hydroxycinnamic acids (*9*), sterols (*5*), chlorogenic acid, caffeine, trigonelline (*7*, *20*), amino acids (*7*, *10*), metals (*21*), fatty acids (*6*, *11*), polysaccharides (*12*), tocopherols (*4*, *8*), and diterpernoids (*16*, *27*, *28*). The second class is based on spectroscopic techniques, mainly using mid-IR (*22*, *23*) and near-IR (*23*−*26*) spectroscopy, which have been proven to be useful for discrimination between roasted Arabica and Robusta coffees. In particular, in combination with spectral pattern recognition methods, a reliable distinction between Arabica and Robusta coffees was achieved. In addition, IR spectroscopy in combination with principal component analysis (PCA) has been shown to distinguish between Arabica and Robusta instant coffees (*29*), and even "Timor Hybrid" (Híbrido de Timor, HdT), which is a crossbreed of Arabica and Robusta coffees, was correctly identified by this method (*15*).

The two classes of analytical approaches are associated with specific advantages and disadvantages. Chemical methods rely upon different chemical compositions in Arabica and Robusta coffees, but the quantitative analysis requires time-consuming and costly sample processing in an adequately equipped chemical laboratory. On the other hand, the previously employed spectroscopic methods were applied to ground roasted or green beans without further chemical extraction procedures. However, the spectral analysis relies upon statistical evaluation procedures, which sensitively depend on the calibration model. Moreover, sample preparation has to follow a precise protocol because, for instance, water content and grain size may affect the spectra and thus the PCA (*13*, *14*).

The present Fourier-transform (FT) Raman spectroscopic approach is capable of overcoming the drawbacks associated with the chemical and IR-spectroscopic techniques because it represents a fast procedure applicable to ground as well as whole beans. High-quality Raman spectra are obtained, which allow identification of the characteristic vibrational bands of kahweol, which in a previous study were detected in chemical extracts of processed green and roasted beans (*16*). Due to the different contents of this diterpenoid in Arabica and Robusta (*27*−*30*), these two coffee species can readily be distinguished on the basis of the kahweol Raman bands without sophisticated spectral analysis. The present work is dedicated to demonstrating that this approach allows for determining the kahweol content of coffee beans without mechanical and chemical processing, such that it may be developed toward a reliable and fast analytical technique for in situ quality control of coffee.

MATERIALS AND METHODS

Samples. Green coffee beans from different origins were obtained through the University of Lavras in Minas Gerais, Brazil, University of Hawaii in Manoa, Novadelta, S.A. (Portugal), and from a local contact in Dili, East Timor, as described in detail in the Supporting Information (Table S1). The country of origin of all samples is known. Exact geographical location coordinates are indicated whenever available. The beans were used either directly for FT Raman spectroscopic characterization or after grinding to a fine powder in a mill (Restch, Germany) with a grain size of < 1 mm as described previously (*19*). Kahweol acetate (CAS Registry No. 81760-47-6) was purchased from LGC Standards (Wesel, Germany) and used without further purification.

Fourier-Transform Raman spectroscopy. Raman spectra were recorded with a Bruker RFS 100/S Fourier-transform spectrometer with 1064 nm excitation and a spectral resolution of 4 cm^{-1} (*31*). The accumulation time for each spectrum was ca. 6 min. All experiments were carried out at ambient temperature using a laser power of 300 mW, which did not cause any damage to the sample as checked by repetitive measurements and measurements as a function of the laser power. No background correction was applied to the spectra prior to spectral analysis.

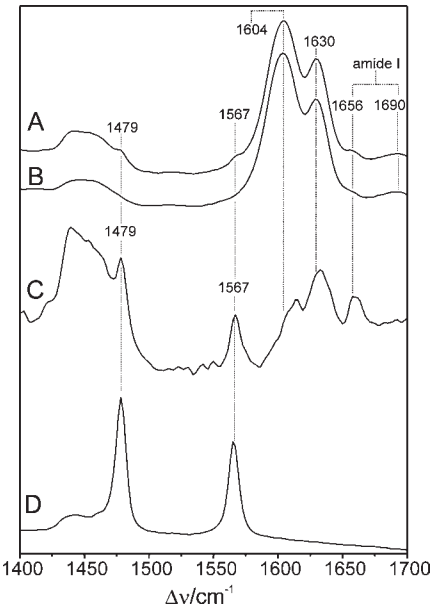

Figure 1. Raman spectra of a whole green bean from Arabica (sample 28; **A**) and from Robusta (sample 21; **B**). Spectrum **C** represents the difference of **A** − **B** to show more clearly the Raman bands of kahweol. The experimental Raman spectrum of neat kahweol is shown in trace **D**. All spectra were obtained with 1064 nm excitation.

RESULTS AND DISCUSSION

The Raman spectra of all whole and ground coffee beans display a quite similar overall vibrational band pattern. A characteristic example (Arabica sample 28) is shown in **Figure 1A**. In the region between 1400 and 1700 cm^{-1}, the spectrum is dominated by a prominent band pair at 1604 and 1630 cm^{-1}, originating from the aromatic and C═C stretchings of polyphenols and phenolic (chlorogenic) acids, which are important constituents of coffee beans with up to 10% w/w of the dry mass (*7*, *9*). The high intensities of these bands, which are evidently not proportional to the relative concentrations of these constituents, result from the relatively large Raman cross sections of these modes and a preresonance enhancement even at 1064 nm excitation. Conversely, Raman bands of proteins are relatively weak and give rise to a weak peak at 1690 cm^{-1} and a shoulder at 1656 cm^{-1}, originating from the amide I bands of β-sheet and α-helix structures, respectively.

The overall spectral similarity holds for the Raman spectra of typical Arabica (**Figure 1A**) and Robusta (**Figure 1B**) beans. However, a closer inspection reveals differences related to two weak peaks at 1479 and 1567 cm^{-1} in the spectra of Arabica samples. These two peaks, which can be more clearly identified in the difference spectrum of Arabica minus Robusta (**Figure 1C**), are at the positions of the most prominent bands of kahweol as demonstrated by the comparison with a spectrum of neat kahweol (**Figure 1D**), in agreement with the previous study by Rubayiza and Meurens (*16*). These findings reflect the ca. 10 times higher kahweol content in Arabica (0.11−0.35%) as compared to Robusta (< 0.01%) (*16*, *27*, *32*). Again, the detectability of the kahweol bands benefits from the relatively large Raman cross section of these modes at 1064 nm excitation.

Analysis of the Raman spectra. For a quantitative analysis, we have employed a component analysis in which the experimental

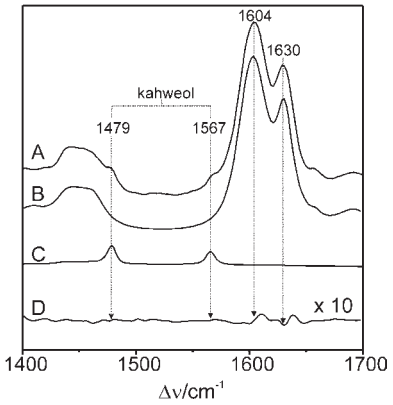

Figure 2. Raman spectra of a whole green bean from Arabica (sample 28; **A**; same as **Figure 1A**) and the synthetic component spectra of the coffee "background" (**B**) and kahweol (**C**). Trace **D** represents the residuals of a fit of the component spectra **B** and **C** to the experimental spectrum **A** (see text for further details).

Raman spectra are simulated by the superposition of the spectra of individual components (*33*). In this approach, we first used component spectra of kahweol and of the remaining spectral contribution, denoted "background" (**Figure 2A−C**). These component spectra were constructed by combining several individual bands with Lorentz and Voigt profiles obtained from a band fitting to the experimental spectra of neat kahweol and of a typical Robusta sample lacking the kahweol bands. Using a linear combination of these two "synthetic" component spectra, it was possible to achieve a satisfactory description of the experimental Raman spectra obtained from different coffee samples. However, such highly constrained fits in which the amplitudes of the two component spectra were the only two adjustable parameters afforded relatively high residuals in the region between 1590 and 1650 cm^{-1}, reflecting slight differences of the polyphenol and chlorogenic acid content in the various samples as compared to the reference to which the "background" component spectrum refers. In the second step, the intensity of the prominent 1604 cm^{-1} band of the background as well as the relative intensities of the two kahweol bands was taken as an additional independent variable. In this way, the overall quality of the fits was improved (**Figure 2D**). On the basis of these fits, the relative spectral contribution of kahweol in coffee samples was expressed in terms of the amplitude ratio of the kahweol (1479 cm^{-1}) and "background" (1630 cm^{-1}) component spectra and denoted the spectral kahweol index σ_{ka}. The results were essentially the same as for the pure component analysis (first step, vide supra) and the mixed component/band fitting analysis (second step).

Raman Spectroscopic Analysis of Ground Beans. The Raman spectroscopic determination of σ_{ka} was found to be highly reproducible as demonstrated by repetitive measurements of the same powder samples. The average mean standard deviation of these measurements was determined to be 3.5%. We have then applied the analysis first to 125 ground samples, taken from 25 different origins of both Arabica and Robusta types (**Table 1**). From each origin, five samples from different beans were prepared. The individual σ_{ka} values are plotted in **Figure 3** (top), demonstrating that Arabica and Robusta samples can unambiguously be distinguished. All Arabica samples exhibit σ_{ka} values of >10, whereas in the case of Robusta values between 0 and 5 are determined. The only deviation refers to the five beans assigned to sample 19, that is, to an Arabica type coffee. However, the very low σ_{ka} values determined for these beans clearly show that this classification is not correct and that they must originate from Robusta coffee beans. In contrast to the other samples, coffees 18 and 19 were obtained directly from a local roasting facility in Dili, East Timor, with little information about their type. Considering that HdT coffee has begun to replace the local Arabica in Timor and that crosses between *C. arabica* var. *caturra* and HdT generated a population called Caturra highly cultivated in that region, the hypothesis that sample 19 is a misidentified Arabica coffee has to be considered. For all other samples, the original classification is confirmed by the present analysis.

Table 1. Average Spectral Kahweol Indices σ_{ka} of Ground Coffee Beans

origin[a]	sample	type	σ_{ka}[b]
Brazil	1	Arabica	18 ± 5
	2	Arabica	33 ± 5
	3	Arabica	30 ± 6
	4	Arabica	28 ± 8
	5	Arabica	23 ± 8
Hawaii	6	Arabica	20 ± 5
	7	Arabica	27 ± 2
	8	Arabica	19 ± 2
	9	Arabica	31 ± 4
	10	Arabica	23 ± 9
Kenya	11	Arabica	19 ± 4
	12	Arabica	19 ± 1
	13	Arabica	19 ± 2
	14	Arabica	24 ± 6
	15	Arabica	19 ± 4
East Timor	16	Arabica	27 ± 4
	17	Arabica	26 ± 4
	18	Arabica	24 ± 4
	19	Arabica	1 ± 1
	20	Arabica	20 ± 4
India	21	Robusta	2 ± 2
	22	Robusta	1 ± 1
	23	Robusta	1 ± 2
	24	Robusta	1 ± 2
	25	Robusta	3 ± 2

[a] Details of the origin of the coffee are given in the Supporting Information (Table S1). [b] The σ_{ka} values were obtained from various ground green coffee beans of the same type and origin as described in the text. Standard deviations typically refer to measurements of five different ground beans.

The σ_{ka} values of the individual Arabica samples reveal a remarkable scattering ranging from 10 to 40. These variations not only refer to Arabica samples from different origins but also samples prepared from beans of the same origin exhibit σ_{ka} values in a relatively wide range as expressed by the standard deviations listed in **Table 1**. These standard deviations are larger by a factor of nearly 10 than the reproducibility of the spectroscopic analysis for an individual sample (3.5%; vide supra). Thus, these findings indicate a considerable bean-to-bean heterogeneity of the kahweol content, which can be expected given the long phenological cycle of these plants (8−9 months). Not only do coffee fruits take several months to ripen, but also fruits of the same coffee plant do not necessarily ripen at the same time. Due to uneven ripening, a tree will have fruits with different degrees of ripeness. It is quite common to see immature, mature, and overripe fruits simultaneously on the same branch. Hence, environmental conditions (e.g., temperature, humidity, water availability, light exposure) will influence the metabolism of such fruits at distinct developmental stages. It is also important to note that the beans analyzed in this study were obtained from the same geographical origin, and in some cases from the same plantation, but most probably from different coffee plants. This "biological" heterogeneity

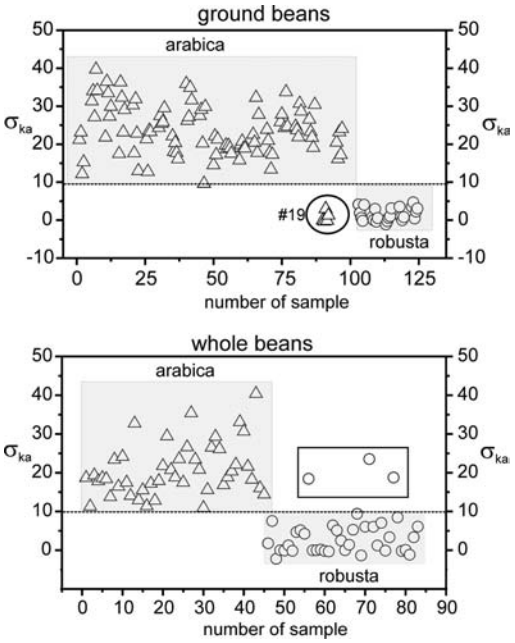

Figure 3. Spectral kahweol index σ_{ka} determined from the Raman spectra of various coffee samples: (top) σ_{ka} values obtained from 125 powder samples (**Table 1**), including 25 different types and origins, 5 different beans of each; (bottom) σ_{ka} values obtained from 83 whole bean samples (**Table 2**), including 12 different types and origins, 5−10 different beans of each. Samples originally classified as Arabica and Robusta are represented by triangles and circles, respectively. The characteristic range of σ_{ka} for Arabica and Robusta is indicated by shaded areas. Values that fall outside the expected range are indicated by the circular and rectangular frame. Sample numbers are indicated by "#" (see **Tables 1** and **2**).

Table 2. Average Spectral Kahweol Indices σ_{ka} of Whole Coffee Beans

origin[a]	sample	type	σ_{ka}[b]
Kenya	15	Arabica	18 ± 5
East Timor	20	Arabica	25 ± 6
Papua New Guinea	26	Arabica	22 ± 5
Peru	27	Arabica	23 ± 9
Panama	28	Arabica	23 ± 6
Honduras	29	Arabica	22 ± 11
India	21	Robusta	3 ± 2
India	23	Robusta	3 ± 4
Cameroon	30	Robusta	1 ± 4
Cameroon	31	Robusta	4 ± 6
Papua New Guinea	32	Robusta	4 ± 8
Laos	33	Robusta	7 ± 8

[a] Details of the origin of the coffee are given in the Supporting Information (Table S1). [b] The σ_{ka} values were obtained from various whole green coffee beans of the same type and origin as described in the text. Standard deviations typically refer to measurements of 5−10 different beans.

might account for the differences observed in chemical composition of various beans, for example, the kahweol content determined here.

Raman Spectroscopic Analysis of Whole Beans. In the second step we have applied the Raman spectroscopic analysis to 83 whole beans, including samples of four different origins for which also ground beans have been analyzed (**Table 2**; **Figure 3**, bottom; **Figure 4**, top). Here we note a good agreement when comparing the average σ_{ka} values determined for four ground and four nonground (whole) beans of the same type and origin. For Arabica coffees 20 and 15 the average σ_{ka} values of the ground

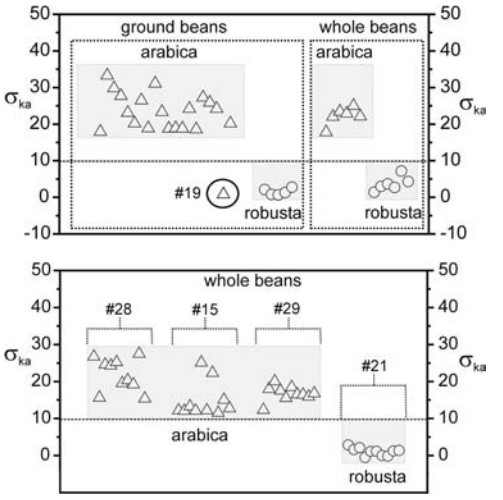

Figure 4. (Top) Average spectral kahweol index σ_{ka} obtained from the data in **Figure 3** upon averaging over the values of the various beans of the same type and origin; data referring to powder and whole bean samples are shown in the left and right dotted frames, respectively. The characteristic range of σ_{ka} for Arabica and Robusta is indicated by the shaded areas. The value for sample 19 unambiguously points to a Robusta coffee, although it was classified as Arabica upon delivery. (Bottom) σ_{ka} values obtained for 10 measurements from a single bean. The data refer to three Arabica and one Robusta sample. Sample numbers are indicated by "#" (see **Tables 1** and **2**).

(whole) beans were determined to be 20 ± 4 (25 ± 6) and 19 ± 4 (18 ± 5), respectively. Values for the Robusta coffees 21 and 23 were found to be 2 ± 2 (3 ± 2) and 1 ± 2 (3 ± 4), respectively. However, we note that the standard deviation is systematically larger for whole beans than for ground beans. In the case of Arabica coffee, the average standard deviations for individual beans of coffee from the same origin were determined to be 27 and 31% for ground beans and whole beans, respectively.

To identify the origin of the larger standard deviation for whole bean measurements, we have analyzed four different samples, that is, Arabica samples 28, 15, and 29 and Robusta sample 21, by using a single bean for 10 measurements upon refocusing the laser on different spots of the bean. **Figure 4** (bottom) displays the respective spectral kahweol indices σ_{ka} of 22 ± 4, 15 ± 5, and 17 ± 2 for Arabica beans 28, 15, and 29, respectively, whereas for Robusta bean 21 σ_{ka} is determined to be 1±1. Again, the standard deviations are nearly 1 order of magnitude larger (22%) than that of the reproducibility of the spectroscopic analysis (3.5%, vide supra). This finding implies that the scattering of σ_{ka} noted for a whole bean at randomly chosen different spots cannot be attributed to intrinsic error of the measurements and the data analysis but instead points to a heterogeneity of the kahweol distribution in the bean.

This heterogeneity has an impact on the Raman spectroscopic distinction between Arabica and Robusta coffees using whole bean measurements. In general, the data allow for a correct classification in terms of Arabica and Robusta on the basis of the σ_{ka} values of single beans (**Figure 3**, bottom; **Figure 4**, top). However, there are remarkable exceptions referring to three beans of three different Robusta batches (31−33; **Table 2**). In these cases, the σ_{ka} values are outside the normal variations of Robusta coffee (vide supra) and fall into the range indicative for Arabica (**Figure 3**, bottom). These unexpected values were found to be reproducible in independent measurements from the same beans. Because in these specific cases erroneous classification by the supplier is not very likely, the data indicate that individual

beans of Robusta batches may possess unusually high kahweol contents. This finding is quite surprising and has, to our knowledge, not yet been reported before because classical analytic approaches usually sample a large number of beans.

The observed heterogeneity could be related to differences in fruit maturation in time and to the impact of the biochemosphere. Further studies are necessary to evaluate the influence of environmental factors on kahweol content of green coffee beans. In this respect, the present Raman spectroscopic approach offers the opportunity to explore the parameters controlling the production of kahweol in coffee beans of the same type and origin. Comparing ground and whole beans in a systematic manner, one may clarify whether unusually high σ_{ka} values specifically for Robusta beans (vide supra; **Figure 3**, bottom) are partially or entirely the result of the (accidental) match of the laser spot with a region of high kahweol concentrations.

Potential of the Approach for Routine Analyses of Coffee. The spectral kahweol index introduced in this work to characterize the various samples and to distinguish between Arabica and Robusta is proportional to the actual kahweol content (e.g., in w/w of dry mass). The determination of the proportional factor requires calibration of the spectral index on the basis of samples of known kahweol content via chemical analyses. Such a calibration is a prerequisite for developing the present Raman spectroscopic approach toward a highly accurate and rapid analytical tool for coffee quality control. However, already the spectral kahweol index that is readily derived from the Raman spectra represents a reliable criterion to classify green coffee beans if the kahweol content is considered to be an adequate marker. In fact, previous chromatographic analyses have demonstrated that the kahweol content in green coffee beans varies between 0.11 and 0.35% of the dry mass for Arabica, whereas kahweol could be detected only as traces in Robusta (*16*, *27*, *32*, *34*). Particularly high kahweol contents have been determined for Arabica coffee from Brazil with ca. 0.6% in fresh fruits (*34*). These data are in qualitative agreement with the average kahweol indices for Arabica of 23 ± 5 and Robusta of 3 ± 3, as derived from all samples studied in this work (**Tables 1** and **2**). Among them, the Arabica coffees from Brazil exhibit a mean kahweol index that is higher than the average value of 23.

On the basis of the present results, one may estimate the detection limit for Robusta contaminations in ground and whole bean measurements. For powder measurements, the detection limit critically depends on the knowledge of the expected kahweol content for the pure Arabica batch. Assuming a 10 times higher kahweol content in Arabica than in Robusta and taking into account the accuracy of an individual measurement of 3.5%, the theoretical detection limit of Robusta in a sample of coffee powder is evaluated as ca. 4%. However, particularly in view of the quite substantial bean-to-bean variation of the kahweol content in coffee even of the same origin, an accurate reference value for the kahweol content of a specific Arabica sample will usually not be available. Then the accuracy of this procedure is determined by the variations of the kahweol content in Arabica coffees in general and thus will not be better than 10%. In this respect, analytical procedures that rely upon more than just one marker are more accurate (*28*, *32*).

The situation is different for the analysis on the basis of whole beans because the distinction between Arabica and Robusta is possible for individual beans given that a few measurements are carried out for the same bean to account for the heterogeneous intrabean kahweol distribution. Here the average kahweol indices for Arabica of 23 ± 5 and Robusta of 3 ± 3 can be considered as sound criteria. Then, the detection limit for Robusta contaminations in a batch of Arabica coffee solely depends on the number of beans investigated and thus may well compete with certified chromatographic procedures associated with a detection limit of 1% (*28*, *32*). Moreover, in contrast to the present approach, conventional analytical techniques, which lack this single-bean sensitivity and, instead, average over many beans, cannot distinguish between contaminations of coffee samples by Robusta beans and samples of pure Arabica coffee with somewhat lower kahweol content.

The main advantage of the present spectroscopic approach is that it can be performed without any time-consuming chemical processing of the coffee beans and, in the case of whole bean measurements, even without mechanical pretreatment. Furthermore, this approach does not rely on pattern-matching spectral techniques, which depend upon spectral differences regardless of the molecular origin or are sensitive to the sample (pre)treatment (e.g., water content). Instead, it is based on the relative contribution of a specific chemical ingredient of the coffee beans (i.e., kahweol) and thus may be applied as an accurate tool in all cases when the kahweol content is a classification criterion, that is, distinction between Arabica and Robusta, detection of admixtures of Robusta to Arabica, and possibly also the identification of different origins (*27*). A single Raman spectroscopic measurement of a coffee sample (powder or bean) does not require more than 6 min. Thus, the technique is much less time-consuming and less costly compared to chemical methods such that a reliable analysis of a given coffee sample can be obtained within less than an hour for powder samples and for whole beans within a few hours depending on the desired accuracy. Improved technical adaptation of the equipment to the needs of these specific experiments and optimization of the evaluation software are expected to further reduce the times required for the spectroscopic analysis. Fields of applications are, for example, quality control by coffee-roasting companies and coffee dealers or monitoring of the growth and development of beans at coffee plants.

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Supporting Information Available: Further details on the origin of the coffee beans (Table S1) and a representative overview Raman spectrum of a coffee bean. This material is available free of charge via the Internet at http://pubs.acs.org.

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