JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

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Quantification of the Robusta Fraction in a Coffee Blend via Raman Spectroscopy: Proof of Principle

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ABSTRACT: Among the 100 different known *Coffea* species, *Coffea arabica* L. (Arabica) and *Coffea canephora* Pierre (Robusta) are the only two of commercial interest. They differ in a range of agronomic, genetic, and chemical properties. Due to the significant price difference between Arabica and Robusta, there is an economic incentive to illicitly replace Arabica with Robusta. Therefore, it is crucial to have accurate methods to determine the Robusta-to-Arabica-ratio in blends. This paper presents the proof of principle of a new and fast approach to determine the Robusta fraction in a blend based on Raman spectroscopy. The oils of two references (a pure Robusta and pure Arabica coffee) and six blends thereof consisting of different Robusta and Arabica fractions were extracted using a Soxhlet system. The solutes were analyzed by means of Raman spectroscopy without further workup. Using the intensity ratio between two Raman peaks, one characteristic for kahweol and one characteristic for fatty acids, allowed determinination of the Robusta content in a given mixture. The intensity ratio is linearly dependent on the Robusta content of the compound. Above a Robusta content of 75 wt %, kahweol was not detectable. The Raman data are in agreement with results obtained from the very time-consuming multistep DIN 10777 procedures based on HPLC.

KEYWORDS: Raman spectroscopy, coffee, discrimination, Arabica, Robusta

INTRODUCTION

Rubiaceae is one of the largest botanical families of flowering plants and can be subdivided into three subfamilies: Rubioideae, Cinchonoideae, and Ixoroideae. The family consists of 43 tribes, 611 genera, and 13143 species.[1] All coffee plants belong to the tribe Coffea of the subfamily Cinchonoideae and comprise approximately 100 species. Only two of them are of commercial importance and are used for the beverage coffee: *Coffea arabica* L. (commonly known as Arabica) and *Coffea canephora* Pierre (commonly known as Robusta). Arabica and Robusta each account for approximately 65 and 35% of the world's production, respectively; other species with not much commercial value such as *Coffea liberica* and *Coffea excelsa* represent only 1%.[2]

Coffee is of high economic importance to more than 40 developing countries, and it is estimated that between 75 and 125 million people make their living in this sector (International Coffee Organization, ICO). Remarkably, the prices for coffee dramatically increased in 2010. According to the ICO's composite price indicator, the coffee price in the global market topped 170 cents per pound by the end of 2010 and has continued to increase in 2011, compared to the average price of 121 cents per pound recorded in October 2009. Moreover, the price gap between Arabica and Robusta coffee has significantly widened. The difference in price between Arabica and Robusta is caused by many differences between the two species, for example, the genetic makeup and physical and morphological properties. These lead to characteristic differences in flavor profiles between the species and cause a higher price for Arabica. One explanation for the widening of the price gap is that Robusta grows on flat land, whereas Arabica grows on mountain slopes. As a consequence, productivity advances have been faster in the Robusta sector due to an easier mechanization of the production. Another reason is the higher demand for high-quality (specialty) products mainly consisting of Arabica beans, which finally results in elevated Arabica prices.

One consequence of the increased price gap is a growing financial incentive to unlawfully replace Arabica with Robusta. It is therefore important to have precise and simple methods to determine the Robusta-to-Arabica ratio in coffee blends. In the form of green or roasted beans, the Robusta-to-Arabica ratio can be estimated visually (counting beans) with an approximate precision of about 5%. Green coffee beans are relatively simple to differentiate due to their size, as Arabica beans are normally larger than Robusta beans. Also, differences in the shape and color are normally visible. In the case of roasted coffee beans a size-based discrimination might lead to misleading results. Once ground, the Robusta fraction can be estimated only by either sensory or chemical means. In sensory assessments, an experienced taster can give at best an estimation with a precision of 20% for the Arabica-to-Robusta mix.

The alternative to sensory evaluation is chemical analysis.[3] Nowadays, chromatographic techniques are well-established methods to distinguish the two species (see DIN 10777[4]). The chemical composition of Arabica and Robusta differs in a variety of compounds including the following: tocopherol,[5,6] chlorogenic acid, caffeine, free amino acids,[7] fatty acids,[8] and polysaccharides.[9] Besides the chromatographic approaches, also spectrographic methods such as Fourier transform infrared spectroscopy[10] are able to discriminate Arabica and Robusta beans. Esteban-Daiz et al.[11] showed that near-infrared spectroscopy (NIRS) is capable not only of distinguishing the two species

Received: May 14, 2011
Revised: August 9, 2011
Accepted: August 10, 2011
Published: August 10, 2011

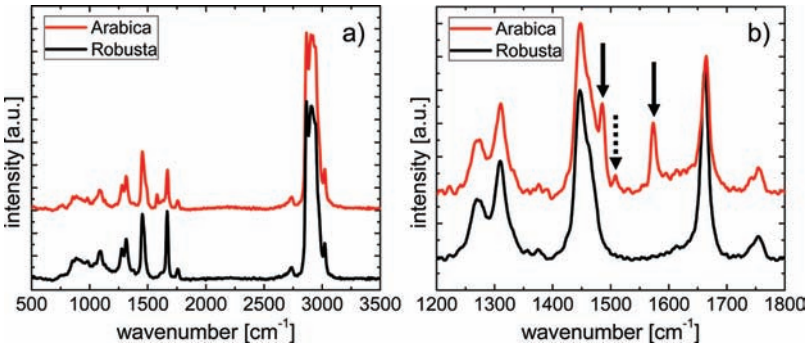

Figure 1. (a) Raman spectra of extracted oil from Robusta and Arabica beans. (b) Close-up of the same spectra. The solid arrows at 1485 and 1570 cm^{-1} as well as the dotted arrow at 1507 cm^{-1} mark peaks appearing only in the spectrum of Arabica.

but also of measuring the Robusta content in a Robusta/Arabica blend.

In addition to the above-mentioned components, the lipid fractions of green and roasted Arabica and Robusta coffee beans are rich in two diterpenes specific for coffee: cafestol and kahweol.[12,13] The total diterpene content ranges from 1.3 to 1.9% (w/w) in green Arabica beans and from 0.2 to 1.5% in green Robusta beans.[10−12] Rubayiza and Meurens showed in 2005[2] that it is possible to discriminate the extracted lipid fraction of Arabica and Robusta by means of Raman spectroscopy due to kahweol, which is almost exclusively present in Arabica beans. Recently, Keidel et al. demonstrated that Fourier transform Raman spectroscopy can be also applied to identify the species in ground coffee or whole beans.[3] The advantage of this approach is that time-consuming chemical or even mechanical processing is obsolete.

In general, Raman spectroscopy allows the identification of chemical compounds due to characteristic Raman bands. In this work, we demonstrate that the relative intensities (ratios) of specific bands in the Raman spectra of coffee oil can be used to quantitatively analyze the Arabica-to-Robusta ratio in a blend. The main purpose of the work is to establish a new and simple process with minimal sample workup and relatively fast analysis that allows accurately determination of the Arabica-to-Robusta ratio and provides a proof of principle for the approach developed here. Not addressed in this paper is the question of the variability of these ratios with different coffee species and processing; this will the subject of future studies.

EXPERIMENTAL PROCEDURES

Sample Preparation. Roasted coffee beans from Vietnam (Robusta) and Brazil (Arabica) were examined. Besides references of pure Arabica and Robusta, Robusta/Arabica mixtures with Robusta contents of 5, 10, 25, 33, 50, and 75 wt % were used. Ten grams of ground coffee of each mixture (including pure Arabica and Robusta) and 20 g of sodium sulfate were comminuted in a mortar. The powder was transferred into a Soxhlet filter and extracted with *tert*-butyl methyl ether (tBME) at 55 °C for about 5 h. Approximately 3 mL of the solution was used for the Raman analysis. After extraction, the tBME was completely removed under vacuum. An additional 5 mg of sodium L(+)-ascorbate was added to the residue; 80 mL of an ethanolic KOH solution (10 g of KOH in 90% v/v ethanol/water) was added, and the mixture was saponified for 2 h under reflux. After saponification, the ethanolic KOH solution was concentrated in vacuo. With portions of 80 mL of hot water, the residue was transferred in a 500 mL separating funnel. The flask was rinsed with 50 mL of methanol, and 20 mL of a 10% sodium chloride solution was added to the separating funnel. The solutions were mixed with an additional 100 mL of tBME. After separation of the organic layer, the procedure was repeated. The collected organic layers were solvent extracted with 100 mL of a 2% sodium chloride solution. The organic layers were dried with 7.5 g of sodium sulfate for 10 min. The dried layer was filtered and transferred in a round-bottom flask. The tBME was completely removed under vacuum, and the residue was transferred with dichloromethane to a reaction tube. In the following the dichloromethane was removed in a stream of N_2. Finally, the obtained residue was dissolved in 1.5 mL of acetonitrile and filtered through a syringe filter (0.45 μm). The sample solution was transferred in a HPLC vial and injected.

Raman Spectroscopy Experiments. The extracts of the Soxhlet procedure were analyzed with a confocal Raman microscope (alpha 300, WITec, Germany) equipped with a green laser of 532 nm and a 20×x objective. The samples for the Raman experiments were prepared by putting one drop of the Soxhlet solution onto a glass slide. While the tBME evaporated, the extracted substances remained on the glass slide. For every sample at least seven Raman spectra were recorded. Each spectrum was recorded at a different area.

All mixtures and references were measured 0, 2, 7, and 10 days after conclusion of the Soxhlet extraction. The samples were stored in solution and at ambient conditions in the absence of light. Additionally, the aging behavior of the coffee extracts was examined. For this purpose the Raman spectrum of Arabica oil stored in light was compared to those of an Arabica oil stored in the dark for 0, 4, 7, or 10 days.

Quantitative Analyses of 16-Methyl-*O*-cafestol with HPLC. The chromatographic analyses were performed with an Agilent 1200 series HPLC equipped with an EC Nucleosil C18, 5 μm, 100 Å column (125 mm × 4 mm i.d., Machery Nagel, Switzerland). Detection was done with a DAD at 223 nm. Mobile phase A was water, and mobile phase B was acetonitrile. The gradient mode was initially set at 50% B for about 25 min and then linearly increased to 100% B at 25−35 min and held to 45 min.

The flow rate was 0.8 mL/min, and the injection volume was 50 μL. Concentrations of 16-methyl-*O*-cafestol were calculated using the linear regression equation of their concentration in coffee bean mixture and peak area. Identification was done with a 16-methyl-*O*-cafestol standard, comparing the retention time.

RESULTS AND DISCUSSION

Raman. Figure 1 illustrates the Raman spectra of the lipid fraction of Arabica and Robusta references. Figure 1a shows the complete Raman spectrum, whereas Figure 1b is a close-up of the fingerprint region. The spectra of Robusta and Arabica are similar and show a number of identical peaks. The intense Raman bands in the region of 2800−2900 cm^{-1} correspond to symmetric and asymmetric C—H stretching vibrations,[14,15] whereas the small peak at 3014 cm^{-1} originates from stretching vibrations

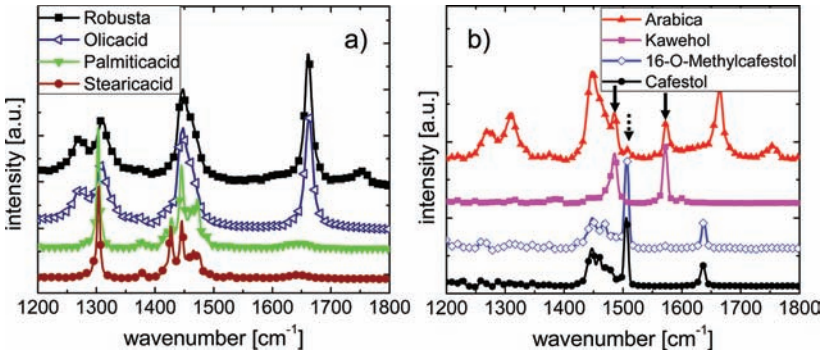

Figure 2. (a) Raman spectra of oleic, palmitic, and stearic acid and pure Robusta. The strong correlation between oleic acid and Robusta indicates that oleic acid is the main constituent of the extracted oil. (b) Comparison between the spectra of Arabica, kahweol, cafestol, and 16-methyl-*O*-cafestol. The two peaks in the Arabica spectrum marked by solid arrows are due to kahweol. The small peak (dotted arrow) can be assigned to cafestol.

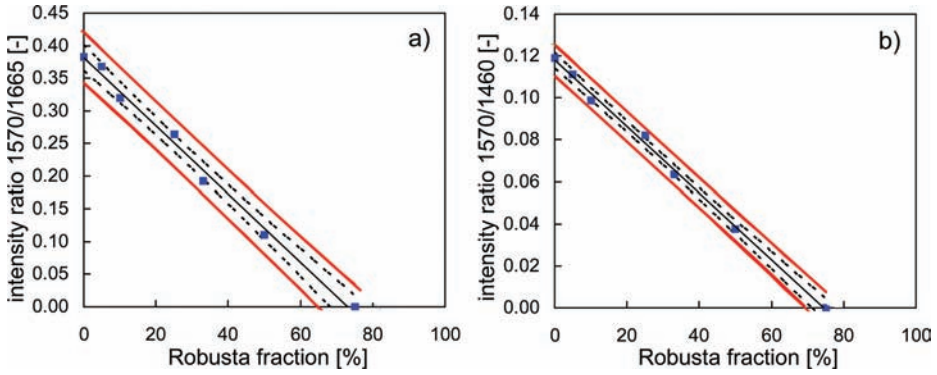

Figure 3. Intensity ratios between (a) the Raman peaks at 1570 and 1665 cm^{-1} and (b) the Raman peaks at 1570 and 1460 cm^{-1} as a function of the Robusta fraction. The black lines are a linear regression of the data. The black dotted lines mark the 95% tolerance interval, whereas the red lines correspond to the 95% confidence interval.

of an ethylenic group. At about 1750 cm^{-1} a peak due to C═O stretching vibrations appears. The peak at 1665 cm^{-1} is caused by the carbon C═C stretching vibration. The bands at 1460 and 1310 cm^{-1} correspond to methylene scissoring deformation vibration and in-phase methylene twist vibration, respectively. The peak at 1270 cm^{-1} can be assigned to a ═CH vibration.[12,16−18]

Figure 1b highlights two major peaks at 1485 and 1570 cm^{-1} (solid arrows) and one minor peak at 1507 cm^{-1} (dotted arrow) that appear only in Arabica. The two major peaks allow an explicit discrimination between Arabica and Robusta by means of Raman spectroscopy.

The lipid fraction of coffee consists of a mixture of different fatty acids.[19] To identify the main constituents of the extracted oils, the spectrum of Robusta was compared with the spectra of oleic, palmitic, and stearic acid (see Figure 2a). The spectrum of Robusta and oleic acid are nearly identical. Therefore, the extracted oil of Robusta consists mainly of oleic acid or fatty acids very similar to oleic acid. Figure 2b compares Arabica with the Raman spectra of kahweol, cafestol, and 16-methyl-*O*-cafestol, which are naturally occurring diterpenes found in coffee.[14] The plot illustrates that the peaks at 1485 and 1570 cm^{-1} (solid arrows) in Arabica are due to kahweol. These peaks are absent in the spectrum of Robusta as Robusta contains only small traces of kahweol. The peak at 1507 cm^{-1} in the Arabica spectrum (dotted arrow) can be assigned either to cafestol or to 16-methyl-*O*-cafestol. As 16-methyl-*O*-cafestol is generally present only in Robusta beans,[13,20] it is highly plausible that this peak corresponds to cafestol.

Table 1. Values of Axis Intercepts and Slopes of the Linear Fits of the Measurements Performed 0, 2, 7, and 10 Days after Extraction

	intensity ratio 1570/1665 cm^{-1}		ontensity ratio 1570/1460 cm^{-1}	
days after extraction	axis intercept	slope ($\times 10^{-3}$)	axis intercept	slope ($\times 10^{-3}$)
0	0.382	−5.23	0.118	−1.58
2	0.346	−4.37	0.116	−1.41
7	0.310	−4.15	0.120	−1.58
10	0.308	−3.88	0.119	−1.41

The Raman peaks corresponding to kahweol in the spectra of Arabica enable a quantitative determination of the respective fraction in a Robusta/Arabica mixture. One approach to calculate the proportion of a certain mixture is analyzing the intensity ratio between the kahweol peak at 1570 cm^{-1} and the Raman peaks of the fatty acids at 1460 or 1665 cm^{-1}. Plotting the intensity ratios rather than the absolute values has the advantage that artifacts such as the focus and the intensity of the laser are negligible. Figure 3 shows (a) the 1570/1665 cm^{-1} intensity ratio and (b) the 1570/1460 cm^{-1} intensity ratio. In both panels, the black

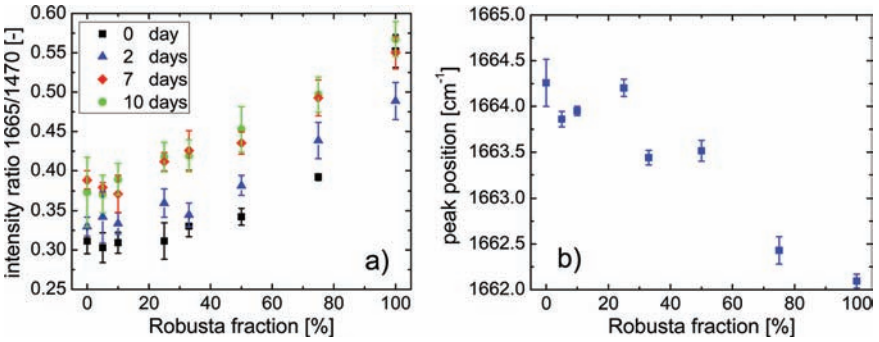

Figure 4. (a) 1665/1460 cm^{-1} intensity ratio with increasing fraction of Robusta measured 0, 2, 7, and 10 days after extraction of the oil. (b) Shift of the peak position at 1665 cm^{-1} depending on the Robusta content.

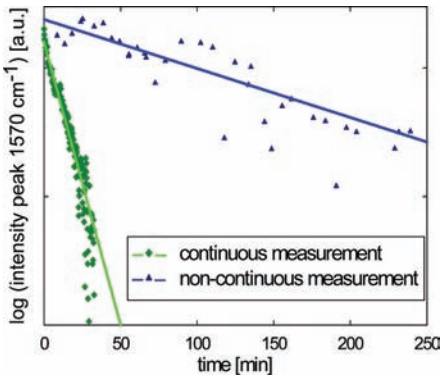

Figure 5. Intensity of the kahweol peak at 1570 cm^{-1} for "continuous measurement" performed at one spot (green diamonds) and for "noncontinuous measurement", where every data point was acquired at a different spot of the sample (blue triangles).

lines correspond to the linear regression of the data. The black dotted lines mark the 95% tolerance interval, whereas the red lines correspond to the 95% confidence interval. Both plots show the same behavior: a linear decrease of the intensity ratio with increasing Robusta content. The highest Robusta content in a mixture was 75 wt %. This seems to be near the minimal Arabica concentration still detectable. According to the confidence interval (red lines), the detection limit in the case of the 1570/1665 cm^{-1} intensity ratio is approximately 7.5%, and it is 4.9% for the 1570/1460 cm^{-1} intensity ratio.

The results were fitted linearly by means of a least-squares method. Table 1 lists the values of the axis intercept and the slope of the linear fits measured after 0, 2, 7, and 10 days of storage. In the case of the 1570/1665 cm^{-1} intensity ratio the axis intercept and slope decrease with increasing storage time. In the measurement for 0 days the axis intercept and the slope are 0.382 and -5.23×10^{-3} wt %$^{-1}$, respectively. After 10 days, the axis intercept has decreased to 0.308, whereas the slope dropped to -3.08×10^{-3} wt %$^{-1}$. This behavior might be caused by a decreased kahweol concentration as it is chemically not stable[18] or due to an increased peak at 1665 cm^{-1} due to oxidation processes.[19] The trend of decreasing slopes and axis intercepts with increasing storage time is not detectable for the 1570/1460 cm^{-1} intensity ratio. As a consequence, the trend visible for the 1570/1665 cm^{-1} intensity ratio is caused by an increased intensity of the peak at 1665 cm^{-1} due to oxidation processes.

Figure 4a shows the intensity ratio between the C═C peak at 1665 cm^{-1} and the CH_3 band at 1460 cm^{-1}. The samples were measured 0, 2, 7, and 10 days after extraction. Robusta contains proportionally more unsaturated fatty acids than Arabica,[21,22] which causes the increase of the 1665/1460 cm^{-1} ratio with increasing Robusta content. Spectra acquired after a specific storage time exhibit the same trend: the intensity ratio is constant up to a Robusta concentration of 10 wt %, whereas it shows a linear increase of the ratio for higher Robusta contents. With increasing storage time of the samples, the ratio for a specific mixture shifts to higher values. The shift in the first two days is stronger than that between 7 and 10 days. The increase of the ratio is probably due to oxidation processes, which causes an increase of the intensity of the peak at 1665 cm^{-1}.

Figure 4b displays the position of the peak at around 1665 cm^{-1} as a function of the Robusta fraction. The peak positions were obtained by applying Gaussian fits to the data. With an increasing amount of Robusta, the peak position is shifted to lower wavenumbers. The shift between pure Robusta and pure Arabica is approximately 2 cm^{-1}. This shift might be caused by a change in the distribution among different unsaturated lipids in Robusta compared to Arabica.[22,23] Although the scattering of the data is rather large, the shifts are significant and reproducible.

Kahweol and cafestol are known to be sensitive to acids, heat, and light.[24] As Raman spectroscopy is a light-based method, it is crucial to analyze the influence of the laser on kahweol. Figure 5 illustrates the time-dependent decrease of kahweol. In the experiment using "continuous measurement", the Raman spectrum was collected continuously at one specific spot of a coffee oil stain. In the experiment using "noncontinuous measurement", a spectrum was collected approximately every 10 min, always at a different spot of the coffee oil stain. Between measurements the laser was turned off. During both experiments the samples were exposed to daylight. In continuous measurement the kahweol peak decreases rapidly. After approximately 30 min, the Raman signal has vanished. In noncontinuous measurement the decay is significantly slower. Even after 240 min the kahweol peak is still detectable. Exposure to light leads to a degradation of kahweol and therefore influences negatively the accuracy of the measurements. The influence of the light on the results of the samples presented in Figure 3 is marginal. The acquisition time for one spectrum was only 10 s. Furthermore, each spectrum was measured at a different spot on the coffee oil stains and between measurements the laser was turned off. The maximal light exposure time of the samples was 5 min. The noncontinuous

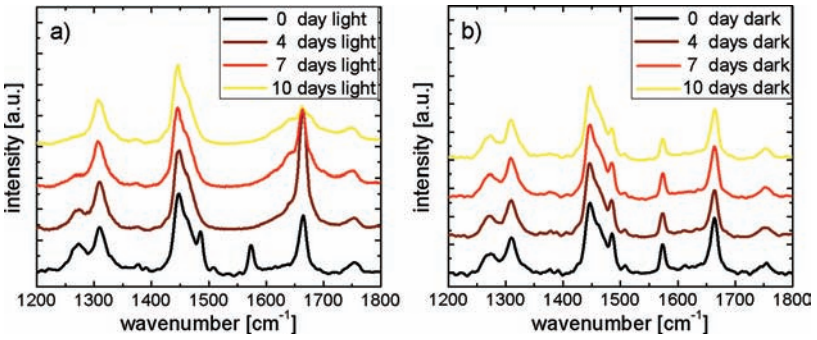

Figure 6. Raman spectra of solid Arabica oil measured 0, 4, 7, and 10 days after the extraction in the case of samples stored (a) in light or (b) in the dark. The arrows on both figures highlight peaks that alter with increased storage.

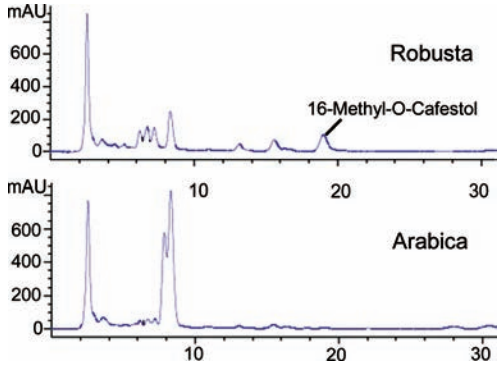

Figure 7. Typical HPLC chromatograms of Robusta and Arabica coffee extracts. Only the Robusta extract shows a 16-methyl-*O*-cafestol peak.

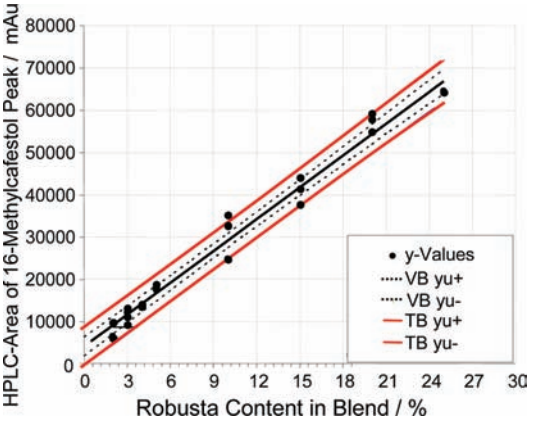

Figure 8. Intensity of the 16-methyl-*O*-cafestol peak depending on the Robusta content of the coffee blend: linear regression with 95% confidence and 95% tolerance intervals.

measurement shows that such a short time has only a minor influence.

Table 1 and Figure 4a indicate that the oil oxidizes during storage. Figure 6 presents Arabica spectra collected during 10 days. The samples were stored as solutions, under ambient conditions in daylight (Figure 6a) or in the absence of light (Figure 6b). Exposure to daylight alters the spectra of Arabica significantly. After 4 days, two main changes occur. First, the two kahweol peaks (marked by solid arrows) vanish completely. Second, the intensity of the peak at 1665 cm^{-1} drastically increased, which is due to oxidation processes. It is assumed that it derives from conjugated dienes that are formed during the oxidation process of oils. They can occur in different isomeric configurations and lead to strong Raman peaks.[23] Over storage time, the intensity of each of the two peaks, at 1665 cm^{-1} and at 1270 cm^{-1}, decreases. The intensity of both peaks (dotted arrows in Figure 6a) correlates directly to the C=C bond concentration. Therefore, the oxidation leads to a diminishing of unsaturated fatty acids. The samples stored in the dark show only minor changes. The intensity of the kahweol peaks (see solid arrows in Figure 6b) slightly decreases with time. Nevertheless, even after 10 days, the peaks are clearly visible.

HPLC. HPLC is a well-established method for the analysis of coffee beans based on their chemical composition. This makes it the perfect technique to verify the results obtained by Raman spectroscopy. Robusta can be detected and quantified by using the Arabica 16-methyl-*O*-cafestol peak at approximately t_r = 19.1 min, which appears only in the Robusta samples (see Figure 7).

Figure 8 illustrates the intensity of the 16-methyl-*O*-cafestol peak measured by means of HPLC depending on the Robusta content of the coffee blend. The detection limit of HPLC is in the range of 3.7 wt % Robusta in the blend. The result of the HPLC measurement shows a similar linear behavior of the Robusta content in the blend as in the Raman measurements. Hence, the results obtained by HPLC are a good verification of the reliability of the results based on Raman microscopy.

The results presented here demonstrate that Raman spectroscopy is a valuable tool for analyzing the mixing ratio of Robusta and Arabica. Here we have demonstrated that a series of ratios of Raman peaks, measured in extracted coffee oil, can provide the Arabica-to-Robusta ratio in a blend of known species. Using Raman spectroscopy has several advantages compared to alternative techniques:

- Raman spectroscopy enables the measurment of the mixing ratio of a blend via several approaches. The most promising approach is measuring the relative kahweol content (intensity ratio). Furthermore, the intensity ratio between peaks from C=C vibrations at 1665 cm^{-1} and CH_3 vibrations at 1460 cm^{-1} can be used as a second measure for the same ratio. Finally, the peak position at 1665 cm^{-1} of a pure Arabica is shifted to lower wavenumbers with increasing Robusta content due to the higher content of unsaturated fatty acids in Robusta compared to Arabica, providing a third measure for the Arabica-to-Robusta ratio. Because all three values are measured in the same Raman spectrum, they can be combined to increase the accuracy of the results.

• Sample preparation and measurements are relatively fast and uncomplicated. Time-consuming and work-intensive preparation of the samples, which is needed for HPLC experiments, is greatly reduced.

• The measurements give additional information about the composition of the fatty acids such as the content of unsaturated fatty acids.

• Samples stored in the dark at room temperature are stable. Therefore, measurements can be repeated after several days without the preparation of new samples.

Drawbacks of the methods are the following:

• Kahweol is not stable in light. Long laser irradiation of the samples has to be avoided as it would negatively influence the measurements. To gain comparable results with high accuracy, the measured samples must have the same sample treatment history.

• The detection limit, which in the presented study is in the range between 4.9 and 7.5%, to improve the accuracy of measurements.

The work presented here was conducted on known blends of Arabica and Robusta species to develop the method and evaluate its accuracy. In subsequent projects we will explore the variability of the proposed values as a function of coffee species and processing. This will help to better define the scope and applicability of the methods in situations when the coffee species and/or the postharvest treatments are not known.

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ACKNOWLEDGMENT

We acknowledge Calo Delfs from Strauss Commodities AG for providing the different coffee samples. Furthermore, we thank Roman Büttiker for his valuable input and thoughts regarding the HPLC measurements.

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