

Characterization of Peppercorn, Pepper Oil, and Pepper Oleoresin by Vibrational Spectroscopy Methods

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NIR-FT-Raman as well as ATR-IR and NIR spectroscopy in combination with efficient chemometric algorithms was applied for rapid determination of piperine in black and white ground pepper and green whole pepper berries as well as pepper oleoresins. Most of the well-resolved Raman signals detected in the spectra of pepper and the related oleoresins can be assigned to piperine, which is known to be the main pungent principle in these products. On the basis of the specific key bands of piperine, also selective Raman mappings were successfully performed to determine the in situ distribution of the alkaloid in the whole green berry and the dried peppercorn as well. It was found that piperine occurs more or less in the whole perisperm of the green fruit. Furthermore, the content and composition of the volatile fraction in various pepper samples were determined by applying the mentioned vibrational spectroscopy techniques. Whereas only NIRS measurements present sufficient reliability to predict the main essential oil substances directly in ground black and white pepper, all spectroscopy methods applied in this study can be used to analyze individual terpenoids in the hydrodistilled oil. It can be assumed that some of the new, efficient vibrational spectroscopy methods have the potential to replace the standard analysis procedures presently applied for the quality control of peppercorns, pepper extracts, and pepper oil.

KEYWORDS: NIR-FT-Raman; ATR-IR; NIRS; piperine; pepper essential oil; quality; chemometrics

INTRODUCTION

The most important exporters of black pepper are India (Malabar and Tellicherry pepper), Indonesia (Lampong pepper), and Brazil and Malaysia (Sarawak pepper). Pepper cultivars grown in the provenances of Tellicherry and Malabar are known for their excellent aroma and pungency properties. Whereas the flavor of Lampong pepper is more or less similar to the *Malabar* type, the taste of Malaysian and Brazilian varieties is comparatively milder. Black pepper varieties obtained from other countries are usually less valued, as they vary in heat and mostly lack the complex aroma found in Indian and Malaysian cultivars. The most important source of white pepper is the small Indonesian island of Bangka, located southeast of Sumatra; the peppercorns obtained from this area are named Muntok after the island's main port (1).

The main pungent principle in the green berries of pepper (*Piper nigrum* L.) is piperine. At least five other alkaloids,

structurally related to piperine, also occur in smaller amounts. "Black pepper" is obtained from unripe green berries on sun-drying, whereas fully ripe dried fruits devoid of pericarp form the commercial "white pepper". Generally the piperine content of black or white peppercorns lies within the range of 3–8 g/100 g, whereas the content of the minor alkaloids piperidine and piperettine have been estimated as 0.2–0.3 and 0.2–1.6 g/100 g, respectively (1). The aroma of pepper is determined by the composition of the essential oil usually obtained by steam distillation of black peppercorns (2). In the past several years also supercritical fluid extraction (SCFE) of black pepper using liquid CO₂ as solvent has received much attention; it has been found that this extraction technology is an efficient process resulting in high yields and authentic aroma profiles (3, 4). According to former studies (5–7) the volatile fraction of pepper oil is dominated by monoterpene hydrocarbons (30–70%) such as α - and β -pinene, limonene, sabinene, myrcene, α -phellandrene, and δ -3-carene. Sesquiterpenes such as α - and β -caryophyllene and β -farnesene make up ~25–45% of the total essential oil content, whereas oxygen-containing sesquiterpenes such as caryophyllene oxide occur in amounts of 4–14%. It is well-known that the composition of black pepper oil can vary considerably relating to the origin (cultivar), harvest year, and

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ripening stage (5, 6). The pinenes possess a turpentine–eucalyptus-like smell, whereas the odor of the other monoterpenes is described as fresh and pine-needle-like. An optimal pepper aroma quality is obtained if the content of monoterpenes (excluding α - and β -pinene) is high and at the same time the amount of pinenes is low. Generally the so-called “top-peppery-note” is assigned to the monoterpene group; the “pepper odor” is related to the sesquiterpenes, and the oxygen-containing sesquiterpenes are made responsible for the “body” of the pepper aroma.

The International Organization for Standardization (ISO) (8), the American Spice Trade Association (ASTA) (9), and the Association of Official Analytical Chemists (AOAC) (10) have published spectroscopic methods for piperine determination, involving UV–vis absorbance measurements at the piperine absorption maximum near 343 nm. To get a more specific determination of piperine (excluding the other pepper alkaloids), a number of HPLC methods have been described (11–13). However, all methods presently available for the estimation of piperine in pepper and its products are more or less time-consuming and need the use of organic solvents. Therefore, the aim of this study was to develop rapid and nondestructive vibrational spectroscopy methods for the rapid determination of pungency. Furthermore, it was aimed to use these spectral data simultaneously for classifications of pepper oils according to their different monoterpene and sesquiterpene compositions.

MATERIALS AND METHODS

Reagents and Plant Material. The analyzed pepper samples were obtained from the following spice companies: Symrise GmbH & Co. KG, Holzminden Germany; Gewürzmüller GmbH, Stuttgart, Germany; Raps & Co., Kulmbach, Germany. Pure standard substances (piperine, α -pinene, β -pinene, myrcene, limonene, δ -3-carene, sabinene, β -caryophyllene, caryophyllene oxide, α -phellandrene) were purchased from Sigma-Aldrich (Taufkirchen, Germany) and Roth (Karlsruhe, Germany). The other reagents were used without further purification.

Sample Preparation and GC Measurements. The ground pepper samples were hydrodistilled according to the official ASTA method 5.0 (9), and the received essential oils were subsequently analyzed by gas chromatography by applying a Hewlett-Packard gas chromatograph 5890 series II, fitted with an HP 5 column (50 m \times 0.32 mm i.d.; film thickness = 0.52 μ m) and a flame ionization detector (detector and injector temperatures were set at 280 and 250 °C, respectively). The following oven temperature program was used: linear increase from 60 to 220 °C at a heating rate of 10 °C/min; the final temperature was held for 10 min. Carrier gas was nitrogen with a constant flow rate of 1 mL/min (split 1:40).

GC-MS analyses of the isolated essential oils were performed using an Agilent MSD 5972/HP 5890 series plus 2, equipped with a 30 m \times 0.25 mm i.d., 0.25 μ m CP-Sil8 Chrompack column. The oven temperature was programmed from 70 to 220 °C at a heating rate of 4 °C/min, and the final temperature was held for 20 min. Injector and detector temperatures were set at 250 and 280 °C, respectively. Carrier gas was helium with a constant flow rate of 1 mL/min (split 1:20). The ionization energy was set at 70 eV. Identification of the detected compounds was based on their relative retention time and their mass spectra in comparison with those observed by the above-mentioned pure standard substances. The other compounds were tentatively identified by using the NBS75K and Wiley 138 library databases of the GC-MS system. The percentage composition was computed from the GC peak areas according to the 100% method without using any correction factors.

Reference HPLC Analysis of Piperine. Approximately 50 mg of the powdered pepper seeds, accurately weighed, was extracted with 10 mL of 96% ethanol using an Ultra-Turrax at 13500 rpm. The resulting extract was centrifuged at 3000 rpm, decanted in a 10 mL flask, and filled to the mark. An aliquot of the filtered solution was directly used for the subsequent HPLC analysis. The HPLC separation

was performed using a 3.5 μ m C18 column (150 \times 3.0 mm i.d., Zorbax SB). The column was kept at 30 °C using a column oven. Eluents were (A) 50% acetic acid/water (1:99, v/v) and (B) 50% acetonitrile. Flow rate was 0.6 mL/min, and UV detection was set at 343 nm.

Attenuated Total Reflectance (ATR)-IR Spectroscopy. Mid-infrared analyses of ground peppercorns and the related hydrodistilled pepper oils were carried out on a portable ATR/FT-IR spectrometer (Resultec Analytic Equipment, Oberkirchberg, Germany) using a single reflection cell. Approximately 5–10 μ L of the individual pepper oil was placed directly on the surface of the diamond–Zn–Se ATR crystal. For measurements of the solid samples \sim 2–5 mg of powdered pepper was taken; an intimate contact between the sample and the sensing device could be achieved by the use of a load-restraining pressure applicator. An overpressure restraint and a visual display of the actually existing pressure prevented the risk of overpressurization and ensured that a reproducible amount of pressure was applied from sample to sample.

NIR Spectroscopic Measurements. Measurements on ground peppercorns (51 different samples) were performed by an FT-IR spectrometer system IFS 55 Equinox (Bruker GmbH, Ettlingen, Germany) equipped with a light source, beam splitter, detector, and y-shaped fused silica fiber suitable for the NIR region. Approximately 1–2 g of powdered peppercorns was transferred into an open 30 mm diameter spinning sample cup. The fiber optics probe was placed with a constant distance above the sample material, so that the measuring spot had a diameter of \sim 4 mm. In all cases the spectrum was averaged from 12 interferometer scans. The wavenumber region for the analysis was 4000–12000 cm^{-1} . The isolated pepper oils (minimum sample amount = 600 μ L) were analyzed in the transfection mode using quartz cuvettes (path length = 0.5 mm).

Raman Measurements. Raman spectra were recorded by applying an NIR-FT-Raman spectrometer of Bruker (model RFS 100) equipped with a diode-pumped Nd:YAG laser, emitting at 1064 nm, and a germanium detector cooled with liquid nitrogen. The following parameter set was used: laser power = 50 mW, spectral resolution = 4 cm^{-1} , number of accumulated scans = 128. The Raman mapping was performed by using an xy stage directly connected with the Raman spectrometer. For mapping studies the laser energy was reduced to 30 mW and the individual spectra were recorded at a resolution of 4 cm^{-1} , accumulating four scans per spectrum. The mapping areas were 5500 \times 5500 μ m for black peppercorns and 6500 \times 6000 μ m for green pepper fruits, applying 150 μ m increments each time.

Chemometrics. Chemometric analyses of the ATR-IR and NIR spectra were performed using a commercial software program (Opus/Quant 2.0, Bruker GmbH, Rheinstetten, Germany), whereas interpretation of the NIR Raman spectra was carried out with the commercial statistic program GRAMS (Galactic Ind., Salem, NH).

The spectral data were transformed with first-derivative processing (IR, NIR) as well as MSC (Raman) and subsequently mean centered. The following wavenumber ranges were used for the development of calibration equations: 4000–12000 cm^{-1} (NIR), 700–4000 cm^{-1} (IR), and 200–2000 cm^{-1} (Raman).

The calibration program was set up using the partial least-squares (PLS) algorithm. All data in the calibration set were checked carefully to detect and eliminate outlier samples.

Generally, statistical accuracy is described by the coefficient of determination (R^2) and the standard error of cross-validation (SECV). The optimum number of PLS factors for each component was determined by application of the predictive residual error sum of squares (PRESS) calculation.

RESULTS AND DISCUSSION

The FT-Raman spectra obtained from green pepper berries, ground black pepper, and black pepper oleoresin, presented in **Figure 1**, predominantly show significant key signals of piperine. Apart from the intense –C–H stretching vibrations between 2800 and 3100 cm^{-1} , the main Raman signals occur in the fingerprint range between 1100 and 1630 cm^{-1} . The spectra of black pepper and the related oleoresin present very

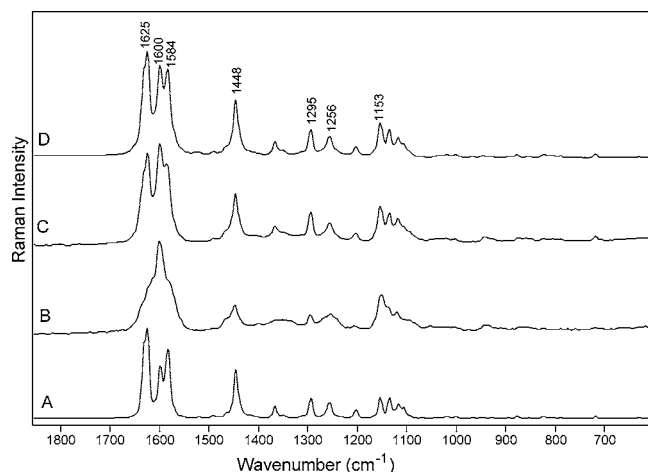


Figure 1. FT-Raman spectra of pure piperine (A), intact green pepper berries (B), ground black pepper (C), and black pepper oleoresin (D).

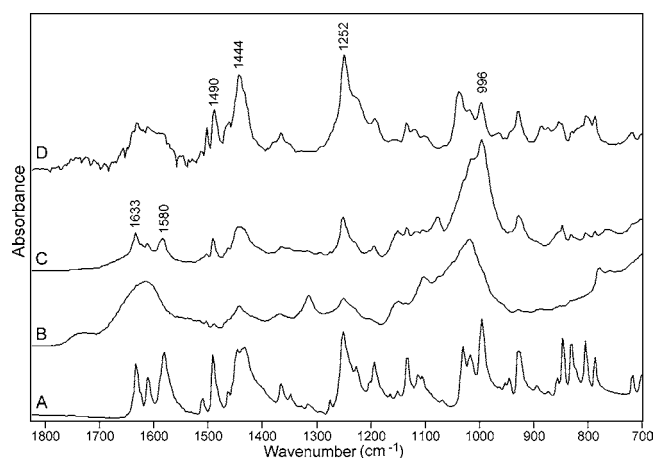


Figure 2. FT-IR spectra of pure piperine (A), green pepper berries (B), ground black pepper (C), and black pepper oleoresin (D).

good resolution of the aromatic and aliphatic $\text{C}=\text{C}$ as well as $\text{>N}-\text{C}=\text{O}$ stretching vibrations detected between 1580 and 1635 cm^{-1} . The signal observed at 1448 cm^{-1} is assigned as a $-\text{CH}_2$ bending vibration, whereas the other bands in the range of 1100 and 1400 cm^{-1} are mainly due to $-\text{C}-\text{C}-$ stretching (1153 cm^{-1}) as well as $-\text{CH}_2$ twisting and rocking vibrations (1295 and 1256 cm^{-1}) of the piperine molecule. The corresponding IR spectra of pepper fruit and pepper oleoresin (Figure 2) show mainly signals due to $\text{C}=\text{O}-\text{C}$ stretching vibrations at 1194 and 1252 cm^{-1} as well as CH_2 wagging vibrations at 996 cm^{-1} ; compared with the related Raman spectra, the IR absorptions are less resolved. Whereas the IR spectrum of green pepper is dominated by broad water bands (e.g., around 1600 cm^{-1}) as well as signals related to other main components (e.g., hydrocarbons), the IR spectrum of ground black pepper reveals several specific piperine signals (e.g., detected at 1633, 1580, 1252, and 996 cm^{-1}). Due to the significantly higher concentration of pepper alkaloids found in the oleoresins ($\sim 30\%$) the corresponding IR spectrum presents most of the characteristic IR signals of the pure piperine standard (Figure 2D).

On the basis of the individual key bands in the range between 1580 and 1635 cm^{-1} it is possible to determine the distribution of piperine in a peppercorn in situ by NIR-FT-Raman microspectroscopic mapping. As shown in Figure 3, the pungent principle is distributed more or less in the whole perisperm of the green fruit; only the endosperm, located in the center of the berry, contains lower amounts of piperine. Also, the black

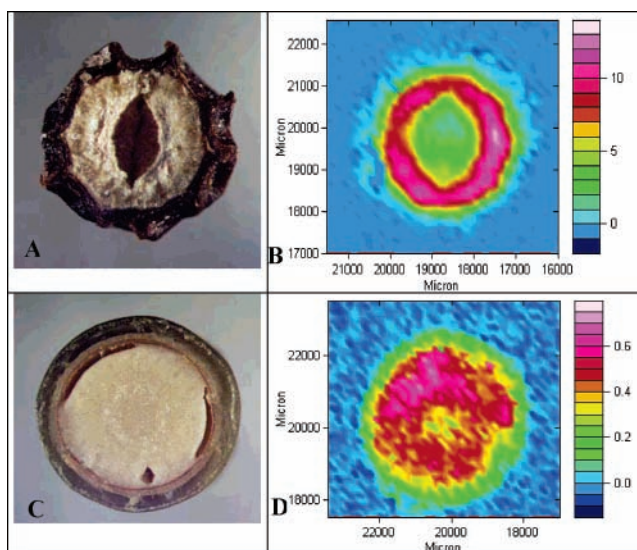


Figure 3. Microscopic images of black peppercorn (A) and green pepper fruit (C) as well as the related Raman maps (B, D) showing the distribution of piperine in the plant tissue. The relative concentration of piperine was determined according to the intensity in the frequency range between 1547 and 1659 cm^{-1} .

Table 1. Typical Gas Chromatographic Composition (Mean GC Peak Area Percentages for Each Component) of the Individual Pepper Oils Analyzed in This Study^a

component	sabinene/ caryophyllene type	caryophyllene type	δ -3-carene/ caryophyllene/ limonene type
α -pinene	4.3–5.4	nd–2.3	2.5–5.0
β -pinene	6.5–9.8	0.4–4.2	5.1–10.4
sabinene	9.34–20.9	nd–3.6	nd–3.2
myrcene	1.4–2.1	nd–1.4	0.6–2.6
α -phellandrene	0.6–1.9	nd–1.2	1.6–5.9
δ -3-carene	1.4–7.4	0.6–6.8	13.9–32.7
limonene	11.8–17.0	1.2–8.8	10.9–19.5
β -caryophyllene	17.6–34.6	39.8–65.7	13.9–46.6
caryophyllene oxide	0.7–1.7	1.2–14.2	0.3–4.1

^a nd, not detectable.

peppercorn shows clearly the presence of piperine directly beneath the skin of the fruit.

In Table 1, the individual pepper oil components with an amount of $>0.1\%$ (total GC peak area percentage) are presented. The molecular structures of these volatile components are shown in Figure 4. According to their different amounts of the main essential oil components caryophyllene, sabinene, δ -3-carene, and limonene, in this study three pepper types could be clearly discriminated. Generally, all analyzed pepper oils show characteristic fingerprints in the related Raman and ATR-IR spectra (Figures 5 and 6). The corresponding Raman spectrum of the so-called “caryophyllene type” (Figure 5B) shows the comparatively strong $\nu_{\text{C}=\text{C}}$ stretching vibration modes at 1632 and 1671 cm^{-1} and a broad band at 1446 cm^{-1} that is assigned to CH_2 deformation vibrations. Also in the Raman spectrum of the “sabinene/caryophyllene type” (Figure 5D) these key bands of caryophyllene can be identified, but additionally the characteristic bands of sabinene ($\nu_{\text{C}=\text{C}}$ at 1653 cm^{-1} and δ_{CH_2} at 1415 cm^{-1}) are observed. Furthermore, several sabinene bands of minor intensity occur in the range between 600 and 1000 cm^{-1} ($\delta_{\text{C}-\text{C}}$ at 950 cm^{-1} and ring deformation vibration at 652 cm^{-1}); the Raman spectrum of the third pepper oil type, which is represented by more or less equal amounts of δ -3-carene (key

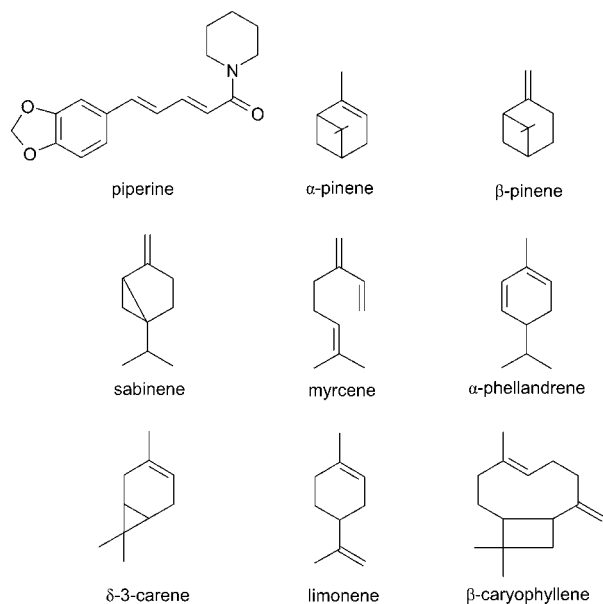


Figure 4. Molecular structures of some main volatiles detected in the analyzed pepper oils.

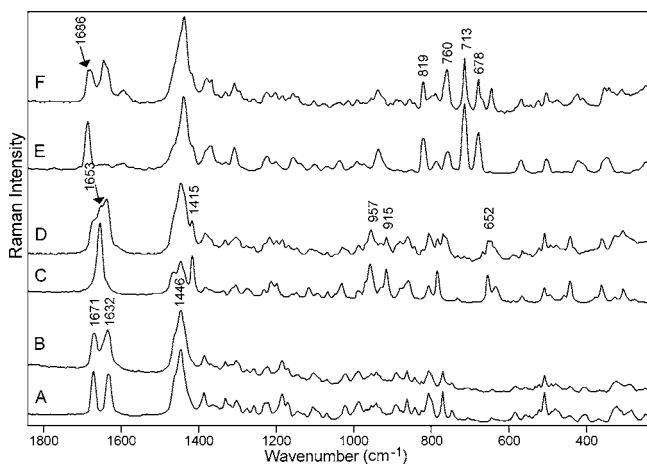


Figure 5. Raman spectra of different pepper oil types isolated by hydrodistillation from various commercial pepper samples: (A) pure caryophyllene standard; (B) pepper oil (caryophyllene type); (C) pure sabinene standard; (D) pepper oil (sabinene/caryophyllene type); (E) pure δ -3-carene standard; (F) pepper oil (δ -3-carene/caryophyllene/limonene type).

bands at 1686, 819, 713, and 678 cm^{-1}), caryophyllene, and limonene (key band at 760 cm^{-1}), is generally dominated by very strong signals of δ -3-carene (**Figure 5F**). The corresponding ATR-IR spectra are also useful for the discrimination of the three pepper oil types analyzed in this study. Whereas the spectrum of the “caryophyllene-type” shows nearly the same signals as the pure standard (**Figure 6B**), the ATR-IR spectrum of the “sabinene/caryophyllene type” presents the individual key bands (**Figure 6D**) of the main oil components in the range between 850 and 900 cm^{-1} , which can be interpreted as out-of-plane bending vibrations of the terminated methylene groups at 885 cm^{-1} (caryophyllene) and 862 cm^{-1} (sabinene). The third pepper oil type is mainly characterized by signals of caryophyllene (registered at 875 and 885 cm^{-1}) as well as δ -3-carene (out-of-plane bending vibration at 785 cm^{-1}).

In accordance with former NIRS studies of various essential oils (14–17) the obtained spectra are dominated by overtones and different combinations of $-\text{C}-\text{H}$ stretching and bending

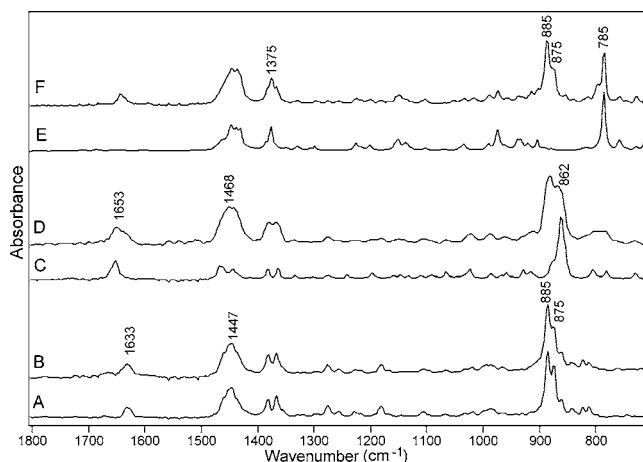


Figure 6. ATR-IR spectra of different pepper oil types isolated by hydrodistillation from various commercial pepper samples: (A) pure caryophyllene standard; (B) pepper oil (caryophyllene type); (C) pure sabinene standard; (D) pepper oil (sabinene/caryophyllene type); (E) pure δ -3-carene standard; (F) pepper oil (δ -3-carene/caryophyllene/limonene type).

vibration modes, which can be found as comparatively broad absorptions between 1600 and 1800 nm as well as between 2200 and 2500 nm.

The heterogeneity of the measured Raman spectra obtained from the different black and white pepper samples is demonstrated in **Figure 7**. The spectral distances were calculated with the standard algorithm after the application of vector normalization. Cluster analysis was performed for the wavenumber range from 200 to 2000 cm^{-1} . When Ward’s algorithm was applied for cluster analysis, a clear discrimination of the three above-mentioned pepper oil types could be achieved, but it was not possible to distinguish the essential oils of black and white pepper within these subgroups.

On the basis of the ATR-IR, NIR, and Raman measurements performed on 51 pepper samples, chemometric equations have been developed for the individual quality parameters (**Table 2**). As can be seen, generally good calibration results were obtained for piperine, total essential oil, and the main terpenoids present in the volatile fraction. Some volatiles show lower prediction quality, which could be due to the low concentration level of these substances in the peppercorn or to coincidences with the spectral contribution of other plant constituents. Especially, Raman measurements on peppercorns and pepper oleoresins result in a high response for piperine but show comparatively low intensity for terpenoid substances. Only a few monoterpenes (α - and β -pinene as well as limonene) present characteristic Raman bands at lower wavenumbers; this might be the reason that these substances can be analyzed with higher prediction quality ($R^2 = 0.82\text{--}0.84$) because the individual key bands do not coincide with other vibration modes of the plant matrix. Contrary to that, very good correlation statistics were obtained for the vibrational spectroscopic analysis of individual essential oil components (**Table 3**). The multiple coefficients of determination (R^2) reach values of >0.95 , representing a very high correlation to the GC reference data. Also, the SECV values are comparatively low and guarantee reliable predictions of the individual essential oil profiles.

In conclusion, feasibility studies performed to predict the individual piperine and essential oil content in various ground pepper samples by using ATR-IR, NIR, and Raman spectroscopy methods demonstrate high correlation statistics between the vibrational spectroscopic data and the chromatographic

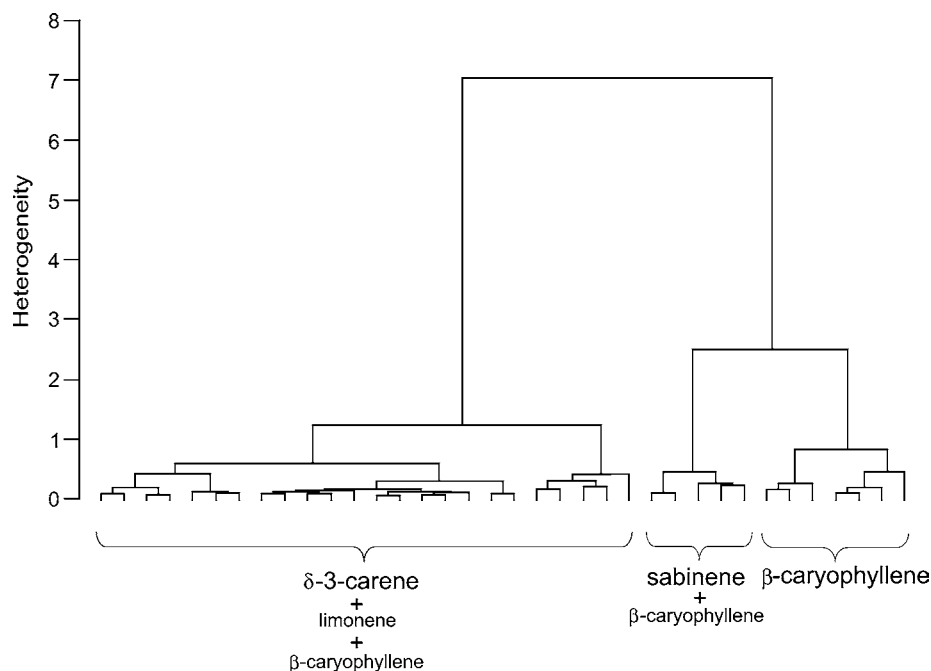


Figure 7. Cluster analysis based on Raman spectra of different black and white commercial pepper samples.

Table 2. ATR-IR, NIR, and Raman Correlation Statistics for Piperine, Essential Oil, and Pepper Oil Substances in Various Black and White Pepper Samples ($N = 51$)^a

parameter	range	ATR-IR			NIR			Raman		
		SECV	R^2	OL	SECV	R^2	OL	SECV	R^2	OL
piperine (mg/100 g)	2.75–5.07	0.19	0.86	8	0.21	0.83	6	0.23	0.84	5
essential oil (mL/100 g)	0.80–3.60	0.32	0.88	0	0.27	0.91	2	0.40	0.82	5
α -pinene (mL/100 g)	nd–0.18	0.019	0.91	0	0.013	0.96	5	0.025	0.82	6
β -pinene (mL/100 g)	0.003–0.29	0.019	0.97	3	0.019	0.97	5	0.044	0.83	8
sabinene (mL/100 g)	nd–0.75	0.062	0.65	5	0.070	0.82	0	0.147	0.32	5
myrcene (mL/100 g)	nd–0.08	0.008	0.94	5	0.007	0.95	4	0.015	0.76	5
α -phellandrene (mL/100 g)	nd–0.16	0.015	0.93	4	0.018	0.90	5	0.037	0.56	6
δ -3-carene (mL/100 g)	0.005–0.80	0.100	0.87	1	0.071	0.94	4	0.174	0.62	6
limonene (mL/100 g)	0.01–0.52	0.050	0.93	2	0.040	0.96	3	0.075	0.84	4
β -caryophyllene (mL/100 g)	0.26–1.22	0.134	0.69	5	0.102	0.82	8	0.130	0.60	7
caryophyllene oxide (mL/100 g)	0.005–0.14	0.012	0.66	8	0.009	0.83	8	0.016	0.65	6

^a OL, number of outliers; nd, not detectable.

Table 3. ATR-IR, NIR, and Raman Correlation Statistics for Individual Terpenoids Detected in Hydrodistilled Pepper Oils^a

oil component	range (mL/100 g)	ATR-IR		NIR		Raman	
		SECV (mL/100g)	R^2	SECV (mL/100g)	R^2	SECV (mL/100g)	R^2
α -pinene	0.26–5.39	0.45	0.92	0.45	0.65	0.36	0.95
β -pinene	0.69–11.96	0.35	0.98	0.65	0.87	0.50	0.97
sabinene	nd–20.9	0.20	1.00	0.40	1.00	0.43	1.00
myrcene	0.21–3.26	0.05	0.99	0.12	0.93	0.08	0.99
α -phellandrene	nd–6.61	0.18	0.99	0.21	0.98	0.24	0.98
δ -3-carene	0.72–32.65	0.91	0.99	1.18	0.98	1.00	0.99
limonene	1.64–22.48	0.59	0.98	0.64	0.94	0.86	0.95
β -caryophyllene	12.84–65.68	1.73	0.98	1.65	0.96	1.03	0.99
caryophyllene oxide	0.22–8.35	0.38	0.97	0.15	0.80	0.43	0.96

^and, not detectable.

reference values. On the basis of such calibration equations it is possible to perform routine quality analysis of pepper and pepper extracts nondestructively in <1 min. Furthermore, Raman mappings obtained from green pepper berries as well as black peppercorns allow the distribution of piperine to be

determined in situ. It has been shown that the pungent principle is distributed more or less over the whole perisperm of the green fruit.

The composition of pepper oil, varying according to provenance (cultivar), climate, harvest year, and ripening stage, can

be reliably determined on the basis of the obtained spectral data. Thus, the described vibrational spectroscopy methods offer a very useful approach for an efficient quality control of peppercorns and pepper extracts as well as pepper oil processed in industry.

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