JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Rapid Classification of Basil Chemotypes by Various Vibrational Spectroscopy Methods

H. SCHULZ,*,† B. SCHRADER,‡ R. QUILITZSCH,† S. PFEFFER,† AND H. KRÜGER†

Federal Center for Breeding Research on Cultivated Plants, Institute for Plant Analysis, Neuer Weg 22/23, D-06484 Quedlinburg, Germany, and Institut für Physikalische und Theoretische Chemie, Universität Essen, D-45117 Essen, Germany

The potential of vibrational spectroscopy methods (attenuated total reflectance/Fourier-transform-infrared (ATR/FT-IR), FT-Raman and near infrared (NIR) spectroscopy) for the identification and quantification of valuable as well as carcinogenic substances in different basil chemotypes is described. It is shown that all main volatile components occurring in different basil accessions can be reliably determined in the isolated essential oils or solvent extracts but also in the air-dried herbs. While NIR data can be interpreted only by chemometric methods, IR and Raman spectra present characteristic key bands of the individual volatiles; therefore, in the latter case, a discrimination of basil chemotypes is frequently possible without applying chemometric algorithms. NIR calibrations are successfully established for various terpenoids and phenylpropanoids; on the basis of these data, the content of the two carcinogenic compounds methyleugenol (range: 2−235 μg/100 g) and estragole (range: 34−138 μg/100 g) can be reliably predicted in air-dried basil leaves (R^2 (coefficient of determination) = 0.951; SECV (standard error of cross validation) = 19.1 μg/100 g and R^2 = 0.890; SECV = 12.8 μg/100 g, respectively). The described methods were found to be very useful tools for the efficient selection of special basil single plants, adapted to the new demands set by the legislator and the consumer. Furthermore, they can be applied in industry to very easily control the purifying, blending, and redistilling processes of basil oil.

KEYWORDS: ATR/FT-IR; FT-Raman; NIR; essential oils; *Ocimum*, chemometry

INTRODUCTION

The genus *Ocimum* L. comprises ca. 30 species that occur mainly in tropical and subtropical regions (*1*). Among them, sweet basil (*Ocimum basilicum* L.) is the most popular species; especially the cultivars cv. Genoveser and cv. Genovese Gigante are used as fresh or dried herbs and for the production of pesto, a special Italian sauce with a typical spicy flavor.

Some other basil species such as *O. americanum* L., *O. gratissimum* L. and *O. tenuiflorum* L. have been also grown as medicinal plants, culinary herbs, and insect-controlling agents, especially in the Mediterranean area.

There are only two basil oil types that are commercially important: The "Réunion type", which is obtained by steam distillation of the flowering tops or whole plants of *O. basilicum* and the so-called "European type", which is predominantly produced in France, Italy, Egypt, and South Africa. Whereas the Réunion type contains mainly estragole, which may be 80% or more, major components of the European type are linalool (35−50%) and estragole (15−25%). Both essential oils are used for food flavoring as well as in perfumery. Detailed specifications for these basil oils are described in the Food Chemical Codex (FCC) as well as in the individual documents of the French National Standard (Norme Française) and the International Standard Organization (ISO) (*2*−*5*).

Small amounts of a Bulgarian or a so-called "cinnamon basil oil", rich in methyl cinnamate are also traded as a natural source for this aroma substance.

The aromatic character of each basil chemotype depends mainly on the individual composition of the essential oil fraction; generally chemotypes are classified on the basis of prevalent compounds or components with an amount higher than 20% in adult plants (*6*). However, it is also known that the composition of the essential oil is largely influenced by the vegetative stage of the basil plant. In cv. Genovese Gigante, the methyleugenol content was found to decrease during ontogenesis, whereas eugenol increased at the same time. This aspect is of special importance, because human exposure to this compound is of toxicological concern, due to the structural similarity to known carcinogenic phenylpropanoids such as estragole and safrole (*7*). For several years, it already has been known that estragole is a naturally occurring genotoxic and carcinogenic flavoring substance. 1-Hydroxyestragole, the supposed proximate carcinogen, has been found in the urine of men dosed with 1 μg/kg body weight, and the induction of liver tumors seems to depend on

* To whom correspondence should be addressed. E-mail: h.schulz@bafz.de.
† Institute for Plant Analysis.
‡ Universität Essen.

10.1021/jf021139r CCC: $25.00
Published on Web 04/01/2003

Table 1. Typical Gas Chromatographic Composition of the Investigated Basil Chemotypes (13)[a]

chemotype	ocimene	1,8 cineole	linalool	camphero	estragole	citral	thymol	eugenol	methyl cinnamate	α-bergamotene	methyleugenol	β-elemene	β-caryophyllene	β-bisabolene	elimicin
bisabolene type		2.61	1.15		8.07		5.16	4.22		5.94	2.82		2.68	47.17	
camphor type		18.71	2.70	53.16									0.73		
caryophyllene type								29.59			0.78	10.00	47.89		
citral type		0.73	13.00			41.96				1.34			5.52		
elimicin type	16.34	1.75	0.65								10.8		3.58		56.77
estragole type					97.44						0.49		0.83		
eugenol type	17.38	0.71	0.50					66.41					1.70		
methyleugenol type								0.34			83.44	4.19	11.17		
linalool type		0.73	90.26	0.43				0.94		1.66					
thymol type		0.86	1.82		0.55		44.88	0.67		0.39	1.82		2.43		
methyl cinnamate type		3.95	1.10	0.45	1.63		0.23		79.85	0.98					

[a] Results are expressed as relative areas in percentage of the total peak areas. Tentative identifications of α-bergamotene, β-elemene, β-bisabolene and elimicin were based on comparison of GC/MS data with those of Wiley and NBS computer mass libraries. Other identifications were performed by comparison of GC/MS data and retention indices with those of authentic samples.

formation of this metabolite of estragole. Both estragole and its 1′-hydroxy metabolite induced unscheduled DNA synthesis in rat hepatocytes in vitro, and estragole also did the same in vivo (*8*). This is the reason the Council of Europe presently recommends that methyleugenol should not be detectable and that estragole should not exceed a limit of 0.05 mg/kg in food products (*9*, *10*).

This new legislative status will require enormous additional research activities soon, to select suitable new basil cultivars meeting the high quality standard set by the upper limits for methyleugenol and estragole on one side and the aroma properties expected by the consumer on the other side.

In addition to the commercially used basil cultivars mentioned above, several other chemocultivars varying in the essential oil composition have been selected over the years by natural evolutionary processes or bred by crossing with other cultivars or closely related species. Because morphological characters alone appear to be insufficient to describe different forms of *O. basilicum*, Paton and Putievsky (*11*) suggested to use the different essential oil profiles as additional characters to describe the taxonomy of the individual basil types. For this purpose, numerous accessions of *O. basilicum* and other species of this genus were analyzed for their essential oil composition by GC-mass spectrometry (*6*, *12*, *13*). These data provide a great deal of very useful information for the identification of the individual chemotype and the precise quantification of valuable essential oil components. However, regarding the aspects of quality control and "Good Manufacturing Practice", gas chromatography is time-consuming, comparatively expensive, and requires highly qualified technicians. Therefore, the aim of this study was to develop new spectroscopical methods for the classification of basil oils and micro-extracts as well as rapid evaluation of the most important volatile substances directly in the drug as a useful alternative for usually applied chromatography techniques.

In recent years, several near-infrared (NIR), infrared (IR) and Raman spectroscopy methods have been developed and successfully applied with respect to quality control purposes as well as with the aim to distinguish different species/chemotypes of various spice plants and to efficiently predict the content of valuable plant substances in teas and herbs or in the isolated volatile fractions (*14*−*21*). However, up to now, no attempts have been made to use these efficient methods for the determination of monoterpenes and phenylpropanoids in basil drug or basil oil.

MATERIALS AND METHODS

The analyzed basil accessions, containing 11 different chemotypes, were cultivated in the experimental garden of the Federal Center of Breeding Research on Cultivated Plants (BAZ) in Quedlinburg (Germany) as well as in the gene bank of the Institute for Plant Genetics and Crop Plant Research (IPK) at Gatersleben (Germany).

Pure standard substances (1,8-cineole, linalool, estragole, thymol, eugenol, β-caryophyllene, methyleugenol) were purchased from Roth (Karlsruhe, Germany) and Sigma-Aldrich (Taufkirchen, Germany), respectively. The other reagents were of analytical grade and were used without further purification.

Reference GC and GC-MS Analysis. The air-dried and crushed basil herbs were hydro-distilled according to the standard method described in the European Pharmacopoeia (*22*), and the received oils were analyzed by gas chromatography/flame ionization detector (GC/FID) using an Hewlett-Packard gas chromatograph 5890 series II, fitted with an HP-5 column (50 m × 0.32 mm i.d.; film thickness, 0.52 μm). Detector and injector temperatures were set at 280 °C and 250 °C, respectively. The following oven temperature program was used: 80 °C for 1 min, then 10 °C/min up to 220 °C; this final temperature was held for 9 min. Carrier gas was nitrogen with a constant flow rate of

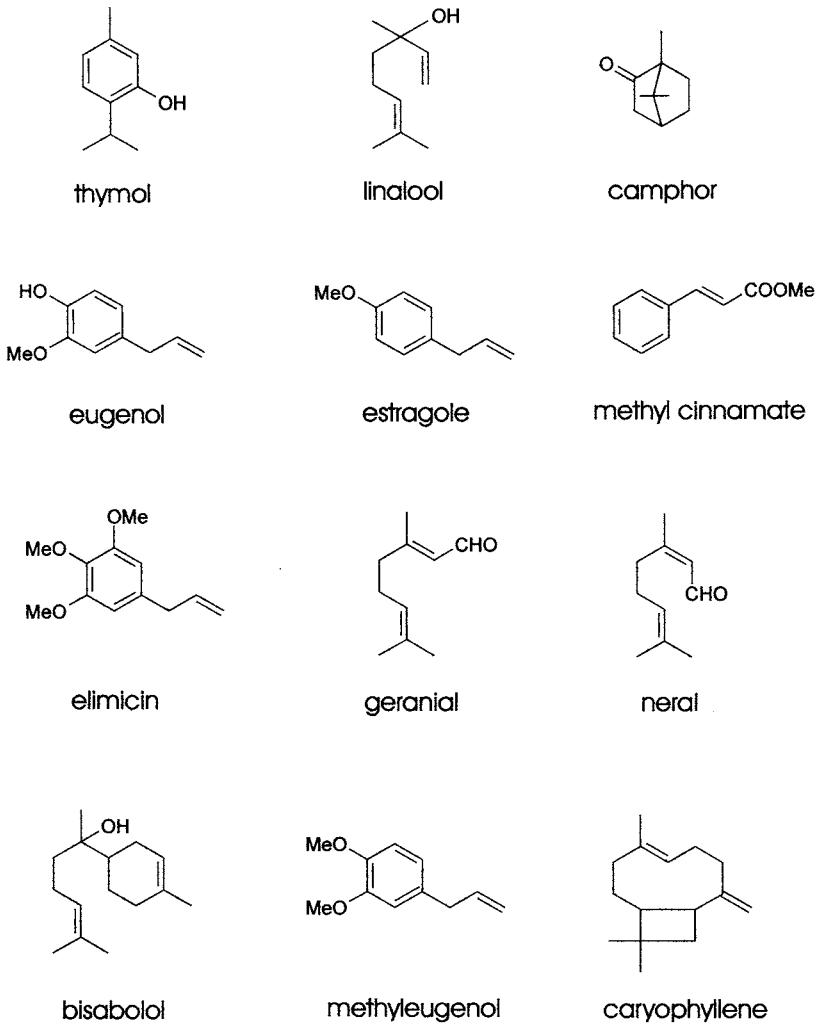

Figure 1. Molecular structures of some main volatiles detected in the analyzed basil chemotypes.

1 mL/min (split, 1:40). GC−MS analyses of the isolated essential oils were performed using an Hewlett-Packard MSD 5972/HP 5890 Series plus 2, equipped with a 15 m × 0.25 mm i.d., 0.25 μm Permabond OV-1-DF column. Column temperature was set at 60 °C for 2 min, then programmed from 60 to 100 °C at a rate of 2 °C/min, and finally increased to 270 °C at 10 °C/min and held for 6 min. Injector and detector temperatures were set at 250 and 280 °C, respectively. 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.

NIR Spectroscopic Measurements. Measurements on basil drugs (72 samples) were performed by a dispersive NIR System 5000 (Foss Instruments Inc., Hamburg, Germany). A few grams of the crushed sample were transferred into rectangular cups (51 × 64 mm, 11 mm depth) placed in a transport unit, moving the sample up and down at right angles to the incident radiation. Each sample was measured twice with 32 scans each time. The isolated basil oils (minimum sample amount, 600 μL) were analyzed in the transflection mode using quartz cuvettes equipped with a diffuse gold reflector (path length, 2 × 0.2 mm).

ATR (Attenuated Total Reflectance)-IR Spectroscopic Measurements. The mid-infrared analyses were carried out on a portable ATR/FT-IR spectrometer (Resultec Analytical Equipment, Garbsen, Germany) in a single reflection configuration. Approximately 100 mg of the homogenized drug sample was extracted with 1 mL of CCl_4 in a 5 mL stainless steel capsule, and 5 μL of the resulting extract was placed on the surface of the diamond-ZnSe ATR crystal. The measurement was started after 20 s, when the main part of the solvent is evaporated (controlled by the characteristic double band of CCl_4 at 762 and 790 cm^{-1}). The spectrometer can be operated by a 12 V car battery and is fitted with a Michelson interferometer and a DLATGS detector. The wavenumber region used for the analysis was 650−3500 cm^{-1} with a spectral resolution of 2 cm^{-1}.

NIR-FT Raman Spectroscopy Measurements. The Raman spectra were recorded on a Bruker RFS 100 with a diode-pumped Nd:YAG laser, emitting at 1064 nm, and a germanium detector cooled with liquid nitrogen. All spectra were measured with a resolution of 4 cm^{-1} in the range of 200−3700 cm^{-1} with a laser power of ca. 350 mW supplied by a nonfocused laser beam. The individual samples were transferred into a 6-mm-diameter test tube and placed at the center of a hemispherical mirror, and the spectra were accumulated from 159 scans collected during 5 min (*23*).

Chemometrics. Chemometric analyses of the ATR-IR and NIR-Raman spectra were performed using a commercial software program (Opus/Quant 2.0, Bruker GmbH, Rheinstetten, Germany).

Statistical accuracy is described by R^2, SEC, and SECV, using the leave-one-out procedure. The spectral data were transformed with first derivative processing and subsequently mean centered. The whole IR wavenumber range from 650 to 2000 cm^{-1} was used for the partial least squares (PLS) calibration. The optimum number of PLS factors for each component was determined by application of the predictive residual error sum of squares calculation.

Development of appropriate chemometric NIRS methods was carried out with the commercial statistic program WINISI (Infrasoft International Inc., Port Matilda, USA). To reach optimal correlation values,

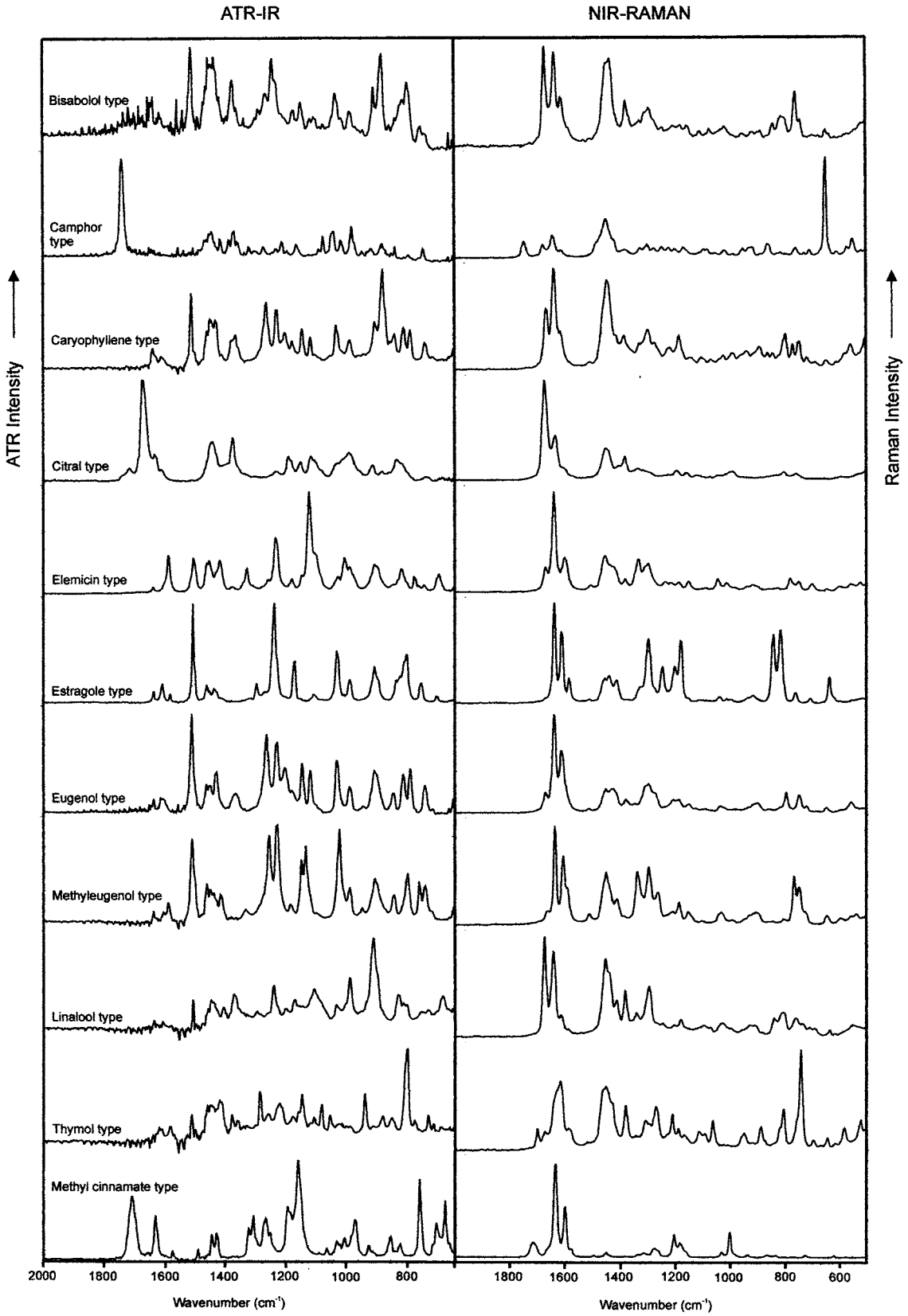

Figure 2. ATR-IR and Raman spectra of individual essential oils isolated by hydrodistillation from different basil genotypes.

the spectral data were pretreated with weighted multiplicative scatter correction and were transformed individually with first and second derivative processing. The calibration program was set up with the whole wavelength range (1100−2500 nm with a data interval of 2 nm), using the PLS algorithm. All data in the calibration set were checked carefully to detect and eliminate outlier samples by applying a special algorithm of the WINISI software.

RESULTS AND DISCUSSION

In **Table 1**, the values obtained for the main volatile components (>0.1% of total GC peak area percentages) occurring in the analyzed basil samples are presented. It can be seen that each chemotype shows a special chemical fingerprint of substances in the volatile fraction. The molecular structures

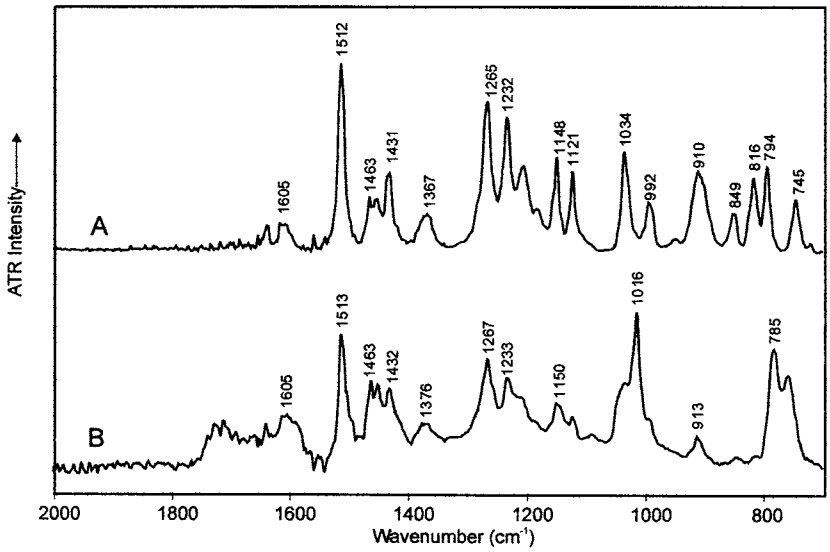

Figure 3. ATR-IR spectra obtained from the essential oil (A) and the CCl_4-microextract (B) of air-dried basil leaves (eugenol type).

Table 2. ATR-IR Correlation Statistics for Most Valuable Substances in the Essential Oils of Various Species Belonging to the Genus *Ocimum* ($N = 73$)

oil component	range (g/100 g)	SECV (g/100 g)	R^2
1,8-cineole	0.36 – 27.70	1.48	0.93
linalool	0.20 – 90.26	1.77	1.00
estragole	0.55 – 98.09	1.74	1.00
thymol	0.23 – 44.88	1.57	0.98
eugenol	0.25 – 66.41	0.87	0.99
methyleugenol	0.30 – 83.44	1.87	0.99
β-caryophyllene	0.36 – 47.89	2.22	0.96

of some characteristic volatile substances occurring in the analyzed basil plants are presented in **Figure 1**.

ATR/FT-IR and Raman Spectra. Generally, all vibrational spectroscopy methods used in this study were useful for rapid and reliable discrimination of the described 11 basil chemotypes. **Figure 2** shows the individual IR and Raman fingerprints of the essential oils, isolated from the different basil plants.

The IR spectrum of the thymol type shows the characteristic signals at 739 and 807 cm^{-1}, which are assigned to ring vibrations to be detected in this basil accession. In the corresponding Raman spectrum, this vibrational mode appears at 740 cm^{-1}. Recently, similar observations were also described for different oregano chemotypes, presenting characteristic peaks at 759 cm^{-1} (carvacrol type) and 740 cm^{-1} (thymol type) (*18*). The camphor type is characterized by the strong IR carbonyl band at 1743 cm^{-1} and the intense Raman signal at 650 cm^{-1}, which is assigned as ring breathing vibration of the bicyclic compound (*24*). IR and Raman spectra obtained from the citral type look very similar; the two main essential oil substances (geranial and neral) show sharp bands of the aldehyde group at 1674 cm^{-1} and specific bands at 1377 and 1446 cm^{-1} assigned to CH_2-deformation modes. The FT-Raman spectrum of the linalool type shows the typical ν_{C-C} vibration modes at 1643 and 1675 cm^{-1}. Contrary to that, the corresponding IR spectrum is characterized by out-of-plane C−H bending vibrations at 918 and 994 cm^{-1}. Estragole is the main component in some basil plants that are consumed as culinary herbs or spices (*Ocimum basilicum* L., cv. Genovese Gigante) (*25*). The isolated essential oil of this chemotype presents the characteristic IR and Raman bands of medium intensity due to out-of-plane stretching vibrations of the hydrogen atoms at the aromatic ring (*26*). In accordance with their similar molecular structure the eugenol, methyleugenol, elimicin and estragole types show comparable spectral profiles. Nevertheless, there exist some specific signals that allow a reliable distinction of these different chemotypes (*27*). With respect to the high content of methyl cinnamate (ca. 82%) the Raman spectrum of this chemotype is dominated by the very strong ester peaks occurring at 1717, 1638, and 1602 cm^{-1}. The corresponding IR absorption bands are to be seen at 1713 and 1635 cm^{-1}. Furthermore, several intense bands occur in the region between 1100 and 1320 cm^{-1} that can be assigned to C−O−C vibration modes of the ester group. Also, the monosubstituted benzene ring shows characteristic stretching and bending IR and Raman vibrations of lower intensity between 1000 and 1050 cm^{-1} that provide more reliable conclusions concerning the substitution pattern (*26*). Whereas all above-mentioned chemotypes are characterized by one major essential oil component, the so-called "cineole type" contains nearly the same proportions of 1,8-cineole, eugenol, bisabolene, and estragole. According to that, equally strong bands of these four essential oil components contribute in a similar way to the spectrum obtained from this special basil chemotype.

Along with the characteristic absorptions in the fingerprint region, typical bands relating to various C−H stretching vibrational modes are observed between 2850 and 3100 cm^{-1}. These IR and Raman bands are not shown here, and they were also not used for the calibration models presented in **Table 2**. The prediction quality was found to be slightly lower when the chemometrical interpretation was based on all spectral data points registered in the whole measuring range from 650 to 3500 cm^{-1}. As demonstrated in **Figure 3**, the ATR−IR spectra obtained from the essential oil and the microextract, which have been prepared from the same basil plant (linalool type), look very similar. Therefore, it is possible, in principle, to apply the more efficient extraction procedure as an appropriate cleanup step for subsequent ATR−IR analyses of the described basil chemotypes.

NIR Spectra. For NIR transflection measurements on essential oils and extracts, sample amounts of approximately 600 μL were found to be sufficient. According to former studies performed with marjoram and citrus oils (*15*, *28*), the NIR spectra of the analyzed basil oils are dominated by overtones or different combinations of C−H-stretching and bending

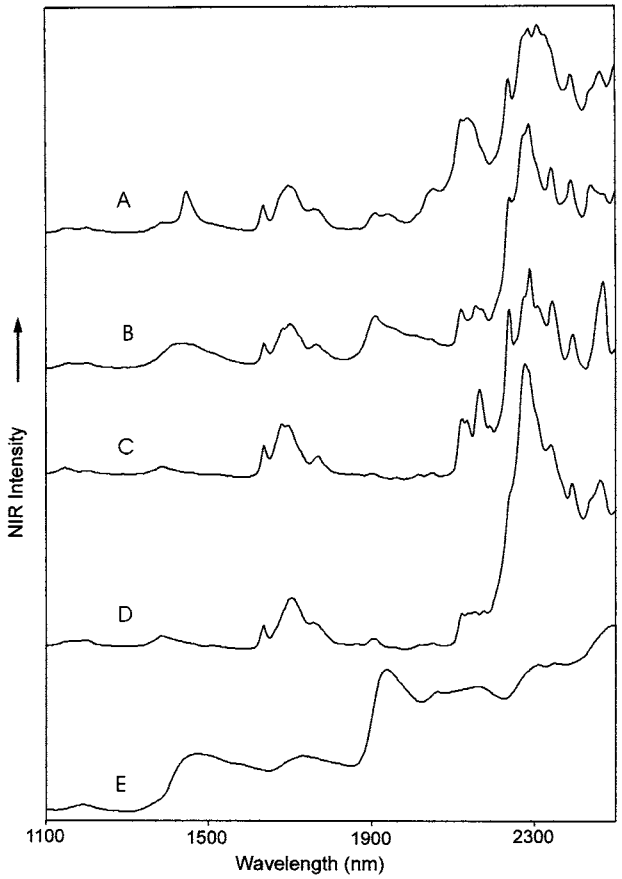

Figure 4. Near-infrared spectra obtained from four different basil oils (A = *Ocimum seilol* Benth. (elimicin type), B = *Ocimum tenuiflorum* L. (β-caryophyllene type), C = *Ocimum tenuiflorum* L. (methyleugenol type), D = *Ocimum gratissimum* ssp. Iringense Ayobangira ex Paton (eugenol type)) and one NIR spectrum representative for basil drug (E = *Ocimum basilicum* L. cv. Genoveser).

vibrations occurring between 1600 and 1800 nm and 2200−2500 nm, respectively. Although the NIR absorptions are comparatively broad, generally distinctive differences between the individual chemotypes can be seen, which is exemplarily demonstrated for four basil accessions presented in **Figure 4** (parts **A**−**D**).

Table 3. NIRS Correlation Statistics for Most Valuable Substances in the Essential Oils of Various Species Belonging to the Genus *Ocimum* ($N = 73$)

oil component	range (g/100 g)	SECV (g/100 g)	R^2
1,8-cineole	0.36 – 27.70	1.2	0.99
linalool	0.20 – 90.26	0.9	1.00
estragole	0.55 – 98.09	1.0	1.00
thymol	0.23 – 44.88	1.4	1.00
eugenol	0.25 – 66.41	0.7	1.00
methyleugenol	0.30 – 83.44	0.9	1.00
β-caryophyllene	0.36 – 47.89	0.8	0.99

Table 4. NIRS Correlation Statistics for the Total Amount of GC Volatile Components and Content of Main Valuable Substances in Air-Dried Basil Leaves of cv. Genoveser (Linalool Type)

	N[a]	range (μg/100 g)	SEC[b] (μg/100 g)	R^2	SECV (μg/100 g)
GC volatiles	72 (69)	150 – 460	23.7	0.904	36.8
1,8-cineole	72 (69)	13 – 67	3.5	0.923	6.9
linalool	72 (70)	7 – 196	7.8	0.967	14.3
estragole	30 (30)	34 – 138	8.5	0.890	12.8
eugenol	72 (72)	1 – 139	7.9	0.939	14.2
methyleugenol	68 (68)	2 – 235	12.6	0.951	19.1

[a] N = number of measured samples, data in brackets correspond to the number of samples used for the calibration process (elimination of outliers). [b] SEC = standard error of calibration.

Contrary to that, the spectra received from air-dried basil herb predominantly show strong bands of water at 1450 and 1940 nm; Along with the spectral response of other main plant constituents (e.g. cellulose), some smaller absorptions of the volatile fraction occur between 2250 and 2350 nm (**Figure 4E**).

Chemometrics. Generally, feasibility studies performed to characterize the isolated essential oil profiles from various basil gene bank accessions demonstrate high correlations between vibrational spectroscopical data and GC reference values for the most relevant components (**Tables 2** and **3**). Applying the PLS algorithm, very good calibration results could be obtained; the individual SECV is in the same order of magnitude as the standard error of the described reference GC method. As can be seen in **Table 4**, the total amount of GC volatiles, as well as the content of main valuable volatile substances (1,8-cineole,

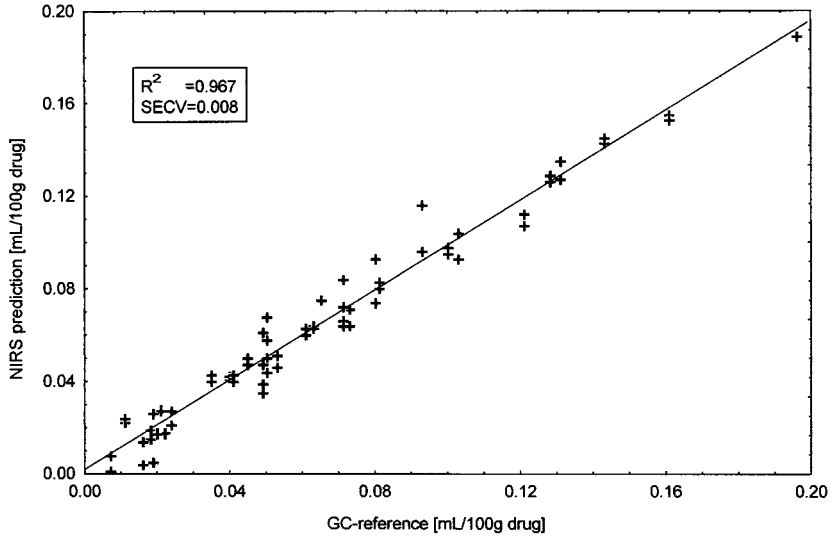

Figure 5. Reference GC values vs NIRS prediction of the linalool content for air-dried basil leaves of cv. Genoveser ($N = 72$). SECV = standard error of cross validation, R^2 = coefficient of determination.

linalool, estragole, eugenol, methyleugenol) occurring in basil leaves of cv. Genoveser (linalool type), can be reliably predicted by NIRS spectroscopy measurements. In **Figure 5** the high correlation between reference GC values and the predicted values received from the NIRS calibration set is exemplarily shown for the linalool content determined in cv. Genoveser.

CONCLUSION

A good opportunity exists to very easily determine the seasonal and genetic variations to be observed during cultivation of basil plants. To use the presented NIRS calibration tentatively established for air-dried basil herb for routine analysis in practice, more detailed calibration work must be performed over several cultivation years.

The main advantages of the presented methods are in the field of applied breeding research and quality assurance. The ability to monitor rapidly wild basil populations as well as progenies of crossing experiments makes it possible to use the described rapid methods for the efficient selection of high-quality single plants. Furthermore, the described spectroscopy techniques may well be appropriate for the evaluation of the optimal harvest time (with special respect to aroma profile and level of estragole and methyleugenol). NIR and Raman calibrations, developed for the essential oils, can also be used in industry for the continuous controlling of the distillation process.

ACKNOWLEDGMENT

The authors are grateful to Cornelia Helmund and Christine Langanke for carefully performing ATR-IR and NIR measurements. Thanks are also due to Dr. Manfred Feustel (Resultec Analytic Equipment in Garbsen, Germany) for supplying the Travel-IR instrument.

LITERATURE CITED

(1) Paton, A. A synopsis of *Ocimum* L. (*Labiatae*) in Africa. *Kew Bull*. **1992**, *47*, 405−435.
(2) *Food chemical codex* (FCC), 4th ed., National Academy Press: Georgetown, 1996.
(3) Norme Française (NF) 1992, No.: T75-244. Huile essentielle de basilic, type linalol (*Ocimum basilicum* L.).
(4) Norme Française (NF) 1991, No.: T75-357. Huile essence de basilic, type méthylchavicol (*Ocimum basilicum* L.).
(5) International Standard Organization (ISO) 1997, No.: 11043. Oil of basil, methylchavicol type (*Ocimum basilicum* L.).
(6) Grayer, R. J.; Kite, G. C.; Goldstone, F. J.; Bryan, S. E.; Paton, A.; Putievsky, E. Infraspecific taxonomy and essential oil chemotypes in sweet basil, *Ocimum basilicum*. *Phytochemistry* **1996**, *43*, 1033−1039.
(7) Phillips, D. H. DNA adducts derived from safrole, estragole and related compounds, and from benzene and its metabolites. *DNA Adducts*: *Identification and Biological Significance*; IARC Scientific Publications: International Agency for Research on Cancer: Lyon, France, 1994; No. 125, pp 131-140.
(8) Sangster, S. A.; Caldwell, A. J.; Hutt, A.; Anthony, A.; Smith, R. The metabolic disposition of [*methoxy*-^{14}C]-labeled *trans*-anethole, estragole, and *p*-propylanisole in human volunteers. *Xenobiotica* **1987**, *17*, 1223−1232.
(9) Council of Europe, 2001. Opinion of the scientific Committee on Food on methyleugenol (4-allyl-1,2-dimethoxybenzene). SCF/CS/FLAV/FLAVOUR/4 ADD1 FINAL.
(10) Council of Europe, 2001. Opinion of the Scientific Committee on Food on estragole (1-allyl-4-methoxybenzene). SCF/CS/FLAV/FLAVOUR/6 ADD2 FINAL.
(11) Paton, A.; Putievsky, E. Taxonomic problems and cytotaxonomic relationships between and within varieties of *Ocimum basilicum* and related species (*Labiatae*). *Kew Bull*. **1996**, *51*, 509−524.
(12) Pushpangadan, P.; Bradu, B. L. In *Advances in Horticulture*. Vol. 11, Medicinal and Aromatic plants; Chadha, K. L., Gupta, R., Eds.; Malhotra Publishing House: New Delhi, 1995; pp 627−657.
(13) Krüger, H.; Wetzel, S. B.; Hammer, K.; Zeiger, B. The chemical variability of *Ocimum* L. species. *J. Herbs, Spices, Med. Plants* **2002**, *9*, 335−344.
(14) Schulz, H.; Schrader, B.; Quilitzsch, R.; Steuer, B. Quantitative analysis of various citrus oils by ATR/FT-IR and NIR-FT Raman spectroscopy. *Appl. Spectrosc*. **2002**, *56*, 117−124.
(15) Steuer, B.; Schulz, H.; Läger, E. Classification and analysis of citrus oils by NIR spectroscopy. *Food Chem.* **2002**, *72*, 113−117.
(16) Schulz, H.; Drews, H.-H.; Krüger, H. Rapid NIRS determination of quality parameters in leaves and isolated essential oils of *Mentha* species. *J. Essent. Oil Res*. **1999**, *11*, 185−190.
(17) Rösch, P.; Popp, J.; Kiefer, W. Raman and surface enhanced Raman spectroscopic investigation on *Lamiaceae* plants. *J. Mol. Struct*. **1999**, *480*−*481*, 121−124.
(18) Schrader, B.; Klump, H. H.; Schenzel, K.; Schulz, H. Nondestructive NIR FT Raman analysis of plants. *J. Mol. Struct*. **1999**, *509*, 201−212.
(19) Schulz, H.; Quilitzsch, R.; Drews, H.-H.; Krüger, H. Estimation of minor components in caraway, fennel, and carrots by NIRS − comparison of results from dispersive and Fourier transform instruments. *Int. Agrophysics* **2000**, *14*, 249−253.
(20) Schulz, H.; Engelhardt, U. E.; Wegent, A.; Drews, H.-H.; Lapczynski, S. Application of near-infrared reflectance spectroscopy to the simultaneous prediction of alkaloids and phenolic substances in green tea leaves. *J. Agric. Food Chem*. **1999**, *47*, 5064−5067.
(21) Daferera, D. J.; Trantilis, P. A.; Polissiou, M. G. Characterization of essential oils from Lamiaceae species by Fourier Transform Raman spectroscopy. *J. Agric. Food Chem*. **2002**, *50*, 5503−5507.
(22) European Pharmacopoeia, Part 1, 1983; p. V.4.5.8, Maisonneuve SA, Sainte Ruffine.
(23) Andreev, G. N.; Schrader, B.; Schulz, H.; Fuchs, R. Nondestructive NIR−FT-Raman analyses in practice. Part 1. Analyses of plants and historic textiles. *Fresenius J. Anal. Chem.* **2001**, *371*, 1009−1017.
(24) Freeman, S. K. *Applications of Laser Raman Spectroscopy*; John Wiley & Sons: Hoboken, NJ, 1974; p 148.
(25) Miele, M.; Dondero, R.; Ciarallo, G.; Mazzei, M. Methyleugenol In *Ocimum basilicum* L. cv. Genovese Gigante. *J. Agric. Food Chem*. **2001**, *49*, 517−521.
(26) Schrader, B. *Infrared and Raman Spectroscopy*, VCH Verlagsgesellschaft mbH: Weinheim, Germany, 1995; p 208.
(27) Dollish, F. R.; Fateley, W. G.; Bentley, F. F. *Characteristic Raman frequencies of organic compounds*; John Wiley & Sons: Hoboken, NJ, 1974; p 183.
(28) Schulz, H.; Krüger, H.; Steuer, B.; Pank, F., 1999. Bestimmung von Inhaltsstoffen des Majorans (*Origanum majorana* L.) mittels Nah-Infrarot-Spektroskopie. *Z. Arzn. Gew. Pfl*. **1999**, *4*, 62−67.

Received for review November 20, 2002. Revised manuscript received January 31, 2003. Accepted February 1, 2003.

JF021139R