Available online at www.sciencedirect.com
SCIENCE DIRECT®
Vibrational Spectroscopy 35 (2004) 81–86
VIBRATIONAL SPECTROSCOPY
www.elsevier.com/locate/vibspec

Chemotaxonomic characterisation of essential oil plants by vibrational spectroscopy measurements

Hartwig Schulz[a,*], Malgorzata Baranska[a,b], Hans-Hermann Belz[c], Petra Rösch[d], Marion A. Strehle[d], Jürgen Popp[d]

[a]*Federal Centre for Breeding Research on Cultivated Plants (BAZ), Institute for Plant Analysis, Neuer Weg 22-23, D-06484 Quedlinburg, Germany*
[b]*Faculty of Chemistry, Jagiellonian University, Ingardena 3, 30-060 Cracow, Poland*
[c]*Thermo Nicolet, Werner-Heisenberg-Strasse 2, D-63263 Neu-Isenburg, Germany*
[d]*Institut für Physikalische Chemie, Friedrich-Schiller-Universität Jena, Helmholtzweg 4, D-07743 Jena, Germany*

Received 10 March 2003; received in revised form 21 November 2003; accepted 1 December 2003

Available online 1 February 2004

Abstract

The essential oils isolated from basil (*Ocimum* sp.), chamomile (*Chamomilla recutita* L.), thyme (*Thymus vulgaris* L.) and oregano (*Origanum* sp.) were analysed rapidly and non-destructively applying different vibrational spectroscopic methods (ATR-IR, NIR and Raman spectroscopy). Whereas NIR spectroscopic data can be interpreted only by application of chemometric algorithms, IR and Raman spectra obtained from the essential oils present characteristic key bands which can be used as marker bands to discriminate different plant chemotypes. The methods described will be very useful in the flavour and fragrance industry to control very easily the purifying, blending, and redistilling processes of essential oils.

Keywords: Essential oil; Raman; ATR-IR; Chemometrics; Quality control; Breeding

1. Introduction

Essential oils are complex mixtures of various terpenoids, aromatic substances, aldehydes, ketones, alcohols and esters. Usually the fragrance and flavour substances are isolated by hydro-distillation from the dried or fresh plant material (e.g. leaves, seeds, fruits, stems, bark or wood). Most of the oils are used in perfume compositions as well as for flavouring of food-stuffs or mouth care products. Some essential oils containing a high phenol content such as thyme and oregano oil are also used in phytopharmaceutical products or as additives for pet food relating to their antibiotic properties. Since several volatile plant components are thermolabile, sensitive to acids or easily hydrolysed, the composition and quality of the obtained essential oils depends not only on the individual raw material but also on the distillation procedure applied.

In order to ensure the quality of essential oils used in fragrances, cosmetic materials and aroma preparations, usually gas chromatography combined with flame ionisation or mass spectrometry detection is applied. Today, a large number of commercial essential oils have been described by the ''International Organization for Specification (ISO)'', the ''Food Chemical Codex'' and the ''European Pharmacopoeia'' [1,2]. During the last decades, the economic importance of essential oils has led to systematic breeding activities; in this context numerous new cultivars were obtained by alternation of generations or by vegetative routes. Higher yields of essential oil and particularly changed profiles of the oil are primary targets, leading to deviations from the standardised product quality.

Since most of the methods presently applied for quality control purposes and selection of high-quality plants are very time-consuming, some attempts have been made to find alternative analytical options. In this context some new vibrational spectroscopic methods in combination with sophisticated chemometric algorithms were successfully introduced (NIRS, ATR-IR and Raman) for an efficient and mostly non-destructive determination of secondary metabolites occurring in different parts of various medicinal and aromatic plants [3–11,26]. Whereas NIRS data can be interpreted only by application of statistical methods, IR and Raman spectra in most cases present characteristic key bands of the individual volatile fraction and therefore in principle allow the discrimination of different essential oil

* Corresponding author. Tel.: +49-3946-47-231; fax: +49-3946-47-234.
E-mail address: h.schulz@bafz.de (H. Schulz).

0924-2031/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.vibspec.2003.12.014

profiles of the individual oil plants among the same species (chemotypes) without applying any chemometric algorithms [14–25].

2. Experimental

2.1. Sample material and reference analysis

The essential oil plants were cultivated in the experimental garden of the Federal Centre for Breeding Research on Cultivated Plants (BAZ) in Quedlinburg (Germany). The air-dried drug material was hydro-distilled according to the standard method described in the European Pharmacopoeia. The isolated essential oils were analysed by GC/FID using a Hewlett-Packard chromatograph 6890 series, fitted with a HP-5, 50 m × 0.32 mm fused silica column (film thickness 0.52 μm). The percentage composition was computed from the GC peak areas without using any correction factors.

Pure standard substances (α-bisabolol, chamazulene, thymol, carvacrol, linalool, myrcene, *p*-cymene, α-terpinene, γ-terpinene, ocimene, sabinene) were purchased from Roth (Karlsruhe, Germany) and Sigma–Aldrich (Taufkirchen, Germany), respectively. The other analytes were tentatively identified by using the NBS75K and Wiley 138 library databases of the GC–MS system. The individual percentage composition was calculated from the GC peak area applying no correction factors.

2.2. Vibrational spectroscopic measurements

NIRS measurements on essential oils were performed on a dispersive near-infrared spectrometer NIRSystem 5000 of Foss Instruments in the range between 1100 and 2500 nm with a spectral resolution of 2 nm. The samples were analysed in the transflection mode using quartz cuvettes, equipped with a diffuse gold reflector (path length: 2 mm × 0.2 mm).

Mid-IR spectra were recorded in the range between 650 and 4000 cm^{-1} with a portable diamond ATR/FT-IR spectrometer "TravelIR" of Resultec Analytical Equipment in a single reflection configuration. Approximately 5–10 μl of the essential oil were placed on the surface of the diamond-ZnSe-ATR crystal.

Raman spectra were recorded both on a Bruker RFS 100 with a diode-pumped Nd:YAG (neodymium doped yttrium aluminium garnet) laser emitting at 1064 nm (laser power: maximum 100 mW) and a LabRam HR invers (Jobin-Yvon Horiba) equipped with a diode laser emitting at 830 nm (laser power: approximately 4 mW at the sample). The spectra were measured with a resolution of 4 cm^{-1} in the range of 200–3700 and 200–2000 cm^{-1}, respectively. Micro-Raman spectra obtained from peppermint leaves were measured on a Raman 960 of Thermo Nicolet, equipped with an InGaAs detector and a micro-sampling unit. The laser power (emission at 1064 nm) was limited to 400 mW.

2.3. Chemometrics

Chemometric analyses of the ATR-IR spectra were performed using a commercial software programme (Opus/Quant 2.0 Bruker Optics GmbH, Ettlingen, Germany). The whole individual wavenumber range was used for the partial least square (PLS) calibration; the optimum number of PLS factors for each component was determined applying the PRESS (predictive residual error sum of squares) calibration. The accuracy of each calibration statistics was characterised by the overall error between modelled and reference data, the root mean square error of cross validation (RMSECV) and the multiple coefficient of determination (R^2). Development of NIRS methods was carried out with the commercial statistic programme WINISI (Infrasoft International, Port Matilda, USA) in the range between 1100 and 2500 nm with a data interval of 2 nm using also a PLS algorithm. To reach optimal correlation values, the spectral data were pre-treated with weighted multiplicate scatter correction and were transformed individually with the first and second derivative processing.

3. Results and discussion

It has been found that the distribution and content of main volatile substances in hydro-distillates or solvent extracts isolated from various essential oil plants can be predicted by the different spectroscopy methods described. Based on the spectral data sets, cluster analysis can be established presenting the specific differences of the individual chemotypes. As demonstrated in Fig. 1 the cluster analysis established on the basis of ATR-IR data of various chamomile oils shows a remarkable variation relating to the different genetic background of the plants (chemotypes) and special manufacturing processes, respectively. Furthermore, it was found that the individual GC composition of the investigated oils (Table 1) correlates very well with the statistic results (Ward's algorithm) calculated from the spectral data.

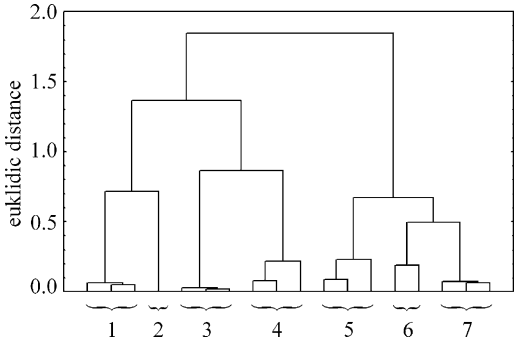

Fig. 1. Cluster analysis (Ward's algorithm) based on the ATR-IR spectra (measurement range: 650–4000 cm^{-1}) of different chamomile chemotypes and manufacturing processes (GC composition range of each analysed cluster is given in Table 1).

Table 1
Gas chromatographic composition of the investigated chamomile essential oils obtained from different chemotypes and manufacturing processes

Cluster	β-Farnesene	α-Bisabolol	Bisabolol oxide A	Bisabolol oxide B	Bisabolone oxide A	Chamazulene
1	4.2–8.2	0–0.3	0	0–0.7	0	0
2	3.9	1.5	17.6	34.4	15.7	8.1
3	46.9–47.3	28.2–28.8	0	1.1–1.2	0	6.9–7.2
4	10.9–14.5	48.8–55.5	0	1.4–1.7	0	15.4–21.2
5	3.1–9.0	5.7–7.3	12.4–21.8	46.3–49.2	2.2–2.9	1.9–5.1
6	6.9–9.0	2.2–3.4	53.3–54.3	8.1–8.4	8.1–8.2	2.6–3.3
7	15.4–15.8	5.8–11.9	21.6–27.3	13.2–16.3	3.7–5.3	10.8–12.9

Tentative identifications of bisabolol oxides A and B, bisabolone oxide A and β-farnesene were based on comparison of GC–MS data with those of Wiley and NBS computer mass libraries.

According to former studies performed on various marjoram, peppermint and citrus oils [3,5,7] the NIR spectra of the analysed essential oil samples are dominated by overtones and different combinations of C–H-stretching and bending vibrations occurring between 1600–1800 and 2200–2500 nm, respectively. Although the NIR absorptions are comparatively broad, the spectra of different basil chemotypes present characteristic key bands which can be used for discrimination purposes (Fig. 2). According to the results of a recently published study [27] it was found that these four basil types possess remarkable differences with regard to the composition of the essential oil fraction; applying GC the following main substances have been detected in the individual oils: type A, approximately 57% elimicin; type B, approximately 48% caryophyllene; type C, approximately 83% methyleugenol; type D, 66% eugenol. Corresponding to this fact, the different main substances occurring in the individual essential oils influence principally the resulting NIR spectra.

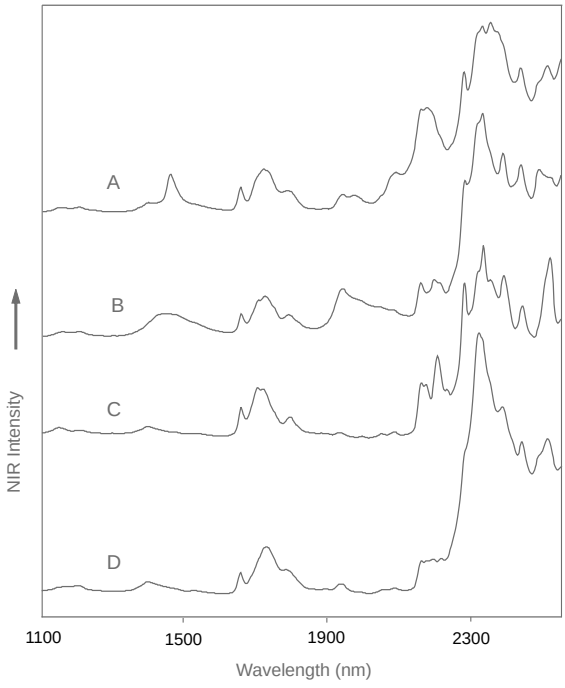

Fig. 2. Near-infrared spectra obtained from essential oils of different basil chemotypes: (A) *Ocimum seilol* Benth. (elimicin type); (B) *Ocimum tenuiflorum* L. (β-caryophyllene type); (C) *Ocimum tenuiflorum* L. (methyleugenol type); (D) *Ocimum gratissimum* ssp. Iringense Ayobangira ex Paton (eugenol type).

Fig. 3 presents the Raman spectra obtained from chamomile oil using laser excitations at 830 and 1064 nm, respectively. It can be seen that the signals acquired with the Nd:YAG laser show higher intensity in the range between 1500 and 2300 cm^{-1}, whereas in the lower frequency range (500–1300 cm^{-1}) the 830 nm diode laser supplies more sensitivity. Note this observation is due to the characteristic optical response of the spectrometer and the detector used in both experiments. The decrease of the Raman intensity detected in the spectra is caused by the sensitivity decrease of the silicon CCD detection with regard to NIR radiation. Therefore, applying the exciting radiation with lower quantum energy, the characteristic Raman bands of the polyacetylenes occurring at 478, 1628 and 2236 cm^{-1} (*cis*- and *trans*-spiroether) can be analysed at optimal conditions. Some less intense bands in the spectra are related to α-bisabolol which is present in the oil in amounts of approximately 45% ($\nu_{C=C}$ at 1673 cm^{-1}, δ_{CH_2} at 1380 and 1433 cm^{-1}, δ_{C-H} at 753 cm^{-1}). Relating to Raman measurements of pure chamazulene it can be assumed that the spectral contribution of this essential oil component to the spectrum of the chamomile oil is very low.

In Fig. 4 Raman spectra of thyme oil (obtained by excitation at 830 and 1064 nm, respectively) are shown. Both spectra present the characteristic key bands of thymol (733 cm^{-1}), carvacrol (751 cm^{-1}) and *p*-cymene (799 and 1204 cm^{-1}). These results are in good agreement with former studies performed on Lamiaceae plants [12,13]. Fig. 5 presents Raman spectra of an origanum oil obtained from a special genotype with high thymol content (approximately 66%). Generally, the spectral fingerprint is very similar to that of a special chemotype of basil which has been recently described in detail [8]. The most intense signal at 733 cm^{-1} is assigned to ring stretching vibrations of the aromatic substance; other less intensive bands to be observed at 1376 and 1610 cm^{-1} are due to vibrational modes of the ring system.

In some instances a chemotaxonomic identification can be tentatively also obtained directly from the living plant

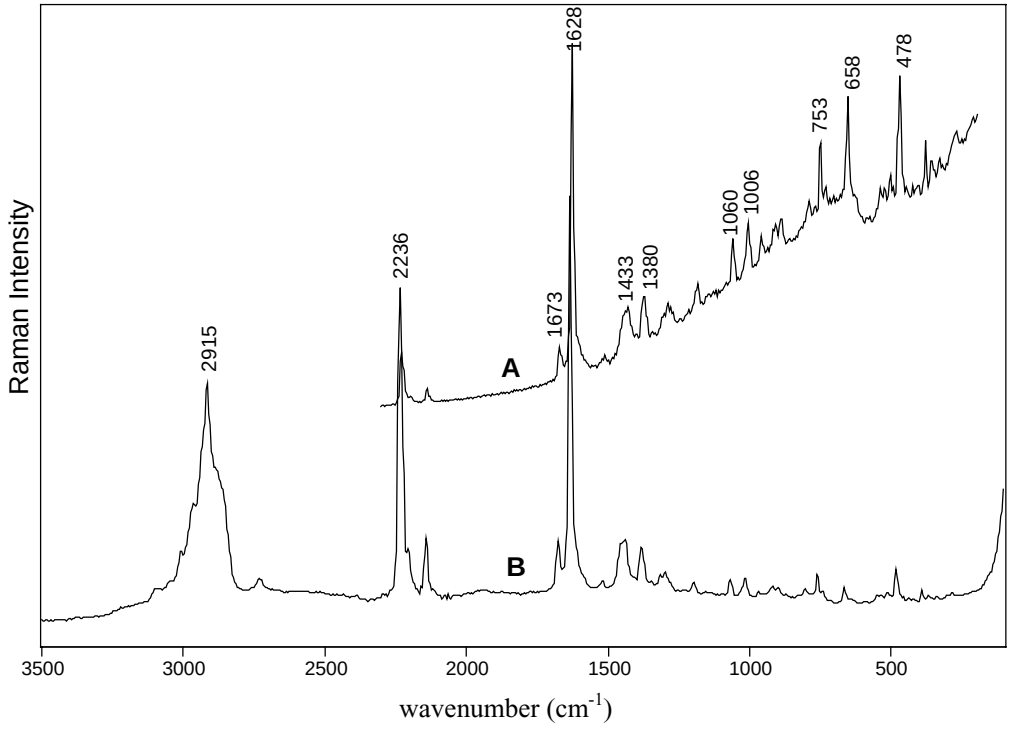

Fig. 3. Unmanipulated Raman spectra obtained from the essential oil of chamomile (content of main components: 45% bisabolol, 18% chamazulene, 14% β-farnesene, 9% *cis*-spiroether) acquired with a 830 nm diode laser (A) and a Nd:YAG-laser operating at 1064 nm (B).

[13,14]. As presented in Fig. 6 micro-Raman measurements performed on the essential oil cells of peppermint leaves (*Mentha piperita* L.) allow to get a general impression of the individual essential oil composition which in this case looks very similar to the Raman spectrum obtained from a pure isolated peppermint oil. In order to reduce spectral influences of other cell areas, it is necessary to build the difference spectrum, calculated from measurements of the

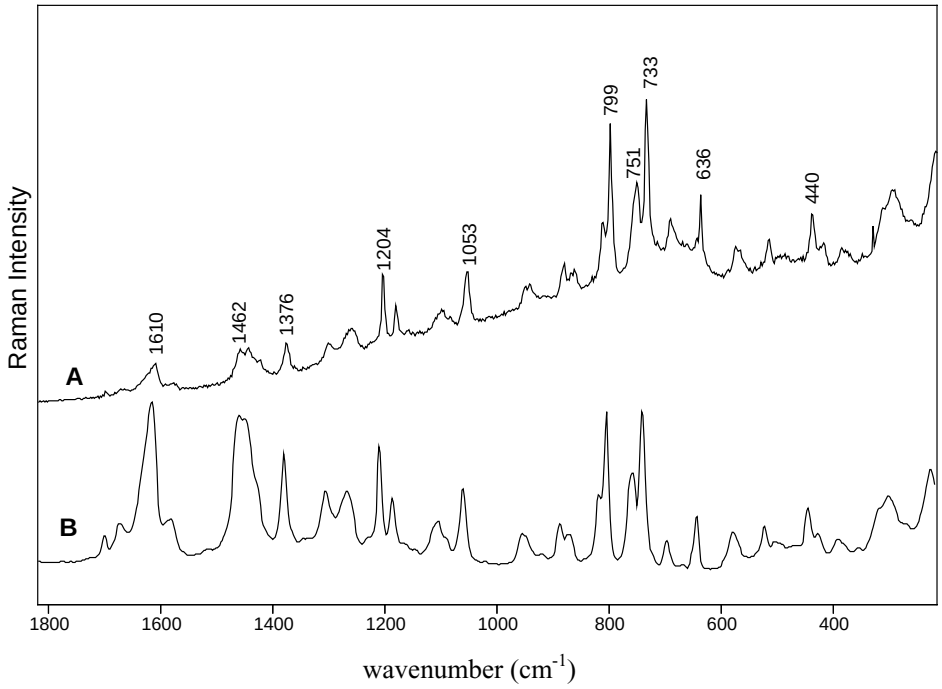

Fig. 4. Unmanipulated Raman spectra obtained from the essential oil of thyme (content of main components: 29% *p*-cymene, 24% thymol, 19% carvacrol) acquired with a 830 nm diode laser (A) and a Nd:YAG-laser operating at 1064 nm (B).

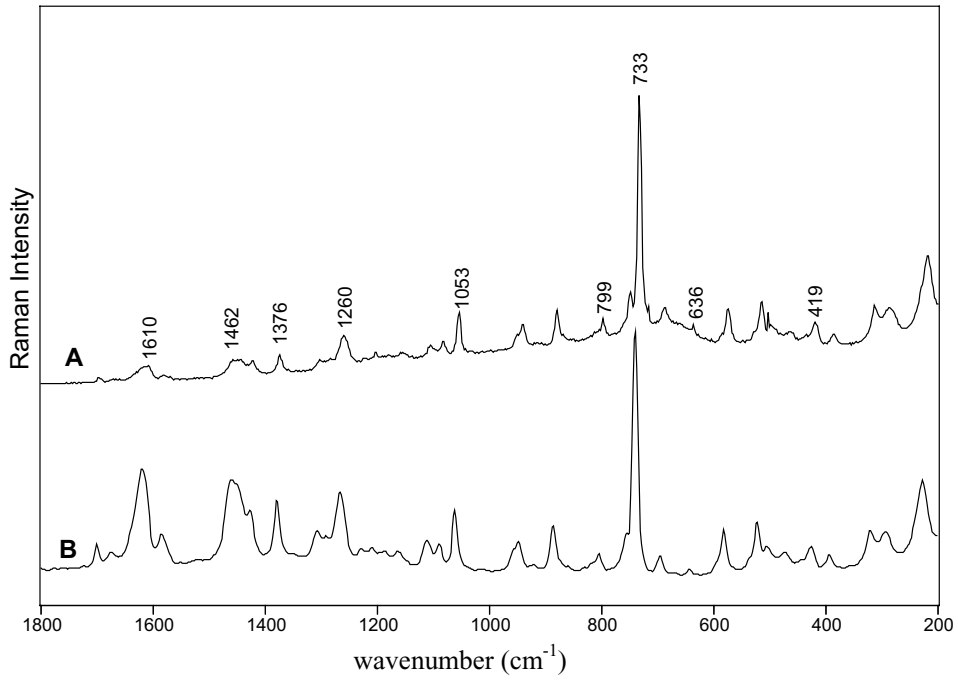

Fig. 5. Unmanipulated Raman spectra obtained from the essential oil of origanum (content of main components: 66% thymol, 10% γ-terpinene, 5% *p*-cymene) acquired with a 830 nm diode laser (A) and a Nd:YAG-laser operating at 1064 nm (B).

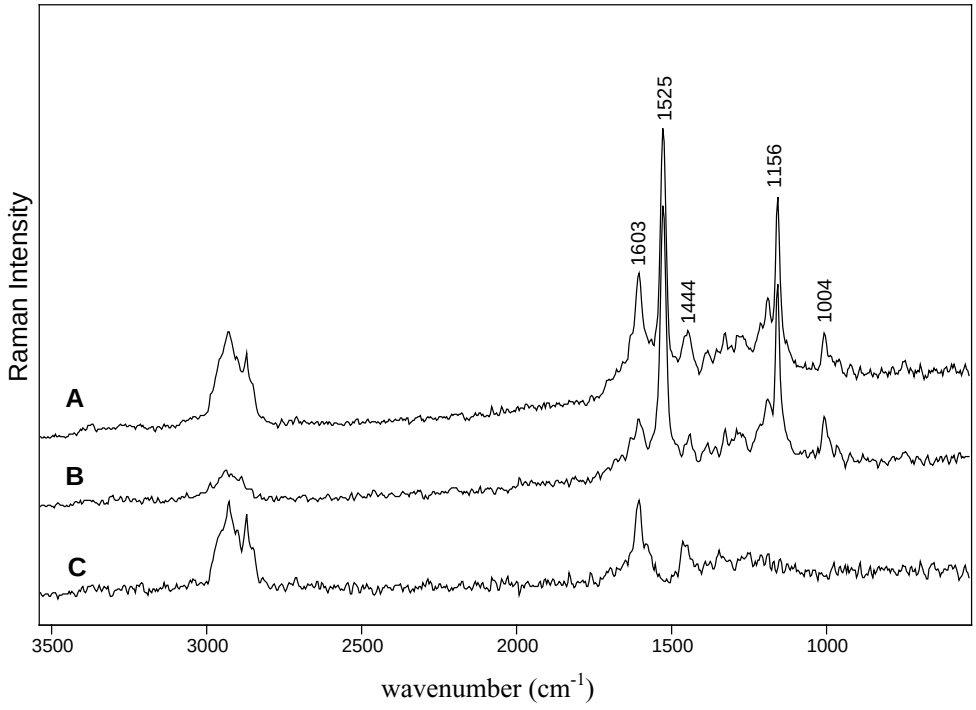

Fig. 6. NIR FT-Raman measurements on peppermint leaves: (A) Raman spectrum of the essential oil cell; (B) Raman spectrum of the plant matrix; (C) difference spectrum of (A) and (B).

essential oil cell and the surrounding matrix. Whereas the bands occurring at 1004, 1156 and 1525 cm^{-1} are assigned to carotenoids, the signals at 1600 cm^{-1} and in the region between 2800 and 3000 cm^{-1} are related to terpenoid vibrations of the peppermint oil present in the oil glands.

4. Summary

The quality control of natural products such as essential oils is one of the most challenging tasks in modern analytics. Vibrational spectroscopy has proven to be a non-destructive

and fast method requiring both minimal sample preparation and minimum amount of analyte. In this paper we have shown various examples of application using different vibrational spectroscopy methods for the evaluation of some essential oil producing species belonging to the Asteraceae and Laminaceae families.

The ability to monitor rapidly various essential oils makes it possible to efficiently select high-quality single plants from wild populations as well as progenies of crossing experiments. Furthermore, the vibrational spectroscopy methods described here can also be used in the flavour and fragrance pharmaceutical industry in order to perform fast quality checks of incoming raw materials and continuous controlling of distillation processes.

Acknowledgements

The financial support of the ''Deutsche Forschungsgemeinschaft (DFG)'' in Bonn, Germany (grant nos. Schu 577/7-1 as well as Po 563/4-1) is gratefully acknowledged.

References

[1] Food Chemical Codex (FCC), 4th ed., National Academy Press, Georgetown, 1996.
[2] European Pharmacopoeia, Part 1, Maisonneuve SA, Sainte Ruffine, 1983, p. V.4.5.8.
[3] H. Schulz, H.-H. Drews, H. Krüger, J. Essent. Oil Res. 11 (1999) 185.
[4] H. Schulz, R. Quilitzsch, H.-H. Drews, H. Krüger, Int. Agrophys. 14 (2000) 249.
[5] B. Steuer, H. Schulz, E. Läger, Food Chem. 72 (2002) 113.
[6] H. Schulz, B. Schrader, R. Quilitzsch, B. Steuer, Appl. Spectrosc. 56 (2002) 117.
[7] H. Schulz, H. Krüger, B. Steuer, F. Pank, Z. Arzn. Gew. Pfl. 4 (1999) 62.
[8] H. Schulz, B. Schrader, R. Quilitzsch, S. Pfeffer, H. Krüger, J. Agric. Food Chem. 51 (2003) 2475.
[9] H. Schulz, R. Quilitzsch, H. Krüger, J. Mol. Struct. 661–662 (2003) 299.
[10] H. Schulz, NIR News 13 (2002) 10.
[11] H. Schulz, ISHS Acta Hort., in press.
[12] D.J. Daferera, P.-A. Trantilis, M.G. Polission, J. Agric. Food Chem. 50 (2002) 5503.
[13] P. Rösch, J. Popp, W. Kiefer, J. Mol. Struct. 480–481 (1999) 121.
[14] P. Rösch, W. Kiefer, J. Popp, Biopolymers (Biospectroscopy) 76 (2002) 358.
[15] A. Herisset, J. Jolivet, P. Rey, Plant. Med. Phytol. 5 (1971) 188.
[16] J. Jolivet, P. Rey, M.-F. Boussarie, Plant. Med. Phytol. 5 (1971) 199.
[17] A. Herisset, J. Jolivet, P. Rey, Plant. Med. Phytol. 5 (1971) 305.
[18] A. Herisset, J. Jolivet, P. Rey, Plant. Med. Phytol. 6 (1972) 11.
[19] A. Herisset, J. Jolivet, P. Rey, Plant. Med. Phytol. 6 (1972) 137.
[20] A. Herisset, J. Jolivet, P. Rey, Plant. Med. Phytol. 6 (1972) 194.
[21] A. Herisset, J. Jolivet, P. Rey, Plant. Med. Phytol. 6 (1972) 281.
[22] A. Herisset, J. Jolivet, P. Rey, Plant. Med. Phytol. 7 (1973) 37.
[23] S. Gunasekaran, R. Rajkumar, Asian Chem. Lett. 3 (1999) 195.
[24] A. Herisset, J. Jolivet, P. Rey, M. Lavault, Plant. Med. Phytother. 7 (1973) 306.
[25] M. Sawamura, Recent Res. Develop. Agric. Food Chem. 4 (2000) 131.
[26] G. Lösing, M. Degener, G. Matheis, Dragoco Rep. 45 (1998) 180.
[27] H. Krüger, S.B. Wetzel, K. Hammer, B. Zeiger, J. Herbs, Spices, Med. Plants 9 (2002) 335.