

## **P 18: Discrimination of fennel chemotypes applying IR and Raman spectroscopy – discovery of a new $\gamma$ -asarone chemotype**

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### **Abstract**

Various vibrational spectroscopy methods have been applied to classify different fennel chemotypes according to their individual profile of volatile substances.

Intact fennel fruits of different chemotypes could be successfully discriminated by Attenuated Total Reflectance Fourier transform Infrared (ATR-FTIR) and Near Infrared (NIR) spectroscopy. Solvent extracts (CCl<sub>4</sub>) of the considered fennel fruits showed characteristic fingerprints with marker bands related to the individual volatile components (*trans*-anethole, fenchone, estragole, piperitenone oxide,  $\gamma$ -asarone, limonene) for ATR-FTIR and FT-Raman spectroscopy.

Especially  $\nu$ C=C and  $\nu$ C=O absorption bands contribute to the different spectral profiles. Based on hierarchical cluster analysis, the considered fennel accessions were classified according to gas chromatographic (GC) and vibrational spectroscopic data. Furthermore, even a discrimination of "sweet" and "bitter" fennel fruits, both belonging to the *trans*-anethole chemotype, could be successfully performed. All vibrational spectroscopical techniques used in this study are rapid and easy to apply. Hence, they allow different fennel chemotypes to be reliably distinguished and can also be used for on-site measurement in free nature.

Keywords: ATR-FTIR, FT-Raman, NIRS, *Foeniculum*, chemometry,  $\gamma$ -asarone

### **Introduction**

Fennel (*Foeniculum vulgare* Mill.) is cultivated worldwide for its characteristic aromatic leaves and fruits and represents the sole plant species in the genus *Foeniculum*.

The species shows a large diversity in morphology and chemical composition of essential oil (BERNATH and NEMETH, 2007; BERNATH et al., 1996; CHUNG et al., 1999; GUDI et al., 2014; KRÜGER and HAMMER, 1999). Nevertheless, a distinct discrimination of different chemotypes only by visual evaluation is highly defective and represents the major challenge for goods receipt and quality control.

The application of GC/MS methods provides reliable information for the identification of the individual chemotype and the precise quantification of the related essential oil components. Due to the laborious and expensive extraction steps needed followed by chromatographic separation, gas chromatography is not suitable enough for large sample sets and routine screening.

Especially for breeding purposes, destructive analysis like GC would destroy the opportunity of subsequent cultivation. Hence, more efficient, rapid and robust analytical methods directly applicable to intact plant material like near infrared (NIRS) or mid infrared (MIRS) spectroscopy are demanded.

Therefore, the aim of this study was to develop new vibrational spectroscopy methods for a fast and non-destructive classification of fennel fruits and related micro-extracts as well as a rapid determination of the most important volatile substances directly in the intact fennel fruit. The

results were already published as full paper in the Journal of Agricultural and Food Chemistry (DOI: 10.1021/jf405752x) (GUDI et al., 2014).

## Materials and Methods

Fruits of different fennel accessions were provided by the Institute of Plant Genetics and Crop Plant Research in Gatersleben (IPK) and of the Federal Plant Variety Office in Dachwig (Germany). Further botanical information together with detailed experimental description for GC-FID, GC-MS, infrared and Raman spectroscopic measurements and nuclear magnetic resonance (NMR) spectroscopy are given in the appropriate literature (GUDI et al. 2014).

Solvent extracts of fennel fruits in CHCl<sub>3</sub> were investigated by GC-MS for qualitative description of the volatile organic fraction and quantification of the individual components was performed by GC-FID.

Intact fennel fruits were analyzed by attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR, portable ATR diamond crystal infrared spectrometer Alpha, Bruker Optics GmbH, Ettlingen, Germany) according to GUDI et al. 2014.

Variability of the fennel samples was investigated by hierarchical cluster analysis (Ward's algorithm) and by principal component analysis (PCA) under usage of the instrument software OPUS 6.5 (Bruker Optics GmbH, Germany). Therefore, relevant spectral ranges were determined by comparison of sample spectra with those of appropriate standards.

## Results

GC-MS/ GC-FID analyses of solvent extracts from the investigated fennel genotypes revealed highly diverse qualitative and quantitative composition of the volatile fractions. Except one accession (FOE 25), all considered types contained between 6.29 and 35.08 % fenchone and are dominated by at least one phenylpropanoic structure as the main component. In contrast, FOE 25 is dominated by nearly 47 % of limonene and 29 % of piperitenone oxide and therefore, has to be assigned to the piperitenone/ piperitenone oxide chemotype according to literature (BADOE et al., 1994).

Two other accessions (FOE 86 and 87) were dominated both by a phenylpropanoid but neither *trans*-anethole nor estragole could be detected. Based on <sup>1</sup>H- and <sup>13</sup>C-NMR spectroscopy in combination with MS this structure could be identified as  $\gamma$ -asarone (Figure 1), a hitherto not described component in *Foeniculum*.

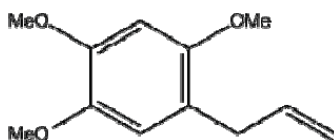


Fig. 1 Molecular structure of  $\gamma$ -asarone according to MS, <sup>1</sup>H- and <sup>13</sup>C-NMR spectroscopic analysis of volatile fractions from FOE 86 and FOE 87.

Different vibrational spectroscopy methods have been applied for intact fennel fruits and solvent extracts. ATR-FTIR showed to provide good separation of all different fennel chemotypes according to the appropriate GC-FID data. Figure 2 presents the results of hierarchical cluster analysis for the 10 different fennel accessions based on GC-FID and ATR-FTIR of intact fennel fruits.

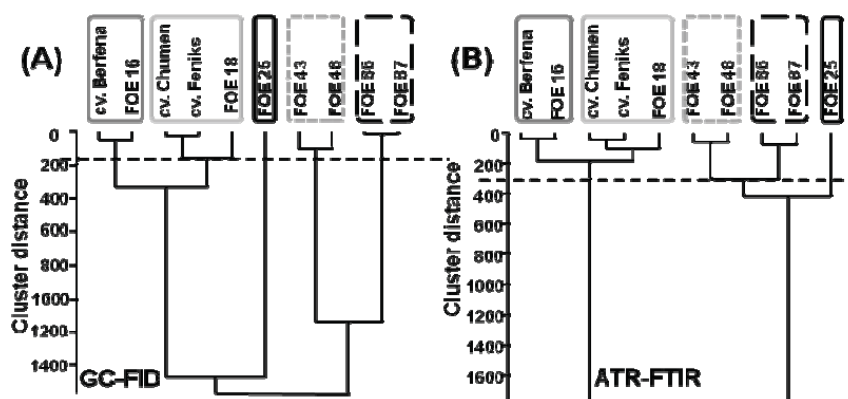


Fig. 2 Hierarchical cluster analysis of 10 different fennel accessions based on the GC-FID data (A) and ATR-FTIR spectra of intact fruits (B). The dashed lines mark the lowest level of complete discrimination of the individual chemotypes. Adapted to and reprint with permission from (Gudi et al., 2014). © 2014 American Chemical Society.

As shown in Figure 2 different fennel accessions could be successfully discriminated according to the chemical profile of the volatile fraction determined by GC-FID. The separation into chemotypes was based on the major component in the volatile fraction which is *trans*-anethole for FOE 16, FOE 18 and the cultivars 'Berfena', 'Chumen' and 'Feniks', whereas estragole represents the major component in FOE 43 and FOE 48. As described above, FOE 86 and FOE 87 are characterized by high amounts of  $\gamma$ -asarone in contrast to FOE 25, which mostly contained piperitenone derived structures and limonene.

Whereas the *trans*-anethole content resulted in discrimination of fennel cultivars conforming to EuPharm from those not consumable cultivars (separation into two big clusters at cluster distance level of 1700), the ratio of *trans*-anethole and fenchone was used for differentiation of sweet and bitter fennel (two clusters of 'Berfena'/FOE 16 and 'Feniks'/'Chumen'/FOE 18 at cluster distance level of 200). This differentiation by ATR-FTIR is accessible due to the characteristic absorptions for the C=O bond in fenchone and the conjugated C=C system in anethole.

In conclusion, ATR-IR offers for intact fennel fruits a tool for fast discrimination of different chemotypes according to their chemical profile of the volatile fraction, additionally enabling a fast differentiation of sweet and bitter fennel in between the *trans*-anethole-chemotype. A comprehensive overview about experimental data and complete description of the results can be found in Gudi et al. (2014).

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