Raman Analysis of Caraway (*Carum carvi* L.) Single Fruits. Evaluation of Essential Oil Content and Its Composition

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FT-Raman spectroscopy was applied for in situ analysis of single and intact fruits obtained from a diverse caraway (*Carum carvi* L.) collection. The Raman spectra showed characteristic bands that could be assigned to lignin, unsaturated fatty acids, and polysaccharides. Additionally, the essential oil composition was determined by gas chromatography (GC), which showed a great variation in carvone and limonene content among accessions obtained from European botanical gardens as well as advanced breeding materials. GC data and information obtained from Raman measurements were correlated and allowed development of calibration models for essential oil yield and carvone and limonene content (R^2 = 74%, 81%, and 75%, respectively). The congruence between results obtained by both techniques indicates FT-Raman spectroscopy to be a valuable screening tool for quality control and selection of fruits with desired essential oil composition, which can be performed without fruit destruction.

KEYWORDS: Caraway; carvone; limonene; GC; medicinal plant; in situ analysis

INTRODUCTION

Essential oil analysis is usually performed by using different spectrophotometric or chromatographic methods, among which gas chromatography (GC) and high-performance liquid chromatography (HPLC) are mostly used due to their high reproducibility and low detection limit. However, in most cases the use of these techniques requires a long process of sample preparation and therefore alternative fast and simple methods for plant analysis are of great interest. The first applications of FT-Raman spectroscopy to nondestructive measurements of various plant tissues were found to be very promising, and this method has been therefore used extensively for this purpose in the past few years (*1*).

Generally, in FT-Raman spectroscopy a sample is radiated with monochromatic near-IR light emitted from a laser, which probes molecular vibrations, and consequently information about the structure of chemical components present in the investigated sample is gathered. Functional groups of the molecules can be identified by their unique pattern of light scattering (Raman spectrum), and the intensity of the signals may be used for calculation of their relative concentration in the sample. One measurement provides the whole spectrum with information about all components present in the sample; however, usually only those at higher concentrations can be recognized and identified directly. Nevertheless, the use of chemometric methods and calibration models allow minor components to be analyzed as well (*2–4*).

Caraway (*Carum carvi* L.) is one of the most important medicinal plants. This biennial crop is cultivated mainly in European countries with temperate climates: i.e., The Netherlands, Germany, Finland, Czech Republic, Slovakia, and Hungary. In Poland caraway is cultivated on 4000–8000 ha. An increase in cultivation area in Canada, the USA, Syria, and Marocco has been recently observed as well. Production of caraway fruits is estimated as 10 000–15 000 tons per year and caraway essential oil as 30 tons, which is the fifth highest among species of the *Apiaceae* family. Caraway fruits (*Carvi fructus*) are rich in essential oils composed mainly of cyclic monoterpenes, i.e. carvone and limonene, which make up over 95% of the total essential oil (*5,6*). They are used in the pharmaceutical industry as antispasmodic, carminative, bactericidal, and diuretic agents as well as promoters of milk secretion. Fruits are also used as a valuable spice for meat, vegetables (potato and cabbage), cheese, and alcoholic beverages. Caraway essential oil is also used in the cosmetic industry for toothpaste, mouthwash, soap, cream, and perfume production. Carvone, obtained from caraway essential oil, applied to potato tubers decreases fungal infestation and inhibits potato sprouting during storage (*7*).

The aim of this work was to apply FT-Raman spectroscopy for in situ analysis of single caraway fruits, particularly in order to assess the oil content and its composition. Calibration models were established by using gas chromatography as a reference method.

MATERIALS AND METHODS

Plant Material. In the study a collection of 25 caraway (*Carum carvi* L.) accessions was used to evaluate a diversity of the initial materials for caraway breeding program. The collection consisted of accessions obtained from 18 European botanical gardens, 7 accessions of advanced breeding material, i.e. the Czech cultivar "Rekord", 2 strains of the Polish cultivar "Konczewicki" developed through repeated reproduction during maintenance breeding, and 4 new advanced breeding lines (9/2, 9/7, 9/12, 60/8) developed at the Institute of Medicinal Plants of Poznan (**Table 1**).

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Table 1. Main Components of Caraway Fruit Essential Oil Determined by GC (%)

location/accession	essential oil content	carvone	limonene	dihydro-carvone	carveol	α-pinene	total of GC profile
			Accessions from Botanical Gardens				
Bayreuth (DEU)	2.1	77.9	20.7	<0.1	<0.1	nd[a]	98.74
Berlin (DEU)	3.5	66.0	30.2	<0.1	nd	<0.1	96.27
Bonn (DEU)	3.6	59.2	37.0	<0.1	<0.1	<0.1	96.27
Cluj (ROM)	3.9	62.7	33.7	<0.1	nd	<0.1	96.53
Göttingen (DEU)	4.5	62.7	30.5	nd	0.15	nd	93.35
Jena (DEU)	4.0	61.0	35.7	<0.1	nd	<0.1	96.73
Krakow (POL)	5.0	62.0	36.0	0.10	<0.1	<0.1	98.18
Lausanne (CHE)	2.6	65.5	31.2	<0.1	nd	<0.1	96.74
Nantes (FRA)	2.9	77.2	22.5	nd	nd	nd	99.70
Poznan (POL)	2.3	63.6	32.1	<0.1	<0.1	<0.1	95.80
Prague (CZE)	5.0	63.1	34.5	<0.1	<0.1	<0.1	97.70
Reykjawik (ISL)	3.9	68.8	26.0	nd	nd	nd	94.80
Riga (LVA)	3.0	77.1	20.7	nd	nd	nd	97.80
Salzburg (AUT)	3.2	65.6	31.7	<0.1	nd	<0.1	97.32
Ulm (DEU)	3.1	60.2	36.2	0.11	nd	nd	96.51
Warsaw (POL)	5.8	63.7	34.4	<0.1	<0.1	<0.1	98.14
Wisley (GBR)	2.5	73.0	23.2	<0.1	<0.1	<0.1	96.25
Wroclaw (POL)	2.5	83.2	16.6	nd	nd	nd	99.80
			Advanced Breeding Materials				
"Rekord"	4.2	64.6	30.7	<0.1	nd	<0.1	95.40
"Konczewicki" 6	2.8	69.8	24.9	nd	nd	nd	94.70
"Konczewicki" 7	3.1	61.5	34.6	<0.1	nd	<0.1	95.13
line 9/10	2.8	63.0	33.5	0.11	nd	nd	96.61
line 9/12	4.3	72.1	23.7	<0.1	nd	nd	95.81
line 9/13	3.0	73.5	23.1	<0.1	nd	nd	96.61
line 60/8	2.7	74.0	22.2	<0.1	nd	nd	96.23

[a] nd = not detected.

The field caraway collection was established in 2007 in the experimental station of the Institute of Medicinal Plants in Plewiska near Poznan, Poland. In April seeds of all accessions were sown in a greenhouse. Plantlets with 5–8 leaves were planted in the field at the beginning of May, 25–140 plants per plot. Mature fruits were collected from 10 plants of each accession, separately from each plant, by hand in June 2008. After harvest fruits were allowed to dry in an open air shed.

Reference Analysis. The content of the essential oil was estimated after hydrodistillation from a mixture of caraway fruits (10 g) of each accession in two replications using Dering's apparatus by following the methods recommended by the Polish Pharmacopoeia VI (2002). The hexane solution of the oil (1:10) was analyzed by gas chromatography using a Perkin-Elmer Clarus 500 system under the following conditions: chromatographic column, Elite 1 (30 m × 0.32 mm × 0.25 μm); volume of each injected sample, 1 μL; injector temperature, 200 °C; carrier gas. helium; helium flow, 1 mL min^{-1}; FID detector temperature, 220 °C. The times of component retention of the tested solution are as follows: carvone, 45.17 min; limonene, 15.69 min; α-pinene, 9.16 min; dihydrocarvone, 38.04 min; carveol, 49.91 min.

FT-Raman Measurements. FT-Raman spectroscopy was used to evaluate the composition of individual fruits. The Raman spectra were recorded on a Nicolet NXR 9650 spectrometer with a diode-pumped Nd: YAG laser emitting at 1064 nm and a germanium detector cooled with liquid nitrogen. The instrument was equipped with an x-y-z automatic "MicroStage", a mirror objective, and a prism slide for redirection of the laser beam. In contrast to the standard vertical sampling arrangement, the samples were mounted horizontally and visualized before and during the measurements through a CCD camera. Such a configuration allowed us to choose the spot of laser illumination with high precision. With a point mapping accessory an automated measurement from the set of seeds was performed.

Samples of 10 fruits (one fruit per single plant) of each accession were placed directly on the stage. All spectra were measured in the range from 100 to 4000 cm^{-1} with an unfocused laser beam of about 1 mm diameter and with 100 mW power; 256 scans were collected with a spectral resolution of 4 cm^{-1}.

Chemometric Algorithms. The development of appropriate chemometric methods for calibration of the main components of caraway oils was carried out with the commercial statistic software OPUS (Bruker, Germany). A partial least-squares (PLS-1) algorithm, available in the Quant 2 package of the OPUS software, was used with an optimum number of PLS factors. The number of PLS factors in the models was determined on the basis of an F-test on PRESS (sum of square error of prediction) values in the cross validation. The calibration accuracy was described by the coefficient of determination (R^2) and the overall error between modeled and reference values (root mean standard error of cross-validation, RMSECV).

All data in the calibration set were preprocessed by background correction; the average spectrum for each accession was calculated and checked carefully to detect and eliminate outlier samples.

RESULTS AND DISCUSSION

GC Analysis of Caraway Oil. The mean oil content in the investigated caraway fruits determined using gas chromatography was 3.5% dry matter but varied considerably among accessions from 2.1% (Bayreuth) to 5.8% (Warsaw) (**Table 1**). High variation between accessions is similar to values reported by Bailer et al. (8), who evaluated eight caraway cultivars (3.0–5.1%), and those reported by Seidler-Lozykowska (6), who analyzed the content of essential oils (3.5–5.9%) in a provenance collection consisting of 22 origins. Among advanced breeding material, only the cultivar "Rekord" and line 9/12 were characterized by a higher amount of oil than the average, but they still possessed lower oil amounts than accessions obtained from botanical gardens in Warsaw (5.8%), Krakow (5.0%), and Prague (5.0%). A substantial difference between the cultivars "Rekord" and "Konczewicki" was noticed as well as between two "Konczewicki" populations, which contained much less than 4% essential oil as was provided in the breeder's description for this cultivar (9).

Article

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Carvone and limonene were the main components of the essential oil obtained from all accessions, contributing from 93.2% to 99.8% of the GC profile. The amount of carvone was 1.6−3.8-fold higher than that of limonene and varied from 59.2% (Bonn) to 83.2% (Wroclaw) of the total essential oil. The content of carvone for 13 accessions was higher than ever reported in caraway fruits so far: i.e., >65% of total oils (*6*, *10−13*). Limonene content was inversely proportional to the content of carvone (Pearson correlation $r = -0.98$, $p < 0.001$) and varied from 16.6% (Wroclaw) to 37.0% (Bonn). The detected amounts were much lower than those presented in previous reports (33.5−51.7%) (*6*, *10−13*). Other determined essential oil components such as dihydrocarvone, carveol, and α-pinene constituted less than 0.2% of the total GC profile, with dihydrocarvone being the most common.

The presented results obtained from a large and diverse collection confirms that the essential oil yield and the amounts of individual components highly depend on genetic background (*8*). However, a substantial difference in carvone and limonene content between presented data and the previous reports is noticeable. It is well documented that the content of volatile compounds can be highly influenced by climate conditions. The most crucial factor is light intensity, through its effect on assimilates availability, which determines the essential oil content and its quality. Sunny weather enhances limonene-6-hydroxylase activity, and as a result the content of carvone can increase 2-fold in comparison to limonene content (*14*). Moreover, agronomical aspects such as harvest date and plant density are also significant (*8*).

The registered cultivars and advanced lines developed for high oil content differed considerably in oil composition, which indicate potential improvement that can be achieved through breeding programs. Among only four chemotypes, differences between the lines reflecting the lowest and the highest carvone and limonene contents (lines 90/10 and 60/8) were 17% and 51%, respectively (**Table 1**). It is also noticeable that some botanical gardens possess accessions exhibiting a higher level of either carvone (e.g., Wroclaw, Bayreuth) or limonene (Bonn) than for cultivars and lines used in this study; thus, they may be a valuable material for breeding chemotypes rich in a desired compound.

Spectral Features of Single Caraway Fruit. FT-Raman spectroscopy was already successfully applied to a nondestructive analysis of single fruits from plants belonging to the *Apiaceae* family (*15*). Main seed components such as fatty acids (FA), polysaccharides, proteins, and lignin were identified on the basis of the obtained Raman spectra. The application of cluster analysis discriminated among most of the species evaluated and grouped them according to their taxonomical classification (*15*). Also, some micro FT-Raman experiments for the characterization of natural compounds in fennel fruits (*Foeniculum vulgare* Mill.) (*16*) or rape seeds (*17*) were reported. Raman mapping was used for the investigation of terpenoids occurring in fennel essential oil in single fruits. In situ measurements allowed localization of the essential oil cells; however, the analysis was limited only to oil detection, while the quantification of its components was not performed (*16*).

In this work, Raman spectra taken directly from single caraway fruits showed some distinctive bands which can be assigned mainly to primary plant metabolites (**Figure 1**). The most characteristic signal at 1606 cm^{-1} can be observed due to lignin, which is a main component of cell walls present in hard pericarp. This band is associated with stretching of aromatic rings of phenolic compounds composing the polymeric structure of lignin. In fact, in this range lignin gives a doublet band, with one maximum at about 1600 cm^{-1} (aromatic vibrations) and a peak of lower intensity at higher wavenumbers in the range of 1630−1660 cm^{-1}.
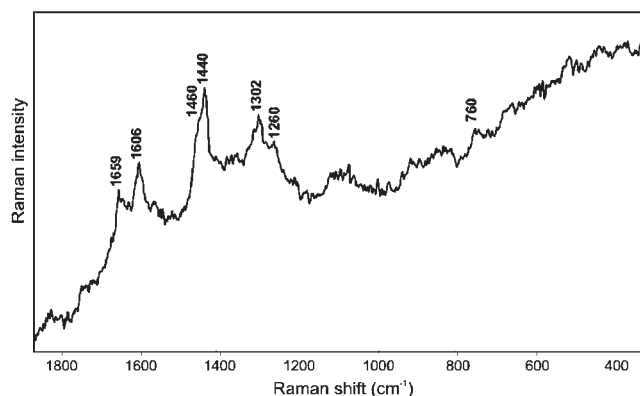

Figure 1. FT-Raman spectrum obtained from single caraway fruit.

The latter band coincides with the C=C stretching mode of FA identified by a signal at 1659 cm^{-1}. This mode can be assigned as the ν(C=C)$_{cis}$ stretching vibration of unsaturated FA, whereas the intense band near 1440 cm^{-1} is due to a CH$_2$ scissoring deformation vibration. The degree of FA unsaturation can be estimated from the peak area of the bands at 1260 and 1302 cm^{-1}, which are due to an in-phase =CH− symmetric rocking and a methylene twisting vibration, respectively. The estimated ratio of the discussed bands indicates a low degree of FA unsaturation that is additionally confirmed by a narrow band at 3008 cm^{-1} (not included in the spectral range presented in **Figure 1**). This conclusion is in agreement with previous GC reports describing oil composition and showing that 18:2 and 18:3 FA contribute below 35% to the total FA in essential oil (*18−20*).

Some bands in the spectrum of caraway fruit can also be assigned to polysaccharides: i.e. near 1460 cm^{-1} due to a −O−CH$_3$ stretch. This mode slightly overlaps with previously described CH$_2$ scissoring deformation of fatty acids observed at 1440 cm^{-1}. Polysaccharides, mainly β(1−4)-mannan, are stored in the cell walls of caraway fruit (*21*).

Determination of the Main Components of Essential Oils. Essential oil components can be recognized by Raman spectroscopy when they occur in the oil in higher amounts and when the measurement is taken from an isolated oil (*22*, *23*). Some data refer also to the chemotaxonomic analysis of essential oils based on in situ Raman measurements of various parts of aromatic plants (*24*), but up to now caraway fruits were not a subject of such analysis.

The main components of the investigated caraway oil samples estimated by GC, carvone and limonene, have characteristic bands in Raman spectra, which can be used for their unequivocal identification. Generally, monocyclic monoterpenes show intense Raman bands due to stretching vibrations of C=C bonds at about 1670 cm^{-1} (*25*). In the case of limonene two signals can be recognized in this range: 1678 cm^{-1} due to ν(cyclohexene C=C) and 1645 cm^{-1} due to ν(ethylene C=C), whereas for carvone these bands occur at 1670 and 1644 cm^{-1}, respectively. Most monocyclic terpenes demonstrate also a strong ring deformation vibration in the FT-Raman spectrum between 740 and 760 cm^{-1}. For limonene this band is situated at 760 cm^{-1} (*26*), but carvone, with keto substitution in the ring, can be characterized by two deformation modes of cyclohexene with frequencies of 700 and 680 cm^{-1}. These low-wavenumber signals can be used as spectral markers for those components.

Data reported before in the literature show that vibrational spectroscopy can be used not only for chemotype classification of various essential oils but also for identification of their major oil constituents, when the oil is isolated from the analyzed plant (*22*).
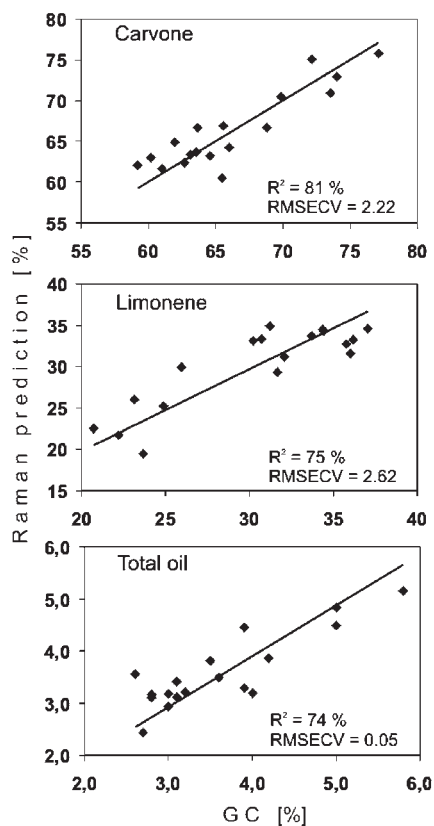
Figure 2. Reference GC values vs Raman predictions of carvone, limonene, and total oil content in caraway fruits.

Here in situ measurement of intact caraway seeds did not show signals due to either carvone or limonene, as they were obscured by other plant components (**Figure 1**). However, chemometric methods enabled discrimination between accessions possessing various amounts of essential oil components. The obtained set of Raman data consisted of spectra recorded between 100 and 4000 cm^{-1}, but for chemometric analysis the fingerprint range from 100 to 2000 cm^{-1} and high wavenumber range between 3600 and 3000 cm^{-1} were taken, which contain the most characteristic modes of the individual molecules. The average spectra from 10 measurements of single fruits for each accession were correlated with GC data using the PLS algorithm with an optimal number of factors. The best models obtained showed GC and Raman results to be congruent for carvone (R^2 = 80.5%, 2.22 RMSECV) and limonene (R^2 = 75.0%, 2.62 RMSECV) as well as for the oil content (R^2 = 74.0%, 0.05 RMSECV) (**Figure 2**). Other minor components, α-pinene and dihydrocarvone, occurring in the caraway oil also correlated with GC data but with lower precision (R^2 = 72.6% and 65.8%, respectively) and provided information only on the presence or absence of the analyte. Thus, on the basis of the Raman measurements of single fruits and the obtained models with low standard errors of cross validation, it is possible to estimate the composition of caraway fruits with high accuracy. The obtained results indicate that Raman spectroscopy can be a valuable tool in fast screening of small samples and selection of fruits with desired composition for further breeding purposes.

SUMMARY

In this work the application of FT-Raman spectroscopy for the analyses of caraway fruits was shown. The measurements employing mapping accessory and point acquisition mode allowed us to accelerate Raman measurements and obtain reliable data from a whole set of samples. A high congruence between data obtained from Raman measurements and reference GC data indicate that the former has the potential to be applied for quality control in the flavor and fragrance as well as pharmaceutical industry in order to perform fast quality checks of incoming raw materials and continuous control of distillation processes. Moreover, it may allow single fruits of desired essential oil composition to be selected without component extraction. Thus, nondestructively assessed and alive fruits can be used in further breeding programs for the development of new advanced chemotypes or cultivars.

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