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Rapid Discrimination of Closely Related Seed Herbs (Cumin, Caraway, and Fennel) by Direct Analysis in Real Time Mass Spectrometry (DART-MS)

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Direct analysis in real time mass spectrometry (DART-MS) was applied as a rapid method for the discrimination of the spices and traditional medicines cumin (*Cuminum cyminum* L.), caraway *(Carum carvi* L.), and fennel (*Foeniculum vulgare* Mill.). The seeds of these plants were analyzed without sample preparation by DART ion source coupled with quadrupole time-of-flight (QTOF) tandem mass spectrometry. The relatively clean DART spectra showed characteristic patterns, fingerprints, for each herb. It was found that a marker compound can be assigned to each species that can identify unambiguously these plants. Principal component analysis has also been used to analyze the DART-MS data of these seed herbs. Crispanone, carvone, and fenchone are the dominant compounds in the positive DART spectra of cumin, caraway, and fennel, respectively. Crispanone was first time identified as a constituent of cumin. Furthermore, the collision-induced dissociation (CID) behavior of the $[M+NH_4]^+$ ion of crispanone was also described.

Keywords Caraway, crispanone, cumin, direct analysis in real time tandem mass spectrometry (DART-MS/MS), fennel, principal component analysis (PCA)

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Introduction

The seed herbs cumin (*Cuminum cyminum* L.), caraway (*Carum carvi* L.), and fennel (*Foeniculum vulgare* Mill.) are closely related members of the parsley family (*Apiaceae* L.). The plentiful seeds of these plants are used as spices in cuisine all over the world. Cumin, caraway, and fennel are also used in traditional oriental medicine and other folk medicines. The essential oils of these plants show several similar biological activities including antibacterial, antifungal, anticancer, and antioxidant effects.[1-8] They are also employed beneficially in indigenous medicine for treating stomach and digestive problems.[9-11] In addition, they are used as constituents in cosmetics and flavoring products.[6,9,12]

Cumin is often confused with caraway or fennel due to the morphological similarities. Moreover, these spices are mentioned under various and confusing names, *e.g.* caraway is also known as meridian fennel or Persian cumin, or the term sweet cumin is used for fennel (In Hungarian, all three herbs are referred to as "*kömény*" distinguished by attributives, such as "*római kömény*", "*fűszer kömény*", and "*édes kömény*" for cumin, caraway, and fennel, respectively). These similarities, namely biological activities, morphology, and name, initiated our research to find a quick method to distinguish these plants unambiguously. Gas chromatography (GC), liquid chromatography (LC) and chromatography coupled with mass spectrometry (GC-MS, LC-MS) are conventionally used for the analysis of the essential oils of cumin, caraway, and fennel.[6,9-20] The ambient mass spectrometric techniques, *e.g.* direct analysis in real time mass spectrometry (DART-MS), enables the examination of plant samples in the open environment without sample preparation.[21] The DART ion source can ionize molecules directly from the surface of the different parts of the plants. DART-MS in combination with a multivariate analysis such as principal component analysis (PCA) is, therefore, an ideal tool for the rapid chemical profiling of plant species and for the identification of herbal products.[22-30] To the best of our knowledge, there have been no studies about the discrimination of these traditional herbs cumin, caraway and fennel by DART-MS technique.

Experimental

Materials

Commercially available cumin, caraway, and fennel seeds were analyzed. For identification of plant-drugs (the plant parts containing the bioactive compounds), morphological and histological investigations were conducted. In our study, 50 seeds per sample were measured for calculating the average weight of seeds. Their length and width were measured on mm-scale. Shape and color were investigated through stereo-microscope (Wisilight CL-30, VWR) equipped with a digital camera (Nikon D5100, Japan). Seeds were sectioned

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Table 1 Identification of the samples on the basis of morphological and histological investigations

No.	Species	Drug name	English name	Cultivated
1	*Cuminum cyminum* L.		Cumin, cummin, Roman caraway	Austria
2	*Cuminum cyminum* L.		Cumin, cummin, Roman caraway	Syria
3[a]	*Cuminum cyminum* L.		Cumin, cummin, Roman caraway	Syria
4	*Cuminum cyminum* L.		Cumin, cummin, Roman caraway	India
5	*Carum carvi* L.	Carvi fructus	Caraway	Austria
6[a]	*Carum carvi* L.	Carvi fructus	Caraway	Austria
7	*Carum carvi* L.	Carvi fructus	Caraway	Finland
8	*Carum carvi* L.	Carvi fructus	Caraway	Egypt
9	*Carum carvi* L.	Carvi fructus	Caraway	Czech Rep.
10	*Foeniculum vulgare* Mill.	Foeniculi fructus	Fennel	Hungary
11	*Foeniculum vulgare* Mill.	Foeniculi fructus	Fennel	Egypt
12[b]	*Carum carvi* L.	Carvi fructus	Caraway	Austria

a. The packet has been open for half a year. b. It was bought as fennel in a herb store.

by hand. The transverse sections of seeds were analyzed with the bright field facilities of an Olympus Provis BX50 microscope equipped with an Olympus DP80 digital camera. For identification figures and descriptions of relevant features, scientific articles and books were used.[31-36] All the samples were saved and registered in the Plant-Drug Collection of the Department of Botany (University of Debrecen, Hungary), numbered 2016.001 – 2016.011. The properties of the samples are compiled in Table 1, and the details of the identification are presented in Supporting Information. Ten different samples were examined. Sample 3 is the same as sample 2 except the packet was opened a half year before the experiments. The same is true for samples 6 and 5.

Quadrupole time-of-flight mass spectrometry

Measurements were performed with a MicroTOF-Q type Qq-TOF MS instrument from Bruker (Bruker Daltoniks, Bremen, Germany). For MS/MS experiments, nitrogen gas was used as the collision gas and the collision energies of 7 and 12 eV (in the laboratory frame) were used. The pressure in the collision cell was determined to be ~1.2×10^{-2} mbar. The precursor ions for MS/MS were selected with an isolation width of 4 *m/z* unit. All of the spectra were recorded by a digitizer at a sampling rate of 2 GHz. The mass spectra recorded were evaluated by the DataAnalysis 3.4 software from Bruker.

Ion source for direct analysis in real time (DART)

A DART SVP source was purchased from IonSense (IonSense, Inc., Saugus, MA). The solid samples were manually introduced into the DART gas stream. The gap between the ion source and the spectrometer inlet was 2.5 cm. Samples were inserted into the middle of the gap. The DART system was operated in the positive mode at 350°C with helium 5.0 (purity > 99.999%).

Calculation of the spectral similarity values

The similarity measure was calculated on the basis of correlation coefficient *r*, given by Eq. (1):

$$r = \frac{\mathbf{x}_A^T \cdot \mathbf{x}_B}{\|\mathbf{x}_A\| \cdot \|\mathbf{x}_B\|} \quad (1)$$

where $\mathbf{x}_A$ and $\mathbf{x}_B$ are column vectors representing the compared mass spectra *A* and *B*, respectively. The Euclidean norm $\|\mathbf{x}\|$ is equivalent to the length of the vector, given by the square root of the sum of the squared intensities. *r* is in the range 0 to 1. For better readability, the spectral similarity value *S* was scaled to the range 0 – 100 ($S = 100r$).[37]

Principal component analysis (PCA)

PCA analysis was carried out using Minitab 15 (Minitab Inc., USA) statistical analysis software (Trial Version).

Results and Discussion

Figure 1 shows the DART-MS spectra of: a. cumin (*Cuminum cyminum*), sample 1, b. caraway (*Carum carvi*), sample 5, and c. fennel (*Foeniculum vulgare*), sample 10 seeds recorded in positive-ion mode. The spectra were processed by subtracting the background spectrum of the DART source.

As seen in Fig. 1, the resulting spectra are relatively clean and simple despite the complex nature of the samples. As it will be detailed later, even the spectrum of cumin (Fig. 1a) contains peaks which can be assigned to only a few constituents.

DART-MS analysis of cumin

As Fig. 1a shows, the compound at *m/z* 352 (**1**) dominates the DART-MS spectrum of cumin. The appearance of the dimer of compound **1** at *m/z* 686 (**1′**) reveals the formation of $[M+NH_4]^+$ and $[2M+NH_4]^+$ ions. Ammonium adduct ions are frequently observed in DART-MS spectra. The ammonium ions may either be contained as an impurity of the sample or be generated from traces of ammonia in the laboratory atmosphere.[38] Mass accuracy and isotope distribution indicate that the elemental composition of compound **1** is $C_{20}H_{30}O_4$ (both the measured and calculated monoisotopic *m/z* values for the $[M+NH_4]^+$ ion is 352.248). To our best knowledge, no study has reported the detection of a constituent with elemental composition $C_{20}H_{30}O_4$ in cumin. In order to explore the structure of compound **1** and to identify it, tandem mass spectrometric (MS/MS) experiments were performed. On the basis of DART-MS/MS measurement, compound **1** was identified as crispanone and it will be discussed more in detail in the next section. Furthermore, MS/MS experiments of the ion at *m/z* 352 revealed that the peaks **1a**, **1b**, **1c**, **1d**, and **1e** in the DART-MS spectrum of cumin (Fig. 1a) originated from compound **1** as fragments created in the DART ion source.

The peaks **2** at *m/z* 151 and **3** at *m/z* 149 can be attributed to the polar constituent of the essential oil of cumin with the elemental composition $[C_{10}H_{14}O+H]^+$ and $[C_{10}H_{12}O+H]^+$, respectively. The major constituents are cuminic alcohol, safranal, and myrtenal with the composition $C_{10}H_{14}O$, and cuminaldehyde for $C_{10}H_{12}O$.[12,14] Of course, there are several other constituents in the essential oil of cumin, *e.g.* pinenes, phellandrenes, limonenes,[12] *etc.*, but these nonpolar compounds

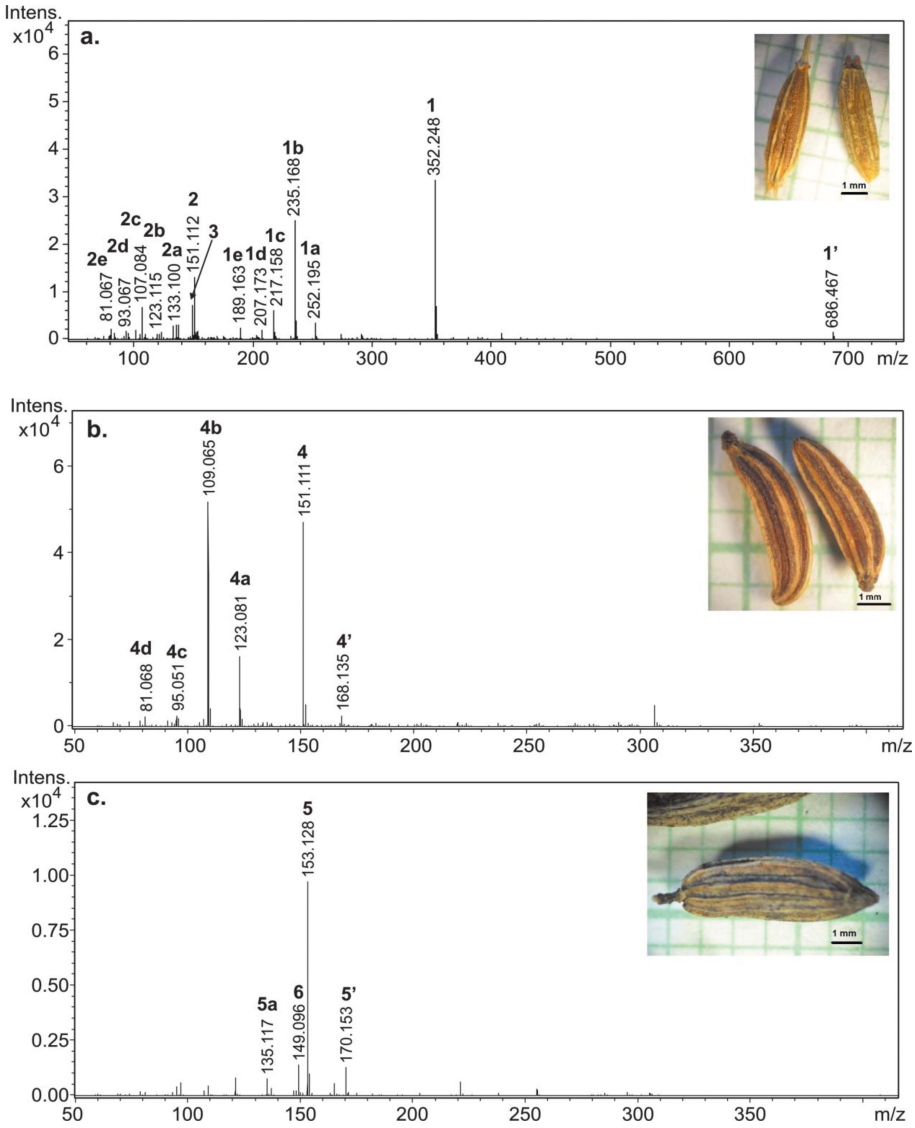


Fig. 1 DART-MS spectrum of; a, cumin (*Cuminum cyminum*), sample 1; b, caraway (*Carum carvi*), sample 5; c, fennel (*Foeniculum vulgare*), sample 10 (subtracting the background spectrum of the DART source).

can hardly be ionized by the DART technique. The MS/MS experiments of peak **2** showed that the peaks **2a**, **2b**, **2c**, **2d**, and **2e** originated from compound **2** as fragment ions created in the DART ion source.

DART-MS/MS analysis of crispanone

As seen in Fig. 1a, compound **1** at *m/z* 352 is the most intense peak in the DART-MS spectrum of cumin. As it was discussed previously, the elemental composition of the $[M+NH_4]^+$ ion of compound **1** was determined as $C_{20}H_{30}O_4$.

Figure 2 shows the DART-MS/MS spectrum of cumin with the precursor ion *m/z* 352. The neutral loss *m/z* 100.053 helped us to identify compound **1**, because it can be assigned to $C_5H_8O_2$ (tiglic acid).[39] It was found that the fragmentation of the $[M+NH_4]^+$ ion of crispanone can yield the product ions shown in Fig. 2. Scheme 1 shows the proposed fragmentation pathways of crispanone.

Even though no study has reported crispanone as a constituent of cumin (to the best of our knowledge) the presence of crispanone in parsley can justify the identification.[40,41]

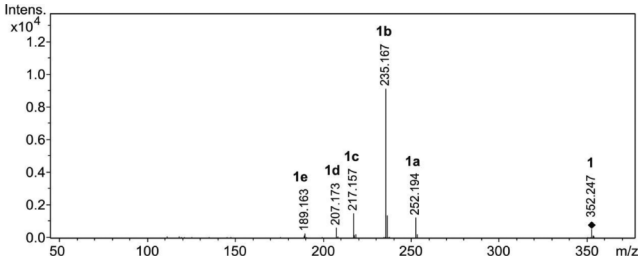
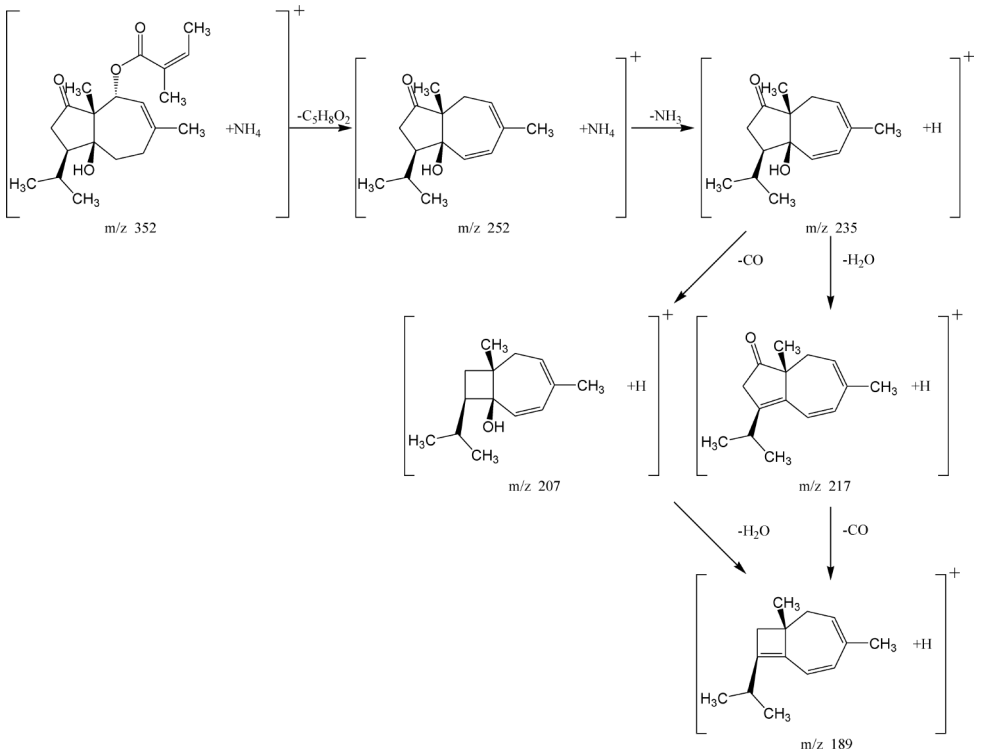


Fig. 2 DART-MS/MS spectrum of crispanone detected from cumin seed, recorded at a laboratory frame collision energy of 7 eV.

Crispanone is a daucane derivative, a class of sesquiterpenes widespread in the plants of the Apiaceae (or Umbelliferae) family.[42,43] Cumin also belongs to this family of aromatic plants. To confirm the identity of compound **1** detected in cumin and the well known constituent of parsley the DART-MS/MS



Scheme 1 The proposed fragmentation pathways of crispanone detected from cumin seed.

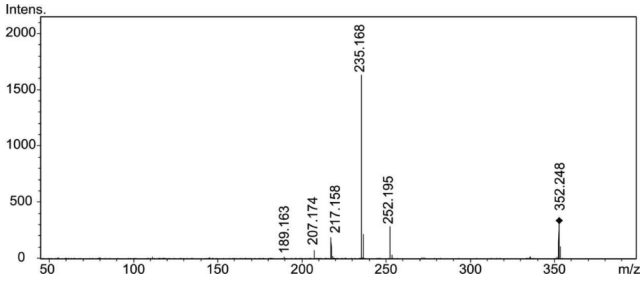


Fig. 3 DART-MS/MS spectrum of crispanone detected from parsley seed, recorded at a laboratory frame collision energy of 7 eV.

spectrum of parsley seed was also recorded (Fig. 3).

Comparing Figs. 2 and 3, the similarity is obvious; a spectral similarity value 99 was obtained, as detailed in the Experimental section.

DART-MS analysis of caraway

The main components of the essential oil of caraway seeds are carvone (40 – 70%) and limonene (25 – 35%).[16-19] As Fig. 1b shows, the DART-MS spectrum of the caraway seeds is very clean, all the remarkable peaks can be assigned to carvone. Peaks **4** and **4′** correspond to the $[M+H]^+$ and $[M+NH_4]^+$ adduct ions of carvone, respectively. MS/MS experiments revealed that the peaks **4a**, **4b**, **4c**, and **4d** originated from carvone (**4**) as fragments created in the DART ion source. Ground seeds were also examined and their DART-MS spectra (see Fig. S1 in Supporting Information) were identical to the spectra of the whole seeds. The DART-MS/MS spectrum of caraway selecting peak **4** (*m/z* 151) as the precursor ion is presented in the Supporting Information as Fig. S2. As seen in Fig. S2, two main product ions at *m/z* 123 and 109 are observed. The dominant product ion at *m/z* 109 (**4b**) is generated by the loss of the isopropenyl group of carvone. The proposed fragmentation mechanism for the formation of the product ion at *m/z* 109 from the protonated carvone by CID (collision-induced dissociation) is shown in Supporting Information as Fig. S3. This product ion (**4b**), together with the protonated molecular ion of carvone (**4**) can serve as marker peaks for the identification of caraway by DART-MS. Besides, the product ion 4b can be used as a characteristic product ion for distinguishing carvone from the components in cumin with the same elemental composition $C_{10}H_{14}O$ (*e.g.* cuminic alcohol, safranal, or myrtenal, peak **2** in Fig. 1a).

DART-MS analysis of fennel

The three major components in the essential oil of fennel are anethole, estragole ($C_{10}H_{12}O$), and fenchone ($C_{10}H_{16}O$),[13] which correspond in the DART-MS spectrum of the fennel seeds to peaks **6** and **5**, respectively (Fig. 1c). The protonated adduct of fenchone dominates the spectrum, and fenchone appears also as $[M+NH_4]^+$ adduct ion at *m/z* 170 (peak **5′** and with an H_2O neutral loss at *m/z* 135 (peak **5a**).

Rapid identification and/or quality control of cumin, caraway, and fennel

Our goal was also to find simple criteria for distinguishing between the closely related seed herbs cumin, caraway, and fennel. DART-MS offers a very fast analysis and, as it was discussed previously, resulting relatively simple mass spectral fingerprints in positive ion mode. Based on the results of DART-MS experiments, it was found that a marker compound can be assigned to each herb and monitored to identify unambiguously the species. In our study, 30, 40 and 10 measurements were performed for cumin, caraway and fennel,

Table 2 Marker compounds for the identification of cumin, caraway, and fennel by DART MS in positive ion mode

Species	Characteristic component	Peaks (*m/z*)
Cumin (*Cuminum cyminum*)	Crispanone	**352**, 686, 252, 235, 217, 207, 189
Caraway (*Carum carvi*)	Carvone	**151**, 168, 123, 109, 95, 81
Fennel (*Foeniculum vulgare*)	Fenchone	**153**, 170, 135

All the peaks listed in a row belong to the same characteristic compound. The more intense molecular adduct ions are in bold, the fragments created in the DART source are in italics.

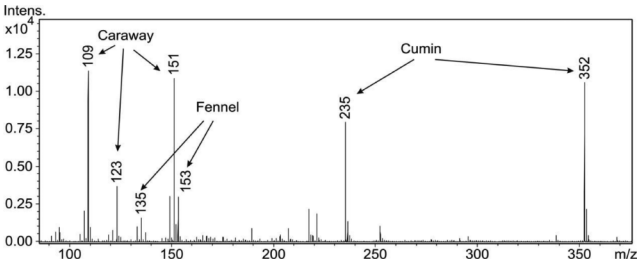


Fig. 4 DART-MS spectrum of a mix of the three herbs cumin, caraway, and fennel (samples 1, 5, and 10) (subtracting the background spectrum of the DART source).

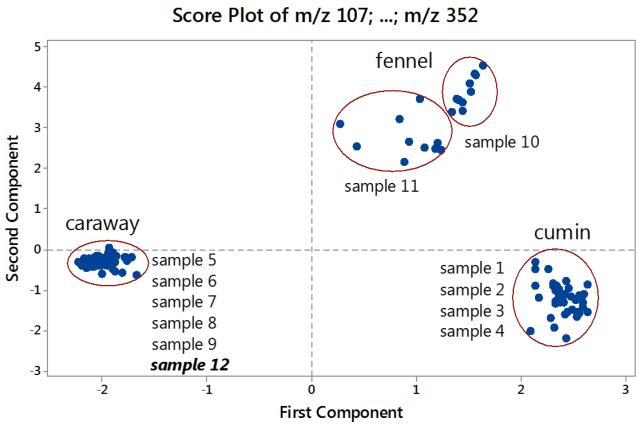


Fig. 5 PCA score plot from DART-MS spectra of cumin, caraway, and fennel on the basis of the abundance of various ions.

respectively. The average relative intensities and standard deviations (std. dev.) of the characteristic peaks were 100% (std. dev., 0.0%), 88.5% (std. dev., 10.6%), and 79.3% (std. dev., 20.9%) for cumin, caraway and fennel, respectively. The criteria for identification are summarized in Table 2.

One of our fennel samples (sample 12), bought in a herb store, happened to be a good test for our distinguishing criteria unintentionally (see Fig. S4 in Supporting Information). As Fig. S4 shows, the characteristic peaks at *m/z* 151, 123 and 109 unambiguously identify the sample as caraway, and the mass peak of fenchone at *m/z* 153, which is the characteristic compound of fennel that could not be detected in the DART-MS of the sample. This identification, on the basis of DART-MS, agrees with the taxonomic identification (see Table 1 and Supporting Information).

A mix of cumin, caraway, and fennel seeds was also tested to determine whether the components could be identified by our criteria. Figure 4 shows the DART-MS spectrum of the seed mix (samples 1, 5, and 10). As seen in Fig. 4, the characteristic peaks (see Table 2) can clearly identify all the three herbs.

Multivariate statistical analysis of the DART-MS spectra

DART-MS in combination with a multivariate analysis such as principal component analysis (PCA) can serve as an efficient tool for classification of natural origin samples. The DART-MS data of cumin, caraway and fennel (10 sample sets for each sample in Table 1) were subjected to PCA using 9 variables (the abundances of the ions at *m/z* 107, 109, 123, 135, 149, 151, 153, 235, and 352). The PCA score plot clearly shows clustering of the data according to the species (see Fig. 5). It is, therefore, evident that DART-MS followed by PCA is an appropriate method for the unambiguous identification of the species cumin, caraway and fennel. As Fig. 5 shows, intra-species variations can also be observed; PCA clearly groups the two fennel samples (samples 10 and 11) according to the cultivation areas. As it was mentioned previously, sample 12 was bought as fennel cultivated in Austria (see Table 1). But, the PCA scores of all the 10 independent DART-MS fingerprints of sample 12 unambiguously classify this sample as caraway (see Fig. 5), in agreement with our previous conclusion and with the taxonomic identification.

Conclusions

Our results show that DART-MS can provide a rapid method for the differentiation of cumin, caraway, and fennel. It is especially important and useful when ground seeds have to be distinguished or identified. A dominant compound appears in the positive ion mode DART spectra for each herb. These marker compounds can unambiguously identify the species. The fragment ions of these major compounds created at the relatively high temperature of the DART ion source can also support the identification of the plants. DART-MS fingerprints combined with PCA analysis can provide a useful method for the rapid identification of cumin, caraway and fennel. Our method may also be applied in the quality control of functional foods and medicines containing these herbs as active ingredients. The collision induced dissociation of crispanone, the dominant compound in the DART spectrum of cumin, was also explored. The fragmentation pattern, reported in the present study, can be used as a library spectrum for the identification of crispanone, which is widespread in the plants of the parsley family.

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Supporting Information

This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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