Received: 19 November 2019 | Revised: 17 December 2019 | Accepted: 24 December 2019

DOI: 10.1002/jms.4501

RESEARCH ARTICLE

Journal of MASS SPECTROMETRY WILEY

Characterization of volatile metabolites formed by molds on barley by mass and ion mobility spectrometry

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Funding information
Federal Ministry of Food and Agriculture, Grant/Award Number: 2814801811; Federal Office for Agriculture and Food, Grant/Award Number: 2814801811

Abstract

The contamination of barley by molds on the field or in storage leads to the spoilage of grain and the production of mycotoxins, which causes major economic losses in malting facilities and breweries. Therefore, on-site detection of hidden fungus contaminations in grain storages based on the detection of volatile marker compounds is of high interest. In this work, the volatile metabolites of 10 different fungus species are identified by gas chromatography (GC) combined with two complementary mass spectrometric methods, namely, electron impact (EI) and chemical ionization at atmospheric pressure (APCI)-mass spectrometry (MS). The APCI source utilizes soft X-radiation, which enables the selective protonation of the volatile metabolites largely without side reactions. Nearly 80 volatile or semivolatile compounds from different substance classes, namely, alcohols, aldehydes, ketones, carboxylic acids, esters, substituted aromatic compounds, alkenes, terpenes, oxidized terpenes, sesquiterpenes, and oxidized sesquiterpenes, could be identified. The profiles of volatile and semivolatile metabolites of the different fungus species are characteristic of them and allow their safe differentiation. The application of the same GC parameters and APCI source allows a simple method transfer from MS to ion mobility spectrometry (IMS), which permits on-site analyses of grain stores. Characterization of IMS yields limits of detection very similar to those of APCI-MS. Accordingly, more than 90% of the volatile metabolites found by APCI-MS were also detected in IMS. In addition to different fungus genera, different species of one fungus genus could also be differentiated by GC-IMS.

KEYWORDS

APCI, fungus, gas chromatography, ion mobility spectrometry, mass spectrometry, mold, soft X-ray

1 | INTRODUCTION

Barley, as one example of a cereal grain, can be infested by molds on the field or in storage, leading to the spoilage of grain and the production of mycotoxins. This contamination causes major economic losses in malting facilities and breweries.[1] One example of a field pest is the genus *Fusarium*, whereas *Aspergillus* is an example of storage pests. *Penicillium* can be attributed to both categories depending on the exact species. *Fusarium* is among the most important grain pathogens. Besides harvest losses, a major issue is the formation of mycotoxins. In addition to the nonvolatile mycotoxin metabolites, a wide spectrum of 50 volatile and 53 semivolatile metabolites (microbial volatile organic compounds [mVOC]) is also produced by different *Fusarium* species.[2-4] An important class of semivolatile metabolites are the sesquiterpenes, which serve as building blocks in the biosynthesis of mycotoxins.[5-7] Other fungi, encountered as field or storage pest alike, are various *Alternaria*,[8-11] *Aspergillus*,[12-14] and *Penicillium* species.

In the laboratory, the detection and identification of fungi is based on cell cultivation, which is usually time-consuming. Thus, approaches based on the detection of molecules, such as MALDI-MS[15] and subsequent database matching of the fingerprint spectra, are being developed. An alternative is the search for characteristic volatile marker compounds in the headspace above the fungi. Advantages of this method are the possibility of detecting hidden fungus contaminations in grain storages and contributing to the characterization of the metabolome of the fungi. The search for the characteristic volatile markers is based on active or passive headspace sampling and subsequent analysis by gas chromatography (GC) and electron ionization (EI)-mass spectrometry (MS).[8,16] The identification of the metabolites occurs mainly via the fragment ion patterns by NIST database matching. However, for many substances, no molecular ion peak is found, reducing the reliability of the assignment. This issue is addressed by atmospheric pressure chemical ionization (APCI),[17] where protonated molecular ions are predominantly formed. In this regard, EI and APCI are complementary ionization methods. Commercial APCI sources in MS are based on corona discharges. Although the corona discharge source is inexpensive, it has some disadvantages such as additional, competing ionization processes and the limited lifetime of the corona needle. Our group already demonstrated the application of an alternative APCI source based on soft X-radiation in MS. In two publications detailing the detection of explosives in the negative mode[18] and the detection of volatile metabolites of fungi in the positive mode,[19] we could show that the underlying ionization mechanism is more selective. These miniature X-ray sources, which are not subject to any legal regulations in Germany (photon energy $E_{X\text{-}ray} < 5$ keV), have already been introduced as alternatives for radioactive ^{63}Ni sources in ion mobility (IM) spectrometers, eg, by the company Bruker Daltonics (Leipzig, Germany).

Since APCI mass spectrometers are instruments usually confined to the laboratory, mobile instruments are required for on-site analyses of grain stores. Ion mobility spectrometry (IMS) is an analytical method with the potential for headspace (HS) screening of environmental and biological samples, which has already been demonstrated.[20] Additionally, handheld IM spectrometers are commercially available. One application is the HS investigation of olive oils by IMS, which allows the detection of components (terpenes) in olive oil,[21] the classification of olive oils,[22] and nontargeted olive oil profiling.[23] Other examples are the detection of odors in the environment,[24] off-flavors in foods,[25] fungal infestations of wood,[26] the identification of human pathogenic bacteria and fungi,[27,28] and breath analysis.[29]

In this work, our previous HS-GC-EI/APCI-MS investigations of different fungi on agar[19] were extended to fungus-contaminated barley grains. The aim was the identification of volatile fungus metabolites. In a second step, the MS method was transferred to IMS, and HS-GC-APCI-IM spectrometric investigations of fungi-contaminated barley grains were carried out. These experiments demonstrated the potential of IMS for on-site monitoring of hidden fungus contaminations in grain storages. The application of the same GC and X-ray-based APCI source in both hyphenation methods allows a straight method transfer.

2 | EXPERIMENTAL PART

2.1 | Microbiological sample preparation

The investigated samples were composed of sterilized barley grain inoculated with a spore suspension of the corresponding fungus. The grain contained about 30% of water. After the inoculation, the sample vials were rotated for 15 minutes to ensure even distribution of the spore suspension; 6 g of the sample were placed inside a HS vial. The suspension was produced from different breeding media (each including a fungus) that were suspended in a 0.9% NaCl solution and filtered subsequently. The fungus cultures used were obtained from culture collections (Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures; VLB-Brewery Research and Education Center Berlin) or were isolated from contaminated grain samples. The investigated field pests were *Fusarium culmorum* (DSMZ 62191), *Fusarium graminearium* (VLB reference stocks), *Fusarium* sp. (isolate from deoxynivalenol-contaminated diastase wheat, called here *F*. DW 14), and *Alternaria alternata* (isolate from brewing barley). The investigated storage pests were *Aspergillus niger* (DSMZ 22593), *Aspergillus ficuum* (DSMZ 932/NRRL 3135), *Aspergillus versicolor* (DSMZ 63292), and three different *Penicilium* spp. (isolate from brewing barley, called here *P*. Pen A, *P*. Pen 14, and *P*. Pen R). Through DNA analysis, *P*. Pen R was found to be either *Penicillium camenbertii* or *Penicillium griseofulvum* and *A. alternata* was identified. This analysis was carried out at the Research Center Weihenstephan for Brewing and Food Quality (Technical University of Munich) via polymerase chain reaction (PCR) sequencing of the rDNA and comparison with the Blast Search database.

2.2 | HS characterization

After a growth period of 8 days, the HS vials were hermetically sealed and left for another 48 hours, stopping the fungus growth. A solid-

phase microextraction (SPME) fiber, coated with divenylbenzene, carboxen, and polydimethylsiloxan, was used for sampling. The volatile compounds in the HS were adsorped onto the fiber over a period of 1 hour at a temperature of 45°C. Desorption was performed in the GC injector at 250°C for 1 minute.

HS investigations of fungi were carried out with GC-EI-MS (7890A GC, 5975C MSD, Agilent Technologies) and GC-APCI-MS (5890 Series II GC, Hewlett Packard, LTQ XL, Thermo Fisher Scientific), which were already described in detail previously.[19] For both MS methods, the same type of GC columns, but different inner diameters (15 m × 0.25/0.32 mm × 1.0 μm), containing poly(5% diphenyl-95% dimethylsiloxan) as the active phase was used for the preseparation of the substances. The same GC temperature program was used as well: initial period of 5 minutes at 32°C followed by a heating phase with 10°C/min and a final period at 200°C for 8 minutes.

The handheld IM spectrometer (Roadrunner, Bruker Daltonics, Leipzig) has an APCI source based on soft X-radiation (miniature X-ray tube, 40 mm length, rhodium target on a beryllium window), with an energy of 2.7/2.8 keV (L_α/L_β-transitions of Rh) and is therefore exempt from registration in Germany. The same type of source was used in the APCI-MS system (LTQ XL, Thermo Fisher Scientific), where it was integrated into a home-built ionization chamber.[18,19] The original application scenario of the Roadrunner instrument is the detection of explosives and drugs on surfaces. In order to couple the spectrometer (95 mm length of the drift tube) to a GC (the same GC as used in GC-APCI-MS), modifications had to be made. The original thermal desorption unit was replaced by a home-made heated (180°C) inlet system for the GC capillary. Additionally, the internal drift gas cycle was modified, and an external drift gas (nitrogen, 400 mL/min) was applied. The resolution of the spectrometer in the GC configuration is around 40. The Roadrunner spectrometer was set to the maintenance mode, which allows long-term GC measurements. The temperature program in the GC was slightly changed in order to improve the resolution: initial period of 30°C prior to a heating phase with 20°C/min and a final period at 200°C for 10 minutes. A custom python script was written for format conversion of the Bruker data file to the data formats of OpenMS and Origin where the data evaluation and visualization was performed.

The spectra resulting from GC-EI-MS, GC-APCI-MS, and GC-APCI-IMS measurements were correlated using the GC retention time. Because an alkane standard generally applied for determination of retention indices cannot be used in APCI-MS or APCI-IMS, a fatty acid methyl ester mixture consisting of $C_nH_{2n+1}COOCH_3$ (n = 8-20 for even n) was used. Furthermore, GC-APCI-MS and GC-APCI-IMS were characterized by a mixture of representative volatile fungus metabolites. This mVOC standard contained 3-methylbutanol (a, 2mM), 2-hexanone (b, 50μM), α-pinene (c, 300μM), benzaldehyde (d, 50μM), 1-octen-3-ol (e, 200μM), 2-phenylethanol (f, 150μM), longipinene (g, 20μM), and caryophyllene (h, 20μM). All substances were purchased from Sigma-Aldrich.

3 | RESULTS

3.1 | Characterization of the GC-IM spectrometer

The aim of this work was the characterization of the mVOC spectrum in the HS above barley grains contaminated with fungi by a mobile analytical instrument based on GC-IMS. In order to enable a direct method transfer from previous GC-MS experiments, the same gas chromatograph, column, and method parameters (flow rates, temperature program) were used in GC-IMS. This allows the simple assignment of mVOC already identified by GC-MS to the peaks in the 2D-GC-IM spectra through correlation of the retention indices. Furthermore, the method was characterized by a standard that contains representatives (volatile metabolites) of the most important substance classes in the fungi HS. The IM spectrometer used was a handheld

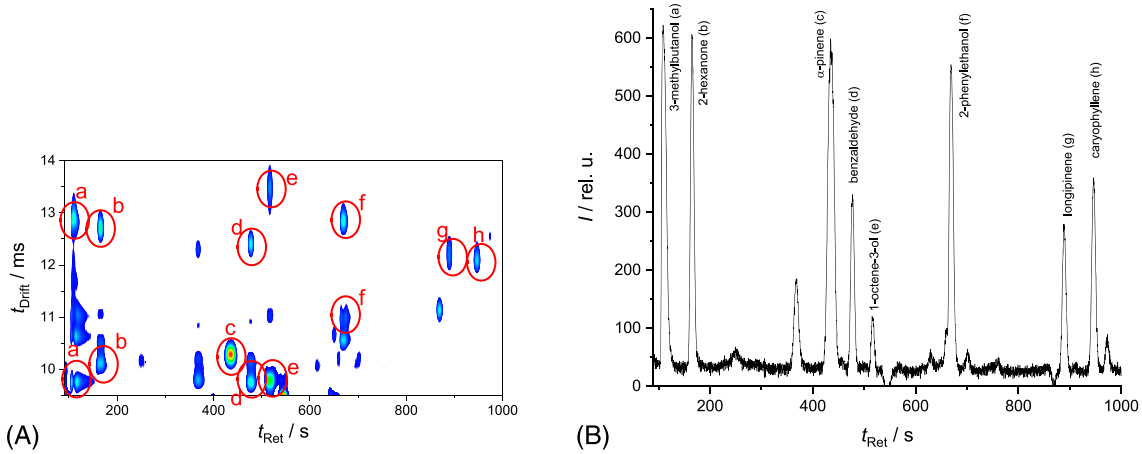

FIGURE 1 (A) Two-dimensional spectrum (drift time t_{Drift} vs retention time t_{Ret}) of the representative volatile fungus metabolites mixture and (B) GC-chromatogram computed by summation of GC traces at various drift times (corresponding to substance maxima in IM spectra) of the same mixture. GC, gas chromatography; IM, ion mobility [Colour figure can be viewed at wileyonlinelibrary.com]

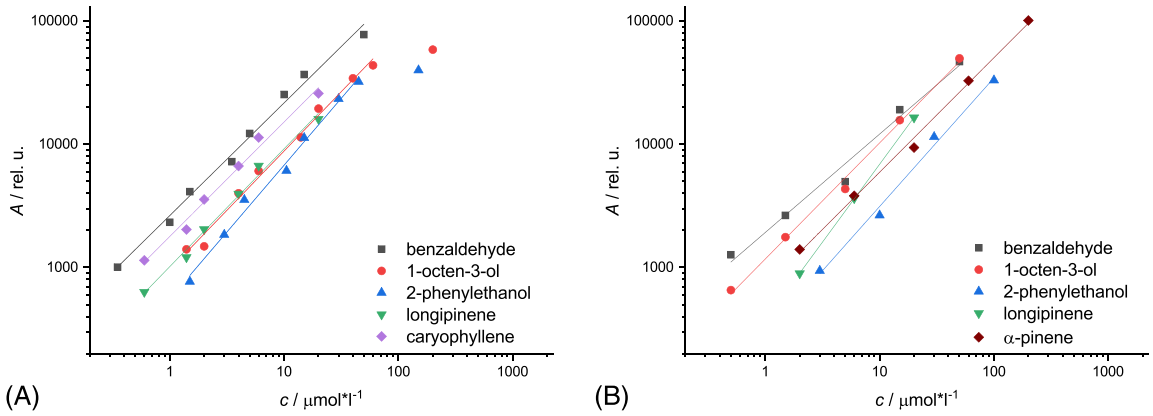

FIGURE 2 Calibration plots (double-log representation) of selected compounds of the mVOC mixture injected into the GC-APCI-IMS (A) as 1-µL liquid sample and (B) after desorption of the SPME fiber in the injector after total evaporation of 2-µL liquid sample. *A* refers to the peak area, *c* refers to the concentration in the injected liquid, and refers c_{sol} to the concentration in the solution below the headspace. APCI, atmospheric pressure chemical ionization; GC, gas chromatography; IMS, ion mobility spectrometry; mVOC, microbial volatile organic compounds [Colour figure can be viewed at wileyonlinelibrary.com]

instrument equipped with the same soft X-radiation source that was previously utilized in APCI-MS. This allows a simple transfer of method parameters from the mass spectrometer to the IM spectrometer. Because the original application purpose of the highly specialized handheld instrument is the detection of explosives and narcotics in baggage check-ins of airports, which are sampled by swipes, a GC connection is not intended in the instrument. Therefore, the inlet part and internal gas flows of the instrument had to be modified. While the focus of the modification of the inlet part was on the prevention of cold spots, the optimization of the gas flows improves sensitivity and resolving power of the instrument. The sampling procedure is based on the adsorption of volatile compounds on a SPME fiber.

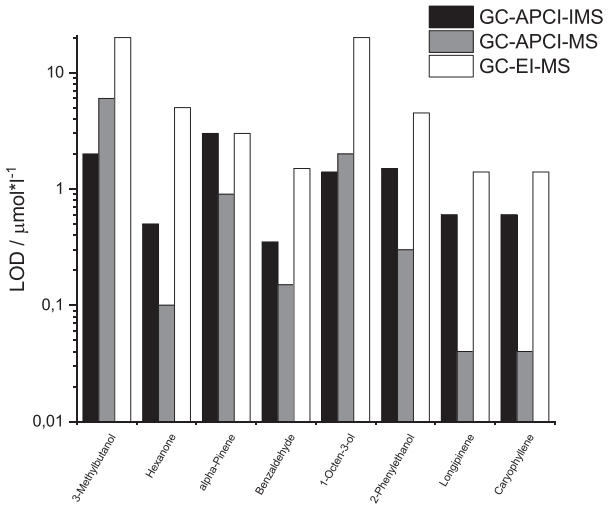

FIGURE 3 LOD of eight mVOC for three detection methods: GC-EI-mass spectrometry (MS), GC-APCI-MS, and GC-IMS. APCI, atmospheric pressure chemical ionization; EI, electron impact; GC, gas chromatography; IMS, ion mobility spectrometry; LOD, limits of detection; MS, mass spectrometry; mVOC, microbial volatile organic compounds

Figure 1A shows the 2D spectrum of the representative volatile fungus metabolites mixture, which includes mVOC of the most important substance classes. The 2D spectrum consists of the two dimensions retention time and drift time. For most compounds, two peaks

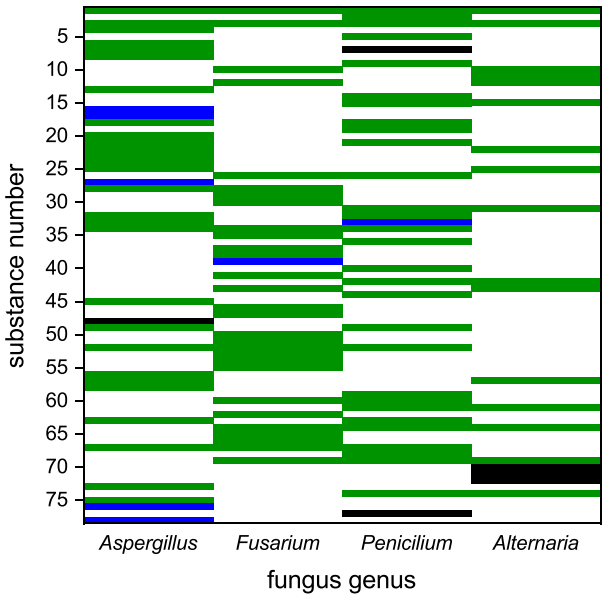

FIGURE 4 Heatmap of the mVOC detected in the headspace of four fungus species belonging to different genera, color code: green—detection by EI-MS and APCI-MS, black—detection by APCI-MS only, and blue—detection by EI-MS only; substance numbers according to the following substance classes: 1-5 alcohols, 6-8 aldehydes, 9-12 ketones, 13 carboxylic acid, 14-16 esters, 17-34 substituted aromatic compounds, 35-36 alkenes, 37-40 terpenes, 41 oxidized terpenes, 42-72 sesquiterpenes, 73-74 oxidized sesquiterpenes, and 75-78 other compounds; refer to Table S1. APCI, atmospheric pressure chemical ionization; EI, electron impact; IMS, ion mobility spectrometry; MS, mass spectrometry; mVOC, microbial volatile organic compounds [Colour figure can be viewed at wileyonlinelibrary.com]

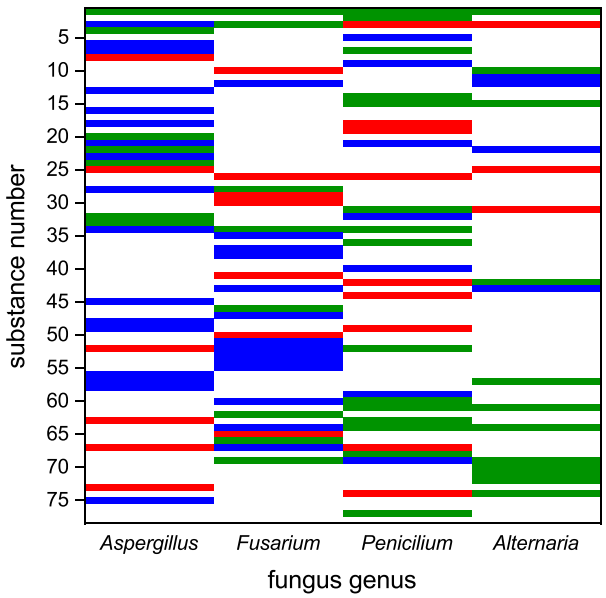

FIGURE 5 Heatmap of the mVOC detected by APCI-MS in the headspace of four fungus species belonging to different genera, color code: red—major components, blue—minor components, and green—traces. APCI, atmospheric pressure chemical ionization; MS, mass spectrometry; mVOC, microbial volatile organic compounds [Colour figure can be viewed at wileyonlinelibrary.com]

are found, which can be assigned to the protonated monomer and dimer ions. All peaks are well separated and have a symmetric shape in both dimensions. The signal intensity (current *I*) as sum of the selected ion traces in the retention time dimension is displayed in Figure 1B. The GC peaks are highly symmetric, which is an indication of the absence of memory effects in the IM spectrometer.

The formation of mVOC as volatile metabolites of fungi can occur over a wide and mostly unknown concentration range. Another challenge is the strong variation of the response values of the compounds in APCI.[19] Therefore, a quantitative description of the mVOC space would require an extensive calibration of each substance. However, many compounds are not commercially available. Calibration plots of five representative mVOC deriving from GC-APCI-IMS measurements are shown in Figure 2, as examples of the concentration ranges and sensitivities of the mVOC detection. The calibration plot after direct injection of liquids is shown in Figure 2A, and the calibration plot after total evaporation of 2-µL solution and subsequent adsorption of the mVOC on an SPME fiber is displayed in Figure 2B. While the different calibration curves in Figure 2A are the result of different response factors of the APCI source, the calibration curves in Figure 2B additionally include the effect of different adsorption equilibria of the mVOC on the SPME fiber. Linear detection ranges cover two orders of magnitude. The estimated limits of detection (LOD) are slightly lower for direct injection of liquids and are in the upper nanomolar range. The small differences of both curves indicate efficient sampling of the mVOC by SPME.

The mVOC were identified by GC-EI-MS and GC-APCI-MS investigations. The results of the MS methods can only be transferred to IMS if the detection ranges of both methods are similar. Furthermore, IMS is only useful for on-site detection and identification of fungi if the LOD are low enough. In Figure 3, the LOD of eight mVOC measured by the mass- and IMS-based methods are compared. The most important result is that the LOD of APCI-MS and APCI-IMS are in the same range except for the sesquiterpenes. It is interesting to note that both in-house modified APCI instruments are more sensitive than the commercial EI-MS instrument. This is very likely an effect of the different ionization efficiencies of the two sources.

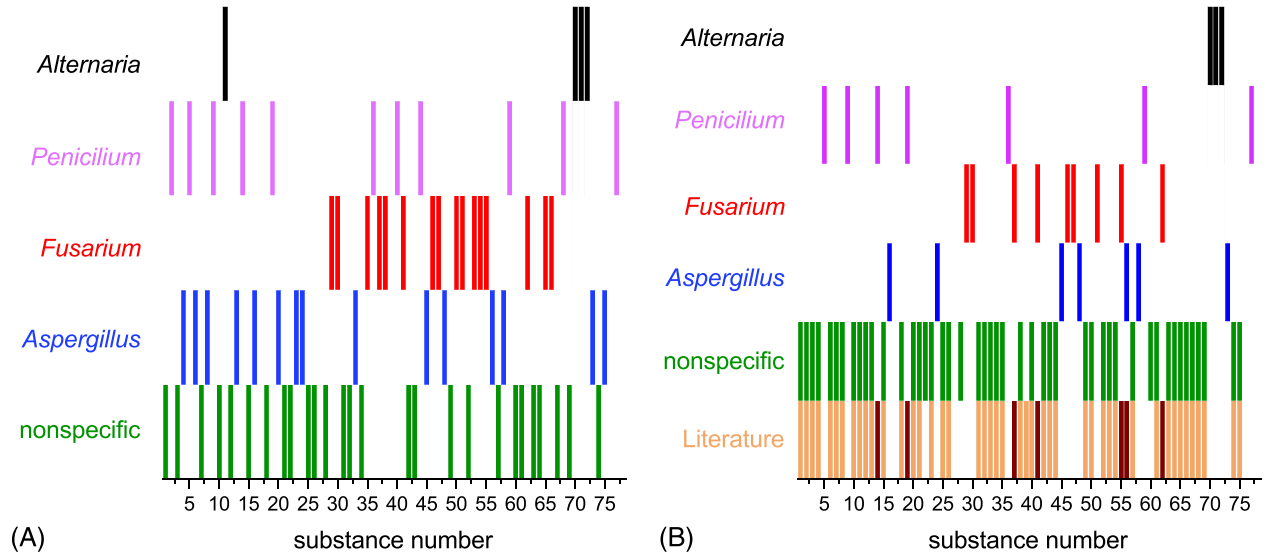

FIGURE 6 Representation of specific and nonspecific metabolites found by APCI-MS with regard to (A) the results of this work and (B) the literature. Seven of the found substances were described as specific in the literature as well (dark brown bars). APCI, atmospheric pressure chemical ionization; MS, mass spectrometry [Colour figure can be viewed at wileyonlinelibrary.com]

3.2 | MS investigation of mVOC formed by fungi on barley grain

In our previous publication,[19] the results of HS monitoring of fungi on different agar substrates by MS were reported. The result of the complementary EI/APCI investigation was an overview of all detected mVOC sorted by substance classes. Marker substances allowing the specific detection of individual fungi were searched in this dataset. In the current work, these investigations were additionally performed for barley grains contaminated by fungi. In the first step, the mVOC were identified by both GC-EI-MS and GC-APCI-MS measurements. Specific marker substances were identified from the resulting mVOC lists. These experiments were extended to IM spectrometric measurements in order to verify the potential of this mobile on-site analytical method for monitoring grain stores. In addition to the differentiation of fungus genera, the potential of IMS for the differentiation of several species of one fungus genus was explored.

The mVOC detected by EI-MS and APCI-MS are presented in a heatmap (Figure 4). The four columns represent the detected volatile metabolites for the different fungus species *Aspergillus*, *Fusarium*, *Penicillium*, and *Alternaria*, which all belong to different genera. The color code indicates the MS methods used to detect the mVOC. Seventy-eight substances were found and are arranged according to their substance classes. These substance classes include alcohols (five mVOC), aldehydes (three mVOC), ketones (four mVOC), carboxylic acids (one mVOC), esters (three mVOC), substituted aromatic compounds (18 mVOC), alkenes (two mVOC), terpenes (four mVOC), oxidized terpenes (one mVOC), sesquiterpenes (31 mVOC), oxidized sesquiterpenes (two mVOC), and additional nonidentified substances. A detailed list of all compounds can be found in Table S1. Most substances (91%) were detected by both ionization methods. Another heatmap providing a semiquantitative representation of the APCI-MS results is shown in Figure 5. Due to the varying and often unknown response factors, the mVOC concentrations were determined only semiquantitatively and classified as major components (red), minor components (blue), and traces (green) in this work.

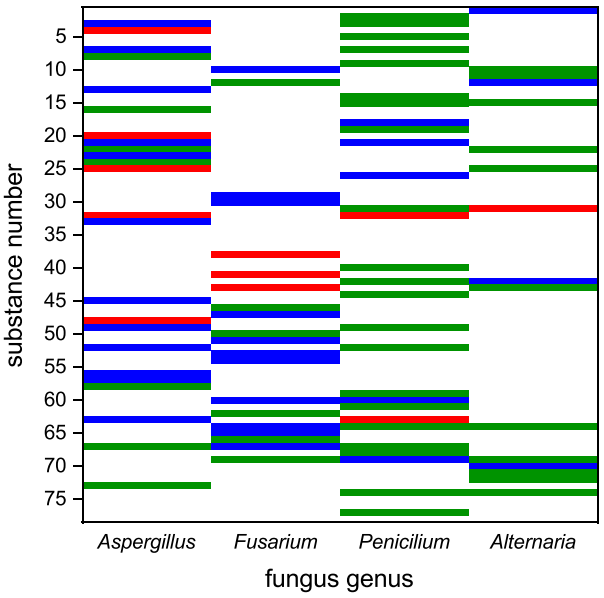

FIGURE 8 Heatmap of the mVOC detected by APCI-IMS in the headspace of four fungus species belonging to different genera, color code: red—major components, blue—minor components, and green—traces. APCI, atmospheric pressure chemical ionization; IMS, ion mobility spectrometry; mVOC, microbial volatile organic compounds [Colour figure can be viewed at wileyonlinelibrary.com]

Compared with the HS measurements on agar,[19] the number of detected mVOC has slightly changed due to the influence of the different substrates. In detail, the transition from different agar

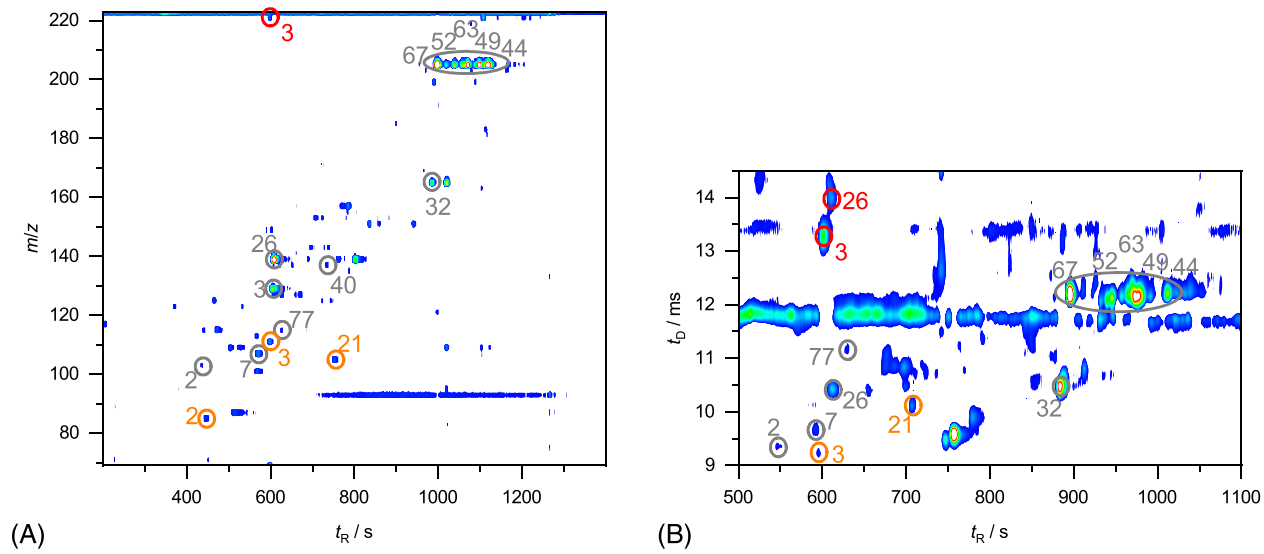

FIGURE 7 Two-dimensional spectra of the mVOC from *P.* Pen 14 obtained by (A) GC-APCI-MS and (B) GC-APCI-IMS (gray—$[M + H]^+$, orange—$[M - OH]^+$, and red—$[2M + H]^+$). APCI, atmospheric pressure chemical ionization; GC, gas chromatography; IMS, ion mobility spectrometry; MS, mass spectrometry; mVOC, microbial volatile organic compounds [Colour figure can be viewed at wileyonlinelibrary.com]

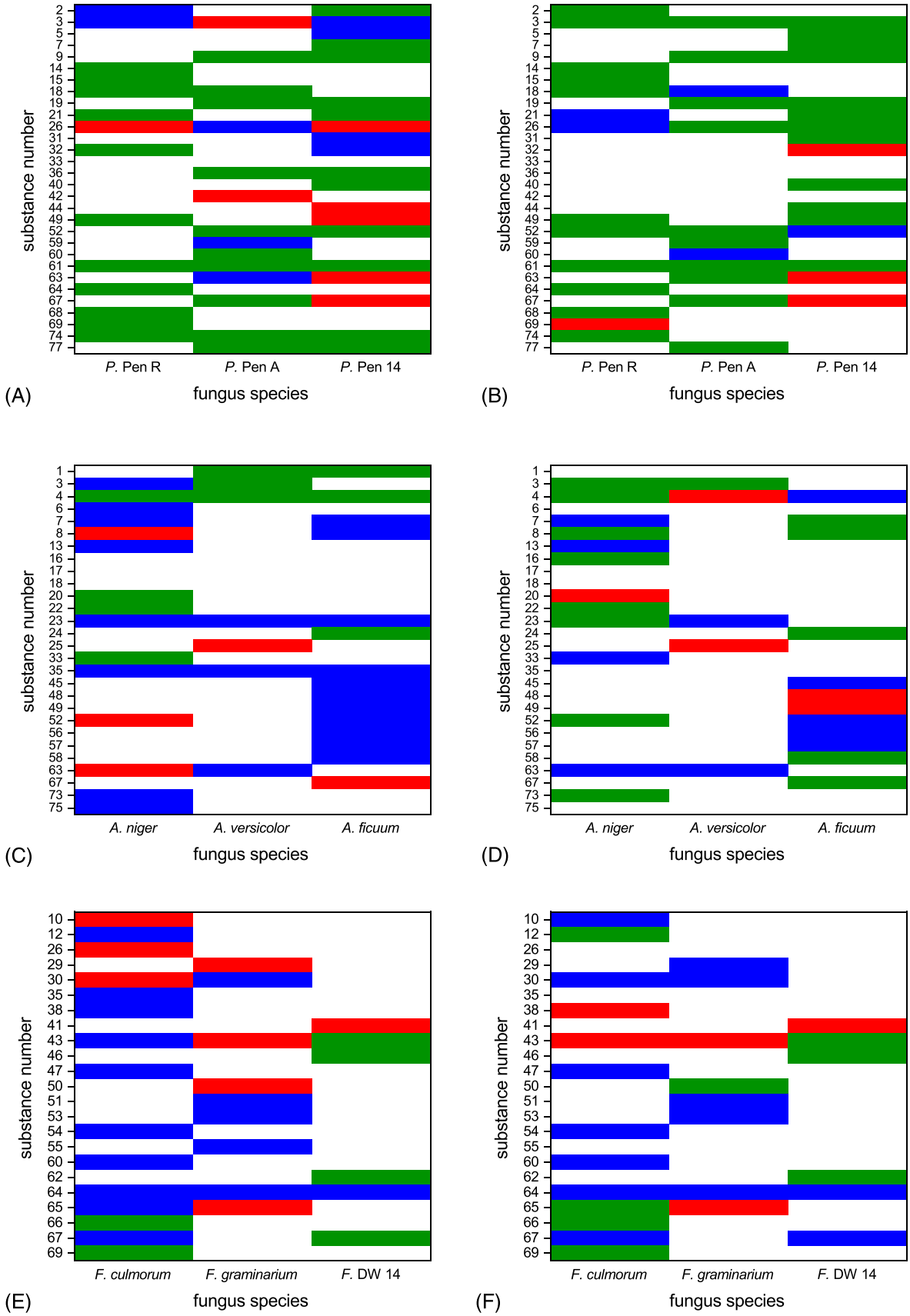

FIGURE 9 Heatmaps for three *Penicillium* species detected by (A) APCI-MS and (B) APCI-IMS; three *Aspergillus* species detected by (C) APCI-MS and (D) APCI-IMS; and three *Fusarium* species detected by (E) APCI-MS and (F) APCI-IMS. APCI, atmospheric pressure chemical ionization; IMS, ion mobility spectrometry; MS, mass spectrometry

substrates to barley results in a decrease of the total number of mVOC detected by GC-EI-MS from 86 to 78, while the total number of mVOC detected by GC-APCI-MS increases from 67 to 73. This finding indicates that the number of unpolar mVOC decreases and the number of the more polar substances slightly increases.

A brief glance already reveals that the mVOC for the four fungus species form characteristic patterns that differ significantly from each other. Within the framework of the four fungus species investigated, 50 mVOC of all 78 mVOC detected by EI-MS and 45 mVOC of all 73 mVOC detected by APCI-MS are specific to the four fungi and can thus potentially be used as marker substances. More details are shown in Figure 6A for the APCI-MS experiments. Fifteen mVOC are specific to *Aspergillus* spp., 16 mVOC are specific to *Fusarium* spp., 11 mVOC are specific to *Penicillium* spp., and four mVOC are specific to *Alternaria* spp. The remaining mVOC were found in the HS of at least two fungi and were classified as nonspecific. Several substances classified as specific in this work were reportedly detected in the HS of other microorganisms in the literature (see Table S2) and have to be reclassified as nonspecific. Therefore, the number of specific substances is strongly reduced. Then, as shown in Figure 6B, seven mVOC are specific to *Aspergillus* spp., nine mVOC are specific to *Fusarium* spp., seven mVOC are specific to *Penicillium* spp., and three mVOC are specific to *Alternaria* spp. In the future with continuing research worldwide, the number of substances specific to a fungus species will furthermore decrease since more mVOC will be discovered in the HS of other microorganisms, which are now classified as specific mVOC. Otherwise, this procedure of comparison with literature results is conservative because the number of nonspecific substances found represents an upper limit, since not all microorganisms will occur together at one place in the field or in storage.

3.3 | Detection of fungi by IMS based on mVOC

HS investigations were also carried out by GC-IMS. Analogous to the 2D GC-APCI-MS spectra (m/z vs t_{Ret}), two-dimensional (t_{Drift} vs t_{Ret}) spectra are also obtained in GC-APCI-IMS; 2D spectra comparing GC-APCI-MS and GC-APCI-IMS for one species are shown in Figure 7. IMS has a lower resolution than MS, but in connection with the GC preseparation, most marker peaks can reliably be separated from surrounding mVOC peaks.

A detailed examination shows that 92% of the mVOC peaks found in APCI-MS are also detected by APCI-IMS. The mVOC detected in the HS of the four fungi can be summarized in a heatmap (Figure 8).

Similar to Figure 5, different mVOC patterns measured by APCI-IMS were obtained for the four fungus species belonging to different genera. Furthermore, characteristic marker substances were identified that only appear in the HS of the corresponding fungus species. Regarding the four fungi, 43 of 66 mVOC found in APCI-IMS are potential specific marker compounds. In detail, 14 specific mVOC for *Aspergillus* spp., 13 specific mVOC for *Fusarium* spp., 11 specific mVOC for *Penicilium* spp., and five specific mVOC for *Alternaria* spp. were found by APCI-IMS.

The mVOC patterns of fungi belonging to different genera feature strong variations. Contrary to this, the distinction of different fungus species of one genus should pose a larger challenge. This question was investigated for the example of three species, each of the three fungus genera *Penicillium*, *Aspergillus*, and *Fusarium*, applying both APCI-MS and APCI-IMS. APCI-IMS is able to detect most of the compounds, which are detected by APCI-MS. In detail, the match between mVOC detected by IMS and MS is 88% for *Aspergillus*, 87% for *Fusarium*, and 93% for *Penicillium*. This finding is also supported by Figure 9. The left column shows the mVOC patterns detected by APCI-MS, and the right column show the corresponding mVOC patterns detected by APCI-IMS. Both are very similar.

A brief survey of the mVOC signatures of the three fungus genera (see Figure 9A,C,E) shows strong differences as was discussed in detail above. As expected, the differences become smaller if the respective three species of each fungus genus *Penicillium*, *Aspergillus*, and *Fusarium* (eg, in Figure 9A,C,E) are compared. This finding is especially pronounced in Figure 9A. Of the 15 compounds detected for *P.* Pen A, three mVOC are also observed in the HS of the other two *Penicillium* species, nine mVOC are also observed in the HS of one of the other two *Penicillium* species, and three mVOC are only observed for *P.* Pen A. Thus, the latter three compounds can be regarded as potential marker compounds for *Penicillium* Pen A. Summarizing all heatmaps in Figure 9, for *Aspergillus* 28% of the mVOC are potential markers for species *A. niger*, 4% for species *A. versicolor*, and 32% for species *A. ficuum*. For *Fusarium*, 43% of the mVOC are potential markers for species *F. culmorum*, 22% for species *F. graminarium*, and

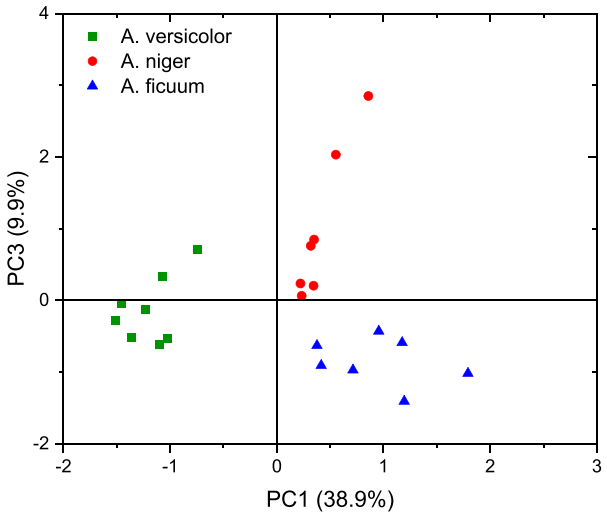

FIGURE 10 Score plot of the PCA of the GC traces (GC-IMS) of the three *Aspergillus* species (color code: *Aspergillus ficuum*—blue triangles, *Aspergillus niger*—red circles, and *Aspergillus versicolor*—green squares). GC, gas chromatography; IMS, ion mobility spectrometry; PCA, principal component analysis. [Colour figure can be viewed at wileyonlinelibrary.com]

13% for species *F.* DW 14. Finally, for *Penicillium*, 17% of the mVOC are potential markers for species *P.* Pen R, 10% for species *P.* Pen A, and 17% for species *P.* Pen 14.

As an alternative to the very time-consuming marker search, principal component analysis (PCA) of the GC traces of three fungus species was carried out. The aim was to establish a fast, non-supervised classification method for fungi based on the GC traces of the GC-IMS measurements, without detailed time-consuming evaluation of all spectra. This analysis based on PCA was demonstrated for the three *Aspergillus* species as shown in Figure 10. In the score plot, the first and third principal components are displayed, which together account for 49% of the variance. Each point in the score plot represents one fungus sample. The following color code was applied: *A. ficuum* (blue), *A. niger* (red), and *A. versicolor* (green). The different fungus samples cluster in three different groups according to the corresponding three *Aspergillus* species. These three different clusters are clearly separated. Therefore, unknown samples can potentially be classified by PCA using the GC traces without further time-consuming data evaluation. It is worth noting that the PCA classification was possible for fungus species of one genus and that fungi of different genera could also be differentiated.

4 | CONCLUSIONS

The investigation of volatile and semivolatile metabolites in the HS above barley allows the detection and identification of fungi. The metabolites were identified by complementary GC-EI-MS and GC-APCI-MS investigations. The mVOC profiles of the fungi investigated have a different pattern, allowing their differentiation. In these mVOC patterns, characteristic marker substances could be found for each fungus. The detection of these marker substances provides a reliable method for the identification of the corresponding fungus. IMS, a technique that can be performed with commercially available handheld instruments, potentially allows the on-site detection of fungus contaminations in grain stores. Based on the application of the same GC parameters and APCI source, a simple method transfer from MS to IMS is possible. Despite the lower resolution of IMS in comparison to MS, most mVOC could be completely separated in GC-APCI-IMS. A characterization of GC-APCI-IMS yields comparable LOD to GC-APCI-MS. This high sensitivity is also reflected in the detection of nearly 90% of the mVOC detected by GC-APCI-MS by GC-APCI-IMS. In addition to the distinction of different fungus genera, different species of one fungus genus can be distinguished by GC-APCI-based IM spectrometry.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support for this research received from the German Federal Ministry of Food and Agriculture and the administrational support from the Federal Office of Agriculture and Food (BLE, grant no. 2814801811).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Erler A, Riebe D, Beitz T, et al. Characterization of volatile metabolites formed by molds on barley by mass and ion mobility spectrometry. *J Mass Spectrom.* 2020;55:e4501. https://doi.org/10.1002/jms.4501