APPLICATION NOTE

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Early-State Detection of Fungi Infection of Tobacco Leaves

Introduction

One of the main purposes of analytical instrumentation is about enabling an impartial product quality control, fulfilling governmental regulations as well as maximizing the consumer's experience.
Tobacco leaves are grown and harvested in huge plantations and the key to first class product quality is about applying strictly the quality control measures for tobacco leaves which includes applying defined and stable storage conditions. Without keeping temperature and humidity controlled, several problems like fungal contamination can occur that impede their further processing. Going down the value chain from tobacco harvesting to the distribution the first important subject to ensure a high tobacco quality is the recognition of potential fungal infection of the harvested leaves at early stages.

With its FlavourSpec G.A.S. developed a highly selective and sensitive measuring system for QC-related analysis of tobacco mouldiness.

GC-IMS: Innovative Quality Control on Tobacco

Experimental Conditions

Table 1: GC Conditions	
Column	FS-SE-54-CB-0.5 15m ID:0.53mm
Temperature	50 °C (isothermal)
Flow program 20 min	linear 5 → 100 mL/min
Carrier gas	N_2

Table 2: IMS Conditions	
Radiation source	Tritium
Temperature	45 °C
Flow rate	150 mL/min
Carrier gas	N_2
Mode	positive

Table 3: Sampling Conditions	
Incubation	none
Sample volume	1 mL (splitless headspace injection)
Syringe temperature	80 °C
Injection speed	0.5 mL/sec

Table 4: Samples	
Samples	1. Brazil-Dark Air Cured 2. India-Sun Air Cured 3. Brazil-Flue Cured Virginia 4. Malawi-Burley
Sample preparation	1 g of each tobacco sample was transferred into a 20 mL headspace vial. In order to induce moulding 1 g of each tobacco species was moistened (10% w/w).

Samples as-is

Figure 1: A small amount of tobacco leaves was directly transferred into the headspace vial. A further sample pre-treatment is not necessary.

Preface

The typical value chain of the tobacco industry reveals the importance of assuring good raw material quality, the dried tobacco leaf. A major risk affecting all tobacco raw materials is the infection by mould, a microbial or fungal infection. As it is demonstrated in biochemistry, early infection is showing almost no visible difference between the good and the future deteriorated product while the symptoms are very clearly visible by the human eye when an infection is in an advanced stage. To prove the latter there is no need for a sophisticated analytical instrumentation: an inspection by an expert supports the task of separating the good from the non-infected batches. The question about a potential infection however is not always easily answered, nor symptoms visible at first sight.

However with respect to manufacturing it has to be decided quickly and reliably when raw material is added to a new production batch. Several microbial volatile organic compounds (MVOCs) indicating microbial contamination have been identified, which are generated by commonly reported fungal species found on moulded tobacco samples (s. Table 1).[1] Fiedler et al. cultured 12 different fungal species on several substrates.[3] The headspace was subsequently analysed for MVOCs by using solid-phase microextraction, whereat 150 volatile compounds were identified.

Each of the analyzed fungal species exhibited a specific pattern of MVOCs based on external factors such as the used substrates, but nearly all species generated the following substances, which can be defined as highly potential fungal markers:

- Oct-1-en-3-ol
- 2-methylbutan-1-ol
- 3-methylbutan-1-ol

Table 5: Potential MVOC marker identified for commonly detected fungal species.[1,2]

Potential MVOC Marker
Hexan-2-one
3-Methylbutan-1-ol
Pentan-2-one
Butan-2-one
2-Methylbutan-1-ol
Oct-1-en-3-ol
Heptan-2-one
2-Hexen-1-ol

RESULTS

In the first step the potential funghi markers listed in table 5 were analysed as pure substances and added to the G.A.S. GC-IMS database. Thereby easy and fast identification of these compounds in unknown samples were enabled by using the GCxIMS Library Search Software by G.A.S.

In order to induce the moulding process one gram of each tobacco species (s. table 4) was humidified by 10 % w/w water. After 10 days the mycelium could be clearly seen (s. figure 2).

Figure 2: Sample as-is after moistening and 10 days incubation. The generated mycelium can be clearly seen (red circle).

[1] Meruva NK, Penn JM, Farthing DE. J Ind Microbiol Biotechnol. 2004;31(10):482-488
[2] Kaminski E, Stawicki S, Wasowicz E. Appl Microbiol. 1974;27(6):1001-1004
[3] Fiedler K, Schütz E, Geh S, International Journal of Hygiene and Environmental Health, 2001;204(2-3):111-121
[4] Shannon U. Morath, Richard Hung, Joan W. Bennett. Fungal Biology Reviews, 2012;26(2-3):73-83

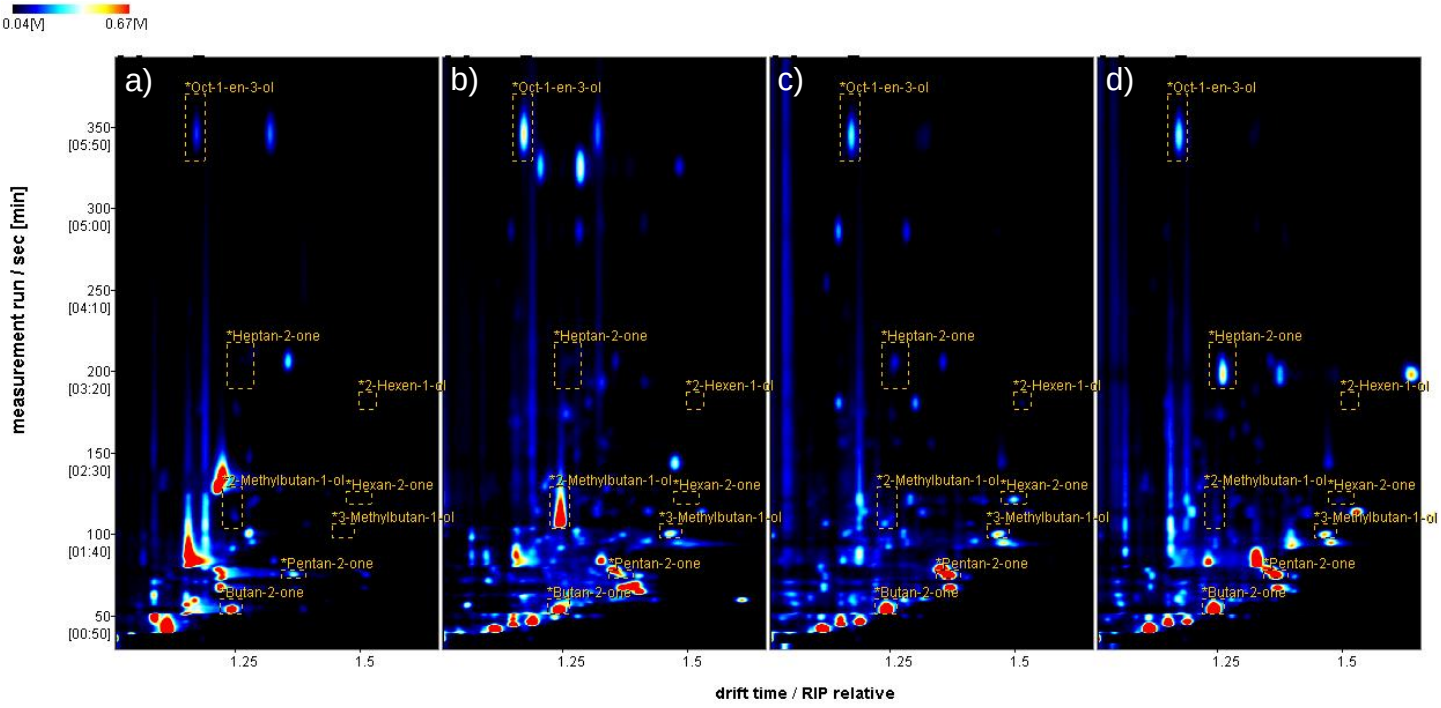

Figure 3: GC-IMS headspace measurements of moulded tobacco leafs. Potential funghi markers are marked (dashed yellow rectangle). Brazil-Dark Air Cured (a), India-Sun Air Cured (b), Brazil-Flue Cured Virginia (c), Malawi-Burley (d).

The moulded tobacco samples were analyzed using the G.A.S. FlavourSpec, in which the complete cycle could be optimized to 7 min, only. The obtained 2-dimensional GC-IMS data clearly show a complex fingerprint for each tobacco species, (Figure 3), representing the rich composition of the tobacco leaves headspace. In order to find signals representing tobacco mouldiness, a multiplicity of the observed fingerprint signals were marked by manually set areas (figure 3, dashed rectangles).

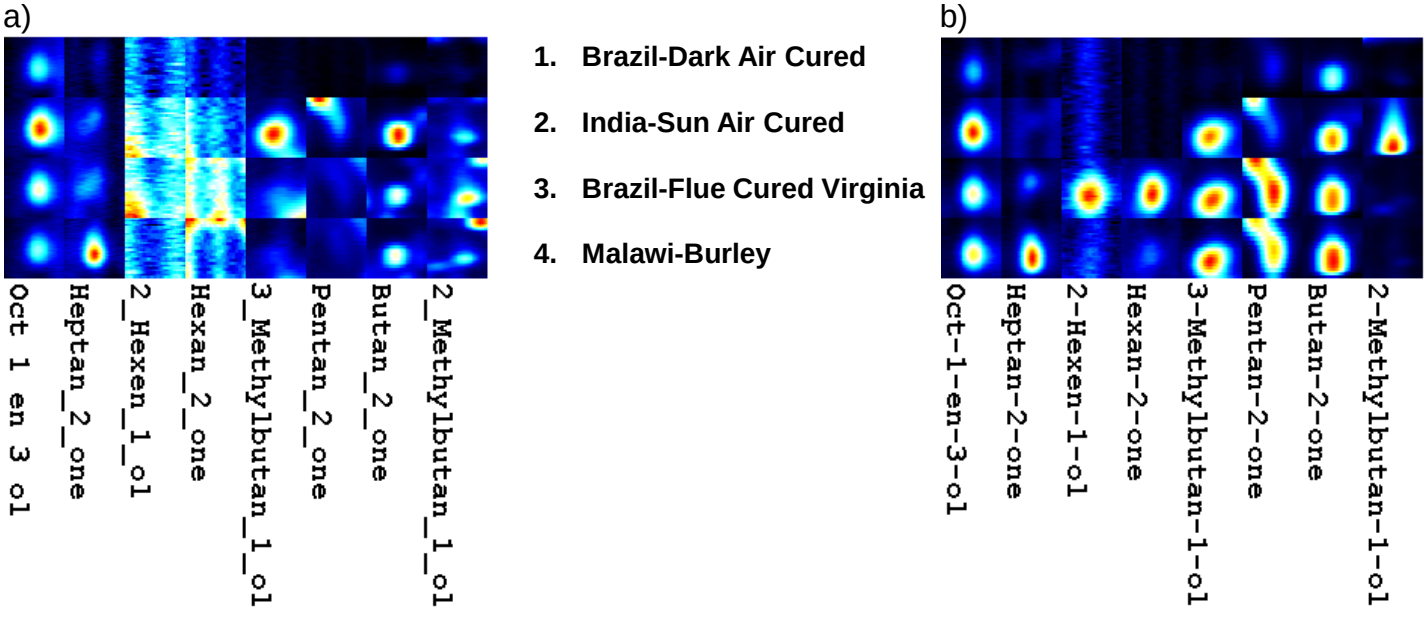

Figure 4: Gallery Plot highlighting the potential fungi marker of moistened tobacco leaves after one (a) and 10 days (b).

It could be proved that all potential fungi markers were detectable in the analyzed moulded tobacco leaves, although not each of them exhibited every fungi marker (figure 4 B). Only Oct-1-en-3-ol and Butan-2-one are observable in every sample after 10 days incubation. A possible explanation is that fungal species form different compounds depending on there habitat and other external circumstances. Already after one day of incubation several samples exhibit fungal markers (figure 4 A). Furthermore a plurality of non-identified signals, only occuring after moulding, were observed, maybe also indicative for moulded samples.

Conclusions

Using the FlavourSpec made by G.A.S. a fast method for measuring the headspace of tobacco leaves, without any need for sample pre-treatment, was established. Furthermore several well-known MVOCs, indicative for fungal attack, were added to the GCxIMS database and therefore are available for identification in unknown samples henceforward. The headspace of all tobacco species exhibited a complex headspace composition of various volatile organic compounds. Analytical results clearly illustrate that an infection of mould could be detected by analysis of the signals representing the presence of potential fungi markers

Even at very early stages, when human sensory inspection might be unspecific, fungal marker signals could be detected.
Not only the presence of mould/fungi related markers but also several other signals occurring/disappearing after moulding were detected and are maybe of interested for QC-related tobacco leaf analysis.

Figure 5: The FlavourSpec made by G.A.S. offers an orthogonal separation by gas-chromatography and ion mobility spectrometry while the latter is used as detector at the same time. The built-in agitator and coupled autosampler enable an efficient and fast workflow.

Related Information

For further information material concerning this application please click on the links below:

- FlavourSpec
- Laboratory Analytical Viewer
- GCxIMS Library Search Software

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