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Identification of fungi with analytical pyrolysis and thermally assisted hydrolysis and methylation

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Abstract

Different fungi (poisonous, edible and wood decaying) were analyzed with pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) and thermally assisted hydrolysis and methylation (THM) and differentiated according to characteristic fragments. Besides the identification by direct comparison of the pyrograms it was possible to arrange fungal species into groups using principal component analysis (PCA). In some cases, it was also possible to identify specific marker compounds, which are characteristic for one single type of fungi, such as the poison α-amanitin in the death cup (*Amanita phalloides*), which could be detected in levels below 3.5 ng, and β-dopa in *Cortinarius violaceus*.

Keywords: Fungi; Pyrolysis; THM; Fatty acid methyl esters; Saccharinic acids

1. Introduction

Fungi are omnipresent in nature and part of many meals. It is very popular for many people to search and cook fungi themselves. Unfortunately, it may occur that different fungi optically very much look alike and inexperienced collectors sometimes mix up the good and the bad ones. This is displeasing, especially if the fungus is a poisonous one, such as the death cup (*Amanita phalloides*), which is often mixed up with the meadow mushroom (*Agaricus campester*). Just one of these specimens contains enough poison to kill a man and almost all fungus poisoning with deadly outcome result from *A. phalloides*.

But fungi can also be varmints and decay wood, which looses mechanical strength as well as optical performance. To fight such an infestation in time it is necessary to identify the fungus correctly and as early as possible.

The identification of fungi is nowadays mostly done utilizing biological methods, such as the microscopic determination of spores. But fungi can also be differentiated because of their chemical constituents. A first approach to characterize fungi with analytical pyrolysis was done in 1980 by Benoit-Guyod et al. [1], but was not very successful because of the rather poor chromatographic performance of the system used. By hyphenation of analytical pyrolysis to modern gas chromatography/mass spectrometry (GC/MS) systems a technique was established, whose excellence in the characterization of for example lignin [2], proteins [3] and carbohydrates [4], as well as biological samples [5–7] was shown. In preliminary experiments [8] it could be shown that fungi can be differentiated with analytical pyrolysis as well as with thermally assisted hydrolysis and methylation (THM), a pyrolysis technique using tetramethylammonium hydroxide as coreagent [9]. THM is especially useful to convert polar components, such as carbohydrates and fatty acids, into more volatile compounds, which can be used for the classification of different fungal species.

It is the aim of this paper to demonstrate the benefits of pyrolytic methods over traditional techniques of natural products chemistry, such as ease of sample preparation, low sample amounts and short analysis time compared to, e.g. solvent extraction and LC/MS analysis. A pyrolysis and THM method for the identification and categorization of some important fungi (poisonous, edible, dye containing and wood decaying) will be presented.

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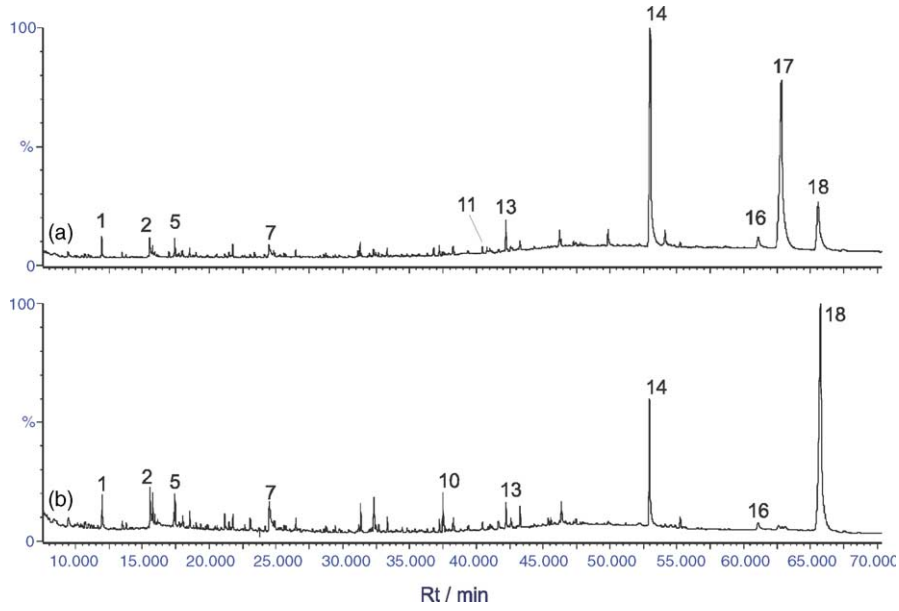

Fig. 1. Chromatograms of (a) *Amanita phalloides* and (b) *Agaricus campester*; numbers refer to compounds listed in Table 1.

2. Experimental

2.1. Chemicals

Tetramethylammonium hydroxide (25% aqueous solution) and α-amanitin were obtained from Fluka. *Amanita citrina*, *A. phalloides*, *Amanita muscaria*, *Amanita pantherina*, *A. campester*, *Fomes fomentarius*, *Trametes versicolor*, *Gloeophyllum odoratum*, *Gloeophyllum sepiarium* and *Serpula lacrymans* were provided by Mag. Stephan Weigl from the Biology Center of the Landesmuseum Linz, Austria; *Cortinarius violaceus*, *Lactarius necator*, *Austroboletus gracilis*, *Boletus retipes* and *Spongiporus caesius* were kindly provided by Prof. Wolfgang Steglich from the Ludwig Maximilian University Munich, Germany.

Table 1
Main products from the pyrolysis of fungi

Number	Compound
1	Hydroxyacetone
2	Acetic acid
3	Pyruvic acid methyl ester
4	2-Furfural
5	Pyrrole
6	Furfurylalcohol
7	Acetamide
8	2-Hydroxy-3-methyl-2-cyclopentenone
9	2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone
10	Anhydromannitol
11	Glycerol
12	1,4:3,6-Dianhydroglucopyranose
13	1*H*-indene
14	16:0 Fatty acid
15	16:1 Fatty acid
16	18:0 Fatty acid
17	18:1 Fatty acid
18	18:2 Fatty acid
19	Levoglucosan (1,6-anhydroglucopyranose)

2.2. Pyrolysis-GC/MS

Typically 100 μg of sample (fungi tissue or reference substance) was placed into a quartz tube sealed with quartz wool on one side. Analyses were carried out with a CDS 2000 pyrolyzer coupled to a Fisons GC 8000/MD 800 system. The samples were pyrolyzed at 500 °C for 10 s and the volatile compounds were separated on a Chrompack CB 52 CP column (60 m, i.d. 0.25 mm, 0.25 μm film) using helium 4.6 as carrier gas (200 kPa) and identified by comparison of their EI mass spectra with NIST 98, Wiley and NBS electronic libraries, literature data and reference compounds. The pyrolysis and GC/MS interface were kept at 250 °C, the GC was programmed from 35 to 90 °C at a rate of 10 °C min^{-1}, and then raised to 250 °C with a rate of

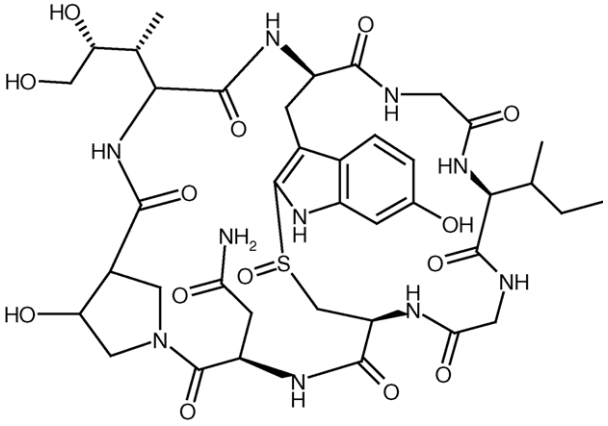

Fig. 2. Structure of α-amanitin, the major toxin of *Amanita phalloides*.