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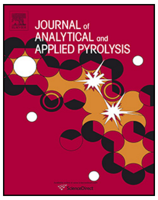

Pyrolysis-GC–MS–Olfactometry: A new approach to identify thermally generated odorants in frankincense

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ABSTRACT

Pyrolysis-GC–MS coupled with olfactometric detection represents a promising new instrumental approach for observing the generation of odor-active compounds from the non-volatile fraction of complex sample materials. The possibility to eliminate the volatiles already present in the sample by a thermal desorption prior to pyrolysis is particularly beneficial with the use of a filament-type pyrolyser. This is exemplified by an investigation of frankincense, the resin of *Boswellia sacra* Flueck. A variety of thermally generated odor active substances, absent in the native material, could be detected by this approach, which would otherwise be covered by volatiles from the sample material. A fractionation of the material by solvent extraction and high vacuum distillation allowed to further narrow down the origin of the pyrolytically generated compounds. Additionally, repeated thermal desorption at mounting temperatures from 120 to 300 °C followed by a pyrolysis run at 500 °C monitored the release and generation of compounds from the sample. This new approach is highly efficient and convenient not only for the investigation of incense materials, but also for off-odor generation in thermally strained materials, investigation of smoke flavor generation and related topics.

1. Introduction

Pyrolysis-GC–MS (Py-GC–MS) has developed to be a well-established method over the last decades. Its particular focus and main application has been, for the most part, the fingerprinting of polymer material through thermal degradation and detection of its monomers and pyrolysis products [1–3]. By that approach, the identification of polymeric materials is feasible on a microgram-scale of sample material. The methodology has found widespread application in polymer chemistry, analysis of biological polymers such as lignin or cellulose [4,5], identification and analysis of laquer, varnish and other materials in both art history and archeology [6–9], environmental analysis (f.ex. [10]), and many other fields.

We now suggest a new field of application for Py-GC–MS by the addition of an olfactometric detection port to the instrumental setup, further on referred to as Py-GC–MS/O. In this study, we demonstrate the key advantages and the application potential of this new approach with the example of frankincense.

Frankincense is the dried gum resin obtained by tapping trees of the genus *Boswellia*. *Boswellia sacra* Flueck., also called the frankincense tree, is native to Oman and Somalia. Several other *Boswellia* exsudates, mainly from *Boswellia papyrifera* Hochst. and *B. frereana* Birdw., are also traded as frankincense. These species grow along the coastal areas of the Red Sea whereas *Boswellia serrata* Roxb. is native to some parts of India and yields another typical resin known as Indian frankincense. *B. sacra* is typically considered to yield the highest quality of frankincense. It has been subject to a large number of studies considering its volatile fraction [11] as well as potential bioactive constituents, such as boswellic acids [12,13]. However, the most common form of application for frankincense, i.e., as smoke resulting from heating on a glowing charcoal or by other means, has received considerably less attention. This may be due to the fact that the analytical methods to investigate smoke are not as well established and notably more complicated. Smoke is, by its nature, a highly complex aerosol made up of particulate matter, volatile substances and gases. Smoke from incense preparations like sticks and cones was already investigated with different methods and, for the most part, under purely analytical or health-related aspects [14–17], yet in almost all cases without a consideration of odor activity.

The smoke of pure frankincense resin was investigated first by Pailer et al. [18–20], and later on by Basar [21]. Pailer et al. used a vacuum pyrolysis setup, in which a diethyl ether extract of the resin was heated under vacuum and the pyrolysis

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products were condensed. The pyrolysis temperatures of 250–310 °C were chosen according to olfactory observations [19]: at these temperatures, pleasant olibanum–smoke-like odor qualities were observed. Reportedly, higher temperatures gave unpleasant tarry notes, lower ones resembled too much the essential oil of the resin. After further fractionation, a variety of compounds was identified by GC–MS. By contrast, Basar [21] applied a solid phase adsorbent technique to collect volatiles emitted from a resin sample placed on a glowing charcoal in a censer. Several new nortriterpenoic compounds, most probably resulting from a thermal decarboxylation of boswellic acids, were detected, isolated and described for the first time in this study.

However, both used indirect methods to trap the smoke and analyze it subsequently. Additionally, their investigations lacked a consideration of odor activity for the observed compounds. Odor active compounds in *B. sacra* resins have recently been investigated in our laboratory [22]. In relation to these findings, we now aim at investigating methodologies for a convenient, realistic and efficient analysis of frankincense smoke.

Py-GC–MS/O, as proposed in this study, presents a highly efficient and direct way to investigate pyrolysis products from materials such as frankincense. It eliminates the need for tedious workup procedures and avoids potential loss of highly volatile or reactive products from the pyrolytic process. The conditions can be well controlled and reproduced. Furthermore, repeated thermal desorption and/or pyrolysis steps enable the monitoring of release dynamics of specific compounds at defined temperatures.

2. Materials and methods

2.1. Sample material

Four different samples of frankincense from three different species were used in this investigation; detailed information is given in Table 1.

All four samples were investigated by headspace solid-phase-microextraction gas chromatography/mass spectrometry (HS–SPME–GC/MS) according to a method by Hamm et al. [23] to ensure sample authenticity. Sample composition was in good agreement with literature data from [23].

2.2. Fractionation of sample material

100 g of powdered resin material from *B. sacra* sample A (BS) was subjected to exhaustive Soxhlet-extraction with 250 ml of dichloromethane. The remaining white powder contains mostly polysaccharides and is further on referred to as the gum fraction (GF). The soluble fraction from the extraction was then distilled in a SAFE apparatus (solvent assisted flavor evaporation [24]) to obtain a volatile fraction (VF) and a nonvolatile resin fraction (RF). The resin fraction appeared to hold back a large part of the semivolatiles, as yields of the volatile fractions were unexpectedly low (<1% of total).

2.3. Sample preparation for measurements

5–10 g of resin raw material from samples A to D were each homogenized by crushing the resin to a fine powder in a mortar filled with liquid nitrogen. While storing the powder at −20 °C for one week until the analysis, it formed a homogenous block of resin, from which small scraps were cut with a scalpel for analysis. Of these scraps, approximately 50 μg were filled into a round-bottom quartz-glass vial and covered with a small layer of quartz glass wool.

GF was dried from residual solvent at room temperature over night and homogenized in a mortar. Approximately 50 μg were transferred into a quartz glass vial and then covered with a small amount of quartz glass wool.

As the dosage of sample material from a solution of defined concentration is easier to realize, VF and RF were diluted in dichloromethane to approximately 10 and 50 μg/μl, respectively. Of these solutions, 1.0 μl were transferred into a round-bottom quartz glass vial, left open to evaporate the solvent for 5 min, and then analyzed without further delay.

The quartz glass vials were mounted onto transport adapters for the autosampler and subjected to Py-GC–MS/O.

2.4. Instrumental setup

The analytic setup consisted of an Agilent GC 7890 coupled to an Agilent MS 5975 quadrupole mass spectrometer (Agilent Technologies, Santa Clara, USA), equipped with a Gerstel Cooled Injection System (CIS 4) PTV-type injector (Gerstel, Mühlheim a.d. Ruhr, Germany), a Gerstel MPS2-XL autosampler and Gerstel Thermal Desorption Unit (TDU). The TDU contained the Gerstel PYRO pyrolysis module, a platinum filament type pyrolyzer with a temperature range from 350 to 1000 °C. The injector contained a liner filled with glass wool and was run in split mode. Both the TDU and the CIS were run at a split ratio of 1:4, adding up to a total split ratio of 1:16. A retention gap of deactivated fused silica capillary (3 m × 0.53 mm) was used to avoid contamination of the main column, an Agilent J&W DB-5 (30 m × 0.32 mm × 0.25 μm). After the main column, the gas stream was split in an approximately 1:1 ratio through a Y-glass-splitter, and directed toward the MS and the odor detection port (ODP) by deactivated fused silica capillaries (2.15 and 1.15 m, respectively, both 0.15 mm inner diameter). Temperatures of system components in this setup were: 120 °C as standby for the thermal desorption/pyrolysis unit, 320 °C for the TDU transfer line, 300 °C for the CIS as well as the ODP, 250 °C for the MS transfer line, 230 °C in the EI ion source and 150 °C for the quadrupole. The ODP was custom made from aluminum and PTFE, inserted into the FI detector slot and used the heating electronics of the FID. Oven temperature was programmed to start at 40 °C for 2 min, was then raised at 6 °C/min to 200 °C, at 40 °C/min to 300 °C and held for 10 min. The carrier gas was helium.

As the gas flow could not be accurately measured, the column head pressure was adjusted in order to achieve an average velocity of approximately 30 cm/s, measured by injection of air. Simultaneous signals at both MS and ODP were assured by analyzing a mixture of odorant reference compounds covering most of the typical retention time frame.

2.5. Pyrolysis method

To begin a measurement, the TDU was actively cooled to 50 °C. After sample introduction and pressure equilibration, it was then heated at a rate of 12 °C/s to the final temperature of the thermal desorption. Each sample was investigated repeatedly at mounting temperatures starting from 120 to 150, 200, 250 and 300 °C. In the final run, with the TDU at its maximum temperature of 300 °C, the pyrolyzer was additionally heated to achieve a temperature of 500 °C for 12 s. The final temperature of the TDU was held for 1.25 min. Then, the oven temperature program and MS acquisition (*m*/*z* range: 45–500) was started. The sample tube was retained in the TDU during the GC run at 120 °C. Blank measurements were run regularly to avoid and track potential carry-over effects.

2.6. Determination of typical surface temperatures of glowing charcoal tablets for incense use

Self-igniting charcoal tablets for incense use from four different suppliers (listed in the legend of Fig. 2) were lit according

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