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DOI
10.1016/j.jaap.2022.105845

Publication date
2023

Document Version
Final published version

Published in
Journal of analytical and applied pyrolysis

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Citation for published version (APA):

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Optimising the analysis of Anacardiaceae (Asian lacquer) polymers using pyrolysis-gas chromatography-mass spectrometry

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ARTICLE INFO

Keywords:
Pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS)
Online derivatisation
Asian, Anacardiaceae, lacquer polymers
Tetramethylammonium hydroxide (TMAH)
Hexamethyldisilazane (HMDS)

ABSTRACT

The identification and differentiation of Anacardiaceae or Asian lacquer polymers is a challenging task, however, the characterisation of these lacquers is highly relevant, especially there is a need to understand the degradation of the polymers and the effects of conservation treatments. To improve the characterisation of artificially light aged polymers from Gluta usitata, Toxicodendron succedaneum and Toxicodendron verniciflum, using pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS), the effects of pyrolysis temperature and the use of online derivatisation were studied in detail. Derivatisation was based on trimethylsilylation with hexamethyldisilazane (HMDS) and hydrolysis and methylation with tetramethylammonium hydroxide (TMAH). The results showed that stepwise increasing the flash pyrolysis temperature is helpful to differentiate between primary pyrolysis reactions and the formation of secondary products. This approach was invaluable to reconstruct the polymeric structure of these lacquers, based on the identified pyrolysates, and the peak areas obtained at specific pyrolysis temperature ranges. Derivatisation of polar compounds in the samples proved a requirement to obtain reliable data. Even though the HMDS based results were found to be inferior to the obtained TMAH data, in terms of derivatisation efficiency and overall analytical repeatability, HMDS was more selective towards alkenylcatechols. This appeared primarily for alkenylcatechols comprising the T. succedaneum polymer and for the analysis of carbohydrates, in samples of all polymeric types. Lowering the split flow, showed that the derivatisation efficiency with HMDS was much improved. Using the reduced split flow method also allowed for e.g., better identification of resorcinol isomers in G. usitata polymers.

1. Introduction

Anacardiaceae Asian lacquer saps, used for thousands of years as organic coating materials to decorate furniture, utilitarian objects, weapons, armour and architecture in East- and Southeast Asian countries, are, once polymerised, characterised by their deep black colour,1 high gloss and inertness against acids, alkalis, and solvents [1–3].

Since the late 16th century an increasing number of objects decorated with Asian lacquer have reached Europe through overseas trade routes. These objects were and, are, highly appreciated in Europe and soon became valued luxury items from the far East. Due to the cultural and historical value of those objects, the majority of the surviving early pieces are preserved in museum collections [4,5].

Asian lacquers are natural polymers excreted as saps by trees of the Anacardiaceae family, indigenous to the East, that harden upon exposure to air [6,7]. Even though the major types of Anacardiaceae polymers are commonly referred to as urushi, laccol and thitsi denoting polymers or lacquers from Toxicodendron verniciflum (Stokes) F.A.

https://doi.org/10.1016/j.jaap.2022.105845
Received 11 April 2021; Received in revised form 16 October 2022; Accepted 20 December 2022
Available online 5 January 2023
0165-2370/© 2022 Published by Elsevier B.V.
Barkely (formerly \emph{Rhus verniciflua} (Stokes)), \emph{Toxicodendron succedaneum} (L.) Kuntze (formerly \emph{Rhus succedanea} (L.)) and \emph{Gluta usitata} (Wall.) Ding Hou (formerly \emph{Melanorrhoea usitata} (Wall.), these terms do not accurately describe the lacquer saps. \emph{Urushi} refers for example to the general term ‘lacquer’ in the Japanese language, similarly \emph{thit-si} is the term used in Burmese [7–9], while \emph{laccol} is used to refer to characteristic molecular compounds of \emph{T. succedaneum} lacquer [8]; hence, the botanical nomenclature of the various lacquer types, deriving from the lacquer trees are better used to designate accurately the lacquer saps or polymers deriving from these lacquer saps.

Lacquer saps are complex water in oil emulsions and consist of water, 30 %, polysaccharides, 7 %, glycoproteins, 2 %, enzymes, 1 %, and a mixture of substituted catechols, 60–65 %, the ratios of which can differ qualitatively, based on the specific botanical type and quantitatively, by the time of harvesting the sap [8–10].

Lacquer producing trees are indigenous to the temperate and subtropical zones of East and Southeast Asian countries. \emph{T. vernicifluum} originates from Japan, China and Korea and its lacquer sap is characterised by \emph{urushiol}, a mixture of catechol derivatives with C15 hydrocarbon substitutions at the 3 position of the catechol ring. The side chain of the catechol nucleus can be saturated or unsaturated with monoene, diene, and triene unsaturations [11–15]. \emph{T. succedaneum} is native to Vietnam, Taiwan, and parts of China. The main compounds in the composition of the lacquer sap are referred to as \emph{laccol}, which are 3-substituted catechols with C17 hydrocarbons, which show 0–3 unsaturations in the side chains [8]. \emph{G. usitata} trees originated from Myanmar.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{diagram.png}
\caption{Exemplary enzyme catalysed dimer structures in mildly oxidised Anacardiaceae lacquer saps as proposed by Kumanotani and Lu et. al [8,11,23].}
\end{figure}
and the North of Thailand. Sap deriving from the *Gluta* genus presents few differences in the chemical constitution of the lacquer sap. The distinctive compounds are named *thitsiol*, which is a mixture of 3- or 4-substituted catechols, 5-substituted resorcinols and 3-substituted phenols with C15 and C17 hydrocarbon chains, or alkylphenyl side chains with C10 or C12 alkyl chain lengths [8,16,17]. Note that the strict geographic categorisation of Anacardiaceae tree distribution, should be referred to as predominant occurrence and usage of lacquer saps in these countries as the situation is more complex with the trees being more widely distributed [18,19], as reviewed to date [2021] by Tamburini [10].

Dimerisation in the mildly oxidised lacquer sap was determined using chromatographic, spectroscopic, and nuclear magnetic resonance (NMR) techniques and follows a complex oxygen oxidative mechanism catalysed by peroxidase and stellacyanin, but mainly by laccase [20,21]. Stellacyanin and laccase are copper containing enzymes that oxidise the hydroxyl groups of the catechols, creating a radical structure, which is converted to reactive semiquinone radicals [8,11]. The radicals react with each other to form carbon to carbon (C-C) bound biphenyls, or they attack the methylene groups in the alkenyl side chains to produce a conjugated methylene to form covalent C-C or carbon to oxygen (C-O-C) bonds with the side chains. Both *urushiol* and *laccol* compounds form biphenyl type dimers which may re-oxidise by repeated laccase catalysed oxidation to form dibenzofurans in the case of *urushiol* dimers [8,11]. Nucleus side chain interactions for *laccol* were characterised by C-C links, while for *urushiol* both C-C and C-O-C can occur [8,22]. Unsaturations in *thitsiol* side chains are typically fewer compared to *urushiol* and *laccol* compounds. As a result of the fewer double bonds in *thitsiol* side chains mostly C-C linked biphenyl dimers form from *thitsiol* monomers [23]. Typical dimers formed by enzymatic catalysis in Asian Anacardiaceae polymers are shown in Fig. 1.

Enzyme catalysed dimerisation, as described here, is the main route to form dimers, and trimers to oligomers in Anacardiaceae lacquer saps. The side chain double bonds are in parallel autoxidised by environmental oxygen to form allyl radicals. Rearrangement of neighbouring double bonds then gives rise to a stable radical site, which reacts with oxygen to give peroxy radicals. Side chain to side chain coupling progresses when the peroxy radicals attack the double bonds of other side chains [8,11], as shown in Fig. 2.

In the process of transforming lacquer sap into a film, by repeated enzymatic and oxidative dimerisations, the polymer becomes progressively insoluble in organic solvents, which severely complicates molecular analysis. The C-C and C-O-C nature of the cross-links also prohibits wet chemical pretreatments to degrade the lacquer polymer to its monomers, making analysis using state of the art NMR, or chromatographic techniques coupled to mass spectrometry extremely difficult. Even though structural analysis of the cross-linked polymer is problematic, broad identifications can still be obtained using direct surface analysis techniques like Fourier transform infrared spectroscopy (FT-IR) [24,25], time of flight- secondary ion mass spectrometry (ToF-SIMS) [26–28] and X-ray photoelectron spectroscopy [29,30]. Those techniques can be utilised to differentiate between the lacquer polymers, but they do not allow us to study oxidative cross-linking or detailed analysis of historical objects decorated with Anacardiaceae lacquer.

Improving the structural analysis of Anacardiaceae lacquers, especially for cultural heritage artefacts, was the main goal of the researchers involved in the current study. This required a dedicated approach to the problem as lacquer formulations on historic objects frequently form complex matrices with other materials, such as inorganic pigments, drying oils, essential oils, resins, proteinaceous materials, and saccharides [9,31–34]. Samples of valuable museum objects, taken for material identification, are often extremely small to limit the damage caused by the sampling. A selective, and at the same time sensitive, technique is, therefore, required to determine the compositions of historic lacquer layers on these minute samples.

Pyrolysis hyphenated with gas chromatography-mass spectrometry (Py-GC-MS) is suitable for this purpose and relies on the introduction of solid samples and online thermal decomposition of the lacquer polymer into small molecules amenable to gas chromatography, followed by mass spectrometry of individually separated compounds [35,36].

Pyrolysis of Anacardiaceae lacquers leads to many polar pyrolysates bearing hydroxylic and carboxylic groups, which are not suitable for gas chromatographic analysis. Their elution usually comes with peak broadening, bad peak shapes, and memory effects [37]. Derivatisation is the common practise used to tackle these problems. In derivatisation hydroxylic and carboxylic groups are substituted into less polar moieties, which increases the volatility of the pyrolysates. Most common derivatisation procedures in use are alkylation and trimethylsilylation (TMS).

Alkylation can be performed using thermally assisted hydrolysis and methylation (THM) with tetramethylammonium hydroxide (TMAH), which methylates the carboxylic and hydroxyl functional groups of compounds present in the sample in combination with

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**Fig. 2.** Side chain autoxidation mechanisms in Anacardiaceae polymers to produce side chain to side chain crosslinks.
transesterification of esters [38–40].

Hexamethyldisilazane (HMDS), N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA), trimethylsilylimidazole (TMSI) and trimethylsilyldimethylamine (TMSDEA) are common silylation reagents used in combination with Py-GC-MS where derivatisation is performed offline or in situ [37,41,42]. Compound identification is generally strong for TMS derivatives, generating very distinctive mass spectra. The drawback of HMDS is however its weak silylating donor capability, especially in combination with the limited contact time during the online pyrolytic process. Steric hindrance problems due to the larger size of TMS groups can also have detrimental efficacy effects on the derivatisation as this creates partially derivatised pyrolysates, resulting in complex pyrograms and less repeatable measurements [37].

Performing pyrolysis and THM using TMAH simultaneously, on samples from cultural heritage objects, is known for its qualitative and reproducible results and is as such favoured in many laboratories. Polysaccharide identification in Asian lacquer with TMAH can, however, be problematic due to the formation of saccharinic acids, which form as a result of carbohydrate reduction and rapid isomerisation [48–50]. Despite unfavourable side reactions, such carbohydrate markers can be found and used for the identification of polysaccharides in T. succedaneum polymers [38]. Identification of carbohydrates in T. verniciflum polymers is, however, much more difficult due to the lower polysaccharide concentration [8] and they are seldom found in G. usitata polymers. Besides detrimental side reactions, inevitably affecting sensitivity of the analyses, saccharinic acids are not as specific as anhydro sugars due to, e.g., loss in stereochemistry. Chirality is not considered detrimental side reactions, such carbohydrate markers can be found and used for the identification of polysaccharides in T. succedaneum polymers [38]. Identification of carbohydrates in T. verniciflum polymers is, however, much more difficult due to the lower polysaccharide concentration [8] and they are seldom found in G. usitata polymers. Besides detrimental side reactions, inevitably affecting sensitivity of the analyses, saccharinic acids are not as specific as anhydro sugars due to, e.g., loss in stereochemistry. Chirality is not considered detrimental side reactions, such carbohydrate markers can be found and used for the identification of polysaccharides in T. succedaneum polymers [38]. Identification of carbohydrates in T. verniciflum polymers is, however, much more difficult due to the lower polysaccharide concentration [8] and they are seldom found in G. usitata polymers. Besides detrimental side reactions, inevitably affecting sensitivity of the analyses, saccharinic acids are not as specific as anhydro sugars due to, e.g., loss in stereochemistry. Chirality is not affected for anhydro sugars which are typical compounds detected upon derivatisation with HMDS, in analyses of for example cellulose [41]. Anhydro sugars are formed by a thermal reaction leading to the elimination of a single water molecule from the sugar hydroxyl groups forming an intramolecular ether bridge [37].

Another important aspect in a Py-GC-MS setup is the pyrolysis temperature. For Anacardiaceae lacquer analysis, pyrolysis temperatures between 400 and 550 °C are reported frequently and seem sufficient for polymer cleavage while preserving monomeric specificity [36, 51, 52]. This general temperature range allows for qualitative identification and distinction between Anacardiaceae lacquer types; however, it does not take into account the specific research questions to be answered: these may require single molecules or molecular groups to be analysed at their peak area optimum in a repeatable way.

With the research presented here we aimed to improve the identification of the polymeric composition of light aged T. verniciflum, T. succedaneum and G. usitata polymers by evaluating and optimising derivatisation procedures, pyrolytic and chromatographic conditions. The research was conducted within the framework of PhySICAL: the Profound study of Hydrous and Solvent Interactions in Cleaning Asian Lacquer, which is dedicated to investigating the molecular effects of cleaning solvents or aqueous solutions on light degraded and thus extremely sensitive Asian lacquer surfaces. Identification of the, aged, lacquer polymers prior treatment, reported in this work, is a foundation on which to base cleaning methods and studies of the molecular modifications found in Asian Anacardiaceae lacquers in response to, previous, cleaning attempts.

2. Materials and methods

2.1. Reference materials

Japanese lacquer from T. verniciflum was acquired from Watanabe-Shoten, Tokyo, Japan, in 2017. The raw lacquer sap was harvested and kurome processed2 in Japan. Taiwanese T. succedaneum lacquer, processed using a similar technique to kurome, was obtained as samples of high quality lacquer sap and came from JC Cultural Creative Service CO., LTD, Taiwan in 2017. The lacquer sap we used was harvested and processed in Taiwan. Thai G. usitata lacquer was acquired from the Thailand Royal Forest Department in 2017. The raw lacquer sap had not undergone any pretreatment, which is common for G. usitata lacquer, unlike T. verniciflum and T. succedaneum lacquer saps. Dry season, November-March harvest, sap is used, which has less water in its composition for high quality G. usitata lacquer work. The G. usitata lacquer sap was gathered in Mae Hong Son province, Thailand, in January 2016. All the lacquer saps were pure uncoloured reference materials, and before preparing the mock ups, we verified the materials using Py-GC-MS.

2.2. Mock-up production

All the lacquer saps were first filtered using Japanese Miyoshinogami filtering paper, acquired from Watanabe-Shoten, Tokyo, Japan. The lacquer was then applied to 2 mm thick borosilicate glass slides, using an Elcometer 3525 film applicator. The wet film thickness for the lacquer layers was set at 50 μm. To trigger enzyme activity, the lacquer films were polymerised inside airtight polypropylene containers under elevated relative humidity (RH) conditions. The microclimate inside the containers was created using Art Sorb 70 % RH preconditioned silica gel, which was used for both the T. verniciflum and T. succedaneum lacquer mock-ups. Silica gel M, conditioned at 80 % RH, was used for the G. usitata lacquer samples. The temperature inside the containers was maintained at 21 °C and RH and temperature were monitored using calibrated LOG210 dataloggers from Dostmann electronic GmbH. The Art Sorb, Silicagel M and data loggers were acquired from Long Life for Art, Eichstetten, Germany. The mock up samples were left to polymerise over a period of 3 months.

2.3. Artificial aging

Objects decorated with lacquer contain many different layers and materials [31, 34, 53]. Foundation layers typically consist of inorganic clay powders mixed with organic binding media [9, 30], such as Anacardiaceae lacquer, proteinaceous materials or polysaccharides [9, 31, 38]. Additionally, Anacardiaceae lacquer layers may be complemented with drying- and essential oils to modify working properties and to improve the quality of the resulting film, or pigments can be added to alter colour [9].

The natural ageing of the sequential layer structure can take many years. This happens by sunlight irradiation in combination with humidity changes or other environmental influences, by which different ingredients mutually interact, yielding very complex degradation pathways [38, 45, 54].

Single material mock-ups were used to study the chemical changes related to the effects of ageing. As such chemical changes are limited to ageing of this single material avoiding the possible effects of any other material involved. To age the mock-ups a slightly modified form of the method given in Han et al. [54] was used.

Naturally aged lacquered surfaces often exhibit hundreds of years of natural ageing and their exact storage conditions are seldom known. Relating artificial regimes to an equivalent in years becomes therefore difficult. Efforts were made to ‘fingerprint’ the molecular degradation process of lacquers taken from naturally aged surfaces. The mock ups
were aged and stepwise monitored using Py-GC-MS, until the artificial results were comparable to naturally aged object analyses. This resulted in following artificial ageing regime.

A Ci4000 Atlas Weather-ometer system was employed with a water-cooled Xenon arc light source (6500 W). The light source was operated at 0.50 W/m² and fitted with a sodium borosilicate glass inner filter, type S65 to filter out part of the UV light. A sodium lime glass with a CIRA coating outer filter, type S65 was used to block part of the infrared wavelengths. The filter combination allowed for an irradiance range between 340 and 800 nm. The black panel temperature was 40 °C, chamber temperature 30 °C and relative humidity inside the chamber was maintained at 65 %. To control the RH, the system uses a moisture nebuliser, spraying a mist of small water droplets into the chamber that might end up on the lacquer surfaces, causing unfavourable ageing phenomena. Simulating indoor ageing conditions, the samples were protected from water damage by custom made sample holders, covering the lacquer surfaces with 2 mm thick borosilicate glass slides. The samples were treated for a total of 576 light hours, or 1039 kJ resulting in approximately 93.000 lux.

2.4. Chemicals and reagents

A C7-C40 alkanes calibration standard, tridecanoic acid, 98%, tetramethylammonium hydroxide (TMAH) 25 wt% in methanol, hexamethyldisilazane (HMDS), 99,6 %, were purchased from Sigma Aldrich. Terephthalic acid, 98 %, 1,2,4-benzenetricarboxylic acid, > 99 %, and 1,2,4,5-benzenetetracarboxylic acid, > 98 %, were also acquired from Sigma Aldrich. LC-MS Chromasolv™ methanol, 99,9 %, was obtained from Riedel de Haën.

2.5. Sample preparation

Film fragments of the lacquer mock-ups were separated from their glass substrates using a scalpel and added to 1.1 mL glass vials with a 15 µL cone. The film fragments were then ground to fine powder using a custom made glass rod.

2.6. Derivatisation procedures

For all analyses, 80 µg of ground samples were transferred to pyrolysis cups (Eco-Cup SF) from Frontier Laboratories. The samples were weighed on a Sartorius SE2 ultramicrobalance with a minimum readability of 0.1 µg. Each sample was weighed 5 times, yielding an average standard deviation of 0.5 µg.

A 3 µL solution containing reagent and internal standard was used to derivatise the compounds in the sample. For TMAH a 5 wt% in methanol was prepared containing 800 ng/mL tridecanoic acid and to the HMDS reagent 60 µg/mL tridecanoic acid was added.

Even though the use of internal standards in analytical pyrolysis is controversial as part of it is converted into pyrolysis products, the fraction that remained intact was repeatable. The absolute internal standard peak areas could, moreover, differ depending on the sample matrix. These phenomena also applied to the lacquer samples, as detected pyrolysates are no direct reflection of molecules present in the original sample [55,56].

2.7. Analytical methods

Artificially aged mock-up samples, made of T. vernicifluum, T. succedaneum and G. usitata lacquer underwent analysis using Py-GC-MS. All analyses were performed on an EGA-PY-3030D multi-Shot pyrolyser (Frontier laboratories), syphoned with a Trace 1310 gas chromatograph and an ISQ LT single quadrupole mass spectrometer (both Thermo).

2.7.1. Pyrolysis-gas chromatography interfacing

Analytical separations were accomplished on a fused silica SLB-5 ms capillary column, Supelco, 20 m, 0.18 mm i.d. × 0.18 µm film thickness, silphenylene polymer stationary phase. An ITF union ASN (Frontier laboratories) designed for blowing the pyrolysis cups back upward into an autosampler tray, was used as a split device to overcome a liner-on-liner system, limiting detrimental liner absorption and peak broadening defects. This split exit was constructed at the interface of the pyrolyser. The analytical column passed a custom-made heated interface of the GC and was connected directly to the ITF union. All flows were regulated by the digital pressure and flow control (DPFC) of the GC [57].

2.7.2. Pyrolysis

Reactive pyrolysis using TMAH or HMDS was done in a helium saturated atmosphere using single shot, flash pyrolysis mode, at the following fixed temperatures: 270 °C, single measurement and only on the T. vernicifluum polymeric type, 300, 350, 400, 450, 480, 500, 550, 600, 650 and 700 °C. Ultrafast thermal degradation (UTD) was used as an alternative to flash pyrolysis; a method in which temperature is ramped from 350° to 700 °C in 0.98 min [58]. The apparatus interface temperature was set at 290 °C for all analyses.

With exception of the UTD method, which cools down after pyrolysis, the samples analysed with flash pyrolysis are exposed to high temperature during the entire GC-MS run. During flash pyrolysis, the samples experience thermal shock effects, leading to instant fragmentation of the polymeric matrices. Pyrolysis should ideally happen very fast, 0.5–6 s [59]. Longer pyrolysis times generally are accompanied by non-informative thermal degradation products, which complicate interpretation of the results and should be avoided. An autosampler can overcome this problem by blowing cups upwards after pyrolysis. An autosampler was lacking for this research, it should however, be taken into consideration for future studies. A reversed UTD procedure could as an alternative be used, by rapid cooling after flash pyrolysis, which might aid in solving this problem.

2.7.3. Gas chromatography

A quick initial temperature ramp was used to speed up analysis time. Undoubtedly, reducing analysis time means sacrificing resolution of the chromatographic separation. Most peaks in this part of the pyrograms were found to be less significant for the study, while separation of the crucial peaks was sufficient. The MS data were deconvoluted, to aid accurate molecular identification, see Section 2.8.

The initial GC oven temperature was 35 °C for 1.50 min, followed by 60 °C/min gradient until 100 °C was reached. The temperature was subsequently ramped up to 250 °C at 14 °C/min. The temperature was finally raised at 6 °C/min to 315 °C, which was maintained for 1.50 min isothermal.

A constant flow of 0.9 mL/min in combination with a split flow of 27 mL/min, or a ratio 1/30, was applied to study the cumulated integrated signals, reported in Section 3.1. The method was also applied to the molecular comparison between results obtained after treating the samples with TMAH or HMDS, described in Section 3.2.

To optimise the chromatographic separation of compounds in the samples, the column flow rate was operated in gradient mode allowing to employ optimum linear gas velocities over the total GC temperature ramp [60].

The programmed flow method was used in combination with a split flow of 21 mL/min, or a ratio 1/30, and optimal flow rates were calculated using GCCalc software from Enovatia, version 1.2.21. The programmed flow method was used for the HMDS derivatisation optimisation study, which is discussed in Section 3.3. The flow rate gradients were performed in parallel with the GC temperature ramp, described

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3 UTD was configured 350–700 °C, however, the temperature reached after 0.98 min was 668 °C
above. The initial flow rate was set at 0.66 mL/min for 1.50 min followed by a flow rate ramp of 0.148 mL/min until 0.82 mL/min. The flow rate was subsequently increased 0.021 mL/min until 1.040 mL/min and finally a 0.010 mL/min gradient was used to 1.13 mL/min, which was maintained for 1.5 min.

2.7.4. Mass spectrometry
The MS transfer line was set at 270 °C. Electron ionisation was performed at 70 eV and the ion source was maintained at 250 °C. The MS was operated in scan mode, in the range of m/z 29–600, with a cycle time of 0.2 s. The solvent delay was set to 1.10 min for THM analyses and 2.90 min for in situ silylation.

2.8. Data treatment
Interpretation of the pyrograms was semi-automated using the Automated Mass spectral Deconvolution and Identification System (AMDIS), v. 2.73. The AMDIS software scanned the analytical data against dedicated target libraries and calculated the integrated signal value as the area of a given component after deconvolution. Mass spectral identification was performed using the national institute for standards and technology mass spectral library 17 (NIST 17) v. 2.3, and by using spectra compiled within the expert system for characterization using AMDIS plus Excel (ESCAPE), which is a mass spectral interpretation tool developed by experts in the cultural heritage community [31, 35,38,61]. The reference data reported in literature were also used.

Fig. 3. Pyrolytic fragmentations observed after the Py-GC-MS measurements of Anacardiaceae lacquer polymers. Mechanisms Py-1 – 3 and thermal dehydration reactions (TD) were proposed by Miyakoshi and co-workers [63–65], which were recently reviewed by Tamburini [10].
An AMDIS target library was compiled and integrated into ESCAPE for trimethylsilylation analyses, using HMDS as a derivatizing reagent. This was performed using published data [43–45,50,62] and manual interpretation of the mass spectra. Kovats retention indices (RI) were calculated using AMDIS, which used a calibration standards library, based on the separation of a C7–C40 alkanes standard. The retention time (RT) values were interpolated into a RI calibration file and the computed values were subsequently used to adjust the match factor of the compounds subjected for identification, for which the maximum RI penalty was set at 10. The minimum match factor was set at 70.

The data, generated in triplicate, were normalised to correct for sample transfer variability. A two-step normalisation was used; step 1) using sample size normalisation, see Section 2.6, and step 2) using internal standard normalisation. Following the sample size normalisation, all the peaks were corrected using the integrated signals from a tridecanoic acid internal standard. Depending on the lacquer type, the average relative standard deviation of the tridecanoic acid integrated signals was 5%.

3. Results and discussion

3.1. Influence of pyrolysis temperature on the fragmentation of aged Anacardiaceae polymers

This work was carried out with the aim to improve the characterisation of aged G. usitata, T. succedaneum and T. verniciflum polymers by the determination of intermolecular cross-links and the molecular distribution of unpolymerised ‘free’ compounds in the polymeric matrices. THM-Py-GC-MS was used for this purpose. Anacardiaceae polymeric samples were exposed to a series of progressive pyrolysis temperatures, allowing us to evaluate the obtained integrated signals of various compounds.

The samples were taken from the artificially light aged mock-ups and the data, obtained after THM-Py-GC-MS analyses, were processed based on cumulated integrated signals for series of related compounds, e.g., homologous series of compounds or molecules with similar functional groups. The assessment using molecular groups proved to be valuable for determining the temperature ranges of pyrolysis and, hence, of the corresponding release of pyrolysis products from the polymer. This helped us to distinguish between primary pyrolysis and secondary thermal dehydration reactions, which are often difficult to differentiate. The thermally dehydrated products are formed from primary pyrolysis products comprising phenols and catechols resulting in the loss of, respectively, one or two hydroxylic groups, as shown by the mechanisms TD-1a and TD-1b in Fig. 3.

Cumulated integrated signals were distributed between different molecular groups, comprising: alkylcatechols (I), alkylphenols (II), alkylbenzenes (III), fatty acids (comprising monocarboxylic fatty acids, dicarboxylic acids, and glycerol) (IV), hydrocarbons (V), carbohydrates (VI) and proteins (VII). Ageing products consisted of carboxylated benzenes (VIII), acid catechols (IX), acid phenols (X), acid phenyls (XI), acid-oxo-phenyls (XII) and phenylalkylketones (XIII). G. usitata monomers incorporate alkylcatechols (I), phenylalkylcatechols (XIV) and phenylalkylphenols (XV), see Fig. 4. The integrated signals of all molecular groups were plotted as histograms versus pyrolysis temperatures, as depicted in Fig. 5. Note: each bar of the histograms corresponds to the analysis of different samples and that the same sample was not re-pyrolysed using progressive temperatures.

3.1.1. Effects of pyrolysis temperature for the fragmentation of the catechol-based polymer

To differentiate between primary and secondary dehydration products polymeric fragmentation was determined using the histograms shown in Fig. 5. The alkylphenol profiles show, for example, that primary pyrolysis occurs between 450 and 500 °C for T. succedaneum and T. verniciflum polymers. This was supported by the observation that very similar integrated signals were obtained within this temperature range, creating a ‘flat’ profile. In contrast the alkylphenols, shown between 550 and 650 °C, showed progressively intensified signals with increasing pyrolysis temperature. This was observed for all polymeric types. Within this same temperature range (550–650 °C) alkylcatechol signals decrease, suggesting that thermal dehydration of the alkylcatechols occurs, resulting in the formation of alkylphenols. Similarly,
alkylbenzenes were formed from the phenols in *T. succedaneum* polymers at very high temperatures. This illustrates that a comparison of integrated signals obtained after using progressive pyrolysis temperatures, allows for the determination of primary pyrolysis products and secondary thermal dehydration products. This makes it possible to identify the type of cross-links in the structures of the Anacardiaceae polymers.

Thermal desorption of alkylcatechols was observed for all lacquer types between 300 and 350 °C, illustrating the occurrence of an unpolymised fraction. At higher temperatures, pyrolysis of the network occurred, resulting in the formation of alkylcatechols by the cleavage of carbon to carbon (C-C) links as shown in the mechanisms Py-2a and Py-2d in Fig. 3. Alternatively, bonds between the side chains can also be cleaved via mechanisms Py-2b and Py-2c. The Py-2c mechanism was less likely to take place for *G. usitata* polymers as this mechanism involves the presence of an intermolecular oxygen, which is not likely to occur in this polymeric type. Only C-C bonds were determined based on analyses of samples from the *G. usitata* polymer in the temperature range between 450 and 550 °C. The occurrence of C-C intermolecular bonds was shown by the integrated signals of the alkylcatechols demonstrating comparable intensities between 480 and 550 °C for the *G. usitata* and the *T. succedaneum* polymers and between 500 and 550 °C for the *T. vernicifluum* polymer. Similarly, the alkylphenol signals rapidly intensified at higher pyrolysis temperatures, illustrating thermal dehydration of the alkylcatechol pyrolysates. Primary pyrolysates resulting from carbon to oxygen to carbon (C-O-C) bond cleavages were found for *T. succedaneum* and *T. vernicifluum* polymers. These polymers comprise both C-C and C-O-C intermolecular bonds to form their cross-linked structures. The C-O-C linked structures resulted in the formation of alkylphenols (Py-1a), which were observed by the flat profile of the histograms in the temperature range between 450 and 500 °C.

The C-O-C bonds in *T. vernicifluum* are not uncommon. Mechanisms leading to those dimeric products have been documented in literature and result, in particular, from enzyme catalysed monomeric coupling [8, 11,14,15,20,66]. This contrasts to *T. succedaneum* polymers, where C-O-C intermolecular bonds have been determined to be almost non-existent [8,22]. Those studies, however, were performed on mildly oxidised lacquer saps and not on fully polymerised samples. We hypothesise that the presence of C-O-C bonds in the *T. succedaneum* polymer, the intermolecular bonds were formed during a later stage of polymerisation, caused by autoxidation rather than by enzyme catalysed reaction pathways. Once the polymer has formed a dense cross-linked matrix, enzymatic diffusion became likely progressively inhibited and Cu- ions could not easily reach the numerous catechol reaction sites [11]. The C-O-C linked matrix in the *T. succedaneum* polymer is therefore likely to be formed by peroxy radicals prevailing from autoxidation, as shown in Fig. 6.

The alkylcatechol profile of the *T. vernicifluum* polymer suggests polymeric decomposition resulting from ageing based on the earlier pyrolysis onset temperature of 400–450 °C for this polymer compared to the other polymer types. This was also shown by the integrated signals of the carbohydrates, proteins, and ageing products, which correlate to the alkylcatechol profile.

The presence of alkylbenzenes, is a known feature for the identification of *G. usitata* polymers [43,63]. The alkyl benzenes can form by preferential cleavage of the phenylalkylcatechols at the benzylic position. The first pyrolysis range is shown between 450 and 500 °C which is roughly in the same range as the *G. usitata* monomers and represent C-C intermolecular bonds between the thiol monomers. The second pyrolysis range, between 550 and 650 °C, represents C-O-C intermolecular bonds where both hydroxylic groups of the catechols or resorcinols are involved, either interlinked with one other monomeric side chain or linked to two different monomeric side chains. Pyrolytic cleavage results in the loss of both intermolecular oxygens, resulting in alkylbenzene pyrolysis product, see mechanisms Py-4a and Py-4b in Fig. 3. A similar alkylbenzene profile was shown for *T. vernicifluum* between 600 and 650 °C, while for *T. succedaneum* the polymer was almost completely pyrolysed at 550 °C and only showed thermal dehydration at higher
temperatures. We investigated the hypothesis that more cross-linking occurred in *G. usitata* and *T. vernicifluum*, and it appeared to be confirmed after a gravimetric evaluation of the pyrolysis efficiency at 550 °C. This resulted in almost complete pyrolysis of the *T. succedaneum* polymer, 98.93%, while pyrolysis was incomplete for the *G. usitata*, 87.23%, and *T. vernicifluum* polymers, 87.36%. Note that Anacardiaceae lacquer polymers can also contain a small non-pyrolysable inorganic fraction, which we considered to be too small to have an influence on the results obtained. For further details see the Supplementary information.

3.1.2. Distribution of degradation products

Degradation products form on a lacquer surface or within the polymeric matrix after exposure to sunlight, inducing photo oxidation. This irreversibly leads to, e.g., different types of carboxylated compounds. In this part of the paper, we will discuss the degradation products identified during our analysis of Anacardiaceae polymeric samples. The degradation compounds were assigned as unpolymerised ‘free’ compounds or as compounds trapped in the polymeric matrix, or possibly forming covalent bonds to the polymeric structure. These compounds, trapped in the polymeric matrix, required pyrolysis of the polymer to enable their release. Alternatively, the compounds could be covalently interlinked to the polymer, this required pyrolytic cleavage of the intermolecular bonds. We studied for the *G. usitata* polymer carboxylated benzenes (VIII), acid catechols (IX), acid phenols (X), acid phenyls (XI) acid oxo-phenyls (XII) and phenylalkylketones (XIII): for the *T. succedaneum* polymer we studied carboxylated benzenes (VIII), acid catechols (IX) and for the *T. vernicifluum* polymer the molecular groups included carboxylated benzenes (VIII), acid catechols (IX) and acid phenols (X), see Fig. 4 for the structures. The cumulated integrated signals of all grouped degradation products are shown per polymeric type in Fig. 5, and the integrated signal distributions of the various degradation product types, are provided in Fig. 7. The pathways leading to the formation of degradation products, as discussed in the literature, are summarised in the Supplementary information.

The most abundant integrated signals relating to degradation products were observed for the *G. usitata* polymer, followed by the *T. succedaneum* polymer, while the *T. vernicifluum* polymer showed the least photo oxidation. The only exception was found for the carboxylated benzenes, which showed to be most present in samples from the *T. vernicifluum* polymer. The analysis of *T. succedaneum* polymeric samples showed a gradual increase for acid catechols from thermal desorption at 300 °C to pyrolysis between 480 and 550 °C. This increasing integrated signal profile was almost identical to the alkylcatechol profile, see Fig. 5, and demonstrates that the acid catechols are not solely present as ‘free’ unpolymerised compounds as their detection also required pyrolysis of the polymer at high pyrolysis temperatures. A similar relation was found for the *G. usitata* polymeric samples for the detected acid phenyls and alkyl catechols, while phenylalkylketones signals depicted a profile comparable to the alkylbenzenes. The phenylalkylketone compounds are especially abundant in the second pyrolysis range, which required very high pyrolysis temperatures (550–650 °C). The acid phenyls showed thermal desorption between 300 and 350 °C, depicting a fraction of unpolymerised compounds in *G. usitata* samples. It was interesting to observe that the overall integrated signal profile for *T. vernicifluum* showed an opposite, decreasing profile, towards higher temperatures to that of the *G. usitata* and *T. succedaneum* polymers. The compounds identified in the *T. vernicifluum* polymers showed mainly unpolymerised compounds, which are easily desorbed at very low temperatures (300–350 °C). We therefore assume that the ageing of the *T. vernicifluum* polymer takes place mostly at the surface. Note: analyses on *T. vernicifluum* showed less degradation products compared to *G. usitata* in the very high temperature range.
(600–650 °C). A possible explanation for this is that carboxylated benzenes, acid catechols and acid phenols, as typical degradation products in *T. vernicifluum* polymers, are less stable at high temperature.

### 3.2. Comparison between TMAH and HMDS derivatisation reagents

In this section the effectiveness of in situ trimethyl-silylation using HMDS and TMAH-based methylation were assessed for the three Anacardiaceae type polymers.

#### 3.2.1. Qualitative comparison between TMAH and HMDS results

A qualitative comparison for a series of characteristic molecular classes was performed after derivatisation using TMAH and HMDS, as can be seen in Fig. 8 and in Table 1. It was found in general that derivatisation efficiency using HMDS was very poor compared to results obtained using TMAH. We postulate this is due to the sterically hinderance of the larger TMS groups, but it could also be due to the very short contact time used in analytical 'flash' pyrolysis, and maybe due to combination of both the above. Full silylation of compounds with multiple derivatisable functional groups could in many cases not be obtained or were leading to products depicting unacceptable signal variability. Our emphasis was therefore put on the partially trimethylsilylated compounds with enhanced repeatability. All the identified compounds are shown as extracted ion chromatograms (EIC) in Fig. 8. The m/z 91 fragment ion represents a [tropylium]+ ion which was used to visualise the alkylbenzenes and acid phenyls. The m/z 151 represents a [bis (methoxy)-tropylium]+ ion allowing to show the alkyl catechols, acid catechols and alkylphenylcatechols. The m/z 217 [TMSOCH=CH–CHOTMS]+ ion is commonly used to illustrate the occurrence of carbohydrates [67] and the m/z 179 fragment, [trimethylsilyloxytropylium]+ ion, is related to partially TMS derivatised alkylcatechols and alkylphenylcatechols [10,44].

#### 3.2.1.1. Alkylcatechols and *G. usitata* monomers

Series of alkylcatechols, with increasing number of carbon atoms in the hydrocarbon side chains, form after preferential cleavages of those side chains. Peak labels in Fig. 8 depict the predominant compounds.

The *T. succedaneum* data showed that when using TMAH as...
The presence of numerous unsaturated compounds in the polymer samples showed fewer alkylcatechols compared to TMAH derived samples. The data resulting from the TMAH based results, however, only one derivatisable site, could effortlessly be identified using TMAH or HMDS. It was interesting to observe that acid phenols, which comprised two hydroxylic sites and one carboxylic acid reaction site represent an important class of photo degradation products. While quantitation of pyrolysis data is challenging using Py-GC-MS, the use of HMDS was found detrimental as compared to the TMAH procedure. This can be tentatively explained by the enhanced occurrence of the labile unsaturated sites in the side chains of alkylcatechols in the T. succedaneum polymer as compared to the other polymers. Due to the thermochemolysis with TMAH the unsaturated compounds are likely more comparable between TMAH and HMDS results, however, larger integrated signals were obtained when HMDS was used. The G. usitata polymer is upon pyrolysis converted mostly to alkylbenzenes which do not contain derivatisable sites. The side chains also contained minor amount of unsaturations: hence, the results obtained either with TMAH or HMDS were very similar.

3.2.1.2. Degradation products. The use of HMDS was found detrimental for the detection of degradation products incorporating more than one derivatisable site.

Acid catechols, comprising two hydroxyl sites and one carboxylic acid reaction site represent an important class of photo degradation products found in aged Anacardiaceae polymers. Such pyrolysates could readily be identified via the TMAH approach, however, detection of acid catechols proved to be impossible using HMDS derivatisation. As complete, or partial derivatised photo degradation products were not identified using HMDS, it appears that the latter were insufficiently volatile or too unstable for successful subsequent GC-MS analysis. Even though derivatisation of acid catechols was thus not possible, there is evidence in the literature that those compounds can be detectable via silylation [10, 47]. It was interesting to observe that acid phenols, which comprised only one derivatisable site, could effortlessly be identified using TMAH or HMDS.

3.2.2. integrated signal yield versus repeatability using different pyrolysis temperatures

While quantitation of pyrolysis data is challenging using Py-GC-MS, it is nevertheless crucial to obtain insight into the repeatability of the...
Fig. 9. Molecular comparison of substituted catechols by means of integrated signals and standard deviation between analyses on Anacardiaceae polymers.
acquired data. The integrated signals and repeatability resulting from the analyses on the aged Anacardiaceae polymers are discussed in this section.

3.2.2.1. Alkylcatechols and G. usitata monomers. Thermal desorption, between 300 and 350 °C, for G. usitata monomers showed dominantly pentadecylcatechol and also phenyldodecylcatechol in the TMAH and HMDS analyses, see Fig. 9. The ratio between integrated signals of pentadecylcatechol versus phenyldodecylcatechol was much higher when TMAH was used. Repeatability was also better using TMAH: 5% RSD for pentadecylcatechol and 12 % RSD for phenyldodecylcatechol. Pyrolysis, between 480 and 550 °C, also showed acceptable repeatability after using TMAH (≤10 % RSD). In contrast to the TMAH procedure, a very poor repeatability (≥20 % RSD) was obtained when pyrolysing the polymer using HMDS. Enhanced repeatability was only obtained (±/–10 % RSD) when in situ silylation was combined with thermal desorption, between 300 and 350 °C.

What immediately stood out from the measurements performed using TMAH on T. succedaneum polymers was the abundance of dihydroxybenzene obtained. This compound is formed after numerous side chain cleavages of alkylcatechols. Signal variability based on analyses on the T. succedaneum polymer was more similar using either HMDS or TMAH (≤10 % RSD at 500 °C).

Repeatability was optimal for T. vernicifluum alkylcatechols after using a pyrolysis temperature of 500 °C, showing ≤10 % RSD for results using either TMAH or HMDS.

3.2.2.2. Degradation products. Acid phenyls in G. usitata polymers were the only compounds we could use to compare results obtained after treatment with TMAH and HMDS. Even though acid phenyls could be identified when HMDS was used, this generally resulted in much smaller integrated signals compared to results obtained with TMAH, see Fig. 10. The resulting HMDS data was highly variable, ranging between 20 % and 70 % RSD. In contrast, the TMAH data showed a repeatability below ≤20 % RSD when subjected to thermal desorption (350 °C) and ≤10 % RSD upon pyrolysis at 550 °C. UTD proved not suitable for most molecular groups because of reduced integrated signals and poorer repeatability.

The detected acid catechols, using TMAH, on samples of T. vernicifluum and T. succedaneum polymers showed upon thermal desorption (300 °C) RSDs ≤10% and for pyrolysis (480–550 °C) RSDs ≤10–20 %. Even though UTD was not useful for analysing T. succedaneum alkylcatechols, for acid catechols it showed similar signal intensities compared to flash pyrolysis between 450 and 550 °C, with somewhat poorer repeatability, RSD ∼15 %.

3.2.2.3. G. usitata carbohydrates. The HMDS data resulting from the analyses on Anacardiaceae polymeric samples showed a dominant β-D-Galactopyranose-anhydro compound, which is an essential marker of the galactopyran main chain of lacquer polysaccharides [68] upon Py-GC-MS analysis, see Fig. 11. The repeatability for β-D-Galactopyranose-anhydro was found to be problematic. The detection of this compound is, however, relevant as its identification is crucial for the characterisation of polysaccharides in Anacardiaceae polymers, and not detectable using TMAH. The most abundant integrated signals were obtained at 400 °C, however, they were poorly repeatable (70 % RSD). At 600 °C the integrated signals severely decreased but repeatability was much improved (6% RSD). A possible cause for this phenomenon is the three dimensional network of the lacquer polymer, in which the polysaccharides are dispersed, and the low thermal stability of the carbohydrate pyrolysates. Note: the three dimensional structure of thitsiol, laccol and wrathiol macromolecules is composed of phenolic agglomerates, formed after enzymatic polymerisation and autoxidation, see introduction and Section 3.1.1. These agglomerates are covered by polysaccharides and glycoproteins, acting as an adhesive between the agglomerates [8,11]. At the lower pyrolysis temperatures incomplete release of the carbohydrates and proteins was observed, leading to poor repeatability under these conditions. Complete pyrolysis takes place at 600 °C, see Section 3.1.1, resulting in improved repeatability. It is likely because the temperature applied, 600 °C, is beyond the thermal stability of the carbohydrate pyrolysates that they will also thermally degrade, explaining the smaller integrated signals.

3.2.3. Derivatisation efficiency

The efficiency of the derivatisation of compounds in the lacquer samples was assessed by comparing the integrated signals of fully derivatised compounds versus the summed integrated signals of the partially and non-derivatised compounds. Note that thermochemolysis using TMAH is a thermally assisted reaction, which also affects pyrolysis. It is therefore reasonable to postulate that incomplete polymeric fragmentation can also impact the derivatisation yield.

The pyrolysis data at 550 °C for 6 alkylcatechols and for one alkyl-phenylcatechol, thitsiol, monomer is shown in Table 2.

It was observed that for T. vernicifluum polymers conversion efficiencies, towards the double methylated alkylcatechols was around 25% when using TMAH. We propose that this could be a result of incomplete pyrolysis, hence of only partial thermochemolysis with TMAH, see also Section 3.1. In contrast, the T. succedaneum polymer was almost completely pyrolysed at 550 °C and yielded a 100% derivatisation efficiency for all methylated compounds subjected for evaluation, see Table 2. These observations illustrate that, if complete TMAH derivatisation can be obtained, three times higher signal intensities for the fully derivatised compounds in samples from T. vernicifluum polymers are, potentially, possible.

3.3. Optimisation of analyses with HMDS based on molecular selectivity and derivatisation efficiency

Carbohydrates were identified with better selectivity and sensitivity after in situ trimethylsilylation with HMDS compared to TMAH based results. Differentiation between G. usitata, T. succedaneum and T. vernicifluum polysaccharides via silylation based on Py-GC-MS analyses has not been reported to date (January 2023). Below we report the potential of this approach for Asian lacquer identification.

A novel in situ trimethylsilylation procedure has been described, to date (2016), using Py-GC-MS for the identification of polysaccharide materials using a micro reaction capsule, in which samples were simultaneously pyrolysed and derivatised using HMDS [37]. The procedure was shown to improve greatly complete silylation by extending pyrolysis time, providing prolonged derivatisation time. The involved researchers showed in this work how the limited contact time between the HMDS reagent and derivatisable compounds in analytical pyrolysis can have a major detrimental impact on the analytical results.

To increase the contact time of derivatisable compounds using HMDS, in an instrumentally simpler way, we lowered the split flow to 5 mL/min in combination with UTD where pyrolysis of the lacquer macromolecule evolves more slowly compared to flash pyrolysis. This proved to be a simple and practical setup to increase post pyrolytic residence time, improving the efficiency of the derivatisation and appreciably improving the repeatability, selectivity, and sensitivity of the analyses.

Applying low split flows transgresses conventional practice in analytical pyrolysis. The traditional process of pyrolysis creates highly reactive products that should be derivatised and transferred as fast as possible to overcome liner absorption defects, unfavourable molecular recombinations or excessive pyrolytic scission. Despite these drawbacks, the application of low split flows in combination with HMDS appears to be a promising method for the analysis of Anacardiaceae polymers, see the extracted ion chromatograms (EIC’s) shown in Fig. 12, these represent the results for the aged G. usitata polymer after optimisation.
Fig. 10. Molecular comparison of degradation products by means of integrated signals and standard deviation between analyses on Anacardiaceae polymers.
3.3.1. Qualitative interpretation of the HMDS based Py-GC-MS results

In the mass spectra of completely derivatised alkylcatechol, phenylalkylcatechol and acid catechols the m/z 267 fragment ion prevails. This fragment ion comes from cleavage of the alkyl chain at the benzylic position and was determined to be a [bis-(trimethylsilyloxy)-tropylium]⁺ ion [44]. The results show the homologous series of alkylcatechols, propyl- up to nonylcatechol, whereby heptylcatechol was the most abundant compound. Alkylcatechols with side chain lengths of C14 to C17 were also detected. Interestingly next to 3-pentadecylcatechol the 4-pentadecylcatechol isomer was also observed in only HMDS based analyses of the G. usitata polymer. Both the phenyldecyl- and phenyldodecylcatechols were identified with their side chain lengths of C14 and C15, respectively.

Table 2

<table>
<thead>
<tr>
<th>Compound name</th>
<th>G. usitata - n = 3</th>
<th>T. succedaneum - n = 3</th>
<th>T. vernicifluum - n = 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficiency (%) Me-TMS</td>
<td>RSD (%) Me-TMS</td>
<td>Efficiency (%) Me-TMS</td>
<td>RSD (%) Me-TMS</td>
</tr>
<tr>
<td>3-Hexylcatechol</td>
<td>100 - 60,12</td>
<td>N.A. - 11,58</td>
<td>100 - 40,37</td>
</tr>
<tr>
<td>3-Heptylcatechol</td>
<td>100 - 14,92</td>
<td>N.A. - 1,01</td>
<td>100 - 21,57</td>
</tr>
<tr>
<td>3-Octylcatechol</td>
<td>N.A. - 33,59</td>
<td>N.A. - 1,34</td>
<td>100 - 26,48</td>
</tr>
<tr>
<td>3-Nonylcatechol</td>
<td>N.A.</td>
<td>N.A.</td>
<td>100 - 10,99</td>
</tr>
<tr>
<td>3-pentadecylcatechol</td>
<td>21,07 - 1,82</td>
<td>10,53-2,50</td>
<td>N.A.</td>
</tr>
<tr>
<td>3-heptadecylcatechol</td>
<td>N.A.</td>
<td>N.A.</td>
<td>100 - 9,17</td>
</tr>
<tr>
<td>3-Phenyldodecylcatechol</td>
<td>96,16 - 8,62</td>
<td>1,06-9,54</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

Fig. 11. The evaluation of carbohydrate peaks based on integrated signals and standard deviation. The results were obtained from G. usitata polymeric samples after in situ silylation-Py-GC-MS.

Fig. 12. Extracted ion chromatograms obtained from a G. usitata polymer sample with HMDS-Py-GC-MS analysis for completely derivatised compounds. The peak labels correspond to the compounds listed in Table 1.

3.3.1. Qualitative interpretation of the HMDS based Py-GC-MS results

In the mass spectra of completely derivatised alkylcatechol, phenylalkylcatechol and acid catechols the m/z 267 fragment ion prevails. This fragment ion comes from cleavage of the alkyl chain at the benzylic position and was determined to be a [bis-(trimethylsilyloxy)-tropylium]⁺ ion [44]. The results show the homologous series of alkylcatechols, propyl- up to nonylcatechol, whereby heptylcatechol was the most abundant compound. Alkylcatechols with side chain lengths of C14 to C17 were also detected. Interestingly next to 3-pentadecylcatechol the 4-pentadecylcatechol isomer was also observed in only HMDS based analyses of the G. usitata polymer. Both the phenyldecyl- and phenyldodecylcatechols were identified with their side chain lengths of C14 and C15, respectively.
chains anchored at the 3 and 4 positions on the catechol ring. Another isomer, phenyldecylresorcinol, was also identified, see Fig. 13. The m/z 180 fragment, ascribable to the radical \([\text{trimethylsilyloxy-tropylium}]+\) ion was obtained after cleavage of the alkylphenol side chains at their nucleus positions. This MS-fragment ion is formed from silylated alkylphenols and phenylalkylphenols, allowing us to identify phenyldecyl- and phenyldodecylphenols \([40]\). Only propylphenol and pentadecylphenol were observed for the alkylphenols while for measurements with TMAH heptadecylphenol could also be detected.

For aged products, 6-(hydroxybenzene)–3-hexanoic acid, 7-(hydroxybenzene)–3-heptanoic acid, 6-(1,2-dihydroxybenzene)–3-hexanoic acid and 8-(1,2-dihydroxybenzene)–3-octanoic acid were detected; 11-phenylundecanoic acid to 13-phenyltridecanoic acid were identified using the characteristic tropylium ion \((m/z\ 91)\); 8-(1,2-dihydroxybenzene)–3-phenyloctan-1-one to 10-(1,2-dihydroxybenzene)-phenyldecy1-1-one were also found. The decyl form showed two isomeric forms with the side chain attached at the 3 or 4 position of the catechol ring. Carboxylated benzenes could not be identified, at least not with more than one carboxylic group attached to the benzene nucleus. By contrast, in analyses using TMAH up to four carboxylic groups...
attached to the benzene nucleus were identified. As measurements with HMDS only showed an overloaded benzoic acid peak, decarboxylation of multикарboxилированных species might be the reason for this. The alternatively possible route through radical oxidation of the phenylalkylcatechol side chains at the benzylic position also seems less plausible, see Supplementary information. The EIC m/z 217 allowed us to visualise the carbohydrate peaks. Series of monosaccharides could be identified where the β-D-galactose anhydro was the most abundant compound. Acid saccharides and dimeric species were also detected at higher retention times in the resulting pyrograms.

3.3.2. Improved derivatisation efficiency with HMDS

Derivatisation efficiency was evaluated based on the integrated signals of pentadecylcatechol and phenylododecylcatechol. The ratios of the completely derivatised compounds versus the sum of incomplete or underderivatised species were assessed using split flows of 27 mL/min and 5 mL/min, see Fig. 14. Note: we also observed, data not shown, that shorter side chain products, up to C7, could be more easily fully derivatised using both methods, while longer side chains showed incomplete derivatisation. The results shown in Fig. 14 illustrate a considerable increase in derivatisation efficiency when a lower split flow was used: for pentadecylcatechol 2% derivatisation efficiency was observed after using a 27 mL/min split flow, while a derivatisation efficiency of 74% was observed using a 5 mL/min split flow. The efficiency for phenylododecylcatechol was 8% using a 27 mL/min split flow, whereas at 5 mL/min it became 78%. This proves that extending derivatisation time, by lowering split flow, allows for a slower post pyrolytic transfer and longer contact time with the HMDS reactant, in this way increasing the derivatisation yield. It is interesting to note that only fully derivatised compounds were sufficiently volatile for GC-MS based isomer identification, see Fig. 13.

Althought the analyses with HMDS were improved by lowering split flow, TMAH gave superior results, easily reaching 90% derivatisation efficiency accompanied with excellent repeatability (RSD <10%). The optimised HMDS methodology showed, on average, integrated signal repeatability of ~15%.

4. Conclusion

We have shown with this study that pyrolysis temperature optimisation is useful to monitor specific molecules or molecular groups when analysing Anacardiaceae polymers. This optimisation has shown to enhance the characterisation of the polymeric compositions. Flash pyrolytic temperature ranges were determined, allowing us to make a distinction between primary pyrolysis reactions and secondary products resulting to form from primary pyrosylates. This greatly benefitted and improved our identification of the macromolecular matrices. Both G. usitata and T. verniciflua polymers showed highly crosslinked matrices, which we could only pyrolyse at very high temperatures, 550–650 °C. The matrices of the G. usitata and T. verniciflua polymers comprise a high amount of crosslinking, mainly due to the incorporation of both the hydroxyl groups of the catechols in the covalent intermolecular bonds. The T. verniciflua polymer showed depolymerisation, which was determined to be an effect of ageing.

Knowledge of these processes, reported here, should prove useful for future analyses of lacquer samples of unknown composition. We suggest that when carrying out analyses on such samples using flash pyrolysis that fixed temperatures anywhere between 480 and 550 °C should be used. In cases where there is still uncertainty, the pyrolysis temperatures can be modified to highlight specific compounds or processes, such as ageing, polymerisation or depolymerisation.

Online derivatisation of the Anacardiaceae polymers was crucial to obtaining reliable, sensitive, and repeatable analyses: TMAH was generally more robust than HMDS, allowing us to obtain better derivatisation efficiencies for most compounds. The derivatisation efficiency, however, depended greatly on the completeness of the pyrolysis step. This means that for incomplete pyrolysis, which was the case for T. verniciflua polymers, even using TMAH, resulted in lower derivatisation efficiencies.

Use of TMAH proved to be detrimental for alkenylcatechols, especially for T. succedaneum polymers, and carbohydrates for all polymeric types, however, selectivity and sensitivity for those molecules was enhanced when HMDS was used. Degradation products comprising more than one derivatisable site, e.g., acid catechols, carboxylated benzenes, were absent in analyses using HMDS. We found that the unfavourable aspects of HMDS could be minimised by lowering the split flow, which extended the derivatisation time and improved derivatisation efficiency. Resorcinol isomer identification in samples from G. usitata polymers became possible using this technique. The optimised conditions reported here ensured a relatively easy and practical solution to the problem of efficient derivatisation of compounds in the lacquer samples, which we found very much improved the analytical results we obtained on samples of Anacardiaceae polymers.

Acknowledgements

The authors would like to express their deep appreciation to Marianne Webb, Michael Schilling and Jing Han at the Getty Conservation Institute (GCI) for their support on the acquisition of the raw materials used to produce the mock-ups and sharing their experience on the artificial light ageing methodology. The authors express their gratitude to Julie Chang for sharing her contacts on Taiwanese lacquer distributors. Suwan Tangmitcharoen and Kaewnapa Kittibanpacha from the Thai Royal Forest Department were of great help in harvesting the Gluta usitata trees and sending the G. usitata lacquer sap, making this research possible. Finally, the authors would like to express their appreciation to their project collaborators Delphine Mesmaeker and Nathalie Vandeperre at the Royal Museums of Art and History (RMAH), Brussels, Belgium for the fruitful collaboration in the Profound study of Hydrous and Solvent Interactions in Cleaning Asian Lacquer project and Louise Decq, Wim Fremout and Alexia Coudray for their unconditional support. This work was made possible by the Belgian Science Policy Office (BELSPO) through grant number: BR/175/A3/PHySICAL (BRAIN-be project).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jaap.2022.105845.

References


