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BRIEF REPORT

Suberin-related Bands Identified with FTIR are Unreliable to Differentiate Neanderthal Tar Production Strategies

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Abstract

The use of adhesives in Middle Pleistocene hominin tool technology represents a significant technological advance. As early as 200,000 years ago, Neanderthals utilized fire to produce birch bark tar. A recent study proposed underground birch bark tar production, identified by FTIR analysis, indicates evidence of Neanderthal cumulative culture. However, we evaluated new FTIR spectra of experimental birch bark tar and found discrepancies in the proposed link between suberin and underground tar production. Our experiments, replicating condensation, pit roll, and raised structure methods, demonstrate the sensitivity of birch bark tar composition to small production variables. Highly similar condensation production experiments yielded tars with varying peak heights associated with SiO^2 and suberin. This can skew results of principal component analyses and shows suberin bands are not unique to tar produced underground. This challenges previous assertions and emphasizes the need to consider production variables and post-depositional influences. While the patterns identified by previous research are intriguing, our study underscores the importance of accounting for biomolecular variability in birch bark tar to securely identify production methods based on FTIR analysis.

Keywords Technological evolution · Middle palaeolithic · Principal component analysis · Ancient biomarker · Birch bark tar · ATR-FTIR

Introduction

The creation and use of adhesives by Middle Pleistocene hominins marked a significant technological development. Adhesives allowed for the application of greater force and facilitated easier handling and precision. This improved the function and efficiency of stone tools, allowing more work to be done more safely and with less effort (Barham, 2013; Niekus et al., 2019). The production of early adhesives probably required a number of traits, such as abstraction, forward planning, and knowledge of resources, that are important in discussions about the cognitive and behavioural capabilities of Neanderthals and Stone Age *Homo sapiens* (Kozowyk et al., 2017; Wadley, 2010; Wragg Sykes, 2015). As early as 200,000 years ago, Neanderthals used fire to produce tar from birch bark (Mazza et al., 2006). The irreversible transformation from papery white bark to viscous black tar, and the use of heat to melt, manipulate, and reuse the final product has been the subject of considerable discussion among Palaeolithic archaeologists (Fajardo et al., 2023; Kozowyk, 2023; Kozowyk et al., 2020; Niekus et al., 2019; Schmidt et al., 2019, 2020, 2022).

A recent study proposed that birch bark tar production represents the strongest evidence of cumulative culture during the European Middle Palaeolithic (Schmidt et al., 2023). The authors argue that because birch bark tar was produced below-ground and out of sight, Neanderthals were unlikely to understand what was happening and must have relied on high-fidelity copying to transmit the knowledge required for the technology. While this hypothesis is intriguing, the link between the archaeological material and below-ground tar production is problematic. In their study, principal component

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analysis (PCA) of Fourier Transform-Infrared Spectroscopy (FTIR) spectra is used to differentiate tar production methods, and they state that suberin is a proxy for underground tar production. To test this claim, and whether the same methodology also works with attenuated total reflectance (ATR) FTIR, we applied their methodology to our own ATR-FTIR spectra of experimental birch bark tar. We conducted additional PCA to further explore how data processing effects the grouping of the results and determine where the variation between production methods lies. We found their claim that suberin is a proxy for below-ground techniques to be problematic, and we briefly discuss potential avenues of research that will improve the validity of future birch tar production identification methods.

Materials and Methods

We reproduced birch bark tar using condensation, pit roll, and raised structure techniques (Fig. 1) as documented in (Kozowyk et al., 2017; Schmidt et al., 2019); images and videos documenting the methods can be found in the respective accompanying supplementary information.

Condensation: A small piece of birch bark is burned beside a vertical or overhanging stone surface, or on top of a flat stone surface. As the bark burns, tar condenses on the stone surface and is scraped off periodically with a flint flake (Schmidt et al., 2019).

Pit roll: A small roll of birch bark and a birch bark cup is placed in a similar sized pit in the ground. Embers are placed over top of the birch bark. Tar forms inside and can be collected from within the remaining bark roll, and from the cup in the bottom of the pit (Kozowyk et al., 2017).

Raised structure: A roll of birch bark is placed on top of a mesh of twigs overlaying a pit containing a birch bark cup (Kozowyk et al., 2017). An earthen mound is built covering everything, and a fire is lit around the mound and allowed to burn for several hours. Tar is collected from within the birch bark cup in the bottom of the pit.

ATR-FTIR spectra of 87 samples (48 condensation, 30 pit roll, 9 raised structure) was collected using a Bruker ALPHA II Compact FT-IR Spectrometer with Platinum ATR accessory and a diamond crystal within a range of 4000–400 cm^{-1}, a spectral resolution of 2, and 16 accumulations. Experimental tar samples are a thick, sticky paste-like consistency and are spread directly onto the ATR crystal and then pressed lightly with the device clamp to ensure uniform and equal pressure between samples. Sample thickness was in the range of several hundred to one thousand µm; orders of magnitude higher than the typical depth of penetration for ATR-FTIR measurements (Kazarian & Chan, 2013).

ATR-FTIR has become a preferred method over transmission FTIR in a wide range of applications, owing to its fast, non-destructive, and easy preparation and analysis time (Lee et al., 2017). ATR-FTIR results in some differences in the raw spectra when compared directly with transmission FTIR using KBr pellets. For example, relative peak intensities of ATR-FTIR spectra differ from non-ATR spectra as peak height tends to increase from left (high wavenumbers) to right (low wavenumbers) (Monnier, 2018). This should be considered when direct comparisons are made between spectra from both methods. However, ATR-FTIR and KBr transmission FTIR do still produce results that can be successfully compared with one another (Dal Sasso et al., 2016; Liu & Kazarian, 2022; Stathopoulou et al., 2008), but see also (Picollo et al., 2014). Although we are focusing on the regions of the spectra described by Schmidt et al. (2023), we are not drawing conclusions based on a direct comparison of peak intensities from their transmission spectra to our ATR-FTIR spectra. Instead, we are focusing on relative differences between tar production methods observed using ATR-FTIR.

Data processing was first done after Schmidt et al. (2023) using Spectragryph v1.2.15 (Friedrich Menges), spectra were baseline corrected and then normalized to the highest and lowest point in the range of 3100–2700 cm^{-1}. However, better results were obtained by applying a standard normal variate (SNV) algorithm to each spectra (Khalighi et al., 2024; Lazzari

Fig. 1 Different birch tar production methods. From left: vertical condensation, horizontal condensation, pit roll, raised structure

et al., 2018; Lee et al., 2017), before calculating first derivatives of each sample within the spectral range of 1800–400 cm^{-1} (giving 679 variables) and exported as csv files. This region was selected to follow Schmidt et al. (2023) as closely as possible and focuses on the fingerprint region. PCA was then calculated using a covariance matrix in OriginPro v9.9.0.225 (OriginLab Corporation). Sixteen of the highest loading variables for PC1 from the score plot were then taken and an additional PCA was conducted using these values to meet a minimum 5:1 sample to variable ratio (Jason & Anna, 2004) to test the validity of using entire spectrum for the initial analyses. PCA was also conducted on first derivatives of each sample in the spectral range 1475–400 cm^{-1} (giving 619 variables), to exclude the bands at approximately 1710 and 1734, which are heavily affected by thermal decomposition (Schmidt & Koch, 2024). Finally, to assess key differences in the primary FTIR spectra, a heatmap was generated using ClustVis Beta (Metsalu & Vilo, 2015) of 14 selected bands related to birch bark tar (1733, 1710, 1641, 1514, 1456, 1376, 728 cm-1; Cîntă-Pînzaru et al., 2012; Schmidt & Koch, 2024; Schmidt et al., 2023) and silica contamination (1172, 1030, 911, 795, 776, 695 cm-1; Vahur et al., 2011). Kaiser-Meyer-Olkin (KMO) scores were calculated using IBM SPSS Statistics (v. 29.0.0.0) to assess sampling adequacy. ChatGPT-3.5 (OpenAI) was used to help generate the abstract.

Results

FTIR spectra from underground pit roll and raised structure tars are in general agreement with previous findings (Schmidt et al., 2023). They tend to have strong peaks at 1734 and 1710 cm^{-1} owing to C=O stretching vibration associated with ester groups in suberin, and the C=O group of free carboxylic acids (Ferreira et al., 2013). In the majority of samples, the peak at 1734 cm^{-1}

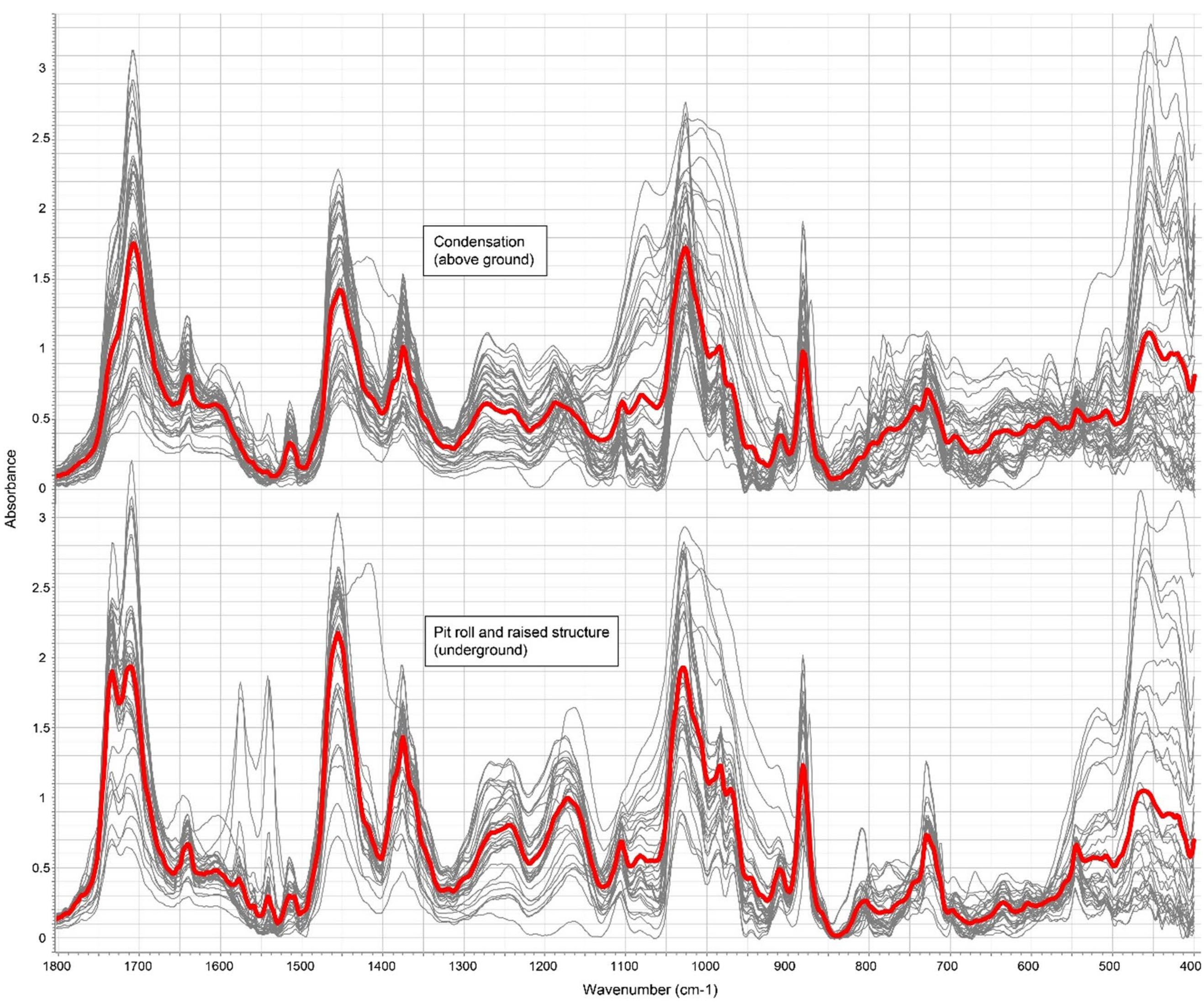

Fig. 2 FTIR spectra of condensation (top) and underground experimental birch tar (bottom) samples. The mean spectra of each are shown in red

is stronger than at 1710 cm^{-1}. However, in 15 out of 39 underground tars, the peak at 1710 cm^{-1} appears stronger than at 1734 cm^{-1} (Fig. 2) suggesting a higher degree of suberin depolymerization (Rizhikovs et al., 2022), and more closely resembles above ground tar. Pit roll and raised structure tars also have identifiable and relatively prominent peaks at approximately 728 cm^{-1} likely corresponding to symmetric and asymmetric C-O and C-H bend associated with vinyl groups (Ferreira et al., 2013), also interpreted as belonging to suberin (Schmidt et al., 2023). In most underground tar samples, peaks attributed to SiO_2 are visible at 795, 777, 695 cm^{-1} (Si-O bending), and a shoulder and weak peak at 1172 and 1081 cm^{-1} (Si-O stretching) (Vahur et al., 2011). However, peaks at approximately 795 and 777 cm^{-1} are absent or very weak in approximately half of the underground samples. Underground tar samples also have a more prominent peak at approximately 804 cm^{-1}, possibly arising from Al–O–Al vibrations, indicating a higher concentration of clays in the contamination (Sontevska et al., 2008), as opposed to silica from the flint in the condensation tars.

FTIR spectra of the condensation tar samples are also in general agreement with previous literature. On average, the 1710 cm^{-1} peak is much stronger than at 1734 cm^{-1}. Only four samples have prominent peaks at 1734 cm^{-1} (Fig. 2), similar to what is seen in below-ground tars. Weak shoulders at 1734 cm^{-1} does not necessarily correspond with weak peaks at 728 cm^{-1}, contrary to what was previously described (Schmidt et al., 2023). All condensation tars have clear peaks likely attributed to SiO_2 at approximately 1081, although this varies significantly in intensity. Only approximately half have clear corresponding SiO_2 peaks at 1164, 795, 777, and 695 cm^{-1} suggesting similar levels of contamination in some condensation tars as those made underground. The relative peak height at 728 cm^{-1} in condensation tars is similar to underground tars. However, the prominence is sometimes masked by peaks on either side. The region between 910 and 650 cm^{-1}, including at approximately 745 cm^{-1}, is characteristic of aromatic compounds (Ku & Mun, 2006; Mouazen et al., 2011; Wang et al., 2023). Al–O–Al vibrations from clay minerals such as muscovite and albite also occur in this region, sharing peak locations at 745 cm^{-1}, as well as SiO_2 peaks at 795 and 777, and 695 cm^{-1} (Martínez Cortizas et al., 2021). The condensation tar was scraped from a quartz river cobble using flint, so contamination from clay minerals with specific peaks at 745 cm^{-1} is unlikely. Despite these differences in this region, more than ten condensation tars show suberin-related bands at 728 of equal or greater intensity than tars made with the pit roll method (Fig. 3). The largest difference on average is the prominence of peaks corresponding to lupenone and betulone at 1734 and 1172 cm^{-1} (Schmidt & Koch, 2024) and the peaks at 1455 and 1375 cm^{-1}, associated with C-H deformation of alkyl groups, present in betulin, betulinic acid, and lupeol (Fig. 3) (Cinta Pinzaru et al., 2012; Tomasini et al., 2012).

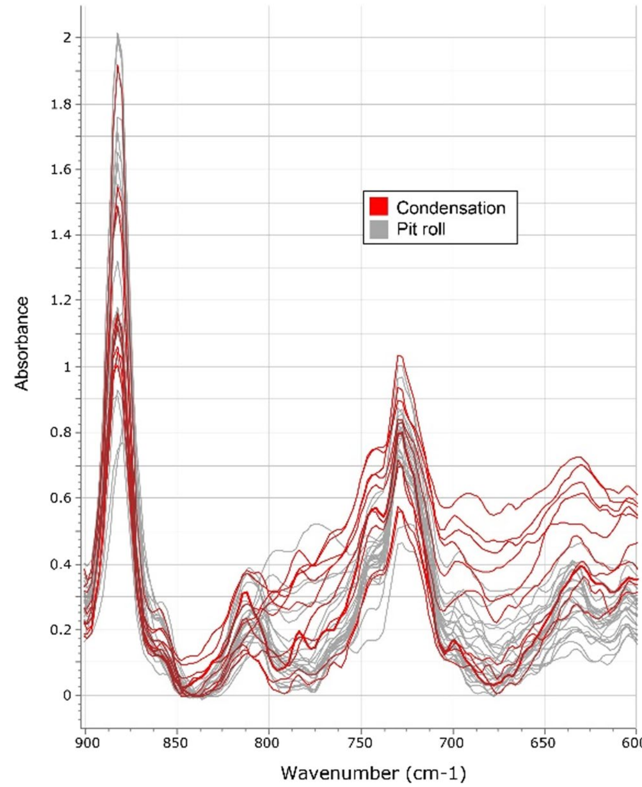

Fig. 3 FTIR spectra of selected ($n=10$) condensation tars (red) with the most prominent peaks at 728 cm^{-1}, compared to pit roll tars (grey) in the spectral region 900–600 cm^{-1}

PCA of the first derivatives in the spectral range 1800–400 cm^{-1} are in agreement with previous research (Schmidt et al., 2023). PC1 and PC2 account for 52.5% and 15.0% of total variance, respectively (Fig. 4a). These fail a KMO measure of sampling adequacy due to the excessively high number of variables producing a matrix that is not positive-definite and do not meet recommended sample to variable ratios for PCA. By selecting the 16 points responsible for the highest loading across the spectra (wavenumbers 1745, 1729, 1714, 1700, 1585, 1535, 1477, 1471, 1444, 1434, 1395, 1049, 1022, 890, 878, 474), the total variance of PC1 and PC2 is increased from 67.5 to 78.2, the KMO score is 0.726, and the overall separation of methods remains (Fig. i4b). The loading plot also shows that the peaks at 1734 and 1710 cm^{-1} are responsible for the largest amount of variance in PC2, which is where the primary separation of production methods appears in the score plot (Fig. 4c). When the region above 1675 cm^{-1} is excluded from the PCA, the separation of methods decreases (Fig. 4d).

Peak intensities, rather than first derivatives, at 14 selected wavenumbers common in archaeological birch bark tar (Vahur et al., 2011) support the general trends seen in the PCA loading and score plots of the first derivatives.

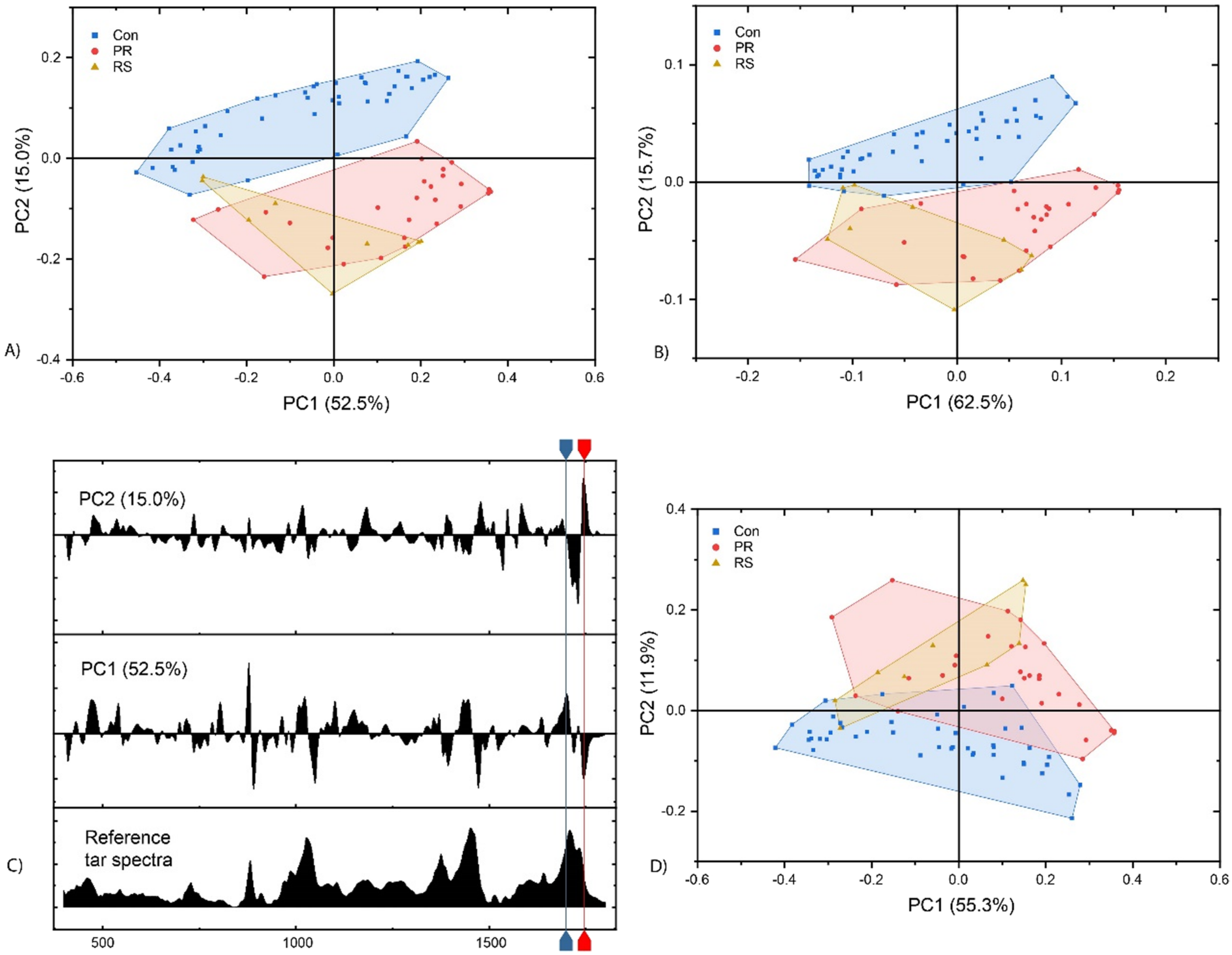

Fig. 4 PCA of experimental birch tar samples. **A** Spectral range 1800–400 cm^{-1}, **B** Spectral values from 16 of the highest loading variables based on the loading plot. **C** Loading plot of PC1 and PC2 compared to a reference condensation tar sample (bottom). **D** Spectral range 1675–400 cm^{-1}. Blue squares indicate condensation tars, red circles indicate pit roll tars, and gold triangles represent raised structure tars

Peaks at wavenumbers 1734, 1456, and 1376 cm^{-1} account for much of the variation directly related to birch bark biomarkers, while peaks at 1172, 1081, 795, 776, 695 cm^{-1} account for differences related to possible SiO_2 contamination (Fig. 5a). PCA of these 14 variables account for 63.2% (PC1) and 17.7% (PC2) of total variance (Fig. 5b) and has a KMO score of 0.689. This method shows only marginal separation, and 1734 and 1710 cm^{-1} are the highest loading variables for PC1, thus contributing again to much of the separation between tars.

Discussion and Conclusion

Previous research has highlighted compositional differences in tars produced by a selection of experimental aceramic techniques. Based on those results, it was suggested that Neanderthals used an underground production process, such as the pit roll or raised structure, to manufacture the birch tar found at Königsaue (Schmidt et al., 2023). Key differences in the FTIR spectra of tars produced via condensation and underground production methods are reported to be the peaks interpreted as suberin at 1734, 1710, and 728 cm^{-1}, as well as contamination possibly arising from the flint scraper (Schmidt et al., 2023). Our results show similar patterns in certain circumstances, yet there remains considerable intra-method variability around these areas, and more work needs to be done to determine if suberin can be used as a marker to differentiate underground production methods.

Our condensation tar samples were only partially distinguishable from underground tars based on suberin and SiO_2 peak intensities. A number of underground tars showed clear peaks associated with SiO_2, and some condensation tars showed very little contamination. As already mentioned

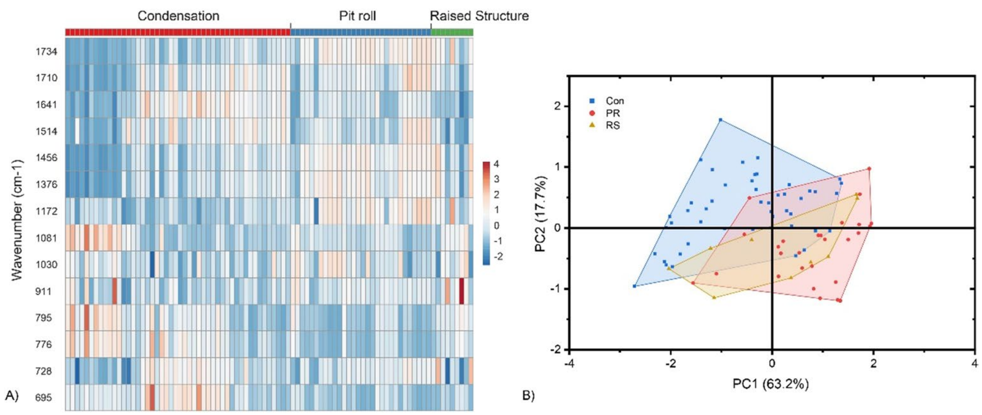

Fig. 5 Heat map and PCA of 14 selected peak heights from FTIR spectra. **A** Primary differences between methods occur in regions associated with degradation products or SiO^2 contamination and are thus unreliable for comparing modern with archaeological materials. **B** PC1 and PC2 of specific peak heights associated with birch tar biomarkers and SiO^2 accounts for 63.2% and 17.7% of total variance, respectively. Marginal separation is visible between condensation and underground tar production methods. Blue squares indicate condensation tars, red circles indicate pit roll tars, and gold triangles represent raised structure tars

(Schmidt et al., 2023), relying on SiO_2 is problematic due to possible contamination from re-use or post-depositional contamination of archaeological samples.

Part of the problem here, however, is that bands in the region of 695–800 cm^{-1} partially obscure the prominence of the suberin band at 728 cm^{-1}. In our experiments, there was not such a clear distinction at 728 cm^{-1} as has been previously reported (Schmidt et al., 2023), particularly in condensation tars that also had lower silica-related peaks. More research should be conducted to determine what causes differences in the suberin peak intensity at 728 cm^{-1}. It could be related to birch bark quality, for example. One study showed that outer birch (*Betula pendula*) bark contains up to 33% by mass of suberin, while inner bark only contains 1.4% (Vedernikov et al., 2011) Other studies suggest approximately 45% in outer bark and 25% in inner bark (Rizikovs et al., 2017). The amount of inner vs. outer bark used, and the suberin content in each, may also be affected by the age of the tree, the season the bark is harvested and whether it is collected from living or dead wood.

Another approach would be to separate the organic from inorganic components and analyse each part independently. Although post-depositional contamination will always be a problem, separating the inorganic content prior to FTIR, and analysing the mineral morphology, grain size, type and ratio separately, may shed more light on how and when the material was added. Which in turn might indicate whether it was part of production or not. For example, silica particles generated by scraping different stone types with flint during collection may be unique in their morphology or composition and be discriminable from other sources. One method would be to embed tar samples into resin to produce thin sections, and compare the mineral particles in tar thin sections with the mineral particles in thin sections of the surrounding sedimentary matrix.

Our PCA only displayed a clear separation between production methods when the peak region 1800–1675 cm^{-1} was included. This is problematic because of the impact heating has on the peaks at 1734 and 1710 cm^{-1}. When tar made with a double-pot process, similar to the raised structure, that had a low 1734:1710 ratio (more similar to condensation tars) was heated for 85 min, the relative peak heights were reversed (Schmidt & Koch, 2024). One reason condensation tars likely have relatively low peaks at 1734 cm^{-1} is because each sample was only produced in a very small quantity over a short period of time. Only enough to conduct the FTIR analysis. It was therefore never exposed to high temperatures for very long. Conducting these experiments for longer or re-using or aging condensation tar may all have an effect on its composition, and thus effect identification using PCA. If this region is removed from the PCA, separation based on production methods becomes more difficult to distinguish. It is unclear what influence this has on the PCA identification of the Königsaue tar done by Schmidt et al. (2023).

In addition to production methods, post-depositional influences also need to be taken into consideration. In another study, modern birch tar reference spectra clustered closer to pine tars and resins than archaeological birch tar, highlighting the potential for burial conditions or aging to affect PCA results of FTIR spectra (Chen et al., 2022a, b). It was suggested that soil contamination in the Königsaue tar is supported by the identification of fatty alcohols C_{28} and C_{30} derived from plant roots (Schmidt et al., 2023). However, this is not considered within the context of their FTIR results. Suberin is a robust macromolecule, and is found primarily in the roots and barks of plants to protect them against water loss and biological attack (A. Chen et al., 2022a, b; Harman-Ware et al., 2021). In some circumstances it is also found in greater relative abundance in deeper peatland soil levels, owing to its robusticity (Balaria & Johnson, 2013; Negassa et al., 2019). Stratigraphic layers above and surrounding the two tar finds from Königsaue consist of peat and gyttja (Koller et al., 2001). It remains untested whether suberin from soil can contaminate archaeological tar in any measurable amount. However, if soil contamination of the Königsaue tar is suggested by the GC-MS results (Schmidt et al., 2023), then the possibility that the suberin detected by FTIR could be a contaminant, rather than a production trace should be considered, if not investigated further. Preservation experiments conducted on reproduced tar buried in different soil types (cf. Langejans, 2010; Monnier & May, 2019) may help determine which FTIR bands are affected by the burial environment.

PCA on full FTIR spectra has been used to successfully differentiate a number of materials (Broderick et al., 2012; Chen et al., 2022a, b; Lazzari et al., 2018). However, it rarely meets recommended sampling adequacy for multivariate or factor analysis (Jason & Anna, 2004). Having 61 samples and 1454 variables (Schmidt et al., 2023) is far from the minimum (3:1) or recommended (5:1, or even 10:1) sample to variable ratio (Cattell, 2012; Jason & Anna, 2004; Velicer & Fava, 1998). The 87 samples and 679 variables presented here also do not meet these criteria. Using an exploratory PCA of the full spectrum and then refining the data based on loadings is one approach to improve the sampling adequacy. Another method is to select peaks with the strongest positive and negative bands in the first derivative spectrum, which yielded similar results (e.g. SI Figure S7; Schmidt et al., 2023). Although there is no definite minimum number of samples required for PCA, more is always better (Jason & Anna, 2004). This is a problem for rare archaeological material, but also for experimental samples which take time to produce. It is therefore paramount that raw data is shared (see Online Resource 1 for our complete spectra), ideally with the same methods of analyses. ATR-FTIR is ideally suited for the analysis of a large number of samples, has already been in a large number of archaeological studies (Chen et al., 2022a, b; Dal Sasso et al., 2016; de Palaminy et al., 2022; Martín Ramos et al., 2018; Martínez Cortizas et al., 2021b; Matheson & McCollum, 2014; Vahur et al., 2011), and reduces the likelihood of having contamination mask important spectral regions. In some cases, archaeological material can also be placed directly on the ATR crystal, making it completely non-destructive and non-invasive. Finally, data processing is still necessary to make the datasets suitable for PCA. This also needs to ensure that variables which are independent of production processes, but that may impact PCA results, are excluded.

The patterns identified by Schmidt et al. (2023) are certainly worth exploring, and clearly show differences in experimental tars based on the production methods they used, some of which are repeated here. However, our results highlight the sensitive nature of the biomolecular make-up of birch bark tar and the wide range of production variables that exist. Small changes to the way that tar is produced, even when using the same general method, can result in considerably different FTIR spectra. In order to securely identify production methods based on FTIR, these variables and taphonomic processes accounting for other molecular pathways need to be accounted for.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s41982-025-00249-8.

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Author Contributions Conceptualization: P.R.B.K. and G.H.J.L. Investigation, Formal analysis, Visualization, Writing – original draft: P.R.B.K. Resources: J.A.P. Writing – review & editing: P.R.B.K., J.A.P., G.H.J.L. Writing – revisions: P.R.B.K. Funding acquisition: G.H.J.L.

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Data Availability All relevant data are supplied in the Supplementary Information file accompanying this manuscript.

Declarations

Competing interests The authors declare no competing interests.

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