Investigating a novel non-destructive identification technique for prehistoric adhesives with dynamic headspace coupled to comprehensive two-dimensional gas chromatography – mass spectrometry

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Abstract

Adhesives were used by prehistoric humans for attaching a handle to a stone tool, to improve tool use. Remains of these adhesives preserve on stone tools until today. Chemical analysis of these residues is essential for an improved understanding of how humans exploited their natural environment, stone tool manufacting and use. However, chemical analysis is not straightforward, the highly degraded residue and the precious artefacts impose limitation. In this study a novel (semi-) non-destructive identification technique for prehistoric hafting adhesives is reported; dynamic headspace sampling coupled to comprehensive two-dimensional GC-MS. The dynamic sampling results in a full characterisation of the volatile profile of the adhesives. A major advantage is that the whole stone tool, with the adhering adhesvie, can be analysed. Moreover, good results are obtained using only slightly elevated temperatures, which avoids heat damage to the stone tools. Nonetheless, the established biomarkers for prehistoric adhesives are not extracted with this method. Therefore, a non-targeted analytical approach combined with multivariate analysis is utilised. In this approach, the chromatogram of a unknown sample is compared to a database of known samples. In this study a start of an adhesive database is made with fourteen different adhesives divided over four adhesive classes. The identification capability of this technique is further evaluated using six experimental stone tools, with different adhesives

adhered to them and subjected to UV-induced degradation. The large stone pieces could not fit in the automated sampling station, thus, a manual sampling set-up was build. It was found that the sampling strategy did not affect the volatiles extracted and that comparison with the database was possible. The tar samples were the least affected and could be easily identified while the resin samples were more degraded and identification was difficult. This technique is promising for non-destructive adhesive identification on prehistoric stone tools.

Keywords

Prehistory, natural substances, multivariate statistics, non-targeted, dynamic headspace, GCxGC

Abbreviations

AG	Animal glue
CIS	cooled injection system
DHS	dynamic headspace
FTIR	Fourier transformation – infrared
FR	Fischer ratio
GC×GC	Comprehensive two-dimensional gas chromatography
HCA	hierarchical clustering analysis
HCMT	hydrocarbon monoterpenoids
HCST	hydrocarbon sesquiterpenoids
HS	headspace
HS-SPME	headspace solid phase microextraction
HCA	hierarchical clustering analysis
LRI	linear retention index
Man-DHS	Manual DHS
MPS-DHS	MultiPurposeSampler DHS
OXMT	oxygenated monoterpenoids
OXST	oxygenated sesquiterpenoids
PCA	principal component analysis
PC	principal component
RF	Random forest
TDU	thermal desorption unit
TOFMS	Time-of-flight mass spectrometry
VOCs	volatile organic compounds

1. Introduction

Adhesives are commonly used in modern societies, but their invention dates back to prehistory, more particularly the Palaeolithic period. In most cases, these adhesives only preserve as small residues on stone tools. The earliest evidence of the use of adhesive dates back approximately 190,000 years before present in what is now Italy [1] and it is, thus, associated with Neanderthals. Evidence suggest that some stone tools only had a protective wrapping made out of adhesives or where hafted in or on a handle manufactured out of wood or other organic material. From about 70,000 years before present, remains of adhesive are more regularly recovered in different areas, associated with both Neanderthals and early modern humans with finds from Europe [2–5], Syria [6,7], and South Africa [8–10]. Prehistoric adhesives can be divided between natural or synthetic adhesives. Natural adhesives are exudates like resins or latex that are directly usable or require little processing. While synthetic adhesives are secondary products following the processing of natural substances, such as in the case of tar made from birch bark. The properties of adheisves can be enhanced with additives such as beeswax, ochre and/or charcoal and variable mixtures have been tested [11,12]. Beeswax may also have been used as an adhesive with addition of charcoal, but there is little evidence [13]. Adhesives have also played an important role in discussion on human evolution in which they have been used as a proxy for complex cognition. This is attributed either to the particular mixture of additives to resins [14–16] or the complexity of the production process as in the case of tar [17,18].

The preservation of adhesives on Palaeolithic stone tools is rare, as the remnants are typically minimal and heavily degraded. While these adhesives were used to glue a stone tool to a handle, it is likely that they were discarded without the handle, because the handle was usually re-used [19,20]. This implies that adhesive remains were left exposed to the elements, which may have favoured degradation. But even in cases where a stone tool would have been discarded within its handle, its organic nature and swift degradation likely offered little protection for the adhesive [21]. Furthermore, certain types of adhesives are more prone to degradation and detachment from stone tools than others [21,22]. The sparse perseverance of the adhesive complicates our understanding of their abundance, manufacturing and use. Moreover, given their rare and precious nature, non-destructive chemical analysis is preferred. However, the severely degraded adhesive pose numerous chemical challenges. Spectroscopy techniques such as (microscopy-) Fourier transformation infrared spectroscopy ((μ-)FTIR) and Raman spectroscopy are (semi) non-destructive but often fail to provide robust data to characterise degraded adhesives. The sample is often too degraded for the specific spectral features to be preserved [23] or directly

analysis on the stone tool is complicated due to the spectral interference of the stone surface [24]. Gas chromatography coupled with mass spectrometry (GC-MS) provides the most accurate chemical data, even for severely degraded adhesives [25] . However, this method is destructive as it requires the adhesive to be extracted from the stone tool and involves an extensive sample preparation. Such a procedure is undesirable from a curation perspective. As a result, GC-MS is only employed in rare cases or for more recent time periods where adhesives are more abundant (e.g., Neolithic period). Hence, there is a clear need for a non-destructive and accurate analytical technique for prehistoric adhesives.

Headspace (HS) analysis of the volatile organic compounds (VOCs) released by adhesives might offer a promising alternative, as it omits direct sample contact and laborious sample preparation. HS-solid phase microextraction GC-MS (HS-SPME-GC-MS) is gaining popularity in cultural heritage research [26–28], but it has seen limited usage within the context of prehistoric (hafting) adhesives [29–31]. Two main reasons can be identified. Firstly, the established biomarkers used for identification with GC-MS are semi-volatile [32] and would require high temperatures to be extracted with HS-SPME, potentially causing visual damage to the stone tool [33]. Secondly, numerous VOCs are trapped with HS-SPME, resulting in very complex chromatograms and good separation is difficult to obtain with GC-MS [29,34]. In recent research the HS-SPME approach is coupled to a comprehensive two-dimensional GC-MS (GC×GC-MS) instrumentation and with extraction temperatures well below the melting point of the adhesives [33,34]. The benefits of GC×GC over one-dimensional GC are the substantial increase in peak capacity and sensitivity and is therefore ideal for the separation of complex samples, such as HS extraction of odorous samples [35–39]. Moreover, in GC×GC analytes with similar chemical properties are eluting in a specific region of the chromatogram which creates a highly structured chromatogram that facilitates in compound identification [40,41]. Even though HS-SPME is a versatile, sensitive and user-friendly sampling technique for VOCs, it is a static extraction and an equilibrium will be established between the fibre and the sample. Furthermore, the polymer coating on the fibre is very thin. Taken together, the HS-SPME extraction is limited by the partition coefficient of the analyte and the coating volume [42]. Active sampling techniques like dynamic headspace (DHS), actively remove the VOCs by purging gas through the HS and are concentrated on a larger volume sorbent, resulting in an exhaustive extraction. After the extraction, the trapped VOCs are injected into a GC by thermal desorption. Coupling of DHS with GC×GC-TOF-MS results in a powerful analytical tool of the whole HS of a sample [37,43].

For the data analysis of VOCs profiles from prehistoric adhesives, targeted approaches based on biomarkers have proven their limitations [29,34]. This is because standardised methods and

validated annotations are usually missing. A non-targeted approach is therefore more suitable [39]. In non-targeted analysis the full chromatogram is analysed and the obtained peak table is subjected to advanced statistical analysis such as principal component analysis (PCA), hierarchical clustering analysis (HCA) and random forest (RF) to identify certain trends in the data [44,45]. These approaches are useful when comparing data sets (good vs. bad) [46] or to classify between different samples [41,47,48]. Unknown samples can be classified by comparing the unknown sample with a collection of known and classified samples e.g., a database [49]. However, the creation of a representative database is time-intensive work, as it requires high-quality chromatograms of numerous samples to cover a broad range of possibilities for the unknown sample. For natural adhesives sources are relatively abundant in nature and vary depending on the geographical region. In this study, the first steps of creating such a database are made, focussing on adhesives available in Western-Europe. After the compilation of this database, the sampling and identification abilities of the DHS-GC×GC-TOFMS method is assessed with experimental samples, from a previous study [50]. In that study, adhesives were deposited on top of the stone tools and subjected exposed to UV-light irradiation to induce degradation.

2. Materials and Methods

2.1. Samples

For the database, fourteen different adhesives from four different classes (resins,including a resin:beeswax mixture; birch tars; animal glues; beeswax) were collected and prepared in-house. The full details of the different samples (e.g., plant and animal species) of the adhesives are listed in Table S1, see the support information. Furthermore, the birch tars were obtained from three different Palaeolithic production methods (raised structure (RS), condensation method (CM), cobble grove method (CG) Lokker et al., in preparation). These adhesives are the most predominant ones found in hafting technologies at Palaeolithic European sites. For each adhesive, three samples of 5 mg were weighted and placed into a 20 mL (22 mm x 75 mm; neck 18 mm) screw-cap headspace vial with a 1.3 mm PFTE septa (Restek® Corp., Bellefonte, PA, USA). For the animal glues, 10 mg of dried flakes were weighted and left overnight with 5 µL MilliQ water, to form a gluey substance. For the case study, six experimental stone tools were analysed (Table S2, SI), which were prepared in a previous study; on each tool a droplet of adhesives was placed and artificially degraded under a UV lamp [50].

2.2. Chemicals

For system suitability testing, the cannabis terpene mix B standard (2000 µg/mL in methanol, Supelco, Merck Life Science BV/SRL, Overijse, Belgium) was used for regular monitoring. The standard was diluted to 20 ppm in methanol (≥ 99.8%, HPLC grade, Fisher scientific, Loughborough, UK) and 2 µL were pipetted into a 20 mL screw-cap headspace vial (see Fig. S1 for the resulted QC chart). For the linear retention index (LRI), a n-alkane standard solution (C7-C30, Sigma Aldrich, Saint louis, USA, 1000 ppm) was diluted with methanol to 20 ppm and 2 µL were pipetted inside a 20 mL vial.

2.3. Instrumentation

2.3.1. Dynamic headspace extraction

For the database acquisition the DHS extraction was automated using a MultiPurposeSampler (MPS, Gerstel K.K, Mülheim an der Ruhr, Germany) which was connected to the GC system, hereafter referred to as MPS-DHS. In Table 1 the extraction parameters are listed of all DHS analysis. The MPS-DHS parameters were optimised via a full factorial design in a previous study [51]. The parameters of the man-DHS were based on the MPS parameters and thus not further optimised. The trap temperature was set to 30°C and the needle temperature to 120°C. The VOCs were trapped on a Tenax TA tube. For the experimental pieces which were too big to fit in a 20-mL vial, a manual set-up was made which uses a bigger jar, hereafter referred to as man-DHS. The jar was closed with a modified lid, in which two holes were drilled. A plastic sheet was placed between the lid and the jar to ensure airtight sealing. A needle was inserted through each hole and through the plastic. One needle was connected to a N_2 bottle and the other to the TD tube. After the TD tube a flow meter and a pump were installed, connected by tubing, to guarantee a stable N_2 flow. Furthermore, the needle which purged the N_2 was placed lower in the jar than the needle connected to the TD tube. This set-up tried to accurately mimic MPS-DHS, only the trap and needle temperature could not be accurately conditioned. The trap was at room temperature and the needle had the same temperature as the inside jar. The other parameters are displayed in Table 1 and Fig. S2, SI depicts the set-up.

Table 1. The extraction parameters used for MPS-DHS and man-DHS.

Parameter	**MPS-DHS**	**411&413**	**410&409**	**408&412**
Incubation time	20 min	30 min	30 min	30 min
Incubation T	50°C	50°C	50°C	50°C
V vial	20 mL	365 mL	232 mL	232 mL

V purge	450 mL	12600 mL	8000 mL	12000 mL
F purge	22.5 mL.min^{-1}	80 mL.min^{-1}	80 mL.min^{-1}	100 mL.min^{-1}

The trapped VOCs were released inside the GC system via a thermal desorption unit (TDU, Gerstel K.K.) connected to a cooled injection system (CIS, Gerstel K.K.). The tubes were heated inside the TDU from 40°C to 280°C at 300°C.min^{-1} and held isothermal for 3 min, the CIS was held at -20°C during desorption. After the TDU desorption, the CIS was rapidly heated to 250°C at 12°C.s^{-1}. The TDU was operated in splitless mode while the CIS split ratio varied between 5 to 20 depending on the VOCs profile of the samples (see Table S1). After each injection the TD tube was conditioned inside the TDU injector at 300°C for 15 min.

2.3.2. Comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry (GC×GC-TOFMS) analysis

All measurements were conducted on a Pegasus GC-4D (LECO® Corp., St. Joseph, MI, USA) GC×GC-TOFMS equipped with a secondary oven and a quad-jet, dual stage thermal modulator. The 1D column was a semi-polar Rxi-624silMS (Restek® Corp.) (30 m x 0.25 mm i.d 1.4 µm df) and the 2D column was a polar Stabilwax (Restek® Corp.) (2 m x 0.25 mm i.d 0.5 µm df), connected to each other with a SilTite µ-union (Trajan Scientific and Medical®, Australia). The initial oven temperature was set at 40°C for 3 min, after which it was ramped to 240°C at 5°C.min^{-1}. The final temperature was held for 2 min. The secondary oven off-set was +5°C in relation to the 1D oven, while the modulator offset was +15°C in relation to the 2D oven. The modulation period was 4 s with a 0.60 s hot pulse time. The flowrate was 1 mL.min^{-1} during the run and the carrier gas was high purity helium (Alphagaz 2, Air Liquide®, Liège, Belgium). A blank was measured at the beginning, after every tenth injection and at the end. The terpenoid standard was measured at the beginning, after the fifteenth injection and end of the sequence. Due to the solid nature of the samples an internal standard could not be added. The terpenoid standard serves therefore also as an external standard.

The temperature of the MS transfer line was 250°C and the ion source was kept at 230°C. The electron ionisation energy was 70 eV. The acquisition mass range was 35 – 550 amu, the acquisition rate was 200 Hz, and the acquisition delay was 300 s. Before the start of each sequence, a mass calibration and tuning were conducted with perfluorotributylamine.

2.4. Data treatment

Data acquisition and analysis were performed with ChromaTOF® (LECO® Corp., St. Joseph, Michigan, USA, v 4.72). The data processing parameters were as follows: baseline offset was 1 (just above the noise), 1D peak width value was 20 s and the 2D value was 0.25 s. A S/N ratio of 50 (expect for birch tar RS 6 the S/N ratio was 100) was used and the area was calculated using a quantitative mass. The database chromatograms were aligned with the Statistical Compare feature of ChromaTOF® (LECO® Corp.). Samples were divided into two classes, being sample and blank, and the chromatograms were aligned under the following criteria: a minimal spectral match of 600 and 1 modulation period difference, a minimum of 3 samples must contain the analyte or minimal 50% of the samples in a class had the analyte. If there was no match in the first peak finding the S/N ratio was reduced to 20. A tentative forward mass library search was performed using the NIST 2017 library, and a tentative name was assigned when the similarity was above 700. Subsequently, the Fischer ratio (FR) was calculated, all analytes with a FR > FR_{crit} or which were undefined, were kept and used for generating the peak table. This approach was already found successful in defining which analytes were present only in the samples and are thus discriminatory [52]. The resulted peak table was exported to Excel. Here, the column bleed and contaminations were removed, and the alignment was checked with the individual processed chromatograms with ChromaTOF®. The analytes were divided in ten different groups (Table 2) and the total area per group was calculated. The experimental peak tables were processed with the same parameters, however, the peak tables were manually matched with the database and where necessary the quantitative mass was recalculated. The resulted peak tables were uploaded to MetaboAnalyst6.0 [53] for multivariate analysis. Here, the peak tables were normalised by sum and auto scaled before the principal component analysis (PCA) and a hierarchical clustering analysis (HCA) were calculated.

For both the database construction and the identification of the experimental adhesives a non-targeted approach based on total peak area per chemical group was adopted for several reasons. First, the lack of LRI for this column set and the similarities between the MS spectra of most compounds complicated the unambiguous assignment of a molecule to each chromatographic peak. However, the advantage of GC×GC-TOFMS is that analytes with similar chemical properties are eluting in a valuable structured and interpretable pattern, often forming distinct bands (or tiles) within the chromatographic space. This unique property allows fast localisation and assignment of a given chemical to a specific chemical group type, e.g. monoterpenoids [41]. Second, the use of total peak area per chemical class minimised the variability between the samples. The natural origin of the samples complicated the achievement of a good RSD for the

individual peaks between the triplicates, even the homogenisation of the sample did not improve (only done for the mix sample). The RSD of the total peak area per chemical class of the replicates varied mostly between 20% - 30%, indicating that acceptable precision levels were obtained when taking the total peak area into account. The RSD of the system was in the similar range as calculated with injection of a terpenes standard (see QC chart Fig. S1). Third, a chemical class is generally better persevered than individual molecules in degraded samples and the chemical deformation pathways are not always known. This is especially helpful when analysing archaeological artefacts, as each sample undergoes a unique degradation pathway depending on the usage during its lifetime and the burial environment.

3. Results and Discussion

3.1. Database adhesives

Fig. 1, a representative chromatogram for each of the adhesive classes is shown, the extraction method resulted in sufficient capturing of VOCs for each class. Beeswax displayed the lowest overall volatility with a total peak area around 10^6 and the birch tar samples were the most volatile with a total peak area between 10^8 – 10^9. This trend was also seen in previous research on similar adhesives analysed with HS-SPME, thus DHS demonstrates similar trapping affinities [33,34].

Furthermore, the non-standard column set resulted in good peak shapes for every peak regardless its polarity. Natural substances like adhesives cover a wide range of polarities, and the exact composition is not known. Moreover, the chemical complexity of the birch tar samples complicated full separation in spite of the enhanced peak capacity and spectral deconvolution offered by GC×GC-TOFMS. The latter characteristics are major advantages and strongly advocate the utilitsation of GC×GC-TOFMS for adhesive characterisation. The structured separation obtained on the chromatograms allow to directly identify trends based on the chemical families present in the sample, even before one dives deeper into the full sample characterisation. For example, Fig. 1C demonstrates sub-groups of terpenoids and sequiterspenoids that is highly specific to resin-based adhesives. Based on this observation, a group-type analysis using chemical families was applied for data processing. This approach has the capacity to be more resilient to single compounds variation, usually observed in (bio)marker approaches.

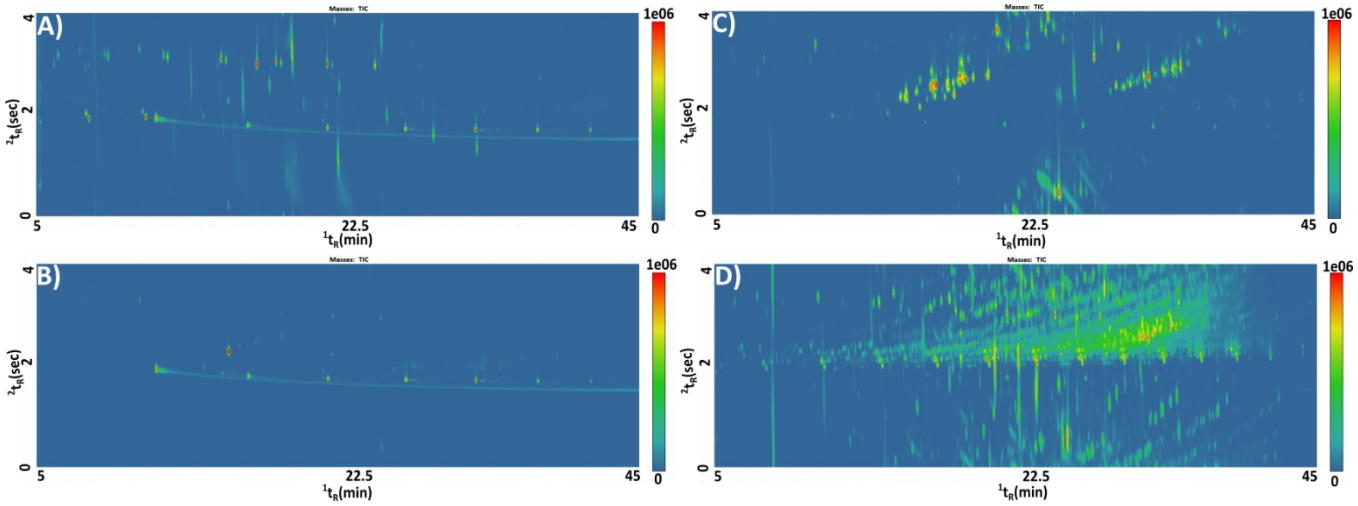

Fig. 1. A chromatogram from a representative sample for each adhesive class A) AG; hide glue (sheep), B) beeswax, C) pine resin; *Picea abies*, D) birch tar; RS6.

The normalised total peak area per chemical class (Table 2) was used to perform multivariate analysis which resulted in a heat map (Fig. 2) and a PCA (Fig. 3). In the heat map, each adhesive of a class is clustered closely together with peers while there is a strong separation between the adhesive classes. The resins are the most distinct in comparison to the other samples, being the only group with an excess in mono- and sesquiterpenoids, as already identified visually in Fig. 1. Also, the mixed sample is firmly placed in the resin branch, meaning that both the heating during adhesive preparation and the addition of beeswax did not severely alter the chemical fingerprint of the resin. This finding is in contrast with earlier research of HS-SPME analysis on adhesives by Cnuts et al., [33], where a separation between a mixed sample and the pure resin was seen in the calculated PCA. However, the different tree species used for the preparation of the mixed sample and the different approach to calculate the PCA might have resulted in a different result (Fig. 2 in [33]). The tars are characterised with an excess of aromatic compounds, hydrocarbons and some sesquiterpenoids. Beeswax mostly contained hydrocarbons, while animal glues (AG) are characterised by the abundance of hetero atoms such as oxygen, nitrogen and sulphur. Even though the main constituents of AG and the beeswax are not volatile, they still present a good VOC profile coming from minor compounds. The PCA, accounting for 70% of the variance over PC1 and PC2, reveals four well-separated clusters corresponding to the adhesive classes, with low intra-class variability (Fig. 3). The same behaviour as in the heat map is seen, with the resins, tars and AGs far apart from each other while the beeswax is placed between the tars and AGs group. By analysing each cluster, it can be seen that the PCA displays also the natural variability within each sample type. For example, in the tar group one sample, RS6, stands out and upon analysing of the chromatogram, its VOC profile contains more VOCs than in the case of the other

three tars. This might indicate that the VOC profile for each tar will slightly change depending on the production environment. Nevertheless, it still clusters within the 95% confidence intervals inside the tar group.

Table 2. Overview of the ten groups in which the analytes of the peak tables were divided.

#	Description	Name
1	Hydrocarbons	Hydrocarbons
2	Hydrocarbons with a nitrogen or sulphur containing functional group	N/S-HCs
3	Hydrocarbons with a oxygen containing functional group	O-HCs
4	Heterocyclic, aromatic	Heterocyclics
5	Hydrocarbon monoterpenoid	HCMTs
6	Oxygenated monoterpenoid	OXMTs
7	Hydrocarbon sesquiterpenoid	HCSTs
8	Oxygenated sesquiterpenoid	OXSTs
9	Aromatic compounds with a functional group	Fun-aromatics
10	Aromatic compounds with alkyl substituent	Alkyl-aromatics

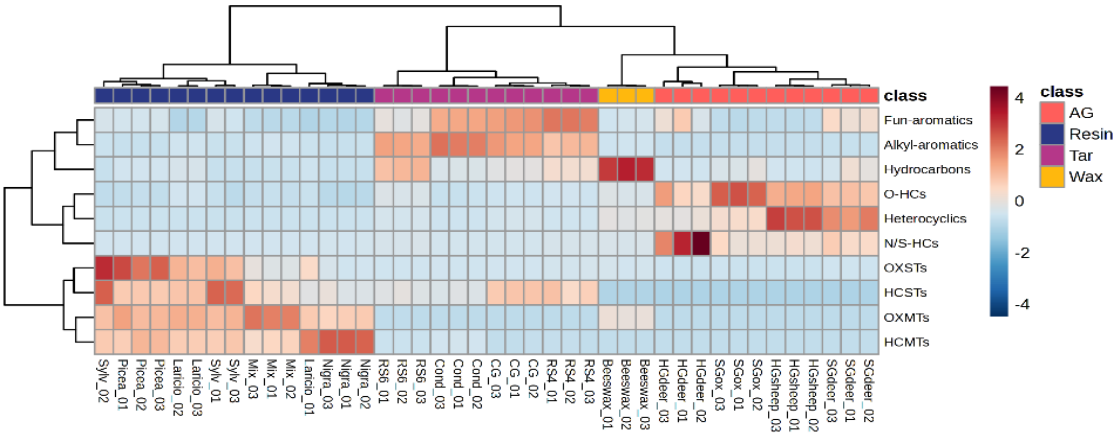

Fig. 2. Hierarchical clustering analysis of the adhesive samples, each adhesive class isforming its own branch in the dendrogram while the samples from the same class cluster together.

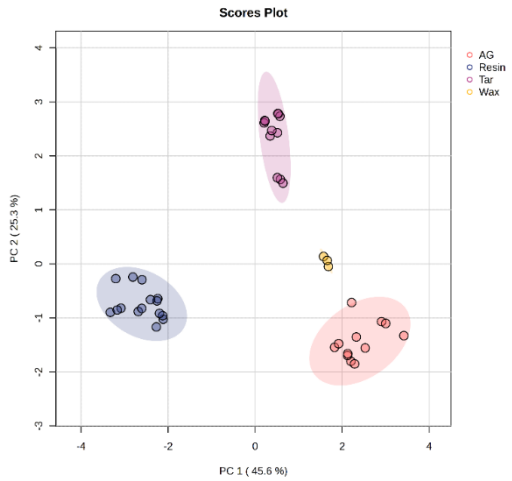

Fig. 3. The PCA of the adhesive samples, each class groups together in a different space of the PCA, PC1 and PC2 are accounting for over 70%.

3.2. Adhesive identification on experimental stone tools

Six experimental stone tools with adhesives were analysed to assess the identification potential of the current database with unsupervised multivariate statistics. The experimental samples were artificially degraded with UV-light in a previous study [50]. While both surfaces of the stone tools contained a few droplets of adhesive, only one surface was exposed to UV-light. Prior to the analyses the stone tools have been stored in closed zip-locked bags for 2 years. Due to the size of the tools, it was not possible to place them in a 20-mL vial used for the MPS-DHS. Therefore, a man-DHS set-up was made with a jar large enough to fit the pieces (Fig. S2).

The obtained chromatograms (Fig. S3-S5) were similar as with MPS-DHS and were,consequently, treated in the same way. The annotated peaks were divided between the ten classes (Table 2) and the total peak area was calculated. This peak table was then combined with the database peak table and used to build a PCA (Fig. 4A). The PCA clearly demonstrates that the experimental samples occupy the same multivariate space as the samples incorporated in the database. This result is an important confirmation of the stability and reliability of the two protocols (Fig. 4A). Indeed, the man-DHS sampling technique did not detrimentally change the VOC profile and reliable comparison with the reference database was therefore possible. Moreover, the special position of the degraded samples provides a first insight for the study of decomposition pathways by combining score and loading plots of the PCA (Fig. 4B).

On the PCA, it can be observed that UV-light degradation influenced the chemical composition of the samples and complicates their identification. The tar samples were the least affected by the degradation. One sample (413) was placed inside the 95% confidence interval of the tar group and the other one (412) right next to the group. The resin:beeswax samples (410 & 411) were more altered by the artificial degradation, however, both were still closer to the resin group than the other groups in the PCA. Therefore, it is possible to conclude that the adhesive is likely a resin with a certain level of confidence, again addition of beeswax was not possible to conclude based on this analysis method. The resin samples (408 & 409) were the most affected by the exposure to UV-light, making it impossible to unambiguously assign them to a specific group in the PCA. However, the accompanying biplot of the PCA (Fig. 4B) aids in understanding the factors that influence the placement of samples within the PCA space. This information is useful to identify differences between the samples and to aid in understanding how the exposure to UV-light altered the VOC profile. It is seen that for the four resins samples the degradation resulted in

more hydrocarbons and aromatic compounds and in less terpenoids. Moreover, there seems to be more sesquiterpenoid present than monoterpenoids. This trend is expected as the most volatile compounds are the first to be removed from the sample (Fig. 4).

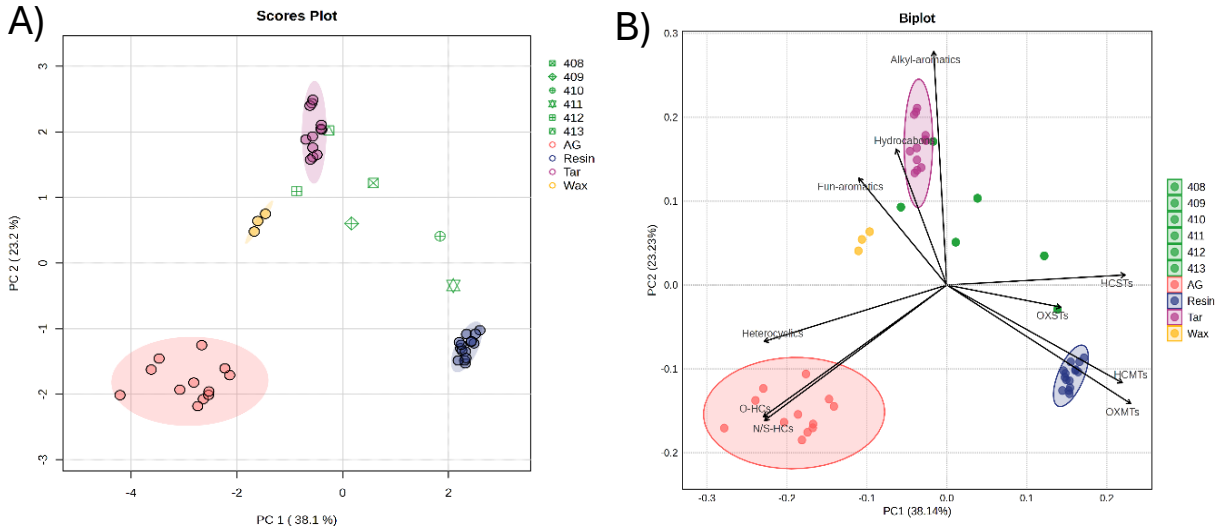

Fig. 4. A) PCA plot of the database adhesives including the experimental samples, note that the resin and AG groups have switched in this PCA compared to Fig. 3, B) the biplot based on the PCA, placement of the samples are the same as in A).

The results of the chemical identification of the experimental samples are also confirmed by the visible observation of the adhesives. Indeed, the tar samples were the best preserved and the distinct tar smell was still slightly present. Moreover, the tar was still sticky in the zip lock bag and also against the walls of the sampling jar. The beeswax in the mixed samples also helped to keep the gluing properties of the resin. The adhesive was not completely dry out and was still adhered to the stone surface. This is probably thanks to the plasticizer properties of the beeswax. By contrast, the pure resin completely dried out and became brittle with minimal adhesion remaining between the adhesive and the stone surface. As a result, hardly any resin was preserved on the stone tools surface and what was left simply fell off the stone following minimal handling. The good preservation of birch tar and resin:beeswax mixtures compared to pure resin has also been observered in another degradation study in which stone tools were buried for 3 years [21]. Consequently, these observations permit to argue for an important bias in survival potential for certain adhesive types in comparison to others, as shown by both taphonomic (burial) degradation and UV-light degradation[54,55]. While this study permitted to touch upon the problem, it provides a basis for more detailed studies of the degradation pathway in the future.

The major advantage of DHS-GC×GC-TOFMS is the ease and the non-destructive character of the sampling. Moreover, in previous research on these adhesives it was found that DHS extraction doubled the total peak area of the VOCs extracted in comparison with HS-SPME. This was especially seen for the polar and least volatile chemical classes (oxygenated mono- and sesquiterpenoids) [51]. Furthermore, headspace sampling preserves the residue on the stone tools which allows further analysis such as (μ-)FTIR spectroscopy or GC-MS [33]. A downside of DHS sampling of the whole stone tool is that the method is not selective for a specific residue on the stone tool. This means that when there are several residues on the stone tool, and perhaps also modern contamination, the DHS captures the VOCs coming from the other residues and the contamination. The experimental tools were cleaned before the artificial degradation experiments, where only handled with gloves and were placed in individual zip-lock bags directly after investigation [50]. As a consequence, contamination from on these tools can be ruled out. For archaeological artefacts good practises recommended that the pieces have been collected in a controlled manner and that the analysis is conducted soon after the excavation [29,34]. As in most cases direct analysis is not possible, efforts are made to prevent contaminations from the environment. These efforts are often based on personal habits and practices at the level of staffs responsible for the storage. It is well known that these practices are very often not ideal and can easily result in the introduction of external VOCs. We are working on the implementation of specific practices for pieces devoted to VOC analyses, but at this stage, we, and any analytical scientist who want to consider VOC analyses on artefacts, has to accept with the risk of being reporting VOCs that might lack of specificity and introduce variations between studies. This is well known in the field and will hopefully get better in the future as VOC analyses will become more accepted.

5. Conclusions

The DHS-GC×GC-TOFMS analysis combined with multivariate statistics demonstrates to be a powerful and non-destructive chemical identification approach for prehistoric hafting adhesives. This technique proves to be valuable because it provides orthogonal information that complements data from standard microscopy and spectroscopy techniques.

The first step of this research involved the development of a database. For this, fourteen different adhesives were divided into four classes (resins, tars, animal glues and waxes), which were analysed with DHS-GC×GC-TOFMS. The VOC profiles between the adhesive types was distinctive enough to be able to obtain a good separation with a PCA. The second step was to test the database against experimental tools which underwent UV-light degradation [50]. The size of

these tools did not permit MPS-DHS sampling and a man-DHS set-up was made. The PCA approach was also used for the identification of these samples. The tar samples could be identified with confidence. The beeswax:resin mix samples were relatively well preserved and could also be identified. The pure resin samples were severely altered due to the exposure of UV-light and it proved complicated to draw firm conclusions from their PCA positions in relation to pure resin samples in the database. More studies on the degradation of adhesives as well as on how taphonomy impacts their survival rate would provide useful insights beneficial for better understanding what to expect for archaeological hafting adhesives.

A subsequent phase of this methodological development involves an application to archaeological artefacts. To investigate whether the approach is sufficiently sensitive for the heavily degraded and minimal surviving traces found on archaeological artefacts. In addition, further efforts will need to be invested to expand the database towards other species and geographical regions. In particular samples from South Africa would be useful given on-going debates on how adhesive production and use may be a relevant proxy for early human behaviour and its complexity.

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Conflict of Interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

The data that support the findings of this study are openly available in Zenodo at 10.5281/zenodo.16742850 and 10.5281/zenodo.16745774

Authors' contribution statement

Anika Lokker: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Visualization, Writing – Original Draft **Pierre-Hugues Stefanuto**: Conceptualization, Methodology, Supervision, Writing – Review & Editing **Dries Cnuts**: Supervision, Writing – Review & Editing **Veerle Rots:** Conceptualization, Data curation, Funding Acquisition, Resources,

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