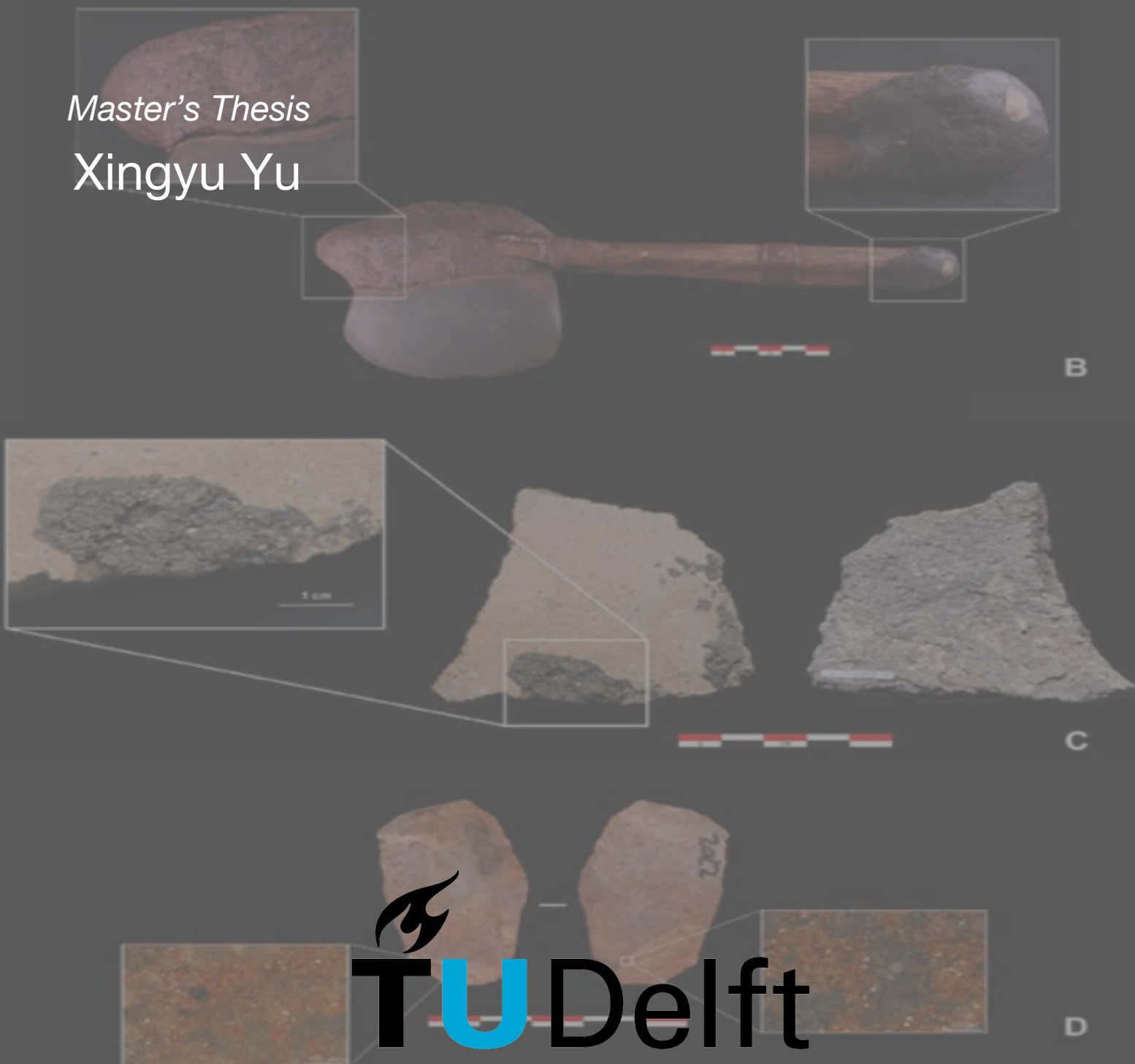


# Using Machine Learning to Study Archaeological Adhesives and Their Production Process

*Master's Thesis*  
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# **Using Machine Learning to Study Archaeological Adhesives and Their Production Process**

by  
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Cover: picture of ancient adhesives taken by Dr. GHJ (Geeske) Langejans (Langejans et al., 2022)



## **Abstract**

Adhesives have played a vital role throughout human history. Studying their composition and production methods offers insight into past technologies and helps reconstruct historical practices. This study focuses on the materials science analysis of *Betula* sp. (birch) bark tar, a widely used adhesive in prehistory times. By examining its molecular composition and production techniques, this research seeks to replicate ancient manufacturing methods using experimentally produced samples.

In this study, Gas Chromatography-Mass Spectrometry (GC/MS) was employed to analyze the chemical composition of the adhesives. To classify different production methods, machine learning techniques—including Principal Component Analysis (PCA), Linear Discriminant Analysis (LDA), and K-Nearest Neighbors (KNN)—were applied. The results indicate that LDA successfully differentiates between production techniques, suggesting its potential for identifying variations in tar preparation. However, since this study is based on experimentally produced samples, its application to archaeological specimens requires further validation.

**Keywords:** archaeological adhesives, GC/MS, machine learning, birch bark tar

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## 1. Introduction: Background of Archaeological Adhesives

Adhesives are substances used to bond materials by forming connections between surfaces, typically through molecular interactions, electrostatic forces, or mechanical interlocking (Pizzi & Mitta, 2003). Their composition varies depending on their intended function but generally consists of three primary components: a base, additives, and solvents. The base provides the fundamental bonding strength and can be either natural or synthetic, including epoxies, polyurethanes, and silicones (Pizzi & Mitta, 2003). Additives, such as fillers, plasticizers, and stabilizers, enhance properties like flexibility, durability, and resistance (Kinloch, 1987). Solvents modify viscosity and facilitate application, evaporating after use to leave a solid bond (Skeist, 1977).

Several key adhesion mechanisms can explain how adhesives form bonds. Mechanical interlocking occurs when an adhesive penetrates surface irregularities and hardens, physically locking the surfaces together, making it particularly effective for porous materials (Pizzi & Mitta, 2003; Kinloch, 1987). Diffusion theory suggests that adhesive molecules infiltrate the substrate, forming an interwoven polymer network that strengthens adhesion (Von Fraunhofer, 2012). Chemical bonding involves covalent, ionic, or hydrogen bonds forming between the adhesive and substrate, increasing bond durability (Kendall, 1994). Adsorption theory describes adhesion through weak physical forces, such as Van der Waals interactions and hydrogen bonding, which create an initial but strong adhesive-substrate interface (Gent et al., 1990; Sharpe & Schonhorn, 1964; Wypych, 2023).

The use of adhesives by humans dates back at least 200,000 years (Mazza et al., 2006). Across cultures and historical periods, various natural materials—including bitumen, starches, natural rubbers, plant resins, and tars derived from bark and wood—served as adhesives (Langejans et al., 2022). Archaeological adhesive residues are typically found as traces on tools and ceramics or as free lumps within sediment layers (Regert et al., 2004). Ancient adhesives existed in diverse forms, such as liquids, pastes, and solids (Pizzi & Mitta, 2003), and were used for a wide range of applications, including hafting stone tools and securing construction materials. These adhesives exhibited properties such as bonding strength, plasticity, and water resistance. Some were also employed for waterproofing, sealing, and artistic applications, including inlays, paints, varnishes, and inks.

In archaeology, the study of adhesives provides valuable information about past technologies, economic systems, and natural resource utilization. The molecular composition of adhesives reflects the technical knowledge of ancient societies and their ability to modify materials for specific purposes (Regert et al., 2004; Rageot et al., 2016; Langejans et al., 2022; Chasan et al., 2024). Additionally, adhesives can offer evidence of trade networks, mobility patterns, and labor organization in prehistoric societies (Langejans et al., 2022).



Figure 1. Five chipped stone points for darts with visible ancient adhesive residue. (Helwig et al., 2014, Figure 1)

This study focuses on *Betula* sp. (birch) bark tar, one of the most commonly used adhesives in prehistorical times. Birch bark tar has been extensively studied due to its strong adhesive properties and frequent use in tool hafting and repair. Following previous studies, this research employs Gas Chromatography-Mass Spectrometry (GC/MS) to analyze the chemical composition of experimental tar samples, as GC/MS is one of the most efficient techniques for characterizing archaeological adhesives.

However, manual interpretation of GC/MS data is time-consuming and prone to error. Recent advances in machine learning provide new opportunities to improve the efficiency and accuracy of GC/MS data analysis. By integrating machine learning models, this study aims to develop a more automated and reliable method for identifying adhesive components, potentially assisting future researchers in reconstructing ancient adhesive technologies.

However, manually interpreting GC-MS data is both time-consuming and prone to error. Recent advances in machine learning provide new opportunities to improve the efficiency and accuracy of GC-MS data analysis. By integrating machine learning models, this study aims to develop a more automated and reliable method for identifying adhesive components, potentially assisting future researchers in reconstructing ancient adhesive technologies.

This thesis has two primary objectives. First, it aims to identify key molecular markers in GC-MS analyses of archaeological adhesives by reviewing and synthesizing existing literature. Second, it seeks to develop machine learning techniques to enhance GC-MS data interpretation. The application of machine learning in archaeological GC-MS analysis remains in its early stages. By exploring its potential, this study aims to bridge the gap between materials science, computational analysis, and archaeological research.

## **2. Prehistoric Aceramic Production Techniques of Birch Bark Tar**

The aceramic (without ceramics) production methods used by European Neanderthals to produce birch bark tar provide valuable insights into the technological and behavioral complexity of prehistoric societies. However, reconstructing these methods remains challenging, as the exact techniques employed by Neanderthals are still unknown. Several hypotheses have been proposed, and this study focuses on four primary techniques: ash mound, condensation, pit roll, and raised structure. Experimental samples were produced using these methods, as illustrated in Figure 2 (Kozowyk et al., 2023, Figure 1).

### **Ash Mound Method**

In this technique, a roll of raw birch bark is buried beneath hot ashes and embers. The residual heat facilitates tar production within the bark roll, which is then excavated and unrolled after 30 to 40 minutes. The tar is scraped and collected using a flint flake or wooden stick. This process occurs in a low-oxygen environment, as both the ashes and the bark itself limit air exposure, preventing excessive combustion (Rageot et al., 2019; Kozowyk et al., 2023; Chasan et al., 2024).

### **Condensation Method**

This method involves burning small amounts of birch bark near a vertical stone surface, allowing the rising smoke to deposit tar on the stone. The tar is then periodically scraped off with a flint flake. Unlike other methods, this process takes place in an open-air environment, making it the least comparable to ceramic tar production techniques. Due to continuous oxygen exposure, tar yield is significantly lower, as much of the organic material is lost through combustion before condensation occurs (Rageot et al., 2019; Kozowyk et al., 2023; Chasan et al., 2024).

### **Pit Roll Method**

In this method, a small pit (~10 cm deep, 7 cm in diameter) is dug, with a birch bark cup placed at the bottom. A tightly rolled piece of raw birch bark is positioned inside, and hot embers are layered on top. As the bark heats, tar drips into the cup while additional residue can be scraped from the bark roll. This method is an intermediate method between single-pot and double-pot ceramic tar production techniques. It allows for semi-controlled oxygen exclusion and efficient tar collection (Rageot et al., 2019; Kozowyk et al., 2023; Chasan et al., 2024).

### **Raised Structure Method**

This method involves digging a small pit (~10 cm × 10 cm) where a birch bark cup is placed at the bottom. Above the pit, a screen of twigs and pebbles are placed to support a roll of birch bark. The entire system is enclosed within a mud dome, with a fire around the structure for several hours. As the bark undergoes pyrolysis, tar drips into the cup, which enables clean collection. This technique resembles a Palaeolithic precursor to the double-pot process. It offers better oxygen control and efficient tar production (Rageot et al., 2019; Kozowyk et al., 2023; Chasan et al., 2024).

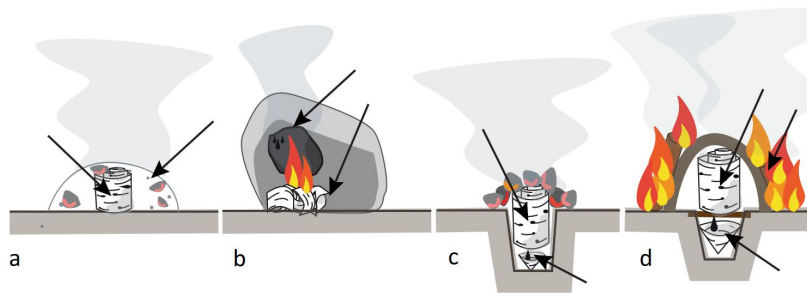


Figure 2. Ceramic birch bark tar production techniques: (a) ash mound (b) condensation, (c) pit roll, (d) raised structure. (Kozowyk et al., 2023, Figure 1).

Each of these techniques varies in key production factors, including oxygen exposure, heating temperature, heating duration, and tar collection method. These variables influence the yield, quality, and chemical composition of the resulting tar.

Oxygen exposure plays a crucial role in pyrolysis efficiency. In low-oxygen environments, such as the pit roll method, tar yield is maximized, as combustion is minimized, allowing the formation of long-chain hydrocarbons (Kozowyk et al., 2023). The raised structure method, which permits moderate oxygen exposure, enables efficient tar production but requires careful monitoring to prevent excessive oxidation. The ash mound method offers limited oxygen control due to its semi-closed environment, sometimes resulting in tar degradation. In contrast, the condensation method exposes the bark to high oxygen levels, causing substantial material loss through combustion before condensation occurs, leading to the lowest tar yield (Rageot et al., 2019). Overall, reduced oxygen exposure enhances tar yield and preserves essential adhesive compounds.

Temperature is another critical factor influencing pyrolysis efficiency. Studies have shown that the optimal temperature range for birch bark tar production is 350–400°C, which enables organic material breakdown while preventing excessive volatilization or combustion (Rageot et al., 2019; Koch et al., 2024; Chasan et al., 2024). Lower temperatures, as seen in the condensation method, lead to incomplete pyrolysis, producing low tar yields with high moisture content. Conversely, temperatures exceeding ~450°C, such as in certain ash mound or raised structure setups, cause tar degradation, leading to the formation of byproducts or complete combustion. The insulating effect of soil and embers in pit roll methods helps maintain a stable temperature, ensuring high-quality tar production.

Heating duration affects both tar yield and quality. Shorter heating times, as seen in the condensation method, result in incomplete pyrolysis, producing low yields with high impurity levels (Rageot et al., 2019). Moderate heating durations, such as in the ash mound and raised structure techniques, lasting several hours, facilitate gradual organic breakdown, yielding higher-quality tar. The pit roll method, which employs prolonged heating under oxygen-restricted conditions, tends to maximize yield by ensuring complete pyrolysis (Chasan et al., 2024). However, extending heating beyond 12–24 hours does not necessarily enhance tar production and may even cause chemical degradation.

The method of tar collection also influences its usability. In the pit roll method, tar accumulates inside both the bark roll and the cup, minimizing loss and simplifying extraction (Kozowyk et al., 2023). The raised structure method allows tar to drip directly into a collection cup, reducing contamination. The ash mound technique, however, presents challenges as tar mixes with ash. Additional purification might be needed. The condensation method is the least efficient, as it involves significant tar loss due to vaporization.

The success of birch bark tar production can also be analyzed at a molecular level, as the decomposition of key organic components (cellulose, hemicellulose, and lignin) varies based on production conditions.

In oxygen-limited environments, such as those in the pit roll and ash mound methods, anaerobic pyrolysis preserves long-chain hydrocarbons and phenolic compounds, such as guaiacol and catechol, which are essential for the adhesive properties of birch tar (Kozowyk et al., 2023). In contrast, high-oxygen exposure, as in the condensation method, promotes oxidative degradation, breaking down organic molecules into CO<sub>2</sub>, CO, and water vapor, which reduces tar yield and alters its chemical composition (Rageot et al., 2019).



Temperature also determines the final chemical composition. At lower temperatures (~250–300°C), incomplete pyrolysis produces low-molecular-weight aldehydes and acetic acid instead of stable hydrocarbons (Chasan et al., 2024). Within the optimal range (350–400°C), lignin decomposes into aromatic hydrocarbons and phenols, forming a viscous, durable tar. Beyond 450°C, desirable compounds degrade into carbonized residues and gases, compromising adhesive quality (Kozowyk et al., 2023).

Heating duration further affects tar consistency. Short heating times yield a high-moisture, low-viscosity product, whereas prolonged heating (12–24 hours) promotes polymer cross-linking, increasing brittleness and reducing adhesion (Rageot et al., 2019).

Finally, tar collection methods influence its chemical purity. Freely dripping tar, as in the pit roll and raised structure techniques, ensures minimal contamination. In contrast, the ash mound method introduces mineral residues, potentially altering pH and reducing adhesive stability (Chasan et al., 2024).

### **3. Characterization Techniques of Archaeological Adhesives**

The chemical analysis of archaeological adhesives requires an understanding of compound aging and comparisons with reference materials (Bonaduce et al., 2017). Over the past few decades, various analytical techniques have been developed to identify and characterize these adhesives. This chapter discusses the most commonly employed methods.

#### **3.1 Macroscopic and Microscopic Observation**

Preliminary analyses of archaeological adhesives often rely on visual and microscopic examination. Observations made with the naked eye or optical microscopes provide information about the general characteristics of ancient adhesives. For example, prehistoric birch bark tar typically appears as a black or brown amorphous organic material, whereas well-preserved plant resins often exhibit a yellow or orange coloration with a glossy surface (Regert, 2004). Optical microscopy enables the examination of morphological features such as surface texture and internal structure, aiding in the classification of adhesives (Ribechini et al., 2009; Aleo et al., 2024).

In some studies, specialized optical microscopy techniques have been employed for more detailed characterization. Polarized light microscopy (PLM), for instance, has been used to analyze small fragments of pigmented adhesives, allowing researchers to distinguish particles based on their size, shape, color, and birefringence (Helwig et al., 2014).

While macroscopic and microscopic observations can help with categorization of adhesives based on their morphological properties, they do not provide molecular-level information. Thus these techniques are insufficient for identifying specific chemical compounds and must be complemented by advanced analytical methods.

#### **3.2 Spectroscopy**

Spectroscopic techniques analyze the interaction between radiation and materials, providing information in elemental composition and chemical bonding. Since each molecule exhibits a unique spectral fingerprint, spectroscopy is particularly useful for identifying adhesives, which are typically complex mixtures.

Fourier-transform infrared spectroscopy (FTIR) is widely employed to identify both organic and inorganic components in adhesives with minimal sample preparation (Ribechini et al., 2009; Helwig et al., 2014; Kaal et al., 2020; Despotopoulou et al., 2024; Aleo et al., 2024). FTIR has proven effective in distinguishing chemically similar adhesives, such as differentiating between spruce resin and spruce callus resin, by comparing spectral data to reference databases (Helwig et al., 2014).

Other spectroscopic techniques, including scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDS), Raman spectroscopy, and X-ray diffraction (XRD), further contribute to adhesive characterization. SEM-EDS facilitates the elemental analysis of small adhesive fragments containing both organic and inorganic materials (Helwig et al., 2014; Despotopoulou et al., 2024). Raman spectroscopy is particularly useful for identifying inorganic components by matching spectral data to reference materials (Helwig et al., 2014). XRD provides detailed mineralogical information on adhesive residues, offering additional insights into their composition (Aleo et al., 2024; Despotopoulou et al., 2024).

Spectroscopic methods offer significant advantages, including molecular-level identification and non-destructive analysis, making them particularly valuable for studying both organic and inorganic materials (Artoli, 2010). However, they are less effective for analyzing complex mixtures, as differentiating individual components from background noise can be challenging. Consequently, spectroscopy is best employed as a complementary technique alongside gas chromatography-mass spectrometry (GC/MS) for a more comprehensive analysis.

### 3.3 Gas Chromatography-Mass Spectrometry (GC/MS) Techniques

Gas Chromatography-Mass Spectrometry (GC/MS) is one of the most widely used techniques for identifying organic compounds in archaeological samples. Its primary advantage lies in its ability to determine the molecular composition of adhesives, as well as assessing their state of preservation and degradation (Andreotti, 2006; Ribechini et al., 2009).

As illustrated in Figure 3 (Emwas et al., 2015, Figure 1), GC/MS integrates two analytical methods. Gas chromatography (GC) separates individual components within a mixture. The sample is vaporized and carried through a capillary column coated with a stationary phase, where compounds are separated based on their interactions with the column material. A carrier gas, such as helium or nitrogen, facilitates the movement of these compounds through the system (Karasek & Clement, 2012; Santos & Galceran, 2003). Each component elutes at a specific retention time (RT)—the time required for it to pass through the column—producing distinct peaks on a gas chromatogram (Hites, 1997).

Mass spectrometry (MS) then identifies each separated compound based on its mass-to-charge ratio ( $m/z$ ). As the compounds enter the mass spectrometer, they are ionized into fragments, which are subsequently sorted and detected. The resulting mass spectrum is unique to each molecule, allowing for precise identification by comparing spectral data with reference libraries such as National Institute of Standards and Technology (NIST) or laboratory-prepared standards (Stashenko & Martínez, 2014).

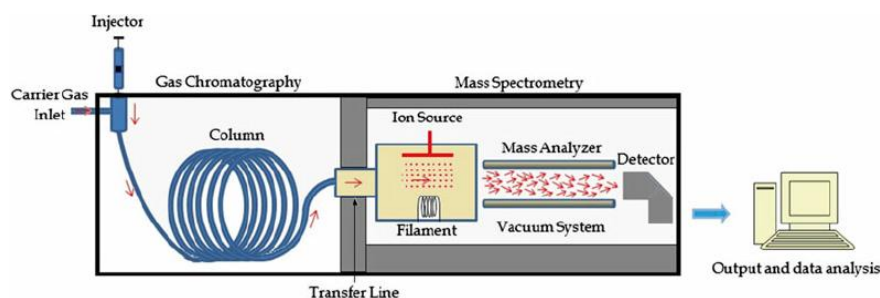


Figure 3. Schematic representation of the main components of a GC-MS instrument (Emwas et al., 2015, Figure 1).

Several factors influence retention time in GC/MS analysis, including the properties of the stationary phase, column thickness, carrier gas flow rate, and temperature settings (Sparkman et al., 2011). Higher temperatures generally decrease retention times by increasing compound volatility, while variations in column polarity affect how strongly a compound interacts with the stationary phase (Hubschmann, 2015). To ensure reproducibility and comparability across studies, standardized GC/MS methodologies are commonly applied in archaeological research (Evershed et al., 1990).

#### 3.3.1 Advantages and Limitations of GC/MS

GC/MS is particularly effective for analyzing complex mixtures of organic adhesives. It allows for the identification of multiple compounds within a single sample, provides semi-quantitative data on relative component proportions, and detects molecular markers characteristic of specific tars (Ribechini et al., 2009; Egenberg et al., 2003; Regert, 2004). In some studies, normalized peak areas have been employed to estimate the relative proportions of different adhesive components, such as conifer pitch and beeswax (Ribechini et al., 2009).

Despite these advantages, GC/MS has several limitations. It is a destructive technique and cannot be performed *in situ*. Additionally, sample preparation is time-consuming, requiring extensive wet chemical pre-treatment before analysis (Ribechini et al., 2009). Furthermore, the quantitative accuracy of GC/MS depends on peak area measurements, which may introduce errors if calibration curves are not properly applied (Bonaduce et al., 2017). Lastly, GC/MS is not well-suited for analyzing non-volatile or thermally labile compounds, as these substances may degrade under standard operating conditions (Sparkman et al., 2011).

### 3.3.2 Supplementary GC/MS-Based Techniques

To address the limitations of conventional GC/MS, several advanced GC/MS-based techniques have been developed.

Pyrolysis-GC/MS (Py-GC/MS) rapidly heats samples to 500–1000°C, breaking down complex molecules into smaller fragments for analysis. This method is particularly useful for identifying non-volatile compounds and materials that are unsuitable for conventional GC/MS (Wampler, 2011; Hubschmann, 2015).

Gas chromatography–isotope ratio mass spectrometry (GC/IRMS) measures stable isotope ratios to determine the origin of organic materials. It has been applied to archaeological adhesives to analyze lipid sources and differentiate between natural and synthetic resins (Bonaduce et al., 2017).

Tandem mass spectrometry (GC/MS/MS) enhances compound differentiation by fragmenting molecules further during analysis. This technique increases sensitivity and selectivity, enabling the detection of trace compounds in complex samples (Long et al., 2019).

Direct Inlet Electron Ionization-MS (DI EI-MS) requires minimal sample preparation, making it particularly valuable for analyzing small or rare archaeological samples (Regert & Rolando, 2002). This method has been employed to detect high-molecular-weight compounds, such as wax fatty esters and triacylglycerols, providing initial insights into adhesive composition before more detailed GC/MS analysis (Rageot et al., 2016).

### 3.4 GC/MS Analysis of Archaeological Adhesives

Archaeological adhesives are complex mixtures containing molecules of varying molecular weights, many of which remain unidentified. Given this complexity, GC/MS is among the most effective techniques for characterizing these materials. It enables researchers to analyze molecular composition, trace degradation pathways, and assess preservation conditions (Bonaduce et al., 2017).

The application of GC/MS in archaeological science was first conducted in 1985, when Evershed et al. used it to investigate the origins of pitch recovered from the *Mary Rose* shipwreck (Evershed et al., 1985). Throughout the late 1980s, its use in archaeology remained limited, primarily for identifying pure substances such as adhesives and sealants (Beck et al., 1989; Heron et al., 1989). A major advancement occurred in 1990, when Evershed et al. introduced high-temperature GC/MS for analyzing complex mixtures in archaeological residues, leading to the establishment of standardized methodologies for ensuring consistency across studies (Evershed et al., 1990). Around the same time, Mills and White conducted pioneering research on the application of GC/MS for identifying organic materials in archaeological contexts (Mills & White, 1994).

Since 2000, GC/MS-based techniques have become widely adopted for identifying organic components in ancient adhesives (Bonaduce et al., 2017). In 2004, Regert et al. applied GC/MS to analyze adhesives preserved under different environmental conditions, adapting GC methodologies to suit the specificity of archaeological materials. By identifying key molecular markers, Regert demonstrated that birch bark tar was widely used during the Neolithic period, providing evidence for variations in tar production techniques (Regert, 2004).

Regert's work laid the foundation for subsequent research in archaeological adhesive analysis. Later studies have further refined GC/MS applications, particularly in differentiating between various birch bark tar production methods (Regert, 2004; Rageot et al., 2019; Kozowyk et al., 2023; Schmidt et al., 2023; Chasan et al., 2024; Koch et al., 2024).

#### 3.4.1 Sample Preparation for GC/MS Analysis

Archaeological adhesive samples typically contain both volatile and non-volatile compounds, with many adhesive components exhibiting low volatility due to their polarity. To address this, chemical or thermal treatments are applied following extraction. While thermal treatments, such as pyrolysis, are occasionally used, wet-chemical derivatization remains the standard for GC/MS analysis. Trimethylsilylation is particularly favored due to its efficiency, speed, and simplicity, as it produces stable, volatile derivatives that improve chromatographic separation (Regert, 2004).

For instance, in Regert's study, ancient adhesive samples were finely ground before undergoing extraction with dichloromethane. The resulting extracts were then subjected to trimethylsilylation, a

derivatization process that enhances volatility and reduces polarity. This was achieved using BSTFA (bis(trimethylsilyl)trifluoroacetamide), a widely adopted protocol in subsequent research (Bonaduce, 2004; Ribechini et al., 2009; Rageot et al., 2016; Helwig et al., 2014; Kaal et al., 2020).

In some cases, the lipid fraction of adhesives is hydrolyzed prior to derivatization to allow for a more comprehensive lipid analysis using an apolar GC column (Stevanato, 1993; Mills, 1994). However, hydrolysis can lead to the loss of structural information from high-molecular-weight compounds, potentially limiting interpretations regarding the adhesive's origin and degradation state (Regert, 2004). To mitigate these issues, researchers have employed high-temperature capillary columns with temperature programs ranging from 50°C to 350°C, thereby avoiding the need for hydrolysis in some cases (Evershed et al., 2002; Regert, 2001, 2004; Charters, 1995; Rageot et al., 2016).

The choice of sample preparation protocol directly impacts the accuracy and reliability of GC/MS results. Researchers must carefully balance the need for enhanced volatility and sensitivity against the risk of losing critical structural information, selecting the most appropriate method based on sample composition and analytical objectives.

### 3.4.2 GC/MS Instrument Setup

Regert (2004) optimized a GC temperature program ranging from 50°C to 350°C with a helium flow rate of 2–6 mL/min for analyzing complex mixtures containing both low- and high-molecular-weight compounds. The study recommended a rapid initial temperature increase of 10°C per minute from 50°C to 150°C, followed by a slower ramp rate of 4°C per minute up to 320°C, with an isothermal hold at 320°C for 20 minutes. This approach proved particularly effective for identifying di- and triterpenoid derivatives in archaeological adhesives. The protocol has since been widely adopted in the field, with studies such as those by Rageot et al. (2016), Ribechini et al. (2009), and Helwig et al. (2014) applying similar methodologies.

Helium is the most commonly used carrier gas in GC/MS analysis, as noted by Regert (2004), Bonaduce and Colombini (2004), Ribechini et al. (2009), and Rageot et al. (2016). Flow rates, however, vary depending on the study objectives. Lower flow rates, such as 1.0 mL/min used by Bonaduce and Colombini (2004) and 1.2 mL/min used by Ribechini et al. (2009), provide better resolution and more stable baselines, though they result in longer analysis times. In contrast, higher flow rates, such as the 2–6 mL/min range recommended by Regert (2004), reduce analysis time but may compromise resolution due to increased peak overlap.

For mass spectrometry detection, Regert et al. (2004) employed electron ionization (EI) mode at 70 eV, using a full scan range of either 50–650 m/z with a 0.75-second scan time or 50–800 m/z with a 0.68-second scan time. This configuration proved effective for identifying high-molecular-weight constituents, with the ion source temperature set to 180°C. Later studies followed similar MS settings, typically using EI at 70 eV and scan ranges between 50–1000 m/z, as demonstrated by Ribechini et al. (2009), Bonaduce and Colombini (2004), and Rageot et al. (2016).

These optimized GC/MS instrument settings have contributed to highly accurate adhesive characterizations, allowing researchers to distinguish molecular markers and degradation products in archaeological samples.

### 3.4.3 MS Identification

Mass spectral identification in GC/MS analysis relies on comparing sample data with reference libraries such as National Institute of Standards and Technology (NIST) or with commercially available standards, including Sigma B9757, Sigma L5632, and Extrasynthèse 0031S (Regert, 2004). This research focuses specifically on birch bark tar, which can be identified based on the mass spectra of its triterpenoid derivatives.

By matching the mass spectra of archaeological samples with these known standards, researchers can determine the chemical composition of adhesives. This technique has proven useful in distinguishing between different tar production techniques and in assessing the degradation processes that ancient adhesives have undergone over time. The ability to identify specific molecular markers provides valuable information about the technological practices of past societies and the environmental conditions that influenced adhesive preservation.

## 4. Molecular Markers

Identifying ancient adhesive samples presents challenges due to alterations in their chemical composition over time. These changes occur both during the production process and as a result of post-depositional degradation. Destructive distillation, a common method for producing adhesives, alters the chemical profile of the material, making it distinct from the raw birch bark. Additionally, environmental factors, including chemical and bacterial interactions in sediments, further modify the composition (Regert, 2004).

Molecular markers help overcome these challenges by providing distinct chemical signatures associated with specific raw materials or production techniques (Evershed, 1993; Smith, 2014; Rageot et al., 2019; Kozowyk et al., 2023; Chasan et al., 2024). Despite these chemical transformations, certain molecular markers remain detectable, offering important information for material identification. These markers have three key roles: (1) linking molecular composition to the original raw materials, (2) distinguishing adhesives derived from different sources, and (3) providing insights into both production techniques and degradation processes (Regert, 2004).

### 4.1 Molecular Markers in Birch Bark Tar: Biomarkers and Degradation Markers

The lupane family, which includes lupenone, lupeol, betulone, betulin, and betulinic acid, is naturally present in birch bark and serves as a primary set of molecular markers for birch bark tar (Regert, 2004; Rageot et al., 2019; Chasan, 2024). Regert et al. identified a characteristic combination of these five triterpenoids as definitive markers for birch bark tar (Regert, 2004). However, subsequent studies have not always detected all five markers, likely due to chemical degradation or variations in production conditions (Rageot et al., 2019; Kozowyk et al., 2023; Chasan et al., 2024).

During the pyrolysis process used in birch bark tar production, some molecules within the lupane family undergo chemical transformation. For instance, betulin can convert into lupa-2,20(29)-dien-28-ol, and lupeol can transform into lupa-2,20(29)-diene. The presence of these compounds signals exposure to heat, making them useful markers for pyrolytic treatment (Regert, 2004; Rageot et al., 2019; Kozowyk et al., 2023; Chasan et al., 2024). However, the origin of allobetul-2-ene remains debated. While Regert (2004) attributed it to natural degradation, Rageot et al. (2019) and Chasan et al. (2024) associated it with heating processes.

Given the complexity of molecular markers in birch bark tar, classification is beneficial. Different studies have categorized these markers in various ways. Regert (2004) initially proposed the following classification, shown in Figure 4:

**Biomarkers** – Indicate the botanical source of the material.

**Transformation markers** – Represent chemical changes from the original biomarkers.

**Degradation markers** – Reflect natural decay over time.

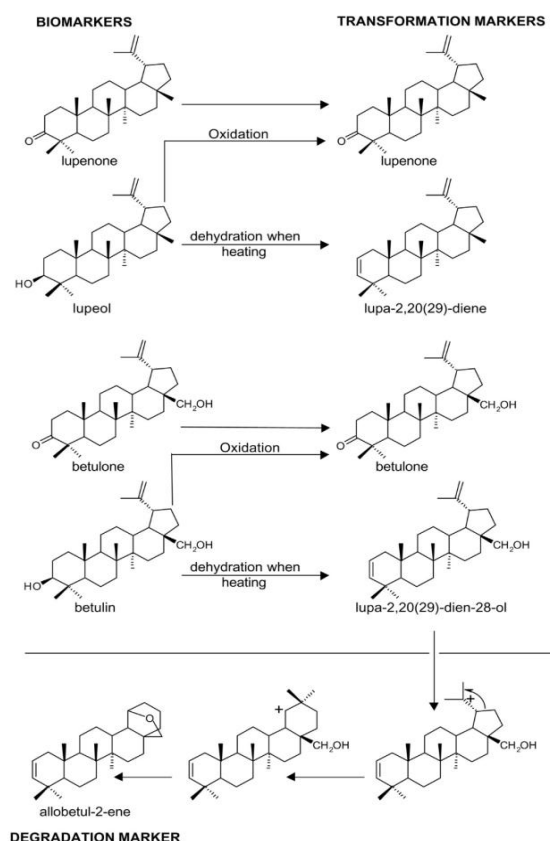


Figure 4. Proposed transformation mechanisms of biomarkers identified in birch bark tar (Regert, 2004; Figure 3).

However, most contemporary research combines transformation markers and degradation markers into a single category. This unified classification is more practical, as heat-induced transformations are a form of degradation (Rageot et al., 2016; Rageot et al., 2019; Niekus et al., 2019). Moreover, some researchers, including Kozowyk et al. (2023), consider breakdown products of betulin and lupeol as biomarkers rather than degradation markers.

Following the framework proposed by Rageot et al. (2019) and other recent studies, this research categorizes molecular markers into two primary groups:

**Biomarkers** – Compounds naturally present in birch bark.

**Degradation markers** – Compounds generated from biomarkers through heating or aging.

Degradation markers can be further divided into soft heating markers and strong heating markers, as classified by Rageot et al. (2019) and Chasan et al. (2024). An overview of these molecular markers is provided in Table 1.

Table 1. Molecular markers of birch bark tar (Rageot et al. 2019, Chasan et al. 2024)

| Biomarkers     | Degradation markers             |   |   |
|----------------|---------------------------------|---|---|
|                | Soft heating markers            | Strong heating markers                          |   |
|                |                                 | Double degraded pentacyclic triterpenoids (DPT) | Hydrocarbon pentacyclic triterpenoids (HPT) |
| Lupeol         | Lupa-2,20(29)-diene             | $\alpha$ -Allobetulin                           | $\alpha$ -Lupane (B337)                     |
| Erythrodiol    | $\alpha$ -Betuline I            | Allobetul-2-ene                                 | $\alpha$ -Lupane (B360)                     |
| Betulin        | Lupa-2,20(29)-dien-28-ol (B395) | 3-Oxoallobetulane                               |   |
| Betulinic acid | Lupa-2,20(29)-dien-28-ol (B396) |   |   |
|                | Olean-2,11-dien-28-oic acid     |   |   |
|                | Betulone                        |   |   |
|                | Allobetulin                     |   |   |
|                | Betulinic, 28-acetate           |   |   |

Soft heating markers result from mild pyrolysis, typically at temperatures below 400°C (Rageot et al., 2019; Chasan et al., 2024). These compounds are primarily influenced by the duration of heating rather than fluctuations in temperature. Some of these markers, such as lupa-2,20(29)-diene, betulone, and lupa-2,20(29)-dien-28-ol, can also form through natural degradation processes (Aveling & Heron, 1998; Rageot et al., 2019; Chasan et al., 2024; Koch et al., 2024).

In contrast, strong heating markers are indicative of prolonged heating or rapid temperature increases exceeding 400°C (Rageot et al., 2019; Chasan et al., 2024). These compounds are categorized into double-degraded pentacyclic triterpenoids (DPT) and hydrocarbon pentacyclic triterpenoids (HPT). DPT markers result from cycloisomerization and alcohol degradation, while HPT markers can sometimes arise from chemical rearrangement (Rageot et al., 2019).

## 4.2 Alteration Process of Birch Bark Tar

Molecular markers not only aid in the identification of birch bark tar but also offer insights into its degradation over time. Researchers have increasingly focused on using these markers to trace degradation pathways and reconstruct production techniques.

### 4.2.1 Molecular Degradation Pathways of Birch Bark Tar

The primary biomarkers and their degradation products formed during tar production are illustrated in Figure 5 (Rageot et al., 2019). Compared to earlier models (Regert, 2004), this updated representation provides a clearer understanding of the degradation pathways. Notably, Rageot et al. classified lupenone and betulone as both biomarkers and degradation markers, highlighting their role both as natural components of birch bark and as products of thermal degradation.

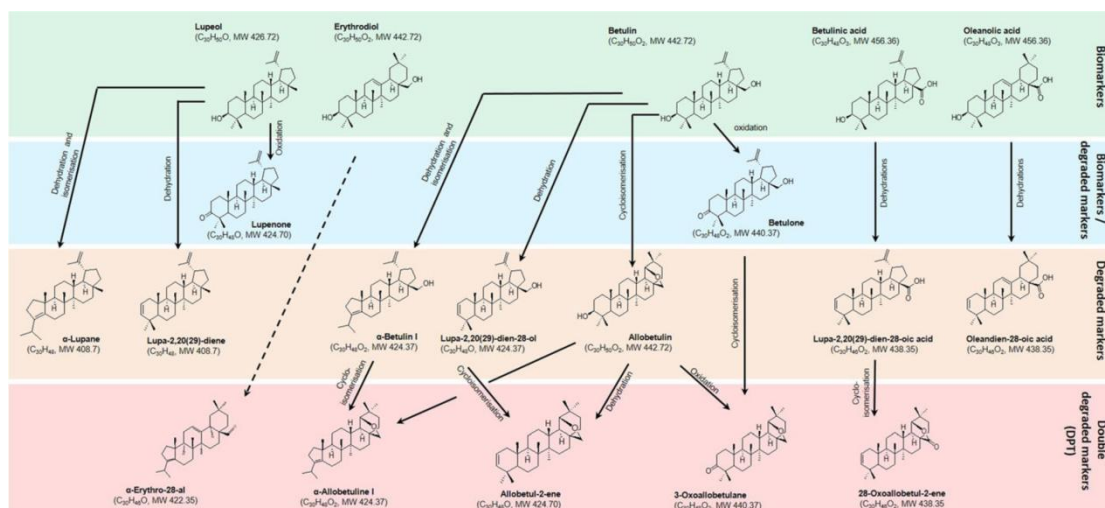


Figure 5. Main biomarkers from birch and their degraded products formed during production (Rageot et al. 2019; Figure 7)

A significant advancement in Rageot et al.'s study was the introduction of the Index of Degradation (ID), a quantitative measure of birch bark tar alteration. The ID is calculated using the following formula:

$$ID = [lupa-2,20(29)-dien-28-ol + betulone] / betuline$$

Since betulin is a stable biomarker, and lupa-2,20(29)-dien-28-ol and betulone are its degradation products, the ID reflects the extent of oxidative and thermal degradation. By comparing these molecular ratios, researchers can evaluate the preservation state of birch bark tar (Rageot et al., 2019; Chasan et al., 2024).

#### 4.2.2 Molecular Study of Production Process

Regert (2004) proposed that comparing the ratios of degradation markers (such as lupa-2,20(29)-diene and lupa-2,20(29)-dien-28-ol) to biomarkers (such as lupeol and betulin) could provide insights into the production methods of prehistoric birch bark tar. However, this approach remained largely theoretical.

Subsequent experimental studies confirmed that double-pot production requires more precise control over raw material quality and heating conditions compared to single-pot methods (Rageot et al., 2019). Rageot et al. also identified specific fatty acids and triterpenic compounds that could differentiate between these production techniques.

More recent research, however, has raised concerns about the reliability of these molecular markers in distinguishing between aceric tar production methods (Kozowyk et al., 2023). The complexity of birch bark tar's composition suggests that observed differences may result not only from production methods but also from post-production alterations, which are still not fully understood (Koch et al., 2024).

## 5. Challenges and Opportunities

### 5.1 Challenges

A significant challenge in archaeological research is the aging and degradation of organic compounds. The preservation of molecular structures is heavily influenced by environmental conditions. Compounds with longer carbon chains degrade more slowly than those with shorter chains, and saturated compounds are generally more stable than unsaturated ones (Eglinton & Logan, 1991). Preservation is most effective in dry, oxygen-free environments, where microbial activity is minimized (Evershed, 1993). For instance, lipids are particularly well-preserved in acidic and anoxic conditions, reducing their susceptibility to microbial degradation (Evershed, 1993; Moucawi et al., 1981). However, degradation processes are complex, and even quantitative methods such as the Index of Degradation (ID) proposed by Rageot et al. (2019) cannot precisely measure the extent of molecular decay.

Interpreting GC/MS results also presents challenges. Some biomarkers overlap across different materials, complicating their differentiation. Furthermore, the molecular profile of the same raw



material can vary depending on the production method employed. While recent studies have successfully differentiated between single- and double-pot ceramic-based tar production techniques using GC/MS (Rageot et al., 2019; Rageot et al., 2021), distinguishing prehistoric aceramic tar production methods is more difficult due to overlapping molecular signatures and variability inherent to these processes (Kozowyk et al., 2023; Chasan et al., 2024). This variability may be due to less control over heating conditions in aceramic techniques, compared to the more stable environments of ceramic-based methods (Kozowyk et al., 2023).

As research progresses, new challenges arise. Experimental archaeology has become a key tool for reconstructing ancient tar production methods, helping researchers link molecular markers to specific techniques. Rageot et al. (2019) compared experimental birch bark tars and identified compounds such as behenic acid, betulinic acid, and various fatty acids and diacids that appeared exclusively in double-pot tars. However, more recent studies by Kozowyk et al. (2023) demonstrated that these markers cannot reliably differentiate aceramic production techniques. Similarly, Chasan et al. (2024) showed that while some molecular markers can help distinguish aceramic tar production, their presence depends more on factors like heating duration and tar collection methods, rather than production technique alone. Both Chasan and Rageot emphasized that GC/MS results from experimental tars cannot be directly applied to archaeological samples without considering preservation conditions. Unlike controlled lab settings, archaeological samples have undergone centuries of environmental exposure, which alters their composition in unpredictable ways (Rageot et al., 2019; Chasan et al., 2024). As a result, broader interpretations tend to be more reliable than specific identifications (Chasan et al., 2024).

Beyond production techniques, preservation and identification biases also present challenges. Taphonomic factors play a significant role in molecular degradation, influencing the accuracy of GC/MS results. Different burial environments produce distinct degradation patterns, which can lead to potential misinterpretations. For example, lipids degrade more slowly in acidic and anoxic conditions, while alkaline and sandy soils foster microbial activity, accelerating molecular breakdown. In such environments, only the most stable biomarkers tend to survive in significant amounts, potentially leading to inaccurate conclusions about the original production method. For instance, degraded tar in alkaline clay may suggest production through condensation or ash mound techniques, due to the selective preservation of certain biomarkers (Chasan et al., 2024).

To mitigate these biases, Chasan et al. (2024) proposed an ID cut-off threshold, enabling researchers to screen for well-preserved samples and reduce the risk of misidentifications. Since ID measures degradation levels, samples below the threshold can be considered reliable, while those above the cut-off should be treated with caution.

A more direct approach to addressing degradation biases involves studying taphonomic processes in controlled experiments. Schmidt et al. (2023) analyzed modern tars to eliminate taphonomic interference, while Kozowyk et al. (2020) conducted a three-year preservation experiment to evaluate the durability of natural adhesives on flint flakes. Their findings indicated that birch bark tar outlasted many other adhesives, while resin, beeswax, and ochre adhesives showed the most consistent preservation across various burial conditions.

## 5.2 Opportunities

A key challenge in improving GC/MS analysis is the limited availability of reference datasets. Current spectral libraries, such as NIST, contain only a fraction of the molecules relevant to archaeological adhesives, leaving many GC/MS peaks unidentified. As more ancient tar samples are analyzed, new molecular markers may be identified, refining existing interpretations.

For example, Chasan et al. (2024) reanalyzed previously published birch bark tar samples (Aveling & Heron, 1998; Charters et al., 1993; Rageot, 2015; Reunanen et al., 1993) and identified additional molecular markers, including Lupa-2,20(29)-dien-28-ol, Olean-2,11-dien-28-oic acid, 3-Oxoallobetulin, and Lupenone. These new findings suggest that expanding GC/MS datasets and creating open-access libraries would significantly improve molecular identification (Reber, 2020; Kozowyk et al., 2023; Chasan et al., 2024). Larger experimental datasets would also increase confidence in linking specific biomarkers to different production techniques.

Traditionally, GC/MS data analysis has been performed manually, requiring expert knowledge and substantial time investment. As datasets grow, manual interpretation becomes increasingly impractical. Machine learning (ML) algorithms offer a potential solution, improving both efficiency and accuracy in data processing.

### 5.3 New Opportunities in Machine Learning

The integration of machine learning (ML) in GC/MS analysis has transformed data processing over the past few decades. Initially, manual peak identification was the only method available, making GC/MS analysis a time-consuming process and prone to human error. As computational technology advanced, statistical techniques such as principal component analysis (PCA) and linear discriminant analysis (LDA) were introduced to help classify and interpret chromatographic data (Brereton, 2003).

Supervised ML models, including support vector machines (SVMs) and decision trees, have since been applied to classify compounds and predict molecular structures based on training datasets (Cortes & Vapnik, 1995; Hastie et al., 2009). More advanced models, such as random forests, have improved classification accuracy, enabling GC/MS data to be analyzed with higher precision (Breiman, 2001).

Recent developments in deep learning, particularly convolutional neural networks (CNNs), have further enhanced GC/MS data analysis. CNNs are effective at recognizing molecular patterns and handling noisy datasets, making them particularly useful for archaeological applications, where sample preservation can vary significantly (Skarysz et al., 2018).

Despite these advancements, the application of ML to archaeological GC/MS analysis is still in its early stages. Some studies have begun using ML to classify biomarkers in ancient residues, such as Lebanov et al. (2020), who applied random forests to analyze essential oils in archaeological contexts. The potential of ML in archaeology is vast, particularly for recognizing degradation patterns, identifying unknown biomarkers, and differentiating ancient production techniques.

However, archaeological samples are often highly degraded and heterogeneous, making accurate model training difficult. Moreover, deep learning models require significant computational resources and expertise. Ensuring that ML-generated results are transparent and interpretable is essential for their acceptance in archaeological research (Rudin, 2019).

With continued advancements, ML could revolutionize archaeological adhesive analysis, allowing for automated data interpretation, more accurate identification of molecular markers, and a deeper understanding of ancient production techniques.

## 6. Materials and Methods of This Research

### 6.1 Materials

This study analyzed 30 experimental samples of birch bark tar, produced at the Vlaardingen (Vla) and Horsterwold (HOR) open-air experimental centers in the Netherlands. Some of these samples have been previously published (Kozowyk et al., 2023; Chasan et al., 2024). The tar was produced using four traditional aceramic techniques: ash mound, condensation, pit roll, and raised structure.

The experimental protocols followed methods established by Kozowyk and Schmidt (Kozowyk et al., 2017; Schmidt et al., 2019). The specific methods for each technique are as follows:

**Ash Mound (N = 4):** A roll of birch bark was buried under hot ash and embers. The heat generated tar inside the roll, which was later collected by unrolling the bark and scraping it with a flint flake or wooden stick.

**Condensation (N = 7):** Small amounts of birch bark were burned near a near-vertical stone surface. The smoke condensed on the stone, forming a tar residue, which was periodically scraped off using a flint flake.

**Pit Roll (N = 7):** A shallow pit (approximately 10 cm deep and 7 cm in diameter) was dug, and a small birch bark cup was placed at the bottom. A roll of birch bark was positioned above the cup, with hot embers placed around it. As the bark was heated, tar dripped into the cup and was also retained within the bark roll. Tar was collected from both the cup and the bark surface.

**Raised Structure (N = 5):** A small pit (10 × 10 cm) was dug, and a birch bark cup was placed at the bottom. A twig screen was placed over the pit, covered by a layer of round pebbles. A roll of birch bark was placed on top, and the entire structure was enclosed in a mud dome. A fire burned around it for several hours, and tar dripped down to collect in the birch bark cup.

In addition to these four aceramic techniques, this study also included laboratory-produced birch bark tar (TLB) samples for comparison. These reference samples (N = 7) were produced using a double-pot system in a PID-controlled muffle furnace (Figure 6). Birch bark was placed in a stainless steel work tube with a perforated base, which was connected to an external glass jar for tar collection. The bark

was heated for 2.5 hours at 350–405°C in an oxygen-limited environment. The extracted tar was then boiled on an electric hot plate until solidified at room temperature, leaving 43% of the original tar mass after evaporation.

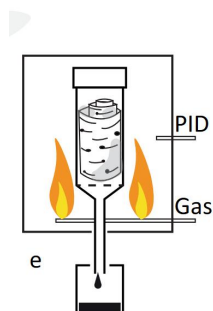


Figure 6. Illustration of laboratory furnace birch bark tar production. (Kozowyk et al., 2023, Figure 1).

## 6.2 Analytical Method: GC/MS Characterization

### 6.2.1 Sample Preparation

Approximately 4 mg of each sample was collected for GC/MS analysis, following the protocol established by Regert et al. (2006). The preparation process consisted of two main steps:

- (1) Lipid Extraction: Each sample was dissolved in 4 mL of dichloromethane (DCM, HPLC grade) and subjected to 30 minutes of ultrasonication.
- (2) Derivatization: The lipid extracts were dried under nitrogen gas at 30°C, then derivatized with the following reagents:

- 50 µL bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane
- 10 µL DCM
- 5 µL pyridine

The reaction was left for 20 minutes at room temperature before drying under nitrogen and reconstituting with DCM.

### 6.2.2 GC/MS Instrumentation

Samples were analyzed using an Agilent 7890B GC system, equipped with a 5977B EI MSD interface and an FID detector. A DB-5 MS column (30 m × 0.25 mm i.d.; 0.25 µm film thickness) was used for separation.

#### (1) GC Parameters:

- Inlet: Splitless mode at 300°C, septum purge flow 3 mL/min
- Carrier Gas: Helium, constant flow 1.6 mL/min (velocity 32.146 cm/s)
- Oven Program: 50°C (2 min hold). Ramp 10°C/min to 150°C. Ramp 4°C/min to 320°C (held for 20 min)

#### (2) MS Parameters:

- Ion Source: 230°C
- Transfer Line: 280°C
- Mass Range: 35–950 m/z
- Electron Ionization: 70 eV

## 6.3 Analytical Method: LDA Analysis

### 6.3.1 Data Extraction from Mass Spectrometry Images

The raw GC/MS data were initially stored as mass spectrometry images (Figure 7, as an example), rather than direct numerical outputs. To extract useful data, an automated Optical Character Recognition (OCR) pipeline was developed. Preprocessing steps were applied to enhance the clarity of the text, including grayscale conversion, binarization using Otsu's thresholding, and morphological operations to remove artifacts. Tesseract OCR was then used to extract mass-to-charge ( $m/z$ ) values and their corresponding intensities. Data cleaning techniques, such as pattern-matching algorithms, were employed to filter out non-numeric artifacts. The extracted values were cross-validated with manually recorded data and structured into a tabular format.

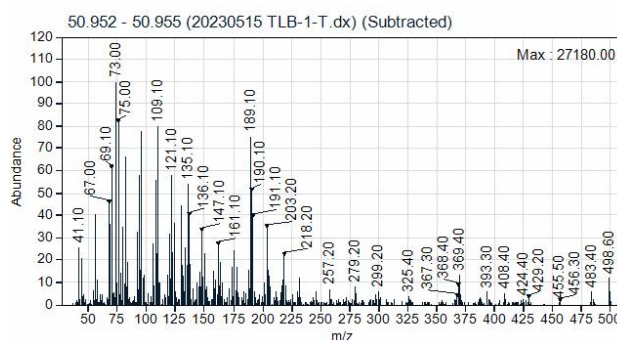


Figure 7. Example of MS data showing a molecule at the retention time of 50.952 min.

### 6.3.2 Data Processing and Matrix Representation of GC/MS Data

A Python-based data processing pipeline was developed to transform raw GC/MS outputs into a structured binary matrix suitable for statistical analysis. The retention time range spanned 4.0 to 58.0 minutes (in 0.1-minute increments), while the mass-to-charge ratio ( $m/z$ ) covered 35.0 to 570.0 (in 0.5  $m/z$  intervals).

As illustrated in Figure 8, retention time values were rounded to two decimal places and assigned to matrix row indices, while rounded  $m/z$  values defined the column indices. Each matrix cell was populated with a binary value: "1" indicated detection of a specific  $m/z$  signal at the corresponding retention time, while absent signals remained "0". The resulting binary matrix was organized into a pandas DataFrame for later machine learning applications.

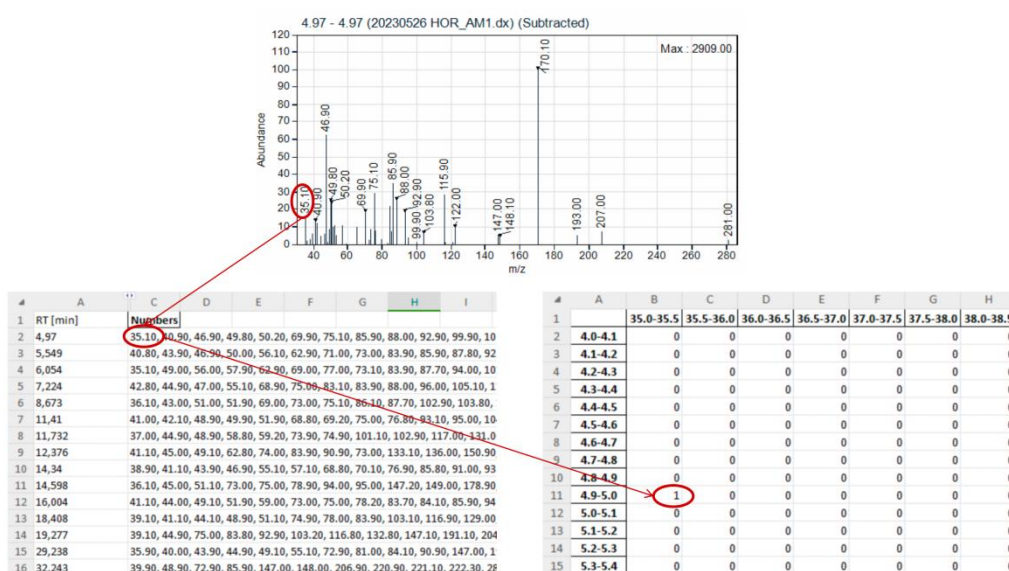


Figure 8. Schematic representation of the GC/MS data processing

### 6.3.3 LDA Classification of GC/MS Data

Based on the matrix developed in Section 6.3.2, we implemented a computational pipeline combining dimensionality reduction (PCA and LDA) with K-nearest neighbors (KNN) classification to differentiate between archaeological adhesive production techniques. The analysis incorporated GC/MS data from five production methods:

- Ash Mound (4 samples)
- Raised Structure (5 samples)
- Pit Roll (7 samples)
- TLB (Lab-produced tar) (7 samples)
- Condensation (7 samples)

Each production method was represented by Excel files containing binary matrices of GC/MS data, which were restructured into numerical feature vectors. These matrices - derived from retention times and m/z values - were flattened into one-dimensional arrays to serve as model inputs.

To address the high dimensionality of the GC/MS data, we employed a two-stage dimensionality reduction approach:

PCA was employed as a preprocessing step to reduce redundancy and extract the most significant features from the high-dimensional GC/MS dataset. The algorithm projects the dataset onto a new coordinate system, where the principal components (PCs) capture the maximum variance in the data. In this study, three principal components (PCs) were retained, as they effectively preserved the majority of the dataset's variance while reducing computational complexity.

Mathematically, PCA transforms the original dataset  $X$  into a lower-dimensional representation  $X'$  using the equation:

$$X' = XW$$

where  $W$  is the matrix of eigenvectors corresponding to the top principal components. This transformation allowed for more efficient feature extraction and noise reduction in the dataset.

Following PCA, Linear Discriminant Analysis (LDA) was applied to enhance the class separability of the adhesive production techniques. LDA differs from PCA in that it maximizes the variance between classes while minimizing variance within classes, thus optimizing classification accuracy. In this study, two LDA components were retained, transforming the dataset into a low-dimensional space where samples from different adhesive production methods formed distinct clusters.

The transformation function of LDA is defined as:

$$X'' = X'V$$

where  $V$  represents the linear discriminants that maximize the separation between classes.

This two-step PCA + LDA approach ensured that the most relevant chemical features were preserved while removing noise and redundant information, ultimately improving classification performance.

Once the dataset was reduced to two LDA components, a k-Nearest Neighbors (KNN) classifier was trained to classify the adhesive production methods. KNN is a non-parametric algorithm that assigns a new sample to the majority class of its nearest neighbors in feature space. For this study, the number of neighbors ( $k$ ) was set to 3.

The classifier was trained on the PCA-LDA transformed dataset, where each adhesive sample was assigned a numerical label:

- 0 → Ash Mound
- 1 → Raised Structure
- 2 → Pit Roll
- 3 → TLB
- 4 → Condensation

To illustrate the effectiveness of the classification model, a scatter plot visualization of the LDA-transformed dataset was generated. Each data point was color-coded according to its adhesive production method, and the test sample was plotted as a black point with a yellow outline for emphasis.

## 7. Result

### 7.1 Overview of LDA Classification Results

The Linear Discriminant Analysis (LDA) results provide a visual and statistical representation of how different archaeological adhesive production techniques can be distinguished based on GC/MS data. Five production techniques were analyzed:

- Ash Mound (Red)
- Raised Structure (Blue)
- Pit Roll (Green)
- TLB (Lab produced tar) (Orange)
- Condensation (Purple)

The LDA scatter plots (Figure 9) illustrate the distribution of these adhesive classes in a two-dimensional space, where each point represents an individual sample. The clusters suggest varying levels of separation and overlap among the different techniques.

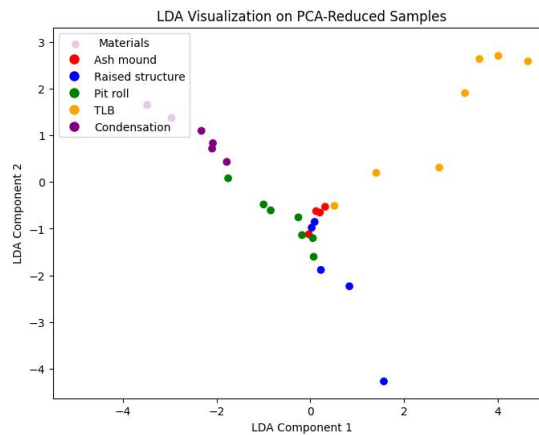


Figure 9. LDA scatter plot of birch bark tar samples

### 7.2 PCA Explained Variance and LDA Coefficients

Before applying LDA, Principal Component Analysis (PCA) was used for dimensionality reduction, transforming the high-dimensional GC/MS dataset into a lower-dimensional space. The explained variance of the first three principal components is:

$$[0.09, 0.07, 0.06]$$

This means that The first principal component (PC1) explains 9% of the total variance in the data. The second principal component (PC2) explains 7% of the total variance. And The third principal component (PC3) explains 6% of the total variance.

While these values indicate that no single component dominates variance, PCA still serves as an effective preprocessing step to remove noise and emphasize key features before applying LDA. LDA was applied after PCA to maximize the separation between classes. The resulting LDA coefficients are:

[[ -0.03 -0.08 -0.01]

[ -0.03 -0.15 0.13]

[ -0.07 -0.00 0.05]

[ 0.30 -0.12 -0.05]

[ -0.19 0.27 -0.09]]

Each row represents one of the five production techniques, while each column corresponds to a principal component. The fourth row (TLB) has the highest positive coefficient (0.30 in PC1), meaning TLB samples contribute strongly to this discriminant function and are highly separable. The fifth row (Condensation) has a strong positive weight (0.27 in PC2), suggesting Condensation adhesives are mainly distinguished along PC2. Pit Roll (third row) has smaller coefficient values, confirming its chemical overlap with other methods, which contributes to higher misclassification rates.

These LDA coefficients confirm that while some adhesives (e.g., TLB and Condensation) are chemically distinct, others (e.g., Pit Roll and Ash Mound) share similar spectral features, leading to classification challenges.

## 7.3 LDA Scatter Plot Interpretation

### 7.3.1 Distinct Clusters for Some Classes

Each class form a compact cluster, suggesting a consistent chemical composition across all samples. Among all the different classes, TLB (Orange) samples are well-separated from other classes, indicating clear chemical distinctions between these adhesive production methods.

### 7.3.2 Overlap Between Certain Classes

Raised Structure (Blue) and Pit Roll (Green) samples partially overlap, suggesting chemical similarities in their tar composition. Condensation (Purple) samples show significant dispersion, indicating high variability in chemical composition, likely due to inconsistencies in the condensation process affecting tar formation.

Moreover, the dispersion of Condensation samples may explain why these adhesives are more difficult to classify, as their chemical signatures appear less consistent than those of other methods.

## 7.4 Classification of Test Samples

To further evaluate the classification model's performance, a total of eight test samples were analyzed using the LDA model. The results of these test are shown as follows.

### Test Sample 1 (Raised Structure): Incorrect Classification

- Predicted Class: Ash Mound (Class 0)
- Expected Class: Raised Structure (Class 1)
- This suggests a degree of chemical similarity between Raised Structure and Ash Mound adhesives, possibly due to compositional overlaps in tar formation.

### Test Sample 2 (Raised Structure): Correct Classification

- Predicted Class: Raised Structure (Class 1)
- Expected Class: Raised Structure (Class 1)
- The correct classification suggests that LDA can separate Raised Structure adhesives when sufficient discriminatory features are present.

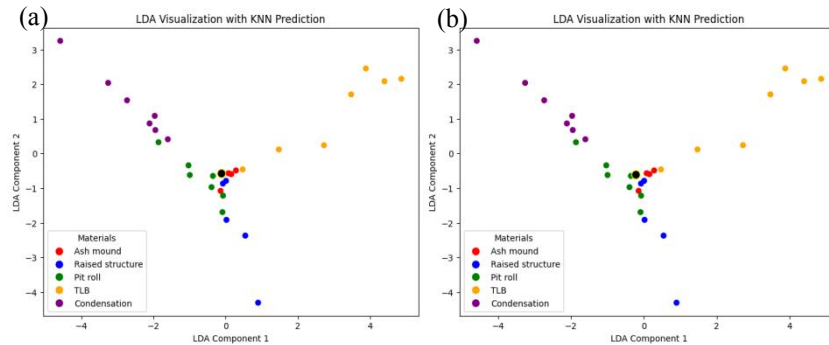


Figure 10. LDA test result of two raised structure samples (black dot). (a) Predicted class of sample 1: Ash Mound (Class 0). (b) Predicted class of sample 2: Raised Structure (Class 1).

### Test Sample 3 (Pit Roll): Incorrect Classification

- Predicted Class: Ash Mound (Class 0)
- Expected Class: Pit Roll (Class 2)
- The classification error suggests that Pit Roll adhesives lack distinct chemical markers in the LDA-transformed space

### Test Sample 4 (Pit Roll): Incorrect Classification

- Predicted Class: Ash Mound (Class 0)
- Expected Class: Pit Roll (Class 2)
- The repeated misclassification of Pit Roll as Ash Mound suggests high chemical similarity between these two methods

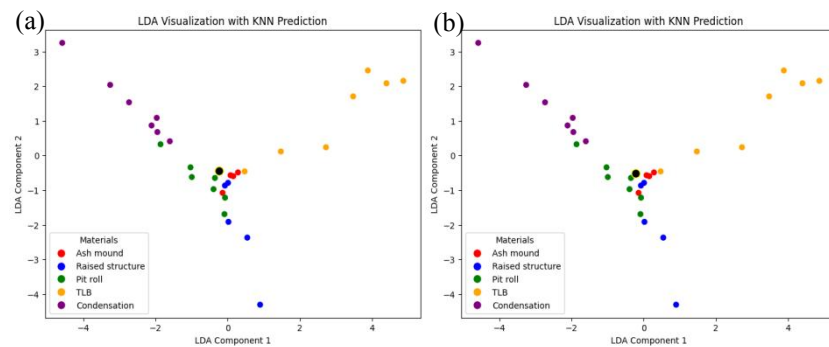


Figure 11. LDA test result of two pit roll samples (black dot). (a) Predicted class of sample 3: Ash Mound (Class 0). (b) Predicted class of sample 4: Ash Mound (Class 0).

### Test Sample 5 (TLB): Correct Classification

- Predicted Class: TLB (Class 3)
- Expected Class: TLB (Class 3)
- The higher classification accuracy for TLB samples suggests that this production technique exhibits unique chemical features.

### Test Sample 6 (TLB): Correct Classification



- Predicted Class: TLB (Class 3)
- Expected Class: TLB (Class 3)
- The consistent correct classification further reinforces the reliability of LDA for identifying TLB adhesives.

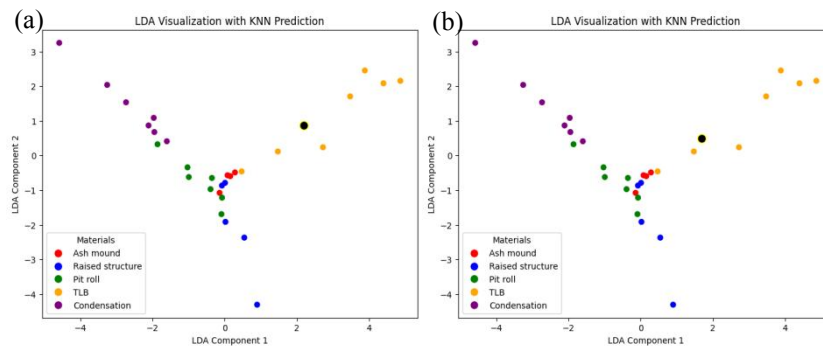


Figure 12. LDA test result of two TLB samples (black dot). (a) Predicted class of sample 5: TLB (Class 3). (b) Predicted class of sample 6: TLB (Class 3).

#### Test Sample 7 (Condensation): Incorrect Classification

- Predicted Class: Ash Mound (Class 0)
- Expected Class: Condensation (Class 4)
- This suggests that some Condensation samples share chemical characteristics with Ash Mound adhesives, making them difficult to differentiate.

#### Test Sample 8 (Condensation): Incorrect Classification

- Predicted Class: Ash Mound (Class 0)
- Expected Class: Condensation (Class 4)
- The repeated misclassification indicates that Condensation adhesives are not clearly separated from Ash Mound adhesives in the LDA feature space.

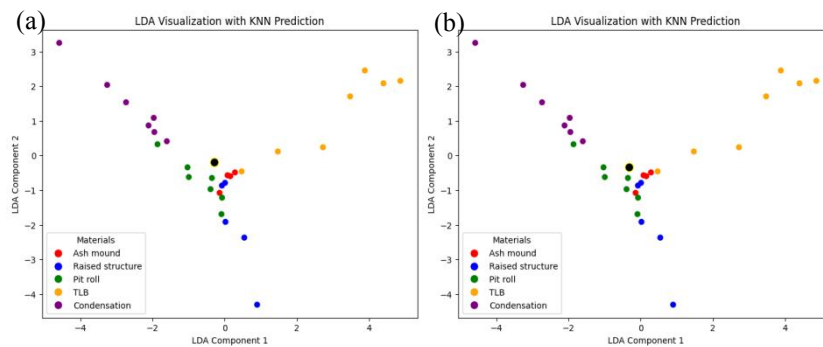


Figure 13. LDA test result of two condensation samples (black dot). (a) Predicted class of sample 7: Ash Mound (Class 0). (b) Predicted class of sample 8: Ash Mound (Class 0).

The LDA classification results confirm that some adhesive production methods exhibit distinct chemical signatures, while others share overlapping features.

TLB adhesives were classified with 100% accuracy, confirming that their chemical profile is highly distinct. Condensation and Pit Roll adhesives were frequently misclassified, likely due to greater chemical variability or compositional overlap with Ash Mound samples. PCA confirmed that no single component dominated variance, suggesting that LDA had to work with subtle chemical differences to achieve classification.

## 8. Discussion

### 8.1 Influence of Production Methods on LDA Separation

As discussed in Chapter 2, the four aceramic production methods (Ash Mound, Condensation, Pit Roll, and Raised Structure) differ in oxygen exposure, heating temperature, heating time, and tar collection methods. These factors influence the chemical composition of the resulting adhesives, which in turn affects their classification in Linear Discriminant Analysis (LDA). A summary of these key differences is presented in Table 2.

Table 2. Summary of key differences of four aceramic birch bark tar production techniques

| Method           | Oxygen exposure | Heating temperature | Heating time             | Tar collection method                                    |
|------------------|-----------------|---------------------|--------------------------|--|
| Ash mound        | Low             | Moderate to High    | Hours to a day           | Tar scraped from the bark                                |
| Condensation     | Open            | Low to Moderate     | Short (minutes to hours) | Tar scraped from the stone surface                       |
| Pit roll         | Low             | High                | Several hours to a day   | Tar collects from the cup and scraped from the bark roll |
| Raised structure | Low             | Moderate to High    | Several hours            | Tar collects from the cup                                |

The Ash Mound method produced a relatively well-defined cluster in LDA space, though some overlap with Pit Roll and Condensation adhesives was observed. Its moderate LDA coefficients suggest that while chemically distinct in some aspects, Ash Mound adhesives share similarities with other methods. This is likely due to its semi-closed heating environment, which limits oxidation while allowing thermal degradation of high-molecular-weight compounds. The long heating duration contributes to gradual tar formation. However, since the tar is scraped directly from the bark, it may contain residual organic material and partially degraded compounds. These factors likely explain its chemical similarities to Pit Roll adhesives, where tar is also collected from the bark surface. Moderate overlap with Condensation adhesives suggests the presence of shared oxidation byproducts, particularly in areas of the ash mound where oxygen exposure is higher. However, these discussions remain tentative, since the amount of Ash mound samples are very limited.

The Condensation method produced the most chemically dispersed cluster, with misclassifications into the Ash Mound category. This aligns with the open-air nature of the Condensation method, where birch bark is heated in an uncovered environment, allowing volatiles to condense onto a cooler stone surface. The relatively low heating temperatures contribute to incomplete pyrolysis, resulting in a higher proportion of oxidation products and semi-volatile compounds. The short heating time further limits thermal degradation, increasing molecular variability. Since the tar is collected from a stone surface rather than within the bark, it is more prone to environmental contamination, which may explain its broader chemical inconsistencies. The misclassification of Condensation adhesives as Ash Mound adhesives suggests that both methods produce oxidation-derived compounds, though Condensation adhesives exhibit greater chemical variability due to open-air exposure.

The Pit Roll method showed overlap with Ash Mound and Raised Structure adhesives, leading to a high misclassification rate. LDA coefficients were relatively low, indicating weak discrimination from other methods. This is consistent with the low-oxygen, high-temperature conditions used in Pit Roll tar production. The tightly rolled birch bark is heated in a pit, creating a partially anaerobic environment that limits oxidation and maximizes tar yield. The high temperature accelerates pyrolysis, breaking down long-chain hydrocarbons into lower-molecular-weight compounds. Because the heating time is relatively long, the resulting tar is highly polymerized and less prone to oxidation. However, tar is collected from both inside the bark roll and a separate collection cup, leading to a mixture of condensed and non-condensed tar fractions. The overlap with Ash Mound adhesives is likely due to similarities in heating duration and oxygen conditions. Misclassification with Raised Structure adhesives may be explained by their shared prolonged heating processes, which contribute to comparable thermal degradation products.

The Raised Structure method formed a distinct cluster, but some overlap with Pit Roll and Ash Mound adhesives was evident. Moderate LDA coefficients suggest that while its chemical profile is somewhat unique, it still retains similarities with other methods. Like Pit Roll tar production, Raised Structure

adhesives are formed in a low-oxygen, moderate-to-high-temperature environment. However, unlike Pit Roll adhesives, which form inside the bark roll, Raised Structure adhesives are collected in a separate cup, reducing contamination from partially decomposed organic matter. The shorter heating time results in less polymerization of the final adhesive, which may contribute to its distinct clustering. Because tar is collected in a more controlled manner, oxidation byproducts are minimized, allowing for a more stable molecular composition. This could explain why Raised Structure adhesives were partially distinct but still misclassified in some cases.

The TLB method produced the most chemically distinct adhesives, forming a compact and well-separated cluster in LDA space with no misclassifications. The highest LDA coefficients, particularly along PC1, indicate a unique chemical signature. This result is expected, as the TLB samples were produced under controlled conditions using a double-pot system in a PID-controlled muffle furnace. The optimized heating temperature, strict oxygen control, and refined tar collection resulted in an adhesive with minimal oxidation byproducts and a highly stable molecular composition. The uniformity of the TLB adhesives is likely due to the absence of environmental variability and post-production inconsistencies seen in the aceramic methods.

In conclusion, the LDA scatter plot and coefficient analysis confirm that production conditions significantly influence the chemical composition of the adhesives, which in turn affects classification in machine learning models. The TLB method produced the most chemically distinct adhesives, leading to perfect classification accuracy in LDA space. In contrast, Pit Roll, Raised Structure, and Ash Mound adhesives exhibited overlapping features, likely due to shared thermal decomposition processes and moderate oxidation conditions. Condensation adhesives were the most chemically inconsistent, leading to frequent misclassification into the Ash Mound category.

## 8.2 Comparisons of Production Processes at the Molecular Level

To analyze the molecular similarities across different classes, a computational approach was applied to match molecules with close retention times and similar mass spectrometry (MS) features. This allowed for a systematic comparison of the chemical compositions of adhesives, helping to identify structural similarities and differences between production techniques.

To ensure accuracy, GC/MS peak data from each sample was processed. A nearest neighbor search algorithm, using a BallTree structure, was implemented to detect molecular similarities between samples from different production methods. The Euclidean distance metric was used to quantify the similarity between GC/MS peak intensity profiles, ensuring only chemically relevant peaks within a controlled range of retention times were compared. A numerical similarity threshold was applied to retain only highly comparable molecular features, reducing noise and weak correlations that could distort the analysis.

Table 3. Average inter-class distance of birch bark tar samples

| Class            | Ash mound | Condensation | Pit roll | Raised structure | TLB  |
|------------------|-----------|--------------|----------|------------------|------|
| Ash mound        | --        | 3.02         | 3.28     | 3.12             | 3.60 |
| Condensation     | 2.84      | --           | 3.06     | 3.12             | 3.47 |
| Pit roll         | 3.19      | 3.08         | --       | 3.25             | 3.60 |
| Raised structure | 3.17      | 3.05         | 3.29     | --               | 3.54 |
| TLB              | 3.60      | 3.49         | 3.61     | 3.56             | --   |

The average inter-class distance between different birch bark tar samples is shown in Table 3.

The strongest molecular similarity was observed between Ash Mound and Condensation adhesives (distance = 2.84), indicating that these two methods generate highly comparable chemical products. This similarity likely arises from moderate oxygen exposure in both processes, which influences the oxidation and degradation pathways of organic compounds. The Ash Mound technique allows limited oxygen interaction through embers, while the Condensation method, an open-air process, facilitates greater oxidation.

In contrast, the largest inter-class distance (3.60) was found between Pit Roll and TLB, highlighting their chemical distinction. Pit Roll involves low oxygen exposure but high temperatures, resulting in tar that retains long-chain hydrocarbons and polymerized structures. TLB, produced under controlled conditions with precise temperature regulation (350–405°C) and post-production refining, yields a

highly purified and stable tar with fewer oxidation byproducts. These stark differences in production conditions explain the molecular divergence between the two.

Among all production methods, TLB consistently exhibited the highest inter-class distances, reinforcing its chemical distinctiveness. Conversely, Condensation tar had lower inter-class distances, suggesting that its chemical composition overlaps more with multiple adhesive types. This indicates that Condensation tar may contain highly variable compounds, making it more prone to molecular similarity with other adhesives.

Table 4. Material Class Counts

| Total Occurrences |     |
|-------------------|-----|
| Ash mound         | 200 |
| Condensation      | 487 |
| Pit roll          | 623 |
| Raised structure  | 326 |
| TLB               | 320 |

The frequency of similarity matches between different adhesive samples provides further insight into their compositional relationships, as is shown in the above Table 4.

Pit Roll had the highest number of similarity matches (623 occurrences), suggesting it shares molecular features with multiple adhesive types. This aligns with the LDA classification results, where Pit Roll adhesives were misclassified due to overlapping chemical signatures.

Condensation adhesives had fewer similarity matches (487 occurrences), indicating a lower frequency of molecular overlaps with other classes. This suggests that Condensation adhesives exhibit greater chemical variability.

TLB, while chemically distinct, still exhibited some molecular overlap (320 occurrences), particularly with Raised Structure tar at specific retention time intervals. Ash Mound had the lowest number of occurrences (200), suggesting a highly specific molecular profile with limited shared features across other production methods.

Table 5. Most Similar Row Pairs

| Class 1          | Sample | Row 1     | Class 2          | Sample | Row 2     | Distance |
|------------------|--------|-----------|------------------|--------|-----------|----------|
| Pit roll         | PR7    | 46.3-46.4 | Condensation     | C2     | 46.3-46.4 | 1.41     |
| Condensation     | C2     | 46.3-46.4 | Pit roll         | PR7    | 46.3-46.4 | 1.41     |
| Condensation     | C5     | 48.5-48.6 | Pit roll         | PR2    | 48.6-48.7 | 1.73     |
| Condensation     | C1     | 48.5-48.6 | Pit roll         | PR2    | 48.6-48.7 | 1.73     |
| Pit roll         | PR2    | 48.6-48.7 | Condensation     | C1     | 48.5-48.6 | 1.73     |
| Pit roll         | PR2    | 48.6-48.7 | Condensation     | C5     | 48.5-48.6 | 1.73     |
| Raised structure | RS5    | 49.4-49.5 | TLB              | TLB1   | 49.4-49.5 | 1.73     |
| TLB              | TLB1   | 49.4-49.5 | Raised structure | RS5    | 49.4-49.5 | 1.73     |
| Ash mound        | AM2    | 53.1-53.2 | Condensation     | C6     | 53.1-53.2 | 1.73     |
| Condensation     | C6     | 53.1-53.2 | Ash mound        | AM2    | 53.1-53.2 | 1.73     |

The most similar molecule pairs and their distances are shown in Table 5.

The strong similarity between Pit Roll and Condensation adhesives at 46.3–46.4 min (distance = 1.41) suggests that both production methods retain similar compounds at this retention time. This range is close to  $\alpha$ -Betulin I, TMS (46.409 min) and Lupa-2,20(29)-diene (46.028 min). These pentacyclic triterpenes are commonly found in birch bark tar and are relatively stable under pyrolysis conditions. The fact that both Pit Roll and Condensation adhesives contain these compounds suggests that their production methods preserve pentacyclic triterpenes, possibly due to similar oxygen exposure and thermal conditions.

Another similar match occurred at 48.5–48.7 min, with Pit Roll and Condensation again showing strong similarities. This range is close to Olean-2,11-dien-28-oic acid, TMS (48.988 min) and

Allobetul-2-ene (49.474 min), indicating moderate oxidation. The presence of these oxygenated triterpenoids suggests that oxygen exposure during production plays a role in their formation.

The Raised Structure and TLB similarity at 49.4–49.5 min (distance = 1.73) suggests both methods preserve Allobetul-2-ene (49.474 min). Despite TLB being chemically distinct overall, this overlap indicates that certain triterpenoid compounds remain stable under specific pyrolysis conditions.

A similar match between Ash Mound and Condensation at 53.1–53.2 min suggests that these methods preserve Betulin, bisTMS (53.200 min) and Betulinic acid bisTMS (53.523 min). Since betulin derivatives are primary components of birch bark, their presence in both adhesives suggests that moderate heating conditions in these methods prevent their complete degradation.

Table 6. Most Frequently Compared Row

| Retention time | Frequency |
|----------------|-----------|
| 50.9-51.0      | 322       |
| 53.1-53.2      | 268       |
| 46.3-46.4      | 159       |
| 48.5-48.6      | 139       |
| 53.2-53.3      | 109       |
| 48.6-48.7      | 94        |
| 46.4-46.5      | 89        |
| 51.0-51.1      | 84        |
| 53.0-53.1      | 59        |
| 49.4-49.5      | 56        |
| 36.5-36.6      | 38        |
| 43.7-43.8      | 36        |
| 47.2-47.3      | 26        |
| 46.0-46.1      | 26        |

The most frequently compared retention time ranges, as is shown in the above Table 6, provide critical insight into which molecular structures appear consistently across different tar production methods.

The most frequently occurring retention time range is 50.9–51.0 min, appearing 322 times, suggesting that a specific molecular structure is consistently present across multiple tar samples. This range is very close to Lupeol, TMS (51.015 min) and Lupenone (50.623 min). Both of these compounds are pentacyclic triterpenoids, which are well-documented components of birch bark tar. Their frequent presence suggests that these triterpenoids are either resistant to thermal degradation or are consistently formed as stable degradation products in different production techniques.

The second most frequently matched range is 53.1–53.2 min, with 268 occurrences. This range is very close to Betulin, bisTMS (53.200 min) and Betulinic acid bisTMS (53.523 min). These molecules are betulin derivatives, which are primary components of birch bark. Their frequent appearance suggests that the conditions in multiple production methods favor the preservation of betulinic acid-related structures. Since betulin derivatives are heat-sensitive, their frequent presence across different tar samples may indicate that the heating temperatures used in these production techniques do not completely degrade these triterpenoids, allowing them to persist as molecular markers of birch bark tar.

The third most frequently occurring retention time range is 46.3–46.4 min, matched 159 times, which suggests that a common compound is present across multiple adhesives. This range is very close to  $\alpha$ -Betulin I, TMS (46.409 min) and Lupa-2,20(29)-diene (46.028 min). Both of these compounds belong to the lupane-type triterpenes, indicating that their presence is a result of partial thermal decomposition of pentacyclic triterpenoids. The consistent occurrence of these compounds across multiple samples suggests that their formation is not limited to a single tar production method but instead represents a common thermal transformation pathway in birch bark pyrolysis.

The 48.5–48.6 min range appeared 139 times. This range is very close to Olean-2,11-dien-28-oic acid, TMS (48.988 min) and Allobetul-2-ene (49.474 min). These oxygenated triterpenoids suggest that controlled oxidation plays a role in the formation of these compounds across multiple production techniques. The frequent detection of these retention times implies that oxygen exposure, even in low-oxygen environments such as Pit Roll or Ash Mound production, is sufficient to generate stable oxygenated triterpenoid byproducts.

Other frequently occurring retention times include 49.4–49.5 min (56 occurrences), 53.2–53.3 min (109 occurrences), 48.6–48.7 min (94 occurrences), and 46.4–46.5 min (89 occurrences). These results further reinforce the idea that certain pentacyclic triterpenoids, including betulin, betulinic acid derivatives, and lupeol-related structures, remain stable across different tar production conditions. The high frequency of these retention times suggests that they serve as molecular markers for birch bark tar, regardless of the specific production technique used.

## 9. Conclusions

This research has explored the classification methods of production techniques of birch bark tar. By applying Gas Chromatography-Mass Spectrometry (GC/MS) and machine learning techniques such as Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA), the study aimed to distinguish between different aceramic production methods. The results have provided new insights into the chemical transformations occurring during tar production and the feasibility of machine learning in archaeological adhesive analysis.

A critical aspect of this research was the comparative analysis of four traditional aceramic tar production methods: ash mound, condensation, pit roll, and raised structure. Each method was found to have different influences on tar composition due to variations in oxygen exposure, heating temperature, heating duration, and tar collection techniques. The ash mound and condensation methods produced chemically similar adhesives, likely due to their moderate oxygen exposure leading to oxidation-derived compounds. In contrast, pit roll and raised structure tars retained more high-molecular-weight triterpenoids, suggesting more controlled pyrolysis conditions with minimal oxidation. The TLB (laboratory-produced) tar exhibited the most distinct chemical profile, demonstrating the impact of precise temperature regulation and post-production refinement on tar purity.

GC/MS analysis revealed that biomarkers and degradation markers serve as reliable indicators for identifying birch bark tar and assessing its production conditions. Key molecular markers such as betulin, lupeol, betulinic acid, and their derivatives remained stable across multiple production techniques, highlighting the robustness of triterpenoids under pyrolysis. Differences in heating intensity and oxygen exposure influenced the formation of soft and strong heating markers, allowing for differentiation between tar production processes. However, interpreting GC/MS data remains challenging due to molecular degradation, environmental contamination, and taphonomic alterations in archaeological samples.

Machine learning techniques, particularly PCA, LDA, and k-Nearest Neighbors (KNN), were applied to categorize tar samples based on their GC/MS data. LDA results demonstrated that while TLB adhesives were chemically distinct, the other production methods exhibited varying degrees of overlap, particularly between pit roll and raised structure adhesives. The use of BallTree-based nearest neighbor analysis further confirmed molecular similarities between pit roll and condensation tar, indicating shared triterpenoid degradation pathways despite differences in oxygen exposure.

Despite these advancements, challenges remain in the study of prehistoric tar production. The influence of taphonomic factors on molecular degradation raises questions about the reliability of experimental tar results when applied to archaeological samples. Additionally, while molecular markers were useful for differentiating tar production methods, their variability across studies suggests that further refinement is needed. The research also highlighted the limitations of manual GC/MS data analysis, reinforcing the need for larger spectral databases and automated analytical pipelines to improve identification accuracy.

Looking ahead, future research should focus on integrating advanced machine learning models to enhance classification performance. Expanding the dataset with additional experimental and archaeological tar samples would provide a broader basis for comparison, allowing for more reliable conclusions about prehistoric adhesive production. Additionally, further studies on the long-term

preservation and alteration of birch bark tar under different environmental conditions would help bridge the gap between experimental and archaeological findings.

In conclusion, this study has demonstrated that GC/MS analysis combined with machine learning techniques can provide insights into the composition and production methods of prehistoric adhesives. The findings contribute to a better understanding of early technological practices and highlight the potential for computational approaches to revolutionize archaeological material analysis. By continuing to refine analytical techniques and expanding experimental datasets, researchers can further uncover the complexities of ancient adhesive production and its role in early human innovation.

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