Globalizing Scents:
Investigating the Trade and Use of Aromatic Plants in the Past using Biomolecular Approaches

Dissertation

der Mathematisch-Naturwissenschaftlichen Fakultät

der Eberhard Karls Universität Tübingen

zur Erlangung des Grades eines

Doktors der Naturwissenschaften

(Dr. rer. nat.)

vorgelegt von

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Tübingen

2023

Gedruckt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät der Eberhard Karls Universität Tübingen.

Tag der mündlichen Qualifikation:	13.02.2024
Dekan:	Prof. Dr. Thilo Stehle
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Summary

Scents and aromatic plants have historically been pivotal in shaping the socio-cultural and economic landscapes of various populations. Beyond their sensory appeal, they intertwine with trade, ritual and daily practices and societal norms. Yet, the ephemeral nature of these "invisible" phenomena presents inherent methodological challenges when attempting to reconstruct the sensory experiences of our ancestors.

This thesis seeks to navigate these challenges and investigates the use, consumption and trade of aromatic substances in ancient Arabia and Egypt in the 2nd and 1st millennia BCE. The research underscores the early globalization processes tied to aromatic products and the vast ancient trade networks driven by the pursuit of these fragrant plants. Beyond commerce, the thesis demonstrates that aromatics permeated daily life, playing pivotal roles in funerary practices, sanitation, preservation, and as symbols of societal distinction.

To identify and reconstruct these ancient aromatics, a multi-analytical approach was adopted, harnessing techniques like gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). These methodologies enabled the identification of aromatic compounds in organic residues from archaeological artefacts associated with aromatic use. The molecular findings were then contextualized with diverse historical datasets, including archaeological contexts, textual records, and botanical and palaeoenvironmental data.

The case studies within the thesis unveil intricate trade networks spanning vast territories. For instance, the analysis of ancient Egyptian mummification balms from the Valley of the Kings, dating to around 1450 BCE, focused on the aromatic substances used in funerary practices of the time. This complex composition of aromatic substances not only underscores the extensive trade connections of the Egyptians in the 2nd millennium BCE but also hints at potential trade links extending to Central Europe and South-East Asia. The second study of mummification balms from ca. 600 BCE emphasized the aromatic and bioactive properties of these substances, such as anti-microbial and anti-fungal activity. The study has uncovered the use of cedar-derived compounds, indicative of cedar tar, and suggests that a dry distillation process was utilized to obtain the tar. This, in turn, sheds light on ancient production techniques for extracting aromatic substances.

Further insights into past aromatic trade emerge from the case study of incense burners from the oasis of Tayma in ancient Arabia. The analysis covered samples with a span of almost 2000 years, revealing a rich history of aromatic use, from coniferous resins and plant oils in the Bronze Age to Pistacia resin in the Early Iron Age and frankincense in later periods. The spatial distribution of these substances at the oasis highlighted their multifaceted roles, ranging from ritualistic applications of certain scents in temples and graveyards to more practical uses of frankincense in homes.

Methodologically, the thesis conducted novel degradation experiments to understand the transformation patterns of secondary metabolites in ancient plant residues. These experiments offered new insights into the preservation dynamics of organic compounds in ancient settings, with broader implications for identifying ancient plants. Moreover, the research identified robust archaeological biomarkers for identifying cedar species in ancient residues.

In conclusion, this thesis has significantly broadened the understanding of the ancient trade and use of aromatics in ancient Egypt and Arabia. It not only identified the substances traded at specific times, but also highlights their complex roles in ancient societies and emphasizes the enduring importance of aromatic plants in shaping human history.

Zusammenfassung

Düfte und aromatische Pflanzen spielten historisch eine wichtige Rolle in der Prägung soziokultureller und wirtschaftlicher Lebenswelten vergangener Gesellschaften. Über ihre sinnliche Anziehungskraft hinaus waren sie eng mit Handel, Ritualen, alltäglichen Praktiken und gesellschaftlichen Normen verknüpft. Doch trotz ihrer Bedeutung stellen diese „unsichtbaren“ Phänomene aufgrund ihrer flüchtigen Natur inhärente methodische Herausforderungen dar, insbesondere bei dem Versuch, die sinnlichen Erfahrungen unserer Vorfahren zu rekonstruieren.

In der vorliegenden Dissertation wurden die Nutzung, der Konsum und der Handel mit aromatischen Substanzen im antiken Arabien und Ägypten während des 2. und 1. Jahrtausends v. Chr. eingehend untersucht. Neben einer Betonung der frühzeitlichen Globalisierungsprozesse, die mit aromatischen Produkten einhergingen, und des antiken Handels, offenbart die Arbeit, wie tief Aromata das alltägliche Leben durchdrangen. Ihre Rolle war entscheidend in Beerdigungsritualen, im Tempelkult, bei der Konservierung und als Symbole gesellschaftlicher Stratifizierung.

Ein multi-analytischer Ansatz, der Techniken wie Gaschromatographie-Massenspektrometrie (GC-MS) und Flüssigkeitschromatographie-Tandem-Massenspektrometrie (LC-MS/MS) nutzte, ermöglichte, aromatische Substanzen in organischen Rückständen aus archäologischen Artefakten zu identifizieren. Die dabei erlangten molekularen Ergebnisse wurden in den Kontext verschiedenster historischer Daten eingebettet – darunter archäologische Kontexte, antike Texte, botanische Daten sowie Informationen zur Paläoumwelt.

Die Fallstudien innerhalb der Arbeit enthüllten komplexe Handelsnetzwerke, die große Distanzen überspannten. So konzentrierte sich zum Beispiel die Analyse der altägyptischen Mumifizierungsbalsame aus dem Tal der Könige, etwa 1450 v. Chr., auf die zu dieser Zeit in den Beerdigungspraktiken verwendeten aromatischen Substanzen. Diese komplexe Zusammensetzung verschiedener Substanzen unterstreicht nicht nur die umfangreichen Handelsbeziehungen der Ägypter im 2. Jahrtausend v. Chr., sondern deutet auch auf mögliche Fernhandelsverbindungen bis nach Zentraleuropa und Südostasien hin. Die zweite Studie über Mumifizierungsbalsame von ca. 600 v. Chr. betonte die aromatischen und bioaktiven

Eigenschaften dieser Substanzen, wie antimikrobielle und antimykotische Aktivität. Die Studie deckte des Weiteren den Einsatz von Zedern-Teer auf, was nahelegt, dass ein Trockendestillationsprozess zur Gewinnung des Teers verwendet wurde, und lieferte dadurch auch Einblicke in antike Produktionstechniken.

Weitere Aufschlüsse über den vergangenen Handel mit Aromata lieferte eine Fallstudie über aromatische Substanzen, die in der Oase Tayma in Arabien im 2. und 1. Jahrtausend v. Chr. verwendet wurden. Diese Studie untersuchte einen Zeitraum von fast 2000 Jahren und offenbarte eine vielfältige Verwendung von aromatischen Produkten, von Koniferen-Harzen und Pflanzenölen in der Bronzezeit bis hin zu Pistazienharz und Weihrauch in späteren Perioden. Die räumliche Verteilung dieser Substanzen in der Oase unterstreichte ihre vielschichtigen Rollen, von rituellen Anwendungen bestimmter Düfte in Tempeln und Friedhöfen bis hin zu alltäglichen Anwendungen von Weihrauch in Wohnhäusern.

Methodisch führte die Arbeit neuartige Degradationsexperimente durch, um die Transformationsmuster sekundärer Metaboliten (Pflanzenstoffe) in antiken Pflanzenresten zu verstehen. Diese Experimente boten neue Einblicke in den Erhaltungszustand organischer Substanzen in antiken Umgebungen mit Implikationen für die Identifizierung antiker Pflanzen. Darüber hinaus konnten durch die Experimente neue archäologische Biomarker für die Identifizierung von Zedernarten in antiken Rückständen gewonnen werden.

Zusammenfassend erweitert diese Arbeit das Wissen um den antiken Handel und die Verwendung von Aromata im antiken Ägypten und Arabien beträchtlich, indem sie nicht nur die spezifisch gehandelten Substanzen identifiziert, sondern auch ihre vielschichtigen Rollen in antiken Gesellschaften beleuchtet. Sie unterstreicht die andauernde Bedeutung aromatischer Pflanzen in der menschlichen Geschichte und eröffnet neue Perspektiven auf die methodologische Herangehensweise an ihre Erforschung.

List of Publications

Accepted Manuscripts (Appendix 1)

Manuscript A - 2022. Huber, B., Larsen, T., Spengler, R. N., Boivin, N. How to use modern science to reconstruct ancient scents. *Nature Human Behaviour* 6, 611–614.

Manuscript B – 2022. Huber, B., Giddings Vassão, D., Roberts, P., Wang, Y. V., Larsen, T. Chemical Modification of Biomarkers through Accelerated Degradation: Implications for Ancient Plant Identification in Archaeo-Organic Residues. *Molecules* 27(10), 3331.

Manuscript C – 2023. Huber, B., Hammann, S., Loeben, C. E., Jha, D. K., Giddings Vassão, D., Larsen, T., Spengler, R. N., Fuller, D. Q., Roberts, P., Devièse, T., Boivin, N. Biomolecular characterization of 3500-year-old ancient Egyptian mummification balms from the Valley of the Kings. *Scientific Reports* 13, 12477.

Manuscripts in preparation (Appendix 2)

Manuscript D – in prep. Huber, B., Hausleiter, A., Dinies, M., Giddings Vassão, D., Säumel, I., Fernandes, R., Roberts, P., Pham, T.L.H. Exploring the aromatic diversity of incense materials at the ancient oasis of Tayma using metabolic profiling.

Manuscript E – in prep. Huber, B., Loeben, C. E., Giddings Vassão, Perruchini, E., Hellwig, F., Arndt, S., Hammann, S., Spengler, R. N., Roberts, P., Boivin, N., Devièse, T. Metabolic profiling reveals the aromatic and bioactive properties of ancient Egyptian mummification balms.

Contributions

Manuscript A – (Huber et al. 2022a)

B. Huber conceived of the idea of the perspective paper, undertook the background research, and developed the workflow model for the scientific reconstruction of ancient aromatics. The original draft was prepared by B. Huber, who then co-wrote the manuscript with N. Boivin, incorporating contributions from R. N. Spengler and T. Larsen.

In total, B. Huber contributed 90% to the research, which forms the methodological and conceptual cornerstone for her PhD research.

Manuscript B – (Huber et al. 2022b)

B. Huber and T. Larsen conceptualized the study. The methodological framework was developed by B. Huber and D. Giddings Vassão and both carried out the experiments as well as the instrumental analysis in the lab. B. Huber performed data analysis and curated the data together with T. Larsen. Statistical analyses were undertaken by T. Larsen and Y.V. Wang. The original draft preparation was spearheaded by B. Huber, with subsequent manuscript reviews and edits contributed by all authors. Graphics were prepared by T. Larsen and B. Huber. The project was supervised was by T. Larsen and P. Roberts.

In total, B. Huber contributed 80% to the research, including conceptualization, laboratory work (set-up of experiments, sample extraction and preparation, GC-MS analysis), data analysis, visualization of data as well as writing the manuscript.

Manuscript C – (Huber et al. 2023)

B. Huber and N. Boivin designed the research. B. Huber, S. Hammann, and D. Giddings Vassão performed the laboratory work. B. Huber, S. Hammann, D. K. Jha, and T. Devièse analysed and interpreted the data. C. E. Loeben provided access to the archaeological material and information on the history of the canopic jars. D. Q. Fuller and R. N. Spengler provided the archaeobotanical background, and D. Q. Fuller prepared the botanical maps. P. Roberts, C. E. Loeben, and N. Boivin advised on the archaeological background. B. Huber prepared the original draft and wrote the manuscript with N. Boivin, including input from all co-authors. P. Roberts, T. Devièse, and N. Boivin supervised the research.

In total, B. Huber contributed 85% to the research, including conceptualization, sampling at the museum, laboratory work (sample extraction and preparation, GC-MS and LC-MS/MS analysis), data analysis, visualization of data and manuscript preparation.

Manuscript D – (Huber et al. in prep a)

B. Huber and A. Hausleiter formulated the research design. B. Huber, D. Giddings Vassão and T.L.H. Pham performed the laboratory work. B. Huber and T.L.H. Pham interpreted the data A. Hausleiter facilitated access to archaeological materials and provided historical context. M.D. contributed the archaeobotanical perspective. B. Huber prepared the original draft and

wrote the manuscript with collaborative input from all co-authors. P. Roberts and R. Fernandes supervised the research.

In total, B. Huber contributed 85% to the research, including conceptualization, sample selection and sampling in Tayma, laboratory work (sample extraction and preparation, GC-MS, and LC-MS/MS analysis), data analysis, visualization of data and writing.

Manuscript E – (Huber et al. in prep b)

B. Huber, N. Boivin and T. Deviese conceptualized the study. B. Huber, S. Hammann, E. Perruchini and D. Giddings Vassão performed the laboratory work. B. Huber and T. Devièse analysed and interpreted the data. C. E. Loeben facilitated access to the archaeological materials and supplied information regarding the history of the canopic jars. F. Hellwig and S. Arndt provided the modern botanical reference material. R. N. Spengler provided guidance on the archaeobotanical context, while P. Roberts and C. E. Loeben contributed insights into the archaeological background. B. Huber prepared the original draft, including input from all co-authors. P. Roberts, T. Devièse, and N. Boivin supervised the research.

In total, B. Huber contributed 85% to the research, including conceptualization, sampling at the museum and at the Botanical Garden, Jena, laboratory work (sample extraction and preparation, GC-MS and LC-MS/MS analysis), data analysis, visualization of data and manuscript preparation.

1. Introduction

1.1. Scent as a 'global' phenomenon in the past

Today, the pervasive influence of scents can be witnessed across cultures and societies worldwide. Transcending their historical and cultural significance, they now form a multi-billion-dollar industry, permeating a wide array of consumer products (Verhoef et al. 2009; Bradford and Desrochers 2009; Rocha et al. 2023). From olfactory marketing for influencing consumer behaviour (Rimkute et al. 2016) to the food and beverage sector (Spence 2015), cosmetic products (Kumar 2005), ambient scents and aromatherapy (Tisserand 1977; Teller and Dennis 2012), all of these industries leverage the power of scent to shape human experience and behaviour (Rao and Jetti 2020). These strategies make use of the direct connection of the sense of smell to certain brain areas, which are responsible for processing memory and emotion (Herz and Engen 1996). By inhaling an odour, the smell molecules are transported to the olfactory epithelium in the nasal cavity, where sensory neurons detect them and transmit signals to the olfactory bulb at the front of the brain (Shepherd 2004). These signals are then relayed to several brain regions, including the amygdala and the hippocampus, which are crucial for emotion processing and memory formation, respectively (Gottfried and Dolan 2003).

Thus, smells have the power to alter human behaviour and physiological responses, serve as alerts to health hazards, and shape choices and decision making. For instance, certain fragrances are utilized in marketing to induce consumer behaviours in stores, such as the scent of freshly-baked goods in supermarkets to stimulate hunger and increase sales (Spangenberg et al. 1996; Pope 2017). Beyond commercial applications, scents have essential roles in public safety and urban planning. For instance, foul odours, typically comprising mercaptans and thiols, are added to natural gas as warning odorants to signal leaks (Wise et al. 2021). Furthermore, scents have historically influenced the spatial arrangement of cities and settlements, often relegating malodorous sectors and industrial areas to the outskirts or downwind from populated areas (Henshaw 2014; Koloski-Ostrow 2015). In the context of evolution, the ability to detect particular scents enabled our ancestors to identify edible food (Stevens and Hume 2004), avoid poisonous plants, and recognize potential predators or dangers (Apfelbach et al. 2005). The olfactory capabilities have also been essential in facilitating social interactions, helping in mate selection through pheromones (Geary et al.

2004; Havlicek and Roberts 2009) and establishing social bonds and hierarchies within communities (Herz 2004). The above-mentioned functions of scent have persisted from our evolutionary past through to the present day (Hoover 2010).

The mass production and distribution of scented products on a global scale began in the late 19th and early 20th centuries, largely as a result of the Industrial Revolution. This era marked the introduction of novel manufacturing processes and the development of technologies for the extraction, synthesis, and production of aromatic compounds, facilitating the production of goods on an unprecedented scale (Brock 1993; Bauer et al. 2008). However, the roots of scent globalization extend back to antiquity, with evidence of various societies engaging in the trade of aromatic materials and the exchange of knowledge pertaining to perfumery, medicine, sanitation – including personal hygiene as well as the association of scent with disease and death – and culinary practices for millennia and on trans-continental scales (Classen et al. 1994; Manniche 1999; Geller 2010; Giachi et al. 2013). In this thesis, the concept of globalization is employed as a theoretical framework to investigate the dispersal of aromatic products in the past, examining their trade, geographic proliferation and direction of trade. While the term globalization encapsulates a modern set of phenomena (Naisbitt 1982), the elements it describes – such as inter-regional connections, trade networks, and the widespread dissemination of goods – are also emblematic of past socio-cultural and economic dynamics (Hodos et al. 2017; Boivin and Frachetti 2018).

Scent as an early 'global' event denotes the ubiquitous presence and cross-cultural significance of aromatic plants and fragrances throughout the Old World, which encompasses Afro-Eurasia. Throughout history, scent has transcended geographic boundaries and fostered cultural connections (Reinarz 2014). For instance, the aromatic resin frankincense, a typical incense material in ancient Arabia, has also been discovered in archaeological sites as disparate as Mersea Island, UK (Brettell et al. 2013) and the Famen Temple, near Xi'an, China (Ren et al. 2022). In both instances, as well as in other examples from ancient Rome (Devièse et al. 2017), frankincense was associated with mortuary practices, thereby illustrating a commonality in cultural usage across diverse regions and societies. The influence of aromatic substances, including incense, herbs, resins, spices, scented oils and woods, extended far beyond their regions of origin (Gilboa and Namdar 2015; Crowther et al. 2015; Scott et al.

2020; Rageot et al. 2023). These aromatic plants were coveted, traded, and transported across vast distances, thereby contributing to early forms of globalization in the ancient world.

1.1.1 Research areas, periods and strategies of this thesis

In this dissertation, focus is placed on investigating the trade and use of aromatic plants in two regions known to have been important hubs and channels for their dispersal and consumption: i) Ancient Arabia and ii) Ancient Egypt. Ancient Arabia, renowned for its abundance of naturally-occurring aromatic plant species with unique scents and bioactive properties (Langenheim 2003), was a critical node for the incense trade, especially in the 1st millennium BCE (Hausleiter 2012; Luciani 2016). This region facilitated the distribution of these valuable materials to the Mediterranean and beyond, via extensive trade networks, such as the famed Incense Road (Fig.1.1) (Macdonald 1997). Conversely, Ancient Egypt, a major consumer of aromatics, was largely reliant on imports due to the geographical constraints of the region (El Hadidi and Hosni 1996; Malleson 2020). Despite this, the specific consumption of aromatic substances, scented oils, embalming materials and perfumes was deeply embedded in the ritual and cultural practices of societies and cultural contexts, with scents also believed to possess spiritual, purifying, and healing powers (Manniche 1999).

Chronologically, the analysis undertaken in this thesis centres on the 2nd and 1st millennia BCE, representing key periods for the exploration of aromatics trade and consumption in both ancient Arabia and Egypt (see sections 1.1.2 and 1.1.3). Within Arabia, previous studies on the aromatics trade have primarily concentrated on the late 1st millennium BCE, a time corresponding to the zenith of incense trade during the Nabataean and Roman eras (Groom 1981; Zimmerle 2014; Bar-Oz et al. 2022). The understanding of traded goods in ancient Arabia, specifically in the context of the aromatics trade, is largely founded on Classical written sources, including works such as Pliny's *Natural History* and *The Periplus Maris Erythraei*. The scarcity of documented evidence for earlier periods has resulted in a significant research gap, particularly in the exploration of the aromatics trade and the traded substances from the Bronze Age to the Roman era. Consequently, this thesis aims to investigate these underexamined periods, with an emphasis on unravelling the types of plants traded as well as their dispersal.

In ancient Egypt, the 2nd and 1st millennia BCE also mark significant periods for a burgeoning aromatics trade. At the beginning of the New Kingdom (1539 – 1077 BCE) in the mid-2nd millennium BCE, large-scale expeditions were initiated to acquire aromatic resins from the land of *Punt*, materials highly valued in cultural practices (Taterka 2016). Apart from written sources, this period is marked by fragmentary material evidence of long-distance spice trade, indicating far-reaching trade networks already in the 2nd millennium, but necessitating further examination regarding the reach and types of products that were acquired as well as their contexts of consumption.

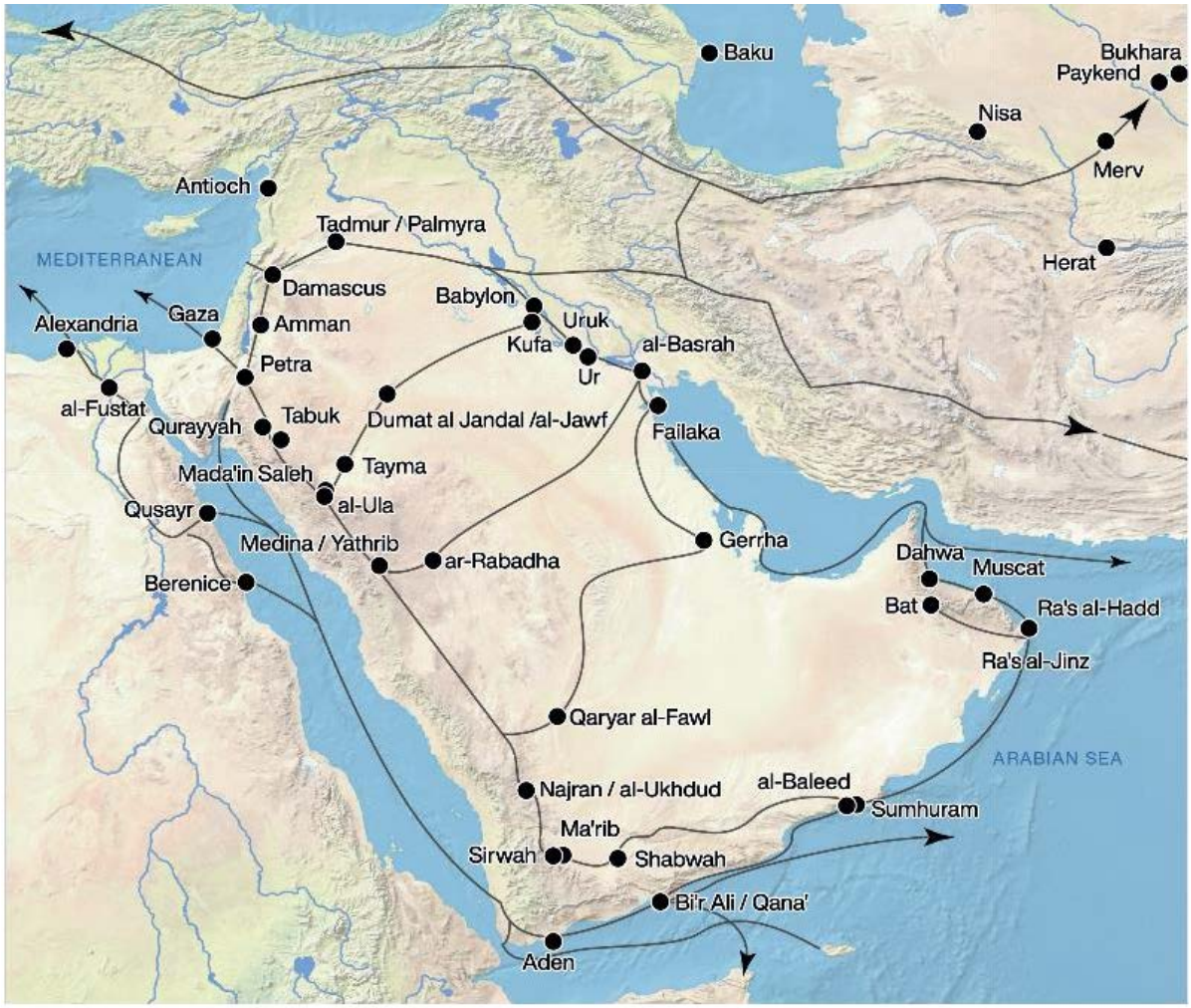

Fig. 1.1 Main stops and oases along the Incense Road in ancient Arabia, connecting South Arabia with ancient Egypt, the Mediterranean, Mesopotamia and beyond (map by Hans Sell, MPI-GEA).

In previous studies, much attention has been devoted to written sources for exploring the distribution of aromatic products (Potts 1988; Avanzini 1997). However, the research strategy of this thesis aims to move beyond this traditional framework, emphasizing the need

to investigate direct material evidence. This approach will particularly focus on organic residues of aromatic substances within archaeological artefacts, seeking to ground-truth existing hypotheses of trade and use (see further chapter 2 and 3). Unlike crops, most aromatic substances are derived from plants that grow in geographically limited areas and cannot be easily cultivated elsewhere, due to specific climatic requirements (Langenheim 2003). Consequently, these plants often have a clear origin and their 'global' availability depends on transport, closely linking aromatics to trade and making them crucial elements for studying their distribution along trade routes.

The intrinsic connection between aromatics and their geographically restricted origins, gives these substances a unique rarity. This rarity, however, extends beyond a simple measure of economic value. It takes on a broader sociocultural dimension, manifesting itself in the form of socioeconomic inequality and specialized status within societal hierarchies (Classen et al. 1994). The constrained availability of aromatic substances, along with their distinctive properties, elevated them to a level beyond mere commodities; they became markers of prestige, luxury, and social differentiation (Classen et al. 1994; Price 2022). This distinction gave rise to an olfactory hierarchy, particularly evident in ancient Egypt, where specific scents were reserved for sacred or elite contexts, such as for the gods, the Pharaoh, or specialized rituals and festivals (Goldsmith 2019, 2021). The importance of aromatics in cultural practices is especially evident in the funerary sphere, where significant efforts were invested in embalming the deceased with numerous odorous substances, most of them imported (Germer 2005; Rageot et al. 2023). These aromatic balms served several purposes: masking the smell of decay, preserving the body for the afterlife, highlighting the social status of a person and connecting the earthly and divine realms, reinforcing the spiritual significance of aromatics. The convergence of economic, social, pharmacological and spiritual roles in the use of aromatics signifies how the scarcity of these substances exerted a profound impact on diverse facets of society, holding a multifaceted importance that reached deep into the fabric of daily life and belief systems.

The selected case studies for this thesis (Manuscripts C to E) are intricately linked with the research areas, time periods, and themes of trade, aromatic dispersal, as well as use and consumption discussed above. Manuscripts C and E specifically delve into the relationship between scent and death, focusing on the utilization of aromatic ingredients in embalming

and ancient Egyptian funerary practices. In contrast, Manuscript D focuses more on the commercial aspect of aromatics, investigating the trade and use of resins at an oasis settlement on the ancient Incense Road in Saudi Arabia (the Manuscripts are explained in more detail in Chapter 2). Collectively, these case studies aim to provide insights into the applications of aromatics but also to enhance our understanding of their dispersal in the ancient world, shedding light on early globalizing processes. As the focal points of investigation in this thesis centre around ancient Arabia and Egypt, the subsequent background section on the early processes of globalization will be anchored around these geographical areas.

1.1.2 The aromatics trade and "Bronze Age globalization"

Although there is evidence for exchange between Mesopotamia and the Arabian coastal regions already as early as the Neolithic (late 6th and 5th millennia BCE), demonstrated by Mesopotamian Ubaid ceramics found at Arabian sites (Potts 1990), the Early Bronze Age (3rd millennium BCE) in West Asia marked a significant shift in the dynamics of trade (Boivin and Fuller 2009). This shift can partly be attributed to the geographical positioning of these regions, which became critical passageways for traders and merchants. This period's concern with movement and connectivity was largely due to the rising importance of bronze and other highly-valued raw materials such as tin, lapis lazuli, and carnelian (Linduff 2004; Haibt 2018; Scott et al. 2020). Notably, carnelian beads and shell cylinder seals originating from the Indus region, modern-day India and Pakistan, were discovered at the Royal Cemetery at Ur in Iraq (Kenoyer 2008), and Indus pottery has been found at Umm an Nar-period sites (2450-2200 BCE) in Oman, for example in Dahwa (Al-Jahwari and Douglas 2021). These findings illustrate the early long-distance trade and exchange relationships that existed between the Indus and Mesopotamian civilizations (Kenoyer 2008), as well as with coastal societies in Oman (Cleuziou and Méry 2002).

During the same period, Oman emerged as a significant player in the copper trade. By the early 3rd millennium BCE, Oman was extensively trading copper, with surplus production being transported to markets in Mesopotamia and North-West Arabian oases (Liu et al. 2015; Luciani 2016; Dumitru and Harrower 2018). This development played a pivotal role in the establishment of broad trade networks across Eurasia, paving the way for the flourishing trade in aromatic plants and spices. During the transition into the Middle and Late Bronze Age in the

2nd millennium BCE, an expansion of bronze production has been observed across Eurasia (Moorey 1999; Linduff 2004). This period also witnessed the emergence of urban societies and early states that were interconnected through these expansive trade routes (Boivin and Fuller 2009). They exhibited a heighted appreciation for exotic items, including aromatics and spices, reflecting the increasing complexity and diversity of trade during this era (Gilboa and Namdar 2015; Scott et al. 2020).

Historical record from this period also mention long-distance trade ventures to distant territories in search of unique commodities, including balsams and resins (Scott et al. 2020; Taterka 2016). A prominent illustration of such expeditions is depicted on the temple walls in Deir el-Bahri, Egypt, constructed during the 18th Dynasty (1539 – 1292 BCE) (Kitchen 2014). This specific expedition, commissioned by the Egyptian Queen Hatshepsut in the 15th century BCE, was directed towards *Punt*, which was likely situated in the Horn of Africa (Scott et al. 2020; Taterka 2016) or in Eritrea (Kitchen 2014). The mission sought to obtain large quantities of resin of the local *'ntyw* (antiu) tree, which can tentatively be translated as the myrrh tree (Germer 2008). The expedition resulted not only in the procurement of aromatic products but also the transportation of living trees back to Egypt (Kitchen 2014). Similarly, Old Babylonian texts dating back to the first half of the 2nd millennium BCE from the Kingdom of Larsa (Iraq) also mention aromatic substances, such as resins and gums, obtained from South Arabia or India being used in sacrifices (Middeke-Conlin 2014). Furthermore, a study by Scott et al. (2020) provides compelling evidence for the early globalization of spices, by demonstrating the presence of South-East Asian spices, such as turmeric, in the Levant during the Bronze and Early Iron Age. These findings, along with the discovery of peppercorns native to southern India in the ancient Egyptian tomb of Ramses II ca. 1200 BCE (Boivin and Fuller 2009; Boivin 2017), and cloves from South-East Asia found at Terqa, Syria, dating to the 18th century BCE (Buccellati and Kelly-Buccellati 2019), underscore the reach and complexity of the ancient aromatics trade in the 2nd millennium BCE.

Nevertheless, despite the growing body of evidence supporting the existence of a long-distance trade in spices during the Bronze Age, direct material evidence remains sporadic and confined to a limited number of examples. It is also noteworthy that a significant portion of these samples were recovered from graves, emphasizing the cultural and ceremonial value of such traded goods. The current body of knowledge shows a vibrant and intricate trans-

Eurasian trade network, suggesting connections and the circulation of goods across vast distances (Scott et al. 2020). However, the specifics of Bronze Age trade in scented substances – such as the particular types and sources of aromatics traded and the socio-cultural practices attached to them – in ancient Arabia and Egypt are still not fully understood. This incomplete understanding extends to the reach of this trade, including the geographical extent and the levels of interaction between different regions, underscoring the need for further research in this area.

1.1.3 The Trade of Aromatics in the 1st millennium BCE and Late Antiquity

The movement of aromatics and spices during the 1st millennium BCE and Late Antiquity played a significant role in the expansion and intensification of trade networks. This period saw the exchange of goods and ideas over vast distances, contributing to the development of interconnected economies and cultures (McLaughlin 2018). The overland and maritime trade routes that facilitated the exchange of aromatics were part of a larger network of interregional connections that later came to be known as the Incense and the Silk Roads (Groom 1981; Macdonald 1997; Frankopan 2016). These routes connected East and West, as well as North and South, facilitating not only the trade of goods but also the exchange of ideas, innovations, technologies, and cultural practices (Spengler 2019; Zimmerle 2021). The Silk Road, for instance, played a crucial role in the diffusion of natron glass from the Mediterranean to East Asia by the 5th century BCE, highlighting the exchange of goods and technologies along this transcontinental connection (Lü et al. 2021). Furthermore, resins endemic to South-East Asia, such as elemi and dammar, have been found in ancient Egypt, in a mummification workshop dating back to the 26th Dynasty, around 600 BCE (Rageot et al. 2023).

On the Incense Routes, the primary sought-after goods moving from South of Arabia to the North were aromatic gum-resins, notably frankincense and myrrh. These goods were harvested from trees of the *Burseraceae* family (from *Boswellia* and *Commiphora* species), which mainly grow in the southern Arabian Peninsula, the Horn of Africa and India (Langenheim 2003). These aromatics were transported across vast distances, reaching as far as northern Europe via the Mediterranean, ancient Egypt, Mesopotamia and even China (Baeten et al. 2014; Middeke-Conlin 2014; Ren et al. 2022). The trade was facilitated by the domestication of the dromedary camel occurring around the turn of the 2nd to the 1st

millennium BCE (Hausleiter 2012), which revolutionized overland trade by enabling the transport of goods across the harsh desert terrains of the Arabian Peninsula. Dromedary camels, inherently resilient to the arid conditions, were capable of traversing extensive distances without the need for frequent hydration, while simultaneously bearing substantial loads (Farsi et al. 2022). This development fostered the establishment of caravan trade. As a consequence, oasis settlements across the Arabian Peninsula, providing essential water sources, became important hubs for the exchange of goods, thereby facilitating the formation of interconnected trade routes (Hausleiter 2012; Luciani 2016; Hausleiter and Eichmann 2018).

The trade of aromatics also had significant economic implications for the region. It facilitated the growth of powerful city-states and kingdoms, such as the Nabataeans, who controlled the incense trade routes (Al-Salameen 2011; Erickson-Gini and Israel 2013). The prosperity of cities, such as Petra in Jordan and al-Ula in Saudi-Arabia, was intrinsically linked to these trade routes, their wealth acting as a testament to the lucrative nature of the aromatic trade (Erickson-Gini and Israel 2013; Rohmer and Charloux 2015; Bar-Oz et al. 2022). The rise of the Roman Empire during the end of the 1st millennium BCE and Late Antiquity further amplified the demand for aromatic substances. The Roman Empire, with its elaborate religious rituals and public ceremonies, was a voracious consumer of incense, particularly frankincense and myrrh, as attested by several Classical sources (e.g. Pliny the Elder, *Natural History*, Bk. XII, Chap. 41). This body of historical references, including documents authored by Greek and Roman scholars, provide evidence for the trade in these highly-desired resins.

Despite this documentation for the later time periods, a noticeable paucity of sources exists for the mid- and early 1st and the 2nd millennia BCE concerning the trade of aromatics (Macdonald 1997). This gap underscores the necessity for a comprehensive investigation into these less-documented periods. A key area of interest lies in exploring local perspectives on incense use, a facet often neglected by extant ancient textual sources that predominantly concentrate on the commerce of aromatics for Greek and Roman consumption. Furthermore, the conventional view of oases as mere transitory points in the desert overlooks their importance as dynamic centres of activity. Far from being just stopping points for travellers, oases have historically been vital hubs of trade, culture, and agriculture (Dinies et al. 2016; Loreto 2017; Hausleiter and Eichmann 2018; Luciani 2021). They served as unique

intersections of diverse influences, facilitating the exchange of goods, ideas, and technologies. Given the lack of contemporary Arabian sources, an investigation into the material evidence present within these oases is important in broadening understandings of the trade and consumption of aromatics (see Manuscript D).

The popularity of aromatics in the past can be attributed to their multifaceted applications. These substances have held a place of great significance, not only for their olfactory appeal, but also for their substantial influence in molding the social, religious, medicinal and economic spheres of past societies (Peacock and Williams 2007; Boivin and Fuller 2009; Baltussen 2015; Clements 2015; Draycott 2015). Their uses ranged from the burning of incense in ritual practices (Baeten et al. 2014; Ren et al. 2022) to being utilized in perfumery and cosmetics. There were also various medical applications and hygienic, sanitary and disinfecting purposes of aromatics (Manniche 1999; Giachi et al. 2013), underscoring the integral role of scent in past societies. Consequently, scent occupied a pivotal position at the intersection of the sacred, therapeutic, cosmetic, and culinary domains, a status it maintained throughout the Middle Ages and Early Modernity into the present day and across various populations (Reinarz 2014; Tullett 2019). It was this multi-use of aromatic substances that made them so popular and contributed scientifically to their widespread dispersal.

1.2 Olfactory perspectives in archaeology and the archaeology of scent

Up until the early 2000s, discussions of the past had remained largely odourless (Bembibre and Strlič 2017). In recent years, however, the archaeology of smell and sensory approaches to archaeology have gained momentum, as researchers recognized the potential of these approaches for understanding past societies (Day 2013a; Hamilakis 2013; Toner 2014; Bradley 2015a; Betts 2017). While archaeological research has predominantly focused on the visual materiality of the past, scents and odours have often been overlooked, mainly due to their transient nature and the challenges inherent in reconstructing ancient aromatics (see also Manuscript A). From a post-processual perspective, it is crucial to acknowledge that the experience of scent is deeply personal and culturally specific, making it uniquely varied across individuals and societies (Hodder 1997). This inherent subjectivity makes the reconstruction and comprehension of ancient olfactory experiences even more challenging. Furthermore, the scarcity of archaeobotanical remains of aromatic plants in archaeological assemblages, and the difficulties associated with characterizing amorphous residues at macroscopic or

microscopic scales, further complicate the identification of ancient aromatic sources and the reconstruction of past olfactory phenomena.

However, there is a burgeoning interest in olfactory approaches in archaeology for determining how past societies used scent and altered, manipulated and tamed their olfactory surroundings. Due to the lack of direct, tangible evidence from archaeological records, most previous studies on ancient scent have relied heavily on ancient written sources or visual representations (Manniche 1999; Day 2013b; Bradley 2015a; Betts 2017; Hawthorn and Rendu Loisel 2019). Most of these studies focused on odours in Greek and Roman literature (Allen 2015; Betts 2017), including studies of the use of odours as cures in medicine (Draycott 2015; Totelin 2015), ritual smells (Clancy 2019), scents in poetry (Butler 2015), ancient philosophizing on the sense of smell (Baltussen 2015), culinary smells (Potter 2015), divine scents (Clements 2015), foul bodies (Bradley 2015b), and the use of odours in entertainment (Day 2017). Apart from classical Antiquity, scholars also studied smells mentioned in ancient Egyptian texts (Manniche 1999; Goldsmith 2021; Price 2022), cuneiform sources (Middeke-Conlin 2014; Hawthorn and Rendu Loisel 2019) and fragrances in Christianity and in the rabbinic world (Green 2015; Toner 2015).

These studies have provided valuable insights into the cultural perception, purpose, and use of smells in the past, but they often present limitations in terms of precise botanical identification of the attested ancient plant names (phytonyms) (Germer 2008; Böck 2011; Pommerening 2016; Geller and Panayotov 2018; Creasman and Yamamoto 2019). This challenge arises due to the fact that identifications of plants from ancient texts are mostly based on philology and ethno-comparisons, which can potentially lead to misleading conclusions (Panayotov 2014; Pommerening 2016; Scott et al. 2020). Moreover, vernacular nomenclature, particularly for plants, is inherently dynamic. As such, meanings can shift or entirely change when transferred through languages or across different regions (Scurlock 2012). Consequently, the specificity of the scents produced by certain aromatic substances, as described in ancient texts, often lacks precision. This aspect is particularly crucial when discussing trade and tracing the origins of the scented substances. For instance, the ancient Egyptian word *snṯr* (senetjer), a term referring to an aromatic substance mentioned in many ancient Egyptian sources, has been translated and identified as frankincense (Baum 1994), the resin of *Pistacia* trees (Serpico and White 2000), or generic incense (Creasman and Yamamoto

2019). This highlights the crucial role of precise identification in understanding the trade and dispersal of these aromatic substances, given the differing geographical origins of frankincense and *Pistacia* trees. While frankincense (obtained from *Boswellia* trees) occurs naturally in South Arabia, India and the regions around the Horn of Africa, *Pistacia* trees are mainly native to the Mediterranean coastal region and Central Asia. Thus, while ancient texts can offer rich information, their limitations necessitate the use of complementary approaches for a more accurate reconstruction of the dispersal of aromatic plants.

1.3 The potential of biochemical and biomolecular approaches in past aroma research

Despite the inherent challenges, the identification of archaeological aromatics is becoming increasingly more achievable through modern biochemical and biomolecular approaches (see also Manuscript A). These techniques, including, for example, gas- and liquid chromatography coupled to mass spectrometry (GC-MS, LC-MS), allow for the investigation and identification of organic residues from archaeological artefacts (see further Chapter 3). By applying these biomolecular approaches to the study of ancient aromatic plants, researchers can elucidate their origins, trade, dispersal and utilization in the past. These methodologies enable the identification and characterization of chemical compounds present in archaeological samples, offering a unique opportunity to reconstruct the olfactory spheres of ancient societies and to trace the movement of aromatic plants across different geographical regions.

Previous organic residue analyses have demonstrated the feasibility of this approach, focusing on the identification of foods (Dunne et al. 2012; Wilkin et al. 2021; Evershed et al. 2022), ritual plants (Baeten et al. 2014; Arie et al. 2020; Ren et al. 2022), cosmetic products (Buckley and Evershed 2001; Ribechini et al. 2011; Riesmeier et al. 2022), narcotic or psychoactive drugs (Zagorevski and Loughmiller-Newman 2012; Tushingham et al. 2018; Ren et al. 2019), and resins (van Bergen et al. 1997; Serpico and White 2000; Arie et al. 2020; Rageot et al. 2023). While a large number of investigations has been conducted previously, biomolecular analyses of incense in ancient Arabia are particularly rare in comparison to other regions and topics, with only a handful of studies carried out so far (Mathe et al. 2007, 2009; Regert et al. 2008). However, despite the demonstrated efficacy of these methods in identifying organic remains, they are not without challenges. A multitude of factors can substantially alter the biomolecular composition of ancient samples over time, especially degradation processes and environmental and taphonomic conditions, potentially leading to

erroneous plant identification if not properly accounted for (see further chapter 3 and Manuscript B).

To target past aromatics and smells, researchers can sample archaeological artefacts associated with the use of aromatic substances, such as incense burners, perfume flasks, cosmetic containers, cooking pots, or storage vessels, in which remains of the contents are still preserved (e.g. as residual crusts on object surfaces or as material-absorbed residues within the porous matrix of objects, such as a ceramic sherd). Larger features with more general uses, such as ash deposits, middens or floor surfaces, or even bodily elements, such as dental calculus can also serve as "scent archives" (Manuscript A), containing the remains of past aromatic substances that can be sampled, analysed, and identified, with the diagenetic pathways being comprehensively reconstructed. Sampling scent archives is also utilized in this thesis and will be further explained in Chapter 3.

A number of projects dedicated to investigating historical smells have emerged over the last 3 years. One of them is the *Odeuropa* project dealing with smell in European history from the 15th- 19th century CE (https://odeuropa.eu/). Researchers in *Odeuropa* utilize cutting-edge AI methods on textual and visual data and aim to identify numerous aspects tied to smells and olfaction (Leemans et al. 2022; Van Erp et al. 2023). The project also aims to compile this diverse data to create a "European Olfactory Knowledge Graph" (https://odeuropa.eu/). Within this framework, it is also planned to carry out investigations on museum objects using sensory and analytical techniques such as GC-O (gas chromatography – olfactometry) and multidimensional GC-MS, although the studies have not been published yet. In addition to *Odeuropa,* other projects investigating this topic have also been established in the last 2 years. This includes the *Odotheka* project (https://hslab.fkkt.uni-lj.si/), which is archiving heritage smells in Poland and Slovenia, and the *Alchemies of Scent* project (https://www.alchemiesofscent.org/), which is using ancient texts and experimental methods to investigate the techniques of ancient Greco-Egyptian perfumery.

2. Aims and objectives of the doctoral research

The primary objective of this thesis is to investigate the use, consumption and trade of aromatic substances in ancient Arabia and Egypt in the 2nd and 1st millennia BCE, thereby examining the role they played in cultural practices, everyday life, and as part of an incipient globalization process. In order to achieve this, biomolecular analyses, including metabolic profiling and lipid residue analysis, are applied to key artefacts associated with the use of aromatics. Examples of these include incense burners, pottery vessels, and canopic jars, in which remnants of ancient aromatics have been preserved. This thesis has two further interlinked aims: firstly, to explore how biomolecular approaches and the study of scent can be used to gain unique insights into the past sensual world, society and human behaviour and, secondly, on a more methodological note, to obtain a fundamental understanding of the chemical alterations associated with degradation processes in ancient samples, and the implications for ancient plant identification.

The first paper of this thesis acts as a programmatic proposal of a framework for a new research field – the biomolecular archaeology of smell, exploring how biomolecular and omics sciences can be leveraged to study the role of scent in the past, and how smell can be used to better understand certain aspects of past lifeways (Manuscript A). The paper puts forward a model that introduces several key themes, such as trade, ritual practices, social hierarchy, pharmacology/medicine, sanitation and culinary practices, where the close investigation of scent and aroma plays a significant role in enriching our understanding of these aspects of past societies. Some of these key themes mentioned above will also be further investigated in the three case studies of the thesis (Manuscripts C - E), analysing organic residues from artefacts associated with the use of aromatics in well-known archaeological contexts (Fig. 2.1).

Manuscript B addresses challenges in the identification of ancient plant species due to degradation and the issues associated with comparing modern plant biomarkers with ancient datasets. The study's primary aim was to understand the transformation patterns of secondary metabolites in plant residues, using *Cedrus atlantica* as a model plant. The experimental design of the study involved a series of degradation experiments, simulating the diagenetic effects potentially catalysed by archaeological materials and natural taphonomic processes on these compounds. Furthermore, a significant aim was to identify robust

archaeological biomarkers for cedar species, which withstand accelerated degradation conditions of the experiments. The findings from this research are expected to serve as a foundation for future studies, and aim to offer insights into the preservation dynamics of organic compounds and their interactions with various archaeological materials.

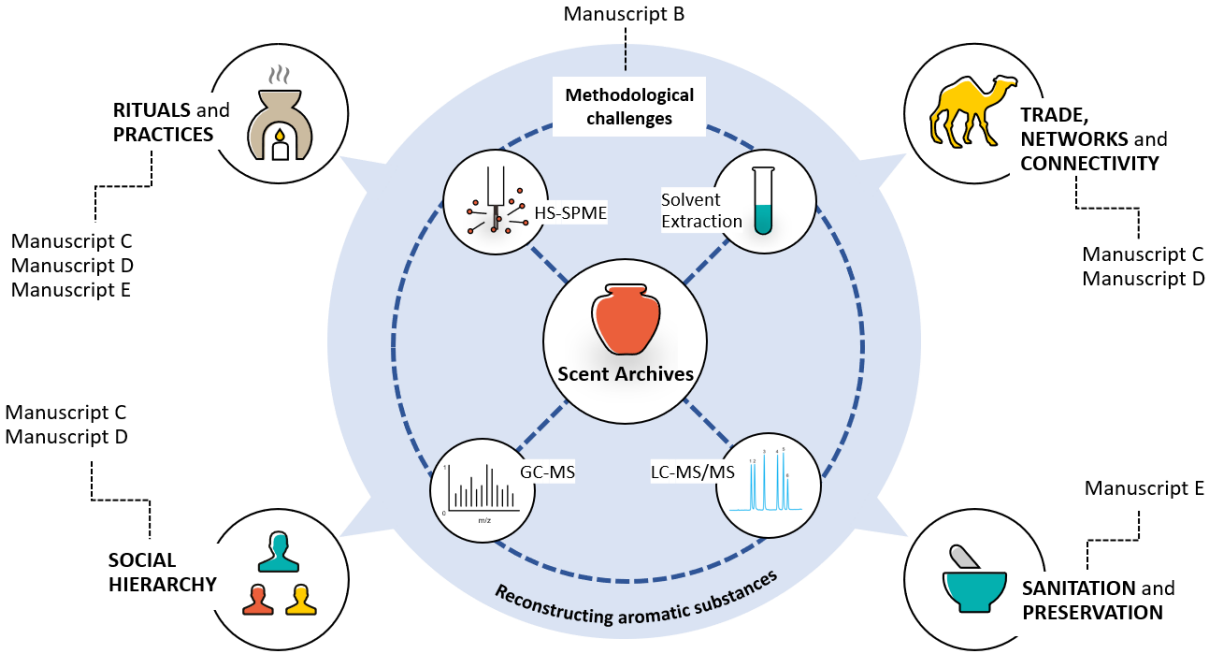

Fig. 2.1 Graphical abstract of the research strategy and methods applied (in blue circle), as well as the key themes investigated in this thesis. All themes are assigned to the respective manuscripts that address these topics.

The first two case studies studies explore the use of scented substances in ancient Egyptian mortuary practices. As delineated in the introductory section of the thesis, the ancient Egyptians exhibited significant consumption of aromatic substances, particularly pertaining to the elite class and in the context of mummification. Manuscript C aims to identify the ingredients of mummification balms used in the embalming of the noblewoman Senetnay in the 18th Dynasty, ca. 1450 BCE, buried in the Valley of the Kings, and to investigate whether the composition of the embalming materials reflects her high social standing. Given the dependency of ancient Egypt on foreign imports of aromatic substances, another objective of this study was to investigate the regions from which these scented products were acquired, thereby examining early trade connections in the mid-2nd millennium BCE.

Manuscript E also conducts an analysis of mummification balms of a high-status woman, but from the 26th Dynasty, ca. 600 BCE, a period often referred to as the 'height of mummification' (Evershed and Clark 2020). The exceptional preservation of the organic residues in these balms provides a unique opportunity to further investigate the odour-active and bioactive compounds contained within them. As many aromatics are composed of a multitude of active ingredients possessing various properties such as antimicrobial, antifungal, and antibacterial activity (Abegaz and Kinfe 2019), the study aims to shed light on the specific bioactivity of the substances. This can help to explain the reasoning behind the ancient Egyptians' selection and manipulation of particular plants for the purpose of preserving the deceased's body, given that achieving eternal life was possible only if the physical integrity of the body was maintained.

The final case study (Manuscript D) focuses mainly on the trade aspects of aromatic resources by investigating the oasis settlement of Tayma in ancient Arabia, an important hub on the incense trade route. The study aims to explore the trade of aromatics in the 2nd and 1st millennia BCE along this prominent route by investigating incense burners found at the site, examining the use and diversity of fragrant materials and their impact on various aspects of society and daily life. The oasis of Tayma was selected as case study, as it demonstrates evidence for continuous burning of incense from the Middle Bronze Age through to Late Antiquity, covering a time span of almost 2000 years. The study, thus, also aims to provide a representative cross-section of incense use in Tayma through time.

Overall, this thesis endeavours to bridge the gaps in our knowledge of ancient aromatic plant usage, to shed light on the complexities of scent-related practices, to contribute to the growing field of biomolecular archaeology, and to provide insights into the historical dynamics of aromatic trade and utilization in Ancient Arabia and Ancient Egypt.

3. Materials and methods

3.1 Sample selection

The sample selection process for the individual studies was guided by specific criteria and objectives. For the ancient Egyptian examples (Manuscripts C and E) the canopic jars from the burials of two high-status women were selected for examination of the mummification balms. One jar originates from the New Kingdom in the mid-2nd millennium BCE, and the other from the Late period in the mid-1st millennium BCE, allowing for a comparative study of the techniques and ingredients used in the mummification balms. Recognizing that these balms were likely to be intricate mixtures and due to complex taphonomic pathways, samples were taken from various positions within each canopic jar to enable a nuanced understanding. The jars from Late period (Manuscript E) were chosen specifically for their exceptional preservation of organic remains, still containing their original scent even after centuries. This unique characteristic provided the opportunity to additionally investigate volatile organic compounds (VOCs), enabling a detailed study of both the odour-active and bioactive properties of the balms.

In the case of the Tayma study (Manuscript D), three essential criteria informed the process: 1. The selection of artefacts that were defined as incense burners based on their form and association with the use of aromatic substances. 2. The consideration of these incense burners across different contexts and time periods was essential to obtaining a chronological understanding of the incense materials used and to study the application of different aromatics in different settings. This led to the choice of burners from the Middle Bronze Age through to Late Antiquity, encompassing the Early, Mid and Late Iron Age, as well as the Nabataean and Roman periods. In terms of contextual investigations, samples were chosen from diverse contexts at the oasis, including temples, public areas, funerary sites, private houses, and storage spaces. 3. The selection process also placed emphasis on choosing objects that exhibited clear traces of burning on the surface or had visible remains and crusts.

3.2 Sampling strategies

For the studies within this dissertation, samples were extracted from ancient Egyptian canopic jars housed at the Museum August Kestner in Hanover, Germany (Manuscripts C and E) as well as from incense burners excavated at the oasis of Tayma, Saudi Arabia (Manuscript D).

The samples from Tayma were collected at the conservation laboratory of the local museum (Tayma Museum of Archaeology and Ethnography). Two primary sampling strategies were employed based on the preservation of the organic remains: 1.) sampling of visible remains, such as resinous layers or crusts adhering to surfaces (Fig. 3.1.A) and 2.) sampling of materials absorbed into the objects, which was typically indicated by surface discolouration, carbonized traces, or soot (Fig. 3.1.B). In the latter case, samples were extracted by drilling into the archaeological objects. However, for two specific objects from Tayma, a solvent wash technique was employed due to drilling restrictions (Fig. 3.1.C). The overarching goal was minimally-invasive sampling. Predefined sampling spots on each object were prepared by removing a thin surface layer using a clean scalpel or modelling drill, thus eliminating surface contamination. The samples for chemical analysis were then taken from these cleaned areas.

Fig. 3.1. Preserved organic remains in archaeological artefacts. (A) Visible crusts within the ancient Egyptian canopic jar; (B) organic residues absorbed into the ceramic matrix of the goblet; (C) applying solvents to the object's surface to recover biomolecules. Photos A and C: Barbara Huber; photo B: Johannes Kramer, DAI Orient Department.

For the first sampling strategy, approx. 100-300 mg of organic remains were collected with disposable scalpels and stored in sterile glass vials. Additional samples from two canopic jars in Hanover (see Manuscript E) were placed directly into sterile headspace SPME vials, which were sealed immediately to preserve volatile compounds and to avoid further handling. For the second sampling strategy, a Dremel drill was used to obtain around 2 g of artefact powder, which we assumed to contain absorbed biomolecules. An area of roughly 1 x 2 cm to a depth of 2-3 mm was drilled with tungsten or diamond grinding bits depending on the

material. The drill bits were cleaned with methanol between sampling to avoid cross-contamination. Control samples, such as solvent swaps or samples from the exterior of the objects, as well as soil controls from the archaeological context, were taken when available to check for contamination. All samples were stored in combusted glass vials or wrapped in combusted aluminium foil for further treatment under clean lab conditions.

3.3 Analytical approach

Organic residue analysis is a field that leverages techniques from organic chemistry and biochemistry to characterize and determine the nature and origin of organic residues, that archaeological methods cannot discern solely through visual traits, as in archaeobotany, for instance (Pollard and Heron 2008; Evershed 2008; Craig et al. 2019). This area of study is built on the understanding that organic materials connected to past human activities can endure and be found in various places and formations in the archaeological record (Evershed 1993, 2008). These organic residues encompass the biomolecular components of natural products, which can be analysed using specific separation methods, such as gas- (GC) or liquid (LC) chromatography, and identification processes, such as mass spectrometry (MS) (Colombini and Modugno 2009). This enables the recovery of both preserved and transformed (either by natural or anthropogenic degradation) biomolecular elements of organic residues.

Upon identifying these elements, the principles of the *Archaeological Biomarker Concept* (Evershed 2008) can be applied to investigate specific chemical characteristics of biomolecules, or their combined 'chemical fingerprint'. Biomarkers are instrumental in identifying substances within archaeological samples, such as aromatic plant products and exudates, and refer to compounds that have distinct chemical characteristics (Peters et al. 2004). The unique chemical markers found in ancient materials can be matched with those in modern plants, allowing the identification and analysis of substances used in the past (Evershed 2008). Plant secondary metabolites (PSMs) and lipids are often used as archaeological biomarkers, due to their inherent resistance to degradation and their source diagnostic capabilities (Modugno et al. 2006; Patalano et al. 2020; Arimura and Maffei 2021). Aromatics contain numerous of PSMs, which are small molecules that are in some cases taxon-specific, making them diagnostic biomarkers for plant identification (Hussein and El-Anssary,

2019; Lukin et al., 2018; Singh, 2016). Among the PSMs, terpenoids are typical compounds in resins, which can be characterized by means of metabolic profiling.

However, identifying the biological origin of archaeological residues using plant biomarkers presents substantial challenges, as various factors may alter the biomolecular composition of ancient samples over time, potentially leading to incorrect plant identification if these structural changes are not considered (Whelton et al. 2021).

3.3.1 Identifying and mitigating analytical challenges and limitations in this thesis

The identification and interpretation of ancient aromatic substances present some challenges, due to the volatility of certain key plant compounds, such as mono- and sesquiterpenoids (Capetti et al. 2020). These compounds are prone to evaporation and alteration over time in archaeological samples, influenced by various factors, such as temperature, drying, light and humidity (Sadgrove et al. 2022; Malik et al. 2023). The disappearance of these diagnostic markers can compromise the ability to distinguish between certain plant species (Salomé-Abarca et al. 2018). For example, distinguishing between most ancient coniferous resins presents a challenge, as the primary surviving and stable compounds, di- and triterpenoids, are commonly shared among various conifers (Modugno et al. 2006; Brettell et al. 2014). Plant products abundant in metabolites, such as resins and spices, can typically be identified to the family or genus level (Brettell et al. 2017b). Achieving more specific identification, such as pinpointing the species, presents a greater challenge. This is primarily due to the fact that the chemical composition of plants within a specific genus can exhibit striking similarities, with overlapping compounds and shared characteristics (Rowe 1989). Furthermore, compounds in archaeological samples can experience natural and anthropogenic chemical transformations. This can be caused by a variety of factors including long-term deposition, exposure to the elements, bacterial degradation, combustion, oxidation, and reduction, all of which may result in chemical alterations (Malainey et al. 1999; Gelbrich et al. 2008; Tamburini et al. 2016). Such processes can fragment the original compounds and generate new derivatives that are not specific to a particular plant species. For instance, while vanillin and vanillic acid are natural constituents of vanilla extracts, they are also typical breakdown products of woody tissue, signalling lignin degradation (Brunschwig et al. 2009; Łucejko et al. 2015).

In addition, chemical reactions may be hastened by catalysis, both organic and inorganic, leading to the alteration of plant substances (see Manuscript B). In an archaeological setting, these reactions might occur when organic materials come into contact with container materials like clay, iron, or bronze, for example. Resulting reactions can involve complex changes such as condensation, addition or removal of oxygen-containing groups, intramolecular migration, functional group substitution, desaturation, or bond cleavage (Duce et al. 2015). All these reactions, and their potential to alter the molecular composition of archaeological plant residues, must be considered in the analysis of biomarkers and the interpretation of data. Despite extensive research on the effects of degradation on the chemical makeup of archaeological materials, most of these studies have primarily focused on the decomposition of lipids (Dudd et al. 1998; Malainey et al. 1999; Hammann et al. 2018) or wood/lignin (Blanchette 2000; Huisman et al. 2008; Colombini et al. 2009; Gjelstrup Björdal 2012; Łucejko et al. 2015). This has left gaps in our understanding of other materials, such as aromatic substances.

Another challenge comes in the form of the investigation of mixtures, which occur for example in perfumes, balms, incense blends, adhesives, ointments and medical remedies (Buckley and Evershed 2001; Giachi et al. 2013; Fulcher et al. 2021; Bertelli et al. 2023). In these cases, different plant products, such as spices, resins, flowers, and herbs, may have been mixed with one another or combined with waxes, fats, or oils. The diversity of certain substances varies widely in terms of their volatility, polarity, solubility and molecular weight, ranging from fairly small and highly volatile compounds to large macromolecules (Nelson et al. 2021). The complexity of the contrasting physicochemical properties of the natural substances makes it difficult to disentangle and identify the individual ingredients within the mixtures (Allwood et al. 2011; Ribechini et al. 2011). Thus, reconstructing the recipe of a perfume or a balm requires a holistic study of all ingredients present in the sample and thus often calls for multi-analytical approaches.

For these reasons, in this dissertation, a comprehensive and in-depth analysis of samples was undertaken using a multi-analytical approach to maximize the extraction of information from the ancient remains. This holistic perspective becomes already evident in the sample preparation and extraction phase, where multiple methods were applied to a single sample, allowing for the sequential extraction of biomolecules with varied chemical

qualities, including volatile organic compounds (VOCs), hydrophobic lipids, and polar metabolites. This methodical procedure aimed to capture the full range of chemical characteristics inherent in a sample (see section 3.4). These specialized preparation methods, combined with sensitive multi-instrumental analyses, facilitated an in-depth investigation of compounds and enhanced the molecular recovery within the samples (see section 3.5).

Although organic materials are subject to chemical changes, significant structural features remain detectable (Regert et al. 2001; Pollard and Heron 2008). Processes like oxidation, combustion, hydrolysis, and even intense heating also leave chemical signatures, that enable the study the ancient processes involved in preparing materials (Malainey et al. 1999; Duce et al. 2015; Tamburini et al. 2016; Hammann et al. 2018) (see also Manuscript E). Rather than mere obstacles, these chemical changes can be leveraged as informative details. Through in-depth analysis, the pathways and transformations of these substances can be explored, revealing ancient technologies and historical preparation methods, for example distillation of plant products (Koller et al. 2003; Regert et al. 2006).

Finally, within the framework of this dissertation, Manuscript B specifically deals with issues of chemical degradation in order to gain a better understanding of the chemical alterations in plant material by conducting accelerated degradation experiments to simulate the conditions that ancient plant residues might have been exposed to. Through the use of catalysts mimicking archaeological materials, such as clay, gypsum, copper or bronze containers, the experiment investigated how different materials influence the degradation and preservation of compounds within plant residues.

3.4 Sample preparation and extraction

In the pursuit to elucidate the nuances of ancient aromatics, different extraction methods were employed, specifically designed to capture and analyse a broad range of compounds from the archaeological samples.

3.4.1 Headspace solid phase microextraction (HS-SMPE)

Recognizing that volatile compounds such as mono- and sesquiterpenes can be lost during solvent extraction and derivatization (Hamm et al. 2004), HS-SPME was employed to specifically target volatile organic compounds (VOCs). This solvent-free extraction technique

uses polymer-coated fibres to trap specific types of molecules, which can subsequently be directly desorbed in a GC injector (Bothe et al. 2003; Schmidt and Podmore 2015) (Fig. 3.2). Extracting molecules involved placing 20-40 mg of sample in a 4 ml HS-SPME glass vial, which was subsequently sealed with PTFE-silicon septa (the samples were place in the HS-SPME vials already in the museum to trap all potential evaporating volatiles). Volatile and semi-volatile compounds were released into the headspace above the sample in the vial. A polydimethylsiloxane (PDMS) fibre with a coating thickness of 100 µm was chosen for trapping analytes. Exposure occurred by injecting the syringe needle through the septum of the vial and releasing the SPME fibre into the headspace above the sample. The fibre was maintained approximately 1 cm above the sample at 60 °C and retracted after 60 min. Subsequently, the analytes were thermally desorbed in the GC injector port.

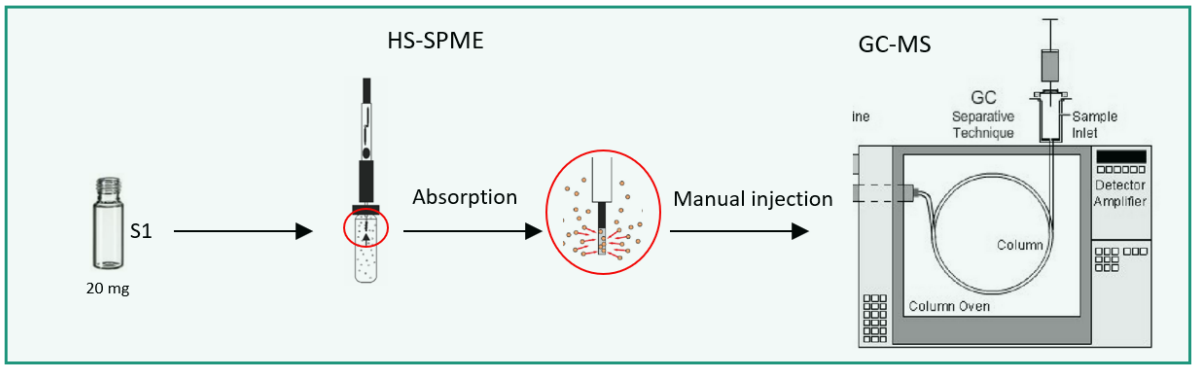

Fig. 3.2. Principles of HS-SPME GC-MS analysis. Volatile organic compounds are trapped in the headspace above the samples by the SPME fibre and desorbed in the GC injector port. Figure has been composed from elements of the following sources: (Hussain and Maqbool 2014; Schmidt and Podmore 2015) *and* https://estanalytical.com/industries/food-flavor-consumer-products/what-is-spme/.

3.4.2 Solvent extraction (Matyash method)

For samples exhibiting visible organic material, a sequential extraction procedure was deployed to isolate distinct classes of biomolecules (i.e. lipids, proteins, and metabolites) from a single sample. This protocol was aligned with methodologies established in prior research (Matyash et al. 2008; Salem et al. 2020), with specific modifications applied to accommodate the peculiarities of extracting from ancient samples. In sum, between 50 to 100 mg of each sample was finely homogenized and subjected to solvent extraction utilizing a methyl tert-butyl ether (MTBE): methanol (MeOH) mixture in a 3:1 volumetric ratio. Following agitation

of the mixture, it was subjected to ultrasonication for a duration of 15 minutes. Subsequently, an aqueous methanol solution in a 3:1 volumetric ratio was incorporated into each sample, and the mixture was thoroughly agitated on a vortex mixer. The samples were then centrifuged at 20,000 x g for 5 min. At this juncture, a dense precipitate of proteins formed at the bottom of the sample, accompanied by the formation of two distinct liquid phases: (1) an upper phase inclusive of hydrophobic lipids, resulting from the lower density of MTBE, and (2) a lower phase containing semi-polar and polar metabolites. Each of these liquid phases were transferred independently into new glass vials, while the remaining protein precipitate was cleansed with methanol and subsequently stored at -80 °C for future palaeoproteomic analysis, pending the future expansion of aromatic plant material in protein reference databases.

Aliquots of samples containing the lipids (upper phase) were subjected to trimethylsilylation via N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA, containing 1% TMCS) for a 60-minute period at 70 °C, prior to gas chromatography-mass spectrometry (GC-MS) analysis. The lower phase enriched with polar metabolites was subjected to liquid chromatography-electrospray Ionization-tandem mass spectrometry (LC-ESI-MS/MS) analysis. Method blanks of each extraction batch were prepared alongside the samples using the same protocol.

3.4.3 Solvent extraction using DCM and MeOH

A separate extraction method was employed for the incense burner samples obtained through drilling, following Evershed et al. (1990) with an additional MeOH step. The extraction of biomolecules was performed using a Dichloromethane (DCM): MeOH (2:1, v/v) solution, followed by a Methanol-only extraction, enabling subsequent analysis by GC-MS and LC-ESI-MS/MS, respectively. The procedure involved the addition of the DCM: MeOH solution to the powdered sample, followed by rigorous mixing and ultrasonication for a 15-minute duration. A centrifugation step at 3000 x g for 10 minutes was employed to partition the solution from the solid matter. The supernatant was transferred to a new vial. To amplify the concentration of the extract, the extraction process was replicated once, with the resulting extracts amalgamated. Furthermore, the procedure was reiterated twice with MeOH solely, aimed at extracting more polar compounds from the solid residue for LC-ESI-MS/MS analysis. For GC-MS analysis, the extracts were derivatized again with BSTFA in the same way as described in

the previous section. Control samples and method blanks were prepared in alignment with the aforementioned protocol to ensure consistency in the experimental procedures.

3.5 Instrumental analysis: chromatography and mass spectrometry

3.5.1 GC-MS and High temperature GC-MS

GC-MS analyses using manual injection (for HS-SPME) and standard liquid injection were executed with an Agilent 8890 GC-System coupled to an Agilent 5977B GC/ Mass Selective Detector (MSD) at the MPI-GEA. Samples (1 µL) were injected onto a HP-5ms capillary column (Agilent, 60 m x 250 µm, 0.25 µm film thickness). For HS-SPME-GC-MS analysis, the PDMS fiber was manually injected into the GC injector port and analytes were thermally desorbed for 5 min at 250 °C with a split ratio of 5:1. Helium was used as carrier gas, maintaining a constant flow rate of 1.0 mL/min and the mass spectrometer was operated in electron impact (EI) mode at 70 eV. The mass scanning range was adjusted from *m/z* 30 to 700 amu for liquid injections and from *m/z* 30 to 400 amu for HS-SPME. The temperatures of the transfer line and source were set at 250 °C and 230 °C, respectively. The GC oven temperature programs and resulting run times were modified between the different studies and injection modes. Detailed information regarding these changes can be obtained by examining the method sections of the associated research papers (Manuscripts B-E). To prevent carryover, injection blanks were inserted between each sample.

High temperature GC-MS analyses were performed on an Agilent 8860 GC coupled to a 5977B mass spectrometer at the Friedrich-Alexander University Erlangen-Nurnberg. Chromatographic separation was accomplished on a DB-1HT column (15 m x 250 µm i.d., 0.1 µm film thickness) using a cool-on-column injector. Helium was used as carrier gas with a constant flow rate of 1.2 mL/min and the transfer line, ion source, and quadrupole temperatures were adjusted to 350 °C, 230 °C, and 150 °C, respectively. Electron ionisation at 70 eV was used and data was recorded in full scan from *m/z* 50 to 800 amu. The GC oven was programmed as follows: a 2-minute hold at 50 °C, followed by an increase to 350 °C at a rate of 10 °C/min, and a final hold duration of 10 minutes at the maximum temperature.

3.5.2 LC-ESI-MS/MS

LC-ESI-MS/MS analyses were conducted on two different platforms: a Shimadzu LCMS-8050 triple-quadrupole system, housed within the MPI-GEA, and an Agilent Technologies 6460,

located at the Technical University Berlin. Chromatographic separation on the Agilent system was achieved using a Kinetex C18 column (100 mm x 2,1 mm, 100A) and on the Shimadzu apparatus on a Restek Raptor Biphenyl analytical column (100mm x 2.1mm, 2.7µm particle size) and a Shimadzu Shimpack Velox SP-C18 column (100mm x 2.1mm, 2.7µm particle size. Both systems used an identical mobile phase, consisting of HPLC-grade water and 0.1% formic acid (mobile phase A) along with acetonitrile (mobile phase B). The column temperature was uniformly held at 25 °C across both systems, and a gradient program was applied with 0.5% B for the initial 1 min, increasing to 80% B at 10 min, 100% B at 15 min with a hold until 17.5 min, and returning to 0.5% B and holding until 20 min. The injection volumes were modulated between 1 and 2 µL, depending on the individual sample concentration. Ionization was performed with an electro spray ionization (ESI) ion source, which operated in both positive and negative modes. All sample were analysed in duplicate for the sake of reliability and precision and MeOH blanks were interspersed between samples.

4. Results

4.1 Manuscript A - How to use modern science to reconstruct ancient scents.

Huber, B., Larsen, T., Spengler, R. N., Boivin, N. 2022. *Nature Human Behaviour* 6, 611–614.

Synopsis

This perspective paper presents a comprehensive exploration of the realm of olfaction and its profound influence on humans throughout history. It investigates how advanced biomolecular and 'omics' sciences, such as the study of secondary metabolites and lipids, palaeoproteomics and -genomics, can be leveraged to gain more direct insights into the past use of smells and aromatic substances. It also offers new avenues for archaeologists to explore critical aspects of ancient society and lifeways, in which scent was significant, such as perfumery, cosmetics and sanitation, but also more general topics such as trade and connectivity.

The paper identified several groups of biomolecules that can be found in organic residues and analysed to reconstruct scented products. First, ancient secondary metabolites, as these compounds are very odour-active and are highly characteristic for identifying resins, gums, essential oils and other scented plants. Second, lipids, primarily water-insoluble molecules like fats, oils and waxes, which very often served as a carrier for ointments, perfumes and cosmetics. Third, the paper also emphasizes the significance of proteins in understanding ancient olfactory practices. Preserved in archaeological specimens, like dental calculus and food residues, these proteins can be very informative about the use of spices and other culinary ingredients. Lastly, ancient DNA offers another avenue of exploration. Ancient DNA, encapsulating the genetic information of life, can be used to identify certain plants, but also to study bacteria and shed light on infections, which are associated with bodily odours and hygienic practices.

Overall, the paper underscores the multitude of information uncoverable from studying past 'invisible phenomena'. The investigation of these biomolecules offers insights into the multifaceted roles of scents in the past and the practices associated with aromatic substances in ritual, culinary, medicinal and sanitary spheres, but also shows how aromatics were integral to trade and shaping intercultural exchanges. Furthermore, scents were intertwined with social identity, often acting as markers of status and societal roles.

4.2 Manuscript B – Chemical Modification of Biomarkers through Accelerated Degradation: Implications for Ancient Plant Identification in Archaeo-Organic Residues

Huber, B., Giddings Vassão, D., Roberts, P., Wang, Y. V., Larsen, T. 2022. *Molecules* 27(10), 3331.

Synopsis

The study delves into the complexities of interpreting ancient plant residues, aiming to study the transformation patterns of plant secondary metabolites by conducting a series of degradation experiments on a model plant (i.e., *Cedrus atlantica).* The experiments aimed to simulate the diagenetic effects that archaeological materials might catalyze in compounds within ancient plant residues. Five different catalysts (bronze, copper, iron, clay, and gypsum) were employed to represent materials historically used for crafting containers that stored organic substances. Additionally, to probe natural taphonomic processes, accelerated redox reactions were induced through oxidizing and reducing agents. Less intense conditions were explored by using ambient air oxygen as the oxidizing agent and nitrogen flushing to establish a non-oxidizing atmosphere. Each catalyst underwent testing at room temperature and at 80 °C over a period of seven days, with each experimental condition replicated five times. Another aim of the study was to identify archaeological biomarkers for cedar species, which can withstand these accelerated degradation experiments.

The results of the degradation experiments were grouped into three categories based on the behaviour of the compounds:

- G1 Compounds: These compounds either disappeared or decreased significantly after seven days of incubation. Given the rapid decline observed under accelerated conditions, it is inferred that these compounds are unlikely to be present in ancient residues that have been deposited for centuries or millennia.
- G2 Compounds: These compounds remained relatively stable or even increased over time. Notably, oxidized and dehydrogenated compounds that lost even numbers of hydrogen atoms to form double bonds were observed. These alterations generally make the compounds more stable, suggesting they might remain in archaeological samples. As such, these compounds could serve as more reliable biomarkers to aid in the identification of archaeological residues.

- G3 Compounds: These compounds were not initially present in fresh cedar but formed during specific experiments, indicating the presence of certain catalysts or storage materials. These compounds can provide insights into the specific materials or conditions the residues might have been exposed to in the past.

The specific compounds identified as potential archaeological biomarkers for cedar species in the study are predominantly himachalenes, particularly α- and γ-dehydro-ar-himachalene, ar-himachalene and β-himachalene oxide. These compounds were chosen due to their resistance against degradation under various experimental conditions. Other markers, such as β-himachalene, which is the most abundant compound in fresh cedar, was rapidly diminished in all experiments, making it unsuitable as a marker of ancient cedar.

The specific catalysts used in the study had varying effects on the degradation of secondary metabolites in fresh cedar. Clay treatments showed minimal variations with temperature, indicating its stable interaction with compounds. Gypsum and metals, especially copper and iron, exhibited pronounced changes in compound degradation, with temperature playing a significant role. Copper, in particular, acted as a potent catalyst, causing substantial alterations in biomolecular profiles. In conclusion, these findings highlight the diverse effects of different archaeological materials on the preservation and transformation of organic compounds in ancient contexts.

4.3 Manuscript C – Biomolecular characterization of 3500-year-old ancient Egyptian mummification balms from the Valley of the Kings.

Huber, B., Hammann, S., Loeben, C. E., Jha, D. K., Vassão, D. G., Larsen, T., Spengler, R. N., Fuller, D. Q., Roberts, P., Devièse, T., Boivin, N. 2023. *Scientific Reports;* 13, 12477.

Synopsis

The study focuses on the examination of ancient Egyptian mummification balms from Tomb KV42 in the Valley of the Kings, dating back to around 1450 BCE. Utilizing modern analysis techniques such as GC-MS, HT-GC-MS, and LC-MS/MS, six balm samples from canopic jars were analysed, that contained the mummified organs (liver and lungs) of the noble lady Senetnay.

The results of the LC-MS/MS analysis revealed a high number of terpenoids, including di- and triterpenoids. Oxidized derivatives were present, like 7-oxo-dehydroabietic acid (7ODHA) and dehydroabietic acid (DHA), together with several resin acids, characteristic of coniferous resins of the Pinaceae family. The presence of the diagnostic compound larixol suggests a *Larix* species as the most likely source for the coniferous resin. Triterpenoids like dammarenolic acid, typically found in dammar resin, were also detected, along with pentacyclic triterpenoids like oleanonic or moronic acid. These compounds have also been associated with *Pistacia* species, but due to the similarity in structure of dammar and *Pistacia* resin and overlapping occurrence, the exact source could not be securely determined. Phenolic and aromatic compounds, including vanillic acid, coumarin, and benzoic acid, were also found. These likely reflect the degradation of woody tissues, and balsamic substances, though their precise origin remains ambiguous. The additional GC-MS analysis of the lipid fraction provided further insights. It revealed saturated straight-chain fatty acids, indicating a mix of plant oils and degraded fats. Detection of n-alkanes as well as hopanes and steranes led to the identification of bitumen. The study confirmed the presence of beeswax, identified by various markers including n-alkanes, long-chain fatty acids, monoesters of palmitic acid and hydroxy wax esters. Its presence provides solid evidence for beeswax being a prominent ingredient in the balms.

Overall, the results revealed a complex combination of natural products and odorous ingredients, including oils, fats, beeswax, bitumen, Pinaceae resins (most likely larch resin), balsamic substances, and possibly dammar or *Pistacia* tree resin. The balm composition varied between jars, suggesting that different balms were applied to different organs, although this remains a tentative hypothesis due to potential degradation and other factors. This composition is the richest and most intricate yet identified in mummification balms from this early time period. It not only highlights Senetnay's exceptional social standing, but also illustrates the trade connections of Egyptians in the 2nd millennium BCE. The possibility of early trade links extending further afield, including the potential use of south-east Asian resins, reflects the far reach of trade networks already in the mid-2nd millennium BCE. Additionally, the presence of the coniferous ingredient suggests trade links with Central Europe, a connection for which we previously had limited evidence. Overall, these insights contribute to our knowledge of ancient Egyptian mummification, demonstrating a complex understanding of embalming practices during the beginning of the New Kingdom.

4.4 Manuscript D – Exploring the aromatic diversity and trade of incense materials at the ancient oasis of Tayma using metabolic profiling.

Huber, B., Hausleiter, A., Dinies, M., Giddings Vassão, D., Säumel, I., Fernandes, R., Roberts, P., Pham, T.L.H. in prep.

Synopsis

This paper focuses on investigating the trade of aromatic substances in ancient Arabia, with particular attention to the oasis of Tayma. Through detailed examination using GC-MS and LC-MS/MS, the results reveal a complex history of aromatics trade, use and consumption over almost 2000 years. In total, the analysis covered 33 incense burners, revealing 23 with clear incense materials, three with only plant oils and fats, and seven with no detectable aromatic substances.

From the Middle Bronze Age, the results revealed the presence of resin acids as well as 7ODHA and DHA, indicative of coniferous plants from the Pinaceae family. Additional substances, including phytosterols and aromatic compounds like benzoic and vanillic acid, were detected, suggesting the presence of plant material and by-products of combustion. The second burner from this period showed no signs of aromatic resins but instead contained fatty acids like palmitic, stearic, and myristic acid, as well as cholesterol and phytosterols, pointing towards plant oils and fats. During the Early Iron Age, samples from incense burners found in the temple revealed triterpenoids characteristic of *Pistacia* tree resin. These substances were identified unambiguously through the presence of diagnostic markers (i.e. moronic, oleanoninc, oleanolic and betulinic, masticadienolic acids and dipterocarpol) and the presence of the specific compound 28-norolean-17-en-3-one, confirming that the resin had been heated. Further complexity in the use of aromatics was found in samples taken from the tombs of the necropolis. Here, greater diversity was uncovered with the use of aromatic blends. A high correspondence with modern *Commiphora* species was discovered, identifying *Commiphora*-type resin as the main ingredient in incense blends. Additional findings revealed mixtures of *Commiphora* with *Pistacia* or coniferous resin and, in one case, Brassicaceae seed oil. In the latter part of the 1st millennium BCE, frankincense was found to be in use, as evidenced by specific markers for *Boswellia* plant exudates, such as boswellic acids and their derivatives. The chemical composition revealed clear proof that frankincense was not just

utilized but burned, and it was detected in various types of burners in houses and storage spaces in the residential area.

The study paints a nuanced picture of aromatic use in ancient Tayma, highlighting the complex cultural, economic, and ritual roles that these substances played. From coniferous resins and plant oils in the Bronze Age to *Pistacia* resin in the Early Iron Age and frankincense in the later periods, the study provides an intricate snapshot of the evolution of aromatic practices, underlining how these substances were not just traded through the oasis, but also important parts of the ceremonial fabric and everyday life of the inhabitants of Tayma. Their spatial distribution at the oasis highlights their multifaceted roles, varying from ritual applications to reflections of wealth, with local and imported resins reflecting both availability and preferences. The scents were not only symbolic, carrying significant cultural meanings in practices such as funerals, but also served practical purposes, such as the use of frankincense in homes to protect stored goods. Ultimately, the study unravels the significance of aromatics in Tayma's ancient society and provides a local perspective on incense use.

4.5 Manuscript E – Metabolic profiling reveals the aromatic and bioactive properties of ancient Egyptian mummification balms.

Huber, B., Loeben, C. E., Giddings Vassão, Perruchini, E., Hellwig, F., Arndt, S., Hammann, S., Spengler, R. N., Roberts, P., Boivin, N., Devièse, T. in prep.

Synopsis

This study examined the composition of ancient Egyptian mummification balms from canopic jars from ca. 600 BCE. It emphasized their bioactive properties and the techniques utilized in their acquisition. Six balm samples, preserved to the extent that they had maintained their aroma for over 2500 years, were subjected to HS-SPME-GC-MS, GC-MS, and LC-MS/MS analyses.

The HS-SMPE-GC-MS analysis focused on the volatile organic compounds within the balms. The results unveiled an exceptional preservation of highly volatile terpenoids, with 62 distinct compounds being identified. The most predominant compounds that emerged from the analysis were sesquiterpenes with the himachalene carbon skeleton. These compounds are specific markers of cedar trees (*Cedrus* species), which were unambiguously identified as

one of the ingredients in the balms. The detected himachalenes were also the ones that proved resilient to degradation (compare with Manuscript B). Furthermore, they provide insights into distinctions between plant components. They are indicative of cedar wood exudates, such as essential wood oils or wood tars, while being notably absent in tissues like leaves or cones. Consequently, the compounds in the ancient samples were pinpointed as originating from a product distilled from cedar wood. The presence of other markers indicative of combustion, such as the carbonaceous wood-tar creosote and naphtalenes, suggests a dry distillation process (e.g. pyrolysis) to obtain a tar rather than steam or hydro-distillation. In this instance, the analysis of the VOCs in the balm samples provided not only evidence of the substances used for mummification, but also illuminated the techniques employed.

The study of the bioactive qualities of cedar tar revealed strong anti-microbial and anti-fungal activity. The detected compound himachalol exhibits particularly strong insecticidal properties and is today deployed to repel beetles and moths and agricultural pests. Furthermore, the effect of cedar tar on bacterial growth clearly demonstrates its strong inhibitory activity on bacterial cells.

A further result was the detection of triterpenoids, suggesting the incorporation of *Pistacia* spp. tree resins in the mummification balms. Several volatile compounds, known to be characteristic of *Pistacia* resin, further supported this conclusion. *Pistacia* resin has also good qualities for aiding in preservation of organic tissue. The solidifying nature of it – from a liquid to a densely compacted solid at room temperature – serves as a moisture-resistant sealant, encapsulating the organs within the balm. Additionally, plant oils and fats were found in the mixture.

Apart from the strong bioactivity of cedar tar, another plausible rationale behind its selection might stem from its potent aroma, adept at both masking the putridity of decay and deterring insects and pests. Already in the past, ancient authors, like Herodotus and Pliny the elder reflected on a “juice” termed *kedros* or *cedrium*, which was so strong that the Egyptians used it for mummification (Pliny the Elder, *Natural history*, Bk. V, Chap. 11; Herodotus, *Historiai*, bk.II). Overall, the effective odour-active and bioactive qualities can explain why the ancient Egyptians deliberately chose this substance in the mummification process and provides insights into the links between bioactive, aromatic and symbolic properties of substances in ancient Egypt as well as ancient techniques.

5. Discussion

5.1. Studying the past through scent: integrating biomolecular, archaeological and paleoenvironmental data for a comprehensive analysis

The examination and identification of ancient aromatic residues through biomolecular methods is the first step in finding tangible evidence of the olfactory dimensions of past lifeways. However, this scientific attempt, while foundational, is merely the beginning of an integrative approach to holistically interpret the socio-cultural and economic ramifications of these aromatic discoveries. Central to this approach is the correlation of molecular findings with diverse historical datasets. This necessitates a synthesis of biomolecular evidence with archaeological contexts, textual records, visual artefacts, and paleoenvironmental data (Fig. 5.1). Such a comprehensive approach not only contextualizes the identified aromatic compounds but also facilitates a nuanced understanding of their role in shaping behaviours and practices, thereby offering a more profound insight into historical anthropological patterns. The study of the oasis of Tayma will serve as an example to illustrate what can be gained with this approach.

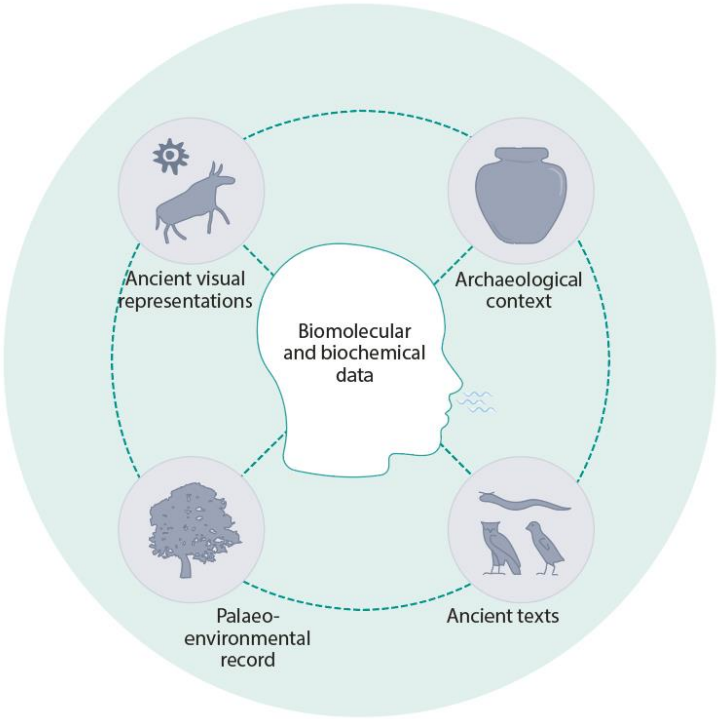

Fig. 5.1 Contextualizing biomolecular data to reconstruct critical aspects of past societies. The analytical results can be contextualized within the framework of relevant historical texts and visual representations, as well as the archaeological and environmental records (modified from Fig. 2 in Manuscript A).

Firstly, the archaeological context is key for understanding the settings in which aromatics were used. It reveals the specific spatial contexts – be it domestic homes, temples, public buildings, or funerary sites, using Tayma as an illustration – where these fragrant substances played a role. This allows scientists to gain more contextual information of incense materials and insights into the socio-economic stratification of their users. Additionally, a temporal analysis is important. It prompts questions regarding the chronological origins of these aromatic traces, their recurrence within archaeological strata, and helps ascertain if particular substances were consistently used for specific purposes. Such inquiries can illuminate the exclusivity or ubiquity of these aromatic substances, offering insights into their socio-economic implications in antiquity. At Tayma, a chronology of incense was established, spanning almost 2000 years, during which distinct trends or 'fashions of use' have been identified.

Environmental data further enriches our understanding by offering a macroscopic perspective on the ecological niches that nurtured these aromatic botanicals. These plants could either be endemic to the region around the archaeological site or native to distant areas, indicative of ancient trade networks. Exploring the native biomes of these botanicals allows us to gain insights into the strategies ancient peoples employed for their procurement (Beck 2013). Additionally, environmental proxies, such as palynological data, enhance our grasp of the historical landscape, shedding light on the relationship between human activities and their respective ecosystems. For instance, in Tayma, the pollen record from the paleolake indicated the presence of *Pistacia* trees within the oasis's catchment area (Dinies et al. 2015, 2016). Consequently, the identified *Pistacia* tree resin, used in the early Iron Age, was likely sourced from local trees.

Textual and iconographic sources can also provide important complementary information. Ancient texts can reveal how societies perceived and described these aromatics, and the sociocultural or sacral roles they played. Visual artefacts further illustrate how these substances were integrated into societal rituals or daily practices. Combining textual and biomolecular evidence can further provide insights into the meaning of words, such as plant names. In the introduction, the difficulty of accurately translating ancient botanical names was highlighted. One potential solution is to examine vessels that both bear inscriptions of their contents and contain organic residues; these dual sources can offer new insights regarding

the meanings of specific terms. For instance, the word '*ntjw*' (antiu) from ancient Egyptian texts is commonly translated as 'myrrh' (Koura 1999; Germer 2008). Yet, a recent study (Rageot et al. 2023) examining the organic residues in Egyptian vessels labelled 'antiu' revealed a mix of coniferous plant products and animal fat—surprisingly, there were no traces of myrrh. This highlights the importance of complementary approaches.

While biomolecular methodologies offer an empirical basis for aromatics used in the past, it is the synergy of these data with archaeological, ecological, and textual sources that truly unravels the complex olfactory narratives that both shaped and were shaped by past societies.

5.2 Key aspects of past societies explored through aromatics

The results from the case studies in this thesis provide a foundation for discussing two primary facets of ancient societies: first, the trade and dispersal of natural products, and second, their consumption and utilization in rituals, sanitation, preservation, and as markers of social status.

5.2.1 Tracing trade and the distribution of aromatics

The case studies presented in this thesis demonstrated the complex trade connections that existed in ancient West Asia and North-East Africa during the 2nd and 1st millennia BCE, suggesting both regional trade and potential long-distance connections on a trans-continental scale for the Old World. A significant indicator of these trade networks is the discovery of aromatic substances in locations where they are not endemic. For example, the majority of aromatics in the ancient Egyptian balms were not indigenous to Egypt, emphasizing their import from other regions (El Hadidi and Hosni 1996; Langenheim 2003). Both studies (Manuscripts C and E) pinpointed the presence of resins from the pine family, which are foreign to Egypt. One study singled out larch resin (*Larix* sp.) as the most likely source, while the other detected tar derived from cedar trees (*Cedrus* sp.).

Within the *Larix* genus, ten species are recognized, yet none are indigenous to Africa or South-West Asia (Farjon 2016; Malleson 2020). The only *Larix* species native in Europe, and the most proximate source to Egypt, is *L. decidua* (Fig. 5.2), predominantly found across the northern Mediterranean and Central European highlands (Tutin et al. 1964). The limited diversity of this genus in Europe can be traced back to the history of this plant lineage, evolving before the Angiosperm Proliferation and subsequently facing competition from the

burgeoning flowering plants during the Eocene (Benton et al. 2022). As a result, only a handful of relic *Larix* species persist today, mainly in colder climate zones where angiosperms have not come to dominate. Therefore, the last refugial populations of the *Larix* genus within Europe that hold out through the Holocene are restricted to insular mountain-top forests. While other *Larix* species exist in Siberia, such as *L. sibirica* and *L. gmelinii*, and others on South Asian mountain ranges like the Himalayas, including *L. potaninii, L. mastersiana, and L. griffithii* (Farjon and Filer 2013), their remote locations make them less likely candidates as the resin source for this study. As a result, *L. decidua* emerges as the most probable source that the ancient Egyptians imported. This is the first time that larch resin has been identified in ancient Egyptian mummification balms, which suggests potential trade connections between Egypt and Central Europe that are so far poorly understood and warrant further research.

Regarding the identified cedar tar, three primary cedar species grow endemically in proximity to Egypt (Farjon and Filer 2013): the Lebanese cedar (*Cedrus libani*), the Atlas cedar (*C. atlantica*), and the Cyprus cedar (*C. brevifolia*). The Lebanese cedar is native to regions along the Mediterranean coast of southern and south-western Anatolia and in Lebanon. In contrast, the Atlas cedar is indigenous to the Rif and Atlas Mountains in Morocco and Algeria. The Cyprus cedar thrives in the Troödos Mountains of central Cyprus, specifically in the Cedar Valley within the Pafos Forest. Given Egypt's historical ties and territorial interests in regions like Canaan (encompassing modern-day Lebanon and Israel), the Lebanese cedar seems the most likely source for the identified tar (Stern et al. 2003; Rageot et al. 2023).

At the oasis of Tayma (Manuscript D), coniferous resin has been identified as well, dating back to the Middle Bronze Age. While this resin could only be classified at the family level (Pinaceae) and not to a specific genus, it is evident that none of the genera within this plant family, such as cedar, pine, fir, larch, or spruce, are native to the Tayma region (refer to Fig. 5.2). Given that these trees predominantly grow in the Mediterranean region, it is highly probable that the coniferous resin was transported from the North down along the Incense Road to the oasis of Tayma. This discovery not only challenges the prevailing notion of the types of incense materials traded along the Incense Road, which predominantly refers to frankincense and myrrh (Groom 1981), it also offers a fresh perspective on the directionality of trade routes. Conventionally, the aromatic trade is perceived to flow from South Arabia to

the Mediterranean, with limited knowledge about the commodities traded in return. A rare piece of evidence that sheds light on this is a cuneiform tablet from the mid-8th century BCE discovered in Iraq. This tablet, a report from the governor of the land of Suḫu and Mari, details an incident where a caravan from the "people of Tema [Tayma] and Saba" was attacked and looted in the governor's territory (Cavigneaux and Ismail Khalil 1990; Macdonald 1997). Notably, the caravan's cargo did not include any aromatics but consisted of wool, iron, and precious stones, suggesting that the caravan might have been returning to Arabia.

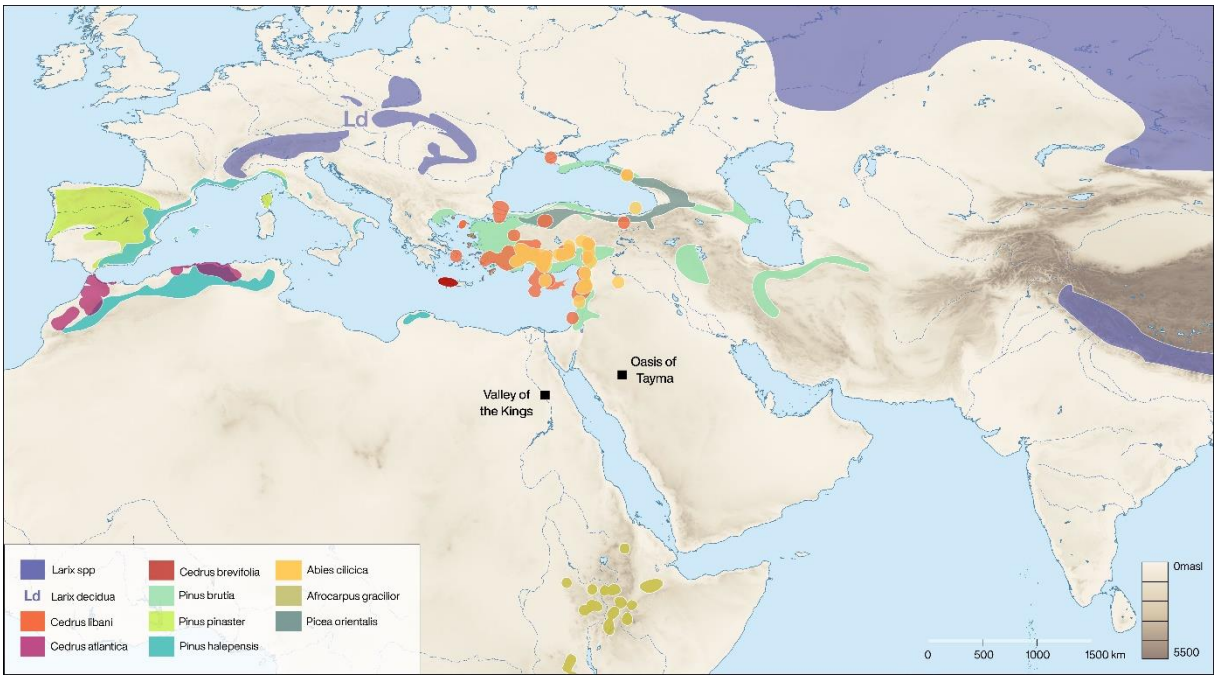

Fig. 5.2 Map showing the distribution of potential conifer resin sources from the Pinaceae family in relation to the Valley of the Kings and the Oasis of Tayma (modified from Fig. 7 in Manuscript C)

In addition to coniferous resins, the resin of *Pistacia* trees has been detected in mummification balms and was used as incense material at Tayma. *Pistacia* trees, notably *P. terebinthus* and *P. lentiscus*, are indigenous to the Mediterranean coastal region stretching from southern Spain to the Levant (Fig. 5.3). Both species have been historically valued for their resin production, yielding turpentine and mastic respectively. While these resins saw extensive use in ancient Egypt (Serpico and White 2000) and were widely utilized across the Mediterranean, as attested by later Classical sources (Theophrastus 1916), they are again not native to Egypt and had to be imported. In contrast, pollen analyses have pinpointed *Pistacia* trees as local sources at Tayma, where the inhabitants seem to have used the locally available

incense. The mummification balm of Senetnay presents a conundrum; the analysis indicates the presence of either *Pistacia* or dammar resin. The latter, produced by trees in the Dipterocarpaceae family, is native to south-east Asian tropical forests (Langenheim 2003). While dammar resin has been identified in a mummification workshop at Saqqara dating to the mid-1st millennium BCE (Rageot et al. 2023), it has not been reported thus far in ancient Egypt in the 2nd millennium BCE. If the substance is indeed dammar resin, it would suggest that ancient Egyptians accessed south-east Asian resins through long-distance trade routes nearly a millennium earlier than previously detected. These findings could strengthen the suspicions of early trade dynamics between Egypt and South-East Asia as discussed in the introduction, showcasing the extensive reach of ancient Egypt in sourcing aromatic products.

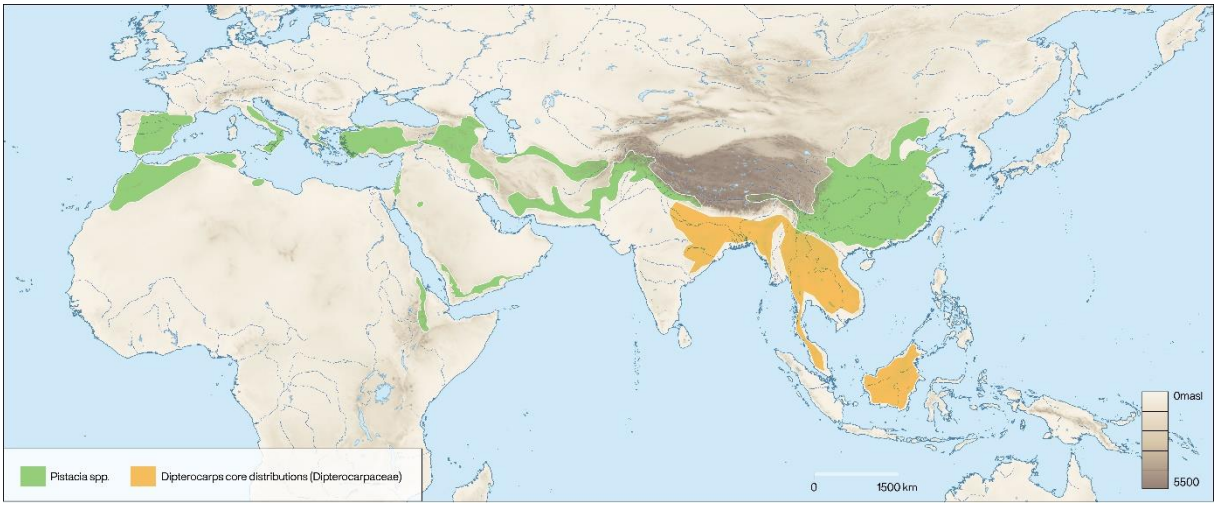

Fig. 5.3 Map displaying the natural habitat of Pistacia spp. and the core distribution of Dipetrocarpus and Hopea (Dipterocarpaceae family), excluding small population in the Western Ghats of South India (modified from Fig. 7 in Manuscript C).

Frankincense, discovered at Tayma, also does not occur naturally in North Arabia. It is derived from *Boswellia* trees, a shrub-like genus. Belonging to the Burseraceae family, *Boswellia* is predominantly an Afro-Indian plant, growing in the dry regions of North-Eastern Africa and mountainous areas spanning from southern Arabia to South-East Asia (Langenheim 2003). The species closest to Tayma is *B. sacra*, predominantly found in South Arabia, specifically in Yemen and Oman. Other species like *B. Serrata* are native to India, while *B. papyrifera, B. neglecta*, and *B. frereana* are commonly found around the Horn of Africa, in countries such as Eritrea, Ethiopia, Sudan, Chad, Somalia, and Kenya (Fig. 5.4) (Bongers et al. 2019). Frankincense began to emerge in Tayma during the latter half of the 1st millennium

BCE, coinciding with the period when the incense trade from South Arabia to the Mediterranean was well-established (Zimmerle 2021). This indicates that the most probable source of frankincense at Tayma comes either from Yemen or the Dhofar region in Oman.

However, trade ties in the aromatic industry also existed between ancient Arabia and the Horn of Africa, particularly Ethiopia, during the 1st millennium BCE (Nebes 2014). The Kingdom of Saba, a notable ancient South Arabian kingdom, was centred around the oasis area of Hadramawt in what is now Yemen (Zimmerle 2021). Its influence stretched to the Dhofar region of Oman and even reached Ethiopia, as evidenced by the presence of the Sabaic language. Notably, inscriptions in the Sabaic language have been discovered not just in Yemen but also in Ethiopia's Tigray region (Japp et al. 2011). This points to a linguistic and cultural exchange, likely driven by trade or migration. The strategic locations of these regions, flanking the Red Sea, facilitated this trade. Consequently, African *Boswellia* (Fig. 5.4) species should also be considered as potential sources. The *Commiphora*-type resin, also identified at Tayma during the mid-to-late Iron Age, might also originate from this area or the broader Horn of Africa region. However, given the vast number of *Commiphora* species (over 200) and their wide distribution from Africa to the western Indian Ocean islands (Langenheim 2003), pinpointing the exact source of the resin remains challenging.

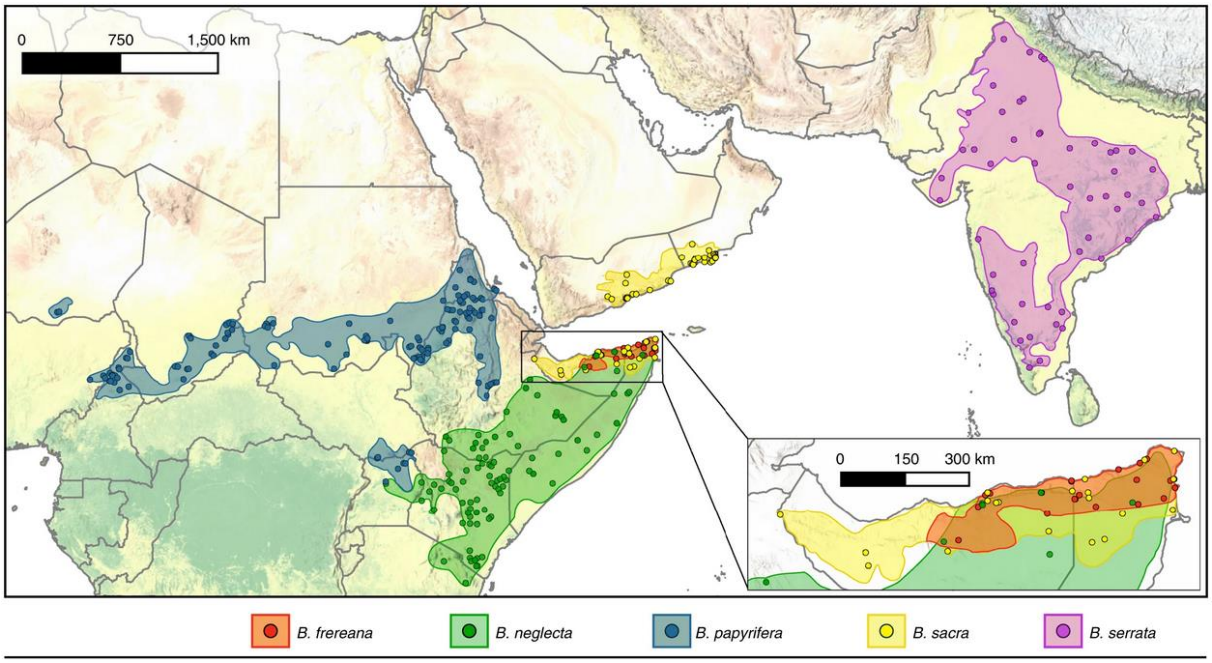

Fig. 5.4 Map showing the distribution of Boswellia species in East Africa, the Arabian Peninsula and India (modified after Bongers et al. 2019).

In conclusion, the case studies in this thesis have underscored that trade networks were not just with neighbouring regions but spanned vast distances, hinting at a 'global' scale of trade for the Old World. They showed the potential expansive reach of ancient trade networks during the 2nd millennium, as well as shorter trade connections into regions where there is limited evidence for trade connections so far, as for example a possible connection between ancient Egypt and central Europe. Additionally, previously-known trade connections, such as the networks between Egypt and the Levant or the inner Arabian trade network were reinforced. The presence of aromatic substances in regions where they did not naturally occur serves as a testament to these trade connections. The findings from this thesis not only shed light on the kinds of aromatic materials that were traded and when, but also challenge and expand the understanding of the directionality and scale of these trade networks. The aromatic trade, with its myriad of substances and sources, serves as a window into the interconnected world of ancient civilizations, their relationships, and their pursuit of valuable commodities.

5.2.2 Exploring consumption, use and the role of scent

This section delves into how aromatic substances have been used in the past and the roles scents played in ancient Arabia and Egypt. By focusing on these substances, three distinct, yet interconnected, aspects of ancient societies are brought to the forefront. Firstly, the significance of scent in funerary contexts is examined, highlighting its importance in rituals, such as mummification and its presence in the Tayma necropolis. Secondly, the narrative shifts to the societal implications of scent, exploring olfactory hierarchies in ancient Egypt and how aromatics might have been markers of social distinction. Lastly, the practical applications of these substances are addressed, encompassing their bioactive properties and their roles in sanitation, preservation and as disinfectants. This comprehensive exploration aims to shed light on the profound influence of aromatic substances in various spheres of ancient life.

Understanding practices and rituals

Scents have historically held an important role in rituals and religious practices. Rituals often employed fragrances to delineate 'special' spaces or events, setting them apart from the mundane and everyday (Bradley 2015a). This not only heightened the sensory experience but also underscored the sanctity and significance of the occasion. Certain smells can become a “signifier” of sacral contexts, bridging the gap between the secular and the divine (Kenna

2005). In the realm of funerary rituals, aromatic substances played a particularly important role in the mummification practices of ancient Egypt. An often-overlooked facet of this intricate process is the profound role of scent (Goldsmith 2019; Price 2022). The ancient Egyptians, with their nuanced and rich olfactory vocabulary, placed a heightened emphasis on the sense of smell in both life and death, distinguishing them from many contemporary societies (Goldsmith 2021). The mummification ritual embraced the olfactive elements of the foul and the divine. Scents served a dual purpose: they masked the pungent aroma of decay and played a pivotal role in the religious belief system with regards to the afterlife (Schiødt 2020). Within this worldview, pleasant fragrances were emblematic of divinity and the soul's purity, while foul odours were harbingers of decay, as demonstrated by ancient written sources. Spell 154 from the ancient Egyptian *Book of the Dead* vividly illustrates this perspective: "His flesh becomes evil (smelling). He stinks. He decays. He turns into countess worms, completely into worms[1]" (BD 154, Book of the Dead of Nu, BM EA10477, London). The text reveals that the ancient Egyptians equated malodorous scents with bodily decomposition – the very phenomenon that mummification should prevent. This underscores the profound importance of the application of aromatic substances, not just as preservatives, but as spiritual safeguards ensuring the deceased's journey to the afterlife remained untainted by the stench of decay.

In funerary rituals, specific scents could have also been chosen deliberately, serving as potent memory triggers and evoking recollections of the deceased. In the graveyard at Tayma, several incense burners containing burned *Commiphora* resin were discovered in Iron Age tombs alongside the deceased. This substance was only used in the context of burials and was found nowhere else at the oasis. In this particular context, this incense material could have been a part of *rites de passage* and an olfactory sign, aiding in the remembrance of the departed (Toner 2015). As previously noted, odours can profoundly enhance our emotional memory (Herz and Engen 1996; Reinarz 2014). This is because the olfactory organ is intricately connected to the amygdala hippocampus complex responsible for processing memory and emotions (Cahill et al. 1995; Zald and Pardo 1997). Consequently, the emotional resonance of certain scents can leave a lasting impression, aiding in the recollection of the ancestors. The

[1] Translated by the author from the German translation by Burkhard Backes: "Sein Fleisch ist zu etwas schlecht (riechendem) geworden. Er stinkt. Er verfault. Er wird ganz zu vielen Würmern, ganz zu Würmern." TM 134299, Papyrus, London; https://totenbuch.awk.nrw.de/spruch/154

employment of *Commiphora* resin, such as myrrh – a species of *Commiphora*, in this context is noteworthy. Apart from ancient Arabia, myrrh has a history of use in funerary practices in later periods. For instance, the Bible recounts that Nicodemus used a mixture of myrrh and aloes to anoint Jesus Christ's body during the burial, highlighting the resin's importance in Judaeo-Christian burial traditions (John 19:39). Similarly, in ancient Greece, myrrh was a valued ingredient in perfumed oils, often used to anoint the body in funerary rites such as the *prothesis*, or laying out of the body (Morris 1992). The consistent use of myrrh across diverse cultures underscores its universal significance in mourning, remembrance, and anointing the dead.

Myrrh was also mentioned in various ancient Egyptian texts in the context of funerary rituals and mummification (Ikram and Dodson 1998). While the word *'ntjw'* (commonly translated as myrrh) frequently occurs in these texts, systematic chemical analyses of mummification balms have not identified its presence in any of the analytical studies (e.g. Buckley et al. 2004; Charrié-Duhaut et al. 2007; Brettell et al. 2017a; Łucejko et al. 2017; Brockbals et al. 2018; Evershed and Clark 2020; Fulcher et al. 2021; Vandenbeusch et al. 2021; Rageot et al. 2023). The resin was also not detected in the case studies of this thesis, although it was specifically screened for. This discrepancy raises significant questions about the historical and linguistic interpretations of ancient Egyptian practices and refers to the debate surrounding the term *'ntjw'* (antiu) (see section 5.1). Traditionally, *'ntjw'* has been translated as myrrh (Germer 2008), but the absence of myrrh in the chemical analyses suggests that this term might denote a different substance altogether or might have a more generic meaning. These challenges in the translation have implications for the understanding of ancient Egyptian ritualistic practices and the specific ingredients they employed. It also emphasizes the importance of aligning philological interpretations with chemical data to ensure a comprehensive and accurate understanding of ancient Egyptian material culture and practices.

However, the consistent identification of coniferous resins in mummification balms from the inception of the practice through to the Graeco-Roman era (Buckley and Evershed 2001; Jones et al. 2014, 2018), and also in both case studies of this thesis, emphasizes the centrality of these resins in the embalming process. These resins were likely chosen initially for their practical benefits in preservation (see section below). Yet, over time, their distinctive

woody and resinous aroma might have taken on a deeper, symbolic significance. This scent could have evolved into an olfactory hallmark of the mummification process. As the practice persisted through generations, the aroma of conifer resin might have come to evoke immediate associations with mummification rituals, becoming emblematic of the journey to the afterlife and embedding itself in the cultural memory and traditions of ancient Egyptian society.

Furthermore, the analysis of the balms from Senetnay's two jars unveiled intriguing differences in their composition, suggesting the potential for organ-specific mummification practices. Specifically, the balm from canopic jar 1, which held Senetnay's lungs, contained the aromatic resin, possibly dammar or *Pistacia*, and the compound larixol, indicative of larch resin. These components were absent in jar 2, which preserved her liver. While the core ingredients of the balms in both jars were largely similar, their ratios also varied. This disparity in balm composition raises the compelling possibility that ancient Egyptian mummification processes might have employed distinct balms tailored for specific organs. This hypothesis needs to be considered with caution, as it is possible that the balms were initially heterogeneous, with ingredients not being uniformly mixed or distributed. Nevertheless, the idea of organ-specific balms finds some resonance in the aforementioned study from the mummification workshop at Saqqara (Rageot et al. 2023). In that case, vessels for preparing the mummification balms for the liver and the stomach also indicated distinct mixtures for specific organs. In the study from Saqqara, however, samples were taken from vessels for balm preparation and not from canopic jars, which contain the "final" balm. Such findings shed new light on mummification rituals, emphasizing the meticulous care and specificity that might have been involved. These observation underscores the importance of further research in this area, particularly the comprehensive analysis of an entire set of canopic jars to investigate the potential organ-specificity of mummification balms.

Understanding status and identity

In ancient societies, the use of aromatic products was not just a matter of personal preference or religious ritual, scents were also symbols of wealth, power, and prestige (Keay 2008). They were deeply intertwined with societal structures and identity, and instrumental in differentiating between various societal groups. Keay's (2008) exploration of spices and aromatics underscores their relationship with power, highlighting how these fragrances were

symbolic of prestige and influence. Furthermore, the distinction between high and low status was often emphasized through the use of specific aromatics. This is exemplified in the case study of Senetnay (Manuscript C), the wet nurse to the future pharaoh Amenhotep II. Given the special treatment her remains received, it is noteworthy that her mummification balm is the most intricate identified from this early period thus far. This complexity and the inclusion of rare and exotic ingredients in her balm underscores her elevated social rank. Other lines of evidence, such as her burial within the Valley of the Kings, a necropolis predominantly designated for pharaohs, signifies an extraordinary privilege, suggesting her pivotal role within the Pharaoh's inner circle. This esteemed position is further emphasized by her title, "Ornament of the King" (Loeben 2005).

The archaeological findings at Tayma (Manuscript D) further emphasize the societal implications of scent usage. The choice of aromatic substances found at this site offers insights into the wealth of the people and the role of the oasis within trade economies. The findings of frankincense in the private houses and storage spaces of the residents suggests that they actively engaged in buying frankincense for personal consumption. This indicates that the oasis served not just as a stopover on the incense trade route but also as an active participant in consumption. A significant number of burners from the residential areas can be traced back to the Nabataean and Roman periods. During this time, frankincense was considered a luxury and was exceedingly expensive, as noted by contemporary Roman author Pliny (*Natural History*, Bk. XII, Chap. 41). This suggests that Tayma's residents had the means to acquire such high-end items, possibly aiming to partake in the use of commodities associated with the elite. Although the exact social standing of these residents remains uncertain based on the available archaeological data, it is evident through the research presented here that they had the resources to indulge in such luxuries. However, it needs to be noted that the cost of frankincense in Tayma might have been more affordable than in distant Rome. The high price of *Boswellia* resin in the Mediterranean was attributed to the multiple tolls collected *en route* (Archibald 2011). With Tayma being closer to the source, it is likely that fewer tolls impacted the final price, making frankincense more accessible. This evidence from Tayma highlights the cultural and societal importance of aromatic substances in shaping identities and lifestyles in antiquity.

Understanding sanitation and preservation

Ancient societies also harnessed the pharmacological and sanitary potential of plants and minerals at their disposal. Aromatics, in particular, held a special place, not just for their fragrant allure, but for their strong pharmacological qualities. The ancient Egyptians had a profound understanding of the medicinal potential of plants (Germer 2008). Among the many natural products utilized, resins, tars, and herbs stood out, which possessed potent bioactive properties, such as antimicrobial, antibacterial and antifungal capabilities (Abegaz and Kinfe 2019). One such substance, cedar tar, was identified in one of the case studies of this thesis as the main ingredient in the mummification balm (Manuscript E). Modern studies have investigated the properties of cedar, revealing that its active compounds, himachalenes, show antimicrobial, antifungal, and insecticidal effects (Saab et al. 2005; Loizzo et al. 2008; Skanderi and Chouitah 2020). In particular, the compound himachalol identified in the case study, exhibits strong insecticidal effects, aiding in repelling pests (Singh and Agarwal 1988). Research has also highlighted cedar tar's inhibitory activity on bacterial growth and its potential to act against various fungi (Pekgözlü et al. 2017; Mercimek Takic et al. 2020).

Recognizing the susceptibility of the deceased body to decay, the Egyptians turned to materials rich in reactive compounds to ensure the long-lasting preservation of biological tissue. These findings demonstrate the ancient Egyptians' botanical knowledge, especially concerning the activity of certain plants and the reason for selecting them. Moreover, cedar tar seems to have served a multifaceted purpose in this practice. Not only did cedar play a role in preservation, but its distinct aroma also had practical benefits, as it naturally acts as a deterrent to moths and insects. This dual functionality, both as a preservative and a repellent, underscores the depth of pharmacological understanding and the strategic use of materials.

Moreover, the prevalent use of frankincense in the residential quarters of Tayma hints at its use in practical and disinfecting purposes. Given the architectural design of these buildings, which often incorporated storage compartments, it is plausible that frankincense was burned as a preventive measure against pests and vermin that could potentially damage stored goods. The bioactive properties of frankincense made it effective in purifying microbially-contaminated air, reducing harmful microbes and creating a fresher and healthier environment (Ljaljević Grbić et al. 2018). This protective use of frankincense in the past is not without precedent. Classical authors (cf. Cato, *De Agri Cultura,* Chap. LXX; Celsus, *De Medicina,*

Bk. V), have highlighted the disinfectant properties of frankincense. It was not merely an aromatic luxury; it played a pivotal role in safeguarding goods and maintaining a hygienic environment. Its dual function, both as a pleasant aroma and as a protective agent, showcases the practical adaptability of such substances in daily life.

In conclusion, the multifaceted roles of aromatic substances in ancient societies, from their spiritual significance in rituals to their practical applications in sanitation and preservation, demonstrate their important role in shaping cultural, spiritual, and daily life. The ancient Egyptians and communities of Arabia harnessed the power of these substances for a myriad of purposes, reflecting their versatility and importance. Whether used in the sacred rites of mummification, as markers of social distinction, or as protective agents in homes, these aromatics were more than just fragrant additions; they were integral parts of the social structure. Their consistent and varied use across different contexts and over long timespans highlights the depth of their significance.

6. Outlook - Future Directions in the Biomolecular Archaeology of Scent

The study of past olfactory phenomena represents a rapidly evolving domain. In just the past couple of years, several interdisciplinary research initiatives on past smells – such as *Odeuropa*[2], *Alchemies of Scent*[3], *SENSIS: The senses of Islam*[4] and *Odotheka*[5] – have surfaced, bridging the disciplines of heritage science, chemistry, computer science, history, and archaeology. At this intersection, diverse research pathways are unfolding, each holding the promise of enhancing the comprehension of the multifaceted interactions past societies maintained with their aromatic environments.

6.1. Methodological outlook

Within this framework, a biomolecular and biochemical approach to ancient aromatics and scents offers a unique lens by transforming the understanding of what are often ephemeral phenomena into more tangible evidence. While the case studies in this thesis predominantly focused on the analysis of secondary metabolites and lipids to elucidate ancient aromatic compositions, there exists potential to broaden this scope. Incorporating the study of other biomolecules, such as ancient DNA or proteins, could further enrich our knowledge. Techniques like genomics and proteomics, which provide high-throughput, comprehensive assessments of specific sets of biological molecules, can be particularly instrumental in this endeavour (for a short overview see also Manuscript A). While the primary focus of proteomics studies in archaeology has often been on animal-derived proteins (Cappellini et al. 2018; Hendy et al. 2018; Wilkin et al. 2021; Bleasdale et al. 2021; Tang et al. 2023), recent advancements have expanded its application to plant-derived proteins as well. For example, Scott et al. (2020) applied proteomics analysis to dental calculus (ancient tartar). Their study identified a vast array of proteins within the dental calculus, revealing also the consumption of spices, such as sesame and turmeric. Such findings can provide direct evidence of the consumption of these plants, shedding light on the culinary aromas, flavours and cuisines.

The application of ancient DNA analysis and genomics shows another avenue for understanding the olfactory past of human ancestors and their adaptive strategies to diverse

[2] https://odeuropa.eu/
[3] https://www.alchemiesofscent.org/
[4] https://sensis.sites.uu.nl/
[5] https://hslab.fkkt.uni-lj.si/

environments. An example of this approach is the study conducted by De March et al. (2023), where they investigated the olfactory capabilities of different hominin populations, including anatomically modern humans, Neanderthals and Denisovans, by analysing ancient DNA sequences associated with thirty odorant receptor genes. Their findings revealed that while Neanderthals and Denisovans exhibited a high degree of conservation in their olfactory receptor sequences, modern humans displayed more variation. These variations might have led to differences in odour detection and perception among these species. Interestingly, while some Neanderthal variants appeared to impair olfactory function, Denisovan variants enhanced sensitivity, particularly to sweet and sulphurous odours. Such genomic insights suggest that while the genus *Homo* shared a core olfactory repertoire, there might have been local ecological adaptations that influenced olfactory capabilities. Such studies underscore the potential of genomics in shedding light on the evolutionary nuances of olfaction, offering a deeper understanding of how our ancestors perceived and interacted with their aromatic surroundings.

In the context of 'omics' technologies for studying olfactory phenomena, the integration of diverse biomolecular techniques offers a potential new direction. Utilizing a multi-omics approach can yield a more holistic and detailed understanding. An example for such an approach is the study of dental calculus samples from an individual in Trondheim, Norway, where proteomics and ancient DNA analysis were employed (Fotakis et al. 2020). The study successfully recovered the genome of *Mycobacterium leprae*, the bacterium responsible for leprosy, which was further validated using mass spectrometry-based proteomics. In addition to detecting the pathogen, the study also provided insights into the individual's diet and health, as well as the phylogenetic placement of the detected strain of *Mycobacterium leprae*. Such multi-omics strategies can also be useful when examining intricate mixtures of aromatic substances, like perfumes and cosmetics. Given the diverse range of ingredients that could be present, spanning from plant-based compounds to animal-derived substances, multi analytical methods might offer more holistic results. In the studies of the thesis, extraction methods were already tailored for multi-omics applications, for example by isolating hydrophobic lipids, polar metabolites, and proteins from a single sample (see Manuscripts C and E). However, due to existing constraints in protein reference sequences for aromatic substances in databases, the proteomic component remains unanalysed, pending further development in this field.

6.2. Beyond Fragrance: Olfactory storytelling in museums

Recreations of smells and olfactory storytelling in museums represent an emerging trend, aiming to immerse visitors in a multisensory experience and foster a deeper connection with exhibits (Marx et al. 2022). Several institutions have already embraced scent as a storytelling medium. For instance, The Jorvik Viking Centre in York is renowned for its recreation of Viking-era York smells[6], while The Rijksmuseum in Amsterdam has offered olfactory tours[7], allowing visitors to experience scents associated with specific artworks. In alignment with this trend, this thesis incorporated an olfactory dimension by recreating the scent of the mummification balm of Senetnay (as detailed in Manuscript C). The biomolecular data from this study yielded the recipe and an 'odour-fingerprint' of the balm. Collaborating with the perfumer Carole Calvez and the olfactory museologist Sofia Colette Ehrich, the recreation, coined "The Scent of the Afterlife", was then transformed into a 'smell-card' (see Appendix 4). The primary aim of this initiative was to showcase a tangible application of ancient scent research, suitable for museums and exhibitions to facilitate multisensory knowledge transfer. Currently, these smell cards are accessible to visitors at the Museum August Kestner in Hannover and the Museu Egipci in Barcelona. Additionally, the ambiance scent is featured in a unique format at the Moesgaard Museum in Aarhus during the special exhibition "Ancient Egypt – Obsessed with Life", running from October 2023 to August 2024.

Making new research results on ancient mummification accessible to a broad public of all ages holds significant societal importance, especially in the realm of communicating science and inclusivity in museums. Such an approach not only bridges a deep temporal divide but also provides an avenue for visually impaired individuals to engage more fully in exhibitions (Verbeek et al. 2022). The recreation of these ancient scents for museum displays offers visitors an immersive, multisensory journey, fostering a connection between past and present through the unique lens of olfaction. Furthermore, the integration of contemporary technological advancements, such as virtual or augmented reality platforms, suggests the potential for innovative 'olfactory tours'. This way we can ensure that history is not just seen or heard, but also felt and smelt.

[6] https://www.jorvikvikingcentre.co.uk/press/norse-ty-niffs-historic-aroma-packages-trialled-bring-vikings-smells-home; accessed 11.10.23

[7] https://www.rijksmuseum.nl/en/whats-on/accessibility/family-tour-for-visitors-with-a-visual-impairment, ; accessed 11.10.23

References

Abegaz BM, Kinfe HH (2019) Secondary metabolites, their structural diversity, bioactivity, and ecological functions: An overview. Physical Sciences Reviews 4:20180100. https://doi.org/10.1515/psr-2018-0100

Al-Jahwari NS, Douglas KA (2021) Umm an-Nar Ritual Building in Dahwa 1 (DH1), Northern Al-Batinah, Oman. Near Eastern Archaeology 84:262–271. https://doi.org/10.1086/716828

Allen K (2015) Stop and Smell the Romans: Odor in Roman Literature. Dissertation, University of Michigan

Allwood JW, De Vos RCH, Moing A, et al (2011) Plant Metabolomics and Its Potential for Systems Biology Research. In: Jameson D, Verma M, Westerhoff HV (eds) Methods in systems biology. Elsevier, pp 299–336

Al-Salameen Z (2011) The Nabataeans and Asia Minor. Mediterranean Archaeology and Archaeometry 11:55–78

Apfelbach R, Blanchard CD, Blanchard RJ, et al (2005) The effects of predator odors in mammalian prey species: A review of field and laboratory studies. Neuroscience & Biobehavioral Reviews 29:1123–1144. https://doi.org/10.1016/j.neubiorev.2005.05.005

Archibald ZH (2011) Hellenistic economies. Routledge, London

Arie E, Rosen B, Namdar D (2020) Cannabis and Frankincense at the Judahite Shrine of Arad. Tel Aviv 47:5–28. https://doi.org/10.1080/03344355.2020.1732046

Arimura G, Maffei M (eds) (2021) Plant specialized metabolism: genomics, biochemistry, and biological functions, First issued in paperback. CRC Press, Taylor & Francis Group, Boca Raton London New York

Avanzini A (ed) (1997) Profumi d'Arabia. Atti del convegno. L'Erma di Bretschneider, Rome

Baeten J, Deforce K, Challe S, et al (2014) Holy smoke in medieval funerary rites: chemical fingerprints of frankincense in southern Belgian incense burners. PLOS ONE 9:e113142. https://doi.org/10.1371/journal.pone.0113142

Baltussen H (2015) Ancient philosophers on the sense of smell. In: Bradley M (ed) Smell and the ancient senses. Routledge, London, pp 30–45

Bar-Oz G, Galili R, Fuks D, et al (2022) Caravanserai middens on desert roads: a new perspective on the Nabataean–Roman trade network across the Negev. Antiquity 96:592–610. https://doi.org/10.15184/aqy.2022.40

Bauer K, Garbe D, Surburg H (2008) Common Fragrance and Flavor Materials: Preparation, Properties and Uses, 3rd ed. John Wiley & Sons, Incorporated, Berlin

Baum N (1994) Snṯr. Une révision. Revue d'Égyptologie 17–39. https://doi.org/10.2143/RE.45.0.2011235

Beck T (2013) Principles of ecological landscape design. Island Press, Washington, DC

Bembibre C, Strlič M (2017) Smell of heritage: a framework for the identification, analysis and archival of historic odours. Herit Sci 5:2. https://doi.org/10.1186/s40494-016-0114-1

Benton MJ, Wilf P, Sauquet H (2022) The Angiosperm Terrestrial Revolution and the origins of modern biodiversity. New Phytologist 233:2017–2035. https://doi.org/10.1111/nph.17822

Bertelli I, Mattonai M, La Nasa J, Ribechini E (2023) Study of thermal behavior and molecular composition of mixtures of resinous materials and beeswax found as adhesives in archaeological finds. Journal of Analytical and Applied Pyrolysis 171:105936. https://doi.org/10.1016/j.jaap.2023.105936

Betts E (2017) Senses of the empire: multisensory approaches to Roman culture. Routledge, London; New York

Blanchette RA (2000) A review of microbial deterioration found in archaeological wood from different environments. International Biodeterioration & Biodegradation 46:189–204. https://doi.org/10.1016/S0964-8305(00)00077-9

Bleasdale M, Richter KK, Janzen A, et al (2021) Ancient proteins provide evidence of dairy consumption in eastern Africa. Nat Commun 12:632. https://doi.org/10.1038/s41467-020-20682-3

Böck B (2011) Sourcing, Organizing, and Administering Medical Ingredients. In: Radner K, Robson E (eds) The Oxford Handbook of Cuneiform Culture. Oxford University Press, pp 690–705

Boivin N (2017) Proto-globalisation and biotic exchange in the Old World. In: Boivin N, Crassard R, Petraglia M (eds) Human Dispersal and Species Movement, 1st edn. Cambridge University Press, pp 349–408

Boivin N, Frachetti MD (eds) (2018) Globalization in Prehistory: Contact, Exchange, and the "People Without History." Cambridge University Press, Cambridge, New York

Boivin N, Fuller DQ (2009) Shell Middens, Ships and Seeds: Exploring Coastal Subsistence, Maritime Trade and the Dispersal of Domesticates in and Around the Ancient Arabian Peninsula. J World Prehist 22:113–180. https://doi.org/10.1007/s10963-009-9018-2

Bongers F, Groenendijk P, Bekele T, et al (2019) Frankincense in peril. Nat Sustain 2:602–610. https://doi.org/10.1038/s41893-019-0322-2

Bothe F, Dettmer K, Engewald W (2003) Determination of perfume oil in household products by headspace solid-phase microextraction and fast capillary gas chromatography. Chromatographia 57:199–206. https://doi.org/10.1007/BF02492103

Bradford KD, Desrochers DM (2009) The Use of Scents to Influence Consumers: The Sense of Using Scents to Make Cents. J Bus Ethics 90:141–153. https://doi.org/10.1007/s10551-010-0377-5

Bradley M (2015a) Smell and the ancient senses. Routledge, London

Bradley M (2015b) Foul bodies in ancient Rome. In: Bradley M (ed) Smell and the ancient senses. Routledge, London, pp 133–145

Brettell R, Martin W, Atherton-Woolham S, et al (2017a) Organic residue analysis of Egyptian votive mummies and their research potential. Studies in Conservation 62:68–82. https://doi.org/10.1179/2047058415Y.0000000027

Brettell R, Schotsmans E, Matin W, et al (2017b) The Final Masquerade: Resinous Substances and Roman Mortuary Rites. In: Livarda A, Madgwick R, Mora SR (eds) The Bioarchaeology of Ritual and Religion. Oxbow Books, pp 44–57

Brettell RC, Stern B, Reifarth N, Heron C (2014) The 'Semblance of Immortality'? Resinous Materials and Mortuary Rites in Roman Britain. Archaeometry 56:444–459. https://doi.org/10.1111/arcm.12027

Brock WH (1993) The Norton history of chemistry, 1st American ed. W.W. Norton, New York

Brockbals L, Habicht M, Hajdas I, et al (2018) Untargeted metabolomics-like screening approach for chemical characterization and differentiation of canopic jar and mummy samples from Ancient Egypt using GC-high resolution MS. Analyst 143:4503–4512. https://doi.org/10.1039/C8AN01288A

Brunschwig C, Collard FX, Bianchini J-P, Raharivelomanana P (2009) Evaluation of Chemical Variability of Cured Vanilla Beans (*Vanilla tahitensis* and *Vanilla planifolia*). Natural Product Communications 4:1934578X0900401. https://doi.org/10.1177/1934578X0900401016

Buccellati G, Kelly-Buccellati M (2019) Terqa: The First Eight Seasons. Annales Archéologiques Arabes Syriens 33:47–67. https://doi.org/10.6084/M9.FIGSHARE.8293511.V1

Buckley SA, Clark KA, Evershed RP (2004) Complex organic chemical balms of Pharaonic animal mummies. Nature 431:294–299. https://doi.org/10.1038/nature02849

Buckley SA, Evershed RP (2001) Organic chemistry of embalming agents in Pharaonic and Graeco-Roman mummies. Nature 413:837–841. https://doi.org/10.1038/35101588

Butler S (2015) Making scents of poetry. In: Bradley M (ed) Smell and the ancient senses. Routledge, London, pp 74–89

Cahill L, Babinsky R, Markowitsch HJ, McGaugh JL (1995) The amygdala and emotional memory. Nature 377:295–296. https://doi.org/10.1038/377295a0

Capetti F, Rubiolo P, Bicchi C, et al (2020) Exploiting the versatility of vacuum-assisted headspace solid-phase microextraction in combination with the selectivity of ionic liquid-based GC stationary phases to discriminate *Boswellia* spp. resins through their volatile and semivolatile fractions. J Sep Sci 43:1879–1889. https://doi.org/10.1002/jssc.202000084

Cappellini E, Prohaska A, Racimo F, et al (2018) Ancient Biomolecules and Evolutionary Inference. Annu Rev Biochem 87:1029–1060. https://doi.org/10.1146/annurev-biochem-062917-012002

Cato MP 234 B.C.-149 B.C (1998) De Agricultura. Prospect Books, Blackawton, Devon

Cavigneaux A, Ismail Khalil B (1990) Die Statthalter von Suhu und Mari im 8. Jh. v. Chr. Baghdader Mitteilungen 21:321–456

Celsus/ transl. W. G. Spencer (1935-1938) On Medicine. Cambridge, MA: Harvard University Press.

Charrié-Duhaut A, Connan J, Rouquette N, et al (2007) The canopic jars of Rameses II: real use revealed by molecular study of organic residues. Journal of Archaeological Science 34:957–967. https://doi.org/10.1016/j.jas.2006.09.012

Clancy D (2019) The Smell of Grief: Odour and Olfaction at the Roman Funeral. THERSITES Journal for Transcultural Presences & Diachronic Identities from Antiquity to Date 89-116 Pages. https://doi.org/10.34679/THERSITES.VOL9.120

Classen C, Howes D, Synnott A (1994) Aroma: The Cultural History of Smell. Routledge, London

Clements A (2015) Divine scents and presence. In: Bradley M (ed) Smell and the ancient senses. Routledge, London, pp 46–59

Cleuziou S, Méry S (2002) In-between the great powers: The Bronze Age Oman peninsula. In: Cleuziou S, Tosi M, Zarins J (eds) Essays on the late prehistory of the Arabian Peninsula. Istituto italiano per l'Africa e l'Oriente : Distributed by Herder, InternationalBook Center, Roma, pp 273–316

Colombini MP, Lucejko JJ, Modugno F, et al (2009) A multi-analytical study of degradation of lignin in archaeological waterlogged wood. Talanta 80:61–70. https://doi.org/10.1016/j.talanta.2009.06.024

Colombini MP, Modugno F (eds) (2009) Organic Mass Spectrometry in Art and Archaeology. John Wiley & Sons, Ltd, Chichester, UK

Craig OE, Saul H, Spiteri C (2019) Residue Analysis. In: Richards M, Britton K (eds) Archaeological Science, 1st edn. Cambridge University Press, pp 70–98

Creasman PP, Yamamoto K (2019) The African Incense Trade and Its Impacts in Pharaonic Egypt. Afr Archaeol Rev 36:347–365. https://doi.org/10.1007/s10437-019-09348-8

Crowther A, Veall M-A, Boivin N, et al (2015) Use of Zanzibar copal (Hymenaea verrucosa Gaertn.) as incense at Unguja Ukuu, Tanzania in the 7–8th century CE: chemical insights into trade and Indian Ocean interactions. Journal of Archaeological Science 53:374–390. https://doi.org/10.1016/j.jas.2014.10.008

Day J (2013a) Making senses of the past: toward a sensory archaeology. Southern Illinois University Press, Carbondale

Day J (2013b) Imagined Aromas and Artificial Flowers in Minoan Society. In: Day J (ed) Making Senses of the Past: Toward a Sensory Archaeology. Southern Illinois University Press., Carbondale, pp 286–309

Day J (2017) Scents of place and colours of smell: Fragranced entertainment in ancient Rome. In: Betts E (ed) Senses of the empire: multisensory approaches to Roman culture. Routledge, London; New York, pp 176–192

De March CA, Matsunami H, Abe M, et al (2023) Genetic and functional odorant receptor variation in the Homo lineage. iScience 26:105908. https://doi.org/10.1016/j.isci.2022.105908

Devièse T, Ribechini E, Castex D, et al (2017) A multi-analytical approach using FTIR, GC/MS and Py-GC/MS revealed early evidence of embalming practices in Roman catacombs. Microchemical Journal 133:49–59. https://doi.org/10.1016/j.microc.2017.03.012

Dinies M, Neef R, Plessen B, Kürschner H (2016) Holocene vegetation, climate, land use and plant cultivation in the Tayma region, northwestern Arabia. In: Luciani M (ed) The archaeology of North Arabia: oases and landscapes; proceedings of the international congress held at the

University of Vienna, 5-8 December, 2013. Austrian Academy of Sciences Press, Wien, pp 57–77

Dinies M, Plessen B, Neef R, Kürschner H (2015) When the desert was green: Grassland expansion during the early Holocene in northwestern Arabia. Quaternary International 382:293–302. https://doi.org/10.1016/j.quaint.2015.03.007

Draycott J (2015) Smelling trees, flowers and herbs in the ancient world. In: Bradley M (ed) Smell and the ancient senses. Routledge, London, pp 60–73

Duce C, Orsini S, Spepi A, et al (2015) Thermal degradation chemistry of archaeological pine pitch containing beeswax as an additive. Journal of Analytical and Applied Pyrolysis 111:254–264. https://doi.org/10.1016/j.jaap.2014.10.020

Dudd SN, Regert M, Evershed RP (1998) Assessing microbial lipid contributions during laboratory degradations of fats and oils and pure triacylglycerols absorbed in ceramic potsherds. Organic Geochemistry 29:1345–1354. https://doi.org/10.1016/S0146-6380(98)00093-X

Dumitru IA, Harrower MJ (2018) From Rural Collectables to Global Commodities: Copper from Oman and Obsidian from Ethiopia. In: Boivin N, Frachetti MD (eds) Globalization in Prehistory, 1st edn. Cambridge University Press, pp 232–262

El Hadidi MN, Hosni HA (1996) Biodiversity in the flora of Egypt. In: Van Der Maesen LJG, Van Der Burgt XM, Van Medenbach De Rooy JM (eds) The Biodiversity of African Plants. Springer Netherlands, Dordrecht, pp 785–787

Erickson-Gini T, Israel Y (2013) Excavating the Nabataean Incense Road. Journal of Eastern Mediterranean Archaeology and Heritage Studies 1:24–53

Evershed R P (2008) Organic Residue Analysis in Archaeology: The Archaeological Biomarker Revolution. Archaeometry 50:895–924. https://doi.org/10.1111/j.1475-4754.2008.00446.x

Evershed RP (1993) Biomolecular archaeology and lipids. World Archaeology 25:74–93. https://doi.org/10.1080/00438243.1993.9980229

Evershed RP, Clark KA (2020) Trends in Use of Organic Balms in Egyptian Mummification Revealed Through Biomolecular Analyses. In: Shin DH, Bianucci R (eds) The Handbook of Mummy Studies: New Frontiers in Scientific and Cultural Perspectives. Springer, Singapore, pp 1–63

Evershed RP, Heron C, Goad LJ (1990) Analysis of organic residues of archaeological origin by high-temperature gas chromatography and gas chromatography-mass spectrometry. Analyst 115:1339–1342. https://doi.org/10.1039/AN9901501339

Farjon A (2016) Conifers of the world. Resources for conifer research.

Farjon A, Filer D (2013) An atlas of the world's conifers: an analysis of their distribution, biogeography, diversity, and conservation status. Brill, Leiden ; Boston

Farsi H, Harti D, Rachid Achaâban M, et al (2022) Seasonal variations in locomotor activity rhythm and diurnal activity in the dromedary camel *(Camelus dromedarius)* under mesic semi-natural conditions. Chronobiology International 39:129–150. https://doi.org/10.1080/07420528.2021.1984936

Fotakis AK, Denham SD, Mackie M, et al (2020) Multi-omic detection of *Mycobacterium leprae* in archaeological human dental calculus. Phil Trans R Soc B 375:20190584. https://doi.org/10.1098/rstb.2019.0584

Frankopan P (2016) The silk roads: a new history of the world, Paperback edition. Bloomsbury, London Oxford New York New Delhi Sydney

Fulcher K, Serpico M, Taylor JH, Stacey R (2021) Molecular analysis of black coatings and anointing fluids from ancient Egyptian coffins, mummy cases, and funerary objects. PNAS 118:e2100885118. https://doi.org/10.1073/pnas.2100885118

Geary DC, Vigil J, Byrd-Craven J (2004) Evolution of human mate choice. Journal of Sex Research 41:27–42. https://doi.org/10.1080/00224490409552211

Gelbrich J, Mai C, Militz H (2008) Chemical changes in wood degraded by bacteria. International Biodeterioration & Biodegradation 61:24–32. https://doi.org/10.1016/j.ibiod.2007.06.007

Geller MJ (2010) Ancient Babylonian Medicine. Wiley-Blackwell, Oxford, UK

Geller MJ, Panayotov SV (2018) Mesopotamian Eye Disease Texts: the nineveh treatise. De Gruyter, Berlin, Boston

Germer R (2005) Mumien. Albatros, Düsseldorf

Germer R (2008) Handbuch der altägyptischen Heilpflanzen. Harrassowitz, Wiesbaden

Giachi G, Pallecchi P, Romualdi A, et al (2013) Ingredients of a 2,000-y-old medicine revealed by chemical, mineralogical, and botanical investigations. Proc Natl Acad Sci USA 110:1193–1196. https://doi.org/10.1073/pnas.1216776110

Gilboa A, Namdar D (2015) On the Beginnings of South Asian Spice Trade with the Mediterranean Region: A Review. Radiocarbon 57:265–283. https://doi.org/10.2458/azu_rc.57.18562

Gjelstrup Björdal C (2012) Microbial degradation of waterlogged archaeological wood. Journal of Cultural Heritage 13:118–122. https://doi.org/10.1016/j.culher.2012.02.003

Goldsmith D (2019) Fish, fowl, and stench in ancient Egypt. In: Schellenberg A, Krüger T (eds) Sounding Sensory Profiles in the Ancient Near East. SBL Press, Atlanta, pp 335–360

Goldsmith D (2021) Smellscapes in ancient Egypt. In: Neumann K, Thomason A (eds) The Routledge Handbook of the Senses in the Ancient Near East. Routledge Taylor & Francis, Abingdon, Oxon ; New York, NY

Gottfried JA, Dolan RJ (2003) The Nose Smells What the Eye Sees. Neuron 39:375–386. https://doi.org/10.1016/S0896-6273(03)00392-1

Green DA (2015) Fragrance in the rabbinic world. In: Bradley M (ed) Smell and the ancient senses. Routledge, London, pp 146–157

Groom N (1981) Frankincense and Myrrh: a study of the Arabian incense trade., 1. publ. Longman, London u.a.

Haibt M (2018) The Eastern Sabkha of Tayma. Analysis of a 4th millennium BC Carnelian Bead Production Centre in Northwest Arabia (master thesis, Freie Universität Berlin).

Hamilakis Y (2013) Archaeology and the Senses: Human Experience, Memory, and Affect. Cambridge University Press

Hamm S, Bleton J, Tchapla A (2004) Headspace solid phase microextraction for screening for the presence of resins in Egyptian archaeological samples. J Sep Science 27:235–243. https://doi.org/10.1002/jssc.200301611

Hammann S, Cramp LJE, Whittle M, Evershed RP (2018) Cholesterol degradation in archaeological pottery mediated by fired clay and fatty acid pro-oxidants. Tetrahedron Letters 59:4401–4404. https://doi.org/10.1016/j.tetlet.2018.10.071

Hausleiter A (2012) North Arabian Kingdoms. In: Potts DT (ed) A companion to the archaeology of the ancient Near East. Wiley-Blackwell, Malden, Mass., pp 816–832

Hausleiter A, Eichmann R (2018) The Archaeological Exploration of the Oasis of Taymāʾ. In: Hausleiter A, Eichmann R, Al-Najem M (eds) Tayma I. Reports 1: Archaeological Research Strategies, Palaeoenvironment, Cultural Contacts. Archaeopress, Oxford, pp 2–55

Havlicek J, Roberts SC (2009) MHC-correlated mate choice in humans: A review. Psychoneuroendocrinology 34:497–512. https://doi.org/10.1016/j.psyneuen.2008.10.007

Hawthorn A, Rendu Loisel A-C (eds) (2019) Distant impressions: the senses in the ancient Near East. Eisenbrauns, University Park, Pennsylvania

Hendy J, Warinner C, Bouwman A, et al (2018) Proteomic evidence of dietary sources in ancient dental calculus. Proc R Soc B 285:20180977. https://doi.org/10.1098/rspb.2018.0977

Henshaw V (2014) Urban smellscapes: understanding and designing city smell environments. Routledge/Taylor & Francis Group, New York

Herodotus / transl. T. Holland (1996) Herodotus: The Histories, Further rev. ed. Penguin Books, London ; New York

Herz RS (2004) A naturalistic analysis of autobiographical memories triggered by olfactory visual and auditory stimuli. Chem Senses 29:217–224

Herz RS, Engen T (1996) Odor memory: Review and analysis. Psychonomic Bulletin & Review 3:300–313. https://doi.org/10.3758/BF03210754

Hodder I (1997) Reading the past: current approaches to interpretation in archaeology, 2 ed., reprint. Cambridge Univ. Press, Cambridge

Hodos T, Geurds A, Lane P, et al (eds) (2017) The Routledge handbook of archaeology and globalization. Routledge, London New York

Huisman DJ, Manders MR, Kretschmar EI, et al (2008) Burial conditions and wood degradation at archaeological sites in the Netherlands. International Biodeterioration & Biodegradation 61:33–44. https://doi.org/10.1016/j.ibiod.2007.06.008

Hussain SZ, Maqbool K (2014) GC-MS: Principle, Technique and its application in Food Science. International Journal of Current Science 13:

Ikram S, Dodson A (1998) The mummy in ancient Egypt: equipping the dead for eternity. Thames & Hudson, New York

Japp S, Gerlach I, Hitgen H, Schnelle M (2011) Yeha and Hawelti: cultural contacts between Saba' and D'MT - New research by the German Archaeological Institute in Ethiopia. In: Institute of Archaeology, British Museum (eds) Proceedings of the Seminar for Arabian Studies. Papers from the forty-fourth meeting of the Seminar for Arabian Studies held at the British Museum, London, 22 - 24 July 2010. Archaeopress, Oxford, pp 145–160

Jones J, Higham TFG, Chivall D, et al (2018) A prehistoric Egyptian mummy: Evidence for an 'embalming recipe' and the evolution of early formative funerary treatments. Journal of Archaeological Science 100:191–200. https://doi.org/10.1016/j.jas.2018.07.011

Jones J, Higham TFG, Oldfield R, et al (2014) Evidence for Prehistoric Origins of Egyptian Mummification in Late Neolithic Burials. PLoS ONE 9:e103608. https://doi.org/10.1371/journal.pone.0103608

Keay J (2008) The spice route: a history, 3. print. Univ. of California Press, Berkeley

Kenna M E (2005) Why does incense smell religious?: Greek orthodoxy and the anthropology od smell. Journal of Mediterranean Studies 15:50–69

Kenoyer JM (2008) Indus and Mesopotamian Trade Networks: New Insights from Shell and Carnelian Artifacts. In: Olijdam E, Spoor RH (eds) Intercultural relations between south and southwest Asia. Studies in commemoration of E.C.L. During Caspers (1934-1996). Archaeopress, Oxford, pp 19–28

Kitchen KA (2014) The land of Punt. In: Andah B, Okpoko A, Shaw T, Sinclair P (eds) The Archaeology of Africa Food, Metals and Towns. Taylor and Francis, Hoboken, pp 587–608

Koller J, Baumer U, Kaup Y, et al (2003) Analysis of a pharaonic embalming tar. Nature 425:784–784. https://doi.org/10.1038/425784a

Koloski-Ostrow AO (2015) Roman urban smells: the archaeological evidence. In: Bradley M (ed) Smell and the ancient senses. Routledge, London, pp 90–109

Koura B (1999) Die "7-Heiligen Öle" und andere Öl- und Fettnamen: eine lexikographische Untersuchung zu den Bezeichnungen von Ölen, Fetten und Salben bei den alten Ägyptern von der Frühzeit bis zum Anfang der Ptolemäerzeit (von 3000 v.Chr. - ca. 305 v.Chr.), Als Ms. gedr. Shaker, Aachen

Kumar S (2005) Exploratory analysis of global cosmetic industry: major players, technology and market trends. Technovation 25:1263–1272. https://doi.org/10.1016/j.technovation.2004.07.003

Langenheim JH (2003) Plant resins: chemistry, evolution, ecology, and ethnobotany. Timber Press, Portland

Leemans I, Tullett W, Bembibre C, Marx L (2022) Whiffstory: Using Multidisciplinary Methods to Represent the Olfactory Past. The American Historical Review 127:849–879. https://doi.org/10.1093/ahr/rhac159

Linduff KM (ed) (2004) Metallurgy in ancient eastern Eurasia from the Urals to the Yellow River. Edwin Mellen Press, Lewiston, NY

Liu S, Rehren T, Pernicka E, Hausleiter A (2015) Copper processing in the oases of northwest Arabia: technology, alloys and provenance. Journal of Archaeological Science 53:492–503. https://doi.org/10.1016/j.jas.2014.10.030

Ljaljević Grbić M, Unković N, Dimkić I, et al (2018) Frankincense and myrrh essential oils and burn incense fume against micro-inhabitants of sacral ambients. Wisdom of the ancients? Journal of Ethnopharmacology 219:1–14. https://doi.org/10.1016/j.jep.2018.03.003

Loeben CE (2005) Canopic jar of Senetnay, "Wet nurse of the king" (Amenhotep II). In: Hornung E, Loeben CE, Wiese A (eds) Immortal pharaoh: the tomb of Thutmose III ; an exhibition of ancient Egyptian objects from Antikenmuseum Basel und Sammlung Ludwig and the Kestner-Museum Hannover with the facsimile of the tomb of Thutmose III. Factum Arte, Madrid, pp 124–127

Loizzo MR, Saab A, Tundis R, et al (2008) Phytochemical analysis and in vitro evaluation of the biological activity against herpes simplex virus type 1 (HSV-1) of Cedrus libani A. Rich. Phytomedicine 15:79–83. https://doi.org/10.1016/j.phymed.2007.03.013

Loreto R (2017) Alle origini degli Arabi: un viaggio nell'archeologia dell'Arabia Saudita, Prima edizione Mondadori Università. Mondadori Università, Milano

Lü Q-Q, Henderson J, Wang Y, Wang B (2021) Natron glass beads reveal proto-Silk Road between the Mediterranean and China in the 1st millennium BCE. Sci Rep 11:3537. https://doi.org/10.1038/s41598-021-82245-w

Łucejko J, Connan J, Orsini S, et al (2017) Chemical analyses of Egyptian mummification balms and organic residues from storage jars dated from the Old Kingdom to the Copto-Byzantine period. Journal of Archaeological Science 85:1–12. https://doi.org/10.1016/j.jas.2017.06.015

Łucejko JJ, Modugno F, Ribechini E, et al (2015) Analytical Instrumental Techniques to Study Archaeological Wood Degradation. Applied Spectroscopy Reviews 50:584–625. https://doi.org/10.1080/05704928.2015.1046181

Luciani M (2021) On the Formation of 'Urban' Oases in Arabia: New Perspectives from the North-west. In: Luciani M (ed) The Archaeology of the Arabian Peninsula 2. Connecting the Evidence. Austrian Academy of Sciences Press, Vienna, pp 89–119

Luciani M (ed) (2016) The archaeology of North Arabia: oases and landscapes; proceedings of the international congress held at the University of Vienna, 5-8 December, 2013. Austrian Academy of Sciences Press, Vienna

Macdonald MCA (1997) Trade routes and trade goods at the northern end of the "incense road" in the first millennium B.C. In: Avanzini A (ed) Profumi d'Arabia. Atti del convegno. L'Erma di Bretschneider, Rome, pp 333–349

Malainey ME, Przybylski R, Sherriff BL (1999) The Effects of Thermal and Oxidative Degradation on the Fatty Acid Composition of Food Plants and Animals of Western Canada: Implications for the Identification of Archaeological Vessel Residues. Journal of Archaeological Science 26:95–103. https://doi.org/10.1006/jasc.1998.0306

Malik TG, Sahu LK, Gupta M, et al (2023) Environmental Factors Affecting Monoterpene Emissions from Terrestrial Vegetation. Plants 12:3146. https://doi.org/10.3390/plants12173146

Malleson C (2020) Flora of ancient Egypt. In: Shaw I, Bloxam E (eds) The Oxford Handbook of Egyptology. Oxford University Press, pp 124–150

Manniche L (1999) Sacred luxuries: fragrance, aromatherapy, and cosmetics in Ancient Egypt. Cornell University Press, New York

Marx L, Ehrich SC, Tullett W, et al (2022) Making Whiffstory. The American Historical Review 127:881–893. https://doi.org/10.1093/ahr/rhac150

Mathe C, Archier P, Nehme L, Vieillescazes C (2009) The study of nabataean organic residues from Madâ'in Sâlih, ancient Hegra, by gas chromatography–mass spectrometry. Archaeometry 51:626–636

Mathe C, Connan J, Archier P, et al (2007) Analysis of Frankincense in Archaeological Samples by Gas Chromatography-Mass Spectrometry. Annali di Chimica 97:433–445. https://doi.org/10.1002/adic.200790029

McLaughlin R (2018) The Roman Empire and the Indian Ocean: the ancient world economy and the kingdoms of Africa, Arabia and India, reprinted. Pen & Sword Maritime, Barnsley

Mercimek Takic H Aysun, Ucan Turkmen F, Sari M (2020) Effect of cedar (Cedrus libani a. Rich) tar on bacterial growth. J microb biotech food sci 9:805–808. https://doi.org/10.15414/jmbfs.2020.9.4.805-808

Middeke-Conlin R (2014) The Scents of Larsa: A Study of the Aromatics Industry in an Old Babylonian Kingdom. Cuneiform Digital Library Journal 1:1–53

Modugno F, Ribechini E, Colombini MP (2006) Chemical study of triterpenoid resinous materials in archaeological findings by means of direct exposure electron ionisation mass spectrometry and gas chromatography/mass spectrometry. Rapid Commun Mass Spectrom 20:1787–1800. https://doi.org/10.1002/rcm.2507

Moorey PRS (1999) Ancient Mesopotamian materials and industries: the archaeological evidence, Repr. Eisenbrauns, Winona Lake, Ind

Morris I (1992) Death-ritual and social structure in classical antiquity. Cambridge University Press, Cambridge ; New York

Naisbitt J (1982) Megatrends: ten new directions transforming our lives. Warner Books, New York

Nebes N (2014) Könige der Weihrauchstraße: zur Geschichte Südarabiens und Äthiopiens im frühen 1. Jahrtausend vor Christus. V & R Unipress Vienna university press, Göttingen [Vienna]

Nelson DL, Cox MM, Hoskins AA, Lehninger AL (2021) Lehninger principles of biochemistry, Eighth edition. Macmillan International, Higher Education, New York, NY

Panayotov SV (2014) A Peculiar Late Babylonian Recipe For Fumigation Against Epilepsy. In: The Recipes Project – Food, Magic, Science, and Medicine

Patalano R, Zech J, Roberts P (2020) Leaf Wax Lipid Extraction for Archaeological Applications. Current Protocols in Plant Biology 5:. https://doi.org/10.1002/cppb.20114

Peacock DPS, Williams DF (2007) Food for the gods: new light on the ancient incense trade. Oxbow Books, Havertown

Pekgözlü AK, Kuştaş S, Mercan B, Biçer A (2017) Chemical Characterization of Lebonan Cedar Tar. Drvna ind 68:107–112. https://doi.org/10.5552/drind.2017.1607

Periplus Mari Erythraei/ transl. L. Casson. Princeton, NJ: Princeton University Press, 1989.

Peters KE, Walters CC, Moldowan JM (2004) The Biomarker Guide: Volume 1: Biomarkers and Isotopes in the Environment and Human History, 2nd edn. Cambridge University Press, Cambridge

Pliny/ transl. J. André et al. 1947-2015. Histoire naturelle. Paris: Les Belles Lettres.

Pollard AM, Heron C (2008) Archaeological Chemistry, 2nd edition. Royal Society of Chemistry, Cambridge

Pommerening T (2016) Wege zur Identifikation altägyptischer Drogennamen – eine kritische Betrachtung. Zwischen Philologie und Lexikographie des Ägyptisch-Koptischen Akten der Leipziger Abschlusstagung des Akademienprojekts “Altägyptisches Wörterbuch” (Abhandlungen der Sächsischen Akademie der Wissenschaften zu Leipzig 82–111

Pope C (2017) Why do we love the smell of bread? UCD scientists find the answer. The Irish Times

Potter D (2015) The scent of Roman dining. In: Bradley M (ed) Smell and the ancient senses. Routledge, London, pp 120–132

Potts DT (1988) Trans-Arabian Routes of the Pre-Islamic Period. Travaux de La Maison de l’Orient 16:127–162

Potts DT (1990) The Arabian Gulf in antiquity: From prehistory to the fall of the Achaemenid Empire. Clarendon Press ; Oxford University Press, Oxford : New York

Price RS (2022) Sensing the Fundamentals: An Examination of Scent as Integral to Ancient Egyptian Society. University of California

Rageot M, Hussein RB, Beck S, et al (2023) Biomolecular analyses enable new insights into ancient Egyptian embalming. Nature 614:287–293. https://doi.org/10.1038/s41586-022-05663-4

Rao KRM, Jetti ER (2020) Scent Marketing – Harnessing the Power of Scents in stimulating senses of Organized Retail Consumers and Employees. IJISRT 5:575–584. https://doi.org/10.38124/IJISRT20SEP134

Regert M, Alexandre V, Thomas N, Lattuati-Derieux A (2006) Molecular characterisation of birch bark tar by headspace solid-phase microextraction gas chromatography–mass spectrometry: A new way for identifying archaeological glues. Journal of Chromatography A 1101:245–253. https://doi.org/10.1016/j.chroma.2005.09.070

Regert M, Colinart S, Degrand L, Decavallas O (2001) Chemical Alteration and Use of Beeswax Through Time: Accelerated Ageing Tests and Analysis of Archaeological Samples from Various Environmental Contexts. Archaeometry 43:549–569. https://doi.org/10.1111/1475-4754.00036

Regert M, Devièse T, Le Hô A-S, Rougeulle A (2008) Reconstructing ancient Yemeni commercial routes during the Middle Ages using structural characterization of terpenoid resins. Archaeometry 50:668–695

Reinarz J (2014) Past scents: historical perspectives on smell. Urbana

Ren M, Ren X, Wang X, Yang Y (2022) Characterization of the incense sacrificed to the sarira of Sakyamuni from Famen Royal Temple during the ninth century in China. Proc Natl Acad Sci USA 119:e2112724119. https://doi.org/10.1073/pnas.2112724119

Ren M, Tang Z, Wu X, et al (2019) The origins of cannabis smoking: chemical residue evidence from the first millennium BCE in the Pamirs. Sci Adv 5:eaaw1391. https://doi.org/10.1126/sciadv.aaw1391

Ribechini E, Modugno F, Pérez-Arantegui J, Colombini MP (2011) Discovering the composition of ancient cosmetics and remedies: analytical techniques and materials. Anal Bioanal Chem 401:1727–1738. https://doi.org/10.1007/s00216-011-5112-2

Riesmeier M, Keute J, Veall M-A, et al (2022) Recipes of Ancient Egyptian kohls more diverse than previously thought. Sci Rep 12:5932. https://doi.org/10.1038/s41598-022-08669-0

Rimkute J, Moraes C, Ferreira C (2016) The effects of scent on consumer behaviour: The effects of scent. International Journal of Consumer Studies 40:24–34. https://doi.org/10.1111/ijcs.12206

Rocha F, Ratola N, Homem V (2023) Fragrances in the Environment: Properties, Applications, and Emissions. Springer Berlin Heidelberg, Berlin, Heidelberg

Rohmer J, Charloux G (2015) From Liḥyān to the Nabataeans: dating the end of the Iron Age in north-west Arabia. Proceedings of the Seminar for Arabian Studies 45:297–319

Rowe JW (ed) (1989) Natural Products of Woody Plants. Springer Berlin Heidelberg, Berlin, Heidelberg

Saab AM, Harb FY, Koenig WA (2005) Essential oil components in heart wood of Cedrus libani and Cedrus atlantica from Lebanon. Minerva Biotecnologica 17:159–161

Sadgrove NJ, Padilla-González GF, Phumthum M (2022) Fundamental Chemistry of Essential Oils and Volatile Organic Compounds, Methods of Analysis and Authentication. Plants 11:789. https://doi.org/10.3390/plants11060789

Salomé-Abarca LF, van der Pas J, Kim HK, et al (2018) Metabolic discrimination of pine resins using multiple analytical platforms. Phytochemistry 155:37–44. https://doi.org/10.1016/j.phytochem.2018.07.011

Schiødt S (2020) Medical science in ancient Egypt: a translation and interpretation of Papyrus Louvre-Carlsberg (pLouvre E 32847 + pCarlsberg 917) / Sofie Schiødt. University of Copenhagen, Department of Cross-Cultural and regional Studies, Copenhagen

Schmidt K, Podmore I (2015) Solid Phase Microextraction (SPME) Method Development in Analysis of Volatile Organic Compounds (VOCS) as Potential Biomarkers of Cancer. J Mol Biomark Diagn 06: https://doi.org/10.4172/2155-9929.1000253

Scott A, Power RC, Altmann-Wendling V, et al (2020) Exotic foods reveal contact between South Asia and the Near East during the second millennium BCE. Proc Natl Acad Sci USA 202014956. https://doi.org/10.1073/pnas.2014956117

Scurlock JA (2012) A plant can have different names in different cultures: A rebuttal to Luc Renaut. Journal des Médecines 19:51–52

Serpico M, White R (2000) The botanical identity and transport of incense during the Egyptian New Kingdom. Antiquity 74:884–897

Shepherd GM (2004) The Human Sense of Smell: Are We Better Than We Think? PLoS Biol 2:e146. https://doi.org/10.1371/journal.pbio.0020146

Singh D, Agarwal SK (1988) Himachalol and β-himachalene: Insecticidal principles of himalayan cedarwood oil. J Chem Ecol 14:1145–1151. https://doi.org/10.1007/BF01019342

Skanderi I, Chouitah O (2020) Chemical Characterization and Antioxidant Activity of Cedrus atlantica Manetti Tar (Atlas Cedar Tar). French-Ukrainian Journal of Chemistry 08:244–255

Spangenberg ER, Crowley AE, Henderson PW (1996) Improving the Store Environment: Do Olfactory Cues Affect Evaluations and Behaviors? Journal of Marketing 60:67–80. https://doi.org/10.1177/002224299606000205

Spence C (2015) Leading the consumer by the nose: on the commercialization of olfactory design for the food and beverage sector. Flavour 4:31. https://doi.org/10.1186/s13411-015-0041-1

Spengler RN (2019) Fruit from the sands: the Silk Road origins of the food we eat. University of California Press, Oakland, California

Stern B, Heron C, Corr L, et al (2003) Compositional variations in aged and heated pistacia resin found in late bronze age canaanite amphorae and bowls from Amarna, Egypt. Archaeometry 45:457–469

Stevens CE, Hume ID (2004) Comparative physiology of the vertebrate digestive system, 2nd ed., 1st pbk. ed. Cambridge University Press, Cambridge ; New York

Tamburini D, Łucejko J, Ribechini E, Colombini MP (2016) New markers of natural and anthropogenic chemical alteration of archaeological lignin revealed by in situ pyrolysis/silylation-gas chromatography mass spectrometry. Journal of Analytical and Applied Pyrolysis 118:249–258. https://doi.org/10.1016/j.jaap.2016.02.008

Tang L, Wilkin S, Richter KK, et al (2023) Paleoproteomic evidence reveals dairying supported prehistoric occupation of the highland Tibetan Plateau. Sci Adv 9:eadf0345. https://doi.org/10.1126/sciadv.adf0345

Taterka F (2016) Hatshepsut's expedition to the land of Punt - novelty or tradition. In: Alvarez C, Belekdanian A, Gill A-K, Klein S (eds) Current research in egyptology 2015: proceedings of the sixteenth annual symposium, University of Oxford, United Kingdom, 15-18 April 2015. Oxbow Books, Oxford Philadelphia, pp 114–123

Teller C, Dennis C (2012) The effect of ambient scent on consumers' perception, emotions and behaviour: A critical review. Journal of Marketing Management 28:14–36. https://doi.org/10.1080/0267257X.2011.560719

Theophrastus (1916) Enquiry into plants. Translated by Sir Arthur Fenton. W. Heinemann, London

Tisserand R (1977) The art of aromatherapy. C. W. Daniel, London

Toner J (2015) Smell and Christianity. In: Bradley M (ed) Smell and the ancient senses. Routledge, London, pp 158–170

Toner J (ed) (2014) A cultural history of the senses in antiquity. Bloomsbury, London ; New York

Totelin L (2015) Smell as sign and cure in ancient medicine. In: Bradley M (ed) Smell and the ancient senses. Routledge, London, pp 17–29

Tullett W (2019) Smell in eighteenth-century England: a social sense, First edition. Oxford University Press, Oxford

Tushingham S, Snyder CM, Brownstein KJ, et al (2018) Biomolecular archaeology reveals ancient origins of indigenous tobacco smoking in North American Plateau. Proc Natl Acad Sci USA 115:11742–11747. https://doi.org/10.1073/pnas.1813796115

Tutin TG, Heywood VH, Burges NA, et al (eds) (1964) Flora Europaea. Volume 1: Lycopodiaceae to Platanaceae. Cambridge University Press

van Bergen PF, Peakman TM, Leigh-Firbank EC, Evershed RP (1997) Chemical evidence for archaeological frankincense: Boswellic acids and their derivatives in solvent soluble and insoluble fractions of resin-like materials. Tetrahedron Letters 38:8409–8412. https://doi.org/10.1016/S0040-4039(97)10234-9

Van Erp M, Tullett W, Christlein V, et al (2023) More than the Name of the Rose: How to Make Computers Read, See, and Organize Smells. The American Historical Review 128:335–369. https://doi.org/10.1093/ahr/rhad141

Vandenbeusch M, Stacey R, Antoine D (2021) Rediscovering Nestawedjat: Embalming residue analyses reunite the mummified remains of an ancient Egyptian woman with her coffins. Journal of Archaeological Science: Reports 40:103186. https://doi.org/10.1016/j.jasrep.2021.103186

Verbeek C, Leemans I, Fleming B (2022) How can scents enhance the impact of guided museum tours? towards an impact approach for olfactory museology. The Senses and Society 17:315–342. https://doi.org/10.1080/17458927.2022.2142012

Verhoef PC, Lemon KN, Parasuraman A, et al (2009) Customer Experience Creation: Determinants, Dynamics and Management Strategies. Journal of Retailing 85:31–41. https://doi.org/10.1016/j.jretai.2008.11.001

Whelton HL, Hammann S, Cramp LJE, et al (2021) A call for caution in the analysis of lipids and other small biomolecules from archaeological contexts. Journal of Archaeological Science 132:105397. https://doi.org/10.1016/j.jas.2021.105397

Wilkin S, Ventresca Miller A, Fernandes R, et al (2021) Dairying enabled Early Bronze Age Yamnaya steppe expansions. Nature 598:629–633. https://doi.org/10.1038/s41586-021-03798-4

Zagorevski DV, Loughmiller-Newman JA (2012) The detection of nicotine in a Late Mayan period flask by gas chromatography and liquid chromatography mass spectrometry methods: Detection of nicotine in a Late Mayan period flask. Rapid Commun Mass Spectrom 26:403–411. https://doi.org/10.1002/rcm.5339

Zald DH, Pardo JV (1997) Emotion, olfaction, and the human amygdala: Amygdala activation during aversive olfactory stimulation. PNAS 94:4119–4124

Zimmerle W G (2014) Aromatics of all kinds: Cuboid incense burners in the ancient Near East from the late Third to the late First Millenia BC. ProQuest Dissertations Publishing

Zimmerle WG (2021) Frankincense and Its Arabian Burner. In: Baird IP, Yağcıoğlu H (eds) All things Arabia: Arabian identity and material culture. Brill, Leiden ; Boston, pp 23–42

Acknowledgements

Completing my thesis would not have been possible without the guidance, support, and encouragement of numerous people to whom I am deeply grateful. My research path led me to the stimulating academic environments of the Max Planck Institute of Geoanthropology and the University of Tübingen. I was more than fortunate in these places – I was embraced by two phenomenal research families, the Department of Archaeology at the MPI, and the Archaeometry Group at Tübingen.

I would like to thank my supervisors, Susanne Greiff and Patrick Roberts, for their mentorship and steadfast support throughout my PhD journey. Susanne, I'm grateful for the warm welcome you extended to me upon joining your group in Tübingen and the fresh perspectives you brought to our discussions. Patrick, I deeply appreciate your faith in my abilities and your unwavering support throughout my time at the MPI. You have taught me not only about being a good researcher, but also about navigating the complex challenges we often face in academia.

I also extend special thanks to my mentors at the MPI, Nicole Boivin and Rob Spengler. Your guidance, constructive criticism, and encouragement have been instrumental in shaping both my research and my academic growth.

Collaboration is the essence of scientific discovery, and I have been fortunate to work with an incredible team. I owe a debt of gratitude to my collaborators all over the world for their invaluable contributions and for challenging me to reach new heights.

To my fellow PhD colleagues, you have made this journey an exhilarating ride! Your support, camaraderie, and humor have made even the most stressful times manageable. A special shout-out to Carli Peters and Traci Billings – your friendship has meant the world to me.

Most importantly, none of this would have been possible without the enduring support of my family. Thank you for your unconditional love, for always having my back and for reminding me of what truly matters.

Completing this PhD has been an incredible journey, and while this chapter may be closing, the memories and friendships I've gained will last a lifetime. Thank you all!

Appendix 1 – Accepted manuscripts

How to use modern science to reconstruct ancient scents

Olfaction has profoundly shaped human experience and behaviour from the deep past through to the present day. Advanced biomolecular and 'omics' sciences enable more direct insights into past scents, offering new options to explore critical aspects of ancient society and lifeways as well as the historical meanings of smell.

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Olfaction has a major effect on how we perceive and navigate the world. When odorant molecules in the air reach our nostrils and bind to the olfactory epithelium, signals are sent directly to the limbic system, the region of the brain involved with processes relating to emotion, cognition and memory[1]. This means that we react to odour stimuli before we think about them. Owing to this neurobiology, smells often float just below the level of our conscious awareness but nonetheless alert us to health hazards, alter our behaviour and physiological responses, shape our actions and choices, guide our emotions, and trigger our memories[1]. This direct link explains why the power of smell is frequently harnessed for commercial purposes, such as in odour management, in which the olfactory environment of places in which people work, consume and/or interact is controlled to elicit particular perceptions and responses[2] — encouraging them to work harder or buy more, for example.

Given the importance of scent in shaping understanding and behaviour, it is likely that it has had an important role throughout human history. The fact that past expeditions of discovery, wars and long-distance exchange often hinged on attempts to acquire obscure materials with strong olfactory properties — such as incense and spices — hints at this importance. New research on the properties of diverse aromatic compounds also shows us that many of the special substances people in the past sought out, traded and used have bioactive, psychological and/or medicinal properties that are only just beginning to be understood. But beyond this, smells (as with other sensory perceptions) are also fully embedded in social and historical contexts, being as much culturally as neurologically constituted[3]. How certain scents are perceived and responded to thus has as much to do with factors such as identity, belief and

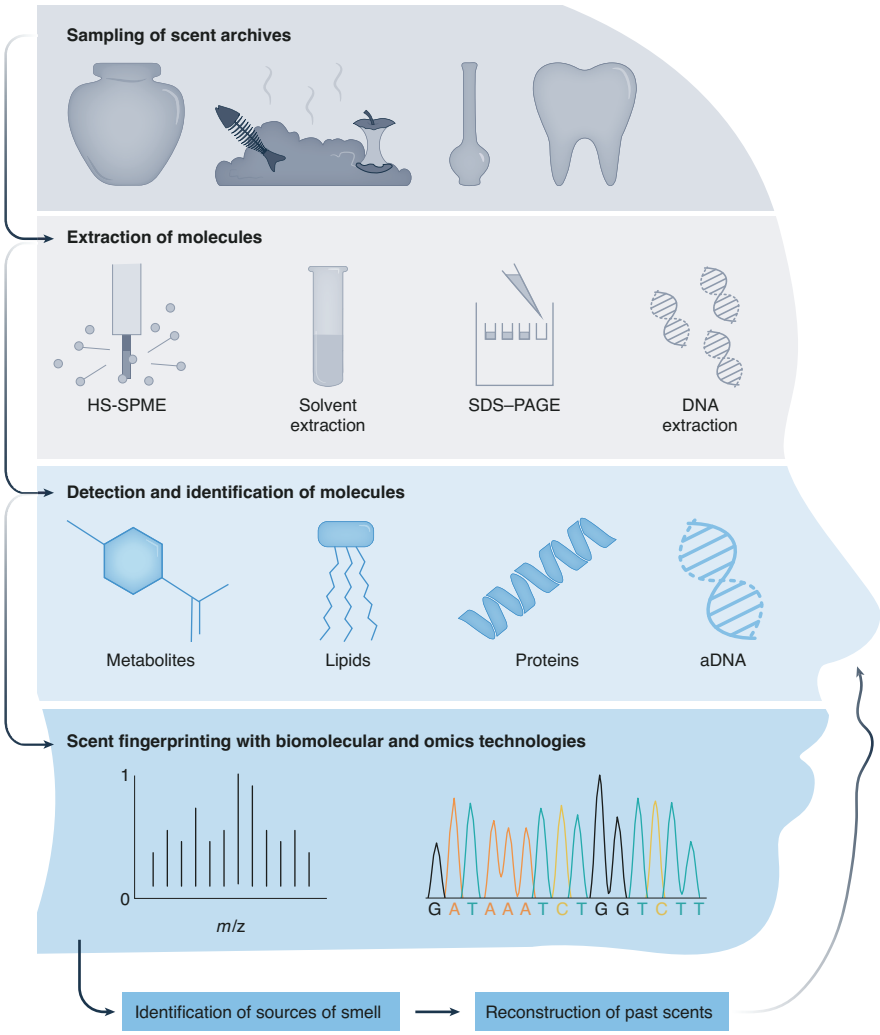

Fig. 1 | Workflow for studying ancient scents using biomolecular approaches. Past scents can be recovered from scent archives by extracting molecules using a variety of different methods, including headspace solid phase microextraction (HS-SPME), solvent extraction techniques, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and DNA extraction techniques followed by amplification (PCR) and/or library preparation for next-generation sequencing. With successful detection and identification of the molecular composition of samples, ancient sources of smell can then be identified. aDNA, ancient DNA. Adapted from an illustration by Michelle O'Reilly, Max Planck Institute for the Science of Human History.

Box 1 | Key biomolecular approaches for studying ancient scents

Analysis of ancient secondary metabolites
Secondary metabolites are important for plant defence, and impart plants with particular scents as well as bioactive properties. They can be extracted from ancient organic residues using a diverse array of methods. The extracts are then analysed using gas or liquid chromatography mass spectrometry, which enables separation and determination of the mass of the individual molecules within a residue. Analytical standards and mass spectral libraries, containing reference mass data, are used for compound identification.

Analysis of ancient lipids
Lipids are mostly water-insoluble molecules, such as fats and oils, and are commonly extracted from archaeological ceramic sherds and sediments using organic solvents or supercritical fluids. Once extracted, lipids can be analysed by gas chromatography–mass spectrometry or liquid chromatography–mass spectrometry, and identified using reference libraries and analytical standards.

Analysis of ancient proteins
Proteins are essential nutrients for humans. They can be found preserved in archaeological specimens such as dental calculus, tissues and food residues. The proteins can be extracted using in-solution or in-gel methods and digested with proteases into smaller peptides. The peptides can then be analysed using a tandem mass spectrometer, and identified by matching against protein sequence databases.

Analysis of ancient DNA
DNA stores the genetic information for life. Ancient DNA can be recovered from archaeological teeth, plants and mummified tissues and subjected to DNA amplification of targeted genetic markers or to DNA library preparation. High-throughput sequencing can be conducted directly on library DNA or after enrichment. The sequencing data can be used for exploratory analyses and modelling.

Bioinformatics
The application of computer technology to the analysis of biological data; now essential because of the huge amount of complex data created through omics methods.

Omics methods
High-throughput methods such as proteomics and genomics that focus on a comprehensive, or global, assessment of a set of biological molecules (for example, all proteins in a sample rather than a single protein).

knowledge as it does with molecules and human physiology. Looking at smell in the past accordingly provides us with fascinating insights into human culture, society and history, as well as the development of knowledge and the ways people learned to apply the properties of smell to shape social interaction, behaviour and health.

Challenges of finding past smells

Despite the physiological and cultural importance of scent, studies and reconstructions of the past remain largely odourless. Although there is a growing interest in studying the senses in archaeology (for example, ref. [4]), past smells are still underexplored and their reconstruction mostly limited to what can be learned from historical documents. This reflects, at least in part, the considerable methodological challenges associated with reconstructing scent. Smell is an ephemeral phenomenon; when we excavate sites, we find things we can touch and see, that were seen by ancient eyes and touched by ancient hands. But we cannot do the same for smell.

The main obstacle in developing an archaeology of scent is the volatile nature of odour compounds: once their source is gone, they too disappear, evaporating and dissipating on the air. Furthermore, most smells — whether fragrant or foul — stem from natural organic sources, such as plants, resins, food, and human and animal bodies and their emissions. These biological materials normally decay rapidly, quickly losing those components that endowed them with their original smell. Some, such as the botanical remains of aromatic plants, are exceedingly rarely encountered in the archaeological record, whereas others become amorphous residues that are nearly impossible to identify at macroscopic or microscopic scales.

Biomolecular reconstruction of scent

Yet for all these extreme challenges, ancient scents increasingly lie within the grasp of modern science. The key is the application of powerful biomolecular approaches. These draw on chromatography, mass spectrometry, sequencing technologies and modern bioinformatics to recover and analyse rare molecules preserved within ancient 'scent archives'. These archives can be archaeological objects associated with the use of aromatic substances, such as incense burners, perfume flasks, cooking pots or storage vessels. Larger features with more general uses (such as city streets, middens or floor surfaces) or even bodily features (such as dental calculus on teeth or mummified remains) can also function as scent archives. The most important property of such scent archives is that they contain the remains of aromatic substances that can be sampled, analysed and identified (Fig. 1). Although the organic remains in these archives are often invisible to the naked eye, they are nevertheless present on a molecular level — preserved on artefact and mineral surfaces and within the walls of ceramic vessels, for example — and they can provide valuable information about many aspects of the past, including disappeared scents and olfactory landscapes.

The growing sophistication of modern biomolecular approaches can help archaeologists to identify an increasing range and number of compounds that hint at past landscapes of smell, as well as taste and experience, that were previously off limits. Studies of preserved ancient residues have been used, for example, to reconstruct the specific scents and compounds used in ancient rituals, including diverse types of incense and drugs, such as frankincense, myrrh, cannabis and tobacco[5,6]. The most recent additions to this suite of biomolecular approaches are omics technologies, including metabolomics, proteomics and genomics. These large-scale, high-resolution and non-targeted analyses open up a holistic view of the overall biomolecular composition of a sample (Box 1).

Four classes of biomolecules are particularly valuable for studying past smells. Perhaps the most important are secondary (or specialized) metabolites, including a number of diverse odorants and pheromones. Metabolite studies, including palaeo-metabolomics, offer an invaluable tool for identifying ancient aromatic plants and plant products (such as resins, flowers, scented woods, herbs, fruits, spices and fungi) that were used in the past as incenses, beverages, drugs and foods, as well as for creating perfumes, cosmetics, medical remedies and for masking the smell of decomposing corpses[5–7]. For example, metabolite characterization has shown

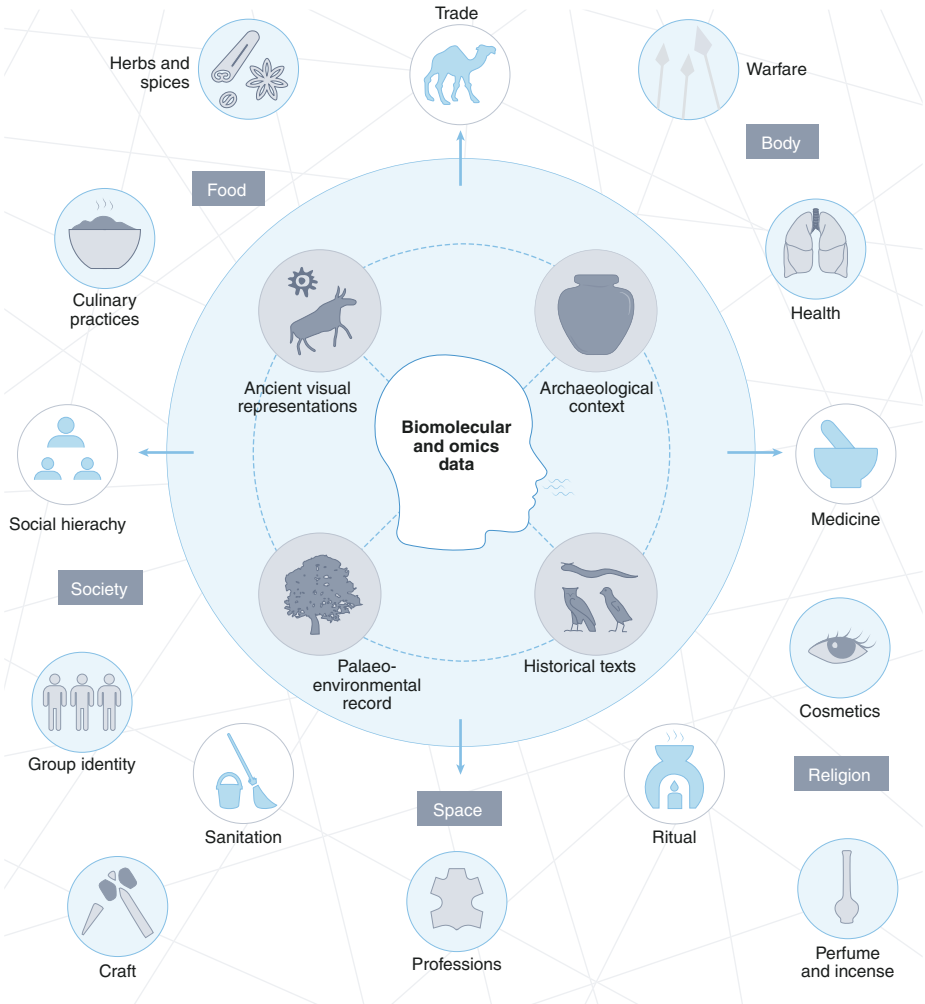

Fig. 2 | Contextualizing biomolecular data to reconstruct ancient experience, behaviour and society. The data produced through the application of biomolecular methods can be contextualized within the framework of relevant historical texts and visual representations, as well as the archaeological and environmental records. Connecting these different sources of information enables the reconstruction of critical aspects of ancient lifeways and society (indicated by arrows emerging from the blue circle). The icons in the outer area of the figure represent examples of some of the themes that can be addressed through this approach (grey boxes indicate broad headings). Adapted from an illustration by Michelle O'Reilly, Max Planck Institute for the Science of Human History.

that dead bodies in late Roman Britain were treated with coniferous Pinaceae resins, Mediterranean *Pistacia*-species resins (mastic or terebinth) and exotic *Boswellia*-species gum resins (frankincense or olibanum) from southern Arabia or beyond[7].

Also of interest to archaeology are lipid molecules. Materials such as oils, waxes and fats contributed in important ways to past smellscapes. They were used as fuel for lamps, were a component of scented ointments, and were coated on human bodies to alter their scent, appearance and temperature, for example[8]. Other lipids and lipid carriers, such as sterols, stanols and bile acids, are useful markers of human or animal faeces[9]. An emerging phase of palaeo-lipidomic research is moving beyond the analysis of a few targeted compounds to the reconstruction of entire lipid assemblages in ancient materials.

Palaeo-proteomic applications are increasingly common in archaeology, and can enable detection of ancient proteins specific to certain foodstuffs — such as spices, meat, dairy products and cereals (for example, ref. [10]) — whose preparation, display and consumption would have contributed substantially to ancient smellscapes. Proteomic approaches (to preserved mummies and dental calculus, for example) have also reconstructed inflammation-related proteins that signal conditions such as infection or gingivitis, associated with strong bodily odours[11].

Archaeogenetic and archaeogenomic approaches have not contributed thus far to identifying past odorants, but microbiome-related studies in the field have particular potential in this regard[12] — especially when we consider that everything from body odours to odorous processes of fermentation and rotting are, in large part, microorganism-driven.

Using smell to understand the past

What can the identification of smells and aromas help us to learn about the past? Here we explore what we see as the future of this research area, examining how biomolecular and omics methods (often in combination and/or in association with information from historical texts, visual depictions and the broader archaeological and environmental records) can be leveraged to provide insight into ancient scents, their roles and the past more broadly. In the following, we address five key themes: ritual, trade, social hierarchy, medicine and sanitation. However, as scents cross-cut multiple spheres of human activity that are themselves fluid and overlapping, our outline of these themes should not be taken as reflective of hard or fixed categories, but rather as heuristic examples within a dynamic web of interlinked lifeways, behaviours and concepts in which scent has had a role (Fig. 2).

Biomolecular methods increasingly allow us to identify the aromatic substances used in past rituals. For example, analyses of secondary metabolites have identified the type of incense burnt during Medieval funerals in Belgium[5]. Research shows that incense has real physiological effects — the burning of agarwood, for example, not only releases a pleasant smell but also boosts serotonin levels to produce anti-depressant and anti-anxiety effects[13]. Linking archaeological, phytochemical and pharmacological research more closely in the future will improve understanding of how scent was used to create distinct sacred spaces and powerfully shape religious experience in the ancient world.

Aromatic products were also a major source of fascination, power and wealth in the past, travelling long distances along trade networks. By applying novel science-based approaches, archaeologists are not only able to more effectively identify these otherwise largely invisible commodities, but also track their movement and exchange.

A multi-method biomolecular study of Medieval resins from Sharma, Yemen, for example, led to the surprising finding that much of the resin from this major frankincense-producing region was, in fact, imported copal from distant Madagascar and East Africa[14], providing critical insight into commercial networks and global exchange in this period.

Meanwhile, research suggests that, similar to today, bodily odours in the past (among other smells) had an important role in both shaping and signalling identity and social hierarchy, with 'bad' odours often associated with lower-status professions and groups. Chemical characterization of ancient 'chewing gum' from sites around the world shows that the sap of diverse tree barks has been used to freshen breath from early times (for example, refs. [12,15]). Historical records warn against simplistic assumptions about its link to social status, however. For example, in Aztec times, use of sapodilla tree bark to freshen breath was considered "a social marker of 'whores' and 'effeminates,' and 'respectable' adults were forbidden to chew in public"[15].

Aromatic substances also served as medicines and, indeed, the boundaries between aromatics, cosmetics, and medicines were often deeply blurred. People in the past appreciated that many substances that possessed strong scents also possessed bioactive, pharmacological and health-related properties. Characterizing the mysterious, often-aromatic substances once held in ancient containers — for example, medicine consisting of pine resin mixed with oil, fat and mineral powders discovered in a tin container aboard an ancient Roman shipwreck[8] — holds the potential to offer new insights into the evolution of medical and drug-related knowledge and practices, as well as the meanings attached to specific scents.

Finally, odiferous substances also offer insight into ancient sanitation, hygiene, and sewage and waste disposal practices, and can be studied through molecular characterization of diverse organic remains. For example, the use of lipid markers to taxonomically characterize palaeofaeces can help to elucidate how household spaces were used by humans and animals: for example, in an Iron Age roundhouse in Scotland, chemical characterization of floor sediments provided insight into living conditions, hygiene practices and the temporary sheltering of animals in human living areas during this period[9].

The powerful role of scent in shaping human experience and behaviour means that it has been important to the human story. The increased accessibility of ancient scents to modern science is allowing researchers to shed new light on how landscapes of smell have been shaped, managed, controlled and altered through human activities over the long term. Such research into smell in the past is also allowing museum curators and others to more-effectively evoke past worlds and experience through the re-creation of scents. Research into ancient scents accordingly enables us not only to better understand our changing societies, but also ourselves as human beings and the ways basic sensory experiences such as smell can profoundly shape our understanding and experience of the world. ❐

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Published online: 28 March 2022

https://doi.org/10.1038/s41562-022-01325-7

References

1. Hoover, K. C. *Am. J. Phys. Anthropol.* **143**(Suppl 51), 63–74 (2010).
2. Goldkuhl, L. & Styvén, M. *Euro. J. Market.* **41**, 1297–1305 (2007).
3. Howes, D. *Sensual Relations: Engaging the Senses in Culture and Social Theory* (Univ. Michigan Press, 2003).
4. Skeates, R. & Day, J. *The Routledge Handbook of Sensory Archaeology* (Routledge, Taylor & Francis, 2019).
5. Baeten, J., Deforce, K., Challe, S., De Vos, D. & Degryse, P. *PLoS ONE* **9**, e113142 (2014).
6. Zimmermann, M. et al. *Sci. Rep.* **11**, 1590 (2021).
7. Brettell, R. C. et al. *J. Archaeol. Sci.* **53**, 639–648 (2015).
8. Giachi, G. et al. *Proc. Natl Acad. Sci. USA* **110**, 1193–1196 (2013).
9. Mackay, H. et al. *J. Archaeol. Sci.* **121**, 105202 (2020).
10. Bleasdale, M. et al. *Nat. Commun.* **12**, 632 (2021).
11. Cappellini, E., Collins, M. J. & Gilbert, M. T. P. *Science* **343**, 1320–1322 (2014).
12. Jensen, T. Z. T. et al. *Nat. Commun.* **10**, 5520 (2019).
13. Kao, W.-Y., Hsiang, C.-Y., Ho, S.-C., Ho, T.-Y. & Lee, K.-T. *J. Ethnopharmacol.* **275**, 114069 (2021).
14. Regert, M., Devièse, T., Le Hô, A.-S. & Rougeulle, A. *Archaeometry* **50**, 668–695 (2008).
15. Mathews, J. P. & Schultz, G. P. *Chicle: The Chewing Gum of the Americas, from the Ancient Maya to William Wrigley* (Univ. Arizona Press, 2009).

Acknowledgements

The authors are grateful to the Max Planck Society for funding this research. B.H. is supported by the Joachim Herz Foundation and holds an Add-on Fellowship for Interdisciplinary Life Sciences. We thank F. Kai Yik Teoh for his feedback and valuable suggestions.

Competing interests

The authors declare no competing interests.

Additional information

Peer review information *Nature Human Behaviour* thanks Joanna Day and Ruth Nugent for their contribution to the peer review of this work.

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Article

Chemical Modification of Biomarkers through Accelerated Degradation: Implications for Ancient Plant Identification in Archaeo-Organic Residues

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Abstract: Biochemical and biomolecular archaeology is increasingly used to elucidate the consumption, use, origin, and trade of plants in the past. However, it can be challenging to use biomarkers to identify the taxonomic origin of archaeological plants due to limited knowledge of molecular survival and degradation for many key plant compounds in archaeological contexts. To gain a fundamental understanding of the chemical alterations associated with chemical degradation processes in ancient samples, we conducted accelerated degradation experiments with essential oil derived from cedar (*Cedrus atlantica*) exposed to materials commonly found in the archaeological record. Using GC-MS and multivariate analysis, we detected a total of 102 compounds across 19 treatments that were classified into three groups. The first group comprised compounds that were abundant in fresh cedar oil but would be unlikely to remain in ancient residues due to rapid degradation. The second group consisted of compounds that remained relatively stable or increased over time, which could be potential biomarkers for identifying cedar in archaeological residues. Compounds in the third group were absent in fresh cedar oil but were formed during specific experiments that could be indicative for certain storage conditions. These results show that caution is warranted for applying biomolecular profiles of fresh plants to ancient samples and that carefully designed accelerated degradation experiments can, at least in part, overcome this limitation.

Keywords: archaeological plant residues; residue identification; secondary metabolites; degradation experiment; catalysis; GC-MS; multivariate analysis

Citation: Huber, B.; Vassão, D.G.; Roberts, P.; Wang, Y.V.; Larsen, T. Chemical Modification of Biomarkers through Accelerated Degradation: Implications for Ancient Plant Identification in Archaeo-Organic Residues. *Molecules* **2022**, *27*, 3331. https://doi.org/10.3390/molecules27103331

Academic Editors: Maria Perla Colombini, Erika Ribechini and Jeannette Jacqueline Łucejko

Received: 4 May 2022
Accepted: 19 May 2022
Published: 22 May 2022

1. Introduction

Biochemical and biomolecular analyses of plant residues from archaeological contexts is a rapidly expanding area of research [1–8]. Within this field, biomarkers are particularly useful indicators for ancient plant identification. Biomarker methodologies rely on the premise that particular biomolecular compounds or fingerprints found in archaeological samples can be linked to the known chemistry of modern plants [9,10]. With instrument advancement and increasing sensitivity of chromatographic and mass spectrometric methods, small molecules, especially secondary or specialized metabolites, including phenolics, alkaloids, benzenoids, and terpenes, are key targets for plant identification [11]. The structures of some of these biomolecules, e.g., terpenoids in plant resins or alkaloids in psychoactive plants, are in some cases, source diagnostic and thus, allow botanical genera and even species identification in comparison to modern plants [12–14]. The application of these methodologies in archaeological contexts has the potential to elucidate the use of material culture, consumption practices, and the origin and trade of certain plants in the past. Coniferous plant products, such as essential oils, resins, tars, and pitches derived from cedar and

pine trees, for example, played an important role as they had multiple applications in the ancient world. These products were used as ingredients in medical remedies, cosmetics and perfumery, as waterproofing agents for containers, as glues, and in rituals, such as mummification [15–18]. However, using plant biomarkers for identifying the biological origin of archaeological residues comes with significant challenges. Numerous factors can substantially affect the biomolecular composition of ancient samples over time and might lead to incorrect plant identification if not considered [19]. It is also absolutely critical that the analyzed biomolecular fingerprints can be assumed to remain largely unaltered or at least predictably altered following their deposition.

A major challenge for identifying and interpreting ancient residues is the volatility of key diagnostic plant compounds, such sesquiterpenoids [14,20]. These volatile compounds can easily evaporate and change in archaeological samples over time, depending, for example, on environmental conditions and thermal fluctuations. The loss of these diagnostic markers can impede the ability to differentiate plant species. Moreover, compounds in archaeological samples can also undergo chemical alteration. Long-term deposition, weathering, biodegradation, combustion, light, oxidation, or reduction have all been demonstrated to lead to chemical transformations [21–23]. These processes may fragment compounds and form new derivatives that are generic for several plant species. For example, the phenolic compounds, vanillin and vanillic acid, occur naturally in vanilla extracts [24,25]. However, they are also common decomposition products of woody tissue and indicative of lignin pyrolysis [19,23,26]. Finally, even unfavorable chemical reactions can be greatly accelerated by catalysis, potentially leading to the modification of plant substances under the influence of organic or inorganic catalysts (see Box 1 for the definition of some chemical terms). In archaeological contexts this may happen when organic substances react with the surfaces or materials of archaeological containers and vessels, such as clay, gypsum, and different metals. These reactions can involve changes in covalent bonds, leading e.g., to condensations, addition, or removal of oxygenated groups, the intramolecular migration or replacement of functional groups (substitution), desaturation reactions, or cleavage of bonds (elimination) [27]. All these reactions and their potential to change the molecular composition of a given residue need to be considered in biomarker analysis and data interpretation. Nevertheless, although the effects of degradation on the chemical compositions of archaeological materials have been subject to many previous studies and experiments, most of these investigations have focused on the degradation of lipids [22,28–30] or wood/lignin [31–35].

To date, there has been relatively little exploration of the potential and nature of secondary metabolite degradation to occur in plant residues, including resins, gums, tars, essential oils, spices, herbs, and psychoactive plant products. This is despite their widespread application in past medicinal, culinary, sanitary, cosmetic, ritual, and economic contexts [1,6,8,36–44]. Here, we investigate the transformation processes influencing the phytochemical composition of secondary metabolites from a selected plant source to gain a fundamental understanding of the chemical alterations associated with chemical degradation processes in ancient samples. We study these alterations by exposing modern reference samples to accelerated degradation treatments and subsequently analyzing them by gas chromatography coupled to mass spectrometry (GC-MS). By applying multivariate analysis to these complex data sets, we identify characteristic biomolecular profiles associated with the various degradation treatments. We use the oil of cedar trees (*Cedrus atlantica*) for the experiment, as coniferous plant products from the Pinaceae family are frequently found in organic residues from archaeological contexts, especially in artefacts from ancient Egypt [17,18,41,45–49]. Cedar essential oil is mainly composed of mono- and sesquiterpenes, with himachalenes as the main components [50]. We highlight the potential of the materials of archaeological containers to affect the chemical composition of their organic contents in diverse ways with some possible biomarkers disappearing from treated oils, while other derivatives are newly formed. Based on these results, we additionally propose valid archaeological biomarkers for identifying cedar plant products

in archaeological residues. We argue that more comprehensive degradation studies are required to better understand the taphonomic pathways of secondary metabolites in different materials and geographical contexts and to develop more rigorous approaches to archaeological interpretation.

Box 1. Glossary for the definition of some chemical terms used in this study.

Activation energy:	**Refers to the minimal quantity of energy required for the reactants to start a chemical reaction.**
Aromatization:	A chemical reaction in which an aromatic system is formed from a single nonaromatic precursor. Typically, aromatization is achieved by dehydrogenation of existing cyclic compounds.
Catalyst:	A substance that increases the rate of a reaction because it lowers the activation energy of the reactants. Catalytic specificity refers to the particular ability of a substance or closely related group of substances to catalyze a given type of chemical transformation.
Functional group:	A structural unit of an atom or group of atoms within an organic compound that has its own characteristic property, regardless of the other atoms in the molecule.
Redox:	A chemical reaction where oxidation and reduction occur simultaneously. An atom is oxidized when it loses electrons and reduced when it gains electrons. Rust is the classic example of oxidation where the reduced iron metal (oxidation state 0) is oxidized to brown iron (oxidation state III) and oxides in the presence of catalysts, such as water, air, or an acid.
Temperature:	An increase in temperature will raise the average kinetic energy of the reactant molecules. As more molecules move faster, the number of molecules moving fast enough to react increases, which results in faster formation of products.

2. Experimental Design

In this study, nine different degradation experiments were designed to mimic the diagenetic effects of different reactions potentially catalyzed by archaeological materials and processes on compounds within ancient plant residues. As catalyst-driven alterations have thus far been insufficiently investigated in archaeological contexts, five different catalysts—bronze, copper, iron, clay (montmorillonite) and gypsum ($CaSO_4$ $2H_2O$)—were selected to simulate archaeological materials. These are among the most common materials used to produce vessels that held organic substances in the past. To evaluate natural taphonomic processes, accelerated redox reactions were also separately induced using ammonium peroxodisulfate and sodium borohydride as oxidizing and reducing agents, respectively. Additionally, milder treatments that simulate these reactions naturally were examined, using air oxygen as an oxidant and nitrogen flushing to remove oxygen from the vial and create a non-oxidizing atmosphere (Table 1). To keep the number of treatments within a manageable range, this study leaves aside the influence of microbial activity as well the impact of exposure to light, which can cause free radical reactions and polymerization of compounds, such as terpenoids and phenolics. To promote different degrees of modification, each potential catalyst was investigated under both room temperature (RT) and at 80 °C ("high temperature", HT) for seven-day periods with five replicates for each experiment. After mixing the catalysts with the oil, the mixtures sat in airtight 4 mL dram vials for the length of the experiments. A set of five untreated cedar oil samples served as the control treatment (see also *Materials and Methods* Section 4).

Table 1. Catalysts used to simulate metabolite reactions with organic compounds caused by archaeological materials (clay, gypsum, bronze, copper, and iron) or redox processes (oxidation, reduction).

Catalyst	Simulated Archaeological Material or Natural Reactions	Sample Code for Room RT and HT Treatments
Montmorillonite KSF clay (SiO_2, Al_2O_3, H_2SO, Fe_2O_3, CaO, MgO)	Clay	ClayRT, ClayHT
Calcium sulfate dihydrate ($CaSO_4$ $2H_2O$)	Gypsum (alabaster)	GypsRT, GypsHT
Bronze powder (Cu:Sn, 90:10)	Bronze	BrRT, BrHT
Copper powder (Cu, 106 µm)	Copper	CuRT, CuHT
Iron powder (Fe, <212 µm)	Iron	FeRT, FeHT
Sodium borohydride ($NaBH_4$)	Reduction	RedRT, RedHT
Flushing with N_2	Non-oxidizing atmosphere	N_2RT, N_2HT
Ammoniumperoxodisulfat ($(NH_4)2S_2O_8$)	Oxidation	OxRT, OxHT
Air	Oxidation	AirRT, AirHT

While we acknowledge that these treatments cannot exactly simulate long-term exposure to a burial environment or the storage within a particular vessel, the study has been designed to assess the major implications of different types of reactions and chemical catalyses for secondary metabolite degradation. As such, the results can provide broad insights into possible pathways of molecular transformation and loss in archaeological samples.

3. Results and Discussion

3.1. GC-MS Results and Multivariate Analyses

We detected a total of 102 compounds across all treatments after seven days of experimental incubation (Tables 2 and S1). Of the 102 detected metabolites, 72 were present in the fresh cedar samples, and the remaining 30 were derivatives formed during the different degradation processes. The absolute peak areas were converted to relative abundances by dividing the area of each peak by the total peak area of a given sample.

The combination of temperature treatments and different potential catalysts simulating archaeological materials had varying effects on the degradation of secondary metabolites in fresh cedar tree oil and on the formation of derivatives. Taken together, we divided the observed compounds into three groups, based on the following criteria: The first group, G1, contains all compounds that were more abundant in the fresh cedar oil than in any of the degradation treatments (with the exception of a few compounds in N_2 treatments and RedRT that were equal to fresh; see Tukey post-hoc multiple comparisons in Table S2). G1 comprised 20 compounds that decreased in the large majority of treatments relative to fresh samples, such as compound #32, whose relative abundance in the CuRT treatment was reduced to one-third compared to fresh. The second group, G2, is composed of 40 compounds that were present in fresh cedar oil, but whose relative abundance appeared to increase during exposure to a catalyst and heat, such as #55, which was 16 times more abundant in FeHT than in fresh oil. The other compound in this group tended to remain relatively stable and only slightly increased. The third and final group, G3, comprised 30 compounds that were absent in fresh cedar oil but present in one or several of the degradation treatments, as well as 12 compounds that were present in fresh cedar oil but in quantities that are barely detectable (<0.02% relative area). These compounds are the result of the chemical reactions during the experiment and are, therefore, likely to be present in archaeological samples. G3 compounds were more difficult to identify, and their relative abundances were generally lower than the G1 and G2 compounds.

Table 2. Identified compounds by GC-MS analysis in the degradation experiments as well as in the fresh samples (29 out of 102). The remaining compounds could not be securely identified. Compounds displayed in bold were confirmed with analytical standards or had a match factor of >900. Compound names given in italic had a match factor >800.

ID	t_r	Compound Identification
#01	8.28	**4-Acetyl-1-methylcyclohexene**
#02	8.46	**3-Cyclohexene-1-methanol, α,4-dimethyl-**
#03	8.94	**Ethanone, 1-(4-methylphenyl)-**
#04	8.99	**α-Terpineol**
#07	11.48	**α-Longipinene**
#10	11.90	*Isolongifolene, 4,5-dehydro-*
#17	12.52	**Longifolene**
#20	12.80	**Vestitenone**
#23	13.07	**Himachala-2,4-diene**
#24	13.24	**α-Himachalene**
#29	13.70	**γ-Himachalene**
#30	13.77	**Himachala-1,4-diene**
#32	14.09	**β-Himachalene**
#33	14.25	**α-Dehydro-ar-himachalene**
#34	14.31	**δ-Cadinene**
#35	14.39	**Calamene** (cis/trans?)
#37	14.49	**α-Bisabolene**
#38	14.58	**γ-Dehydro-ar-himachalene**
#39	14.72	**ar-Himachalene**
#40	14.80	*Calacorene (α/β?)*
#47	15.47	**Oxidohimachalene**
#55	16.32	**β-Himachalene oxide**
#57	16.51	*Epicubenol*
#63	17.03	**Himachalol**
#66	17.26	**Allohimachalol**
#70	17.48	*γ-Atlantone (E/Z?)*
#73	17.70	**2,2,6-Trimethyl-6-(4-methylcyclohex-3-en-1-yl)dihydro-2H-pyran-4(3H)-one**
#74	17.80	**α-Atlantone**
#87	18.79	**Atlantone**

The spread of the various treatments in the PCA plots across each of the three groups illustrates the contrasting effects of the various catalyst and temperature treatments on chemical profiles (Figure 1). Both treatments simulating a non-oxidizing atmosphere (N_2RT and N_2HT) and RedRT resembled the fresh samples in the G1 and G2 groups (Figure 1A,B). It is noticeable that both Cu treatments as well as the OxHT greatly influences chemical composition compared to that of the fresh samples and that the effect of temperature depends mostly on the catalyst used. For example, the effect of high temperature on the abundance of the G1 compounds stands out for Ox and Clay but less so for Air. For the G2 compounds, the effect of high temperature is particularly noticeable for Ox and Red, but not for Br, Cu, and N_2 (except for one outlier sample) (Figure 1B). For the G3 compounds, the effect of temperature is comparatively less noticeable except for Ox (Figure 1C). Interestingly, RT causes more divergent chemical profiles than HT for Cu. The intra-treatment composition was largely consistent, i.e., samples within one treatment fell close to one another in the multivariate space except for the samples in the Ox- and ClayHT and CuRT treatments.

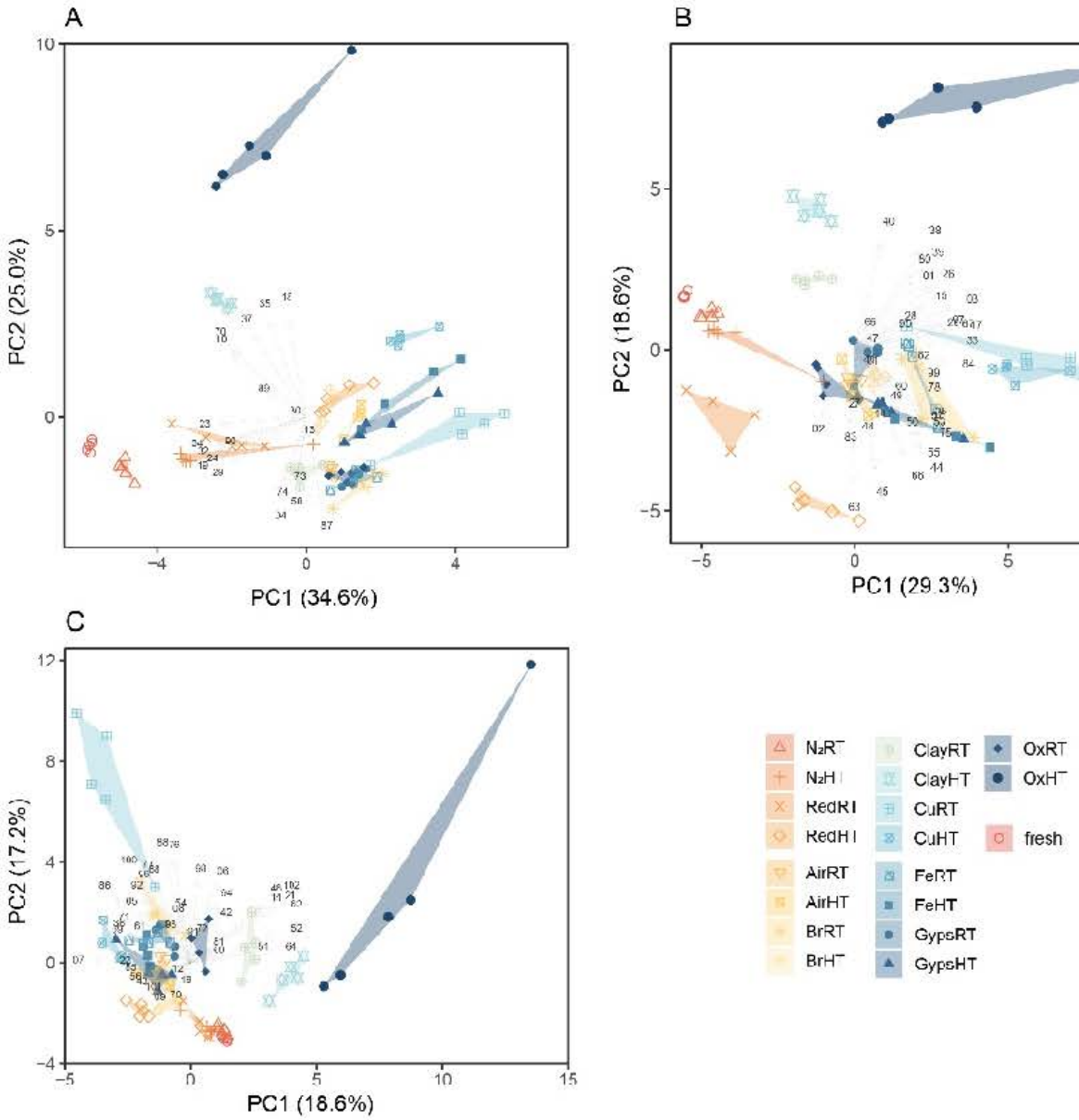


Figure 1. Principal component analyses (PCA) plots based on relative abundance values of each compound with each subplot (**A**–**C**) displaying the first two principal components for the compound groups G1, G2, and G3, respectively. Values in parentheses are the percentage variations accounted by each PC1 and PC2 axis, and the arrows represent the relative weightings of the independent variable, i.e., compound, for creating the PCA. See Supplementary Materials Figure S1 for a visualization of the first four principal components of each compound group.

Within each of the three compound groups, the treatments were divided into six clusters based on their similarities and dissimilarities in compound composition. As illustrated in the clustered heatmaps (Figures 2A, 3A and 4A), the color of a cell represents the relative abundance (% area) of each compound in each degradation experiment as well as the fresh sample. In all three groups, CuRT and OxHT always formed two separate clusters (1, 2) due to their high rate of degrading fresh compounds and forming of new ones. Both clusters showed major reductions in the relative abundance of compounds in G1 and displayed a strong increase in G2 and G3, demonstrating the significant effects of these treatments on the chemical compositions (Table S2).

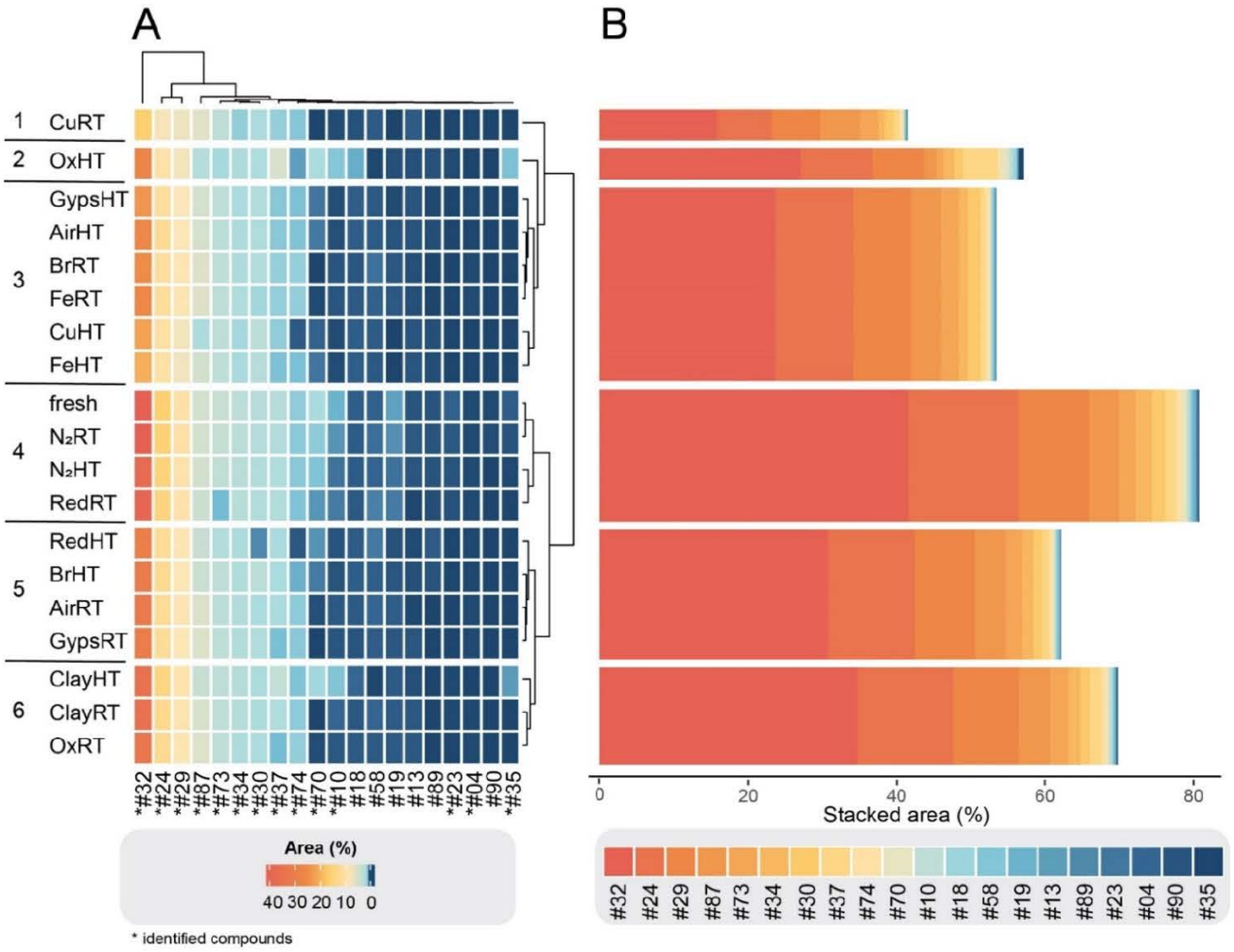


Figure 2. (**A**) Clustered heatmap of G1 compounds in which the color of a cell is proportional to its relative abundance (% area). The length of the branches represents the Euclidean distance or dissimilarity between clusters. The plot comprises 20 detected compounds that had reduced concentrations after the treatment. The horizontal bar plots (**B**) show stacked areas (%) of each of the degraded compounds (average relative abundance of all treatments per cluster).

In G1, fresh samples cluster with both N_2 treatments and RedRT in cluster 4, showing that these treatments affected degradation of fresh G1 compounds minimally (Figure 2A). The clustering in G2 showed a similar distribution, where N_2RT and RedRT were again grouped with the fresh sample (cluster 5; Figure 3A). This trend continued in G3 (Figure 4A) with slight variations in clustering patterns, resulting in both N_2 and Clay treatments clustering with fresh samples (cluster 6) and Red treatments forming a distinct category (cluster 5) next to it.

Throughout all the groups, ClayRT and HT always fell into the same cluster (cluster 6), demonstrating that temperature impacts compounds in clay treatments minimally. More pronounced changes, however, were observed for gypsum and metals. Here, temperature played an important role, dividing these materials into two clusters in G3, placing the RT treatments in cluster 3 and HT treatments in cluster 4. Both clusters showed a substantial increase of certain compounds with very low concentrations or complete absences in the fresh samples, illustrating the impact of heat in catalyzing the formation of novel derivatives in these cases. Copper, iron, gypsum, and AirHT also clustered together in G2 (cluster 3). Again, this cluster was characterized by a high increase of compounds formed during degradation. However, the largest in G2, cluster 4, is composed of a mixture of RT and HT treatments, including both bronze treatments, Fe, Gyps, Air, and OxRT as well as Red and

N_2HT. In G1, the clusters containing the metals and gypsum (3 and 5) were also a mixture of HT and RT treatments. In all three groups, both Air treatments were always clustered together with gypsum and metal experiments. RT and HT Air are always divided into two different clusters, and their distributions resemble those of gypsum throughout all cluster groups.

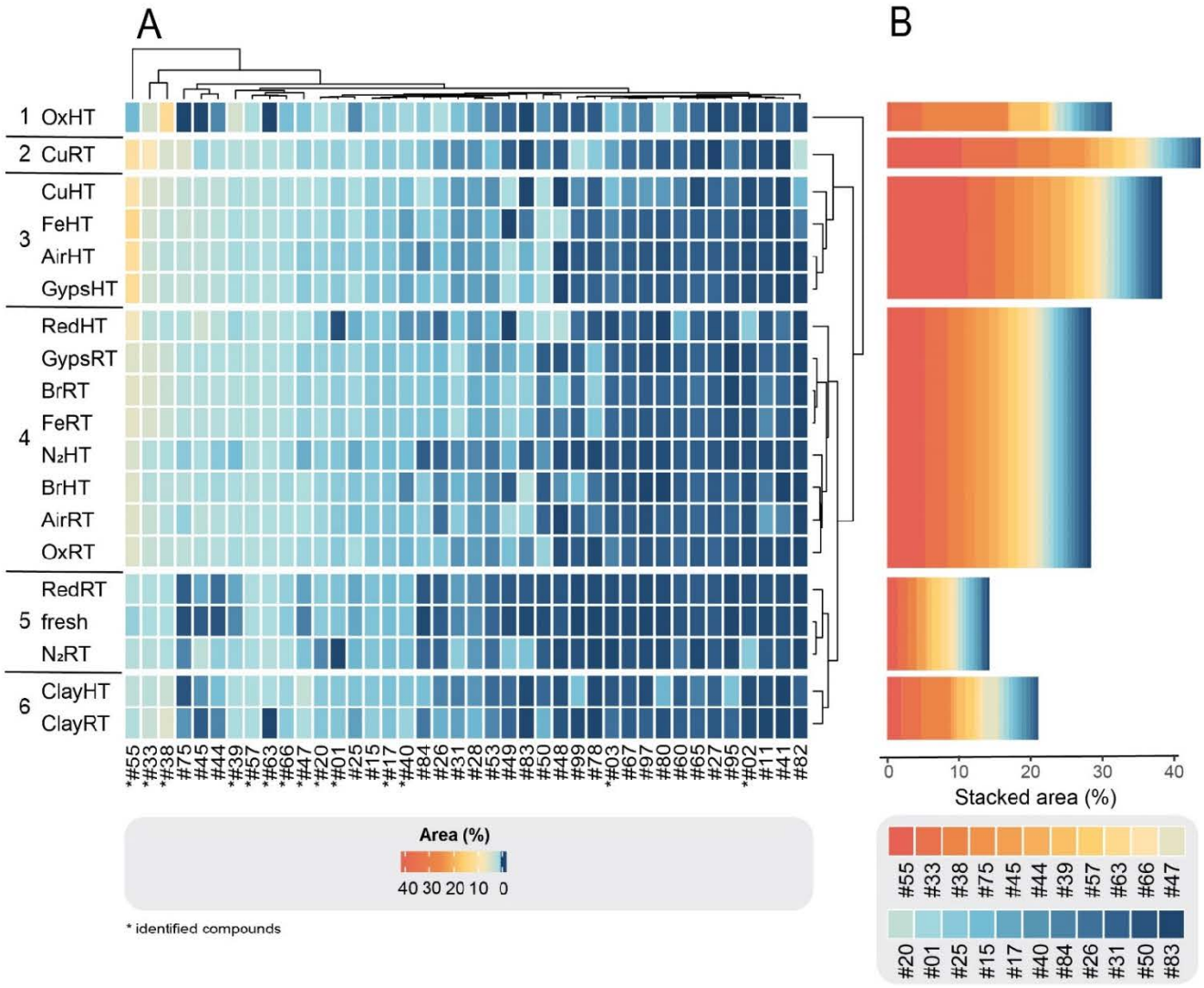


Figure 3. (**A**) Clustered heatmap of G2 compounds. The plot comprises 40 detected compounds that had increased concentrations after the treatment. The horizontal bar plots (**B**) show stacked areas (%) of each of the 20 most abundant degraded compounds.

To quantitatively assess changes in compound composition during degradation, we depicted the stacked areas of the most abundant compounds (for visual purposes only 20 compounds are depicted) for each group (Figures 2B, 3B and 4B). Each compound in the bar plots represents the average relative abundance of all treatments within each cluster. As expected from our criteria for dividing the 102 compounds into three groups, G1 cluster-4 containing the fresh treatment was by far the most abundant group of compounds, comprising stacked areas of 80%. In contrast, the two other clusters with the fresh treatment, G2 cluster-5 and G3 cluster-6, had low stacked areas of 15% and 2%, respectively (the remaining 3% is not visualized). The stacked areas also showed that the CuRT treatment followed by most of the HT treatments caused the greatest change in biomolecular profiles compared to the fresh oil.

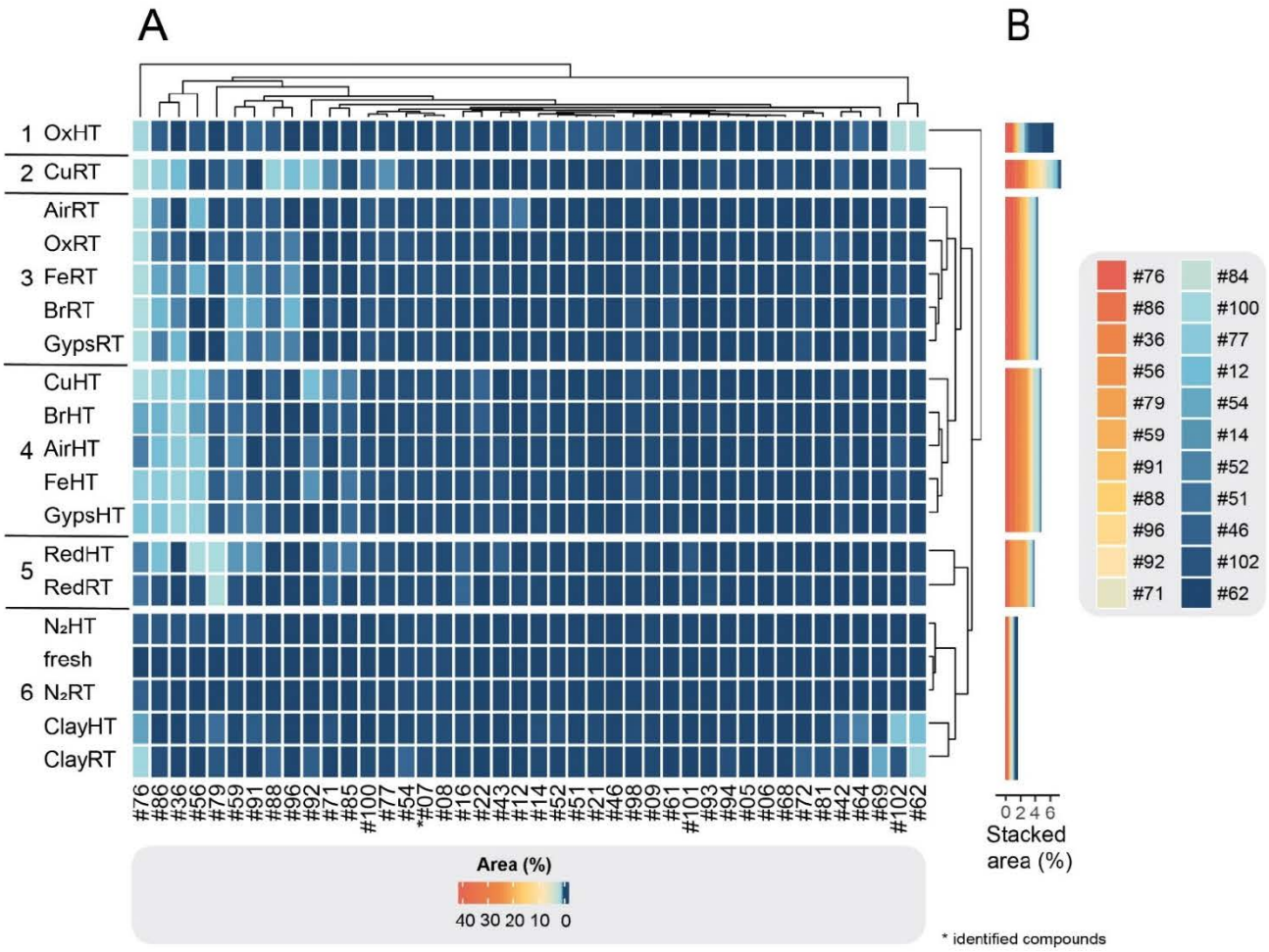


Figure 4. (**A**) Clustered heatmap of G3 compounds. The plot comprises 42 detected compounds that are absent or barely detectable in fresh cedar oil but present in one or several of the degradation treatments. The horizontal bar plots (**B**) show stacked areas (%) of each of the 20 most abundant degraded compounds.

When looking at specific compounds that played a larger diagnostic role among groups, the catalysts had a higher impact on the changes than temperature, as expected. In treatments with the reducing agent or clay, the differences between samples incubated at 80 °C and at RT were minimal for most compounds. For example, 4-acetyl-1-methylcyclohexene (#01, G2) was relatively stable throughout all treatments and in the fresh oils, with the exception of samples containing the reducing agent where this compound almost completely disappeared in both the RT (RedRT) and the HT (RedHT) treatments. The compound 3-cyclohexene-1-methanol, α,4-dimethyl- (#02, G2) can be used to illustrate the opposite effect. This compound had a very low concentration in the fresh sample, which did not change in the other treatments but is drastically increased in both RT and HT reduction treatments. However, some other compounds were also highly affected by temperature, particularly in experiments with the oxidation agent and the copper catalyst.

3.2. Compound Identification

The predominant compounds present in the fresh *Cedrus atlantica* oil were sesquiterpenes with the himachalene carbon skeleton generally found in the genus cedar [50–54], with the bicyclic sesquiterpene hydrocarbons α- γ- and β-himachalenes (#24, #29, #32) forming the largest peaks. Other constituents of the himachalane series, such as himachalene-1,4-diene (#30), α- and γ-dehydro-ar-himachalene (#33, #38), ar-himachalene (#39), oxidohi-

machalene (#47), (#55), and himachalol (#63) were detected in low amounts. Sesquiterpene ketones, such as atlantones, were also present in fresh oil.

Compared to the distributions of compounds from the degradation experiment, all sequiterpenoids from the himachalane series detected in fresh cedar oil were also present in the treated samples, although in variable proportions. Some almost completely disappeared, such as himachala-2,4-diene (#23), or were profoundly reduced in concentrations, such as himachalene-1,4-diene (#30) and β-himachalene (#32) (see G1 compounds). The latter drastically decreased in all samples with the Cu and Fe catalysts as well as in Ox, Gyps, and AirHT (G1, clusters 1–3). All the reduced himachalenes are compounds with nonconjugated double bonds, which makes them more sensitive to chemical alterations [40] and helps to explain the decrease of these constituents. Other compounds were only slightly reduced in abundance, such as the isomers α- and γ-himachalene (#24, #29), but remained relatively consistent throughout all experiments.

The potentially most diagnostic compounds for ancient plant identification purposes are those that remain stable or relatively increased in peak areas after the treatments (i.e., G2 compounds), as they are more likely to be detected in ancient residues. Compared to the fresh oil, α- and γ-dehydro-ar-himachalenes (#33 and #38), ar-himachalene (#39) and oxidohimachalene (#47) increased in all treatments to varying extents. Most of them (#33, #38, #39) contain an aromatic functional group—a benzene ring, which gives the compound an increased thermodynamic and chemical stability (Figure 5). Again, the copper and the oxidizing agent had the strongest effect on the alteration of these compounds. The compound with the sharpest increase in most treatments is β-himachalene oxide (#55). The treatments of G2 clusters 2 and 3 resulted in an over 10-fold larger peak area than in the fresh sample. In contrast to other compounds, β-himachalene oxide also showed variations between RT and HT treatments, demonstrating the impact of temperature on the relative increase of the compound. The only experiment leading to a decrease in concentration of β-himachalene oxide was the oxidizing agent in combination with HT, whereas the same reactant at RT led to a stark increase of the peak.

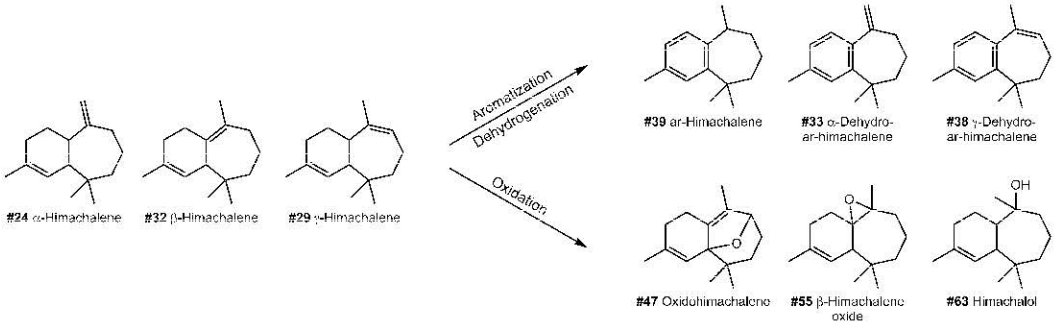


Figure 5. Aromatization, dehydrogenation, and oxidation of himachalenes in *Cedrus atlantica* essential oil.

Apart from the himachalenes, other sesquiterpenes, such as longifolene (#17) and vestitenone (#20), were also relatively stable and resistant to decay. Neither temperature nor catalysts seemed to severely affect the relative abundances of these constituents. By contrast, the ketone atlantone (#87), an abundant compound in the fresh cedar oil, varied in concentration after the experiments, depending on the catalysts as well as temperature. For example, the concentration was reduced severely in the Cu- and OxHT treatments but remained stable or decreased only slightly in the rest of the treatments. Another ketone, γ-atlantone (#70), almost completely disappeared following most treatments, except for the Clay- and OxHT treatment as well as both N_2 treatments.

The most important degradation products that were absent or barely detectable in the fresh cedar samples (i.e., G3 compounds) are presented in Figure 4. These compounds result from of the chemical reactions during the experiment and might, therefore, likely be present in degraded archaeological samples. The clustering of groups shows a clear

division based on the temperature and materials used in the treatment. However, the usefulness of those compounds in diagnosing a source material is limited, as most of them are difficult to detect and identify due to their low abundance. Generally, using degradation compounds as biomarkers requires knowledge of degradation pathways, which we still lack for the majority of plant species.

4. Materials and Methods

4.1. Materials

All solvents used in the experiments were of analytical grade and supplied by Sigma-Aldrich (Munich, Germany) from where calcium sulfate dihydrate ($CaSO_4 \cdot 2H_2O$), sodium borohydride ($NaBH_4$), and montmorillonite KSF (clay) were also purchased. Copper and iron powders (99%, -140 and -70 mesh, respectively) and ammonium peroxodisulfate (98+%) were purchased from Acros (Geel, Belgium), while bronze powder (Cu:Sn; 90:10 wt%, -100 mesh) was obtained from Alfa Aesar (Kandel, Germany). The essential oil of *Cedrus atlantica* was purchased from Florihana Distillerie (Caussols, France). The analytical standards (+)-α-longipinene, (+)-longifolene and (−)-isoledene were supplied by Sigma-Aldrich (Munich, Germany), α-terpineol, bisabolene (mixture of isomers), β-ionone, and ethanone,1-(4-methylphenyl)- by Thermo Fisher Scientific (Kandel, Germany) and 4-acetyl-1-methylcyclohexene by abcr (Karlsruhe, Germany).

4.2. Sample Preparation, Extraction and Analysis

For the experiment, 5 mg of each catalyst were weighed into 4 mL combusted (500 °C for 8 h) glass vials. Subsequently, 25 µL of essential oil of *Cedrus atlantica* were added to each vial. For each catalyst-oil-mixture, two experiments were prepared: (1) a 'heat treatment' where the glass vials were placed in a heating block for seven days at a constant 80 °C; and (2) a treatment of the mixtures at RT for seven days. For each treatment, five replicates were prepared, resulting in a total of 90 samples. All samples were tightly capped before the start of the experiment. For the experiment mimicking a non-oxidizing atmosphere, the vials were flushed with N_2 for circa 5 s and quickly capped thereafter.

After letting all samples incubate for seven days, the samples from the HT treatment were removed from the heating block and centrifuged for 5 min at 500× *g*. Each of the samples was dissolved in 500 µL of CH_2Cl_2 and quickly (~5 s) mixed by vortexing. Essential oils and wood tars are in general completely soluble in CH_2Cl_2, which did not require additional extraction steps [41]. After letting samples rest for 5–10 min for settling of solid remains (catalysts), 10 µL of the extracts were taken out with glass capillaries into 1.5 mL combusted glass vials and diluted with 490 µL of CH_2Cl_2 for GC-MS analysis of the volatile fraction, which was carried out immediately afterwards. The five replicates of fresh, untreated oil were prepared in the same way as the treated samples.

GC-MS analysis was performed using an Agilent 8890 GC-System coupled to an Agilent 5977B GC/MSD. Chromatographic separation was achieved on a HP-5ms 60 m × 250 µm capillary column with a film thickness of 0.25 µm (Agilent, Waldbronn, Germany). The mass spectrometer was operated in electron impact (EI) mode at 70 eV with a scanning range from m/z 30 to 500 amu, and helium was used as a carrier gas. The GC oven temperature was held isothermally for 1 min at 60 °C, ramped to 150 °C at 30 °C/min and held for 1 min, increased at a rate of at 5 °C/min to 200 °C with a 1 min hold and then increased again at 15 °C/min to 320 °C with a final hold time of 1 min. The transfer line and source temperature were set at 250 °C and 230 °C, respectively. The total run time was 25 min with a solvent delay of 7 min. Injection volume was 1 µL with a split ratio of 5:1.

4.3. Data Pretreatment and Statistical Analysis

The Agilent MassHunter Qualitative Data Analysis software 10.0 was used for processing the GC-MS acquisition files. For peak integration, absolute peak area filters ≥1000 counts were used for exporting peak lists. Chromatographic peaks were identified based on comparison with retention times and mass spectra of analytical standards

where available, by comparison to reference mass spectra in the NIST database (NIST 2.2), and with spectra reported in the literature. The peaks were aligned with the R package 'CGalignR' by setting the maximum distance for linear corrections to 0.06 and the minimum expected distance between peaks to 0.04 [55,56]. The chemical profiles (i.e., the relative distribution of compounds) of each replicate within each treatment were largely consistent except for two samples (AirHT.3 and FeHT.5). Since the cause of these very divergent profiles are unknown, we excluded these two outliers from the multivariate analyses. The peak areas of replicates from two metal RT treatments (BrRT replicates 2 and 5 and FeRT replicates 2 and 5) were approximately six time greater than the remaining replicates. However, we kept these four replicates in the multivariate analyses because their relative peak areas resembled the other replicates within their respective treatments. After manually inspecting the peak alignments, removing outlier peaks, and comparing peak areas across all samples, the data were converted to relative abundances by dividing the area of each peak by the total peak area of a given sample.

All statistical analyses were performed in R (version 4.0.1, R-Development-Core-Team, 2017-11-30) with R Studio interface version 1.3.959. The relative abundance of each peak was compared across treatments with a one-way ANOVA with Tukey's HSD test. To identify similarities and differences among the treatments, we applied principal component analysis (PCA). PCA is an unsupervised technique that seeks to maximize variability among samples while reducing the number of dimensions. We rendered clustered heatmaps with the R package 'ComplexHeatmap' to reveal hierarchical clusters, which is a two-way display of a data matrix in which the colour of a cell is proportional to its position along a colour gradient. A group average was used for the cluster algorithms. The length of the branches represents the Euclidean distance or dissimilarity between clusters.

5. Conclusions

Our degradation experiments provide a means of better understanding the complexity of secondary metabolite transformation patterns of ancient plant residues. The careful consideration of catalyst-driven and natural chemical alterations when interpreting the chemistry of plant-derived compounds is expected to lead to a more accurate and rigorous source identification of residues attached to ancient vessels and containers from archaeological contexts. The compounds detected in the degradation experiments described herein were grouped into three categories:

1. G1 compounds disappeared or were decreased after seven days of incubation. If such stark declines were evident after only seven days, albeit under conditions that accelerate degradation, it can be assumed that they are very unlikely to remain in ancient residues, deposited for centuries or millennia. These compounds should, therefore, not be considered as diagnostic biomarkers for ancient plants and plant products under most conditions. Perhaps as importantly, their absence in archaeological samples cannot be considered as useful evidence for the absence of certain plants or for the identification of certain plants over other possible candidates.
2. G2 compounds remained relatively stable or increased over time, particularly oxidized and dehydrogenated compounds that have lost even numbers of hydrogen atoms to form double bonds, which generally makes them more stable and conceivably likely to remain in archaeological samples. These compounds might therefore be considered as more valid biomarkers aiding in the identification of archaeological residues.
3. G3 compounds were not present in fresh cedar oil but formed during specific experiments and are indicative of certain catalysts/storage materials. These compounds could, therefore, also be possible biomarkers for identifying plant materials in archaeological samples provided that these compounds can be identified. In our study, we were only able to securely identify one of the 42 G3 compounds. However, unknown compounds can still provide information regarding the processes involved in the preparation of plant-based products in the past. For example, compound #76 was relatively high in RT treatments in combination with Cu, Fe, and Br, showing that a

high abundance of this compound could be indicative for the contact of cedar residues with metal vessels. Concomitantly, compound #79 appeared in high abundance in both reduction treatments, indicating a reduction process.

While the main purpose of this experiment was to raise awareness of the pitfalls and necessary considerations when using metabolic biomarkers from archaeological specimens for plant identification, it also led us to find robust archaeological biomarkers for the genus *Cedrus*. In general, the himachalenes appear to be good biomarkers for cedar species, as they rarely appear in other plants. We propose α- and γ-dehydro-ar-himachalene (#33 and #38), ar-himachalene (#39), oxidohimachalene (#47), and particularly β-himachalene oxide (#55) as promising molecular markers for identifying archaeological cedar samples, as they increase after redox and catalyst-driven reactions (see G2). Moreover, α-himachalene (#24), himachalol (#63), and allohimachalol (#66) also appear to remain relatively stable. Notably, β -himachalene (#32), the largest component of modern cedar oil, was rapidly reduced in all experiments. This reduction was detected after only seven days; hence, this compound most likely will not be well-preserved in archaeological samples. Similarly, himachala-2,4-diene (#23) and himachalene-1,4-diene (#30) should only be considered as archaeological biomarkers under very limited conditions (see G1). Longifolene (#17) and vestitenone (#20) have proven to be relatively resistant to decay.

Our findings can be compared to results from analyses of archaeological plant residues which were identified as plant products from cedar [41] where dehydrogenated analogues of sesquiterpenoids from himachalenes (e.g., #033 and #038) were also detected, while G1 himachalenes were absent. The similarities between archaeological findings and our accelerated degradation experiments underline the potential for expanding accelerated degradation studies to terpenoids from other plants, as well as to compounds belonging to other natural product classes. It is important to note that degradation conditions can be designed and refined to resemble particular archaeological settings in terms of the catalysts used but also that there is a scope for investigating how biotic conditions, such as bacterial or fungal degradation as well as exposure to light, alter plant metabolic profiles in the archaeological record. These types of data can serve as a cautionary note when identifying plants in ancient samples through comparison with modern materials and can help to avoid misidentification of materials based on common or unstable molecules.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/molecules27103331/s1, Table S1: Absolute peak areas of all samples; Table S2: One-way ANOVA along with the Tukey post-hoc multiple comparisons; Figures S1: PCA plots displaying the first four principal components of each of the G1, G2, and G3 compound groups.

Author Contributions: Conceptualization, B.H. and T.L.; methodology, D.G.V. and B.H.; statistical analysis, T.L. and Y.V.W.; validation, B.H., T.L. and D.G.V.; formal analysis, B.H. and D.G.V.; data curation, B.H. and T.L.; writing—original draft preparation, B.H.; writing—review and editing, B.H., D.G.V., P.R., T.L. and Y.V.W.; visualization, T.L. and B.H.; supervision, T.L. and P.R. All authors have read and agreed to the published version of the manuscript.

Funding: The authors are grateful to the Max Planck Society for funding this research and the publication of this manuscript in open access form under the Projekt DEAL. B. Huber thanks the Joachim Herz Foundation for being awarded an Add-on Fellowship for Interdisciplinary Life Sciences for her PhD research.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data supporting reported results can be found in the Supplementary Materials and be downloaded at: www.mdpi.com/xxx/s1.

Acknowledgments: The authors gratefully acknowledge Max Planck Institute for Chemical Ecology, Department of Biochemistry for providing us with some of the catalysts used in the study.

Conflicts of Interest: The authors declare no conflict of interest.

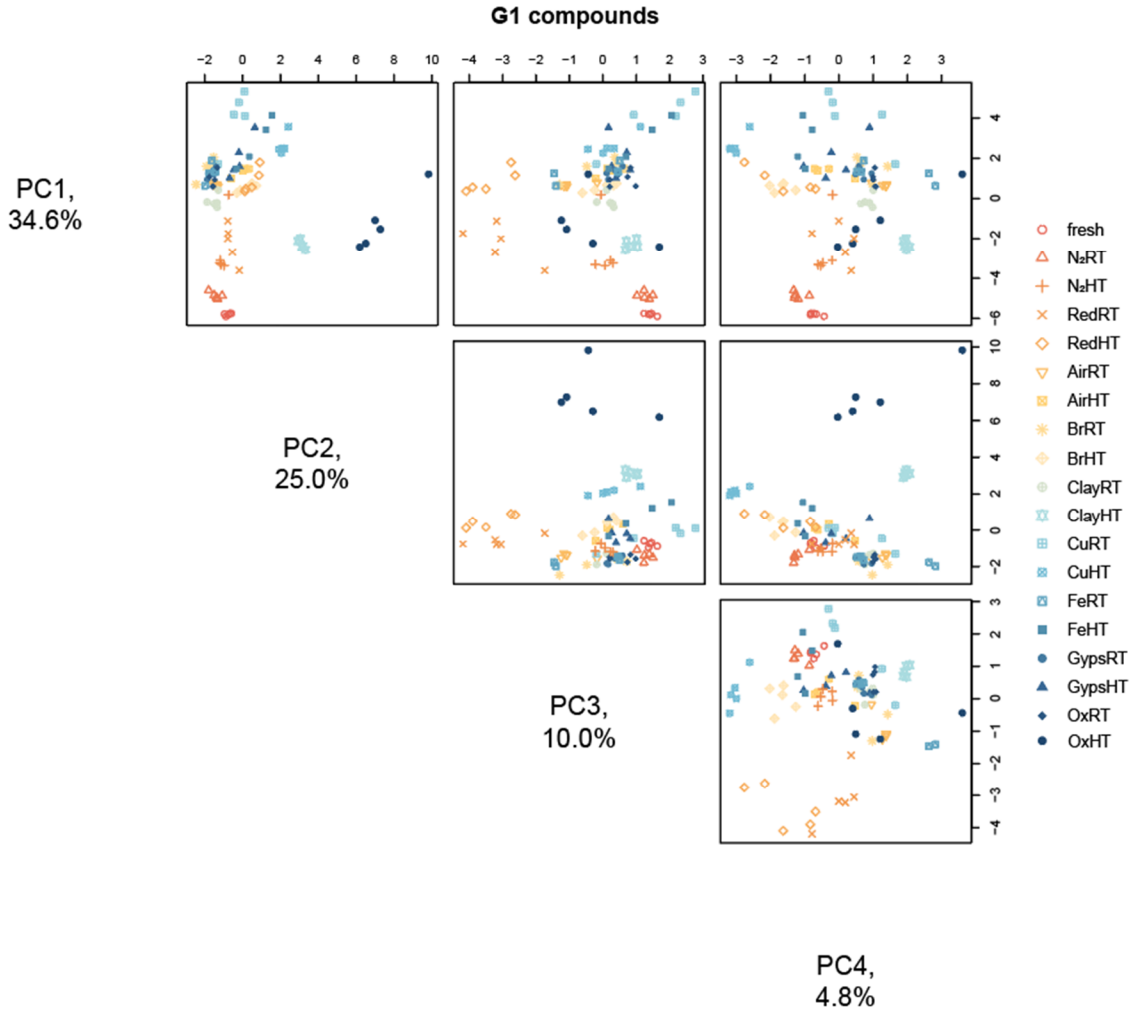
References

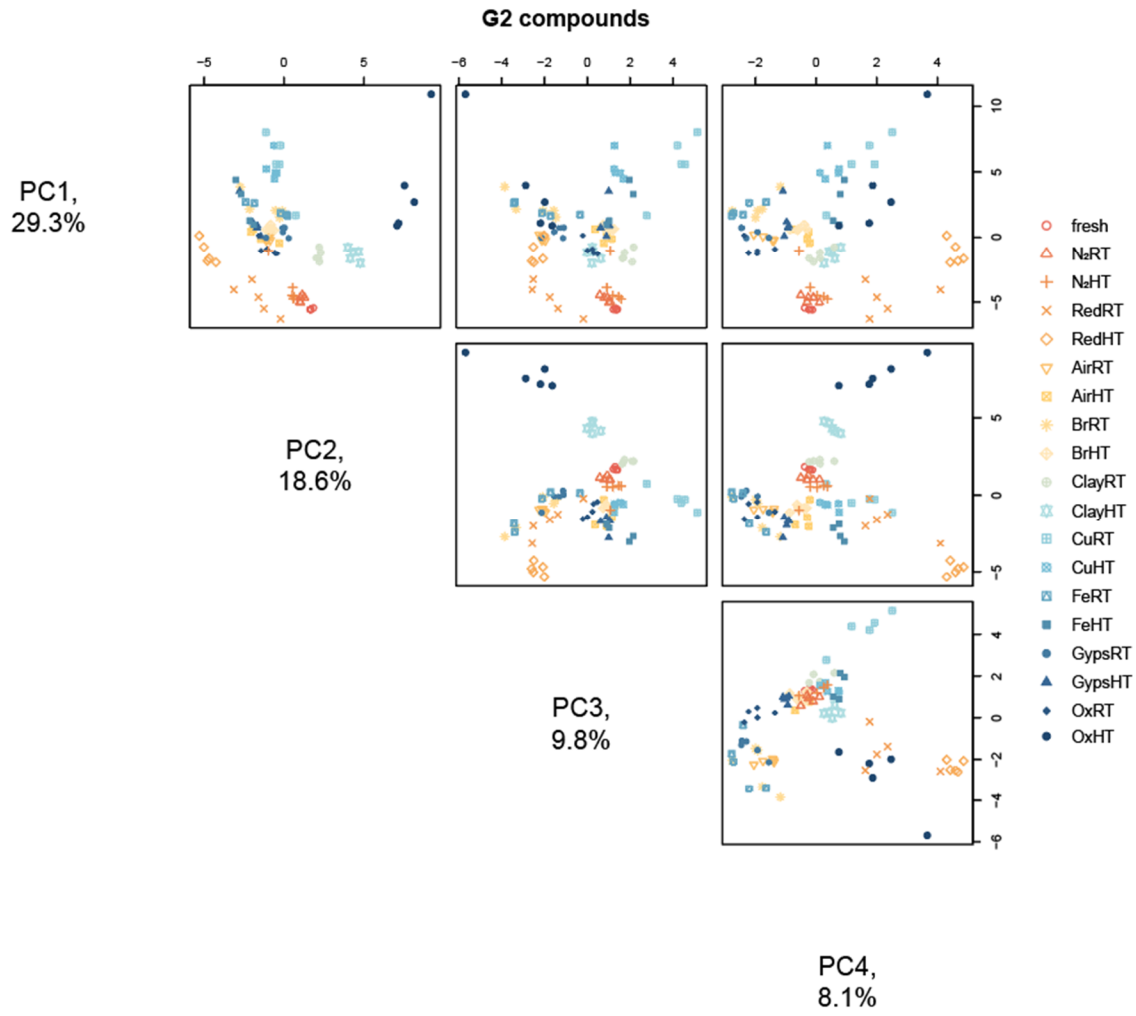
1. Zimmermann, M.; Brownstein, K.J.; Pantoja Díaz, L.; Ancona Aragón, I.; Hutson, S.; Kidder, B.; Tushingham, S.; Gang, D.R. Metabolomics-Based Analysis of Miniature Flask Contents Identifies Tobacco Mixture Use among the Ancient Maya. *Sci. Rep.* **2021**, *11*, 1590. [CrossRef] [PubMed]
2. Brockbals, L.; Habicht, M.; Hajdas, I.; Galassi, F.M.; Rühli, F.J.; Kraemer, T. Untargeted Metabolomics-like Screening Approach for Chemical Characterization and Differentiation of Canopic Jar and Mummy Samples from Ancient Egypt Using GC-High Resolution MS. *Analyst* **2018**, *143*, 4503–4512. [CrossRef] [PubMed]
3. Dunne, J.; Mercuri, A.M.; Evershed, R.P.; Bruni, S.; di Lernia, S. Earliest Direct Evidence of Plant Processing in Prehistoric Saharan Pottery. *Nat. Plants* **2016**, *3*, 16194. [CrossRef] [PubMed]
4. Hendy, J.; Colonese, A.C.; Franz, I.; Fernandes, R.; Fischer, R.; Orton, D.; Lucquin, A.; Spindler, L.; Anvari, J.; Stroud, E.; et al. Ancient Proteins from Ceramic Vessels at Çatalhöyük West Reveal the Hidden Cuisine of Early Farmers. *Nat. Commun.* **2018**, *9*, 4064. [CrossRef] [PubMed]
5. Scott, A.; Power, R.C.; Altmann-Wendling, V.; Artzy, M.; Martin, M.A.S.; Eisenmann, S.; Hagan, R.; Salazar-García, D.C.; Salmon, Y.; Yegorov, D.; et al. Exotic Foods Reveal Contact between South Asia and the Near East during the Second Millennium BCE. *Proc. Natl. Acad. Sci. USA* **2020**, 202014956. [CrossRef] [PubMed]
6. Riesmeier, M.; Keute, J.; Veall, M.-A.; Borschneck, D.; Stevenson, A.; Garnett, A.; Williams, A.; Ragan, M.; Devièse, T. Recipes of Ancient Egyptian Kohls More Diverse than Previously Thought. *Sci. Rep.* **2022**, *12*, 5932. [CrossRef] [PubMed]
7. Miller, M.J.; Whelton, H.L.; Swift, J.A.; Maline, S.; Hammann, S.; Cramp, L.J.E.; McCleary, A.; Taylor, G.; Vacca, K.; Becks, F.; et al. Interpreting Ancient Food Practices: Stable Isotope and Molecular Analyses of Visible and Absorbed Residues from a Year-Long Cooking Experiment. *Sci. Rep.* **2020**, *10*, 13704. [CrossRef] [PubMed]
8. Huber, B.; Larsen, T.; Spengler, R.N.; Boivin, N. How to Use Modern Science to Reconstruct Ancient Scents. *Nature Human Behaviour* **2022**. [CrossRef]
9. Evershed, R.P. Organic Residue Analysis in Archaeology: The Archaeological Biomarker Revolution. *Archaeometry* **2008**, *50*, 895–924. [CrossRef]
10. Evershed, R.P. Biomolecular Archaeology and Lipids. *World Archaeol.* **1993**, *25*, 74–93. [CrossRef]
11. Arimura, G.; Maffei, M. (Eds.) *Plant. Specialized Metabolism: Genomics, Biochemistry, and Biological Functions*; Life Science; First Issued in Paperback: Boca Raton, FL, USA; CRC Press: London, UK; Taylor & Francis Group: New York, NY, USA, 2021; ISBN 978-1-03-209753-4.
12. Paul, M.; Brüning, G.; Bergmann, J.; Jauch, J. A Thin-Layer Chromatography Method for the Identification of Three Different Olibanum Resins (*Boswellia Serrata*, *Boswellia Papyrifera* and *Boswellia Carterii*, Respectively, *Boswellia Sacra*): Thin-Layer Chromatography Identification Of Olibanum. *Phytochem. Anal.* **2012**, *23*, 184–189. [CrossRef] [PubMed]
13. Ribechini, E.; Raffaelli, M.; Colombini, M.P. Botanical and Chemical Characterization of Frankincense Resin from Dhofar. In *A port in Arabia between Rome and the Indian Ocean (3rd C. BC–5th C. AD)*; Avanzini, A., Ed.; Khor Rori Report; "L'Erma" di Bretschneider: Roma, Italy, 2008; pp. 681–686, ISBN 978-88-8265-469-6.
14. Salomé-Abarca, L.F.; van der Pas, J.; Kim, H.K.; van Uffelen, G.A.; Klinkhamer, P.G.L.; Choi, Y.H. Metabolic Discrimination of Pine Resins Using Multiple Analytical Platforms. *Phytochemistry* **2018**, *155*, 37–44. [CrossRef] [PubMed]
15. Degano, I.; Soriano, S.; Villa, P.; Pollarolo, L.; Lucejko, J.J.; Jacobs, Z.; Douka, K.; Vitagliano, S.; Tozzi, C. Hafting of Middle Paleolithic Tools in Latium (Central Italy): New Data from Fossellone and Sant'Agostino Caves. *PLoS ONE* **2019**, *14*, e0213473. [CrossRef] [PubMed]
16. Orengo, H.A.; Palet, J.M.; Ejarque, A.; Miras, Y.; Riera, S. Pitch Production during the Roman Period: An Intensive Mountain Industry for a Globalised Economy? *Antiquity* **2013**, *87*, 802–814. [CrossRef]
17. Buckley, S.A.; Clark, K.A.; Evershed, R.P. Complex Organic Chemical Balms of Pharaonic Animal Mummies. *Nature* **2004**, *431*, 294–299. [CrossRef]
18. Fulcher, K.; Serpico, M.; Taylor, J.H.; Stacey, R. Molecular Analysis of Black Coatings and Anointing Fluids from Ancient Egyptian Coffins, Mummy Cases, and Funerary Objects. *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2100885118. [CrossRef]
19. Whelton, H.L.; Hammann, S.; Cramp, L.J.E.; Dunne, J.; Roffet-Salque, M.; Evershed, R.P. A Call for Caution in the Analysis of Lipids and Other Small Biomolecules from Archaeological Contexts. *J. Archaeol. Sci.* **2021**, *132*, 105397. [CrossRef]
20. Capetti, F.; Rubiolo, P.; Bicchi, C.; Marengo, A.; Sgorbini, B.; Cagliero, C. Exploiting the Versatility of Vacuum-assisted Headspace Solid-phase Microextraction in Combination with the Selectivity of Ionic Liquid-based GC Stationary Phases to Discriminate Boswellia Spp. Resins through Their Volatile and Semivolatile Fractions. *J. Sep. Sci.* **2020**, *43*, 1879–1889. [CrossRef]
21. Gelbrich, J.; Mai, C.; Militz, H. Chemical Changes in Wood Degraded by Bacteria. *Int. Biodeterior. Biodegrad.* **2008**, *61*, 24–32. [CrossRef]
22. Malainey, M.E.; Przybylski, R.; Sherriff, B.L. The Effects of Thermal and Oxidative Degradation on the Fatty Acid Composition of Food Plants and Animals of Western Canada: Implications for the Identification of Archaeological Vessel Residues. *J. Archaeol. Sci.* **1999**, *26*, 95–103. [CrossRef]
23. Tamburini, D.; Łucejko, J.; Ribechini, E.; Colombini, M.P. New Markers of Natural and Anthropogenic Chemical Alteration of Archaeological Lignin Revealed by in Situ Pyrolysis/Silylation-Gas Chromatography mass Spectrometry. *J. Anal. Appl. Pyrolysis* **2016**, *118*, 249–258. [CrossRef]

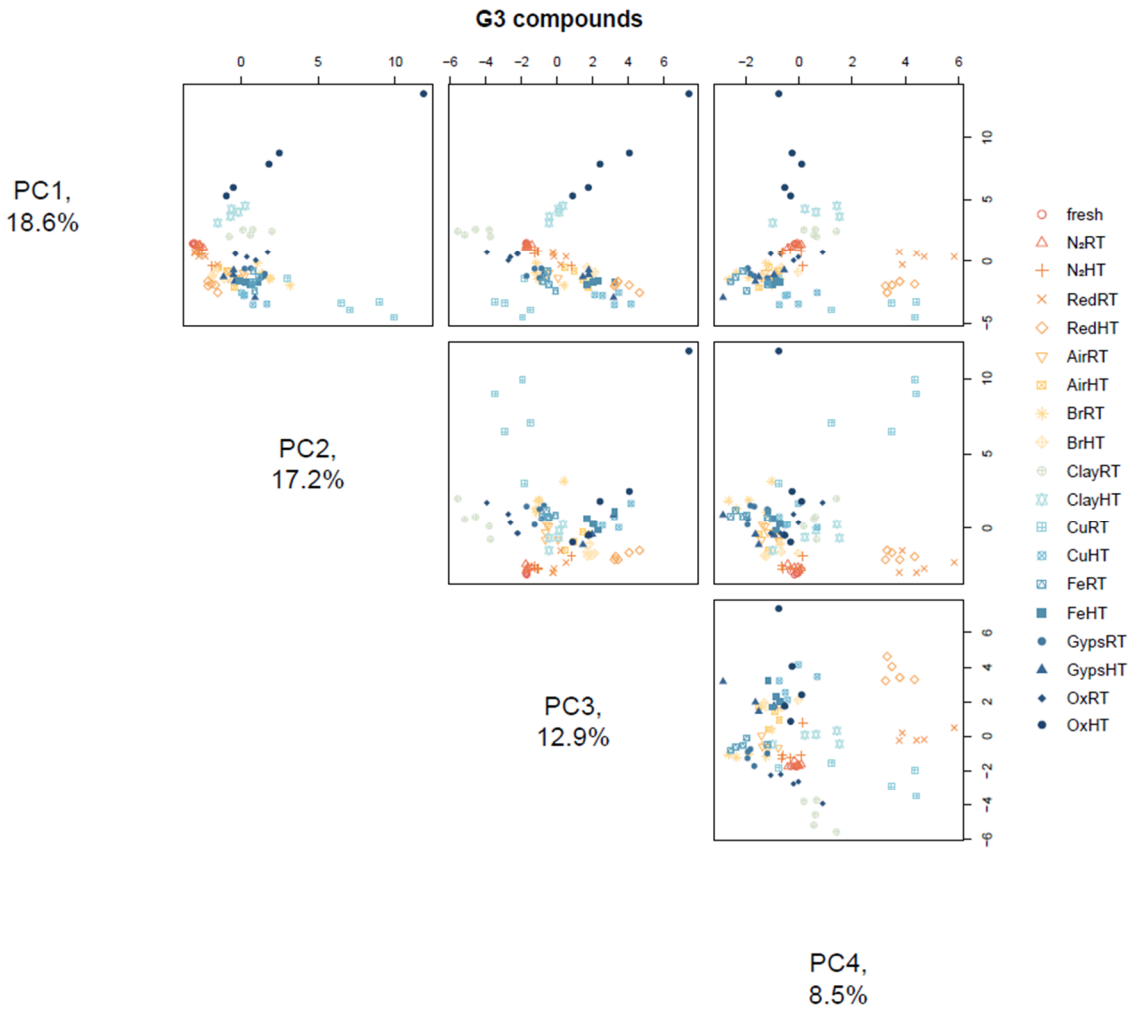
24. Brunschwig, C.; Collard, F.X.; Bianchini, J.-P.; Raharivelomanana, P. Evaluation of Chemical Variability of Cured Vanilla Beans (*Vanilla tahitensis* and *Vanilla planifolia*). *Nat. Prod. Commun.* **2009**, *4*, 1393–1400. [CrossRef] [PubMed]
25. Linares, V.; Adams, M.J.; Cradic, M.S.; Finkelstein, I.; Lipschits, O.; Martin, M.A.S.; Neumann, R.; Stockhammer, P.W.; Gadot, Y. First Evidence for Vanillin in the Old World: Its Use as Mortuary Offering in Middle Bronze Canaan. *J. Archaeol. Sci. Rep.* **2019**, *25*, 77–84. [CrossRef]
26. Lesage-Meessen, L.; Delattre, M.; Haon, M.; Thibault, J.-F.; Ceccaldi, B.C.; Brunerie, P.; Asther, M. A Two-Step Bioconversion Process for Vanillin Production from Ferulic Acid Combining Aspergillus Niger and Pycnoporus Cinnabarinus. *J. Biotechnol.* **1996**, *50*, 107–113. [CrossRef]
27. Duce, C.; Orsini, S.; Spepi, A.; Colombini, M.P.; Tiné, M.R.; Ribechini, E. Thermal Degradation Chemistry of Archaeological Pine Pitch Containing Beeswax as an Additive. *J. Anal. Appl. Pyrolysis* **2015**, *111*, 254–264. [CrossRef]
28. Dudd, S.N.; Regert, M.; Evershed, R.P. Assessing Microbial Lipid Contributions during Laboratory Degradations of Fats and Oils and Pure Triacylglycerols Absorbed in Ceramic Potsherds. *Org. Geochem.* **1998**, *29*, 1345–1354. [CrossRef]
29. Evershed, R.P.; Dudd, S.N.; Copley, M.S.; Berstan, R.; Stott, A.W.; Mottram, H.; Buckley, S.A.; Crossman, Z. Chemistry of Archaeological Animal Fats. *Acc. Chem. Res.* **2002**, *35*, 660–668. [CrossRef] [PubMed]
30. Hammann, S.; Cramp, L.J.E.; Whittle, M.; Evershed, R.P. Cholesterol Degradation in Archaeological Pottery Mediated by Fired Clay and Fatty Acid Pro-Oxidants. *Tetrahedron Lett.* **2018**, *59*, 4401–4404. [CrossRef]
31. Blanchette, R.A. A Review of Microbial Deterioration Found in Archaeological Wood from Different Environments. *Int. Biodeterior. Biodegrad.* **2000**, *46*, 189–204. [CrossRef]
32. Colombini, M.P.; Lucejko, J.J.; Modugno, F.; Orlandi, M.; Tolppa, E.-L.; Zoia, L. A Multi-Analytical Study of Degradation of Lignin in Archaeological Waterlogged Wood. *Talanta* **2009**, *80*, 61–70. [CrossRef]
33. Gjelstrup Björdal, C. Microbial Degradation of Waterlogged Archaeological Wood. *J. Cult. Herit.* **2012**, *13*, S118–S122. [CrossRef]
34. Huisman, D.J.; Manders, M.R.; Kretschmar, E.I.; Klaassen, R.K.W.M.; Lamersdorf, N. Burial Conditions and Wood Degradation at Archaeological Sites in the Netherlands. *Int. Biodeterior. Biodegrad.* **2008**, *61*, 33–44. [CrossRef]
35. Łucejko, J.J.; Modugno, F.; Ribechini, E.; Tamburini, D.; Colombini, M.P. Analytical Instrumental Techniques to Study Archaeological Wood Degradation. *Appl. Spectrosc. Rev.* **2015**, *50*, 584–625. [CrossRef]
36. Devièse, T.; Ribechini, E.; Castex, D.; Stuart, B.; Regert, M.; Colombini, M.P. A Multi-Analytical Approach Using FTIR, GC/MS and Py-GC/MS Revealed Early Evidence of Embalming Practices in Roman Catacombs. *Microchem. J.* **2017**, *133*, 49–59. [CrossRef]
37. Brettell, R.C.; Stern, B.; Reifarth, N.; Heron, C. The 'Semblance of Immortality'? Resinous Materials and Mortuary Rites in Roman Britain. *Archaeometry* **2014**, *56*, 444–459. [CrossRef]
38. Giachi, G.; Pallecchi, P.; Romualdi, A.; Ribechini, E.; Lucejko, J.J.; Colombini, M.P.; Mariotti Lippi, M. Ingredients of a 2,000-y-Old Medicine Revealed by Chemical, Mineralogical, and Botanical Investigations. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 1193–1196. [CrossRef]
39. Regert, M.; Devièse, T.; Le Hô, A.-S.; Rougeulle, A. Reconstructing Ancient Yemeni Commercial Routes during the Middle Ages Using Structural Characterization of Terpenoid Resins. *Archaeometry* **2008**, *50*, 668–695. [CrossRef]
40. Ren, M.; Tang, Z.; Wu, X.; Spengler, R.; Jiang, H.; Yang, Y.; Boivin, N. The Origins of Cannabis Smoking: Chemical Residue Evidence from the First Millennium BCE in the Pamirs. *Sci. Adv.* **2019**, *5*, eaaw1391. [CrossRef]
41. Sarret, M.; Adam, P.; Schaeffer, P.; Ebert, Q.; Perthuison, J.; Pierrat-Bonnefois, G. Organic Substances from Egyptian Jars of the Early Dynastic Period (3100–2700 BCE): Mode of Preparation, Alteration Processes and Botanical (Re)Assessment of "Cedrium". *J. Archaeol. Sci. Rep.* **2017**, *14*, 420–431. [CrossRef]
42. Serpico, M.; White, R. The Botanical Identity and Transport of Incense during the Egyptian New Kingdom. *Antiquity* **2000**, *74*, 884–897. [CrossRef]
43. Stern, B.; Heron, C.; Corr, L.; Serpico, M.; Bourriau, J. Compositional Variations in Aged and Heated Pistacia Resin Found in Late Bronze Age Canaanite Amphorae and Bowls from Amarna, Egypt. *Archaeometry* **2003**, *45*, 457–469. [CrossRef]
44. Tushingham, S.; Snyder, C.M.; Brownstein, K.J.; Damitio, W.J.; Gang, D.R. Biomolecular Archaeology Reveals Ancient Origins of Indigenous Tobacco Smoking in North American Plateau. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 11742–11747. [CrossRef] [PubMed]
45. Bailly, L.; Adam, P.; Charrié, A.; Connan, J. Identification of Alkyl Guaiacyl Dehydroabietates as Novel Markers of Wood Tar from Pinaceae in Archaeological Samples. *Org. Geochem.* **2016**, *100*, 80–88. [CrossRef]
46. Charrié-Duhaut, A.; Connan, J.; Rouquette, N.; Adam, P.; Barbotin, C.; de Rozières, M.-F.; Tchapla, A.; Albrecht, P. The Canopic Jars of Rameses II: Real Use Revealed by Molecular Study of Organic Residues. *J. Archaeol. Sci.* **2007**, *34*, 957–967. [CrossRef]
47. Colombini, M.P.; Modugno, F.; Silvano, F.; Onor, M. Characterization of the Balm of an Egyptian Mummy from the Seventh Century B.C. *Stud. Conserv.* **2000**, *45*, 19–29. [CrossRef]
48. Jones, J.; Higham, T.F.G.; Oldfield, R.; O'Connor, T.P.; Buckley, S.A. Evidence for Prehistoric Origins of Egyptian Mummification in Late Neolithic Burials. *PLoS ONE* **2014**, *9*, e103608. [CrossRef]
49. Łucejko, J.; Connan, J.; Orsini, S.; Ribechini, E.; Modugno, F. Chemical Analyses of Egyptian Mummification Balms and Organic Residues from Storage Jars Dated from the Old Kingdom to the Copto-Byzantine Period. *J. Archaeol. Sci.* **2017**, *85*, 1–12. [CrossRef]
50. Saab, A.M.; Harb, F.Y.; Koenig, W.A. Essential Oil Components in Heart Wood of Cedrus Libani and Cedrus Atlantica from Lebanon. *Minerva Biotecnol.* **2005**, *17*, 159–161.
51. Ainane, T. Chemical Characterization on the Aromatic Composition of Cedrus Atlantica from Morocco in Two Geographical Areas Will Break. *AOICS* **2018**, *2*, 001–004. [CrossRef]

52. Chaudhary, A.; Kaur, P.; Singh, B.; Pathania, V. Chemical Composition of Hydrodistilled and Solvent Volatiles Extracted from Woodchips of Himalayan Cedrus: Cedrus Deodara (Roxb.) Loud. *Nat. Prod. Commun.* **2009**, *4*, 1257–1260. [CrossRef]
53. Oukhrib, A.; Zaki, M.; Benharref, A. The Chemistry of the Himachalenes and Atlantones from Cedrus. *Arkivoc* **2018**, *2018*, 134–178. [CrossRef]
54. Singh, D.; Agarwal, S.K. Himachalol and?-Himachalene: Insecticidal Principles of Himalayan Cedarwood Oil. *J. Chem. Ecol.* **1988**, *14*, 1145–1151. [CrossRef] [PubMed]
55. Ottensmann, M.; Stoffel, M.A.; Nichols, H.J.; Hoffman, J.I. GCalignR: An R Package for Aligning Gas-Chromatography Data for Ecological and Evolutionary Studies. *PLoS ONE* **2018**, *13*, e0198311. [CrossRef] [PubMed]
56. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria; Available online: https://www.R-project (accessed on 21 May 2022).

Figure S1: Principal component analysis (PCA) plots where the first four principal components (PC) are paired against each other for each of the three compound groups (G1, G2 and G3). The percentages of each PC indicate the variability explained by each PC axes.




G2 compounds
PC1, 29.3%
PC2, 18.6%
PC3, 9.8%
PC4, 8.1%
fresh
N₂RT
N₂HT
RedRT
RedHT
AirRT
AirHT
BrRT
BrHT
ClayRT
ClayHT
CuRT
CuHT
FeRT
FeHT
GypsRT
GypsHT
OxRT
OxHT


G3 compounds
PC1, 18.6%
PC2, 17.2%
PC3, 12.9%
PC4, 8.5%
fresh
N₂RT
N₂HT
RedRT
RedHT
AirRT
AirHT
BrRT
BrHT
ClayRT
ClayHT
CuRT
CuHT
FeRT
FeHT
GypsRT
GypsHT
OxRT
OxHT

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Biomolecular characterization of 3500-year-old ancient Egyptian mummification balms from the Valley of the Kings

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Ancient Egyptian mummification was practiced for nearly 4000 years as a key feature of some of the most complex mortuary practices documented in the archaeological record. Embalming, the preservation of the body and organs of the deceased for the afterlife, was a central component of the Egyptian mummification process. Here, we combine GC–MS, HT-GC–MS, and LC–MS/MS analyses to examine mummification balms excavated more than a century ago by Howard Carter from Tomb KV42 in the Valley of the Kings. Balm residues were scraped from now empty canopic jars that once contained the mummified organs of the noble lady Senetnay, dating to the 18th dynasty, ca. 1450 BCE. Our analysis revealed balms consisting of beeswax, plant oil, fats, bitumen, Pinaceae resins, a balsamic substance, and dammar or *Pistacia* tree resin. These are the richest, most complex balms yet identified for this early time period and they shed light on balm ingredients for which there is limited information in Egyptian textual sources. They highlight both the exceptional status of Senetnay and the myriad trade connections of the Egyptians in the 2nd millennium BCE. They further illustrate the excellent preservation possible even for organic remains long removed from their original archaeological context.

Ancient Egyptian society is renowned, in academic and public circles alike, for the complex rituals and extraordinary material culture that it attached to death, particularly amongst ruling social elites[1]. Already by the Late Neolithic, funerary monuments had emerged as central points on the landscape for agricultural groups inhabiting the Nile floodplain[2]. Later, monumental structures, from the earliest built mastabas ca. 3000 BCE to the renowned pyramids of Giza ca. 2600 BCE[3], rose to become key elements of Egyptian religion, economy, society and politics[4]. So important was the elaboration of the funerary sphere in ancient Egyptian culture that its necropolises have been characterized as 'cities of the dead'[2].

At the epicenter of this rich funerary culture were the buried individuals themselves, who were subjected to a highly complex set of postmortem mummification processes that, with the exception of some examples in Chile and China[5–7], are unparalleled in the archaeological record. Ancient Egyptian mummification predates the First Dynasty, as evident in embalming remains found in Late Neolithic burials[8], and continued all the way through to the Greco-Roman period[9], making it a core feature of Egyptian funerary archaeology. Contrasting with natural mummification, which can occur under arid conditions like those found in the Egyptian desert, artificial mummification in Egypt entailed evisceration, and the deliberate desiccation and preservation of the body through the application of various substances[10,11]. The mummification procedure encompassed the meticulous removal of organs such as the lungs, liver, stomach, and intestines, followed by embalming[12]. The organs were frequently,

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but not always, mummified and stored in separate canopic jars. This practice served the purpose of facilitating corporal desiccation by inhibiting bacterial and fungal growth. Its objective was to ensure the long-term preservation of the deceased's body for the afterlife, providing a vessel for the return of the individual's 'souls', in line with Egyptian belief systems[11,13]. The ancient Egyptians held a multifaceted view of the 'soul', conceiving it as a composite of several elements, most notably the *Ka*, *Ba and Akh,* which were associated with notions of the afterlife and funerary rituals[14,15].

Examples of mummified organs were discovered by Howard Carter in the royal tomb "KV (Kings' Valley) 42" in Thebes (now Luxor) in 1900[16]. The viscera he encountered in Tomb KV 42 belonged to the noble lady Senetnay, who lived in Egypt around 1450 BCE. She was the wet nurse of the long-awaited son and heir of Pharaoh Thutmose III, the future Pharaoh Amenhotep II, who was nurtured and breastfed by Senetnay during infancy[17]. After her death, Senetnay's mummified organs were carefully stored in four canopic jars with lids in the shape of human heads (Fig. 1). In order to preserve her remains for the afterlife, they were embalmed, ensuring their long-term conservation, ostensibly for eternity. Two of the jars, those made to contain Senetnay's lungs and liver, are now held in the Egyptian collection of the Museum August Kestner, Hannover (Germany)[17,18]. While the mummified organs themselves have been lost, and the jars are presently empty, residues of the mummification balms are partially preserved as thin coatings on the walls and bases of the jars, as well as permeating into the porous limestone of which the jars are made.

The exact recipes used in ancient Egyptian mummification balms have long been debated due to the paucity of ancient Egyptian texts naming their precise ingredients[19]. Despite the long period over which mummification was practiced (almost 4000 years), there are only a few written sources—such as the *Ritual of Embalming*[20]—that address the mummification process, and none of these texts provide the exact ingredients used in the preparation of the balms. Historical descriptions from much later Greek and Roman sources (e.g., Herodotus, Diodorus Siculus[21,22]) do specify some ingredients, but these were not necessarily the same as those employed more than a millennium earlier. For these reasons, the use of molecular analyses to help in identifying the ingredients of ancient Egyptian embalming materials has been of great interest to scientists since the late 1970s[23]. In particular, technological advances in gas chromatography and mass spectrometry techniques have contributed significantly

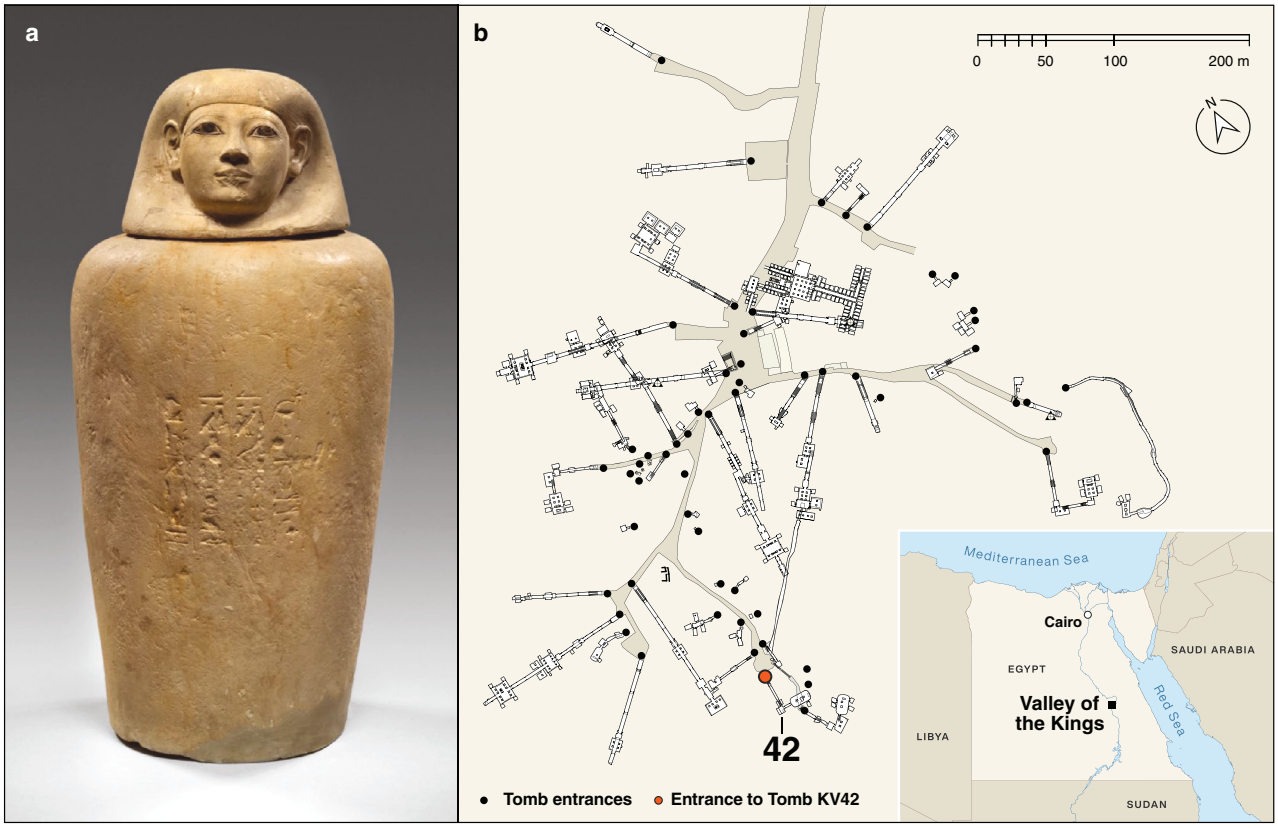

Figure 1. (**a**) Canopic jar of Senetnay, "Wet Nurse of the King" (Amenhotep II), which originally contained Senetnay's mummified lungs, as evident from the inscriptions on the vessel referring to Nephthys, the protective goddess of the lungs. Height of the jar with lid: 42.4 cm; height without lid: 33.7 cm; max. diameter: 21.5 cm. © Museum August Kestner, Hannover (Germany); photo: Christian Tepper (museum's photographer). (**b**) Map of the Valley of the Kings with the location of Tomb KV 42, where the canopic jars were found. Sources of maps: Weeks, Kent R. (ed.). Atlas of the Valley of the Kings (= Publications of the Theban Mapping Project, 1). Cairo: American University in Cairo Press, 2000, 2003. Available online at https://thebanmappingproject.com/sites/default/files/plans/Valley%20of%20the%20Kings.pdf, and Natural Earth vector map data (maps were created using QGIS 3.12 (https://qgis.org/en/site/)).

to elucidating the chemistry of ancient Egyptian balms[24,25]. Previous studies have identified a number of different ingredients that were used in the production of mummification balms, in various configurations, such as oils and fats[9,26–31], beeswax[9,29,30,32,33], bitumen[8,28,34,35], gums and sugars[8,9,32], and resins and tars[8,27,28,31,33,34,36–41]. However, most of these studies focused on embalming materials obtained from the bandages and tissues of mummies themselves, and only a few studies have been carried out on the substances used to embalm the accompanying organs in canopic jars[28,33,42] (see also the Canopic Jar Project at the University of Zurich).

Here, in order to elucidate what broader social, technological and cultural insights can be acquired from the balms used to mummify organs, we investigate balm samples from two of the canopic jars belonging to Senetnay (the other two jars belonging to the assemblage are not available for analysis, as one is housed at the Egyptian Museum in Cairo while the location of the other remains undetermined). The jars analyzed are those that contained Senetnay's lungs and liver, and were initially stored in Egypt, then in the private collection of the Egyptologist Friedrich Wilhelm Baron von Bissing in Munich, then subsequently at the Museum Carnegielaan 12 in The Hague, and finally, since 1935, in the Egyptian collection of the Museum August Kestner in Hannover (with two years of security storage in a salt mine in Grasleben during World War II)[17,18]. At each location, over more than 123 years, the remains were stored under more or less ideal "museum conditions". Understanding the complexity of ancient organic residues, especially mixtures of different products, requires the analysis of multiple compound groups. In recognition of the chemical diversity of the biological components in many analyzed mummification balms, we draw upon a multi-analytical approach combining gas chromatography mass spectrometry (GC–MS), high temperature gas chromatography mass spectrometry (HT-GC–MS) and liquid chromatography tandem mass spectrometry (LC–MS/MS) to differentiate and identify the organic substances contained within Senetnay's canopic jars.

Results

A total of 6 balm samples were selected for analysis, comprising one sample from the bottom and two samples from the inner walls of each of the canopic jars (see Supplementary Figure S1 and Table S1 for exact location and description of samples). The balm samples were subjected to a series of extraction/dissolution steps followed by LC–MS/MS, GC–MS and HT-GC–MS analyses. The results, detailed below, show good preservation of molecules in the samples taken from the interior bottoms of the jars (i.e., AES 062 from jar 1 and AES 067 from jar 2), where residual layers of the embalming material remained adhered. In contrast, residues scraped from the inner walls, which were partially absorbed into the limestone of the jar and barely visible to the naked eye, demonstrated poorer molecular preservation.

LC–MS/MS screening for biomarkers of plant exudates and resins. Three compound groups were identified in the extracts analyzed by LC–MS/MS in multiple reaction monitoring (MRM) mode: terpenoids, phenols and aromatic compounds (Table 1). The LC–MS/MS results showed a high abundance of di- and triterpenoids in the embalming material. Predominant among the diterpenoids is 7-oxo-dehydroabietic acid (7ODHA, Table 1), which was observed in all samples. 7ODHA is an oxidized derivative of the diterpene dehydroabietic acid (DHA), which was also present to a lesser extent. Both compounds are characteristic of coniferous plant products, specifically resins from the Pinaceae family, including pine (*Pinus* spp.), larch (*Larix* spp.) and cedar (*Cedrus* spp.)[8,9,28,34,41]. Other compounds characteristic of Pinaceae resins were also included as analytical standards for the optimization of MRM parameters, notably pimaric, isopimaric, palustric and neoabietic acids[43]. However, due to similar retention times, fragmentation patterns and molecular weights (302 g/mol), LC–MS/MS and detection in MRM mode were not sufficient to differentiate them. Therefore, pimaric acid, isopimaric acid, palustric acid and neoabietic acid are summarized in Table 1 as 'resin acids' (see also Supplementary Table S5), and the samples were additionally analyzed by GC–MS in order to differentiate these compounds (see GC–MS and HT-GC–MS analysis).

	Canopic jar 1			Canopic jar 2		
	AES 062	AES 064	AES 066	AES 067	AES 068	AES 069
Compounds	Interior, bottom	Inner wall	Inner wall	Interior, bottom	Inner wall	Inner wall
Resin acids (pimaric, isopimaric, palustric and neoabietic acids)	✓	Trace	✓	✓	✓	Trace
7-Oxodehydroabietic acid	✓	✓	✓	✓	✓	✓
Dehydroabietic acid	✓	×	✓	✓	✓	✓
Dammarenolic acid	✓	×	×	×	×	×
Oleanonic/moronic acids	✓	×	×	×	×	×
Benzoic acid	✓	✓	✓	✓	✓	✓
Vanillic acid	✓	Trace	Trace	✓	Trace	Trace
Coumarin	✓	Trace	×	✓	×	×

Table 1. LC–MS/MS findings from archaeological samples AES 062, 064 and 066 from canopic jar 1 (containing the lungs) and AES 067, 068 and 069 from canopic jar 2 (containing the liver). Constituents identified based on less than 10,000 counts are indicated as 'trace'. See also Supplementary Table S7 for identified compounds.

The triterpenoids detected in the samples suggest the use of additional scented resinous substances. Dammarenolic acid (Fig. 2a, c), the main secondary metabolite of dammar resin[44], was present in sample AES 062 of canopic jar 1. This triterpenoid compound is a dammarane-type molecule, but with the opening of the A-ring due to oxidation and breakage of the C–C bond, resulting in a carboxyl functional group[45]. Interestingly, this compound was only detected in jar 1 and not in any of the samples taken from canopic jar 2. Our analysis also revealed a peak corresponding to either oleanonic or moronic acid, two pentacyclic triterpenoids that have similar structures and ionization behaviors, and are accordingly difficult to distinguish. This peak was, however, only detected in low abundance (Fig. 2b, d). Oleanonic acid and moronic acid are typical biomarkers for *Pistacia* species and have been previously detected in several ancient Egyptian embalming materials[9,28,36]. However, in combination with dammarenolic acid, oleanonic acid is also a constituent of dammar resin from the Dipterocarpaceae family, among other angiosperm clades[45–47]. Apart from its natural occurrence in dammar resin, dammarenolic acid could also be an oxidation product of the compound dammaradienone, which is present in both dammar and Pistacia resin (Fig. 2e)[46,48]. Previous studies on mummification balms have also noted the overlap between compounds found in dammar and *Pistacia*[28,49]. Hence, based on current evidence, Pistacia and dammar resins cannot be unambiguously differentiated, and both resins are, therefore, considered possible sources for these compounds.

In addition to the terpenoids, phenolic and aromatic compounds were also detected in the balms, including vanillic acid, coumarin, and benzoic acid. Although vanillic acid is found in natural vanilla extracts, in this context it most likely reflects degradation of woody tissue[50–52], and possibly derives from the conifers in the balm. It is more difficult to assign an origin to the aromatic compound coumarin, as it occurs naturally in a wide range of different and disparately related plants, among which are the cinnamons and many Fabaceae. Coumarin has a vanilla-like scent. Another aromatic compound—benzoic acid—was found in all samples. It also occurs in many plant gums and spices, such as gum benzoin, cinnamon and cloves or balsam type plants[32,53]. Given the ubiquity of these compounds in the plant kingdom, we could not assign them to a specific source.

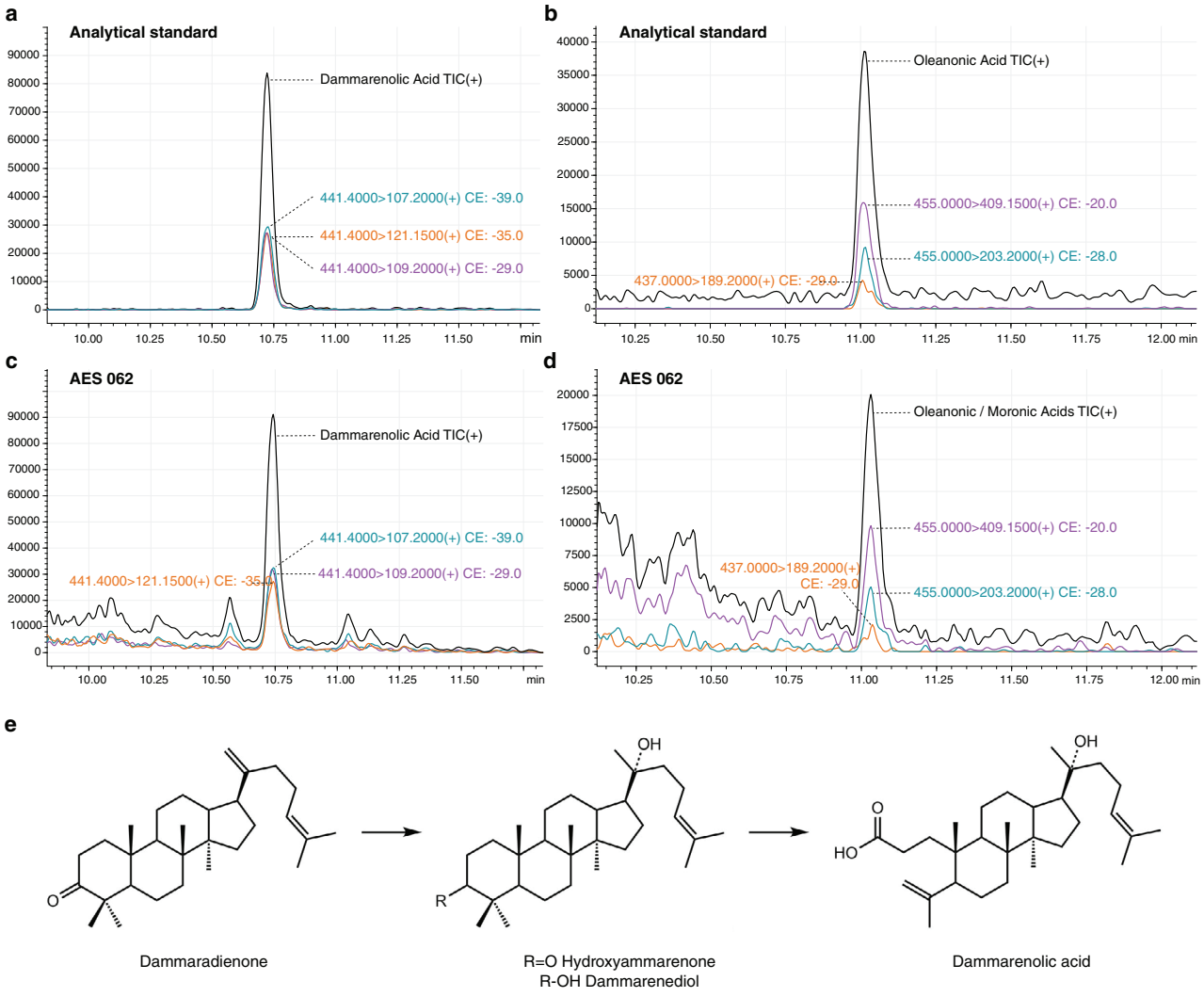

Figure 2. Multiple reaction monitoring (MRM) HPLC chromatograms of the analytical standards dammarenolic acid (**a**) and oleanonic acid (**b**) compared to the presence of these compounds in sample AES 062 of canopic jar 1 (**c,d**). (**e**) Chemical structures of sequential oxidation stages of dammarane-type molecules [after47].

GC–MS and HT-GC–MS analysis. Additional GC–MS measurements of the lipid fraction were carried out to analyze fatty acids and alcohols, *n*-alkanes, and resin acids (Fig. 4a). Similar to the LC–MS/MS results, 7ODHA and DHA were detected in the lipid fraction together with abietic acid, pimaric acid, isopimaric acid and 15-hydroxydehydroabietic acid. The latter is another oxidation product that forms from abietic acid and DHA (Fig. 3). These resin acids were identified only in samples AES 062 and AES 067 taken from the bottom of the jars, but not in the remaining samples, which had an overall lower concentration of organic molecules (less than one tenth compared to AES 062 and AES 067 based on total peak area). All diterpenoids identified occur throughout all genera of Pinaceae, though the abundance of the primary resin acids pimaric acid and abietic acid is more characteristic of resins from *Pinus* species[34]. In addition to these biomarkers for Pinaceae resins, we also detected the compound larixol (Supplementary Fig. S6) in sample AES 062. Like the above-mentioned resin acids, larixol is also present in larch wood resin (*Larix* spp.)[54–57], and is indeed specific to it. Its presence therefore suggests a *Larix* species as a possible source for the Pinaceae resin. However, this finding needs to be treated with caution, as it is based on a single biomarker. Additionally, we also cannot rule out a mixture of different Pinaceae genera, including both pine and larch resins.

Apart from the odiferous resins, the analysis of the lipidic fraction revealed that the balm contained additional ingredients (Fig. 4b). The profile was dominated by high abundances of saturated even-carbon-numbered straight-chain fatty acids, predominantly palmitic acid ($C_{16:0}$) and lignoceric acid ($C_{24:0}$) and, to a lesser extent, behenic acid ($C_{22:0}$) and stearic acid ($C_{18:0}$). These free fatty acids are end-products of the degradation of lipidic substances and can indicate a contribution of either plant oils, or animal/human fats[52,58,59]. The large number of very long-chain fatty acids ($C_{22:0}$–$C_{30:0}$) is characteristic of higher terrestrial plants and epicuticular waxes, as well as beeswax[60]. The odd-carbon-numbered straight-chain components detected in the samples [e.g., pentadecanoic acid ($C_{15:0}$), and heptadecanoic acid ($C_{17:0}$)] are sometimes seen as characteristic for ruminant lipids. However, their low abundance and the absence of corresponding branched isomers instead suggest that these compounds are more likely the result of bacterial degradation[61,62]. The samples also exhibit short-chain homologues from $C_{6:0}$–$C_{10:0}$, which are known to be degradation products caused by oxidation and formed during ageing or drying of organic tissue, for example of plant oil[28,59,63]. Monounsaturated fatty acids are also present in the form of octadecenoic acid ($C_{18:1}$) and hexadecenoic acid ($C_{16:1}$), which are found in vegetable oils and animal fats[52]. Thus, the fatty acid distribution suggests the balms most likely included a mixture of degraded animal fats and plant oils. Some caution in interpretation is required, however, since we cannot distinguish between fat that derives from an added animal ingredient or the human remains themselves.

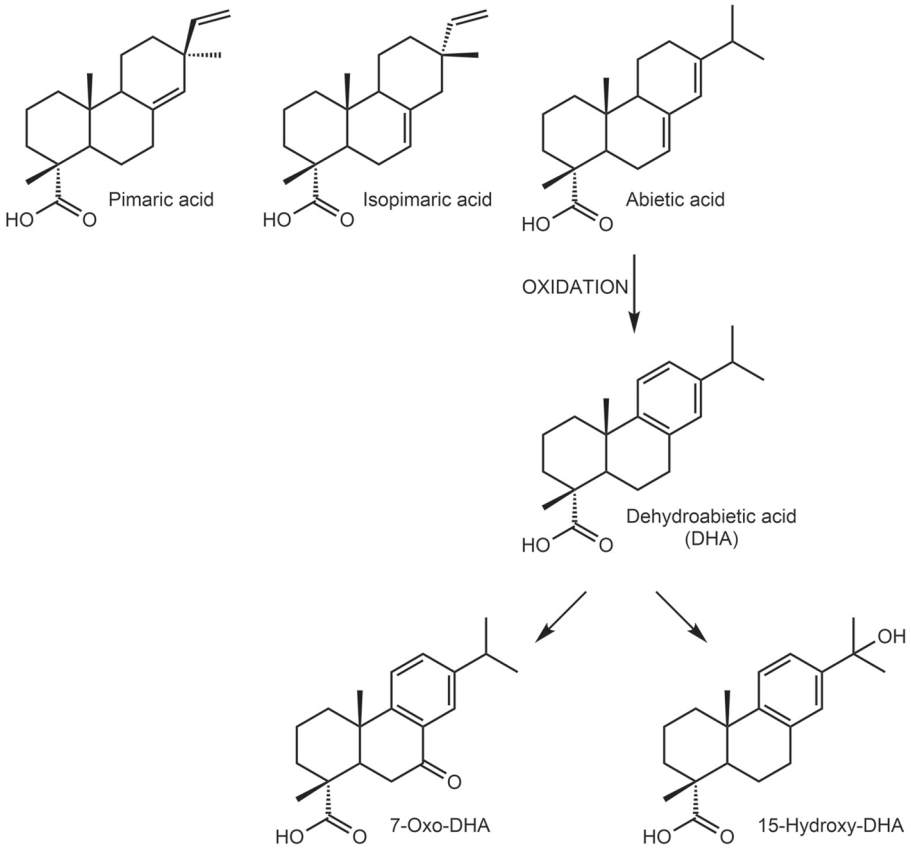

Figure 3. Resin acids present in samples from both canopic jars, and sequential oxidation reactions of abietic acid.

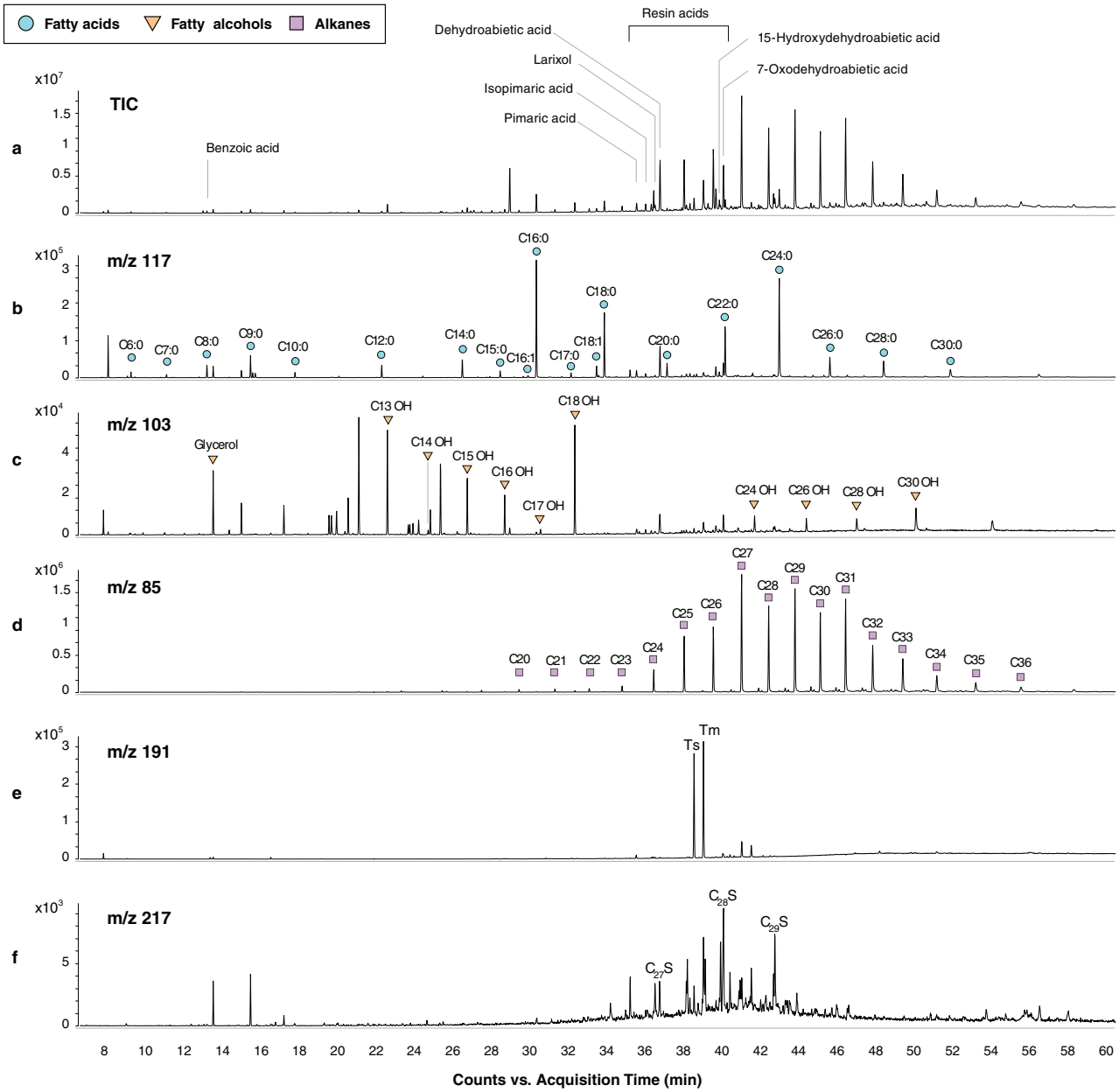

Figure 4. Total ion current (TIC, **a**) and extracted ion chromatograms (EIC, **b–f**) of sample AES 062 displaying ion masses of characteristic fragments from the main compound classes. (**b**) *m/z* 117 displaying fatty acids, n:0 = saturated FA and n:1 = unsaturated FA; (**c**) *m/z* 103 showing the distribution of fatty alcohols; (**d**) *m/z* 85 displaying *n*-alkanes with corresponding carbon numbers and (**e,f**) characteristic fragments of hopanes and steranes. For more detailed information of hopanes and steranes see Supplementary Figs. S4, S5. For identified compounds of samples AES 067 see Supplementary Fig. S2.

Another class of compounds present in the lipid fraction was *n*-alkanes, which represent the most abundant compounds in sample AES 062. The extracts yielded medium and long chain *n*-alkanes, (C_{20}–C_{36}), displaying a slight odd-over-even predominance, with C_{27} as the most abundant *n*-alkane (Fig. 4d). Given the presence of this homologous series of *n*-alkanes, which is characteristic for fossil hydrocarbons, we hypothesized that the *n*-alkanes might reflect bitumen, a substance often associated with Egyptian mummification[28,64,65]. For this reason, we screened for characteristic hopanes and steranes of bitumen (ions *m/z* 191 and *m/z* 217; Fig. 4e, f and Supplementary Figs. S4 and S5). These ions are diagnostic markers for natural petroleum[8,66,67], and were detected in the samples from both canopic jars, thus confirming the presence of bitumen.

n-Alkanes with a chain length from C_{25} to C_{35}, but a strong odd-over-even dominance, are also known to be characteristic of epicuticular waxes of higher terrestrial plants[59,68,69], and of beeswax, which has been reported in previous mummy balm studies[29,36,60]. Beeswax usually consists also of wax esters with a carbon chain length of greater than 40. The samples AES 062 and 067 were additionally analyzed by HT-GC–MS to search for these wax esters. We detected small amounts of monoesters of palmitic acid ranging from C_{40} to C_{50}, as well as the corresponding hydroxy wax esters (Fig. 5) in both samples. The presence of the wax esters, the *n*-alkane distribution

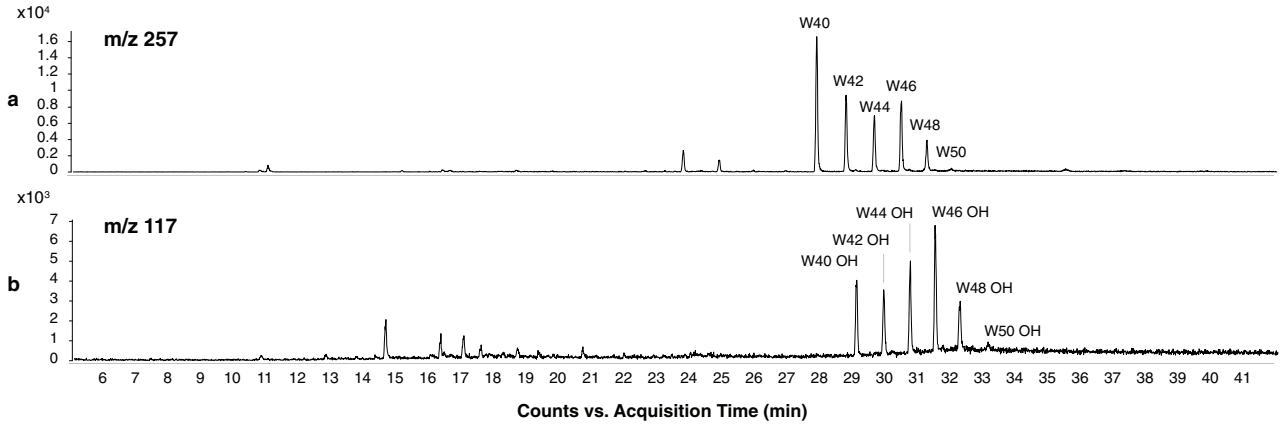

Figure 5. Extracted ion chromatograms (EIC) of HT-GC–MS analyses for *m/z* values 257 and 117 of sample AES 062 displaying monoesters of palmitic acid (**a**) and hydroxy palmitic acid esters (**b**).

with the most abundant peak for C_{27}, the long-chain fatty acids, and the *n*-alcohols (Fig. 4c) provide robust evidence for the use of beeswax as prominent ingredient in the balms.

Discussion

Our analysis shows that rich information is recoverable from the remnants of balms in Egyptian canopic jars, even when such jars have been emptied and transferred between museum collections for more than a century. The samples taken from Senetnay's jars provide evidence for incorporation of a variety of natural products and odiferous ingredients in the balms used to preserve her organs. Oils and fats, together with beeswax and bitumen, seem to have formed the basis of the balms identified in both jars, and our analysis demonstrates that these substances were mixed with coniferous resins, specifically from Pinaceae. Additionally, our analyses revealed the presence of other unidentified plant products containing benzoic acid and coumarin. Previous analyses of other Egyptian balms have also observed benzoic acid, together with phenolic acids, which have been associated with the presence of aromatic plant exudates of balsamic resins or gums[9,10].

Analysis further revealed that the balms from Senetnay's two jars were not identical in composition. The balm of canopic jar 1, which originally contained Senetnay's lungs, included an additional aromatic resin (probably dammar or *Pistacia* resin) that was not found in jar 2 (which contained her mummified liver). Additionally, the compound larixol, suggestive of larch resin, was only detected in jar 1. Apart from these ingredients, the composition of the balms in the two jars appear to have been very similar, although the ratios of the ingredients in each is different. The differences in the balms chemical composition might suggest that balms were organ-specific, highlighting the importance of in-depth investigations of balms from canopic jars. However, given the fact that the samples from Senetnay's canopic jars are almost 3500 years old, and multiple degradation processes likely occurred over the period of deposition and storage, we cannot exclude the possibility that the resinous ingredients were originally the same but have degraded differently through time. Additionally, it is possible that the mummification balm was heterogeneous and that ingredients were not thoroughly mixed or evenly distributed. Nevertheless, we find some support for the notion of organ-specific balm recipes from a recent study of inscribed vessels for the preparation of embalming materials from a mummification workshop at Saqqara, dating to the mid-first millennium BCE[31]. In the Saqqara example, the different mixtures were not found in canopic jars, but rather in vessels in which mummification balms were being prepared for later application to the liver and stomach. In contrast, our study analyzed balms deriving from already embalmed organs and our results provide tentative support for the hypothesis that different balms were applied to different organs.

Our review of the literature on previous balm analyses shows that some of the ingredients we find in the mummification balms used on Senetnay's organs (e.g., bitumen) were not commonly used for embalming in New Kingdom Egypt. Previous analyses suggests that ancient Egyptian mummification balms contained a limited range of ingredients before the Third Intermediate Period (c. 1000 BCE), becoming more complex through time[24]. While analysis of very early balms has revealed the use of multiple ingredients[8], Egyptian balms through the Old and Middle Kingdoms often consisted solely of fats or oils (Fig. 6A). Only in the Second Intermediate Period and New Kingdom (c. 1760–1077 BCE) did balms become more complex, with the introduction of diverse resins, likely reflecting both evolving approaches to mummification, and the increasing ability to acquire ingredients from further afield[24]. In general, in the mid-second millennium BCE, when Senetnay died, only a small number of mummies received this kind of elaborated treatment.

Other examples of sophisticated balms in this period come from the high-status Eighteenth Dynasty (ca. 1479–1424 BCE) burial of a dignitary named Nebiri[49], as well as from the mummies of the royal architect Kha and his wife Merit[70]. When analyzed, these balms were found to contain fats and oils, coniferous resins, and aromatic plant products or gums. The balms from Nebiri and Merit additionally contained *Pistacia* resin and Merit's embalming also had beeswax in it. Senetnay's embalming, also from the Eighteenth Dynasty, and contemporary to or slightly younger than Nebiri's burial, but earlier than those of Kha and Merit, featured another unique and distinctive balm. This included beeswax and fat/oil, as well as an aromatic or balsamic substance, together with

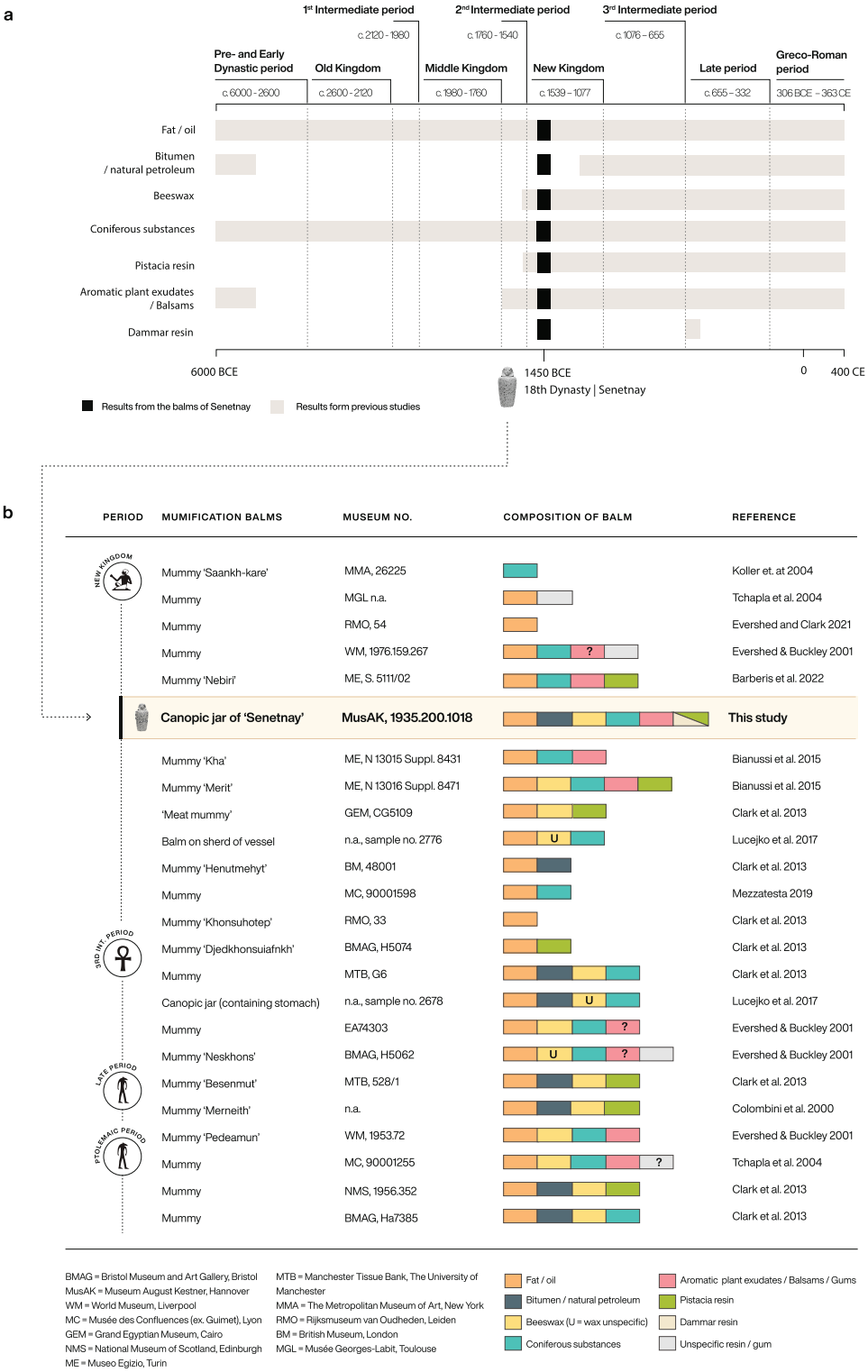

Figure 6. (**a**) Occurrence of reported substances in Egyptian balms through time (sources:[8,9,24,26–29,31,32,35–38,40,41,49,62,70–74]). (**b**) Composition of mummification balms from the New Kingdom, contemporary to Senetnay, and selected balms from the Third Intermediate Period to the Ptolemaic period consisting of 4 or more substances.

coniferous resin (possibly larch resin), and *Pistacia* resin or even a very exotic component in the form of dammar resin. Additionally, Senetnay's balms also contained bitumen, which is evidence of very early use of this natural

substance in the context of mummification. Chemical analyses have not yielded any other example of such a complex balm with 6 ingredients (in jar 1) in the mid-second millennium BCE in Egypt (Fig. 6B). Beeswax and bitumen also only became major ingredients of mummification balms towards the end of the New Kingdom. Overall, the balms used in Senetnay's jars contain ingredients that were commonly employed in Egypt only in later periods, particularly at the "height of mummification" in the first millennium BCE, when balms became more complex and elaborated. Senetnay's balm might therefore be seen as a forerunner for a later trend. It is important to note, however, that the increased number of ingredients identified in Senetnay's balm might simply reflect better preservation and/or our multi-analytical approach, which involved the combined use of GC–MS, HT-GC–MS, and LC–MS/MS, allowing for a more holistic approach to the study of the balm samples. While Fig. 6 synthesizes data from a range of studies, the sample preparation and analytical approaches of previous analytical studies have varied and are not directly comparable. Bitumen in particular, is likely underrepresented due to the necessity of specialized procedures in sample preparation.

Notwithstanding these caveats, Senetnay's remains seem to have received special treatment. The ingredients in her mummification balms give the impression of a woman of exceptional social standing, suggesting, along with other lines of evidence, that she was a highly valued member of the Pharaoh's entourage. The elaborate treatment of Senetnay's remains is echoed in the broader pattern of her burial. Her very presence in the Valley of the Kings, a necropolis normally reserved for pharaohs and powerful nobles[75], points to extraordinary privilege, and the high regard in which Senetnay was likely held by the Pharaoh. Her title, "Ornament of the King"[17], further reinforces the evidence for her special standing.

In keeping with these indications of a woman of prominent status are the origins of the ingredients in the balms employed in Senetnay's canopic jars. Most of the ingredients in her balms were of non-local origin, and, thus, depended on transport to be available in Egypt. Trees of the pine family, for example, are not endemic to Egypt (Fig. 7a). As noted, one possible Pinaceae resin source is larch wood resins from *Larix* species, based on our finding of the compound larixol. Larch resin has also been identified in historical medical remedies in Rome on the basis of the presence of the compound larixol[76]. There are ten recognized species in the *Larix* genus, of which only one is native to Europe (*L. decidua*)[77], while none are native to southwest Asia or Africa[78]. While there are species native to Siberia (*L. sibirica; L. gmelinii)*, and such South Asian mountain chains as the Himalayas (*L. potaninii; L. mastersiana, and L. griffithii*), these are much further from Egypt and thus less plausible sources for the resin in this study. *L. decidua* exists in mountain-top refugial populations across the Pyrenees, Alps, and other western Mediterranean and Central European mountains, and could have been obtained via sea trade, though putative Egyptian trade contacts with Central Europe are poorly understood at present[79].Other Pinaceae sources are also possible though, and those near the ancient Egyptian realm could have included the Cilician fir (*Abies cilicia*), Lebanese and Atlas cedar (*Cedrus libani* and *atlantica*), Asian spruce (*Picea orientalis*), Aleppo pine (*Pinus halepenis*) and the parasol pine (*P. pinea*). The Turkish pine (*P. brutia*) and maritime pine (*P. pinaster*) grow further north in the Mediterranean, notably on many islands and in northern coastal areas. While there is some evidence for population shifts among some of these conifers, notably mid-Holocene range reduction[80], there is no reason to believe that there are any species that existed in Egypt over the past three millennia but are no longer present. Coniferous ingredients within the balms are, therefore, most likely imported products.

Apart from coniferous resins, our analysis also points to the presence of another aromatic plant exudate, which might be either *Pistacia* or dammar resin. *Pistacia* trees, notably *P. terebinthus* and *P. lentiscus*, are native to the Mediterranean coastal region, ranging from southern Spain to the Levant (Fig. 7B). Both of these species have a long history of use for their resins, producing turpentine and mastic resins, respectively. Beyond their use in ancient Egypt[81], later Classical sources show how widely these resins were used across the Mediterranean[82]. Tree species that produce dammars (primarily in Dipterocarpaceae), meanwhile, grow exclusively in southeast Asian tropical forests[83]. Evidence for this type of exotic gum resin is thus unexpected and has not been reported in ancient Egyptian mummification balms from the second millennium BCE. If confirmed, the presence of dammar resin, which has recently been identified in balms from Saqqara, dating to the first millennium BCE[31], would suggest that the ancient Egyptians had access to Southeast Asian resins that arrived in the Mediterranean by long-distant trade almost a millennium earlier. Some support for such long-distance links is perhaps indicated by the finding of peppercorns in the nostrils of the mummy of the pharaoh Ramses II, dated ca. 1200 BCE[84,85]. This spice is endemic only to the wet forests of southern India[86]. Even earlier African-Indian exchange is hinted at by the presence of crops of African origin in the Indian subcontinent by 2000 BCE, where they were being grown on Harappan farms[84]. Nonetheless, these early long-distance trade connections remain very poorly understood, with no associated material culture evidence, and *Pistacia* is the more parsimonious identification at present. If confirmed, this would represent one of the earliest direct identifications of *Pistacia* resin in a mummification balm. Apart from its appearance in the balm of Nebiri, *Pistacia* resin was also used in the preparation of "victual" or food mummies from the late Seventeenth-early Eighteenth Dynasty, when it was applied to some of their wooden coffinets and bandages[74]. Overall, the findings point to early evidence for trade in exotic plants and/or plant substances between Egypt and its near neighbors, with the possibility of early trade links that extended further afield.

Our analysis reveals rich information about social status, technological acumen, and trade that can be obtained from apparently empty archaeological jars excavated more than a century ago. It joins a growing number of studies that highlight the value of applying new methods to investigate trace remains and amorphous residues as well as long-held museum specimens[31,41,53,73,87–90]. Together with these other studies, our findings demonstrate that analytical chemistry is able to shed significant light on the identification of ingredients included in ancient balms, adding substantially to information recoverable from ancient textual sources. At the time that Senetnay's viscera were discovered by Howard Carter, the methods that we have employed in this study would not have been imagined possible. Yet, over 120 years later, the royal tomb known as KV 42 and its contents continue to provide new information about ancient Egyptian cultural practices, society and trade. Our study thus highlights

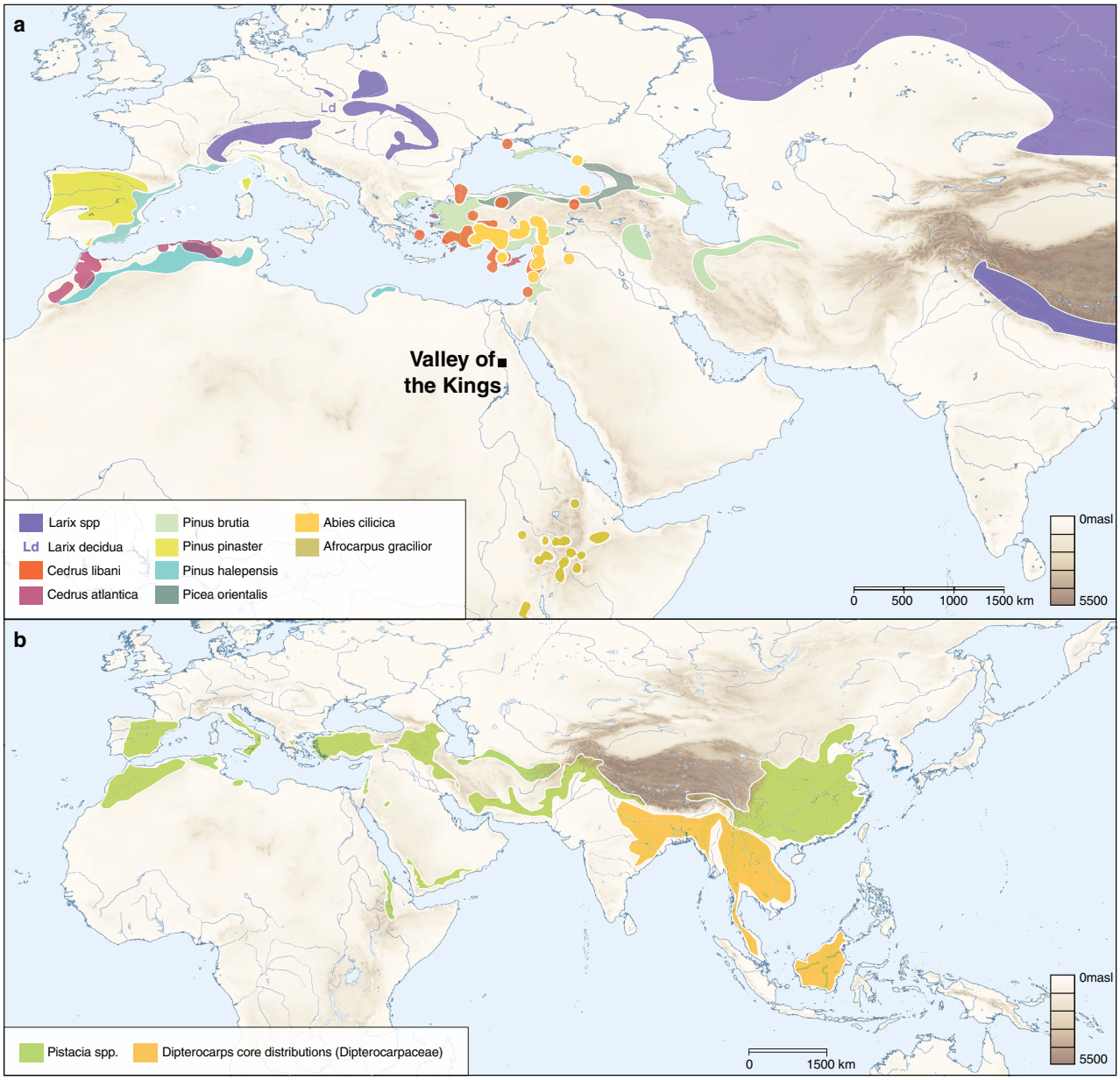

Figure 7. (**a**) Map showing the distribution of potential conifer resin sources in relation to the Valley of the Kings. (**b**) Map displaying the natural habitat of *Pistacia* spp. and the core distribution of Dipetrocarpus and Hopea (Dipterocarpaceae family), excluding small population in the Western Ghats of South India. Conifer and Dipterocarpaceae distributions are based on various sources (see Supplementary Table S6). The maps were created using QGIS 3.12 (https://qgis.org/en/site) and use Natural Earth vector map data from (https://www.naturalearthdata.com/downloads/).

not only the invaluable role of science in archaeological research but also the importance of conserving cultural heritage under optimal conditions over the long term.

Methods

Sampling of ancient mummification balms. The samples of the mummification balm were collected from two ancient Egyptian limestone canopic jars at the Museum August Kestner in Hannover. The jars date to the Eighteenth Dynasty (1450 BCE) and hold the viscera of the noble lady Senetnay. While the jars were empty, a thin layer of organic residue was preserved at the bottom of each. Samples of the embalming material from canopic jar 1 (containing the lungs) and jar 2 (containing the liver) were collected from various parts of the jar (walls and bottom of the jars; see Supplementary Fig. S1). Before collecting these samples, a thin surface layer was removed at the specific sampling spots using disposable scalpels to avoid contamination. Subsequently, samples were taken from below the surface layer with a scalpel. From each spot, ca. 200 mg of residual crust was taken. This was not possible for the remains attached to the walls of the jars, as the layers were very thin and

the residues were mostly preserved within the porous matrix of the limestone. In these cases, the residues were removed with a scalpel without first removing the surface layer to recover enough material (ca. 100–200 mg) for analysis. All samples were immediately placed in glass vials that were previously combusted at 500 °C for 8 h to remove potential contaminants, until further processing under clean lab conditions in the laboratory of the Max Planck Institute for the Science of Human History, Jena, Germany.

Materials. Methanol (MeOH), dichloromethane (DCM), and methyl *tert*-butyl ether (MTBE) used for the analyses, as well as the analytical standards isopimaric acid and vanillic acid, were obtained from Sigma-Aldrich (Munich, Germany). In addition, 7-oxodehydroabietic acid was obtained from Campro Scientific (Berlin, Germany), dehydroabietic acid from Carbosynth (Berkshire, UK), pimaric acid from Abcam (Berlin, Germany), palustric acid from Toronto Research Chemicals (Toronto, Canada), neoabietic acid and oleanonic acid from Santa Cruz Biotechnology (Heidelberg, Germany), dammarenolic acid from Enzo Life Sciences (Lörrach, Germany), moronic acid from TCI chemicals (Eschborn, Germany), coumarin from LGC Standards (Wesel, Germany) and benzoic acid from Agilent Technologies (Frankfurt, Germany). MS-grade formic acid (FA) was purchased from VWR (Leuven, Belgium), while acetonitrile (ACN) and water used for HPLC–MS/MS analyses were purchased from Biosolve (Valkenswaard, Netherlands).

Extraction and analysis. Samples were extracted following established protocols[91,92], with modifications made for the extraction of ancient samples. Briefly, 50–100 mg of the sample were homogenized into a fine powder and solvent extracted using an MTBE: MeOH (3:1, v/v) extraction mixture. After vortexing the mixture and shaking for 45 min, the samples were ultrasonicated for 15 min. Subsequently, a H_2O: MeOH (3:1, v/v) solution was added to each sample and mixed well again. The samples were then centrifuged at 20,000×*g* for 5 min. At this stage, a dense pellet of precipitated proteins formed on the bottom, as well as two liquid phases: (1) an upper phase containing the hydrophobic lipids, which form due to the low density of MTBE and (2) a lower phase with semi-polar and polar metabolites. Each of the two liquid phases were transferred separately to new glass vials, while the remaining pellet was washed with methanol and stored in a −80 °C freezer for future palaeoproteomic analysis, awaiting the development of more plant reference material in protein reference databases. Aliquots of samples with the lipid-containing phase were derivatized with 100 µL *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, containing 1% TMCS, Sigma-Aldrich) for 60 min at 70 °C and then analyzed by GC–MS. The lower phase containing the polar metabolites was dried in a vacuum concentrator and re-suspended in HPLC-grade MeOH before LC–ESI–MS/MS analysis.

GC–MS analyses were performed using an Agilent 8890 GC-System coupled to an Agilent 5977B GC/MSD. Chromatographic separation was achieved on a HP-5ms 60 m × 250 µm capillary column (Agilent) with a film thickness of 0.25 µm. The mass spectrometer was operated in electron impact (EI) mode at 70 eV and helium was used as a carrier gas with a constant flow rate of 1.0 mL/min. The GC oven temperature was set at 60 °C and held for 2 min, then ramped to 120 °C at a rate of 30 °C/min and held for 2 min. The temperature was increased again at 5 °C/min to 320 °C with a final hold time of 15 min. The total run time was 61 min with a solvent delay of 6.5 min. Injection volume was 1 µL and a split ratio of 10:1 was used to improve peak shapes. The scanning range was set from *m/z* 30 to 700 amu. Injection blanks were carried out between each sample to avoid carryover. Transfer line and source temperature were set at 250 °C and 230 °C, respectively.

High temperature GC–MS analyses were performed on an Agilent 8860 GC coupled to a 5977B mass spectrometer. Samples (1 µL) were injected onto a DB-1HT column (15 m × 250 µm i.d., 0.1 µm film thickness) column using a cool-on-column injector. Helium was used as carrier gas with a constant flow rate of 1.2 mL/min. The GC oven was programmed as follows: After 2 min at 50 °C the temperature was increased to 350 °C at a rate of 10 °C/min. This final temperature was held for 10 min. The temperature of the transfer line, ion source and quadrupole were set to 350 °C, 230 °C and 150 °C, respectively, while the inlet temperature was set to track the oven temperature. Electron ionisation at 70 eV was used and data was recorded in full scan from *m/z* 50 to 800 amu after a solvent delay of 5 min.

LC–ESI–MS/MS analysis was carried out using a Shimadzu LCMS-8050 triple-quadrupole system. The HPLC was equipped with LC-30AD binary pumps, a DGU-20A5R solvent degasser, CTO-20AC column oven and a SIL-30AC auto sampler. Chromatographic separation was performed on a Shimadzu Shimpack Velox SP-C18 column (100 mm × 2.1 mm, 2.7 µm particle size) and a Restek Raptor Biphenyl analytical column (100 mm × 2.1 mm, 2.7 µm) particle size. The mobile phase consisted of HPLC grade H_2O and 0.1% FA (mobile phase A) and ACN (mobile phase B). The column temperature was fixed at 25 °C and the gradient program was 0.5% B from 0–1 min, to 80% B at 10 min, to 100% B at 15 min with a hold until 17.5 min, and back to 0.5% B and held until 20 min. The solvent flow rate was maintained at 0.2 mL/min for analyses using the C18 column and 0.3 mL/min for analyses with the biphenyl column, and injection volumes were set at 1 or 2 µL (depending on sample concentration). Ionization was performed with an electro spray ionization (ESI) ion source with detection in both positive and negative modes. All samples were analyzed in duplicates.

Data processing and analysis of GC–MS data was performed using the Agilent MassHunter Qualitative Data Analysis software 10.0. Peak identification was carried out based on comparison with retention times and mass spectra of analytical standards where available, by comparison to the reference mass spectral library NIST (2.2), and with spectra reported in the literature. LC–MS/MS data were collected and processed using LabSolutions software (Shimadzu, Kyoto, Japan). The multiple reaction monitoring (MRM) mode was used for analysis, with authentic analytical standards for the optimization of MRM parameters employed to screen for specific compounds in archaeological samples (see Supplementary Table S3 for list of all compounds, and Table S4 for MRM parameters).

Data availability

All data generated or analysed during this study are included in this published article and its supplementary information files. For any additional information, please contact Barbara Huber (huber@gea.mpg.de).

Received: 28 March 2023; Accepted: 25 July 2023
Published online: 31 August 2023

References

1. Taylor, J. H. *Death and the Afterlife in Ancient Egypt* (University of Chicago Press, 2001).
2. Wengrow, D. *The Archaeology of Early Egypt: Social Transformations in North–East Africa, 10,000 to 2,650 BC* (Cambridge University Press, 2006).
3. Wilkinson, T. A. H. *Early Dynastic Egypt*. (Routledge, 2002). https://doi.org/10.4324/9780203024386.
4. Richards, J. E. *Society and Death in Ancient Egypt: Mortuary Landscapes of the Middle Kingdom* (Cambridge University Press, 2009).
5. Arriaza, B. T. *Beyond Death: The Chinchorro Mummies of Ancient Chile* (Smithsonian Institution Press, 1995).
6. Arriaza, B. *Cultura Chinchorro: Las Momias Artificiales Más Antiguas del Mundo* (Editorial Universitaria, 2015).
7. Zhang, F. *et al.* The genomic origins of the Bronze Age Tarim Basin mummies. *Nature* **599**, 256–261 (2021).
8. Jones, J., Higham, T. F. G., Oldfield, R., O'Connor, T. P. & Buckley, S. A. Evidence for prehistoric origins of Egyptian mummification in Late Neolithic Burials. *PLoS ONE* **9**, e103608 (2014).
9. Buckley, S. A. & Evershed, R. P. Organic chemistry of embalming agents in pharaonic and Graeco-Roman mummies. *Nature* **413**, 837–841 (2001).
10. Jones, J. *et al.* A prehistoric Egyptian mummy: Evidence for an 'embalming recipe' and the evolution of early formative funerary treatments. *J. Archaeol. Sci.* **100**, 191–200 (2018).
11. Ikram, S. & Dodson, A. *The Mummy in Ancient Egypt: Equipping the Dead for Eternity* (Thames & Hudson, 1998).
12. Germer, R. *Mumien* (Albatros, 2005).
13. Abdel-Maksoud, G. & El-Amin, A.-R. A review on the materials used during the mummification processes in ancient Egypt. *Mediter. Archaeol. Archaeom.* **11**, 129–150 (2011).
14. Taylor, J. H. *Journey Through the Afterlife: Ancient Egyptian Book of the Dead* (Harvard University Press/British Museum Press, 2010).
15. Redford, D. B. *The Ancient Gods Speak: A Guide to Egyptian Religion*. (Oxford University Press, 2002).
16. Carter, H. Report upon the Tomb of Sen-nefer found at Biban el-Molouk near that of Thotmes III No. 34. *Ann. Serv. Antiquités l'Égypte* **2**, 196–200 (1901).
17. Loeben, C. E. Canopic jar of Senetnay, "Wet nurse of the king" (Amenhotep II). in *Immortal Pharaoh: The Tomb Of Thutmose III ; An Exhibition of Ancient Egyptian Objects from Antikenmuseum Basel und Sammlung Ludwig and the Kestner-Museum Hannover with the Facsimile of the Tomb of Thutmose III* (eds. Hornung, E., Loeben, C. E. & Wiese, A.). 124–127 (Factum Arte, 2005).
18. Loeben, C. E. Katalog der Ausstellungsstücke aus der ägyptischen Sammlung des Museum August Kestner, Hannover/Catalogue of the exhibited objects from the Egyptian Collection of Museum August Kestner, Hannover. in *Das Geheimnisvolle Grab 63: Die Neueste Entdeckung im Tal der Könige—Archäologie und Kunst von Susan Osgood/The Mysterious Tomb 63: The Latest Discovery in the Valley of the Kings—Art and Archaeology of Susan Osgood* (eds. Dziobek, E., Höveler-Müller, M. & Loeben, C. E.). 142–188 (2009).
19. Germer, R. *Handbuch der Altägyptischen Heilpflanzen* (Harrassowitz, 2008).
20. Töpfer, S. Das Balsamierungsritual: Eine (Neu-)Edition der Textkomposition Balsamierungsritual (pBoulaq 3, pLouvre 5158, pDurham 1983.11 + pSt. Petersburg 18128). (Harrassowitz, 2015).
21. Herodotus. *Herodotus: The Histories*. (Penguin Books, 1996).
22. Diodorus Siculus. *Library of History: Loeb Classical Library. Translated by Oldfather, C. H.* (Harvard University Press, 1935).
23. David, R. *Manchester Museum Mummy Project: Multidisciplinary Research on Ancient Egyptian Mummified Remains*. (Manchester University Press, 1979).
24. Evershed, R. P. & Clark, K. A. Trends in use of organic balms in Egyptian mummification revealed through biomolecular analyses. in *The Handbook of Mummy Studies: New Frontiers in Scientific and Cultural Perspectives* (eds. Shin, D. H. & Bianucci, R.). 1–63 (Springer, 2020). https://doi.org/10.1007/978-981-15-1614-6_9-1.
25. Huber, B., Larsen, T., Spengler, R. N. & Boivin, N. How to use modern science to reconstruct ancient scents. *Nat. Hum. Behav.* https://doi.org/10.1038/s41562-022-01325-7 (2022).
26. Buckley, S. A., Stott, A. W. & Evershed, R. P. Studies of organic residues from ancient Egyptian mummies using high temperature–gas chromatography–mass spectrometry and sequential thermal desorption–gas chromatography–mass spectrometry and pyrolysis–gas chromatography–mass spectrometry. *Analyst* **124**, 443–452 (1999).
27. Charrié-Duhaut, A. *et al.* The canopic jars of Rameses II: Real use revealed by molecular study of organic residues. *J. Archaeol. Sci.* **34**, 957–967 (2007).
28. Łucejko, J., Connan, J., Orsini, S., Ribechini, E. & Modugno, F. Chemical analyses of Egyptian mummification balms and organic residues from storage jars dated from the Old Kingdom to the Copto-Byzantine period. *J. Archaeol. Sci.* **85**, 1–12 (2017).
29. Vandenbeusch, M., Stacey, R. & Antoine, D. Rediscovering Nestawedjat: Embalming residue analyses reunite the mummified remains of an ancient Egyptian woman with her coffins. *J. Archaeol. Sci. Rep.* **40**, 103186 (2021).
30. Marković, M., Mezzatesta, E., Porcier, S., Vieillescazes, C. & Mathe, C. Rethinking the process of animal mummification in ancient Egypt: Molecular characterization of embalming material and the use of Brassicaceae seed oil in the mummification of gazelle mummies from Kom Mereh, Egypt. *Molecules* **27**, 1532 (2022).
31. Rageot, M. *et al.* Biomolecular analyses enable new insights into ancient Egyptian embalming. *Nature* **614**, 287–293 (2023).
32. Tchapla, A., Méjanelle, P., Bleton, J. & Goursaud, S. Characterisation of embalming materials of a mummy of the Ptolemaic era. Comparison with balms from mummies of different eras. *J. Sep. Sci.* **27**, 217–234 (2004).
33. Brockbals, L. *et al.* Untargeted metabolomics-like screening approach for chemical characterization and differentiation of canopic jar and mummy samples from Ancient Egypt using GC-high resolution MS. *Analyst* **143**, 4503–4512 (2018).
34. Brettell, R., Martin, W., Atherton-Woolham, S., Stern, B. & McKnight, L. Organic residue analysis of Egyptian votive mummies and their research potential. *Stud. Conserv.* **62**, 68–82 (2017).
35. Nissenbaum, A. Molecular archaeology: Organic geochemistry of Egyptian mummies. *J. Archaeol. Sci.* **19**, 1–6 (1992).
36. Colombini, M. P., Modugno, F., Silvano, F. & Onor, M. Characterization of the balm of an Egyptian mummy from the seventh century B.C.. *Stud. Conserv.* **45**, 19–29 (2000).
37. Koller, J., Baumer, U., Kaup, Y., Schmid, M. & Weser, U. Analysis of a pharaonic embalming tar. *Nature* **425**, 784–784 (2003).
38. Koller, J., Baumer, U., Kaup, Y. & Weser, U. Herodotus' and Pliny's embalming materials identified on ancient Egyptian mummies. *Archaeometry* **47**, 609–628 (2005).
39. Hamm, S., Bleton, J. & Tchapla, A. Headspace solid phase microextraction for screening for the presence of resins in Egyptian archaeological samples. *J. Sep. Sci.* **27**, 235–243 (2004).

40. Sarret, M. *et al.* Organic substances from Egyptian jars of the Early Dynastic period (3100–2700 BCE): Mode of preparation, alteration processes and botanical (re)assessment of "cedrium". *J. Archaeol. Sci. Rep.* **14**, 420–431 (2017).
41. Fulcher, K., Serpico, M., Taylor, J. H. & Stacey, R. Molecular analysis of black coatings and anointing fluids from ancient Egyptian coffins, mummy cases, and funerary objects. *PNAS* **118**, e2100885118 (2021).
42. Serpico, M. & White, R. Chemical analysis of coniferous resins from ancient Egypt using gas chromatography/mass spectrometry (GC/MS). in *Proceedings of the Seventh International Congress of Egyptology* (ed. Eyre, C.). 1037–1048 (Peeters, 1998).
43. López-Goldar, X., Lundborg, L., Borg-Karlson, A. K., Zas, R. & Sampedro, L. Resin acids as inducible chemical defences of pine seedlings against chewing insects. *PLoS ONE* **15**, e0232692 (2020).
44. Smirnova, I. E. *et al.* Synthesis of dammarenolic acid derivatives with a potent α-glucosidase inhibitory activity. *Med. Chem. Res.* **29**, 94–102 (2020).
45. Franz, M. H. *et al.* Studies of chemical and mechanical properties of hybrid composites based on natural resin Dammar formulated by epoxy resin. *Polym. Bull.* **78**, 2427–2438 (2021).
46. van der Doelen, G. A. *et al.* Analysis of fresh triterpenoid resins and aged triterpenoid varnishes by high-performance liquid chromatography—Atmospheric pressure chemical ionisation (tandem) mass spectrometry. *J. Chromatogr. A* **809**, 21–37 (1998).
47. Peters, K. E., Walters, C. C. & Moldowan, J. M. *The Biomarker Guide: Volume 1: Biomarkers and Isotopes in the Environment and Human History*. Vol. 1 (Cambridge University Press, 2004).
48. Bozorgi, M. *et al.* Five *Pistacia* species (*P. vera*, *P. atlantica*, *P. terebinthus*, *P. khinjuk* and *P. lentiscus*): A review of their traditional uses, phytochemistry, and pharmacology. *Sci. World J.* **2013**, 1–33 (2013).
49. Barberis, E., Manfredi, M., Ferraris, E., Bianucci, R. & Marengo, E. Non-invasive paleo-metabolomics and paleo-proteomics analyses reveal the complex funerary treatment of the early 18th dynasty dignitary NEBIRI (QV30). *Molecules* **27**, 7208 (2022).
50. Lesage-Meessen, L. *et al.* A two-step bioconversion process for vanillin production from ferulic acid combining *Aspergillus niger* and *Pycnoporus cinnabarinus*. *J. Biotechnol.* **50**, 107–113 (1996).
51. Tamburini, D., Łucejko, J., Ribechini, E. & Colombini, M. P. New markers of natural and anthropogenic chemical alteration of archaeological lignin revealed by in situ pyrolysis/silylation–gas chromatography mass spectrometry. *J. Anal. Appl. Pyrol.* **118**, 249–258 (2016).
52. Whelton, H. L. *et al.* A call for caution in the analysis of lipids and other small biomolecules from archaeological contexts. *J. Archaeol. Sci.* **132**, 105397 (2021).
53. Riesmeier, M. *et al.* Recipes of Ancient Egyptian kohls more diverse than previously thought. *Sci. Rep.* **12**, 5932 (2022).
54. Wagner, K. *et al.* Larch wood residues valorization through extraction and utilization of high value-added products. *Polymers* **12**, 359 (2020).
55. Sato, M. *et al.* Comparative analysis of diterpene composition in the bark of the hybrid larch, Larix F1, gmelinii var. japonica × *L. kaempferi* and their parent trees. *J Wood Sci* **55**, 32–40 (2009).
56. Tanaka, R., Ohtsu, H. & Matsunaga, S. Abietane diterpene acids and other constituents from the leaves of *Larix kaempferi*. *Phytochemistry* **46**, 1051–1057 (1997).
57. Ostroukhova, L. A., Raldugin, V. A., Babkin, V. A., Onuchina, N. A. & Levchuk, A. A. Investigation of the chemical composition of larch wood resin. *Russ. J. Bioorg. Chem.* **38**, 775–779 (2012).
58. Roffet-Salque, M. *et al.* From the inside out: Upscaling organic residue analyses of archaeological ceramics. *J. Archaeol. Sci. Rep.* **16**, 627–640 (2017).
59. Dunne, J., Mercuri, A. M., Evershed, R. P., Bruni, S. & di Lernia, S. Earliest direct evidence of plant processing in prehistoric Saharan pottery. *Nat. Plants* **3**, 16194 (2016).
60. Regert, M., Colinart, S., Degrand, L. & Decavallas, O. Chemical alteration and use of beeswax through time: Accelerated ageing tests and analysis of archaeological samples from various environmental contexts. *Archaeometry* **43**, 549–569 (2001).
61. Dudd, S. N., Regert, M. & Evershed, R. P. Assessing microbial lipid contributions during laboratory degradations of fats and oils and pure triacylglycerols absorbed in ceramic potsherds. *Org. Geochem.* **29**, 1345–1354 (1998).
62. Łucejko, J. J., Lluveras-Tenorio, A., Modugno, F., Ribechini, E. & Colombini, M. P. An analytical approach based on X-ray diffraction, Fourier transform infrared spectroscopy and gas chromatography/mass spectrometry to characterize Egyptian embalming materials. *Microchem. J.* **103**, 110–118 (2012).
63. Dunne, J. *et al.* First dairying in green Saharan Africa in the fifth millennium bc. *Nature* **486**, 390–394 (2012).
64. Clark, K. A., Ikram, S. & Evershed, R. P. The significance of petroleum bitumen in ancient Egyptian mummies. *Philos. Trans. R. Soc. A* **374**, 20160229 (2016).
65. Connan, J. Use and trade of bitumen in antiquity and prehistory: Molecular archaeology reveals secrets of past civilizations. *Philos. Trans. R. Soc. Lond. B* **354**, 33–50 (1999).
66. Barakat, A. O., Mostafa, A., Qian, Y., Kim, M. & KennicuttIi, M. C. Organic geochemistry indicates Gebel El Zeit, Gulf of Suez, is a source of bitumen used in some Egyptian mummies. *Geoarchaeology* **20**, 211–228 (2005).
67. Fulcher, K., Stacey, R. & Spencer, N. Bitumen from the dead sea in early iron age Nubia. *Sci. Rep.* **10**, 8309 (2020).
68. Drieu, L. *et al.* A Neolithic without dairy? Chemical evidence from the content of ceramics from the Pendimoun rock-shelter (Castellar, France, 5750–5150 BCE). *J. Archaeol. Sci. Rep.* **35**, 102682 (2021).
69. Maurer, J., Möhring, T., Rullkötter, J. & Nissenbaum, A. Plant lipids and fossil hydrocarbons in embalming material of Roman Period mummies from the Dakhleh Oasis, Western Desert, Egypt. *J. Archaeol. Sci.* **29**, 751–762 (2002).
70. Bianucci, R. *et al.* Shedding new light on the 18th Dynasty mummies of the royal architect kha and his spouse merit. *PLoS ONE* **10**, e0131916 (2015).
71. Clark, K. A., Ikram, S. & Evershed, R. P. Organic chemistry of balms used in the preparation of pharaonic meat mummies. *Proc. Natl. Acad. Sci. USA* **110**, 20392–20395 (2013).
72. Mejanelle, P., Bleton, J., Goursaud, S. & Tchapla, A. Identification of phenolic acids and inositols in balms and tissues from an Egyptian mummy. *J. Chromatogr. A* **767**, 177–186 (1997).
73. Buckley, S. A., Clark, K. A. & Evershed, R. P. Complex organic chemical balms of Pharaonic animal mummies. *Nature* **431**, 294–299 (2004).
74. Ikram, S. A re-analysis of part of Prince Amenemhat Q's eternal menu. *J. Am. Res. Center Egypt* **48**, 119–135 (2012).
75. Hornung, E. *Das Tal der Könige* (Verlag C.H. Beck, 2010).
76. Izzo, F. C., Lodi, G. C. & Vázquez de Ágredos Pascual, M. L. New insights into the composition of historical remedies and pharmaceutical formulations: The identification of natural resins and balsams by gas chromatographic–mass spectrometric investigations. *Archaeol. Anthropol. Sci/* **13**, 2 (2021).
77. *Flora Europaea. Volume 1: Lycopodiaceae to Platanaceae*. (Cambridge University Press, 1964).
78. Farjon, A. *Conifers of the World. Resources for Conifer Research*. (2016).
79. Drosou, K., Collin, T. C., Freeman, P. J., Loynes, R. & Freemont, T. The first reported case of the rare mitochondrial haplotype H4a1 in ancient Egypt. *Sci. Rep.* **10**, 17037 (2020).
80. Hunt, C. O. Quaternary deposits in the Maltese Islands: A microcosm of environmental change in the Mediterranean lands. *GeoJournal* **41**, 101–109 (1997).
81. Serpico, M. & White, R. The botanical identity and transport of incense during the Egyptian New Kingdom. *Antiquity* **74**, 884–897 (2000).
82. Theophrastus. *Enquiry into Plants. Translated by Sir Arthur Fenton*. (W. Heinemann, 1916).

83. Langenheim, J. H. *Plant Resins: Chemistry, Evolution, Ecology, and Ethnobotany*. (Timber Press, 2003).
84. Boivin, N. & Fuller, D. Q. Shell middens, ships and seeds: Exploring coastal subsistence, maritime trade and the dispersal of domesticates in and around the Ancient Arabian Peninsula. *J. World Prehist.* **22**, 113–180 (2009).
85. Boivin, N. Proto-globalisation and biotic exchange in the Old World. in *Human Dispersal and Species Movement* (eds. Boivin, N., Crassard, R. & Petraglia, M.). 349–408 (Cambridge University Press, 2017). https://doi.org/10.1017/9781316686942.015.
86. Fuller, D. Q., Boivin, N., Hoogervorst, T. & Allaby, R. Across the Indian Ocean: The prehistoric movement of plants and animals. *Antiquity* **85**, 544–558 (2011).
87. Hammann, S. *et al.* Neolithic culinary traditions revealed by cereal, milk and meat lipids in pottery from Scottish crannogs. *Nat. Commun.* **13**, 5045 (2022).
88. Giachi, G. *et al.* Ingredients of a 2,000-y-old medicine revealed by chemical, mineralogical, and botanical investigations. *Proc. Natl. Acad. Sci. USA* **110**, 1193–1196 (2013).
89. Devièse, T. *et al.* A multi-analytical approach using FTIR, GC/MS and Py-GC/MS revealed early evidence of embalming practices in Roman catacombs. *Microchem. J.* **133**, 49–59 (2017).
90. Regert, M., Devièse, T., Le Hô, A.-S. & Rougeulle, A. Reconstructing ancient Yemeni commercial routes during the Middle Ages using structural characterization of terpenoid resins. *Archaeometry* **50**, 668–695 (2008).
91. Matyash, V., Liebisch, G., Kurzchalia, T. V., Shevchenko, A. & Schwudke, D. Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics. *J. Lipid Res.* **49**, 1137–1146 (2008).
92. Salem, M., Bernach, M., Bajdzienko, K. & Giavalisco, P. A simple fractionated extraction method for the comprehensive analysis of metabolites, lipids, and proteins from a single sample. *JoVE* https://doi.org/10.3791/55802 (2017).

Acknowledgements

The authors are grateful to the Museum August Kestner in Hannover for providing samples from both of Senetnay's canopic jars for analysis, and to the Max Planck Society for funding this research and the publication of this manuscript in open access form under the DEAL Project. B. Huber thanks the Joachim Herz Foundation for the award of an Add-on Fellowship for Interdisciplinary Life Sciences for her PhD research.

Author contributions

B.H. and N.B. designed the research. B.H., S.H. and D.G.V. performed the laboratory work. B.H., S.H., D.K.J., and T.D. analyzed and interpreted the data. C.E.L. provided access to the archaeological material and information on the history of the canopic jars. D.Q.F. and R.N.S. provided the archaeobotanical background, and D.Q.F. prepared the botanical maps. P.R., C.E.L. and N.B. advised on the archaeological background. B.H. prepared the original draft, and wrote the manuscript with N.B., including input from all co-authors. P.R., T.D. and N.B. supervised the research. All authors have read and agreed to the published version of the manuscript.

Funding

Open Access funding enabled and organized by Projekt DEAL.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-023-39393-y.

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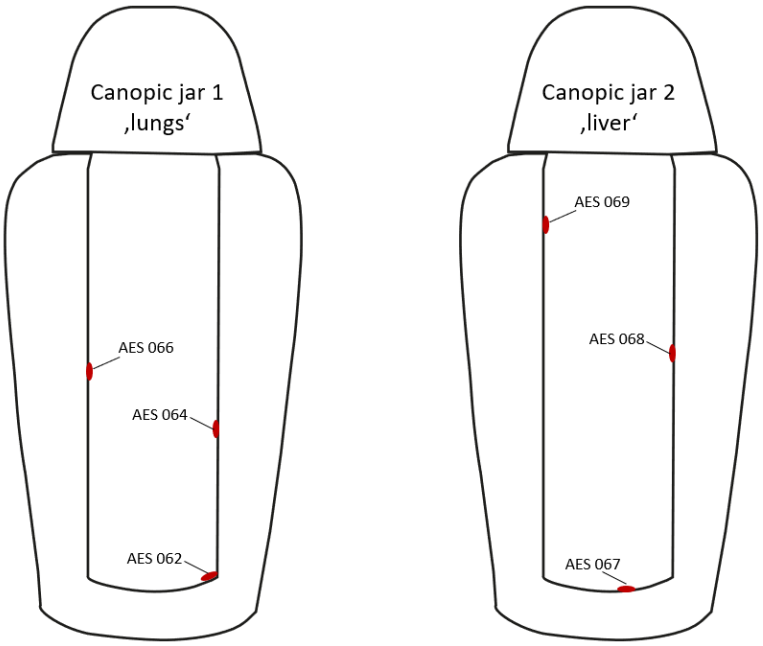
SUPPLEMETARY INFORMATION

Biomolecular characterization of 3500-year-old ancient Egyptian mummification balms from the Valley of the Kings

Huber, B., Hammann, S., Loeben, C. E., Jha, D. K., Vassão, D. G., Larsen, T., Spengler, R.N., Fuller, D. Q., Roberts, P., Devièse, T., Boivin, N.

Content

Supplementary Figure S1



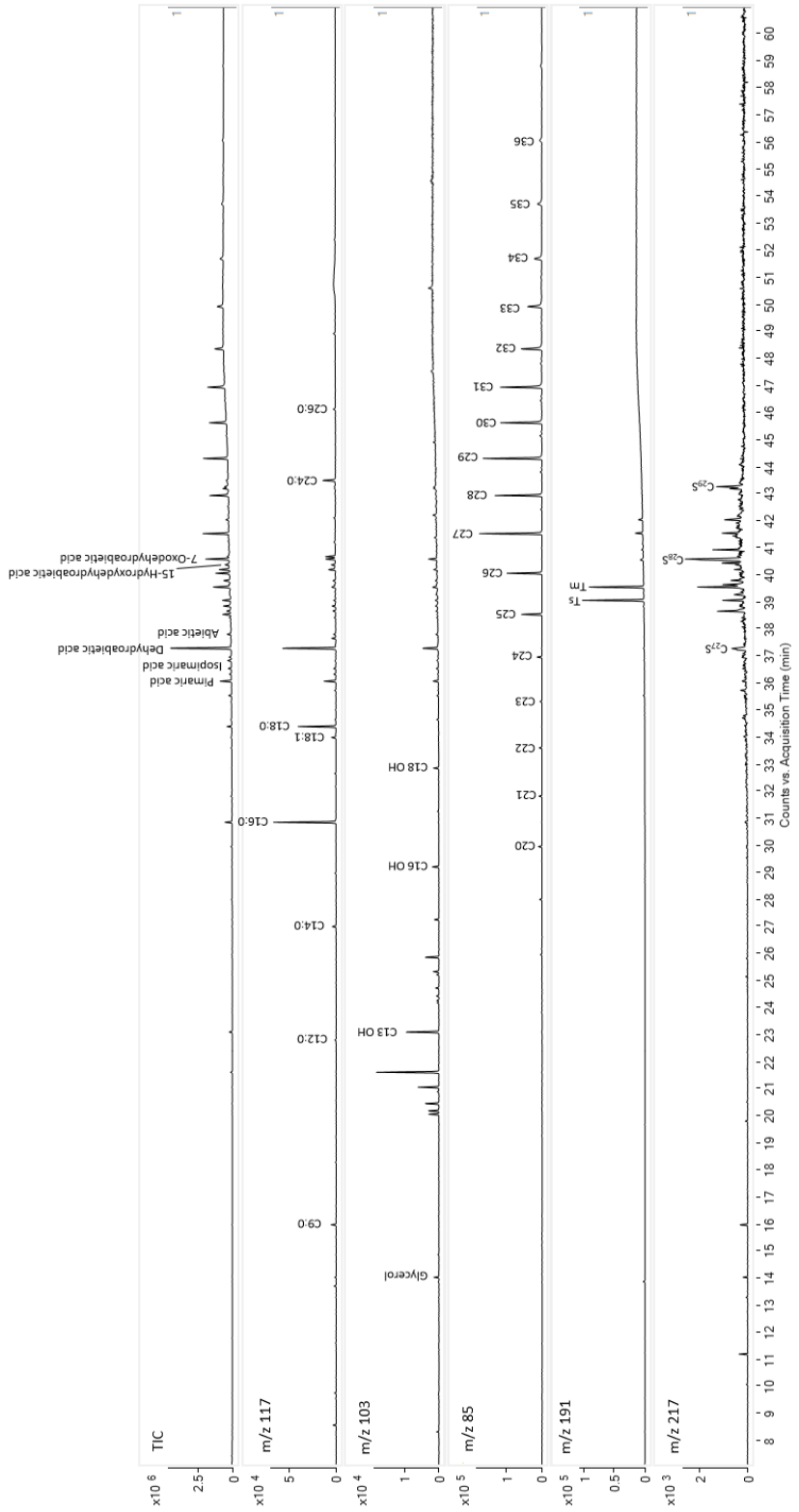
Schematic drawings of Senetnay's canopic jars 1 and 2 from the August Kestner Museum, Hannover, Germany. The red dots indicate the location from which each sample was taken.

Supplementary Table S1

Lab sample no.	***Sample location***	***Description of sample***	***Object no.***	***Dating***	***Context***
DA-AES 062	Interior, bottom of jar	Black, thin remnant of balm on the bottom of the jar	Acq. No. 1935.200.1018, Canopic jar of Senetnay (lungs)	New Kingdom, c. 1450 BCE	Tomb 42, Valley of the Kings, West Thebes
DA-AES 064	Interior, wall of jar	Thin black spot of residue, partially absorbed within the porous material of the limestone	Acq. No. 1935.200.1018, Canopic jar of Senetnay (lungs)	New Kingdom, c. 1450 BCE	Tomb 42, Valley of the Kings, West Thebes
DA-AES 066	Interior, wall of jar	Thin black spot of residue, partially absorbed within the porous material of the limestone	Acq. No. 1935.200.1018, Canopic jar of Senetnay (lungs)	New Kingdom, c. 1450 BCE	Tomb 42, Valley of the Kings, West Thebes
DA-AES-067	Interior, bottom of jar	Black residual spot in the middle of the bottom of the jar	Acq. No. 1935.200.0253, Canopic jar of Senetnay (liver)	New Kingdom, c. 1450 BCE	Tomb 42, Valley of the Kings, West Thebes
DA-AES 068	Interior, wall of jar	Thin black spot of residue, partially absorbed within the porous material of the limestone	Acq. No. 1935.200.0253, Canopic jar of Senetnay (liver)	New Kingdom, c. 1450 BCE	Tomb 42, Valley of the Kings, West Thebes
DA-AES 069	Interior, wall of jar	Thin black spot of residue, partially absorbed within the porous material of the limestone	Acq. No. 1935.200.0253, Canopic jar of Senetnay (liver)	New Kingdom, c. 1450 BCE	Tomb 42, Valley of the Kings, West Thebes

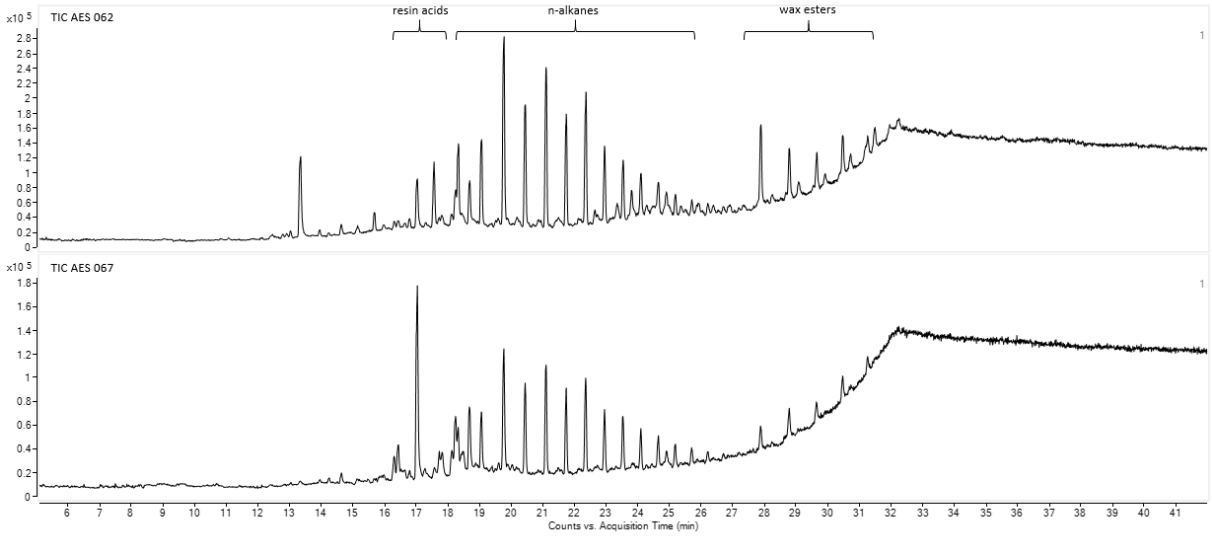
The table provides a description of the individual samples with all relevant information regarding the museum objects from which they derive.

Supplementary Figure S2



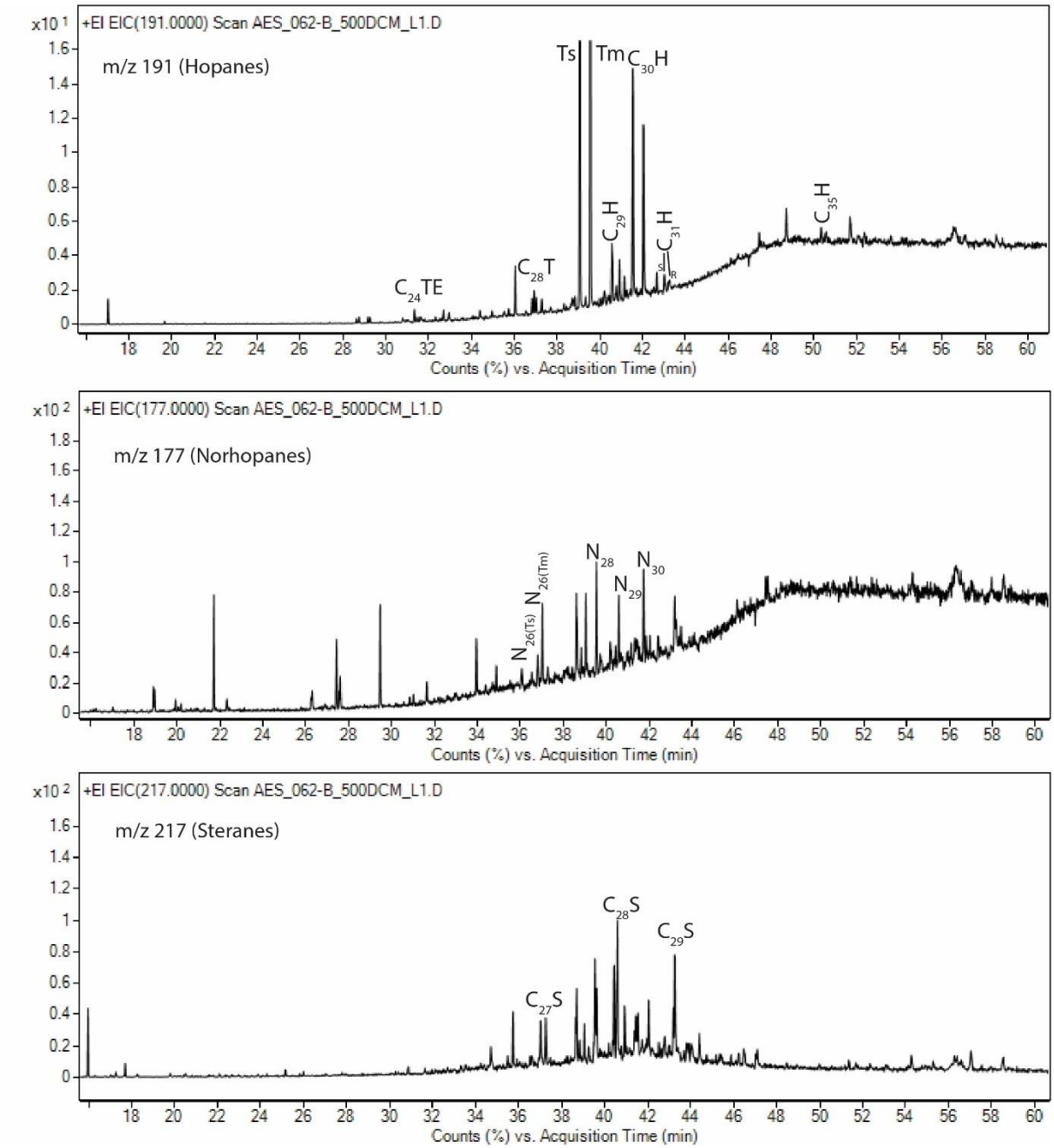
Total ion current (TIC) and extracted ion chromatograms (EIC) of sample AES 067 displaying fatty acids (*m/z* 117; n:0 = saturated FA and n:1 = unsaturated FA), fatty alcohols (*m/z* 103), n-alkanes (*m/z* 103), hopanes (*m/z* 191) and steranes (*m/z* 217). For detailed identification of hopanes and steranes in this sample see Supplementary Figure S5.

Supplementary Figure S3



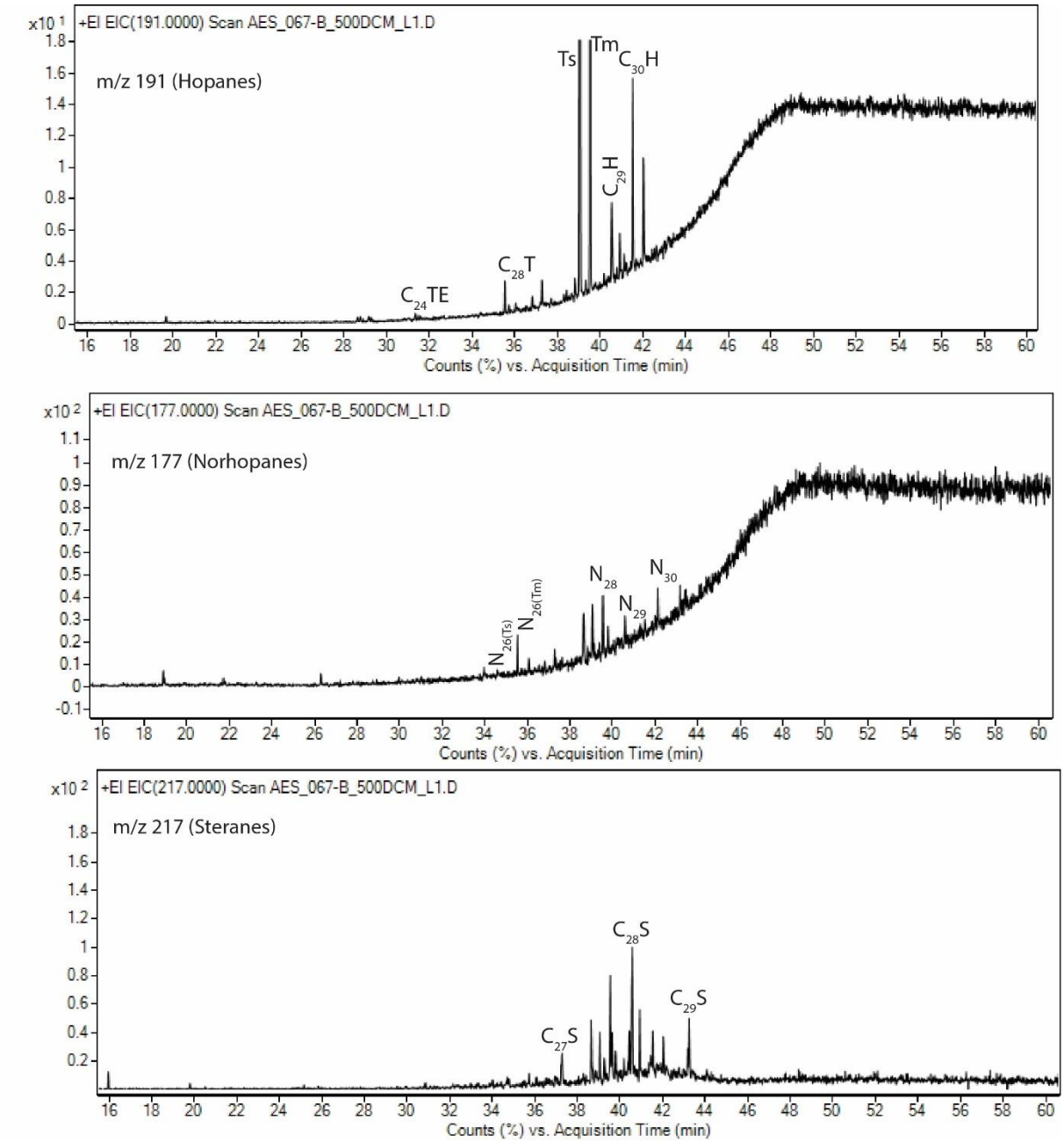
Additional TIC chromatograms of samples AES 062 and AES 067 obtained by HT-GC-MS showing the presence of resin acids, *n*-alkanes and wax esters. For detailed identification of the individual monoesters of palmitic acid and hydroxy wax esters see Figure 5.

Supplementary Figure S4



The hopane and sterane compounds identified in the sample AES 062. Their presence indicates use of bitumen in the balm. The presence of norhopanes (*m/z* 177) in the sample suggests degradation of these compounds. (The compounds are identified using parent and daughter ions (*m/z*) in EIC mode and some compounds were validated in SIM mode in GCMS). For the names of compounds, please see the Supplementary Table S2. Some compounds, which are crucial for understanding the provenance of the bitumen, were only present in low concentrations [1–4]. Therefore, we omitted the ratio and provenance discussion to prevent potential inaccuracies in conclusions.

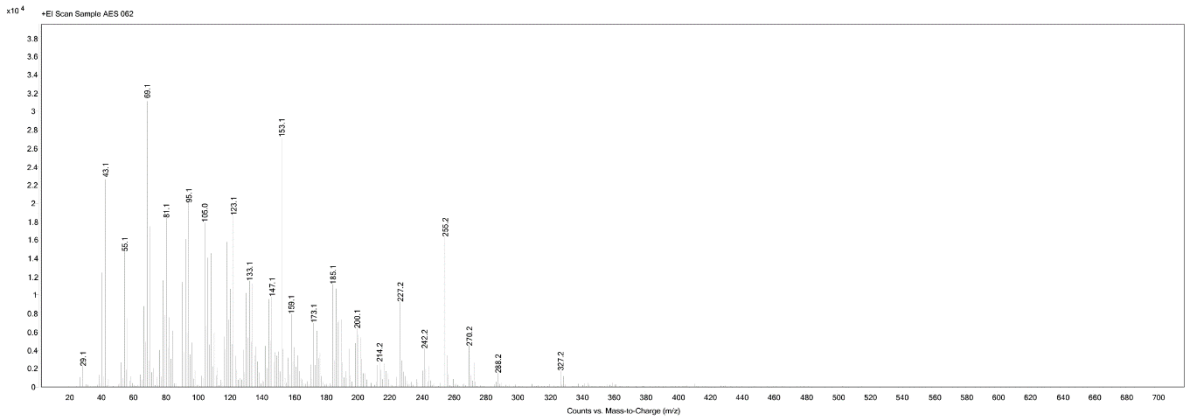
Supplementary Figure S5



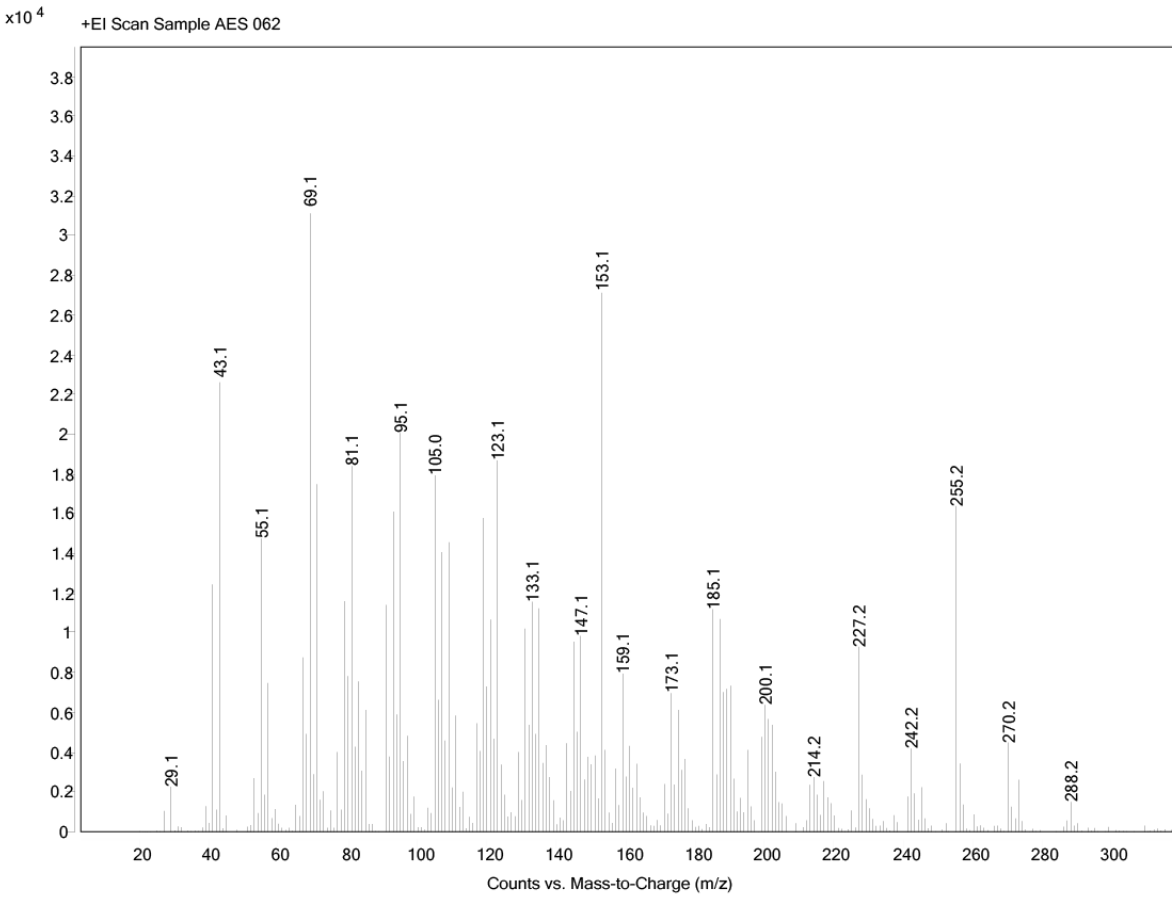
The hopane and sterane compounds identified in the sample AES 067. The compounds are similar to those identified in sample AES 062, but less in abundance. For the names of compounds, please see the Supplementary Table S2.

Supplementary Figure S6

a



b



(a) Mass spectrum of the compound (rt: 37,017) identified as larixol and (b) partial mass spectrum of the same compound with more details.

Supplementary Table S2

List of hopane and sterane compounds identified in samples AES 062 and AES 067. The identification was based on the combination of parent and daughter ions (*m/z*) extracted from the TIC. The ions were also verified using selected ion monitoring (SIM) method.

Compounds	Taget ions	
	Precursor (*m/z*)	**Product (*m/z*)**
Tricyclic terpanes (C28 T)	388	191
Tetracyclic terpane (C24 TE)	330	191
Regular 17α-hopane series		
18α(H),21β(H)-22,29,30-trisnorhopane (Ts)	370	191
17α(H),21β(H)-22,29,30-trisnorhopane ('Tm)	370	191
C29 Hopanes (C29 H)	398	191
C30 Hopanes (C30 H)	412	191
C31 Homohopanes (C31 H)	426	191
C35 Homohopanes (C35 H)	482	191
Norhopane series		
N26 Norhopanes (Ts and Tm)	388	177
N28 Norhopanes (N28)	384	177
N29 Norhopanes (N29)	398	177
N30 Norhopanes (N30)	412	177
Steranes		
C27 steranes (C27 S)	372	217
C28 steranes (C28 S)	386	217
C29 steranes (C29 S)	400	217

Supplementary Table S3

List of authentic analytical standards for the optimization of MRM parameters employed to screen for specific compounds in archaeological samples (for more detailed MRM parameters, such as collision energy (V) and dwell times for precursor and product ions see Supplementary Table S4 – separate excel file).

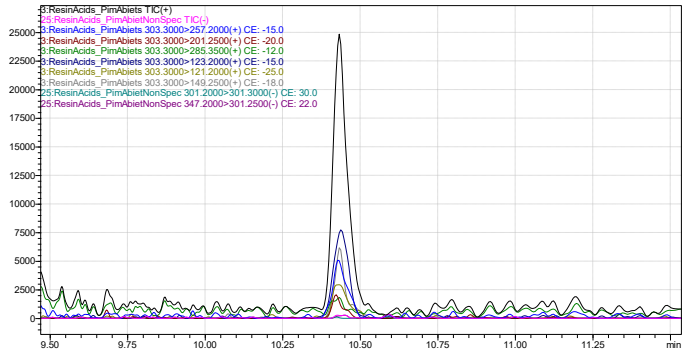
Compound	**Column**	**Ret. Time (min)**
Amygdalin	Velox SP-C18	5.834
Anabasine	Velox SP-C18	1.573
Artemisinin	Velox SP-C18	10.677
Asiatic Acid	Velox SP-C18	9.967
Benzoic Acid	Velox SP-C18	7.418
Betulinic Acid	Velox SP-C18	14.410
Caffeine	Velox SP-C18	6.097
Cinnamic Acid	Velox SP-C18	8.217
Cotinine	Velox SP-C18	5.158
Coumarin	Velox SP-C18	8.069
Curcumin	Velox SP-C18	10.065
Demethoxycurcumin	Velox SP-C18	9.935
Didemethoxycurcumin	Velox SP-C18	9.799
Ferulic Acid	Velox SP-C18	6.830
(E)-Guggulsterone	Velox SP-C18	11.676
(Z)-Guggulsterone	Velox SP-C18	12.176
Harmaline	Velox SP-C18	6.405
Harmane	Velox SP-C18	6.157
Harmine	Velox SP-C18	6.449
Hydrocotarnine	Velox SP-C18	5.935
Incensole	Velox SP-C18	13.904
Meconic Acid	Velox SP-C18	5.240
Nicotine	Velox SP-C18	1.560
Nicotinic Acid	Velox SP-C18	1.564
Oleanolic Acid	Velox SP-C18	14.647
Opianic Acid	Velox SP-C18	6.530
Quinine	Velox SP-C18	5.978
Theobromine	Velox SP-C18	5.553
ar-Turmerone	Velox SP-C18	12.066
Vanillic Acid	Velox SP-C18	6.221
Zingerone	Velox SP-C18	7.475
Resin Acids (pimaric acid, isopimaric acid, palustric acid and neoabietic acid)	Biphenyl	10.400
7-Oxodehydroabietic Acid	Biphenyl	9.411
Dehydroabietic Acid	Biphenyl	10.091
α-Boswellic Acid	Biphenyl	11.101
β-Boswellic Acid	Biphenyl	11.296
Acetyl α-Boswellic Acid	Biphenyl	11.935
Acety β-Boswellic Acid	Biphenyl	12.159
Keto β-Boswellic Acid	Biphenyl	10.414
Acetyl Keto β-Boswellic Acid	Biphenyl	11.309
Cholesterol	Biphenyl	12.292

Campesterol	Biphenyl	12.493
β-Sitosterol	Biphenyl	12.727
Brassicasterol	Biphenyl	12.378
5α-Cholestanol	Biphenyl	12.544
Stigmasterol	Biphenyl	12.681
Sitostanol	Biphenyl	12.998
Cholestanone	Biphenyl	13.376
α-Amyrin	Biphenyl	12.871
β-Amyrin+Lupeol	Biphenyl	12.620
Benzoic Acid	Biphenyl	6.141
Ferulic Acid	Biphenyl	5.876
Dammarenolic Acid	Biphenyl	10.723
Masticadienolic Acid	Biphenyl	10.912
Moronic+Oleanonic Acids	Biphenyl	11.010
Dipterocarpol	Biphenyl	11.475
Urs-12-en-3-one	Biphenyl	13.538

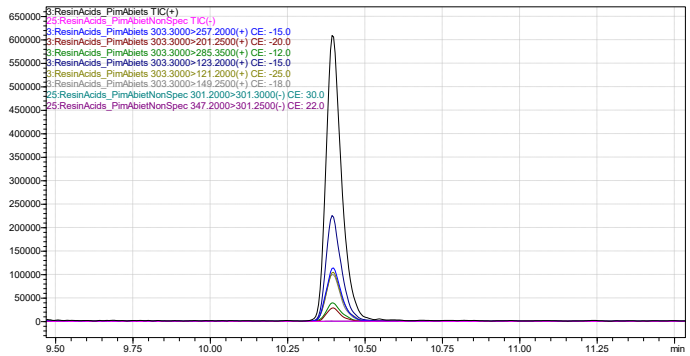
Supplementary Table S5

Multiple reaction monitoring (MRM) chromatograms of the analytical standards neoabietic acid, palustric acid, pimaric acid and isopimaric acid summarized as ‘resin acids’.

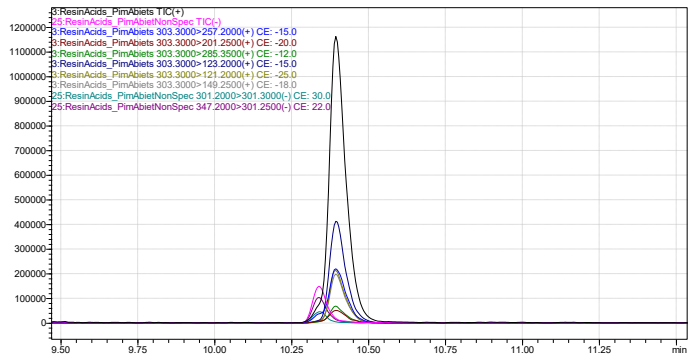
Neoabietic acid



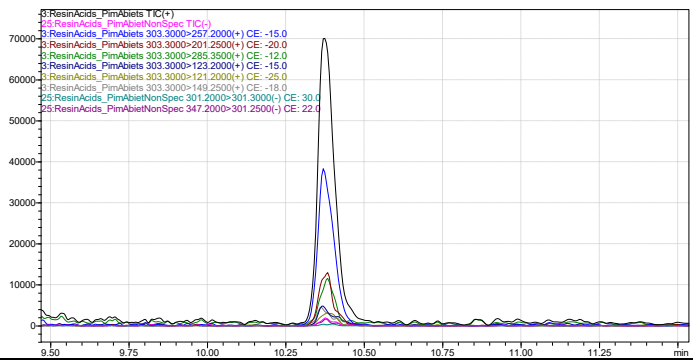
Palustric acid



Pimaric acid



Isopimaric acid



Supplementary Table S6

Sources of occurrence data for candidate conifers (Fig. 7, A)

Taxon	**Plants of the World Online [POWO; kew.org]**	**Farjon 2017; and other sources**	**GBIF.org (04 February 2023)**
Larix decidua Mill.	Austria, Czechoslovakia, France, Germany, Italy, Poland, Romania, Switzerland, Ukraine, Yugoslavia	Europe: Alps, Carpathians, Slovenian moutnains, S. Poland (Wista river); see also Karlman (2010).	https://doi.org/10.15468/dl.pz45jj [but includes many planted populations]
Abies cilicica (Antoine & Kotschy) Carrière	Lebanon-Syria, Turkey	Turkey: Anatalya and Konya (Isaurian Taurus)	https://doi.org/10.15468/dl.f59dkt
Afrocarpus gracilior (Pilg.) C. N. Page	Ethiopia, Kenya, Sudan, Tanzania, Uganda	Ethiopia, Kenya, South Sudan, Tanzania, Uganda	https://doi.org/10.15468/dl.uuyyxh
Cedrus libani A.Rich.	Cyprus, Lebanon-Syria, Turkey	Lebanon, Syria (Djebel el ANsiriya), Turkey (Taurus and Anti-Taurus mountains), Cyprus (Troodos Mountains, Mt. Triphylos)	https://doi.org/10.15468/dl.fvks6u
Cedrus atlantica (Endl.) Manetti ex Carrière	Algeria, Morocco	Algeria, Morocco (Atlas Mountains)	https://doi.org/10.15468/dl.gsk6mt
Juniperus excelsa M.-Bieb.,	Albania, Bulgaria, Cyprus, Greece, Krym, Lebanon-Syria, North Caucasus, Palestine, Transcaucasus, Turkey, Yugoslavia	SE Europe, Central Asia, Middle East, Northwest India (Kashmir), Oman, Leabnan, Syria, Turkey, Cyprus	
Juniperus procera Hochst. Ex Endl.	Djibouti, Eritrea, Ethiopia, Kenya, Malawi, Saudi Arabia, Somalia, Sudan, Tanzania, Uganda, Yemen, Zaïre, Zimbabwe	Ethiopia, Eritrea, Sudan (Red Sea hills), Somalia Yemen, Saudi Arabia (Asir mountains), elsewhere in tropical Africa (Tanzania, Uganda, NE Zimbabwe, Malawi, Congo)	https://doi.org/10.15468/dl.zs9qha
Juniperus indica Bertol	China South-Central, East Himalaya, Nepal, Pakistan, Tibet, West Himalaya	N Pakistan, Kashmir, Himalayas, from Himachal Pradesh eastwards into Yunnan and SW Sichuan	https://doi.org/10.15468/dl.ke9r9h
Picea orientalis (L.) Peterm.	North Caucasus, Transcaucasus, Turkey	Caucasus, North Turkey: coastal mountains	https://doi.org/10.15468/dl.gsk6mt
Pinus halepensis Mill.	Albania, Algeria, Baleares, Corse, East Aegean Is., France, Greece, Italy, Lebanon-Syria, Libya, Morocco, Palestine, Sardegna, Sicilia, Spain, Tunisia, Turkey, Yugoslavia	Mediterranean: From Morocco and Spain to Greece, Israel, Lebanon, SW Syria, Libya: Jabal al Akhdar; also, Critchfield and Little (1966).	https://doi.org/10.15468/dl.vh5hju
Pinus brutia Ten.	Bulgaria, Cyprus, East Aegean Is., Greece, Iran, Iraq, Kriti, Krym, Lebanon-Syria, North Caucasus, Transcaucasus, Turkey, Turkey-in-Europe	East Mediterranean, circum-Black sea, Turkey: Mugla Province Caucasus, NW Iran, N Iraq, Azerbaijain, Georgia, Afghanistan; also, Critchfield and Little (1966).	https://doi.org/10.15468/dl.n47ghf

Pinus pinea L.	Albania, Baleares, Corse, Cyprus, East Aegean Is., France, Greece, Italy, Kriti, Lebanon-Syria, Portugal, Sicilia, Spain, Turkey	Mediterranean Europe and Near East, original native range unclear, but Critchfield and Little (1966) infer a native range only on the Iberian Peninsula)	https://doi.org/10.15468/dl.xpv569 [but includes many planted, and anthropogenic populations]
Pinus pinaster Aiton	Algeria, Baleares, Corse, France, Italy, Morocco, Portugal, Sardegna, Sicilia, Spain, Tunisia	Western Mediterranean: France, Spain, Baleraic islands, Italy, Malta; ; also, Critchfield and Little (1966).	https://doi.org/10.15468/dl.taxaw9

Sources:

Farjon, Aljos (2017) *A Handbook of the World's Conifers.* Leiden: Brill; GBIF= https://www.gbif.org/ ;
POWO= Royal Botanic Gardens Kew (2023) Plants of the world Online. http:// https://powo.science.kew.org/ . Online sources accessed 04 Feburary 2023.
General distribution of other *Larix* spp. from Karlman (2010); *Pinus* spp. from Critchfield and Little (1966):

- Karlman, Lars (2010) Genetic variation in frost tolerance, juvenile growth and timber production in Russian larches (*Larix* Mill.)- implications for use in Sweden. PhD Dissertation, Swedish University of Agricultural Sciences, Umeå. *Acta Universitatis Agriculturae Sueciae* 2010: 30
- Critchfield, William B. and Little, E. L. (1966) *Geographic distribution of the pines of the world*. Washington, D.C, U.S. Dept. of Agriculture, Forest Service

References for these distributions of Pistacia species (Fig, 7B):

- Kozhoridze, G., Orlovsky, N., Orlovsky, L., Blumberg, D. G., & Golan-Goldhirsh, A. (2015). Geographic distribution and migration pathways of Pistacia–present, past and future. *Ecography*, *38*(11), 1141-1154.
- Paludosi, S., Hadj-Hassan, A., eds. (2001) Towards a comprehensive documentation and use of Pistacia genetic diversity in Central and West Asia, North Africa and Europe. Rome: IPGRI
- Dinies, M., Neef, R., Plessen, B., Kürschner, H. (2016). Holocene vegetation, climate, land use and plant cultivation in the Tayma region, northwestern Arabia. In: Luciani, M. (ed). The archaeology of North Arabia: oases and landscapes; proceedings of the international congress held at the University of Vienna, 5-8 December, 2013. Austrian Academy of Sciences Press, Wien, 57–77
- Dinies, M., Plessen, B., Neef, R., Kürschner, H. (2015). When the desert was green: Grassland expansion during the early Holocene in northwestern Arabia. Quaternary International 382:293–302.

References for dipterocarps core distributions (Fig, 7B):

- Deb, J. C., Phinn, S., Butt, N., & McAlpine, C. A. (2017). The impact of climate change on the distribution of two threatened Dipterocarp trees. *Ecology and Evolution*, *7*(7), 2238-2248.
- Maury-Lechon, G., Curtet, L. (1998) Biogeography and Evolutionary systematics of Dipterocarpaceae. In: Appanah, S. and Turnbull, J. M. (eds.) A review of Dipterocarps: taxonomy, ecology and silviculture. Bogor, Indonesia: Center for International Forestry Research. Pp. 5-44
- With consultation of data in gbif.org

Supplementary Table S4

Compounds	Column	Precursor m/z	Product m/z	Dwell time (ms)	Q1 pre-bias (V)	Collision energy (V)	Q3 pre-bias (V)
Benzoic Acid (-)	Velox SP-C18	121.2	76.9	3	28	15	17
Coumarin (+)	Velox SP-C18	147.3	91.2	3	-12	-24	-19
Coumarin (+)	Velox SP-C19	147.3	103.1	3	-13	-20	-10
Vanillic Acid (-)	Velox SP-C18	167.2	152.0	3	16	18	14
Vanillic Acid (-)	Velox SP-C19	167.2	108.0	3	12	16	10
Resin Acids (+)	Biphenyl	303.3	257.2	5	-15	-15	-17
Resin Acids (+)	Biphenyl	303.3	201.3	5	-15	-20	-21
Resin Acids (+)	Biphenyl	303.3	285.4	5	-15	-12	-13
Resin Acids (+)	Biphenyl	303.3	123.2	5	-15	-15	-26
Resin Acids (+)	Biphenyl	303.3	121.2	5	-15	-25	-24
Resin Acids (+)	Biphenyl	303.3	149.3	5	-15	-18	-15
Resin Acids (-)	Biphenyl	301.2	301.3	5	20	30	13
Resin Acids (+)	Biphenyl	347.2	301.3	5	23	22	12
7-Oxodehydroabietic Acid (+)	Biphenyl	315.3	187.2	5	-16	-22	-19
7-Oxodehydroabietic Acid (+)	Biphenyl	315.3	117.2	5	-15	-39	-27
7-Oxodehydroabietic Acid (+)	Biphenyl	315.3	213.2	5	-10	-27	-23
7-Oxodehydroabietic Acid (-)	Biphenyl	313.1	313.2	5	11	10	11
7-Oxodehydroabietic Acid (+)	Biphenyl	359.3	269.3	5	16	28	12
7-Oxodehydroabietic Acid (+)	Biphenyl	359.3	267.3	5	22	29	11
Dehydroabietic Acid (+)	Biphenyl	301.2	173.2	5	-13	-14	-17
Dehydroabietic Acid (+)	Biphenyl	301.2	133.3	5	-14	-24	-28
Dehydroabietic Acid (+)	Biphenyl	301.2	255.4	5	-23	-11	-27

Dammarenolic Acid (+)	Biphenyl	441.4	121.2	5	-10	-35	-22
Dammarenolic Acid (+)	Biphenyl	441.4	107.2	5	-10	-39	-22
Dammarenolic Acid (+)	Biphenyl	441.4	109.2	5	-18	-29	-22
Oleanonic and Moronic Acids (+)		455.0	409.2	5	-18	-20	-14
Oleanonic and Moronic Acids (+)		455.0	203.2	5	-22	-28	-14
Oleanonic and Moronic Acids (+)		437.0	189.2	5	-22	-29	-22

Appendix 2 – Manuscripts in preparation

MANUSCRIPT D

Exploring the aromatic diversity of incense materials at the ancient oasis of Tayma using metabolic profiling

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Abstract:

Ancient culture in Arabia is strongly connected to the smell of burning frankincense, an emblematic fragrance that wafts through historical narratives. While frankincense was a significant natural resource in the past, it represents just a fraction of the olfactory tapestry of ancient Arabia. This paper investigates the aromatic diversity at the ancient oasis of Tayma, unveiling the complexity of sensory experiences at this important hub on the Incense Road. Employing metabolic profiling through GC-MS and LC-MS/MS techniques, we unravel the biomolecular composition of these ancient substances, which sheds light on a broader range of aromatics with distinct applications over time. The results underscore the pivotal role of olfactory elements, which shaped the cultural and economic landscapes of ancient Arabia, thereby painting a more comprehensive picture of their influence.

Keywords

Ancient Arabia, Biomolecular Archaeology, Metabolic profiling, Olfactory Culture, Aromatics

Introduction

Common perceptions of ancient cultures in Arabia are often closely linked to the burning of incense and the aromatic substances that moved along the ancient trade routes, such as the Incense Road (Groom 1981). In ancient Arabia, incense had important cultural, religious and commercial roles and a shared history of use by different groups and societies of the Peninsula (Baird 2021). These traditions continue to this day, with incense burners acting as a material manifestation of this legacy. The burners remain popular items in homes and places of worship, and significantly contributed to shaping local identity and collective practices over time (Baird 2021). In the past, incense smells were used for a variety of reasons, including religious ceremonies, to mask malodors, and to provide a pleasant fragrance (Theophrastus, *De Odoribus*; *The Book of the Dead*). Incense was also believed to have healing, purifying and disinfecting properties, and was applied for personal hygiene, to protect against diseases and to repel pests (Celsus, *De Medicina*; Dioscorides, *Materia Medica*).

Among the incense smells, frankincense was one of the most iconic scents of Arabia (Zimmerle 2021). The scent-intensive resin was obtained from wild shrub-like *Boswellia* trees by tapping into the bark of the trees to produce exuded gum resin. Frankincense played an important role in Arabia's economy and was a key commodity of the aromatics trade in the 1st millennium BCE (Avanzini 1997). The fragrant qualities of frankincense and other aromatics were highly desired by the elites in neighboring regions, e.g. in ancient Egypt, Mesopotamia, Greece and Rome. According to Pliny the Elder, incense, and especially frankincense was burned on a lavish scale by certain Roman Emperors (*Natural History*, bk. XII, ch. 41, 82–84), and also Neo-Assyrian kings, such as Esarhaddon and Tiglath-Pileser III obtained vast quantities of Arabian aromatics (Leichty 2011), which demonstrates the high demand for these substances and the large amounts that have been transported.

Frankincense was not the sole aromatic of cultural and economic significant in ancient Arabia, however. Indeed, the olfactory landscape of Arabia was very diverse with the introduction and use of a number of different aromatics, ranging from resins, balsams, scented woods, herbs, oils and spices (Regert et al. 2008; Mathe et al. 2009). For instance, South Arabian incense burners bear Sabaic inscriptions that can be interpreted as aromatic substances (Biella 1982; Nebes 2014). The inscriptions reveal the words *qsṭ* (likely Indian costus, an aromatic root), *rndm* (nard, an essential oil used as a perfume or incense), as well as the words *kmkm* and *ldn*,

associated with aromatic resins[8] (Biella 1982). Additional inscriptions on incense burners include aromatic names like *lbny* (possibly referring to frankincense or styrax) (Zimmerle 2021). These aromatics encompassed a broad range of commodities including cosmetics, medicines, food flavourings and products for sanitation and disinfection as well as ritual practices.

Although the Sabaic sources mention a variety of names for aromatic substances, accurately translating these plant names continues to be a challenge, as exemplified above. Most of these names can only be tentatively translated. Generally, ancient written sources, especially cuneiform and Egyptian texts, provide limited information on the exact botanical source of aromatic substances, making precise identification of the kind of incense materials difficult (Germer 2008; Böck 2011; Geller and Panayotov 2018). This is due to the fact that identifications of plants from ancient texts are mostly based on philology and ethno-comparisons, which can be misleading (Panayotov 2014; Pommerening 2016). Furthermore, vernacular nomenclature, especially for plants, is dynamic and, when transferred through languages, sometimes shift meaning, or entirely change over time and across regions (Scurlock 2012). As a result, although ancient texts provide valuable insight into the cultural perception and use of aromatics and smells, they often lack specificity regarding the actual aromatic substances. Thus, their limitations necessitate the use of complementary approaches for a more accurate reconstruction of the aromatic plants used. Furthermore, tangible evidence of aromatics in archaeobotanical assemblages is also rare, as burnt incense products leave almost no macroscopic traces (Scott et al. 2020).

Nevertheless, modern analytical methods have the potential to overcome the challenges in identifying aromatic substances (Huber et al. 2022a). Biochemical and biomolecular analyses can be employed in archaeology to explore and identify organic residues from archaeological artefacts. These analyses allow us to discern between different aromatics and give us hints as to how these plants were further processed. One way to target past use of aromatics is to specifically sample archaeological artefacts associated with the use of fragrant substances, such as incense burners. Traces of the burned aromatics can survive as residual crust on object surfaces and even as “invisible residues”, which are absorbed by the porous matrix of the

[8] The Sabaic Online Dictionary of the Friedrich-Schiller Universität Jena has been used for translations of sabaic names. http://sabaweb.uni-jena.de/Sabaweb/Suche/Suche: last access 16.10.2023

incense burner material (Evershed 2008; Roffet-Salque et al. 2017). However, to date, the majority of chemical analyses of organic residues has been done on food-related vessels and jars for unguents and medical remedies as well as on mummies rather than incense burners. Furthermore, these analyses mostly concerned ancient Egypt and Classical Antiquity (Colombini et al. 2005a; Charrié-Duhaut et al. 2007; Dunne et al. 2012; Giachi et al. 2013; Brettell et al. 2017; Evershed and Clark 2020). As a result, in Arabia, analytical studies of organic residues remain relatively rare.

In this paper, we study the diversity of aromatics used at the ancient oasis of Tayma, a large settlement from the Early Bronze Age onwards in NW Arabia, through metabolic profiling of organic remains within incense burners. Located on a branch of the Incense Road in an arid landscape, the oasis was a major hub of the caravan trade (Macdonald 1997; Hausleiter and Eichmann 2018) and an important intersection between the southern parts of the Arabian Peninsula, Egypt, the Levant, Assyria and Babylonia, as well as eastern Arabia and the Gulf regions (Hausleiter 2012) connecting producers and customers of aromatics. Throughout excavations at the site, numerous incense burners were discovered showing both traces of burning and residues of resinous substances on the interior (Huber 2020). Applying a multi-analytical approach, including gas chromatography mass spectrometry (GC-MS) and liquid chromatography tandem mass spectrometry (LC-MS/MS), we analyzed amorphous residues and material absorbed remains from these objects as well as the archaeological and palaeoenvironmental contexts of the site. These traces yielded an extensive data set for discussing aromatic diversity, trade, consumption and use of scented products.

Archaeological contexts and uses of incense burners at the oasis of Tayma

The Tayma oasis settlement (Fig. 1) was established by a sedentary group, which was in contact with surrounding mobile groups and spans over more than 9 square kilometres (Eichmann et al. 2006, 2010, 2011, 2012; Hausleiter 2011, 2014, 2019; Hausleiter and Eichmann 2018). The establishment of a substantial wall enclosure of the oasis during the 3rd millennium BCE (Hausleiter 2018) indicates the presence of a settlement of considerable size, which was permanently occupied over several millennia, from the 3rd millennium BCE onwards until modern times (Hausleiter and Eichmann 2018; Hausleiter 2019). The production of ceramics may have began in the late 4th / early 3rd millennium BCE (Tourtet et al. 2021; Huber

2020) and during the Late Bronze Age and Early Iron Age, the oasis might have also established its own metallurgical production (Liu et al. 2015). Archaeometallurgical studies of objects found at Tayma demonstrated long-distance contacts to Oman, as well as Cyprus to obtain raw materials, such as copper ores, suggesting a dynamic metal trade networks on the Arabian Peninsula during these times (Liu et al. 2015; Renzi et al. 2016). From the Early Iron Age onwards, a large irrigation system with several canals, basins and wells was constructed to supply the subsistence economy of the oasis with groundwater (Hausleiter and Eichmann 2018).

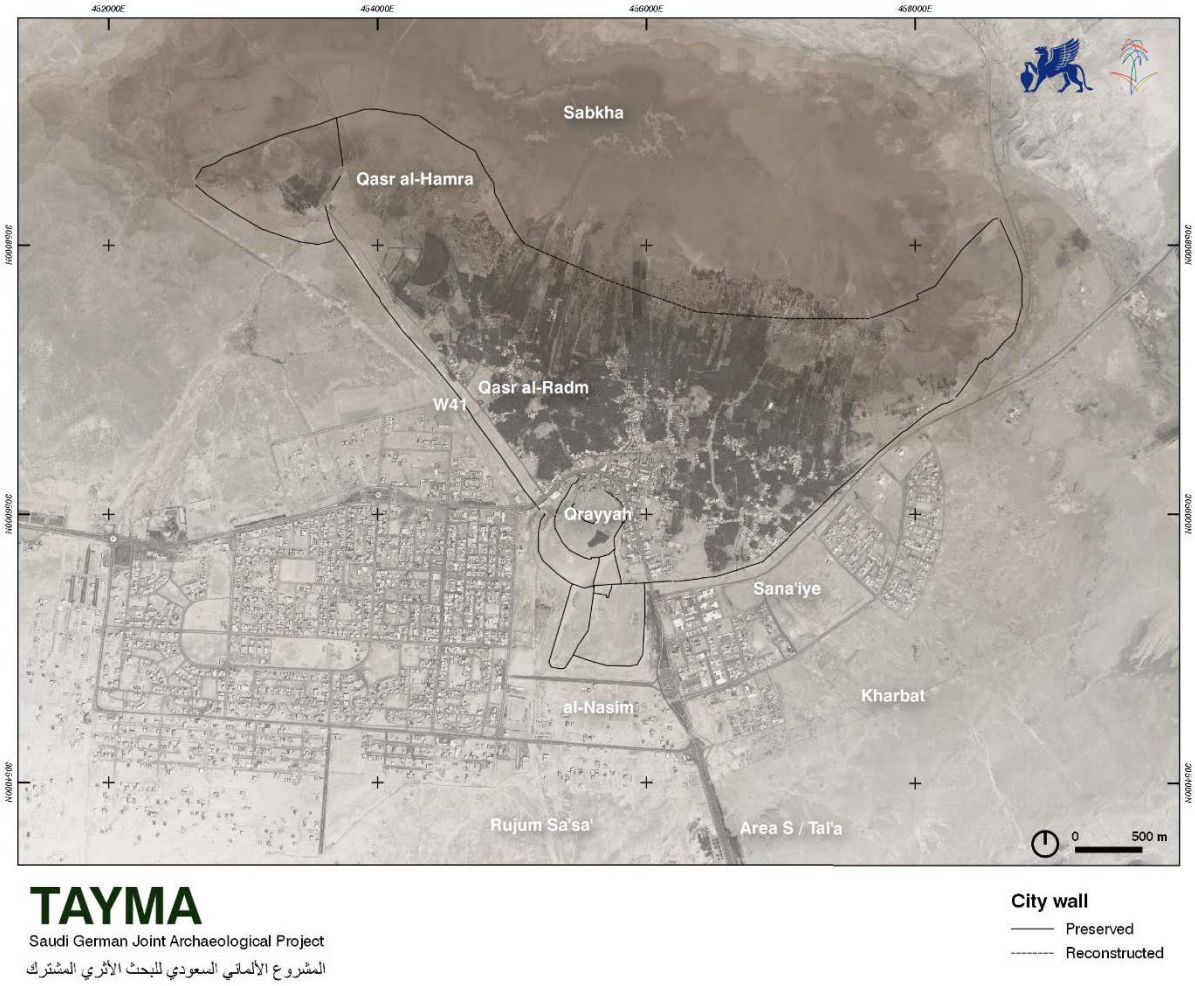


Fig. 1. The oasis settlement of Tayma enclosed by the city wall (map: DAI Orient Department, Sebastiano Lora; source: Huber 2020).

Evidence for the use of incense at the oasis of Tayma can be found in various different archaeological contexts inside and outside the enclosed oasis: in temples and representative, public buildings, in private houses and storage rooms at the domestic quarter and within the tombs in graveyards to the South of the settlement (Huber et al. 2018). Chronologically, the

earliest indicators of incense burning at Tayma trace back to the Middle Bronze Age (1st half of the 2nd millennium BCE), with continues evidence of incense burners until Late Antiquity (Fig. 2).

The two earliest incense burners at Tayma from the Middle Bronze Age come from different contexts at the settlement (Huber 2020). The first one was found within a tower of the western branch of the oasis wall, whose two building stages can be dated to the end of the first / beginning of the second half of the 2nd millennium BCE (Hausleiter 2014). The context of the second one remains undetermined, as discovered in secondary deposition. The burners are pottery vessels coated with a reddish slip, which was subsequently burnished (Tourtet et al. 2021; Huber 2020). These characteristics classify them as Red Burnished Ware (Supplementary Fig. S1).

Incense burners also have been found in public contexts, such as within a large Early Iron Age complex with a main temple building (O-b1) with adjacent rooms (Intilia 2011, 2012). In this complex, a high amount of luxury goods – many Egyptian imports such as faïence figurines of goddesses and an Udjat-Eye as well as metal objects, combs of bone and ivory and cowry-shells were found (Intilia 2011, 2012; Sperveslage 2013). The great amount of prestigious goods and the architectural layout of the whole complex, as well as the absence of domestic pottery, indicates a representative function of the entire complex (Huber 2020). The incense burners found within the temple in Area O are small goblets made of pottery with red and brown painted decorations on the outside (Supplementary Fig. S2). They have been made of a fine, mineral tempered fabric and are characteristic of the so-called Tayma Early Iron Age Ware (Tourtet et al. 2021).

An incense burner from the 1st millennium BCE was found within another temple at the oasis. This large building (E-b1), spanning roughly 500 square meters, was the main temple of the central area of Tayma (Lora 2017). It was most likely built in the 4th or 3rd century BCE. This temple saw significant changes during the Nabataean and Roman eras but was continuously used up until the Late Antiquity (Lora 2017). Within the temple, one artefact – a round stone vessel with three feet – was identified as an incense burner (Supplementary Fig. S3).

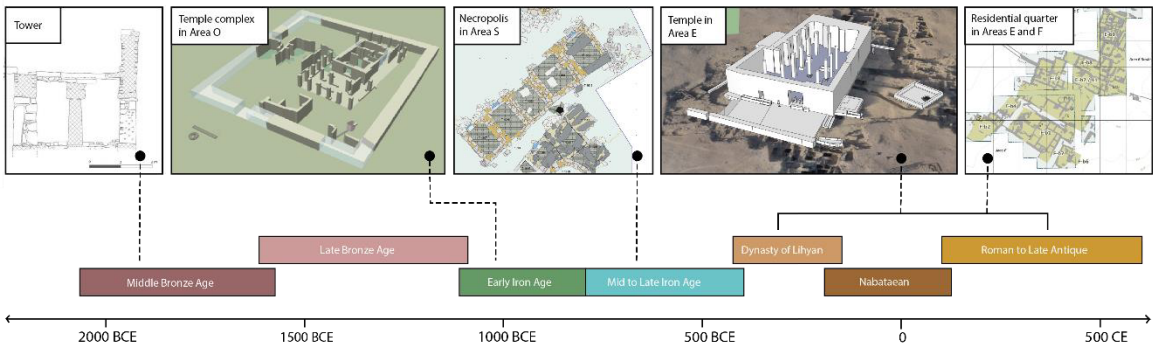
Furthermore, incense burners have also been discovered at the necropolis of Tal'a (Area S), located outside the enclosed oasis settlement of Tayma (Huber 2020). Within this burial ground, southeast of the settlement, 15 tombs have been excavated (Beuger 2010). At the

Tal'a necropolis, tombs were stone-built with entrances to the north-west and were capped with large stones, which often held multiple bodies. These burial site was used between the 9th and 5th centuries BCE (Lora et al. 2010; Petiti et al. 2014; Hausleiter and Zur 2016). Similar to the incense burners of the Early Iron Age, theses burners were pottery vessels with a painted decoration. These containers are conical cups, crafted on a potter's wheel, with flat, circular bases and are characterised by their painted decoration on pale clay. The style of decoration is called Sana'iye Painted Ware (Tourtet et al. 2021), consisting of dark brown and red geometric motifs (Supplementary Fig. S4).

Moreover, incense burners were unearthed in the residential area, situated to the south of temple E-b1, which was inhabited from the latter part of the 1st millennium BCE to the Early Byzantine period (Weigel 2019, 2020). This quarter, covering around 1,600 square meters, showcases dense clusters of multi-storeyed houses and tight alleyways. The houses consisted of numerous small storage niches and spacious chambers (Tourtet and Weigel 2015; Weigel 2019, 2020). The majority of incense burners found in the residential quarter are small, cuboid containers with four feet, which were carved from sandstone blocks (Huber 2020; Supplementary Fig. S5). However, other designs, such as triangular vessels and more intricate structures like shaft-burners with decorative "crowns" and inscribed variants, were also uncovered (Supplementary Fig. S6-S7).

In order to obtain a chronological sequence of the used scented substances at Tayma and to map the contextual distribution of aromatics at the site, 33 incense burners have been selected for analysis from public, funerary and domestic contexts, ranging from the Middle Bronze Age to Late Antiquity (see Fig.2). To identify the aromatics which created scents in the past, organic residues from both the 33 ceramic and sandstone incense burners were collected for analysis. Additionally, 8 control samples, taken either from the exterior parts of the incense burners (e.g. from foots or stands) or from soil samples were prepared to monitor contamination.

A



B

Dating	Period	Context	Area, buildings	Incense burner material	Incense burner shape	No. of burners	No. of samples analysed
Mid - 2nd mil. BCE	MBA-LBA	Public, representative and temple contexts	Tower Area E-East	Pottery	Goblet Round vessel	1 1	1 1
12th – 9th cent. BCE	EIA		Area O	Pottery	Goblets	7	7
2nd half 1st mill. BCE – L. Antiquity	Dynasty of Lihyan, Nabataen, Roman		Temple E -b1	Sandstone	Round vessel	1	1
9th – 5th cent. BCE	MIA-LIA	Funerary contexts	Necropolis of Tal'a (Area S)	Pottery	Conical cups	10	7
2nd half 1st mill. BCE – L. Antiquity	Dynasty of Lihyan, Nabataen, Roman	Domestic context	Houses and storage rooms (Areas E-South /F)	Sandstone	Cuboid burners	13	8
					Burners with 'crowns'	4	2
					Rectangular burners with inscriptions	4	4
					Triangular containers	4	2

Fig. 2. Contexts, dating, materials and shapes of incense burners at Tayma as well as number of analysed samples.

Metabolic profiling of ancient organic residues

Given that accurately identifying ancient resins based on macroscopic observations is unfeasible, biomolecular analyses were carried out to characterize the organic residues of the sampled artefacts. Resins, gums and balsams are non-cellular natural plant exudates, which are produced to protect trees or shrubs from excessive water loss when they are damaged, e.g. through tapping (Langenheim 2003). From the secretory structures of the tree, such as resin ducts, a sticky substance is released, which seals the wound of the plant and serves as a barrier against herbivores and pathogens, and can also contain bioactive compounds, which help to prevent infections (Pollard and Heron 2008). These natural plant products contain numerous of plant secondary (or specialized) metabolites (PSMs), which are small molecules that are in some cases taxon specific, making them diagnostic biomarkers for plant identification (Singh 2016; Lukin et al. 2018; Hussein and El-Anssary 2019). Among the PSMs, terpenoids are typical compounds in resins. Metabolic profiling can identify the PSMs present

in biological samples, such as resins, and involves the identification and quantification of these compounds using analytical techniques such as gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) (Colombini and Modugno 2009).

The molecular composition of resins can vary widely between different plant genera and even between different species (Brettell et al. 2017). Chemical characterization the different metabolite profiles of certain resins can be used to identify and discern different aromatic substances (Evershed 2008; Devièse et al. 2017). For example, boswellic acids and their derivatives are highly characteristic markers for *Boswellia* plant exudates and are relatively resistant to decay (Evershed et al. 1997; cf. van Bergen et al. 1997; Culioli et al. 2003; Mathe et al. 2004; Regert et al. 2008; Ribechini et al. 2008; Paul 2012). They can, therefore, be considered as characteristic compounds for identifying archaeological frankincense. However, despite the source-diagnostic properties of PSMs, not all biomarkers of modern plants can withstand millennia of taphonomic alteration. Therefore, when analyzing archaeological residues, aspects such as degradation and chemical alteration over long timespans need to be considered as well (Huber et al. 2022b). Furthermore, the process of burning incense leads to oxidation reactions, the production of pyrolysis products and the evaporation of volatile compounds (Niebler and Buettner 2016; Niebler et al. 2016).

In order to screen for different incense raw materials, we created a database, consisting of biomarkers that are representative for certain natural products commonly used as incense materials in the past and possess high chemical stability. The database was composed based on our investigation of modern botanical reference samples as well as on a survey of the literature of previous studies (see Huber et al. 2023 Supplementary Table S3 for list of all compounds, and Table S4 for MRM parameters). Analytical standards of these characteristic compounds have been obtained and were used to create the LC-MS/MS method in Multiple Reaction Monitoring (MRM) mode to screen for the presence of these compounds in archaeological samples (see method section). MRM is a targeted analytical mode of operation that is a highly sensitive and specific and allows for the detection of low levels of analytes in complex samples, as is the case for most archaeological samples. Since this approach only screens for target compounds from the database, we additionally carried out LC-MS and GC-MS analyses in full scan mode (a non-selective mode), which record all compounds present in the samples, although with lower sensitivity.

Results of the metabolic profiling of incense residues

The analysis of the two earliest incense burners, dating back to the Middle Bronze Age, showed the presence of organic matter. However, only the sample from the incense burner discovered in the tower (DA-TA-26) yielded aromatic compounds upon analysis. The LC-MS/MS results of sample DA-TA-26 showed a high abundance of diterpenoids, predominantly 7-oxo-dehydroabietic acid and, to a lesser extent, dehydroabietic acid and resin acids (pimaric, isopimaric, palustric and neoabietic acids; see Fig. 3A). These resin acids possess similar structures and ionization behaviours, making them difficult to identify separately and, thus, we grouped them as "resin acids". All of the abovementioned compounds are diagnostic for coniferous plant products, particularly for pine, spruce, cedar, juniper, larch and fir resin (Sato et al. 2009; Pekgozlu et al. 2017; Salomé-Abarca et al. 2018). Apart from the terpenoids, the phytosterols β-sitosterol and campesterol were also detected in the sample. Phytosterols (or plant sterols) are compounds that naturally occur in numerous plants, meaning they are considered clear markers for the presence of plant material (Gylling and Simonen 2015). However, due to their widespread occurrence in the plant kingdom, phytosterols are rather generic and non-diagnostic compounds. Furthermore, small amounts of the aromatic compounds benzoic and vanillic acid, which also occur in many aromatics and balsams, were detected in the sample (Tchapla et al. 2004; Riesmeier et al. 2022). In this case, however, these compounds are most likely formed as by-products of combustion during the burning of incense (Tamburini et al. 2016).

The sample from the second Middle Bronze Age burner (DA-TA-03) did not show any of the characteristic compounds associated with coniferous resin, but results from the GC-MS analysis showed a large amount of saturated straight chain fatty acids, predominantly palmitic acid (C16:0), stearic acid (C18:0) and myristic acid (C14:0), as well as the odd-carbon-numbered components pentadecanoic acid (C15:0) and heptadecanoic acid (C17:0), which are compounds found in degraded oils and fats (Fig. 3B). Additionally, the LC-MS/MS results detected several phytosterols, such as campesterol, β-sitosterol, and stigmasterol as well as the compounds cholesterol and cholestanol, confirming the presence of plant oils and fats (Evershed et al. 2002; Gylling and Simonen 2015). The absence of any aromatic markers in this sample implies that this object may have contained only oils and fats. However, it is important to consider the possibility that aromatic substances may have been added to oils in the past,

but their presence could have gone undetected due to lack of preservation. Therefore, the molecular compositions of incense materials in the Middle Bronze Age at Tayma indicate the use of coniferous resins as well as a blend of plant oil and animal fat (with the possibility of additional additives).

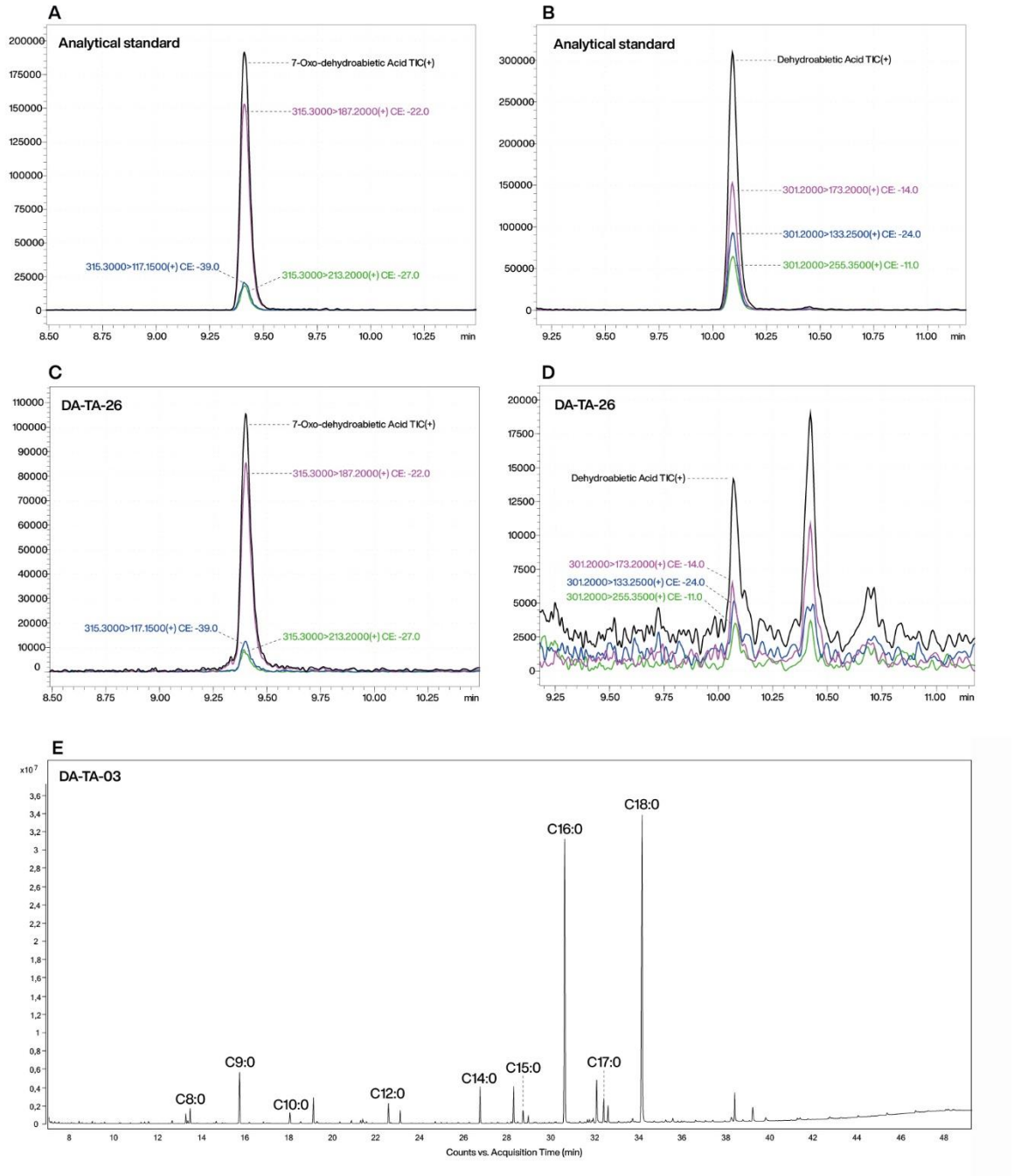


Fig. 3. Multiple reaction monitoring (MRM) HPLC chromatograms of the analytical standards 7-oxo-dehydroabietic acid (A) and dehydroabietic acid (B) compared to the presence of these compounds in the archaeological sample DA-TA-026 from the MBA incense burner (C, D). TIC Chromatogram of sample DA-TA-03 containing saturated fatty acids (E).

The results from the incense burners found within the Early Iron Age complex in Area O demonstrate the use of other scented resinous substances. The samples (*n*=7) taken from the small goblets inside and outside the public building O-b1 all showed a similar molecular composition. Several triterpenoids were detected in the samples through LC-MS/MS and GC-MS analysis, notably moronic, oleanoninc, oleanolic and betulinic acids as well as masticadienolic acid and its related derivatives. Furthermore, the compounds β-amyrin, lupeol, dipterocarpol and 28-norolean-17-en-3-one were present in the sample in small abundances (Fig. 4). These compounds are characteristic biomarkers for the resin of *Pistacia* species (Assimopoulou and Papageorgiou 2005; Sharifi and Hazell 2011; Xynos et al. 2018) and have been reported in previous studies of organic residues, for example in ancient Egyptian vessels from Amarna (Serpico and White 2000; Stern et al. 2003). Based on this evidence, we can unambiguously identify the incense material of the Early Iron Age incense burners as resin of *Pistacia* trees. Moreover, the presence of the compound 28-norolean-17-en-3-one indicates that the resin was heated (Stern et al. 2003), confirming the burning of the resin.

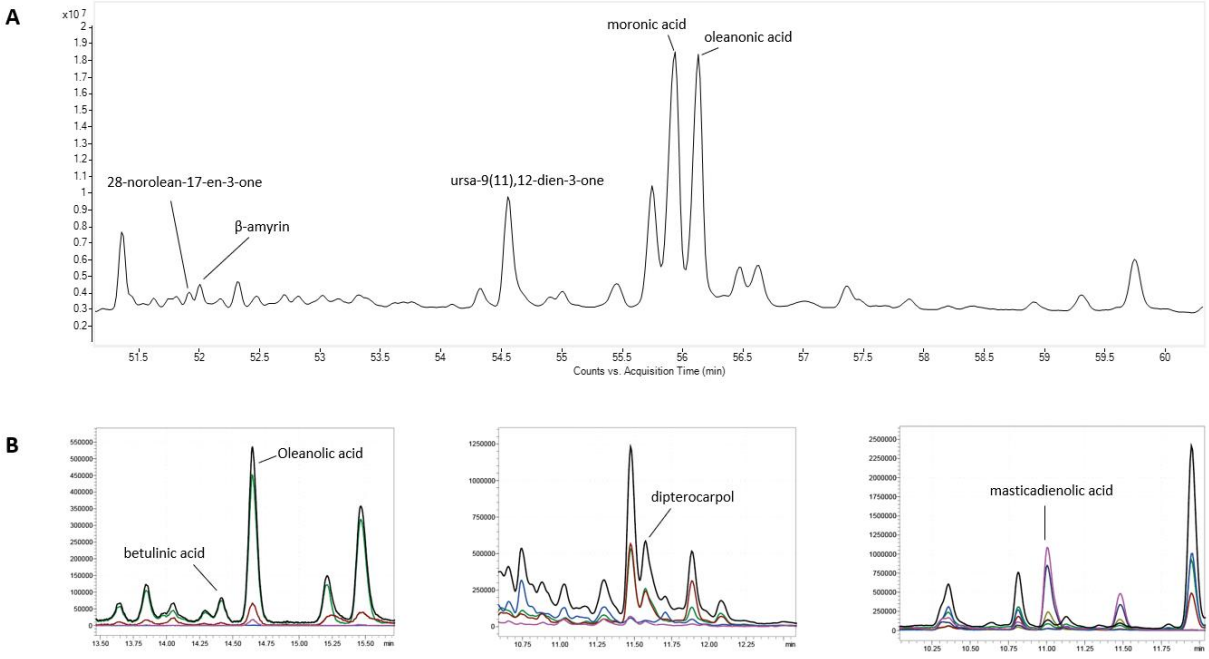


Fig. 4. (A) Partial TIC chromatogram of the archaeological sample DA-TA-16, showing characteristic compounds of Pistacia resin. (B) MRM HPLC chromatograms of compounds present in sample DA-TA-16.

In contrast to Area O, samples collected from incense burners inside the tombs of the necropolis (*n*=7) exhibited a greater diversity of substances due to the use of aromatic blends. The LC-MS/MS results revealed that certain compounds were present in all samples,

originating from a main ingredient of the incense mixture, while others were only present on an occasional basis. All samples contained high abundances of the triterpenoids α-amyrin and β-amyrin/lupeol, as well as a number of sterols and stanols. Furthermore, the GC-MS results revealed the presence of additional compounds, notably the sesquiterpenes β-eudesmol, as well as epi-bicyclosesquiphellandrene. Although these individual compounds can naturally occur in a number of different plants, taken together, all of these compounds haven been reported in studies of *Commiphora* species (for example myrrh or bdellium), which could be a possible source of the main ingredient (Marcotullio et al. 2009; Batiha et al. 2023). To further test this hypothesis, we additionally compared the molecular profiles of the archaeological sample with different modern resin reference samples. Direct comparison with modern *Commiphora* species (*C. opobalsam* and *C. myrrha*) showed a high correspondence of peak distribution, retention time and fragmentation patterns. Due to the presence of *Commiphora* markers and good matching with *Commiphora* reference profiles, the main ingredient in the incense blends is identified as a *Commiphora*-type resin.

In some samples taken from the graveyard, the identification of additional compounds was made. Specifically, samples DA-TA-24 and DA-TA-25 contained moronic and oleanonic acids, which are, as mentioned above, typical triterpenoids found in *Pistacia* resin. In contrast, samples DA-TA-30, DA-TA-31 and DA-TA-04 contained dehydroabietic acid, 7-oxo-dehydroabietic acid, and resin acids, which are characteristic of coniferous resin. Sample Ta-DA-04 additionally contained the phytosterol brassicasterol, which occurs in oils of the Brassicaceae family, also known as cruciferous vegetables, and likely derived from seeds of Brassicaceae plants (Colombini et al. 2005b; Marković et al. 2022). Based on these results, it can be concluded that the Tayma community used either *Commiphora* resin alone or a mixture of *Commiphora* with *Pistacia* or coniferous resin for funerary purposes in order to pay homage to their ancestors, and in one case with a further addition of Brassicaceae seed oil.

Finally, the results from samples collected from the residential area as well as the temple E-b1 from the 2nd half of the first millennium showed clear evidences for the use of frankincense. The analyses revealed highly-specify markers for *Boswellia* plant exudates, including 24-norursa-3,9(11),12-triene, 24-noroleana-3,12-diene, 24-norursa-3,12-diene and 24-Norursa-3,12-dien-11-one, which were the major constituents in the profiles (Fig. 5). These compounds are thermal decomposition products (pyrolysates) of α- and β-boswellic acids and derivatives

(van Bergen et al. 1997; Baeten et al. 2014; Ren et al. 2022), which were present in small amounts in the samples as well. Apart from the boswellic acids, the compounds α-Amyrenone, α-Amyrin, lupeol and insensole were also detected (the latter only in two samples, however). The chemical composition of these profiles shows clear evidence for the use of frankincense and the 24-nor-pyrolysates provide additional information, demonstrating that the resin was burned. Frankincense was detected in cuboid containers, in incense burners with inscriptions as well as in shaft-burners with “crowns”.

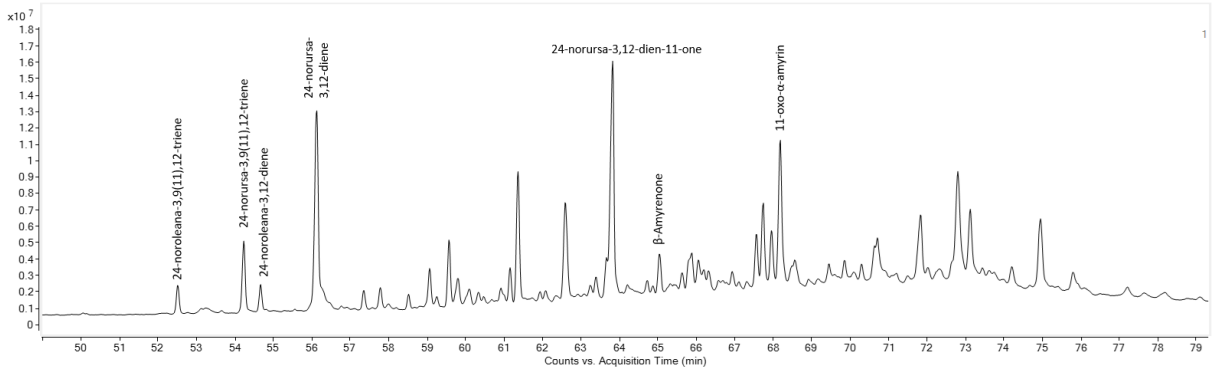


Fig. 5. Partial TIC of sample DA-TA-45 with the identified pyrolysates of boswellic acids and other markers characteristic for Boswellia plant exudates.

The systematic analysis of incense burners from the residential area also included the examination of samples taken from small, triangular sandstone containers with carbonized traces on the surface, designated as DA-TA-52 and DA-TA-56. These particular artifacts also underwent testing to check for aromatic compounds. The chemical profiles, however, identified only significant concentrations of phytosterols, cholesterols, and cholestenol. The substantial presence of these lipid compounds demonstrates that the contents of these containers were predominantly composed of plant-derived oils and fats. The absence of aromatic residues and the lipid composition strongly suggest a functional reassignment of these objects, leading to the conclusion that they were more likely utilized as lamps rather than incense burners.

In summary, out of the 33 analyzed incense burners, 23 clearly contained incense materials, 3 exhibited only plant oils and fats, and in the remaining 7, no aromatic substances were detected (see Table 1). None of the control samples contained traces of incense. However, some of the controls revealed the presence of plasticizers, such as phthalates, indicating plastic contamination. Importantly, these modern contaminants can be clearly differentiated from ancient organic remains.

Table 1. Archaeological samples of organic residues from incense burners and identified substances.

Sample no.	Lab no.	Period	Material of burner	Archaeological context	Identification
TA 18902	DA-TA 02	EIA	Pottery	Public context	Pistacia
TA 18903	DA-TA 03	MBA	Pottery	unknown	Plant oils and fats
TA 18904	DA-TA 04	MIA/LIA	Pottery	Funerary context	Commiphora mix
TA 18905	DA-TA 05	MIA/LIA	Control	Funerary context	Control sample
TA 18908	DA-TA 08	EIA	Pottery	Public context	Pistacia
TA 18909	DA-TA 09	EIA	Pottery	Public context	Pistacia
TA 18910	DA-TA 10	EIA	Pottery	Public context	Pistacia
TA 18911	DA-TA 11	EIA	Control	Public context	Control sample
TA 18914	DA-TA 14	EIA	Pottery	Public context	Pistacia
TA 18916	DA-TA 16	EIA	Pottery	Public context	Pistacia
TA 18917	DA-TA 17	EIA	Control	Public context	Control sample
TA 18921	DA-TA 21	EIA	Pottery	Public context	x
TA 18924	DA-TA 24	MIA/LIA	Pottery	Funerary context	Commiphora mix
TA 18925	DA-TA 25	MIA/LIA	Pottery	Funerary context	Commiphora mix
TA 18926	DA-TA 26	MBA/LBA	Pottery	Tower	Coniferous resin
TA 18927	DA-TA 27	MBA/LBA	Control	Tower	Control sample
TA 18928	DA-TA 28	MIA/LIA	Pottery	Funerary context	Commiphora
TA 18929	DA-TA 29	MIA/LIA	Pottery	Funerary context	Commiphora
TA 18930	DA-TA 30	MIA/LIA	Pottery	Funerary context	Commiphora mix
TA 18931	DA-TA 31	MIA/LIA	Pottery	Funerary context	Commiphora mix
TA 18937	DA-TA 37	MIA/LIA	Control	Funerary context	Control sample
TA 18938	DA-TA 38	Late Roman	Sandstone	Domestic context	x
TA 18940	DA-TA 40	Late Roman	Sandstone	Domestic context	x
TA 18941	DA-TA 41	Late Antiquity	Sandstone	Domestic context	Boswellia
TA 18942	DA-TA 42	Late Antiquity	Sandstone	Domestic context	x
TA 18944	DA-TA 44	Late Antiquity	Sandstone	Domestic context	x
TA 18945	DA-TA 45	Late Roman	Sandstone	Domestic context	Boswellia
TA 18946	DA-TA 46	Late Roman	Sandstone	Domestic context	Boswellia
TA 18947	DA-TA 47	Late Roman	Control	Domestic context	Control sample
TA 18948	DA-TA 48	Late Roman	Sandstone	Domestic context	Boswellia
TA 18949	DA-TA 49	Late Roman	Control	Domestic context	Control sample
TA 18950	DA-TA 50	2nd half 1st mill.	Sandstone	Temple E-b1	Boswellia
TA 18952	DA-TA 52	Late Antiquity	Sandstone	Domestic context	Plant oils and fats
TA 18956	DA-TA 56	Late Antiquity	Sandstone	Domestic context	Plant oils and fats
TA 18960	DA-TA 60	Late Roman	Sandstone	Domestic context	x
TA 18961	DA-TA 61	Nabataean	Sandstone	Domestic context	x
TA 18962	DA-TA 62	Nabataean	Sandstone	Domestic context	Boswellia
TA 18963	DA-TA 63	Nabataean	Sandstone	Domestic context	Boswellia
TA 18964	DA-TA 64	Nabataean	Sandstone	Domestic context	Boswellia
TA 18965	DA-TA 65	Nabataean	Sandstone	Domestic context	Boswellia
TA 18968	DA-TA 68	Nabataean	Control	Domestic context	Control sample

Discussion

Our results demonstrate the use of several incense materials over time at the oasis of Tayma, including coniferous resins, plant exudates of *Pistacia* trees, frankincense, a *Commiphora*-type resin as well as mixtures of different aromatic substances. Connecting these results with the

archaeological context, a chronological and contextual pattern of aromatic use was observed (Fig. 6). Regarding the chronology of aromatic substances at Tayma, the earliest incense burned in the Middle Bronze Age was coniferous resin, while *Pistacia* resin was the only incense employed in the Early Iron Age. During the Mid-to Late Iron Age, the application of aromatic blends started, with Commiphora as the main ingredient and the occasional addition of *Pistacia* and conifer exudates. Interestingly, frankincense was only detected in the archaeological samples from the second half of the 1st millennium BCE, and from then onwards, it seems to have been utilized exclusively.

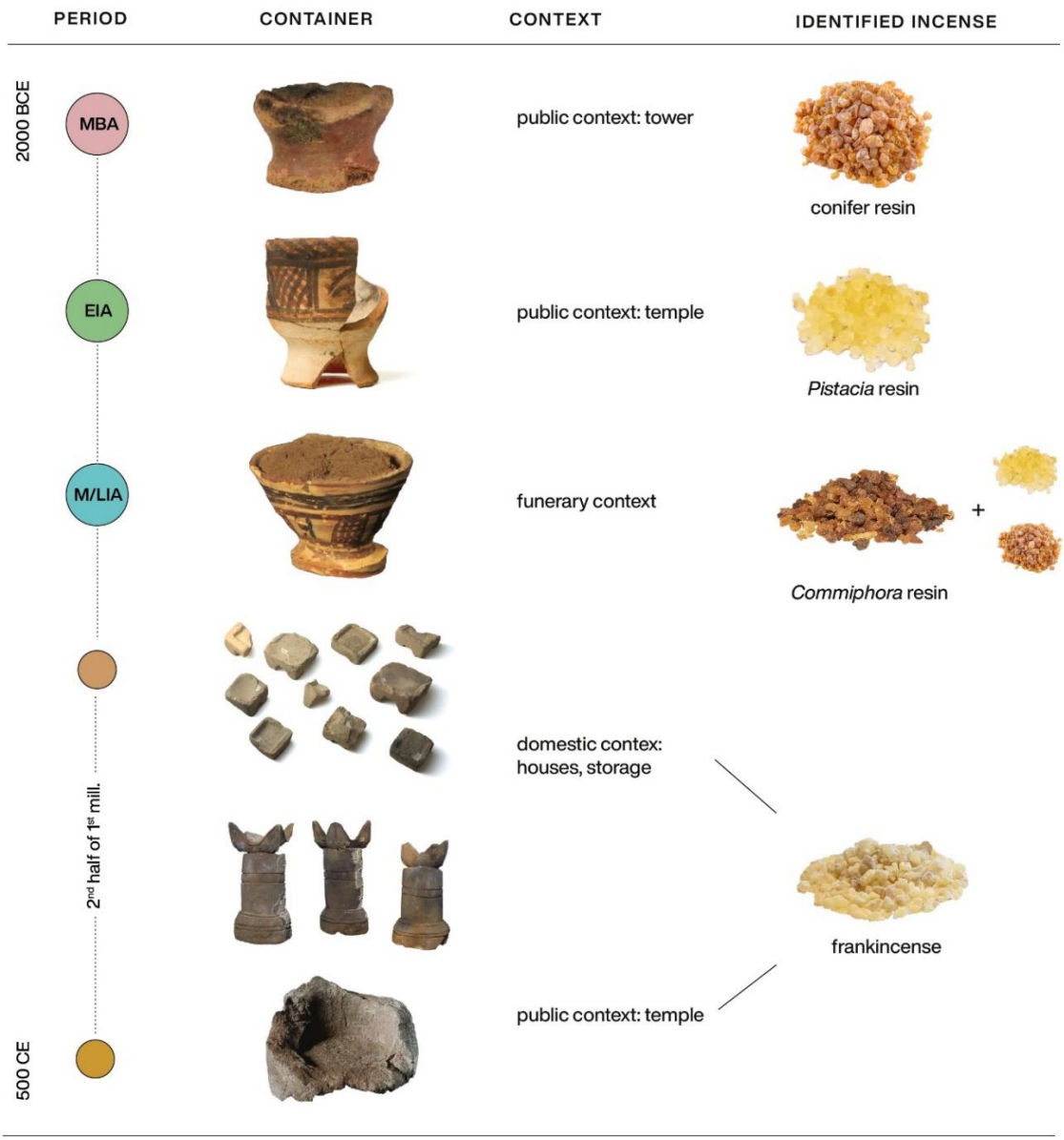


Fig. 6. Summary of aromatic substances discovered in different contexts at Tayma from the Middle Bronze Age to Late Antiquity.

The spatial distribution highlights the diversity of aromatic expression at the community level, as revealed through smells whose functions varied based on different contexts. For example, *Commiphora* was used specifically at the graveyard for burial practices, and was not found at other contexts at the oasis. Occasionally, other resins were mixed into blends for funerals as well. Regarding the purpose of use, the scent of burning *Commiphora* resin is clearly associated with funerary rituals at Tayma. In this context, this particular scent could have been used as an olfactory sign of mourning and memory of the deceased (Huber 2020). Olfactory stimuli have a strong impact on our memory and emotions due to the connection between the olfactory organ and the amygdala-hippocampus complex in our brain (Zald and Pardo 1997; Herz 2016). As such, odors can serve as effective contextual memory cues that can help a community remember and stay emotionally connected to their ancestors (Herz and Engen 1996; Clancy 2019). Additionally, *Commiphora* resin was likely chosen for funerary purposes due to its strong antibacterial and antifungal properties, as well as its ability to cover or reduce the odor of decomposed bodies.

Another significant finding from the analyses is that the residents of Tayma exclusively burned frankincense in the domestic quarter. Given that these buildings often had storage compartments, one possible reason for burning frankincense was to protect stored goods from pests and vermin. Classical authors have noted the disinfectant properties of frankincense (cf. Cato, *De Agri Cultura*; Celsus, *De Medicina*), which was not only burned to keep pests away but also used for hygienic and sanitary purposes in everyday life. The findings also suggest that the residents of Tayma acquired frankincense for their personal use, indicating that the settlement was not merely a transit point on the incense trade route but an active consumer. The majority of the burners from the private houses date back to the Nabataean and Roman periods, when frankincense was was exceedingly costly according to contemporary Roman writers (cf. Pliny, *Natural History*, chapt. 37, 41). It can be inferred that the inhabitants could afford to purchase precious goods or wanted to participate in the use of goods associated with the elite. While there is no further information on the social status of the residents, they were assumingly wealthy enough to obtain luxury goods. However, frankincense purchased in Tayma was probably less expensive than in Rome, given that *Boswellia* resin was described as costly due to tolls paid along the road upon arrival in the

Mediterranean (Erickson-Gini and Israel 2013; Nebes 2014). Over a shorter distance, fewer tolls would have been paid, potentially making the product less expensive.

The public and temple contexts at Tayma displayed the greatest diversity in terms of the use of aromatics. During the second half of the 1st millennium BCE, frankincense was burned in the main temple, while all samples from the earlier temple context in the Early Iron Age contained *Pistacia* resin, and a coniferous substance was used in the tower. However, it appears that the application of specific scents in public contexts was more dependent on the availability of incense materials at certain times as on contextual preferences. Pollen studies conducted on the ancient salt lake (sabkha) at Tayma indicated the presence of *Pistacia* trees in the region (Dinies et al. 2015, 2016), suggesting that the incense used in the temple probably came from local trees (Fig. 7). At end of the second millennium, foreign imports of incense may not have been that common, but local resources may have been utilized. Subsequently, during the first millennium, as the aromatics trade significantly grew and frankincense became more available and perhaps fashionable, it was preferred as temple incense.

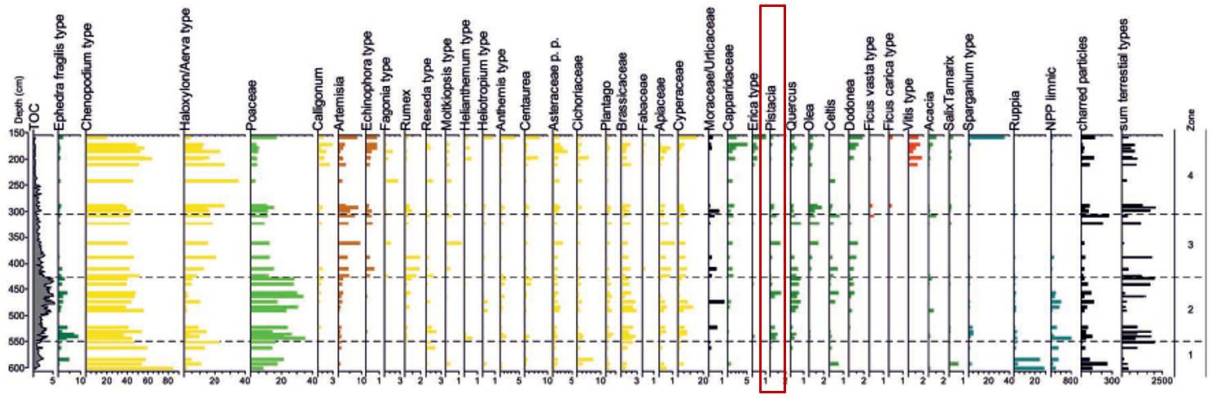


Fig. 7. Diagram showing selected pollen types (percentages calculated against the sum of terrestrial pollen types), TOC and C/N. Pistacia pollen are highlighted in red (source: Dinies et al. 2016).

The question of the origin of natural resources and availability also links aromatics with ancient trade networks beyond the immediate local area, however. Cultural contacts and exchange between Tayma and its neighboring regions are confirmed by archaeological evidence, such as bronze weapons of Syro-Levantine type (al-Hajiri 2011; Hausleiter and Zur 2016) or small faïence figurines of the Egyptian goddesses Isis and Bastet/Sakhmet (cf.

Sperveslage 2013 for the Egyptian influence on Tayma). The presences of incense in the oasis is another indication for trading activities, most likely with South Arabia or eastern Africa, the regions where *Boswellia* and *Commiphora* trees grew. *Boswellia*, a genus of the family of the Burseraceae, is not endemic to North Arabia. It is a typical Afro-Indian plant that grows in arid regions of northeastern Africa and mountain regions from southern Arabia and southeast Asia (Langenheim 2003). While the species *Boswellia sacra* grows mainly in South Arabia (Yemen, Oman) and *B. Serrata* in India, *B. papyrifera, B. neglecta and B. frereana* are species distributed across East Africa (Eritrea, Ethiopia, Sudan, Chad, Somalia, Kenia) (Bongers et al. 2019).

Frankincense began appearing at Tayma in the latter half of the 1st millennium BCE, coinciding with the flourishing South Arabian incense trade (Zimmerle 2021), suggesting its source was likely Yemen or Dhofar, Oman. However, trade also linked ancient Arabia with the Horn of Africa, especially Ethiopia, during this period (Nebes 2014). The influential Kingdom of Saba, primarily located in present-day Yemen, extended its reach to Oman and Ethiopia, with cultural exchanges evidenced by Sabaic inscriptions found in Ethiopia's Tigray region (Japp et al. 2011). This trade was facilitated by the strategic locations adjacent to the Red Sea. Consequently, African *Boswellia* species should also be considered as potential sources too. The *Commiphora*-type resin, identified at Tayma during the mid-to-late Iron Age, might also originate from this area or the broader Horn of Africa region. However, given the vast number of *Commiphora* species (over 200) and their wide distribution from Africa to the western Indian Ocean islands (Langenheim 2003), pinpointing the exact source of the resin remains challenging.

The final type of resin identified at Tayma originates from coniferous trees. Although only identifiable to the family level (Pinaceae) without a specific genus, it is clear that none of the family's genera, like cedar, pine, fir, larch, or spruce, are indigenous to the Tayma area (Farjon and Filer 2013). These trees are mainly found in the Mediterranean, strongly suggesting the resin was transported southward to Tayma along the Incense Road. This find re-evaluates the standard understanding of the incense traded on this route, typically believed to be primarily frankincense and myrrh (Groom 1981), and prompts a reconsideration of trade route directionality. The common view is of aromatic trade moving from South Arabia northward, with little insight into what was traded back.

The findings of this study have revealed the significance of olfactory perception in the ancient oasis of Tayma, a site sitting at the intersection of main trade routes of early scent trade. We demonstrated the use of different resins in various contexts over 2000 years, offering a chronology of aromatic use from the Middle Bronze Age to the Roman Period in Arabia at a site-specific scale. Despite the elusive nature of odours in the archaeological record, it appears that the scent of a place was crucial to the practices, behaviours, and perceptions of the people of Tayma and that aromatics were important trade commodities for ancient economies in Arabia (Nebes 2014; Zimmerle 2021). While the incense road may no longer exist, its legacy and cultural heritage endures through the continued consumption of incense worldwide. Moreover, this study unveils the nuanced evolution of incense use, both temporally and spatially, illuminating local practices that ancient textual sources, typically dominated by accounts of Greek and Roman trade interests, often overlook. This study has also demonstrated the prolonged practice of burning aromatics in an Arabian oasis, well before the extensive trade between South Arabia and the Mediterranean region (Macdonald 1997; Erickson-Gini and Israel 2013; Nebes 2014), highlighting the longevity and cultural embeddedness of this aromatic consumption.

Material and Methods

Sampling:

At Tayma, organic residues were found to have survived in various forms within incense burners: as fills within vessels, as visible residues on the interior surface of objects (i.e. crusts), and as materials absorbed within the porous matrix of the objects. To investigate these residues, we collected samples at the Museum of Tayma's conservation laboratory using three different methods. The first involved mechanical removal of visible crusts using a scalpel, yielding approximately 100-200 mg of material in each case. The second method involved drilling into the surface of the vessels to obtain approximately 2 g of powder from the absorbed residues. The third method involved direct solvent extraction of absorbed residues from the object surfaces, which was used when drilling was not feasible or permitted.

The incense burners were prepared for sample extraction by ensuring that any exogenous contamination was eliminated from their surfaces. Therefore, a thin surface layer was meticulously removed on the sampling spot of each object using a modelling drill.

Subsequently, samples were taken for analysis from these cleaned sampling spots. A Dremel 200 drill, equipped with either a tungsten abrasive bit or a diamond grinding bit, was employed for the destructive sampling of the pottery or stone objects, respectively. In between sampling, methanol was used to thoroughly clean the drill bits to avoid cross contamination. In most cases, an area of approximately 1 x 2 cm was drilled into the object to a depth of 2-3 mm. The powder was deposited onto aluminium foil and subsequently transferred into solvent cleaned glass vials and further processed at the laboratories of the Technical University Berlin (TUB) and the Max Planck Institute of Geonathropology (MPI-GEA).

Materials:

All solvents used for analysis were of analytical grade (GC- or HPLC grade). Dichloromethane (DCM) and methanol (MeOH) were purchased from Sigma-Aldrich (Munich, Germany), acetonitrile (ACN) and ultrapure water from Biosolve (Valkenswaard, Netherlands) and formic acid (FA) from VWR (Leuven, Belgium). The analytical standard isopimaric acid, α- and β-amyrin, α- and β-boswellic acids, lupeol and incensole were procured from Sigma-Aldrich (Munich, Germany), 7-oxodehydroabietic acid and α-Amyrenone from Campro Scientific (Berlin, Germany), dehydroabietic acid from Carbosynth (Berkshire, UK), and pimaric acid from Abcam (Berlin, Germany). Furthermore, palustric acid was purchased from Toronto Research Chemicals (Toronto, Canada), neoabietic acid and oleanonic acid from Santa Cruz Biotechnology (Heidelberg, Germany), moronic acid from TCI chemicals (Eschborn, Germany), coumarin was obtained from LGC Standards (Wesel, Germany), and benzoic acid from Agilent Technologies (Frankfurt, Germany).

Extraction and Analysis

Sample material that was not already in powder form (obtained through drilling), was homogenized into a fine powder using mortar and pestle. Solvent extraction was conducted according to established protocols (Craig et al. 2011; Dunne et al. 2016) using methanol (MeOH) for LC-MS/MS analysis and a DCM:MeOH (9:1, v/v) mixture for GC-MS analysis, followed by 15 minutes of ultrasonication and centrifugation to separate the solution from the solid material. To enhance the extract concentration, the extraction process was repeated three times. The extracts for GC-MS analysis were derivatized with 100 µL of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA, containing 1% TMCS, Sigma-Aldrich) for 60

minutes at 70 °C. Control samples from outside the artefact and method blanks were prepared using the same protocol.

Gas chromatography-mass spectrometry (GC-MS) analyses were conducted using an Agilent 8890 GC-System coupled to an Agilent 5977B GC-MSD at the MPI-GEA. Samples were injected onto a HP-5ms capillary column (Agilent, 60 m x 250 µm, film thickness of 0.25 µm). Helium was used as the carrier gas with a constant flow rate of 1.0 mL/min. The mass spectrometer was operated in electron impact (EI) mode at 70 eV. Initially, the GC oven temperature was set to 50°C for 2 min, then increased to 120 °C at a rate of 30°C/min and held for 2 min. It was then increased at a rate of 15° C/min to 200 °C and at a rate of 2° C/min to 320 °C with a final hold time of 10 min. The transfer line and ion source temperature were set at 250 °C and 230 °C, respectively. 1µL of sample was injected at a split ratio of 10:1. Data was recorded in full scan from *m/z* 50 to 700 after a solvent delay of 6,5 minutes. Injection blanks were carried out between each sample to prevent carryover.

LC-ESI-MS/MS analysis was performed on an Agilent Technologies 6460 (TUB) and on a Shimadzu LCMS-8050 triple-quadrupole system (MPI-GEA). Chromatographic separation on the Agilent system was achieved on a Kinetex C18 column (100 mm x 2,1 mm, 100A) and on the Shimadzu system on a Restek Raptor Biphenyl analytical column (100mm x 2.1mm, 2.7µm particle size) and a Shimadzu Shimpack Velox SP-C18 column (100mm x 2.1mm, 2.7µm particle size. Both systems utilized a mobile phase comprised of HPLC-grade water and 0.1% formic acid (mobile phase A) along with acetonitrile (mobile phase B). The column temperature was maintained at a constant 25 °C, and a gradient program was applied with 0.5% B for the initial 1 min, increasing to 80% B at 10 min, 100% B at 15 min with a hold until 17.5 min, and returning to 0.5% B and holding until 20 min. Injection volumes varied between 1 and 2 µL, depending on the sample concentration. Ionization was performed with an electro spray ionization (ESI) ion source, with both positive and negative modes utilized. MeOH blanks were carried out between each sample, and all samples were analyzed in duplicate.

GC-MS data were analyzed using Agilent MassHunter Qualitative Data Analysis software 10.0 with peak identification based on comparison to standards, the NIST library (2.2), and with spectra reported in the literature. LC-MS/MS data were processed using Shimadzu LabSolutions software in MRM mode, with authentic analytical standards for the optimization of MRM parameters employed to screen for specific compounds in archaeological samples.

Acknowledgements

The authors are grateful to the Saudi Commission for Tourism and National Heritage for providing samples and to the Max Planck Society for funding this research. B. Huber thanks the Joachim Herz Foundation for the award of an Add-on Fellowship for Interdisciplinary Life Sciences for her PhD research.

References

al-Hajiri M (2011) Syro-levantinische Bronzewaffen aus Tayma. In: Franke U, Gierlichs J (eds) Roads of Arabia: archäologische Schätze aus Saudi-Arabien. Wasmuth, Tübingen, p 112

Assimopoulou AN, Papageorgiou VP (2005) GC-MS analysis of penta- and tetra-cyclic triterpenes from resins ofPistacia species. Part II.Pistacia terebinthus var. Chia. Biomed Chromatogr 19:586–605. https://doi.org/10.1002/bmc.484

Avanzini A (ed) (1997) Profumi d'Arabia. Atti del convegno. L'Erma di Bretschneider, Rome

Baeten J, Deforce K, Challe S, et al (2014) Holy smoke in medieval funerary rites: chemical fingerprints of frankincense in southern Belgian incense burners. PLOS ONE 9:e113142. https://doi.org/10.1371/journal.pone.0113142

Baird I (2021) Complex Legacies: Materiality, Memory, and Myth in the Arabian Peninsula. In: Yağcıoğlu H, Baird I (eds) All Things Arabia: Arabian Identity and Material Culture. BRILL

Batiha GE-S, Wasef L, Teibo JO, et al (2023) Commiphora myrrh: a phytochemical and pharmacological update. Naunyn-Schmiedeberg's Arch Pharmacol 396:405–420. https://doi.org/10.1007/s00210-022-02325-0

Beuger A (2010) Salvage Excavations at the burial site of Tala' (Area S). Atlal The Journal of Saudi Arabian Archaeology 20:133–137

Biella JC (1982) Dictionary of Old South Arabic, Sabaean dialect. Scholars Press, Chico, CA

Böck B (2011) Sourcing, Organizing, and Administering Medical Ingredients. In: Radner K, Robson E (eds) The Oxford Handbook of Cuneiform Culture. Oxford University Press, pp 690–705

Bongers F, Groenendijk P, Bekele T, et al (2019) Frankincense in peril. Nat Sustain 2:602–610. https://doi.org/10.1038/s41893-019-0322-2

Brettell R, Schotsmans E, Matin W, et al (2017) The Final Masquerade: Resinous Substances and Roman Mortuary Rites. In: Livarda A, Madgwick R, Mora SR (eds) The Bioarchaeology of Ritual and Religion. Oxbow Books, pp 44–57

Charrié-Duhaut A, Connan J, Rouquette N, et al (2007) The canopic jars of Rameses II: real use revealed by molecular study of organic residues. Journal of Archaeological Science 34:957–967. https://doi.org/10.1016/j.jas.2006.09.012

Clancy D (2019) The Smell of Grief: Odour and Olfaction at the Roman Funeral. THERSITES Journal for Transcultural Presences & Diachronic Identities from Antiquity to Date 89-116 Pages. https://doi.org/10.34679/THERSITES.VOL9.120

Colombini MP, Giachi G, Modugno F, Ribechini E (2005a) Characterisation of organic residues in pottery vessels of the Roman age from Antinoe (Egypt). Microchemical Journal 79:83–90. https://doi.org/10.1016/j.microc.2004.05.004

Colombini MP, Modugno F (eds) (2009) Organic Mass Spectrometry in Art and Archaeology. John Wiley & Sons, Ltd, Chichester, UK

Colombini MP, Modugno F, Ribechini E (2005b) Organic mass spectrometry in archaeology: evidence for Brassicaceae seed oil in Egyptian ceramic lamps. J Mass Spectrom 40:890–898. https://doi.org/10.1002/jms.865

Craig OE, Steele VJ, Fischer A, et al (2011) Ancient lipids reveal continuity in culinary practices across the transition to agriculture in Northern Europe. Proceedings of the National Academy of Sciences 108:17910–17915. https://doi.org/10.1073/pnas.1107202108

Culioli G, Mathe C, Archier P, Vieillescazes C (2003) A lupane triterpene from frankincense (Boswellia sp., Burseraceae). Phytochemistry 62:537–541

Devièse T, Ribechini E, Castex D, et al (2017) A multi-analytical approach using FTIR, GC/MS and Py-GC/MS revealed early evidence of embalming practices in Roman catacombs. Microchemical Journal 133:49–59. https://doi.org/10.1016/j.microc.2017.03.012

Dinies M, Neef R, Plessen B, Kürschner H (2016) Holocene vegetation, climate, land use and plant cultivation in the Tayma region, northwestern Arabia. In: Luciani M (ed) The archaeology of North Arabia: oases and landscapes; proceedings of the international congress held at the University of Vienna, 5-8 December, 2013. Austrian Academy of Sciences Press, Wien, pp 57–77

Dinies M, Plessen B, Neef R, Kürschner H (2015) When the desert was green: Grassland expansion during the early Holocene in northwestern Arabia. Quaternary International 382:293–302. https://doi.org/10.1016/j.quaint.2015.03.007

Dunne J, Evershed RP, Salque M, et al (2012) First dairying in green Saharan Africa in the fifth millennium bc. Nature 486:390–394. https://doi.org/10.1038/nature11186

Dunne J, Mercuri AM, Evershed RP, et al (2016) Earliest direct evidence of plant processing in prehistoric Saharan pottery. Nature Plants 3:16194. https://doi.org/10.1038/nplants.2016.194

Eichmann R, Hausleiter A, Al-Najem M, Al-Said S (2010) Tayma - Autumn 2004 and Spring 2005, 2nd Report on the Joint Saudi-Arabian-German archaeological project. Atlal The Journal of Saudi Arabian Archaeology 20:101–147

Eichmann R, Hausleiter A, Al-Najem M, Al-Said S (2011) Tayma - Autumn 2005 and 2006 (Spring and Autumn) – 3rd report on the joint Saudi Arabian-German archaeological project. Atlal The Journal of Saudi Arabian Archaeology 21:64–118

Eichmann R, Hausleiter A, Al-Najem M, Al-Said S (2012) Tayma 2007 - 4th Report on the Joint Saudi-Arabian-German Archaeological Project. Atlal The Journal of Saudi Arabian Archaeology 22:72–117

Eichmann R, Hausleiter A, Götzelt T (2006) Einstmals an der Weihrauchstraße. forschung 31:4–9. https://doi.org/10.1002/fors.200690044

Erickson-Gini T, Israel Y (2013) Excavating the Nabataean Incense Road. Journal of Eastern Mediterranean Archaeology and Heritage Studies 1:24–53

Evershed R P (2008) Organic Residue Analysis in Archaeology: The Archaeological Biomarker Revolution. Archaeometry 50:895–924. https://doi.org/10.1111/j.1475-4754.2008.00446.x

Evershed RP, Clark KA (2020) Trends in Use of Organic Balms in Egyptian Mummification Revealed Through Biomolecular Analyses. In: Shin DH, Bianucci R (eds) The Handbook of Mummy Studies: New Frontiers in Scientific and Cultural Perspectives. Springer, Singapore, pp 1–63

Evershed RP, Dudd SN, Copley MS, et al (2002) Chemistry of Archaeological Animal Fats. Acc Chem Res 35:660–668. https://doi.org/10.1021/ar000200f

Evershed RP, van Bergen PF, Peakman TM, et al (1997) Archaeological frankincense. Nature 390:667

Farjon A, Filer D (2013) An atlas of the world's conifers: an analysis of their distribution, biogeography, diversity, and conservation status. Brill, Leiden ; Boston

Geller MJ, Panayotov SV (2018) Mesopotamian Eye Disease Texts: the nineveh treatise. De Gruyter, Berlin, Boston

Germer R (2008) Handbuch der altägyptischen Heilpflanzen. Harrassowitz, Wiesbaden

Giachi G, Pallecchi P, Romualdi A, et al (2013) Ingredients of a 2,000-y-old medicine revealed by chemical, mineralogical, and botanical investigations. Proc Natl Acad Sci USA 110:1193–1196. https://doi.org/10.1073/pnas.1216776110

Groom N (1981) Frankincense and Myrrh: a study of the Arabian incense trade., 1. publ. Longman, London u.a.

Gylling H, Simonen P (2015) Phytosterols, Phytostanols, and Lipoprotein Metabolism. Nutrients 7:7965–7977. https://doi.org/10.3390/nu7095374

Hausleiter A (2012) North Arabian Kingdoms. In: Potts DT (ed) A companion to the archaeology of the ancient Near East. Wiley-Blackwell, Malden, Mass., pp 816–832

Hausleiter A (2011) Das antike Tayma: eine Oase im Kontaktbereich der Kulturen - Neue Forschungen an einem Zentralort der Weihrauchstraße. In: Franke U, Gierlichs J (eds) Roads of Arabia: Archäologische Schätze aus Saudi-Arabien. Wasmuth, Tübingen, pp 103–123

Hausleiter A (2014) Pottery Groups of the Late 2nd / early 1st millennia BC in Northwest Arabia and new evidence from the excavations at Tayma. In: Luciani M, Hausleiter A (eds) Recent trends in the study of Late Bronze Age ceramics in Syro-Mesopotamia and neighbouring regions : proceedings of the International Workshop in Berlin, 2-5 November 2006. Leidorf, Rahden/Westf, pp 399–434

Hausleiter A (2019) The Oasis of Tayma: 7000 Years of History. In: Capodiferro A, Colantonio S, Museo nazionale romano (eds) Roads of Arabia: archaeological treasures from Saudi Arabia. Electa, Milano, pp 107–131

Hausleiter A (2018) The outer wall of Taymāʾ and its dating to the Bronze Age. In: Nehmé L, Al-Jallad A (eds) To the madbar and back again: studies in the languages, archaeology, and cultures of Arabia dedicated to Michael C.A. Macdonald. Brill, Leiden, Boston, pp 361–391

Hausleiter A, Eichmann R (2018) The Archaeological Exploration of the Oasis of Taymāʾ. In: Hausleiter A, Eichmann R, Al-Najem M (eds) Tayma I. Reports 1: Archaeological Research Strategies, Palaeoenvironment, Cultural Contacts. Archaeopress, Oxford, pp 2–55

Hausleiter A, Zur A (2016) Taymāʾ in the Bronze Age (c. 2,000 BCE): Settlement and Funerary Landscapes. In: Luciani M (ed) The archaeology of North Arabia: oases and landscapes; proceedings of the international congress held at the University of Vienna, 5-8 December, 2013. Austrian Academy of Sciences Press, Wien, pp 135–173

Herz R (2016) The role of odor-evoked memory in psychological and physiological health. Brain Sciences 6:22. https://doi.org/10.3390/brainsci6030022

Herz RS, Engen T (1996) Odor memory: Review and analysis. Psychonomic Bulletin & Review 3:300–313. https://doi.org/10.3758/BF03210754

Huber B (2020) Incense burners in the Oasis of Tayma, Northwest Arabia: an olfactory perspective. Polish Archaeology in the Mediterranean 29/1:267–294

Huber B, Hammann S, Loeben CE, et al (2023) Biomolecular characterization of 3500-year-old ancient Egyptian mummification balms from the Valley of the Kings. Sci Rep 13:12477. https://doi.org/10.1038/s41598-023-39393-y

Huber B, Hausleiter A, Dinies M, et al (2018) Interdisziplinäre Untersuchungen von Räuchergefäßen zur Rekonstruktion antiker Gerüche. e-Forschungsberichte des DAI 2:120–125

Huber B, Larsen T, Spengler RN, Boivin N (2022a) How to use modern science to reconstruct ancient scents. Nature Human Behaviour. https://doi.org/10.1038/s41562-022-01325-7

Huber B, Vassão DG, Roberts P, et al (2022b) Chemical Modification of Biomarkers through Accelerated Degradation: Implications for Ancient Plant Identification in Archaeo-Organic Residues. Molecules 27:3331. https://doi.org/10.3390/molecules27103331

Hussein R, El-Anssary A (2019) Plants Secondary Metabolites: The Key Drivers of the Pharmacological Actions of Medicinal Plants. In: Builders P (ed) Herbal Medicine. IntechOpen

Intilia A (2011) Area O. In: Eichmann R, Hausleiter A, Al-Najem M, Al-Said S (eds) Tayma - Autumn 2005 and 2006 (Spring and Autumn) – 3rd report on the joint Saudi Arabian-German archaeological project. pp 97–102

Intilia A (2012) Excavations in Area O. In: Eichmann R, Hausleiter A, Al-Najem M, Al-Said S (eds) Tayma 2007 - 4th Report on the Joint Saudi-Arabian-German Archaeological Project. pp 100–106

Japp S, Gerlach I, Hitgen H, Schnelle M (2011) Yeha and Hawelti: cultural contacts between Saba' and D'MT - New research by the German Archaeological Institute in Ethiopia. In: Institute of Archaeology, British Museum (eds) Proceedings of the Seminar for Arabian Studies. Papers from the forty-fourth meeting of the Seminar for Arabian Studies held at the British Museum, London, 22 - 24 July 2010. Archaeopress, Oxford, pp 145–160

Langenheim JH (2003) Plant resins: chemistry, evolution, ecology, and ethnobotany. Timber Press, Portland

Leichty E (2011) The royal inscriptions of Esarhaddon, king of Assyria (680-669 BC). Eisenbrauns, Winona Lake, Ind

Liu S, Rehren T, Pernicka E, Hausleiter A (2015) Copper processing in the oases of northwest Arabia: technology, alloys and provenance. Journal of Archaeological Science 53:492–503. https://doi.org/10.1016/j.jas.2014.10.030

Lora S (2017) A religious building complex in the ancient settlement of Tayma (North-west Arabia) during the Nabataean period: changes and transformations. Syria 94:17–39

Lora S, Petiti E, Hausleiter A (2010) Burial contexts at Tayma, NW-Arabia – Archaeological and anthropological data. In: Weeks L (ed) Death and Burial in Arabia and Beyond: Multidisciplinary Perspectives. British Archaeological Reports, Oxford, pp 237–247

Lukin I, Merz J, Schembecker G (2018) Techniques for the recovery of volatile aroma compounds from biochemical broth: A review. Flavour Fragr J 33:203–216. https://doi.org/10.1002/ffj.3447

Macdonald MCA (1997) Trade routes and trade goods at the northern end of the “incense road” in the first millennium B.C. In: Avanzini A (ed) Profumi d’Arabia. Atti del convegno. L’Erma di Bretschneider, Rome, pp 333–349

Marcotullio MC, Santi C, Oball-Mond Mwankie G Norbert, Curini M (2009) Chemical Composition of the Essential Oil of Commiphora erythraea. Natural Product Communications Vol. 4:1751–1754

Marković M, Mezzatesta E, Porcier S, et al (2022) Rethinking the Process of Animal Mummification in Ancient Egypt: Molecular Characterization of Embalming Material and the Use of Brassicaceae Seed Oil in the Mummification of Gazelle Mummies from Kom Mereh, Egypt. Molecules 27:1532. https://doi.org/10.3390/molecules27051532

Mathe C, Archier P, Nehme L, Vieillescazes C (2009) The study of nabataean organic residues from Madâ’in Sâlih, ancient Hegra, by gas chromatography–mass spectrometry. Archaeometry 51:626–636

Mathe C, Culioli G, Archier P, Vieillescazes C (2004) High-Performance Liquid Chromatographic Analysis of Triterpenoids in Commercial Frankincense. Chromatographia 60:493–499. https://doi.org/10.1365/s10337-004-0417-3

Nebes N (2014) Könige der Weihrauchstraße: zur Geschichte Südarabiens und Äthiopiens im frühen 1. Jahrtausend vor Christus. V & R Unipress Vienna university press, Göttingen [Vienna]

Niebler J, Buettner A (2016) Frankincense Revisited, Part I: Comparative Analysis of Volatiles in Commercially Relevant *Boswellia* Species. Chem Biodiversity 13:613–629. https://doi.org/10.1002/cbdv.201500329

Niebler J, Eslamieh J, Buettner A (2016) Frankincense Revisited, Part II: Volatiles in Rare *Boswellia* Species and Hybrids. Chem Biodiversity 13:630–643. https://doi.org/10.1002/cbdv.201500339

Panayotov SV (2014) A Peculiar Late Babylonian Recipe For Fumigation Against Epilepsy. In: The Recipes Project – Food, Magic, Science, and Medicine

Paul M (2012) Chemotaxonomic investigations on resins of the frankincense species Boswellia papyrifera, Boswellia serrata and Boswellia sacra, respectively, Boswellia carterii: a qualitative and quantitative approach by chromatographic and spectroscopic methodology. Doktors der Naturwissenschaften, Universität des Saarlandes

Pekgozlu AK, Kustas S, Mercan B, Bicer A (2017) Chemical Characterization of Lebonan Cedar Tar. Drvna ind 68:107–112. https://doi.org/10.5552/drind.2017.1607

Petiti E, Intilia A, Hausleiter A (2014) Bioarchaeological investigations at a 4th-3rd century BC cemetery at Tayma, North-West Arabia. Proceedings of the 8th International Congress on the Archaeology of the Ancient Near East 373–392

Pollard AM, Heron C (2008) Archaeological Chemistry, 2nd edition. Royal Society of Chemistry, Cambridge

Pommerening T (2016) Wege zur Identifikation altägyptischer Drogennamen – eine kritische Betrachtung. Zwischen Philologie und Lexikographie des Ägyptisch-Koptischen Akten der Leipziger Abschlusstagung des Akademienprojekts “Altägyptisches Wörterbuch” (Abhandlungen der Sächsischen Akademie der Wissenschaften zu Leipzig 82–111

Regert M, Devièse T, Le Hô A-S, Rougeulle A (2008) Reconstructing ancient Yemeni commercial routes during the Middle Ages using structural characterization of terpenoid resins. Archaeometry 50:668–695

Ren M, Ren X, Wang X, Yang Y (2022) Characterization of the incense sacrificed to the sarira of Sakyamuni from Famen Royal Temple during the ninth century in China. Proc Natl Acad Sci USA 119:e2112724119. https://doi.org/10.1073/pnas.2112724119

Renzi M, Intilia A, Hausleiter A, Rehren T (2016) Early Iron Age metal circulation in the Arabian Peninsula: the oasis of Taymā as part of a dynamic network (poster). Proceedings of the Seminar for Arabian Studies 46:237–246

Ribechini E, Raffaelli M, Colombini MP (2008) Botanical and chemical characterization of frankincense resin from Dhofar. In: Avanzini A (ed) A port in Arabia between Rome and the Indian Ocean (3rd C. BC - 5th C. AD). “L’Erma” di Bretschneider, Roma, pp 681–686

Riesmeier M, Keute J, Veall M-A, et al (2022) Recipes of Ancient Egyptian kohls more diverse than previously thought. Sci Rep 12:5932. https://doi.org/10.1038/s41598-022-08669-0

Roffet-Salque M, Dunne J, Altoft DT, et al (2017) From the inside out: Upscaling organic residue analyses of archaeological ceramics. Journal of Archaeological Science: Reports 16:627–640. https://doi.org/10.1016/j.jasrep.2016.04.005

Salomé-Abarca LF, van der Pas J, Kim HK, et al (2018) Metabolic discrimination of pine resins using multiple analytical platforms. Phytochemistry 155:37–44. https://doi.org/10.1016/j.phytochem.2018.07.011

Sato M, Seki K, Kita K, et al (2009) Comparative analysis of diterpene composition in the bark of the hybrid larch, Larix F1, gmelinii var. japonica × L. kaempferi and their parent trees. J Wood Sci 55:32–40. https://doi.org/10.1007/s10086-008-0988-y

Scott A, Power RC, Altmann-Wendling V, et al (2020) Exotic foods reveal contact between South Asia and the Near East during the second millennium BCE. Proc Natl Acad Sci USA 202014956. https://doi.org/10.1073/pnas.2014956117

Scurlock JA (2012) A plant can have different names in different cultures: A rebuttal to Luc Renaut. Journal des Médecines 19:51–52

Serpico M, White R (2000) The botanical identity and transport of incense during the Egyptian New Kingdom. Antiquity 74:884–897

Sharifi MS, Hazell SL (2011) Isolation, Analysis and Antimicrobial Activity of the Acidic Fractions of Mastic, Kurdica, Mutica and Cabolica Gums from Genus Pistacia. GJHS 4:217–228. https://doi.org/10.5539/gjhs.v4n1p217

Singh R (2016) Chemotaxonomy: A Tool for Plant Classification. Journal of Medicinal Plants Studies 4:90–93

Sperveslage G (2013) Ägyptische Einflüsse auf der Arabischen Halbinsel in vorislamischer Zeit am Beispiel der Oase von Tayma. In: Zeitschrift für Orient-Archäologie. pp 234–252

Stern B, Heron C, Corr L, et al (2003) Compositional variations in aged and heated pistacia resin found in late bronze age canaanite amphorae and bowls from Amarna, Egypt. Archaeometry 45:457–469

Tamburini D, Łucejko J, Ribechini E, Colombini MP (2016) New markers of natural and anthropogenic chemical alteration of archaeological lignin revealed by in situ pyrolysis/silylation-gas chromatography mass spectrometry. Journal of Analytical and Applied Pyrolysis 118:249–258. https://doi.org/10.1016/j.jaap.2016.02.008

Tchapla A, Méjanelle P, Bleton J, Goursaud S (2004) Characterisation of embalming materials of a mummy of the Ptolemaic era. Comparison with balms from mummies of different eras. J Sep Science 27:217–234. https://doi.org/10.1002/jssc.200301607

Tourtet F, Daszkiewicz M, Hausleiter A (2021) Pottery from Tayma: Chronostratigraphy, Archaeometric Studies, Cultural Interaction. In: Luciani M (ed) The archaeology of the Arabian Peninsula 2: Connecting the evidence. Proceedings of the international workshop held in Vienna on April 25, 2016. Austrian Academy of Sciences Press, Vienna, pp 43–88

Tourtet F, Weigel F (2015) Tayma in the Nabataean kingdom and in Provincia Arabia. Proceedings of the Seminar for Arabian Studies 45:385–404

van Bergen PF, Peakman TM, Leigh-Firbank EC, Evershed RP (1997) Chemical evidence for archaeological frankincense: Boswellic acids and their derivatives in solvent soluble and insoluble fractions of resin-like materials. Tetrahedron Letters 38:8409–8412. https://doi.org/10.1016/S0040-4039(97)10234-9

Weigel F (2020) Contrasting Living Spaces at Tayma (Saudi Arabia). In: Otto A, Herles M, Kaniuth K (eds) Proceedings of the 11th International Congress on the Archaeology of the Ancient Near East archaeology: 03-07 April 2018, Munich. Harrassowitz Verlag, Wiesbaden

Weigel FW (2019) Dwelling(s) in a north-west Arabian oasis: decoding residential architecture at Taymāʾ. Arab Archaeol Epigr 30:103–133. https://doi.org/10.1111/aae.12124

Xynos N, Termentzi A, Fokialakis N, et al (2018) Supercritical CO2 extraction of mastic gum and chemical characterization of bioactive fractions using LC-HRMS/MS and GC–MS. The Journal of Supercritical Fluids 133:349–356. https://doi.org/10.1016/j.supflu.2017.10.011

Zald DH, Pardo JV (1997) Emotion, olfaction, and the human amygdala: Amygdala activation during aversive olfactory stimulation. PNAS 94:4119–4124

Zimmerle WG (2021) Frankincense and Its Arabian Burner. In: Baird IP, Yağcıoğlu H (eds) All things Arabia: Arabian identity and material culture. Brill, Leiden ; Boston, pp 23–42

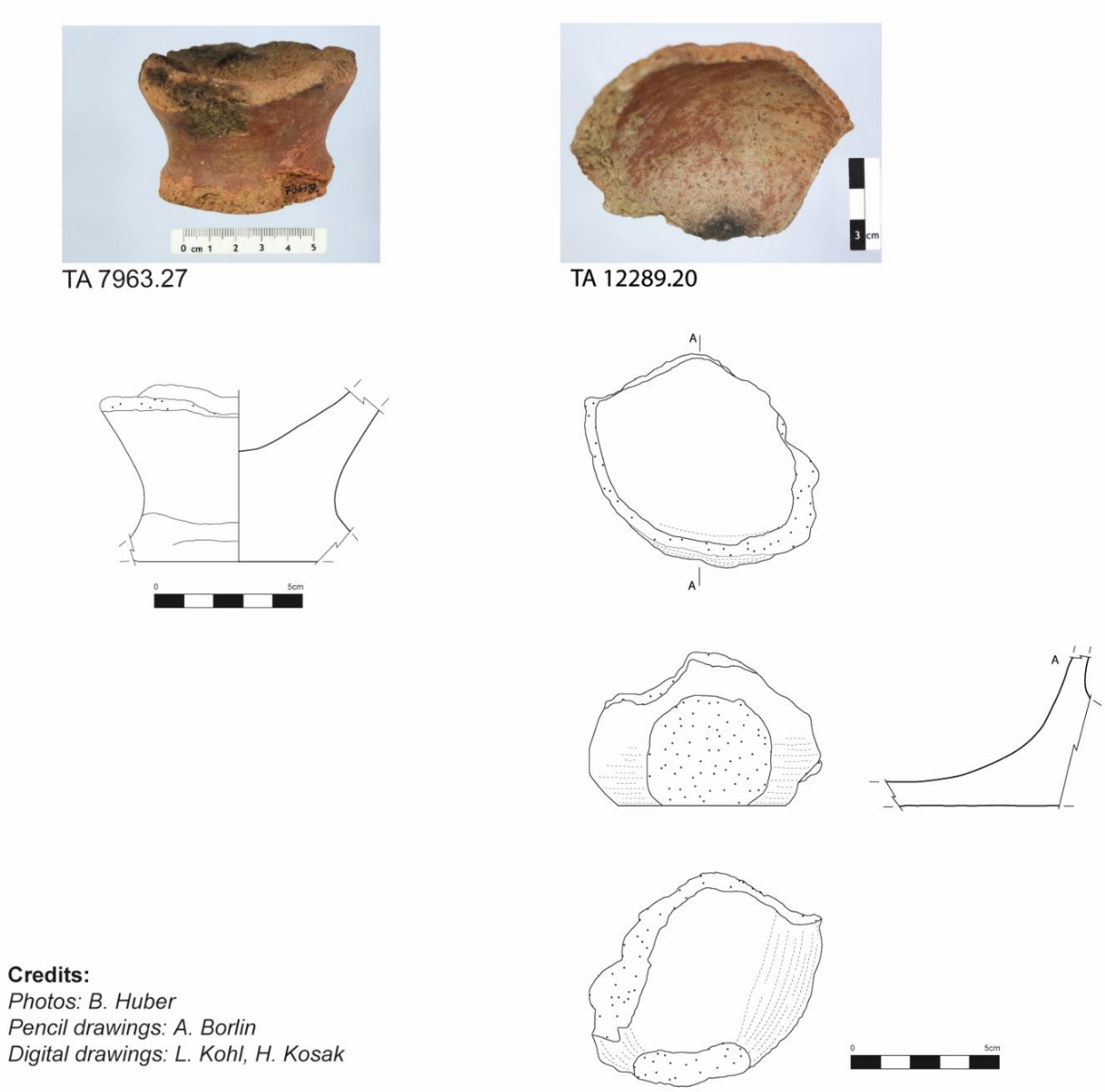
SUPPLEMENTARY INFORMATION

Exploring the aromatic diversity of incense materials at the ancient oasis of Tayma using metabolic profiling

Huber, B.[1,2], Hausleiter, A.[3], Dinies, M.[4], Giddings Vassão, D. [1,5], Säumel, I.[6], Fernandes, R.[1], Roberts, P. [1,7], Pham, T.L.H.[8]

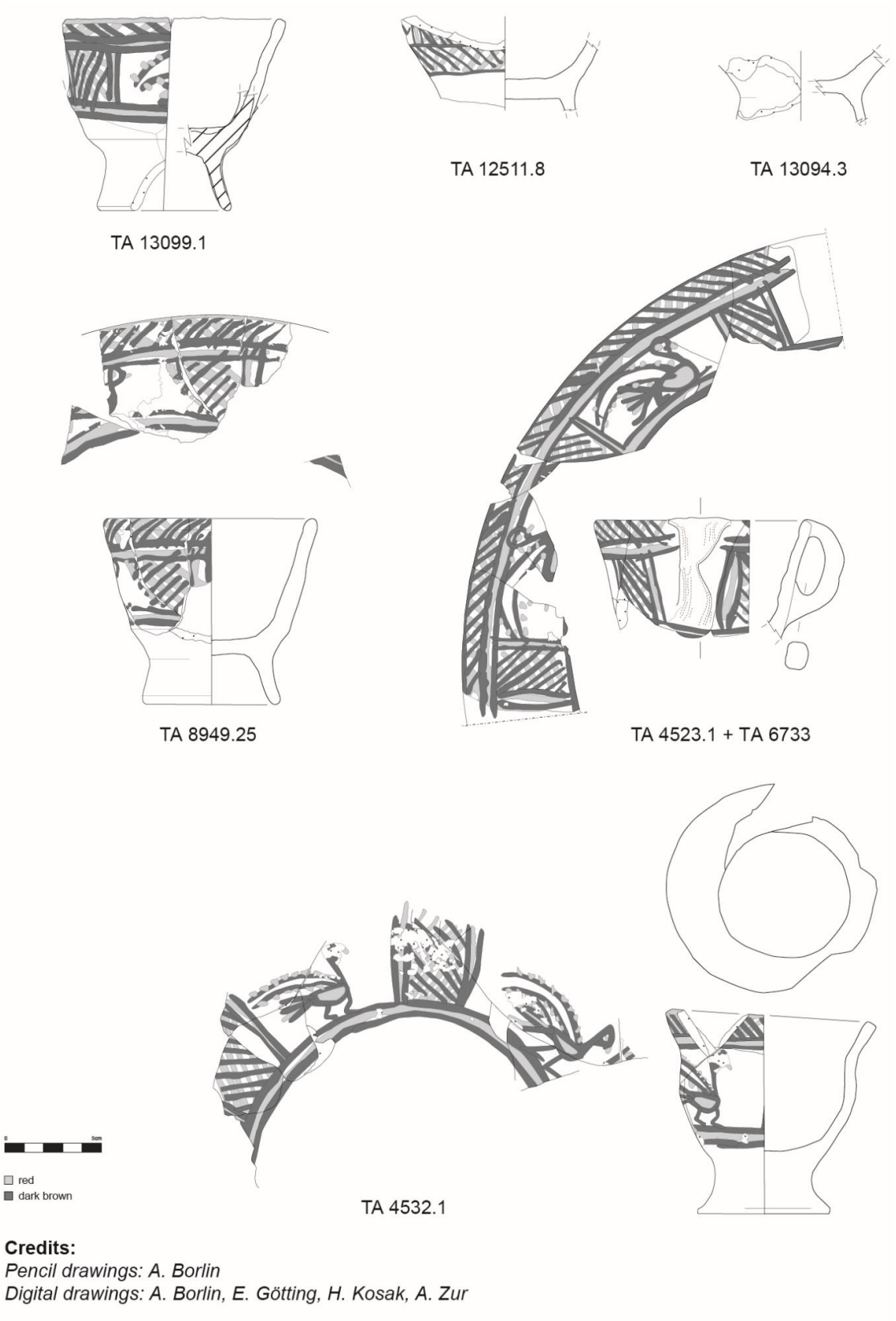
Content

Supplementary Figure S1



Incense burners of the Red Burnished Ware from the Middle Bronze Age (DAI Orient Department; modified from Fig. 3 in Huber 2020) .

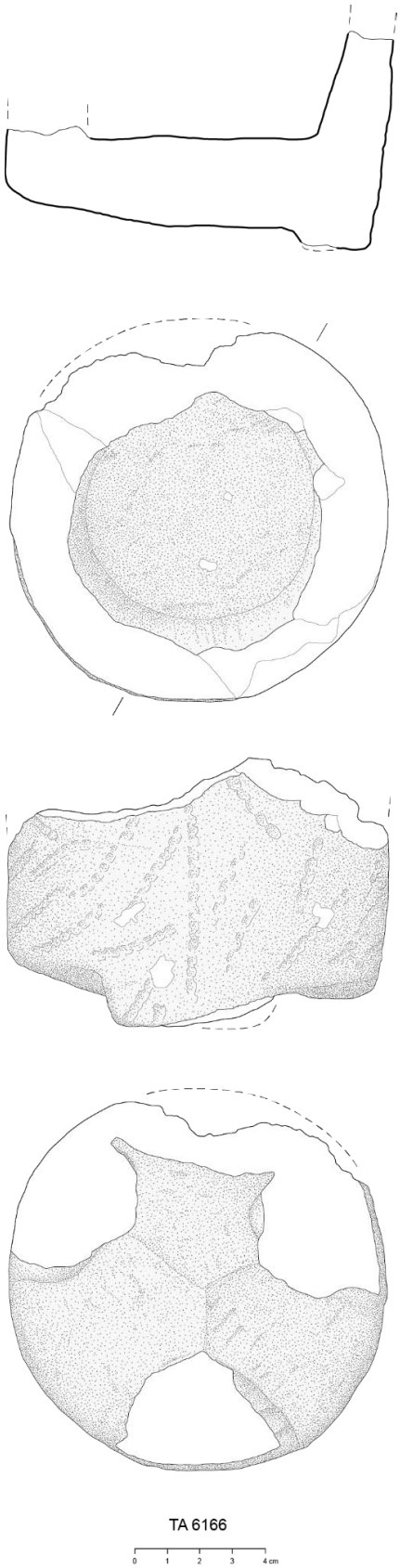
Supplementary Figure S2



Credits:
Pencil drawings: A. Borlin
Digital drawings: A. Borlin, E. Götting, H. Kosak, A. Zur

Pottery incense burners – 'goblets' – from the Early Iron Age temple in Area O (DAI Orient Department; source: Huber 2020).

Supplementary Figure S3

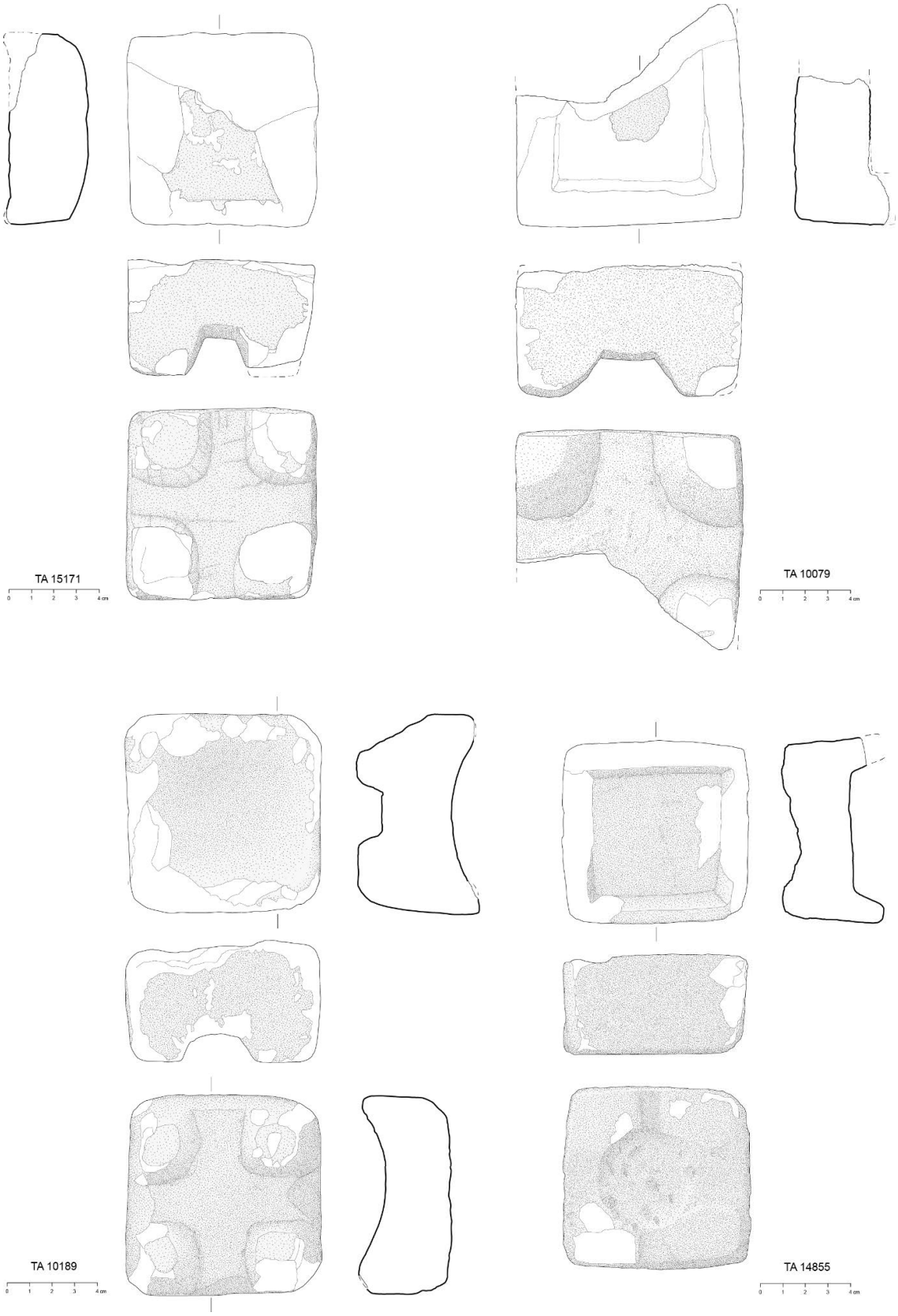


Sandstone incense burner found in temple E-b1 (DAI Orient Department, Pencil drawing: Alessia Borlin; Digital drawing: Helga Kosak).

Supplementary Figure S4

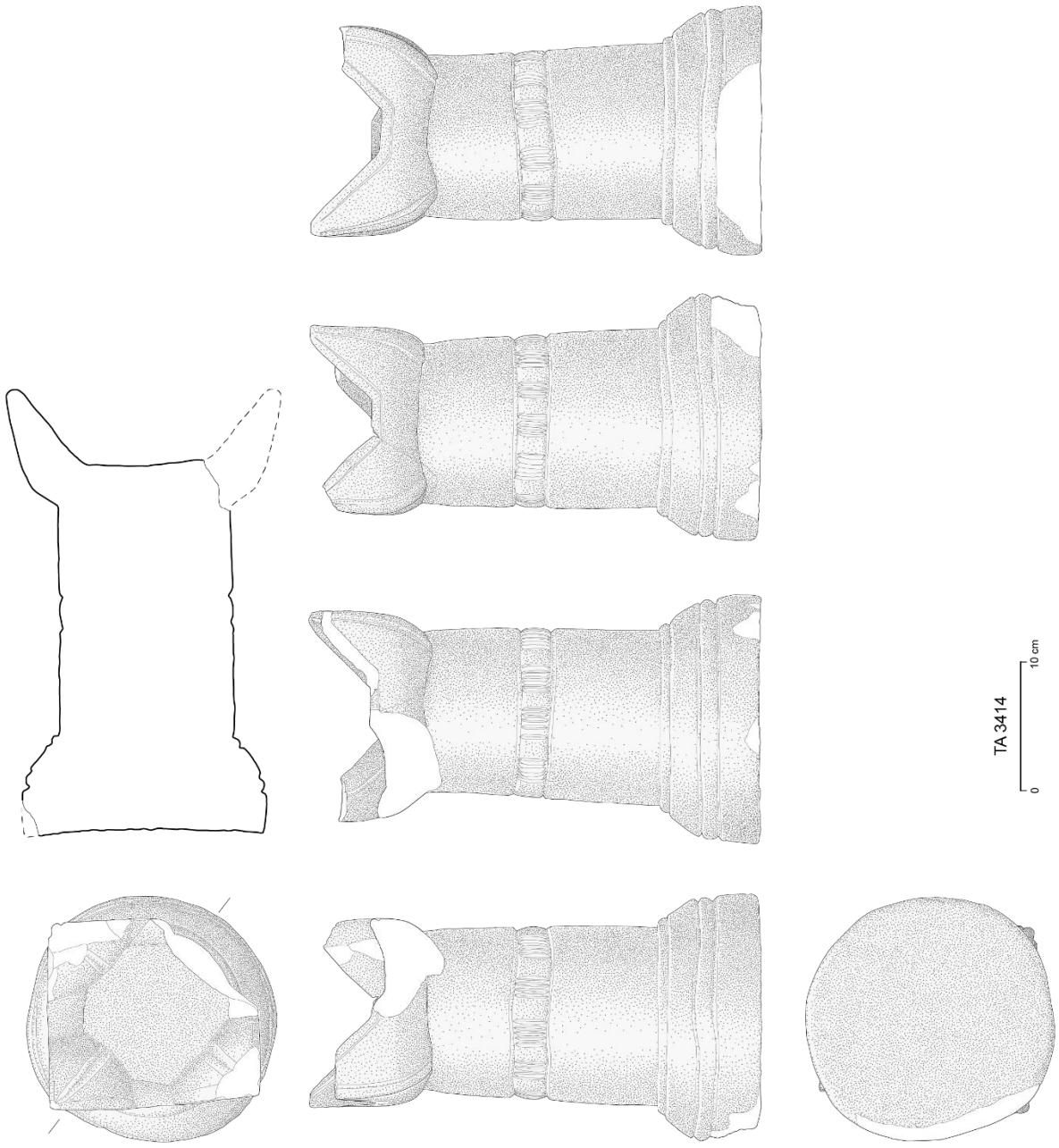
Incense burners found inside the tombs at the necropolis of Tal'a from the Mid-to-Late Iron Age (DAI Orient-Department, Pencil and digital drawing: Alessia Borlin; Photos: Mirco Cusin; modified from Fig. 8 in Huber 2020).

Supplementary Figure S5



Selection of cuboid incense burners from the residential quarter in Area E-South/F (DAI Orient Department, Pencil drawing: Alessia Borlin; Digital drawing: Helga Kosak).

Supplementary Figure S6



Incense burner TA 3414 with 'crown' from Area E-South/F (DAI Orient Department, Pencil drawing: Alessia Borlin; Digital drawing: Helga Kosak).

Supplementary Figure S7

Incense burner TA 0884 with Nabataean inscription (DAI Orient Department, Pencil drawing: Alessia Borlin; Digital drawing: Helga Kosak).

MANUSCRIPT E

Metabolic profiling reveals the aromatic and bioactive properties of ancient Egyptian mummification balms

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Abstract

The ancient Egyptians used the potent effects of plants to great effect, in particular as a key element of processes of embalming and mummification. Aromatics, in particular, held a special place, not just for their fragrant allure, but for their strong pharmacological qualities. Here, we employ headspace solid phase microextraction - gas chromatography mass spectrometry (HS-SPME-GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) to shed light on the selection and transformation of plants exploited to make mummification balms so well-preserved that they have retained their scent for over 2500 years. Balm samples from canopic jars containing mummified viscera, and dating to the mid-first millennium BCE (26th dynasty, ca. 600 BCE), were shown to contain the resin of *Pistacia* trees, bitumen, as well as various animal and vegetable products, most notably cedar tar. The identification of cedar tar sheds light on ancient Egyptian technological acumen, demonstrating that the Egyptians possessed advanced distillation capabilities. We also explore the bioactive properties of ancient Egyptian balms, revealing their anti-microbial, insecticidal an inhibiting property against bacteria. Our analysis highlights the enduring effectiveness of the substance, and provides insights into the links between bioactive, aromatic and symbolic properties in ancient Egypt.

Introduction:

The ancient Egyptians held a profound understanding of the pharmacological potential of plants[1]. Such bioactive attributes of substances were employed across a spectrum of applications. These ranged from cosmetology and perfumery [2], with an emphasis on olfactory aesthetics, to the formulation of medical and sanitary solutions [1,3]. Most intriguingly, these aromatic substances were integral to the intricate process of mummification, where preservative and fragrant characteristics played an important role [4–6]. This procedure, central to ancient Egyptian mortuary practices, involved systematic organ extraction, followed by desiccation and the eventual embalming of the deceased, underscoring the objective of long-term preservation [7]. The latter involved the use of a fragrant balm – a substance composed of many ingredients, including resins, scented oils, tars and herbs, which exhibit significant bioactive capabilities, notably antimicrobial, antibacterial and antifungal characteristics [8]. The embalming fluids not only aided in preservation but also imparted an aromatic quality to the deceased, ensuring a pleasant journey to the afterlife [9].

While previous chemical analyses have predominantly focused on identifying balm ingredients and recipes, primarily through the use of organic residue analysis [e.g. 10–18], remarkably few studies have investigated the bioactive properties of the balms. Several researchers have postulated the potential bioactivity of substances identified in balms [18–21], particularly in coniferous plant products, but only one study has so far actually ventured into an investigation of the bioactive capabilities of these substances [22]. An enhanced comprehension of these properties would not only offer a scientific underpinning for understanding the selection of certain substances for preservation by the ancient Egyptians, but could also reveal the meticulous processes and criteria they likely employed. Delving deeper into these intricacies can illuminate the profound scientific acumen and the botanical knowledge that the ancient Egyptian ritual practitioners potentially held. Moreover, it could unravel the rationale behind the selection, transformation, and application of specific plants in their practices.

In this study, we examine samples of mummification balms from the mid-first millennium BCE. The samples were taken from canopic jars of the 26th Dynasty (ca. 600 BCE), containing the organs of the lady Tas-nechet (T3s-nht). Remarkably, due to high-quality preservation of organic remains, these balm residues still retain their characteristic scent even today. Given this unique case, it presents a compelling opportunity for a detailed investigation of the balms'

bioactivity, preservation qualities and odour-active compounds. It further facilitates an in-depth analysis of specific plant components and tissues, which can provide insights into the extraction processes used to obtain these substances. For our analysis, we employed headspace solid-phase microextraction-gas chromatography mass spectrometry (HS-SPME-GC-MS), a technique particularly suited for detecting volatile secondary metabolites, emphasizing mono- and sesquiterpenes. These compounds are not only integral to the aromatic essence of the balms but also serve as crucial indicators of bioactive effects and can aid in distinguishing between plant species. As a complement to these analyses, we utilized GC-MS and liquid chromatography-tandem mass spectrometry (LC-MS/MS) to analyze both lipids and polar secondary metabolites, aiming to achieve a holistic molecular profile of the balm's composition. Through this rigorous scientific approach, we aspired to also look into synergy effects of the joint use of substances.

Results

Analysis of volatile organic compounds using HS-SMPE-GC-MS

The profiles of volatile secondary (or specialized) metabolites obtained by HS-SPME GC-MS showed exceptional preservation of highly volatile compounds, such as mono- and sesquiterpenes and -terpenoids, which were detected in high abundance and quantity, enabling the identification of 62 compounds (Table 1, Figure 1). We observed that balm samples taken from canopic jar 1, containing a mummified stomach (DA-AES 052-054), showed excellent preservation and had of a large number of monoterpenes, whereas samples of canopic jar 2, holding the liver (DA-AES 057-059), were less well preserved, missing the most volatile constituents.

Table 1. Compound table of archaeological samples DA-AES 052-054 (canopic jar 1) and DA-AES 057-059 (canopic jar 2) obtained by HS-SPME-GC-MS. The compounds are presented in relative peak areas (%) ordered by increasing retention time. Constituents at a trace level < 0.05 % are abbreviated with 'trace'.

Peak	Compounds	RT	052	053	054	057	058	059
1	α-Pinene*	21.28	0.17	0.49	0.37	-	-	-
2	Camphene*	22.332	trace	0.02	0.01	-	-	-
3	β-Pinene*	24.444	trace	0.02	0.02	-	-	-
4	Phenol	24.657	0.01	-	trace	-	-	-
5	γ-Collidine	25.495	-	0.02	0.02	-	-	-

6	p-Cymene	28.017	0.01	0.01	0.01	-	-	-
7	Limonene*	28.317	0.01	0.02	0.01	-	-	-
8	Eucalyptol*	28.547	-	0.01	trace	-	-	-
9	o-Cresol	30.163	0.01	0.02	0.02	-	-	-
10	p-Cresol	31.651	0.09	0.1	0.09	-	-	-
11	Nonanal	33.968	-	0.01	0.02	-	-	-
12	Fenchol	34.712	0.03	0.04	0.04	-	-	-
13	4-Acetyl-1-methylcyclohexene*	35.988	1.91	2.13	1.46	-	-	-
14	Pinocarveol (trans-?)	36.61	0.06	0.08	0.07	-	-	-
15	Verbenol (trans-?)	37.029	0.1	0.12	0.12	-	-	-
16	3-Cyclohexene-1-methanol, α,4-dimethyl-	37.251	0.13	0.12	0.12	-	-	-
17	endo-Borneol	38.594	0.1	0.11	0.1	-	-	-
18	Terpinen-4-ol	39.423	0.09	0.09	0.07	-	-	-
19	p-Cymen-8-ol	39.688	0.03	0.03	0.02	-	-	-
20	Ethanone, 1-(4-methylphenyl)-*	39.902	1.46	1.42	1.01	-	-	-
21	α-Terpineol*	40.363	0.79	1.01	0.89	-	-	-
22	Verbenone	41.757	0.15	0.17	0.15	-	-	-
23	Carveol (cis-?)	42.355	0.05	0.05	trace	-	-	-
24	Homoveratrole	43.706	trace	trace	trace	-	-	-
25	Carvone	44.168	trace	-	trace	-	-	-
26	Camphostene	44.553	-	trace	-	-	-	-
27	p-Ethylguaiacol	46.536	0.33	0.32	0.29	-	-	-
28	β-Methylnaphthalene	47.596	0.09	0.09	-	-	-	-
29	Tridecane	47.853	0.08	0.1	0.04	-	-	-
30	α-Longipinene*	51.589	0.33	0.45	0.35	trace	0.41	0.42
31	Cerulignol	52.478	0.15	0.16	0.13	-	-	-
32	Longifolene-(V4)	53.077	0.14	0.18	0.12	trace	0.35	0.4
33	Benzene, 1-methyl-3,5-bis(1-methylethyl)-	53.368	4.08	3.47	3.28	-	-	-
34	Tetradecane	54.46	0.41	0.09	0.24	-	-	-
35	4,4-Dimethyladamantan-2-ol	54.924	2.28	1.99	1.73	-	-	-
36	Longifolene*	55.266	0.84	0.97	0.79	2.3	3.29	3.57
37	α-Bergamotene	56.967	0.13	0.15	-	-	-	-
38	Dihydro-ar-curcumene	57.591	1.49	1.47	1.16	1.7	2.14	1.7
39	α-Himachalene	58.044	5.77	6.13	5.17	-	-	-
40	α-Curcumene	59.096	0.31	0.45	0.24	-	-	-
41	γ-Himachalene	59.814	4.86	5.13	4.07	-	-	-
42	Pentadecane	60.686	0.34	0.61	0.27	-	-	-
43	Cuparene	61.481	2.35	2.65	2.03	5.41	6.25	6.11
44	α-Dehydro-ar-himachalene	61.994	14.46	13.25	13.21	26.25	26.74	25.27
45	Calamenene	62.456	0.24	0.24	0.28	-	-	-
46	γ-Dehydro-ar-himachalene	62.875	8.42	7.84	7.45	2.85	3.41	2.89
47	aR-Himachalene	63.397	5.66	5.23	5.18	13.92	14.09	13.74
48	10,11-Epoxycalamenene	64.824	-	0.8	0.93	1.75	1.59	1.66
49	Oxidohimachalene	65.602	1.46	1.33	1.81	0.33	0.49	0.52
50	Dihydro-ar-turmerone	66.492	4.52	3.84	4.68	1.36	1.03	0.87

51	Hexadecane		-	-	-	0.27	-	0.3
52	β-Himachalene oxide	67.8	2.11	2.05	2.31	0.37	0.55	0.7
53	Himachalol	69.809	3	2.58	2.89	0.5	0.56	0.7
54	Allohimachalol	70.553	0.78	0.73		-	-	-
55	Cadalene	71.211	0.56	0.5	0.52	1.58	1.49	1.41
56	Heptadecane	72.134	0.35	0.4	0.41	-	-	-
57	2-Butyl-1-methyl-1,2,3,4-tetrahydronaphthalen-1-ol	72.733	1.61	1.52	1.98	-	-	-
58	Cyclopentanone, 3,3,4-trimethyl-4-(4-methylphenyl)-	73.152	1.98	1.87	2.33	-	-	-
59	N-Methyl-N-methoxy-5,6,7,8-tetrahydro-1-naphtamide	74.109	0.95	0.97	1.17	0.97	0.44	0.57
60	Octadecane	77.435	0.15	0.23	0.24	-	-	-
61	3a,9b-Dimethyl-1,2,3a,4,5,9b-hexahydrocyclopenta[a]naphthalen-3-one	80.205	0.77	1.03	1.2	-	-	-
62	Nonadecane	82.497	0.08	0.13	0.14	-	-	-

The predominant peaks detected in the samples are sesquiterpenes with the himachalene carbon skeleton. Notably, these include α-himachalene (39), γ-himachalene (41), α- and γ-dehydro-ar-himachalene (44, 46), ar-himachalene (47), oxidohimachalene (49), β-himachalene oxide (52), and himachalol (53). Such constituents are biosynthesized in high amounts by cedar trees [23–26]. Typically, archaeological samples containing coniferous substances preserve diterpenes [10,15,19]. However, these compounds are common across most coniferous trees, making it a challenge to differentiate among them based solely on diterpenes. In contrast, himachalenes are characteristic of *Cedrus* species, being largely absent in most other conifers. In the few instances they do occur in other species, they do so in very small amounts, as observed in the profiles of the *Abies* and *Juniperus* species (commonly known as fir and juniper, respectively). Since the himachalenes are the most abundant compounds in the mummification balm profiles (Table 1 and Figure 1), they can be confidently identified as cedar-derived substances. Modern degradation experiments underscore the resilience of certain himachalenes, notably α- and γ-dehydro-ar-himachalene (44, 46) and β-himachalene oxide (52), showcasing their stability against degradation [25]. This property renders them as invaluable biomarkers for identifying *Cedrus* species in archaeological materials. Interestingly, the mummification balm samples lacked β-himachalene, a compound that is the most abundant in modern cedar. However, this absence can be explained by the compound's susceptibility to degradation in archaeological contexts, resulting in the swift decomposition of this compound. Given this rapid degradation, its absence in ancient samples is anticipated [25].

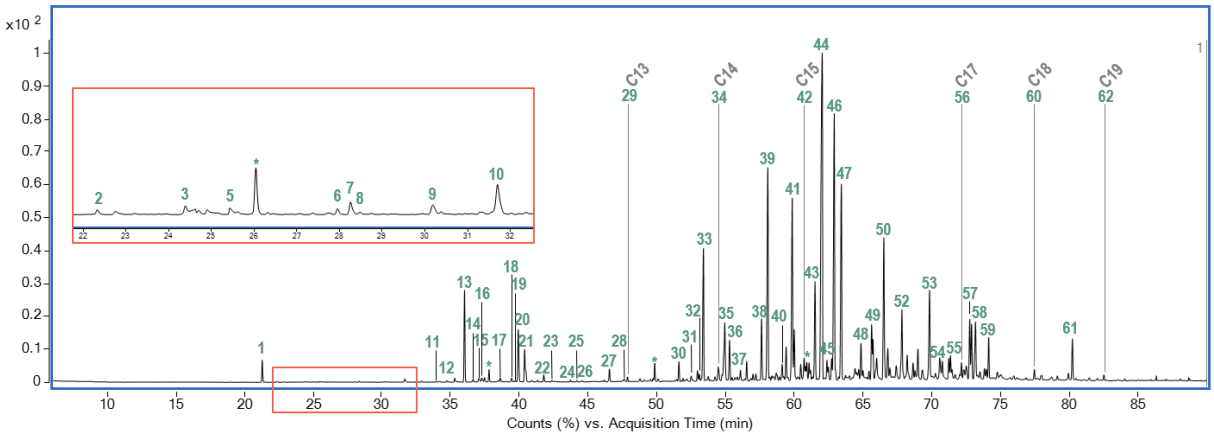


Figure 1. I Total ion current chromatogram of sample DA-AES-053 of the volatile fraction obtained by HS-SPME GC-MS. The numbers refer to compounds in Table 1. C13-19 indicates carbon numbers of n-alkanes. Peaks marked with * are modern contamination. The peaks in the red box show the compounds between retention time 22 and 32 in detail and are obtained in splitless mode.

Moreover, the presence of the identified himachalenes offers nuanced differentiation among plant components [24]. They are indicative of cedar wood exudates, such as essential wood oils or tars, but are conspicuously missing from tissues like leaves and cones [26–28]. Notably, they are also absent in resin (Figure 2) [29]. This suggests that the compounds in the ancient samples predominantly stemmed from products distilled specifically from cedar wood.

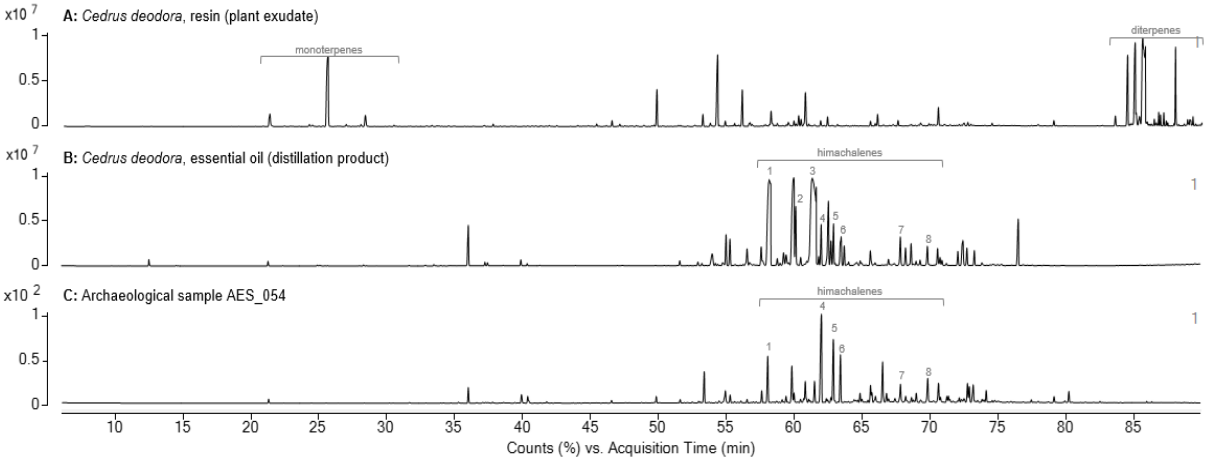


Figure 2. I Total ion current chromatogram of sample DA_AES-054 (C) compared to modern reference samples of *Cedrus* species: A) resin of modern *C. deodora* shows higher abundance of monoterpenes and diterpenes, himachalenes are absent. B) wood essential oil extract of modern *C. deodara*; himachalenes are the major compounds and diterpenes are absent. 1: α-himachalene, 2: Himachalene-1,4-diene, 3: β-Himachalene, 4-5: α- and γ-Dehydro-ar-himachalene, 6: ar-Himachalene, 7: β-Himachalene oxide, 8: Himachalol. Nos. 2 and 3 are only present in the modern samples, all other compounds are present in the archaeological samples as well.

Other significant sesquiterpenes identified in the volatile profiles were turmerones and curcumenes. Predominant among them is dihydro-ar-turmerone (50), while α-curcumene (40) and its structurally related compound, dihydro-ar-curcumene (38), were present in lesser amounts. Although these compounds are abundant in plants in the ginger family (Zingiberaceae), such as *Curcuma* (turmeric) and *Zingiber* (ginger) species, they are also present in cedar wood tar [28,30,31]. Given the clear indicators for cedar (namely, himachalenes) and the absence of other key turmerones typically found in gingers, we argue that these compounds also trace back to cedar plant material. These specific compounds further help distinguish between different products of cedarwood distillation, namely, essential oil and tar, which are produced through different methods. Essential oil is obtained via hydro or steam distillation from cedarwood, whereas tar is produced through dry distillation or pyrolysis. All three compounds do not appear in the chemical profiles of cedarwood essential oils [24], but emerge during wood dry distillation [30] – a procedure wherein cedarwood chips undergo heating in an oxygen-deprived environment, resulting in fumes that condense into a viscous, dark wood tar. The detection of cuparene (43) in the archaeological samples was also noteworthy, acting as an additional constituent found in the tar of cedar [30,31], but absent in the essential oil. The compounds dihydro-ar-turmerone (50), α-curcumene (40) and cuparene (43) have been previously reported in analyses on ancient Egyptian embalming materials [16,22], but were mostly not further discussed.

The hypothesis of dry distillation is further supported by the detection of the carbonaceous wood-tar creosote, such as phenol (5), o-cresol (9) and p-cresol (10), which are usually formed during pyrolysis of plant-derived material for producing tars and pitches [32]. However, it is worth noting that coniferous trees can metabolize small quantities of cresols themselves[33]. Taking together the presence of himachalenes as evidence for cedar wood distillation, and the several molecules pointing to a dry distillation process (turmerones, curcumenes, creosotes and cuparene), we propose the use of cedar wood tar as a key ingredient of the mummification balm. Moreover, dihydro-ar-turmerone (48), alongside markers for *Cedrus* species, emerges as a novel indicator of the dry distillation of cedar wood to produce cedar tar. Analyses of modern cedar species (*C. libani, C. atlantica, C. brevifolia*, and *C. deodara*) did not reveal significant differences in compound composition among species. As a result, the archaeological samples could only be precisely identified at the genus level.

By opting for the very sensitive HS-SPME extraction method, we successfully detected a range of odour-active monoterpenes and -terpenoids in samples 052-054. These include α- and β-pinene (1, 3), camphene (2), limonene (7), 4-acetyl-1-methylcyclohexene (13), pinocarveol (14), terpinen-4-ol (18), ethenone, 1-(4-methylphenyl)- (20), α-terpineol (21), and the sesquiterpenes α-longipinene (30) and longifolene (36). While all these compounds are present in modern cedar species [24,34], they are not exclusive to cedar. This means they could also hint at the inclusion of other terpene-rich plants during the fragrant embalming fluid's preparation. The detection of such volatile and odour-active molecules in archaeological contexts is a rarity. Remarkably, the balm remnants retained their scent even after 2600 years, a testament to their preservation. The balm's matrix likely functioned as a sealant, trapping some of these volatile compounds. In the process of sampling, we carefully removed the balm's uppermost layer, ensuring the sample extracted from within the balm residue still contained these preserved volatile molecules. Leveraging the sensitivity of our extraction method, we managed to capture many of these elusive compounds.

Analysis of solvent extracted semi-polar secondary metabolites and lipids using GC-MS and LC-MS/MS

The analysis of solvent extracts of the mummification balm brought to light more odorous substances. In this step, sub-samples of the embalming material were solvent extracted to analyse the non-volatile compounds of the mixture.

The LC-MS/MS results revealed the presence of several di- and triterpenoids within the semi-polar secondary metabolites. Notably, 7-oxo-dehydroabietic acid (7ODHA, Table 2) stands out among the diterpenoids, being observed in all samples. 7ODHA is an oxidized derivative of dehydroabietic acid (DHA) – a diterpenoid with the abietene-skeleton – which was present in lesser amounts. These compounds act as biomarkers for coniferous plant products, particularly resins from the Pinaceae family, including pine, larch, and cedar [15,17,19]. Given our demonstration of the presence of cedar tar in the sample, these compounds most likely originate from cedar too. Nonetheless, the addition of another Pinaceae resin or tar to the balm cannot be entirely discounted, especially since products from pine have been identified in other ancient Egyptian embalming materials [14,16]. The absence of the defunctionalized diterpenoid retene in the balm samples, which results from the thermal degradation of Pinaceae resin and is a marker for pine pitch, suggests the results are most likely indicative of a resin origin [12,16].

The triterpenoids detected in the samples suggest the use of more resinous substances. We observed the pentacyclic triterpenoids oleanonic acid, oleanolic acid /moronic acid, lupeol / ß-Amyrin as well as the tetracyclic terpenoid dipterocarpol (Table 2) in all of the samples. All these compounds are characteristic constituents of the resin of *Pistacia* spp. trees and have been reported in numerous studies on archaeological residues[10,20,35–37]. Some of the volatile compounds detected with HS-SPME (Table 1, Figure 1) are also present in *Pistacia* resin and might derive from this substance, namely α- and β-pinene (1, 3), camphene (2), p-Cymene (6), Verbenol (15) and Verbenone (22)[38]. Together, these compounds show clear evidence for the incorporation of *Pistacia* resins in the balm.

Table 2. Compound table of archaeological samples DA-AES 052-054 (canopic jar 1) and DA-AES 057-059 (canopic jar 2) obtained by LC-MS/MS.

Compounds	Canopic jar 1			Canopic jar 2		
	DA-AES-052	DA-AES-053	DA-AES-054	DA-AES-057	DA-AES-058	DA-AES-059
Resin acids	✓	✓	trace	×	×	×
7-oxodehydroabietic acid	✓	✓	✓	✓	✓	✓
Dehydroabietic acid	✓	✓	✓	×	×	×
Cholesterol	✓	✓	✓	✓	✓	✓
Campesterol	trace	✓	trace	trace	✓	trace
ß-Sitosterol	✓	✓	✓	✓	✓	✓
ß-Amyrin / Lupeol	✓	✓	✓	✓	✓	✓
Oleanonic acid / Moronic acids	✓	✓	✓	✓	✓	✓
Oleanolic acid	✓	✓	✓	✓	✓	✓
Dipterocarpol	✓	✓	✓	trace	✓	trace
arTurmerone	trace	trace	trace	✓	✓	✓
Vanillic acid	✓	✓	✓	✓	✓	✓

The GC-MS analysis of the lipid-containing phase revealed an array of lipids, encompassing fatty acids, *n*-alkanes, sterols, and phenolic compounds (Figure 3). The balm's constituents stem from both plant and animal origins. The lipid profile was dominated by high abundances of saturated even-carbon-numbered straight-chain fatty acids, with palmitic acid (C16:0) and stearic acid (C18:0) being predominant, and myristic acid (C14:0) in lesser amounts. Monounsaturated fatty acids, such as octadecenoic acid (C18:1), were also abundant in all samples. These fatty acids can arise from both degraded animal fats and plant oils [39–41]. The assumption of the presence of plant oils is strengthened by the detection of phytosterols, including ß-sitosterol and campesterol, both typical markers for plant-derived substances [42].

The samples also presented short-chain homologues, ranging from C6:0 to C9:0. Such short-chain fatty acids, along with identified short-chain α,ω-dicarboxylic acids like azelaic acid (diC9), are recognized as degradation by-products of plants, typically emerging during the aging or drying of plant oil due to oxidation and bond cleavage [14,19,41,43]. The appearance of odd-carbon-numbered straight-chain components, such as C15:0 and C17:0, and their branched isomers in the lipid profile signifies the additional incorporation of animal fat into the mummification balm [39]. This identification is further supported by the elevated levels of sterols like cholesterol and lanosterol, affirming the use of animal fat alongside plant oil [19].

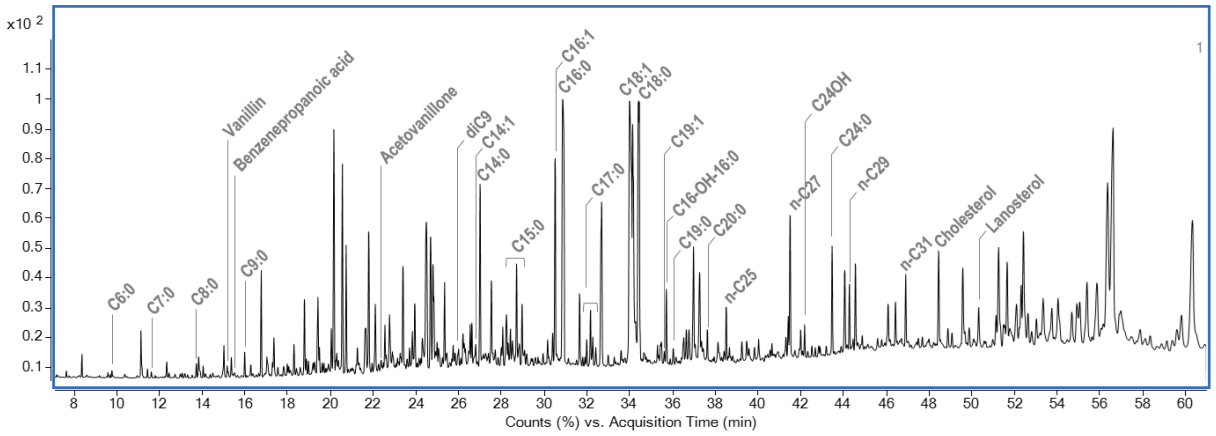


Figure 3 I Total ion current chromatogram of trimethylsilylated TLE extract of sample AES 053 with marked saturated and unsaturated fatty acids, n-alkanes, sterols and phenolic compounds.

The lipid fraction of the samples also yielded medium and long chain odd *n*-alkanes, namely C_{25} – C_{31}, with C_{27} as the most abundant compound (Figure 3). This series of *n*-alkanes is known to be characteristic of plant epicuticular waxes of higher terrestrial plants and beeswax [11,41,44,45]. The concurrent detection of wax esters (C_{40} to C_{50}) and the corresponding hydroxy wax esters through HT-GC-MS unequivocally points to the presence of beeswax in the mummification balms [13]. Medium chain *n*-alkanes, which were identified via HS-SPME GC-MS, appeared in minor concentrations and spanned both even and odd-numbered chains, from 13 to 19 carbon atoms (C_{13} – C_{19}), with the exception of C_{16} (See Figure 1). Moreover, when screening for the distinctive hopanes and steranes associated with bitumen (ions *m/z* 191 and *m/z* 217) – markers indicative of natural petroleum [46,47] – both were identified in the samples from each canopic jar, corroborating the additional incorporation of bitumen.

Phenolic compounds have been detected in the solvent extracts of the balm, namely vanillin, acetovanillone, vanillic acid and benzenepropanoic acid (Figure 3, Table 2). While vanillin, vanillic acid and acetovanillone are found in natural *Vanilla* extracts [48,49], they are also recognized as prevalent decomposition by-products of woody tissue and are indicative of lignin pyrolysis[49–51]. These compounds have been found in a number of previous studies[16,17,19], and Łucejko and colleagues have also postulated that they might indicate the use of wood tar [19]. Since cedar tar and plant oils are present in the sample, it is more likely that the phenolic compounds reflect the production of tar and suggest a degradation process caused by dry pyrolysis of wood.[26]

Discussion

A central discovery from our research was the unequivocal identification of substances derived from cedar, specifically indicating the use of cedar wood tar in the mummification balms. The pronounced presence of himachalenes, which are distinctive for the *Cedrus* species, solidified the argument for cedar use. Additional evidence for the dry distillation hypothesis emerged with the detection of turmerones, curcumenes, and wood-tar creosote. These findings not only facilitate a precise differentiation of coniferous plant materials down to the genus level but also shed light on the specific components of cedar trees employed, namely wood, and the extraction method – pyrolysis – to obtain cedar wood tar. This raises the question as to why the ancient Egyptians opted for cedar tar, especially considering the intricate expertise and effort it necessitates. Delving into the bioactive properties of cedar tar offers a possible rationale behind this choice.

Understanding the body's natural tendency to decompose after death, the ancient Egyptians needed materials abundant in bioactive compounds to guarantee long-lasting preservation of biological tissues. The analysis of the balm yielded a high number of active compounds, predominantly stemming from cedar tar, such as the himachalenes, which possess antimicrobial, antifungal, and insecticidal effects [24,26,30]. In particular, the compound himachalol (53) identified in the case study, exhibits strong insecticidal effects, acting as a deterrent to pests [52]. Additionally, monoterpenes α- and β-Pinene (1, 3) were detected, compounds recognized for their robust antifungal activity [53]. Cedar tar, in particular, has demonstrated significant inhibitory effects on bacterial growth, showcasing its ability to act against a variety of bacteria and fungi [31,54]. Intriguingly, not all plant parts of the cedar tree

contain the reactive himachalenes. This points to meticulous selection by the ancient Egyptians, showcasing their profound botanical knowledge and understanding of the preservative qualities of specific cedar products. While the exact mechanisms through which the ancient Egyptians recognized these properties remain speculative, our findings offer a molecular rationale for their empirical observations.

Beyond the tar's bioactivity, its distinctive and strong aroma offers another potential reason for its choice. This aroma was likely effective in covering the smell of decomposition and warding off insects. Historical figures such as Herodotus and Pliny the Elder have remarked upon a potent "juice" named *kedros* or *cedrium*, so strong (probably in its essence) that the Egyptians utilized it for mummification (Pliny the Elder, AD 77, Nat.hist., B. 5, Chap. 11; Herodotus, Historiai, B.II). Cedar tar would have therefore played a multifunctional role in the mummification process. Besides its preservation properties, its unique scent served practical purposes, acting as a natural repellent against moths and other pests. This combination of preserving and repelling qualities highlights the sophisticated knowledge and deliberate application of materials in ancient times.

While cedar tar seems to be a dominant ingredient, our analyses also emphasized the role of *Pistacia* resin and bitumen in the mummification balm. *Pistacia* resin exhibits antioxidant and antimicrobial activities [55]. Oxidation is a primary degradative process for many organic compounds [25]. Antioxidants might have played a role in scavenging free radicals and reducing oxidative degradation [56]. The solidifying nature of both *Pistacia* resin and bitumen suggest their pivotal role in ensuring moisture resistance, another crucial factor in long-term preservation of organic tissues. This provides further insights into potential synergy effects between the various constituents and the methods of the ancient Egyptians in selecting these specific ingredients for the mixture. *Pistacia* resin and bitumen must have provided an effective seal, preventing the escape of volatile organic compounds and inhibiting the ingress of external contaminants. This protective environment would have further ensured the longevity of the balm's active components.

In conclusion, we argue that the ancient Egyptian embalmers' choices were not arbitrary. They were rooted in a profound understanding of the properties of the materials at their disposal. Our findings underscore this knowledge, highlighting the interplay between the bioactive, aromatic, and symbolic properties of the ingredients. By unravelling the molecular

components of the mummification balms and demonstrating their bioactive capabilities, we gain a clearer window into the sophistication and selection of ancient Egyptian ritual practitioners. It reinforces the narrative of a civilization that went to great lengths to ensure the dignified passage of their deceased, merging spiritual beliefs with empirical knowledge, creating a legacy that has endured millennia.

Methods

Sampling of ancient mummification balms

Samples were taken from a set of canopic jars in the Egyptological collection of the Museum August Kestner, Hannover, Germany. Samples of the embalming material from canopic jar 1 were collected from various parts of the jar (lid, rim, and bottom) to assess variability of the balm material (see also Supplementary Figures S1 and S2 and Table S1). From the second canopic jar, we took samples only from the rim and the bottom of the jar, as the lid did not contain any organic residues. Before collecting the samples, approx. 1 mm of surface layer was removed at the specific sampling spots using disposable scalpels. Subsequently, samples were taken from inside the embalming material, to avoid contamination and them losing their volatile constituents. All samples were kept in glass vials that were previously combusted at 500 °C for 8 h, until further sub-sampling under clean lab conditions in the laboratory of the Max Planck Institute of Geoanthropology (formerly for the Science of Human History), Jena, Germany. For HS-SPME, subsamples were directly placed in a HS vial and tightly capped to retain evaporating volatiles within the vial.

Sampling of modern botanical samples of Cedrus species

Modern botanical reference samples of *C. libani*, *C. atlantica*, *C. brevifolia* and *C. deodara* were obtained from trees at the Botanical Garden Jena (courtesy of Prof. Frank Hellwig and Dr. Stefan Arndt) and dried in the laboratory. Leaves were collected from all cedar species; wood was only available from *C. atlantica* and *C. deodara.* For HS-SPME-GC-MS analysis of the volatile odour profiles of modern cedar species, samples were placed in HS vials directly after harvest and were analysed immediately.

Hydro-distillation of essential oils

Essential oils of the 4 different cedar species were hydro-distilled using a Clevenger apparatus. The plant material (~10 g fresh weight) was introduced into a 1 L round-bottom flask

containing distilled water (250 mL), and the mixture was brought to a boil. The vapours produced by boiling the organic material were condensed (water circulation at 5 °C) and collected in 1 mL of pentane. After 2 h of reflux, the pentane containing the essential oils was recovered using a Pasteur pipette and kept over ~50 mg Na_2SO_4 until analysis.

Materials

Methanol (MeOH), dichloromethane (DCM), and methyl *tert*-butyl ether (MTBE) used for the analyses were of analytical grade and obtained from Sigma-Aldrich (Munich, Germany), as well as the analytical standards α-pinene, (-)-β-pinene, (+)-longifolene, (+)-α-longipinene, campesterol, cholesterol, β-sitosterol, and vanillic acid. α-Terpineol and1-(4-methylphenyl)-ethanone were purchased from Thermo Fisher Scientific (Kandel, Germany), 4-acetyl-1-methylcyclohexene and (+)-(*S*)-ar-turmerone from ABCR (Karlsruhe, Germany), and camphene, eucalyptol and limonene from Agilent Technologies (Frankfurt, Germany). Furthermore, oleanonic acid and dipterocarpol were obtained from Santa Cruz Biotechnology (Heidelberg, Germany), 7-oxodehydroabietic acid from Campro Scientific (Berlin, Germany), oleanolic acid from LGC Standards (Wesel, Germany), moronic acid from TCI chemicals (Eschborn, Germany), and dehydroabietic acid from Carbosynth (Berkshire, UK). MS-grade formic acid was purchased from VWR (Leuven, Belgium), while acetonitrile (ACN) and water used for HPLC-MS/MS analyses were purchased from Biosolve (Valkenswaard, Netherlands).

Extraction and Analysis

Biomolecular characterization and identification were carried out using a dual extraction approach targeting volatile as well as hydrophobic and polar compounds. As most volatile compounds (mono- and sesquiterpenes) can disappear during the solvent extraction process and trimethylsilylation [57,58], HS-SPME was used for detecting organic volatile compounds from a complex matrix (see extraction procedure A). Additionally, for the non-volatile compounds we applied a solvent-extraction procedure that sequentially extracted semi-polar and polar secondary metabolites as well as lipids from the same sample, since most archaeological samples have a limited amount of starting material (see extraction procedure B).

Extraction Procedure A: For this solvent-free sample preparation technique, approx. 20-40 mg of the archaeological samples were placed in an oven-cleaned 4 ml glass vial equipped with screw caps containing PTFE-silicon septa. For the extraction of fresh plant material from cedar

species, approx. 1 g of plant material was used. A polydimethylsiloxane (PDMS) fiber (Supelco, Bellefonte, PA) with a coating thickness of 100 µm was chosen for trapping volatile and semi-volatile analytes. Extraction was performed by injecting the syringe needle through the septum of the vial and releasing the SPME fiber into the headspace above the sample. The fiber was maintained approx. 1 cm above the sample for 45 min at 60°C. Subsequently, the analytes were thermally desorbed for 3 min at 250°C in the GC injector port in splitless mode or at a split ratio of 5:1.

Extraction Procedure B: Subsamples were subjected to an additional sample pre-treatment following established protocols [59,60] with modifications made for the extraction of ancient samples. In summary, approx. 50 mg of subsample was finely pulverized and subjected to solvent extraction using an MTBE: MeOH (3:1, v/v) solution. This mixture was vortexed, followed by agitation for 45 minutes, and ultrasonicated for an additional 15 minutes. A H2O: MeOH (3:1, v/v) solution was subsequently introduced to the sample and once again thoroughly mixed. Centrifugation proceeded at 20,000 x g for 5 minutes, resulting in the formation of a dense protein pellet at the bottom, a lower liquid phase enriched with semi-polar and polar metabolites and an upper phase containing hydrophobic lipids. Both liquid phases were transferred into fresh glass vials. The residual protein pellet was stored for palaeoproteomic analysis. A portion of the samples from the lipid-rich phase underwent derivatization using 100 µl of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA, inclusive of 1% TMCS, procured from Sigma-Aldrich). The sample was kept for an hour at 70°C, and subsequently analyzed by GC-MS. The bottom layer, containing metabolites, was evaporated in a vacuum concentrator and reconstituted in HPLC-grade MeOH before LC-ESI-MS/MS analysis.

GC-MS evaluations were conducted with an Agilent 8890 GC-System paired to an Agilent 5977B GC/MSD, equipped with an HP-5ms capillary column (Agilent, 60 m x 250 µm, 0.25 µm film thickness). The mass spectrometer was used in electron impact (EI) mode at 70eV, with helium as the carrier gas. The GC oven temperature for HS-SPME samples was held isothermally for 2 min at 40°C, ramped to 200°C at 2 °C/min, held for 1 min, increased at a rate of 10°C/min and held at 250°C for 2 min, resulting in a run time of 90 min. The injector was equipped with a narrow-bore SPME inlet liner (0,75 mm Ø, Supelco) to improve resolution. Transfer line and source temperature were set at 250°C and 230°C, respectively.

The mass range was scanned from *m/z* 20 to 400. The fiber was conditioned after each sample and blanks were run thereafter to monitor for cross contamination. For the trimethylsilylated lipid aliquots, the oven temperature was programmed in the following manner: initial temperature was set at 60°C, maintaining this temperature for 2 minutes, then raised to 120°C at a pace of 30°C/min, holding for another 2 minutes. Subsequently, the temperature was raised at a rate of 5°C/min up to 320°C, holding for a final 15 minutes. The entire procedure lasted 61 minutes, with a solvent delay of 6.5 minutes. An injection volume of 1µl was used, and a 10:1 split ratio was applied. The scan range spanned from m/z 40 to 700. To monitor contamination, blanks were injected between each sample. Analysis and interpretation of the GC–MS data utilized the Agilent MassHunter Qualitative Data Analysis software 10.0. Peaks were identified by aligning with the retention times and mass spectra of available analytical standards, referencing the NIST (2.2) mass spectral library, and correlating with published spectra.

Utilizing a Shimadzu LCMS-8050 triple-quadrupole system, LC-ESI-MS/MS analyses were carried out. The chromatographic separation was performed on a Shimadzu Shimpack Velox SP-C18 column (100mm x 2.1mm, 2.7µm) and a Restek Raptor Biphenyl analytical column (100mm x 2.1mm, 2.7µm). The system was equipped with LC-30AD binary pumps, a DGU-20A5R solvent degasser, CTO-20AC column oven, and a SIL-30AC auto sampler. The gradient, which involved mobile phase A (containing HPLC-grade H2O and 0.1% formic acid) and mobile phase B (consisting of ACN), was structured as follows: Beginning at 0.5% B from 0-1 min, it increased to 80% B by 10 min, ascended to 100% B by 15 min, held until 17.5 min, and reverted to 0.5% B by 20 min. The column temperature was consistently set at 25 °C. For the C18 analyses, the solvent flow rate was set at 0.2 mL/min and 0.3 mL/min for the biphenyl evaluations. Depending on the specific sample concentration, injection volumes ranged from 1 to 2 µL. Using an electro spray ionization (ESI) source, ionization was achieved with detection occurring in both positive and negative ion modes. Every sample underwent duplicate analysis to ensure accuracy. The preferred analytical method for these examinations was the multiple reaction monitoring (MRM) approach. Analytical standards were employed to optimize the MRM settings, facilitating the identification of specific compounds within ancient samples (see Supplementary Table S2). All gathered LC–MS/MS data were processed and analysed using the LabSolutions software from Shimadzu, Kyoto, Japan.

References

1. Germer, R. *Handbuch der altägyptischen Heilpflanzen*. (Harrassowitz, 2008).

2. Manniche, L. *Sacred luxuries: fragrance, aromatherapy, and cosmetics in Ancient Egypt*. (Cornell University Press, 1999).

3. Pommerening, T. Wege zur Identifikation altägyptischer Drogennamen – eine kritische Betrachtung. *Zwischen Philologie und Lexikographie des Ägyptisch-Koptischen. Akten der Leipziger Abschlusstagung des Akademienprojekts 'Altägyptisches Wörterbuch' (Abhandlungen der Sächsischen Akademie der Wissenschaften zu Leipzig.* 82–111 (2016).

4. Evershed, R. P. & Clark, K. A. Trends in Use of Organic Balms in Egyptian Mummification Revealed Through Biomolecular Analyses. in *The Handbook of Mummy Studies: New Frontiers in Scientific and Cultural Perspectives* (eds. Shin, D. H. & Bianucci, R.) 1–63 (Springer, 2020). doi:10.1007/978-981-15-1614-6_9-1.

5. Germer, R. *Mumien*. (Albatros, 2005).

6. Abdel-Maksoud, G. & El-Amin, A.-R. A review on the materials used during the mummification processes in ancient Egypt. *Mediterranean Archaeology and Archaeometry* **11**, 129–150 (2011).

7. Ikram, S. & Dodson, A. *The mummy in ancient Egypt: equipping the dead for eternity*. (Thames & Hudson, 1998).

8. Abegaz, B. M. & Kinfe, H. H. Secondary metabolites, their structural diversity, bioactivity, and ecological functions: An overview. *Physical Sciences Reviews* **4**, (2019).

9. Taylor, J. H. *Journey through the afterlife: ancient Egyptian Book of the dead*. (Harvard University Press ; British Museum Press, 2010).

10. Colombini, M. P., Modugno, F., Silvano, F. & Onor, M. Characterization of the Balm of an Egyptian Mummy from the Seventh Century B.C. *Studies in Conservation* **45**, 19–29 (2000).

11. Buckley, S. A., Clark, K. A. & Evershed, R. P. Complex organic chemical balms of Pharaonic animal mummies. *Nature* **431**, 294–299 (2004).

12. Marković, M., Mezzatesta, E., Porcier, S., Vieillescazes, C. & Mathe, C. Rethinking the Process of Animal Mummification in Ancient Egypt: Molecular Characterization of Embalming Material and the Use of Brassicaceae Seed Oil in the Mummification of Gazelle Mummies from Kom Mereh, Egypt. *Molecules* **27**, 1532 (2022).

13. Huber, B. *et al.* Biomolecular characterization of 3500-year-old ancient Egyptian mummification balms from the Valley of the Kings. *Sci Rep* **13**, 12477 (2023).

14. Jones, J., Higham, T. F. G., Oldfield, R., O'Connor, T. P. & Buckley, S. A. Evidence for Prehistoric Origins of Egyptian Mummification in Late Neolithic Burials. *PLoS ONE* **9**, e103608 (2014).

15. Brettell, R., Martin, W., Atherton-Woolham, S., Stern, B. & McKnight, L. Organic residue analysis of Egyptian votive mummies and their research potential. *Studies in Conservation* **62**, 68–82 (2017).

16. Łucejko, J. J., Lluveras-Tenorio, A., Modugno, F., Ribechini, E. & Colombini, M. P. An analytical approach based on X-ray diffraction, Fourier transform infrared spectroscopy and gas chromatography/mass spectrometry to characterize Egyptian embalming materials. *Microchemical Journal* **103**, 110–118 (2012).

17. Fulcher, K., Serpico, M., Taylor, J. H. & Stacey, R. Molecular analysis of black coatings and anointing fluids from ancient Egyptian coffins, mummy cases, and funerary objects. *PNAS* **118**, e2100885118 (2021).

18. Rageot, M. *et al.* Biomolecular analyses enable new insights into ancient Egyptian embalming. *Nature* **614**, 287–293 (2023).

19. Łucejko, J., Connan, J., Orsini, S., Ribechini, E. & Modugno, F. Chemical analyses of Egyptian mummification balms and organic residues from storage jars dated from the Old Kingdom to the Copto-Byzantine period. *Journal of Archaeological Science* **85**, 1–12 (2017).

20. Charrié-Duhaut, A. *et al.* The canopic jars of Rameses II: real use revealed by molecular study of organic residues. *Journal of Archaeological Science* **34**, 957–967 (2007).

21. Koller, J., Baumer, U., Kaup, Y., Schmid, M. & Weser, U. Analysis of a pharaonic embalming tar. *Nature* **425**, 784–784 (2003).

22. Koller, J., Baumer, U., Kaup, Y. & Weser, U. Herodotus' and Pliny's embalming materials identified on ancient Egyptian mummies. *Archaeometry* **47**, 609–628 (2005).

23. Chaudhary, A., Kaur, P., Singh, B. & Pathania, V. Chemical composition of hydrodistilled and solvent volatiles extracted from woodchips of Himalayan Cedrus: Cedrus deodara (Roxb.) Loud. *Nat Prod Commun* **4**, 1257–1260 (2009).

24. Loizzo, M. R. *et al.* Phytochemical analysis and in vitro evaluation of the biological activity against herpes simplex virus type 1 (HSV-1) of Cedrus libani A. Rich. *Phytomedicine* **15**, 79–83 (2008).

25. Huber, B., Vassão, D. G., Roberts, P., Wang, Y. V. & Larsen, T. Chemical Modification of Biomarkers through Accelerated Degradation: Implications for Ancient Plant Identification in Archaeo-Organic Residues. *Molecules* **27**, 3331 (2022).

26. Saab, A. M., Harb, F. Y. & Koenig, W. A. Essential oil components in heart wood of Cedrus libani and Cedrus atlantica from Lebanon. *Minerva Biotecnologica* **17**, 159–161 (2005).

27. Jaouadi, I. *et al.* Chemical variability and antioxidant activity of Cedrus atlantica Manetti essential oils isolated from wood tar and sawdust. *Arabian Journal of Chemistry* **14**, 103441 (2021).

28. Kurt, Y., Suleyman Kaçar, M. & Isik, K. Traditional Tar Production from Cedrus libani A. Rich on the Taurus Mountains in Southern Turkey. *Econ Bot* **62**, 615–620 (2008).

29. Bailly, L., Adam, P., Charrié, A. & Connan, J. Identification of alkyl guaiacyl dehydroabietates as novel markers of wood tar from Pinaceae in archaeological samples. *Organic Geochemistry* **100**, 80–88 (2016).

30. Skanderi, I. & Chouitah, O. Chemical Characterization and Antioxidant Activity of Cedrus atlantica Manetti Tar (Atlas Cedar Tar). *French-Ukrainian Journal of Chemistry* **08**, 244–255 (2020).

31. Pekgözlü, A. K., Kuştaş, S., Mercan, B. & Biçer, A. Chemical Characterization of Lebonan Cedar Tar. *Drvna ind.* **68**, 107–112 (2017).

32. Kester, J. E. Creosote (Coal Tar Creosote and Wood Creosote). in *Encyclopedia of Toxicology* 1055–1060 (Elsevier, 2014). doi:10.1016/B978-0-12-386454-3.00295-5.

33. Fiege, H. Cresols and Xylenols. in *Ullmann's Encyclopedia of Industrial Chemistry* (ed. Wiley-VCH Verlag GmbH & Co. KGaA) 08–25 (Wiley-VCH Verlag GmbH & Co. KGaA, 2000). doi:10.1002/14356007.a08_025.

34. Saab, A. *et al.* Phytochemical Analysis and Cytotoxicity Towards Multidrug-Resistant Leukemia Cells of Essential Oils Derived from Lebanese Medicinal Plants. *Planta Med* **78**, 1927–1931 (2012).

35. Stern, B., Heron, C., Corr, L., Serpico, M. & Bourriau, J. Compositional variations in aged and heated pistacia resin found in late bronze age canaanite amphorae and bowls from Amarna, Egypt. *Archaeometry* **45**, 457–469 (2003).

36. Brettell, R. C., Stern, B., Reifarth, N. & Heron, C. The 'Semblance of Immortality'? Resinous Materials and Mortuary Rites in Roman Britain. *Archaeometry* **56**, 444–459 (2014).

37. Huber, B. *et al.* Interdisziplinäre Untersuchungen von Räuchergefäßen zur Rekonstruktion antiker Gerüche. *e-Forschungsberichte des DAI* **2**, 120–125 (2018).

38. Xynos, N., Termentzi, A., Fokialakis, N., Skaltsounis, L. A. & Aligiannis, N. Supercritical CO2 extraction of mastic gum and chemical characterization of bioactive fractions using LC-HRMS/MS and GC–MS. *The Journal of Supercritical Fluids* **133**, 349–356 (2018).

39. Whelton, H. L. *et al.* A call for caution in the analysis of lipids and other small biomolecules from archaeological contexts. *Journal of Archaeological Science* **132**, 105397 (2021).

40. Roffet-Salque, M. *et al.* From the inside out: Upscaling organic residue analyses of archaeological ceramics. *Journal of Archaeological Science: Reports* **16**, 627–640 (2017).

41. Dunne, J., Mercuri, A. M., Evershed, R. P., Bruni, S. & di Lernia, S. Earliest direct evidence of plant processing in prehistoric Saharan pottery. *Nature Plants* **3**, 16194 (2016).

42. Gylling, H. & Simonen, P. Phytosterols, Phytostanols, and Lipoprotein Metabolism. *Nutrients* **7**, 7965–7977 (2015).

43. Dunne, J. *et al.* First dairying in green Saharan Africa in the fifth millennium bc. *Nature* **486**, 390–394 (2012).

44. Drieu, L. *et al.* A Neolithic without dairy? Chemical evidence from the content of ceramics from the Pendimoun rock-shelter (Castellar, France, 5750–5150 BCE). *Journal of Archaeological Science: Reports* **35**, 102682 (2021).

45. Maurer, J., Möhring, T., Rullkötter, J. & Nissenbaum, A. Plant Lipids and Fossil Hydrocarbons in Embalming Material of Roman Period Mummies from the Dakhleh Oasis, Western Desert, Egypt. *Journal of Archaeological Science* **29**, 751–762 (2002).

46. Clark, K. A., Ikram, S. & Evershed, R. P. The significance of petroleum bitumen in ancient Egyptian mummies. *Phil. Trans. R. Soc. A* **374**, 20160229 (2016).

47. Connan, J. Use and trade of bitumen in antiquity and prehistory: molecular archaeology reveals secrets of past civilizations. *Phil. Trans. R. Soc. Lond. B* **354**, 33–50 (1999).

48. Brunschwig, C., Collard, F. X., Bianchini, J.-P. & Raharivelomanana, P. Evaluation of Chemical Variability of Cured Vanilla Beans (*Vanilla tahitensis* and *Vanilla planifolia*). *Natural Product Communications* **4**, 1934578X0900401 (2009).

49. Lesage-Meessen, L. *et al.* A two-step bioconversion process for vanillin production from ferulic acid combining Aspergillus niger and Pycnoporus cinnabarinus. *Journal of Biotechnology* **50**, 107–113 (1996).

50. Tamburini, D., Łucejko, J., Ribechini, E. & Colombini, M. P. New markers of natural and anthropogenic chemical alteration of archaeological lignin revealed by in situ pyrolysis/silylation-gas chromatography mass spectrometry. *Journal of Analytical and Applied Pyrolysis* **118**, 249–258 (2016).

51. Modugno, F., Ribechini, E. & Colombini, M. P. Aromatic resin characterisation by gas chromatography-mass spectrometry. Raw and archaeological materials. *Journal of chromatography. A* **1134**, 298–304 (2006).

52. Singh, D. & Agarwal, S. K. Himachalol and β-himachalene: Insecticidal principles of himalayan cedarwood oil. *J Chem Ecol* **14**, 1145–1151 (1988).

53. *Flavours and Fragrances: Chemistry, Bioprocessing and Sustainability*. (Springer Berlin Heidelberg, 2007). doi:10.1007/978-3-540-49339-6.

54. Mercimek Takic, H., Aysun, Ucan Turkmen, F. & Sari, M. Effect of cedar (Cedrus libani a. Rich) tar on bacterial growth. *J microb biotech food sci* **9**, 805–808 (2020).

55. Benhammou, N., Bekkara, F. A. & Panovska, T. K. Antioxidant and antimicrobial activities of the Pistacia lentiscus and Pistacia atlantica extracts. *African Journal of Pharmacy and Pharmacology* **2**, 22–28 (2008).

56. Lobo, V., Patil, A., Phatak, A. & Chandra, N. Free radicals, antioxidants and functional foods: Impact on human health. *Phcog Rev* **4**, 118–126 (2010).

57. Hamm, S., Bleton, J. & Tchapla, A. Headspace solid phase microextraction for screening for the presence of resins in Egyptian archaeological samples. *J. Sep. Science* **27**, 235–243 (2004).

58. Capetti, F. *et al.* Exploiting the versatility of vacuum-assisted headspace solid-phase microextraction in combination with the selectivity of ionic liquid-based GC stationary phases to discriminate *Boswellia* spp. resins through their volatile and semivolatile fractions. *J. Sep. Sci.* **43**, 1879–1889 (2020).

59. Matyash, V., Liebisch, G., Kurzchalia, T. V., Shevchenko, A. & Schwudke, D. Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics. *Journal of Lipid Research* **49**, 1137–1146 (2008).

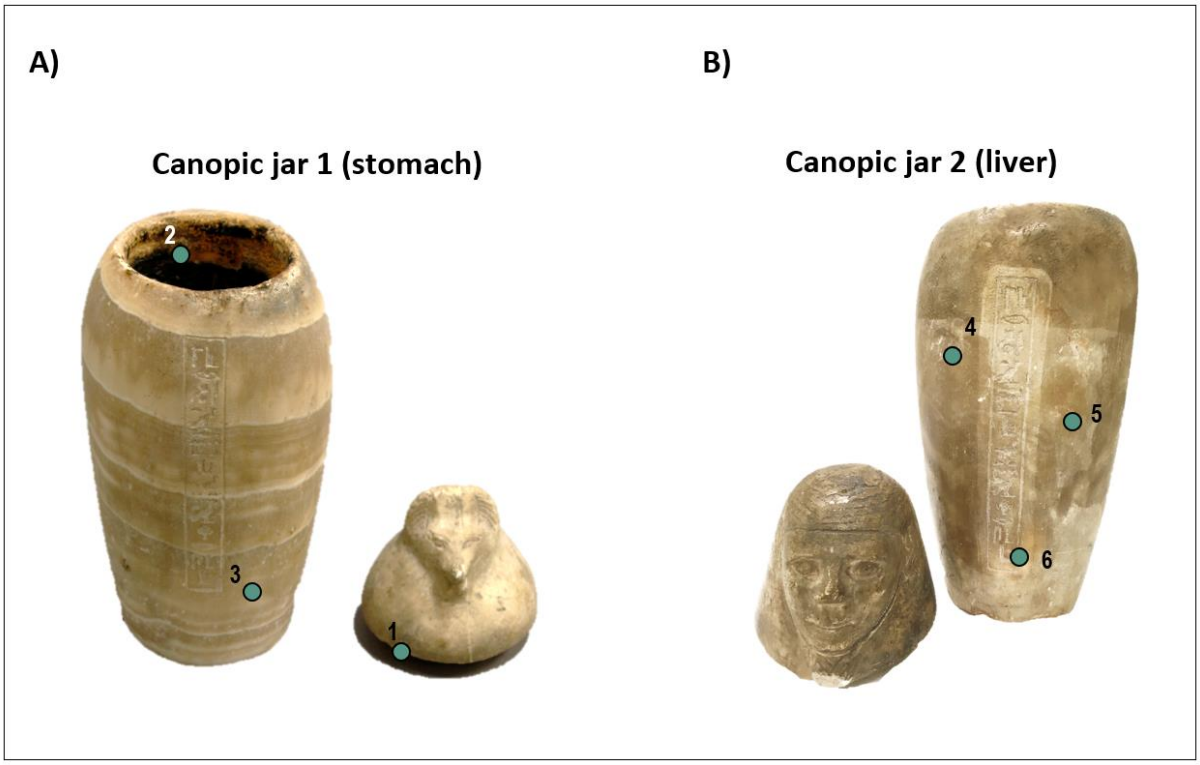
60. Salem, M., Bernach, M., Bajdzienko, K. & Giavalisco, P. A Simple Fractionated Extraction Method for the Comprehensive Analysis of Metabolites, Lipids, and Proteins from a Single Sample. *JoVE* 55802 (2017) doi:10.3791/55802.

SUPPLEMENTARY INFORMATION

Metabolic profiling reveals the aromatic and bioactive properties of ancient Egyptian mummification balms

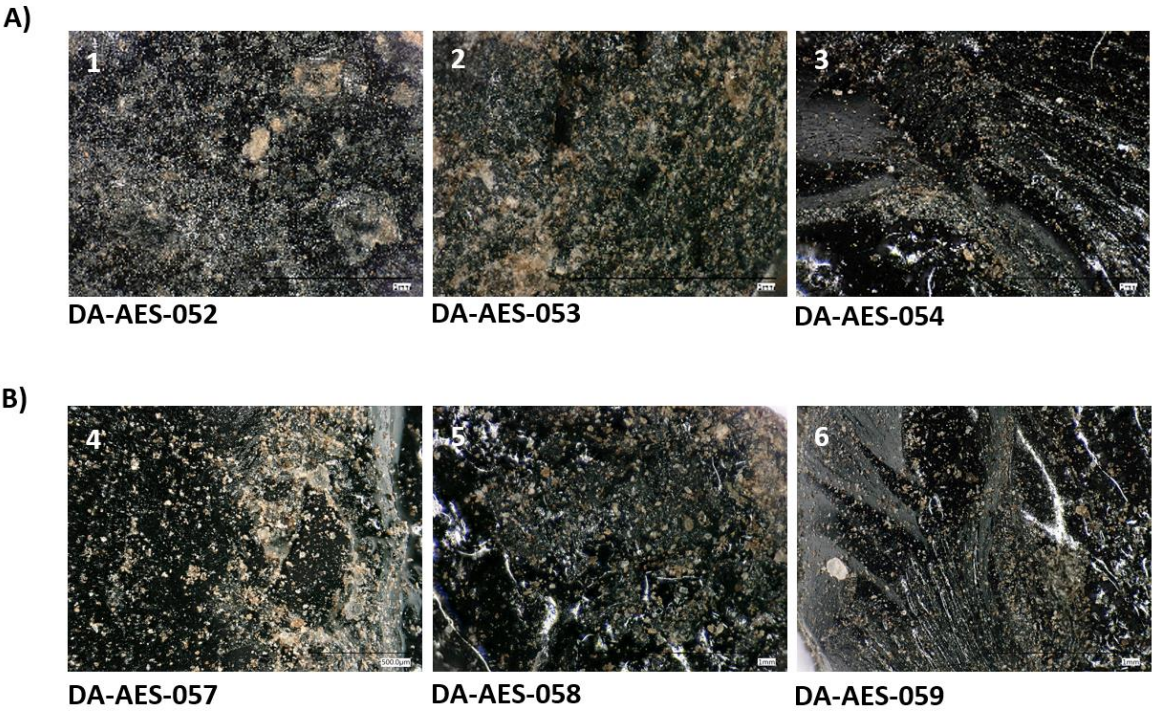
Huber, B., Loeben, C. E., Giddings Vassão, Perruchini, E., Hellwig, F., Arndt, S., Hammann, S., Spengler, R. N., Roberts, P., Boivin, N., Devièse, T.

Supplementary Figure S1



Canopic jars of the lady Tas-nechet (T3s-nht), ca. 600 BCE, 26th Dynasty, at the Museum August Kestner, Hannover, Germany. A) Position of samples taken from the jar and the lid of canopic jar 1, containing the stomach. B) Positions of samples taken from inside the canopic jar 2, which held the liver. For a description and microscope images of the samples see Figure S2 and Table S1.

Supplementary Figure S2



Digital microscope images (Keyence VHX6000) of samples DA-AES-052-054 of canopic jar 1 and samples DA-AES-057-59 taken from canopic jar 2.

Supplementary Table S1

Lab sample no.	***Sample location***	***Reference nos. in Fig. S1***	***Description of sample***	***Object no.***	***Dating***
DA-AES 052	Surface, lid	1	Black, thin remnant of balm on the surface of the lid	Acq. No. 1880, Canopic jar of Tas-nechet (stomach)	Late Period, c. 600 BCE
DA-AES 053	Interior, wall of jar	2	Thick black layer of balm on the wall of the jar	Acq. No. 1880, Canopic jar of Tas-nechet (stomach)	Late Period, c. 600 BCE
DA-AES 054	Interior, bottom of jar	3	Black residual remnants in the middle of the bottom of the jar	Acq. No. 1880, Canopic jar of Tas-nechet (stomach)	Late Period, c. 600 BCE
DA-AES-057	Interior, wall of jar	4	Thick black layer of balm on the wall of the jar	Acq. No. 1881, Canopic jar of Tas-nechet (liver)	Late Period, c. 600 BCE
DA-AES 058	Interior, wall of jar	5	Thick black layer of balm on the wall of the jar	Acq. No. 1881, Canopic jar of Tas-nechet (liver)	Late Period, c. 600 BCE
DA-AES 059	Interior, bottom of jar	6	Black residual remnants in the middle of the bottom of the jar	Acq. No. 1881, Canopic jar of Tas-nechet (liver)	Late Period, c. 600 BCE

The table provides a description of the individual samples with all relevant information regarding the museum objects from which they derive.

Supplementary Table S2

List of authentic analytical standards for the optimization of MRM parameters employed to screen for specific compounds in archaeological samples (for more detailed MRM parameters, such as collision energy (V) and dwell times for precursor and product ions see Supplementary Table S4 – separate excel file).

Compound	**Column**	**Ret. Time (min)**
Amygdalin	Velox SP-C18	5.834
Anabasine	Velox SP-C18	1.573
Artemisinin	Velox SP-C18	10.677
Asiatic Acid	Velox SP-C18	9.967
Benzoic Acid	Velox SP-C18	7.418
Betulinic Acid	Velox SP-C18	14.410
Caffeine	Velox SP-C18	6.097
Cinnamic Acid	Velox SP-C18	8.217
Cotinine	Velox SP-C18	5.158
Coumarin	Velox SP-C18	8.069
Curcumin	Velox SP-C18	10.065
Demethoxycurcumin	Velox SP-C18	9.935
Didemethoxycurcumin	Velox SP-C18	9.799
Ferulic Acid	Velox SP-C18	6.830
(E)-Guggulsterone	Velox SP-C18	11.676
(Z)-Guggulsterone	Velox SP-C18	12.176
Harmaline	Velox SP-C18	6.405
Harmane	Velox SP-C18	6.157
Harmine	Velox SP-C18	6.449
Hydrocotarnine	Velox SP-C18	5.935
Incensole	Velox SP-C18	13.904
Meconic Acid	Velox SP-C18	5.240
Nicotine	Velox SP-C18	1.560
Nicotinic Acid	Velox SP-C18	1.564
Oleanolic Acid	Velox SP-C18	14.647
Opianic Acid	Velox SP-C18	6.530
Quinine	Velox SP-C18	5.978
Theobromine	Velox SP-C18	5.553
ar-Turmerone	Velox SP-C18	12.066
Vanillic Acid	Velox SP-C18	6.221
Zingerone	Velox SP-C18	7.475
Resin Acids (pimaric acid, isopimaric acid, palustric acid and neoabietic acid)	Biphenyl	10.400
7-Oxodehydroabietic Acid	Biphenyl	9.411
Dehydroabietic Acid	Biphenyl	10.091
α-Boswellic Acid	Biphenyl	11.101
β-Boswellic Acid	Biphenyl	11.296
Acetyl α-Boswellic Acid	Biphenyl	11.935
Acety β-Boswellic Acid	Biphenyl	12.159
Keto β-Boswellic Acid	Biphenyl	10.414

Acetyl Keto β-Boswellic Acid	Biphenyl	11.309
Cholesterol	Biphenyl	12.292
Campesterol	Biphenyl	12.493
β-Sitosterol	Biphenyl	12.727
Brassicasterol	Biphenyl	12.378
5α-Cholestanol	Biphenyl	12.544
Stigmasterol	Biphenyl	12.681
Sitostanol	Biphenyl	12.998
Cholestanone	Biphenyl	13.376
α-Amyrin	Biphenyl	12.871
β-Amyrin+Lupeol	Biphenyl	12.620
Benzoic Acid	Biphenyl	6.141
Ferulic Acid	Biphenyl	5.876
Dammarenolic Acid	Biphenyl	10.723
Masticadienolic Acid	Biphenyl	10.912
Moronic+Oleanonic Acids	Biphenyl	11.010
Dipterocarpol	Biphenyl	11.475
Urs-12-en-3-one	Biphenyl	13.538

Appendix 3 – Supplementary datasets

The following datasets can be downloaded at the below links.

Manuscipt B:

https://www.mdpi.com/article/10.3390/molecules27103331/s1

Manuscipt C:

https://static-content.springer.com/esm/art%3A10.1038%2Fs41598-023-39393-y/MediaObjects/41598_2023_39393_MOESM4_ESM.xls

Appendix 4 – Frangrance card