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Analysis of organic residues from the Châteaumeillant oppidum (Cher, France) using GC–MS

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1. Introduction

Roman amphorae were found around the Late Bronze Age, first amongst the Canaanites, then in Egypt and finally, in all regions of the Mediterranean, particularly amongst the Greeks, then amongst the Romans as from 700 BCE. Their main function was the transport and storage of produce and commodities [1], for example, oils, wines, brines, fish and other preparations [2]. Archaeologists are in the privileged position of discovering and classifying the amphorae. The typology of the amphora was first based on one developed by Heinrich Dressel. His study provides information of when and where the produce was grown and harvested [1, 3]. The presence of a stamp or a craftsman's mark on the edge of the amphorae also enables the produce, its quantity and its producer to be identified [4]. However, in the case of an empty amphora, without an inscription and without any observable and recognisable elements, identification of its contents might prove difficult. In addition, the typology and classification are not always significant because an amphora was sometimes re-used, as demonstrated by the amphorology [1]. Chemical analysis in all cases can be useful in providing further information. As for the content, the typology of Dressel I indicates the presence of wine. The first archaeological study of the wine in an amphora was that of a jar from Hajji Firuz Tepe, a Neolithic village in the Zagros mountains of Northern Iran. The estimated period would be 5400–5000 BCE. Patrick E. McGovern and a team from the University of Pennsylvania Museum detected the calcium salt of tartaric acid using Fourier-Transform Infrared spectroscopy (FT-IR) and High-Performance Liquid Chromatography (HPLC) with an Ultraviolet (UV) detector [5, 6]. McGovern's chemical analyses on several archaeological sites have revealed the presence of tartaric acid, one of the main markers of wine [7–10]. It is the most abundant acid found in wine and its role is important in the fermentation process [11]. The chemical analysis of the amphorae must also take into account the presence of an internal waterproofing layer. Since ancient times, manufacturers of amphorae, not unlike boat builders [16], have widely exploited resin for waterproofing purposes [12–15]. During the fabrication of amphorae this sealant was coated with a pitch. The pitch originated from the transformation of a basic "softwood" material (Coniferae order, Pinaceae family, especially *Pinus sp.*) [16, 17]. The conifer tree contains mono-, sesqui-, and diterpenic compounds [18]. Pimaranes and abietanes are notably found in the amphorae study [13, 19]. Chemical skeletons of these main compounds such as abietic, pimaric and dehydroabietic acids are shown in Fig. 1. The techniques of propagating pitch in Roman times are discussed in ancient texts. The pitch, black to light ochre in colour, is not a natural raw material and its chemical composition depends on the heating conditions. The pyrogenation of coniferous wood in a closed environment occurs through methylation of dehydroabietic acid. During the intense heating of coniferous wood in an open environment (incomplete combustion), however, some compounds such as 18-norabieta-8,11,13-trien, 19-norabieta-8,11,13-trien, simonellite, tetrahydrotene and retene are formed. This study was focused on eleven Roman amphorae (Dressel I) coming from the excavation site of the Châteaumeillant oppidum (Cher, France). The aim of this study is to characterise the organic material adsorbed on the internal surfaces of these objects, as well as the amphorae's waterproofing layer and contents. This study also raised the question of the use of resin as additive matter in the case of resinated wine. Gas chromatography-mass spectrometry (GC–MS) analyses were performed on traces of organic matter impregnated on shards of these amphorae. In fact, no visible traces of pitch were visible on any of the studied objects. This analytical technique is frequently applied to archaeological organic residue in order to identify various molecules corresponding to terpenoids, notably diterpenic resinous materials from the Pinaceae family and acid molecules of wine [20–22]. The chemical analyses are based on the identification of diagnostic molecules of each natural product.

Fig. 1. Structure of the diterpenes in this study.

2. Archaeological history of the Châteaumeillant oppidum

The Châteaumeillant oppidum belongs to the territory of the Bituriges the limits of which correspond approximately to those of the current French departments of Cher and Indre, to which must be added part of the departments of Allier and Loir-et-Cher (Fig. 2). Located about 60 km from Bourges- *Avaricum* , the capital of this territory, the *Mediolanum* of the Table de Peutinger occupies a privileged position at the southern end of the Berry region, not far from the borders of the Arvernes and the Lémovices territory. The *oppidum* is installed on the highest part of a rectangular promontory of 60 hectares. orientated north-south, this plateau is framed by two parallel rivers, La Sinaise to the west and La Goutte Noire to the east. Discovered in the 19th century by Émile Chénon, the Châteaumeillant *oppidum* was the focus of major archaeological excavations in the 1950s and 1960s under the direction of Émile Hugoniot and Jacques Gourvest [23] . Completely stopped after the death of the latter in 1984, the excavations were resumed in 2001 thanks to the acquisition of land by the municipality of Châteaumeillant. From 2001 to 2018, the planned archaeological excavations were concentrated in the reserve, exploring the habitat as well as the imposing fortification of the *oppidum* . The excavations revealed a dense and continuous occupation that began around 200 BCE. The settlement grew in size, and as early as 150 BCE, shipments of Italic amphorae arrived in Châteaumeillant. It is in this district of the *oppidum* , marked by handicraft production and commerce, as opposed to residential areas, that, since the 19th century, the famous "amphora cellars" have been discovered. During recent excavations, only one amphora cellar was excavated, cellar 437. Explored in 2016, it contained the remains of 33 Italic amphorae. In spite of its apparently good state of preservation, no amphorae found during the excavation were actually whole; some were broken, some were incomplete and some even showed traces of re-use [24] .

Fig. 2. The oppida of the city of Bituriges in the 1st century BC (DAO : S. Krausz).

3. Experimental

3.1. Archaeological samples

A total of eleven samples from eleven different amphorae (Table 1) were located from "amphora cellars" corresponding to deep cavities (US 3164 and 3165) in which the amphorae seem to have been intentionally stored (Fig. 3). These amphorae, Dressel I from Italy dating from the 2nd century-1st century BC, aligned in rows, vertically upside down, seem to be an exception in Gaul (France) (Fig. 4). The studied shards in various states of fragmentation were not clean. In fact, archaeological samples are sometimes contaminated because of conservation conditions and/or cleaning before restoration. The sampling was carried out during the excavation and the size of the shards varied. The inside surfaces of the amphorae were light brown and no coating material was observed with the naked eye.

Table 1 Identification of organic compounds due to lipid extraction and wine extraction by GC-MS (√ = present, - = no detected, Mw: Molecular weight, RT: Retention time). All of the acids are trimethylsilylation compounds. Sample number 1 2 3 4 5 8 10 16 17 18 26

Fig. 3. South stratigraphic section of cellar 437 and 512 (Relevés et DAO : G. Gouzon, M. Bouchet, C. Millereux) (in Krausz et al. 2021 [24] , Fig. 10).

Fig. 4. Plan view of amphorae at structure 437 (Dessin by F.Olmer in Krausz et al. 2021 [24] , Fig. 10).

3.2. Solvents and chemical reagents

All solvents were of the highest purity grade. Hexane and N, O -Bis (trimethylsilyl) trifluoroacetamide/Trimethylchlorosilane (BSTFA/TMCS) were supplied by Sigma-Aldrich. Methanol and chloroform were supplied by Merck. Ethyl acetate was purchased from SIGMA-ALDRICH. KOH and HCl were supplied by ACROS Organic.

3.3. Standards

Sandaracopimaric, dehydroabietic and 7-oxo-dehydroabietic acids were supplied by Helix Biotech. Isopimaric, syringic acids were purchased from Sigma Aldrich and tartaric acid from Fluka. Concerning commercial resins, sandarac (T. *articulata*) were supplied by EMIGA, S.A. and the colophony (Pinus) by Kremer Pigmente GmbH & Co KG [25] . Van den Berg has reported these identifications in Molart report 10 [26] .

3.4. Sample preparation

1–3 g taken from the centre of shards from the internal face of the amphorae was scraped by scalpel and crushed. Two different procedures were applied to the samples. The first procedure was an extraction with a mixture of 10 mL of methanol and chloroform (1:2 v/v) [27] . The ultrasonic processor (Sonic Vivracell VCX130) was used three times for two minutes each time. The frequency was fixed at 20 kHz. After centrifugation (400 rpm / 15

min), the supernatant was taken and evaporated to dryness under a nitrogen stream. The second procedure was the extraction of the wine acids using 6 mL of KOH (1 M, Panreac, Spain) in water with the ultrasonic VCX130 processor, three times for two minutes [28, 29]. After acidification with HCl (6 M), 3 mL of ethyl acetate was added and

the organic fraction was evaporated to dryness under a nitrogen stream. The derivatisation method consisted of trimethylsilylation, performed with *N, O*-Bis(trimethylsilyl)trifluoroacetamide 99%/Trimethylchlorosilane 1% (BSTFA/TMCS) at 70 °C for 30 min. After trimethylsilylation, the extracts were dried and then dissolved in 1 mL of hexane (Merck, Germany). All the samples were filtered (0.45 µm PTFE filter) before injection into GC-MS.

3.5. GC-MS conditions

The analyses were performed on a Thermo Scientific Focus gas chromatographic system connected to a Thermo Scientific AI 3000 auto-sampler, coupled to an ITQ 700 ion trap mass spectrometer (Thermo Fisher Scientific). The data were treated with Xcalibur software. The splitless injector and interface were maintained at 250 °C and 300 °C respectively. Ion source temperature was 250 °C. Helium was the carrier gas at a constant flow of 1.2 mL/minute. Injections were performed with a 30 m silica capillary column, a 0.25 mm internal diameter wall coated with Thermo TG-5MS, and with a film thickness of 0.25 µm. The chromatographic oven was programmed as follows: 50 °C maintained for 2 min, 50–220 °C at 8 °C • min⁻¹, 220–260 °C at 2 °C • min⁻¹, and 260–330 °C at 10 °C • min⁻¹. Mass spectra were recorded under electron impact ionisation (EI) at 70 eV of ionisation energy and in the *m/z* 50 to 650 range. 1 µL of each solution was injected into the GC-MS apparatus in triplicate to verify the repetitiveness of analyses. The assignment of mass spectra peak was based on a comparison with an internal mass spectrum database (from commercial standards, from fresh and artificially-aged resins) and finally, by comparison with the NIST database (NIST MS Search 2.0).

All of the components were identified according to their specific mass data (base and molecular ions), and their retention times in comparison with standard molecules and specialised literature.

4. Results and discussion

The GC-MS analysis revealed the presence of various organic compounds, as described in Table 1.

Dehydroabietic acid was identified in all of the eleven samples of shards. Several oxidation products such as 3-hydroxydehydroabietic, 7-hydroxydehydroabietic and 7-oxo-dehydroxyabietic acids, characteristic compounds of pitch from Pinaceae resins [30, 31], were also detected. These alteration products could have occurred during the natural ageing process and have already been found in an archaeological context, notably in amphorae [13, 29, 32]. Polyaromatic compounds were observed, such as retene and phenanthrene. During the production of pitch, the heating leads to a change in diterpenic structures via the occurrence of several chemical reactions such as dehydrogenation, aromatisation, demethylation and decarboxylation [28]. Retene is considered to be a marker of anthropogenic degradation, when the resinous material is heated at high temperature [33] or distilled in an environment which is poor in oxygen [13]. The observation of retene indicates that the pathway of transformation is a result of the heating of abietic acid. Moreover, during intense heating exceeding 300 °C, after decarboxylation, dehydroabietic acid gave 18- and 19-norabietatriene [34] respectively. Dehydroabietic acid methyl ester was only detected on the sample from the shard of amphora No. 10. The pyrogenation of coniferous wood in a closed environment results in the esterification of dehydroabietic acid, with the released methanol reacting easily with diterpenic acids [30, 31]. In all of the studied shards, the presence of pitch was therefore characterized in particular by the detection of dehydroabietic acid derivatives and of their degradation compounds. Such products derived from Coniferae indicates the use of waterproofing materials during this period. In the shard of amphora No. 10, the presence dehydroabietic acid methyl ester could indicate the use of wood tar as waterproofing material. Concerning the characterisation of wine, phenolic acids and organic acids must be distinguished. Benzoic acid was present in all samples, which may be explained by the degradation product of anthocyanins by ketones in the wine. Previous chemical analyses of archaeological samples have already revealed the presence of benzoic acid [35, 36]. Syringic acid was identified in five of the eleven amphorae (Fig. 5). This acid is particularly found in red wine [37]. The red colour comes from anthocyanin colorants such as malvidine [38], but also from tannins. Malvidine releases syringic acid during alkaline fusion that degrades the tannins and anthocyanins [39]. In wine, the amount of syringic acid is lower than that of tartaric acid. However, the presence of syringic acid does not indicate the presence of wine and it is absent in white wine [40, 41]. Hydroxybenzoic and syringic acids are obtained during alkaline fusion of malvidin [42]. Vanillic acid was observed only in amphora No.10. Syringic acid is often detected in archaeological residues, as well as tartaric acid [29, 43, 44]. Tartaric acid was not detected in the studied samples, nor were fermentation markers such as fumaric, glutaric and malic acids. With reference to the other wine acids, for example, malic acid, which is one of the major acids, was not detected; its quantity decreases after fermentation. Succinic acid, appearing during acid fermentation, was identified in the majority of samples, as were fumaric, maleic and glutaric acids [45, 42]. As with other results, it is not easy to confirm the

presence of wine in the absence of tartaric acid. This problem may be caused by the extraction step. The extraction with alkaline treatment triggers the release of syringic acid (alkaline fusion), but tartaric acid is not extracted in these conditions with ethyl acetate solvent. This fact can be explained by the low solubility of tartaric acid in ethyl acetate [39]. Unlike acid treatment, the use of BF₃ [46] accelerates the extraction of tartaric acid. In fact, Drieu et al. (2020) reported that 77% amount yield for standard pure tartaric acid was obtained in such conditions. Owing to the limitation of the sample amount available for this study, only one condition was applied. Moreover, syringic acid (derived from malvidin) is also contained in plants, for instance pomegranate (*Punica granatum*) [47], whortleberry, red clover and high mallow [48]. Archaeological research, particularly in the study of amphorae, has not yet confirmed the presence of syringic acid from these other plants. Additionally, the typology of these amphorae is Dressel I, and they were used for wine, based on archaeological knowledge, although tartaric acid was not identified in these study conditions. The amphorae Nos. 2, 10, 15, 17 and 18 can therefore reasonably be considered to have contained wine. In addition, to focus on the results of amphora No. 10, Fig. 6 shows the chromatogram with some specific detected compounds corresponding to pimaranes and labdanes. Sandaracopimaric acid was observed in the highest proportion with other pimaric derivatives, such as isopimaric, hydroxysandaracopimaric and 12-acetoxy-sandaracopimaric acids. Agathic acid was also detected in this amphora and this molecule can be present in resins from two diterpenic botanical families, Cupressaceae and Araucariaceae. However, identified products such as 3-hydroxy-eperuic acid [25] and *trans*-communic acids, together with sandaracopimaric acid and agathic acid (Fig. 7), indicate a diterpenic resin corresponding to *Tetraclinis articulata* belonging to the Cupressaceae family and also named sandarac resin. It is noteworthy that sandaracopimaric acid could also be present in Pinaceae resin with dehydroabietic acid and derivatives [17, 49]. Dexpimaric acid, an isomer compound of sandaracopimaric acid which can be found in the common *Pinus pinaster* variety, was not detected. However, the analysis of amphora No. 10 provided more information than the analysis of the other amphorae, suggesting a difference in the wine production or in its conservation. The identification of two sources of resinous materials from two different botanical families (Pinaceae and Cupressaceae) is especially significant, suggesting a specific use by the Romans. In fact, the purpose of using resin was to actually produce a better conservation of wine. Adding a small quantity of resin during the fermentation process results in an appreciable enhancement of flavour and colour [50]. Furthermore, the resin inhibits many bacteria, especially those of the acetic type (acetobacter), while protecting organic compounds from degradation [6]. The resin in traditional Greek resinated wine comes from the Aleppo pine and the North African sandarac tree [5]. Therefore, the resinous materials observed in this study could have been used either as waterproofing or as an additive to wine.

Fig. 5. Total ion chromatogram (TIC) of sample number 10 with wine extraction by GC MS analysis.

Fig. 6. Total ion chromatogram (TIC) of sample number 10 with lipid extraction by GC MS analysis.

Fig. 7. Chemical structure of sandaracopimaric acid (A), *trans*-communic acid (B), and agathic acid (C).

5. Conclusion

Several organic compounds in the amphorae of the Chateameillant oppidum were identified by GC-MS analysis. Overall, diterpenoids, with abietanes such as retene, dehydroabietic acid and oxidized diterpenic substances, were found in all eleven amphorae. This result indicated that such compounds correspond to pitch from resins of the Pinaceae family. The waterproofing material from amphora No. 10 was probably created by the combustion of softwood. This was identified owing to the presence of methylated diterpenic acids such as methylated dehydroabietic acid. This particular amphora had completely broken during excavation, although the others were conserved in truncated form. A wider range of compounds was also detected inside this amphora. The content or the fabrication process of the pitch coating in amphora No.10 could be considered as different from that of the others. Syringic acid, one of the specific wine compounds, was detected in five amphorae. The presence of tartaric acid is frequently described in wine [7, 10, 51]. However, according to a recent study [52], the detection of these two acids depends on the extraction conditions. This study proved that alkaline treatment with ethyl acetate is not necessarily conducive to the release of tartaric acid. Succinic, benzoic and vanillic acids, which are contained in wine, were detected. Given that the archaeological study identified amphora Dressel I as a wine amphora, the historical context in the Chateameillant oppidum points towards the same being the case for these five amphorae [24]. The chemical analysis provided evidence that the content of the five amphorae (Nos. 2, 10, 15, 17, 18) was indeed wine. Sandarac (Cupressaceae family) found in wine amphora No. 10 is rare because the resinous material in amphorae is usually identified as a Pinaceae family resin, according to the majority of analytical research [14, 29, 51, 53-55]. Only one analysis fails to correspond to the new evidence; it does suggest however, some application possibilities, for example, that the resin was used as an additive to the wine or, alternatively, was applied to the amphora's internal coating.

Author contributions

H.F. analysed all archaeological samples. The interpretation of the results was performed by H.F and C.M. S.K and F.O. performed the archaeological study. C.M. and C.V. supervised the research. H.F., C.M. and C.V. discussed the results. H.F, S.K, C.M and C.V wrote the paper.

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