'Atiqot 114, 2023

The Identification of Ancient Wine through Organic Residue Analysis of Ceramic Vessels

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Wine was a primary commodity in antiquity and prevalent in the Southern Levant Mediterranean-basin regions and Mesopotamia. Therefore, its identification in archaeological artifacts by chemical analysis is of great importance. Many studies of wine residues were conducted on pottery from Mediterranean and Near Eastern sites, using a wide range of approaches to extract and detect organic compounds. This article will evaluate and compare these methods and raise consequent considerations for the interpretation of results to arrive at a historic picture.

Keywords: wine, organic residue analysis, tartaric acid, wine-markers, Southern Levant, ceramic vessels

Introduction

Wine in antiquity was a principal and important commodity in the Southern Levant, the Mediterranean basin and Mesopotamia. This is attested by written and pictorial sources, as well as by archaeological findings of winepresses and botanical grape remains (Palmer 1994; Hamilakis 1996; Wright 2004; Brogan and Koh 2008; Finkelstein, Gadot and Langgut 2022). These testimonies are reenforced by chemical analyses of pottery containers associated with wine, long recognized as potentially offering important information regarding wine production and consumption. This includes identifying evidence for the origins of wine use and wine processing in antiquity, its scale of production and trade, its economic and social importance over time, the circumstances of its consumption, etc. For example, organic residue analysis (ORA) of ceramic vessels from sites in Georgia showed that wine was already produced and consumed in the early Neolithic period (c. 6000–5000 BCE) (McGovern et al. 2017). This recent evidence predates by c. 1000 years the previous earliest identification of wine in a vessel from the Zagros Mountains, Iran, dated to 5400–5000 BCE (McGovern et al. 1996). Many other wine residue studies were conducted on

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pottery from Mediterranean and Near Eastern sites, highlighting wine's important role in many aspects of life (and death) throughout history. Examples of such sites are in Greece (McGovern et al. 2008; Garnier and Valamoti 2016), northern Italy (Pecci et al. 2017), southern France (McGovern et al. 2013), Egypt (McGovern 1997; Guasch-Jané et al. 2004; 2006a; 2006b; McGovern, Mirzoian and Hall 2009), Armenia (Barnard et al. 2011) and Israel (Koh, Yasur-Landau and Cline 2014; Amir et al. 2022; 2023).

Though all studies assert that they have successfully identified wine in ceramic vessels, their analytical methods vary, as does their choice of organic compounds on which their interpretation is based. The main compounds considered as wine-markers in these studies are tartaric acid and/or other diacids, hydroxy acids and hydroxybenzoic acids. However, scholars recently raised doubts concerning these compounds' reliability as wine-markers in archaeological artifacts (Whelton et al. 2021; see below). This argument raises two main questions: Is it indeed possible to identify wine in archaeological findings by chemical analysis? Should the previous organic residue analysis studies of wine in pottery be suspected as unreliable, requiring a revision of historical conclusions? In this article, I will address these questions and discuss whether and how chemical analyses can identify wine in pottery, especially in the Southern Levant.

BIOMOLECULAR EVIDENCE FOR WINE'S PRESENCE

Tartaric Acid

Wine is mainly composed of water and ethanol, though many other molecules are also present in much smaller amounts. Of these, tartaric acid is the most abundant molecule in grapes, together with malic and citric acids (Hale 1962; Ribéreau-Gayon et al. 2006; Drieu et al. 2020). Tartaric acid's preservation in a ceramic matrix was shown to be better than other wine-originated small acids (Pecci et al. 2013) and therefore, tartaric acid has been determined as a potential marker for grape products.

Some researchers (e.g. Barnard et al. 2011; Whelton et al. 2021) have questioned this assumption for two main reasons: (1) tartaric acid's polarity makes it highly soluble in water, and is thus unlikely to survive at its point of deposition in archaeological contexts; (2) tartaric acid is also present in other plants, albeit in much lower concentrations. While there are plants with higher amounts of tartaric acid than grapes, they are not endemic to the Southern Levant. To these concerns must be added the consideration that tartaric acid concentration quantification is currently limited to a small number of plant species. Other plants, not yet studied in detail (and perhaps including kinds endemic to our region) may be found to contain high tartaric acid concentrations in the future (Stern et al. 2008). These considerations would tend to indicate that since tartaric acid is not specific to grapes or to grape products, it cannot reliably attest to the presence of wine in archaeological contexts.

Other scholars, however, do consider tartaric acid and other biomarkers (e.g., succinic, malic, fumaric, citric and syringic acids) as wine identifiers. Though these compounds are indeed hydrophilic and highly soluble, they poses traits that enable their preservation in

the ceramic matrix across the archaeological timescale. Their polar groups may strongly interact with Brønsted or Lewis sites of the ceramic paste, or form hydrogen bonds with silicates (Michel, McGovern and Badler 1993; McGovern, Mirzoian and Hall 2009; Garnier and Valamoti 2016). Additionally, a mechanism in which fatty acids interact with calcium carbonate inclusions in the ceramic matrix was shown to form insoluble calcium fatty acid salts that may be preserved in ceramics over long timespans and may efficiently be recovered from the ceramic sample by acidic extraction (Berner 1968; Frye and Thomas 1993; Hammann et al. 2020). A similar mechanism may also occur between calcium carbonate and the aforementioned compounds. Although these compounds are smaller and more soluble than fatty acids and their salts, their interactions with the ceramic calcium ions may help preserve them in the pottery vessels to some extent.

The extraction method of these wine-markers from the ceramic sample is based on acidic or alkaline treatments, and by the introduction of high energy (by sonication) (Pecci et al. 2013; Garnier and Valamoti 2016; Drieu et al. 2020). None of the published methods is based on washing the sample with water alone (see below). Hence, the extraction procedures illustrate how the polar wine biomarkers are indeed bound to the ceramic matrix by strong interactions and do not easily separate and wash out of it.

While tartaric acid may be found in other plants in the Levant, they contain much lower concentrations compared to grapes (Stafford 1959; DeBolt, Cook and Ford 2006). Therefore, it is likely that tartaric acid from such plants will not be preserved over time at a detectible concentration in archaeological contexts. Those plants containing more tartaric acid than grapes include Chinese hawthorn tree fruits (*Crataegus monogyna*) (McGovern et al. 2004), consumed in the Near Eastern highlands of Anatolia as early as the second millennium BCE (Collins 1990; Ertuğ 2000), tamarind (*Tamarindus indica*), star fruit (*Averrhoa carambola*) and yellow plum (*Spondia mombin*) (Barnard et al. 2011). None of these are native South Levantine plants, and even if imported and present, their usage in this region was certainly not as ubiquitous as the grape. Furthermore, grapes were commonly used and stored as liquid-wine, which interacts with its container over a very large surface area, and thus promotes and eases its detectability. This method of usage is not necessarily the case with the other tartaric acid-containing plants, so that even higher concentrations do not assure leaving detectible traces in the ceramics.

In reviewing the aforementioned data and the present state of research, we may conclude, with due caution, that at least in the Southern Levant, tartaric acid found in archaeological remains is indeed a reliable marker indicating the presence of grape products. That said, one should still be scientifically cautious and acknowledge the possibility that some other plant with a higher tartaric acid concentration, not yet quantitatively studied, may have been the source of the tartaric acid traces detected in a vessel. Therefore, at this time, I consider that a conclusive identification of wine in ceramic vessels should not be determined by tartaric acid's presence alone, but only after identifying a set of biomarkers and considering the archaeological and historical contexts (see below).

Additional Biomarkers

Other compounds present in wine are diacids, hydroxy acids and hydroxybenzoic acids. Among them are malic, citric, maleic, glutaric and syringic acids, originally present in the grapes (Pezzuto 2008; Biasoto et al. 2010; Drieu et al. 2020; Amir et al. 2022), as well as succinic, fumaric, pyruvic, citramalic (α-methylmalic), malonic, acetic and lactic acids, which are formed during the fermentation process (Ribéreau-Gayon et al. 2006; Biasoto et al. 2010). While these molecules were shown to be less preserved than tartaric acid in the archaeological timescale (Pecci et al. 2013), they still appear in chromatographic wine profiles (Fig. 1; Pecci et al. 2013; Garnier and Valamoti 2016; Amir et al. 2022; 2023). None of these compounds can be used as wine markers *per se*, as they also appear in a wide range of plants (Drieu et al. 2020); however, I suggest that their appearance together with tartaric acid does provide a good indication of a wine's presence in the vessel.

Malvidin, or its derivative syringic acid, were proposed as red-wine markers (Guasch-Jané et al. 2004; Stern et al. 2008). Malvidin is an anthocyanidin responsible for the coloring of red wine, which polymerizes with aging into more stable pigments. Releasing syringic acid from the pigment by alkaline treatment of the sample renders it detectable (Guasch-Jané et al. 2004). Syringic acid appears in relatively high amounts in grapes compared to other fruits, and its concentration rises in aging wine. Furthermore, its polymerization enables better preservation in ancient vessels (Singleton 1996; Stern et al. 2008). Nevertheless, its

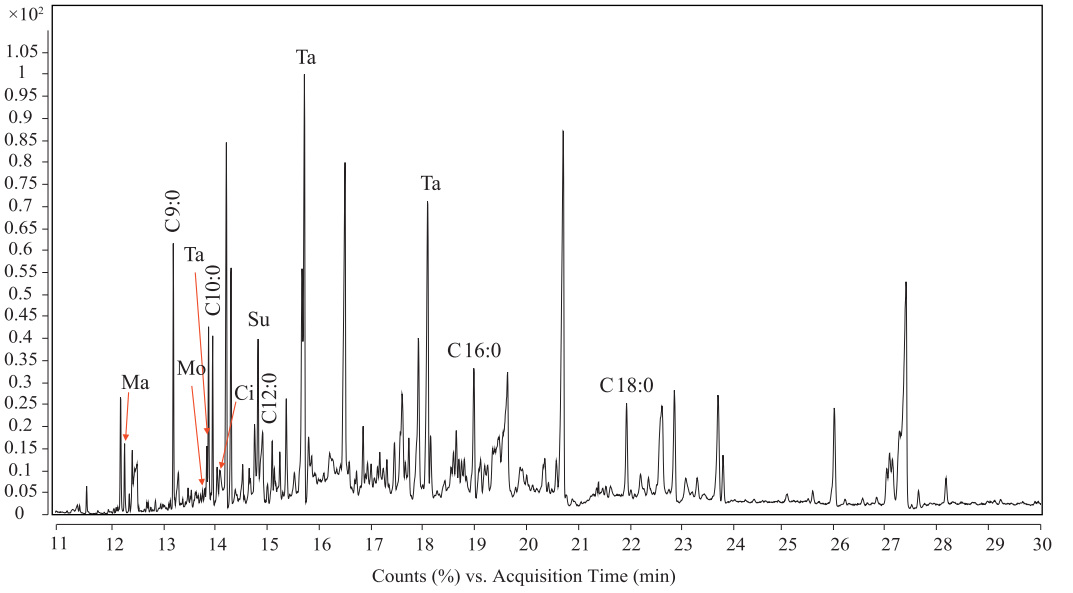

Fig. 1. Representative chromatographic wine profile of a jar from Building 100, Giv'ati Parking Lot excavations, Jerusalem; Cx:y = fatty acid with x carbons in its chain, with y number of double bonds; Cxol = alcohol with x carbons in its chain; Ma = malic acid; Mo = malonic acid; Ta = tartaric acid; Ci = citric acid; Su = succinic acid (see Amir et al. 2022).

utility as a red-wine marker is limited as it is also found in other natural products, in soils and as a degradation product of lignin and cork stoppers (Drieu et al. 2020). Therefore, syringic acid can attest to a red wine's presence only on two conditions: it is demonstrated to have directly derived from malvidin and special care was taken to remove all free syringic acid from the sample prior to the extraction treatment.

It is noteworthy that the absence of syringic acid from a chromatographic wine profile—namely, where tartaric acid and the other wine-associated biomarkers are detected—does not necessarily indicate that white wine was present. Its deficiency may be the result of preservation issues, or due to employing an ineffective extraction method for its release from malvidin.

It is thus reasonable to conclude that wine may be identified in ancient ceramic vessels by biomolecular evidence. The analysis must detect a set of compounds in which tartaric acid is the main acid, together with molecules that are present in grapes or formed during the fermentation process. Assuming a careful, appropriate and well-controlled extraction method that shows that the syringic acid directly derived from malvidin or malvidin polymer precursors, will indicate that the syringic and tartaric acids in a sample derived from red wine. Yet, as abovementioned, the detection of tartaric acid alone, in the absence of syringic acid, cannot prove the presence of white wine.

Besides organic residue analysis, there are also other approaches that may contribute to identifying wine in ancient vessels: DNA sequencing (Cavalieri et al. 2003; Hansson and Foley 2008; Foley et al. 2012), though this method still requires improvement and validation to reliably identify wine (Drieu et al. 2020); and the study of fermented beverage yeasts isolated from ceramic vessels (Aouizerat et al. 2019).

The Analytic Process of Wine Identification

Wine identification consists of two stages: (1) extraction of organic compounds from the ceramic matrix; (2) detection and identification of the compounds. Various methods have been applied for each step (Fig. 2).

Drieu et al. (2020) summarized the published tartaric acid extraction and detection methods and tested two main methods. They concluded that LC-MS/MS and GC-MS are the most suitable detection methods. For tartaric acid extraction, the acidic treatment proposed by Garnier and Valamoti (2016) was found to be the most effective and reliable approach. It was also found useful for extracting other "wine-marker" diacids and hydroxy acids (Amir et al. 2022; 2023). On the other hand, for syringic acid extraction, alkaline treatment (see, e.g., Guasch-Jané et al. 2004; Pecci et al. 2013) was identified as best. However, Drieu et al. (2020) still questioned the reliability of assigning syringic acid to malvidin derived from wine, further asserting that syringic acid detected by alkaline treatment of an archaeological artifact cannot by itself point to an earlier presence of red wine. Therefore, in comparing alkaline and acidic treatments, the acidic treatment seems to be more efficient for the extraction of a broader range of wine markers, and is hence preferable.

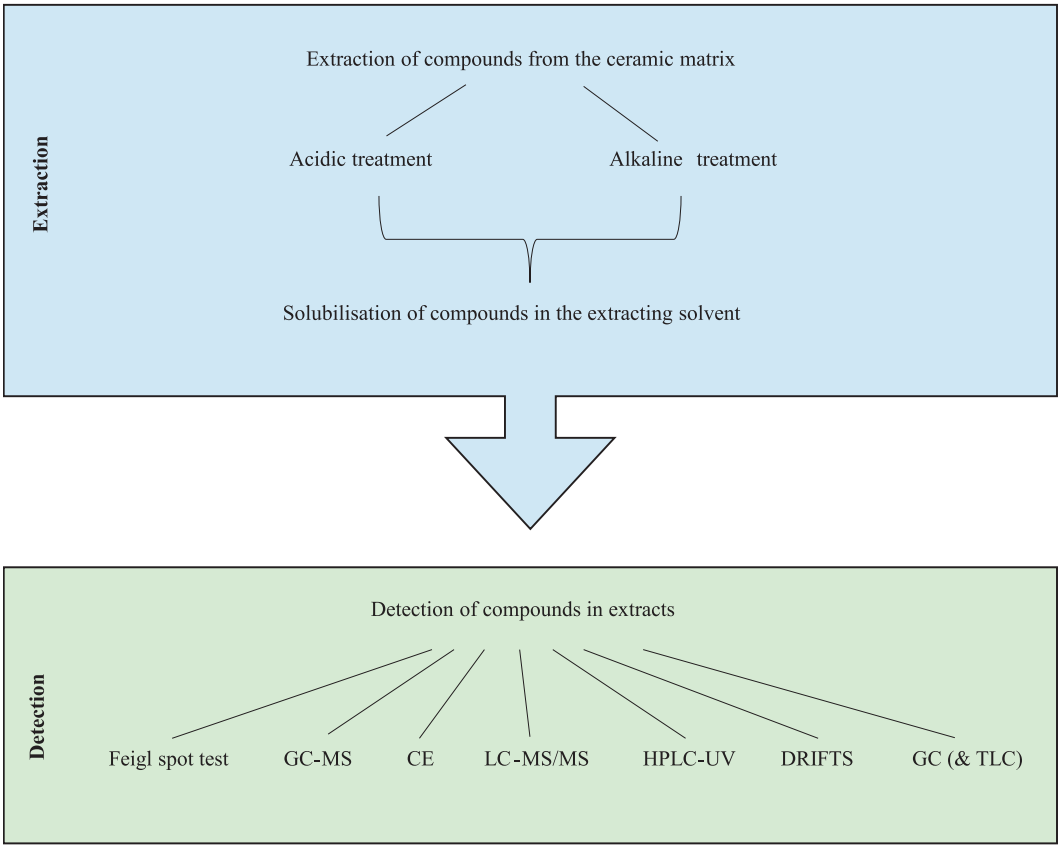

Fig. 2. Summary of chemical processes for the extraction and identification of diacids, hydroxy acids and hydroxybenzoic acids from ceramic vessels.

The reliability of all the abovementioned approaches is predicated upon strict adherence to basic precautions to prevent contamination and misinterpretation, including: (1) avoiding direct contact of the vessel with bare hands, i.e., by wearing gloves throughout its handling stages; (2) avoiding the washing of the vessel; (3) storing the vessel (when sampling is not executed immediately upon exposure) and samples thereof in paper or glass, rather than in plastic containers, as plastic releases phthalates that contaminate the samples and screen the original molecular peaks; (4) sampling and analyzing the sediment from the vessel's immediate vicinity, and/or other vessel forms or parts that are unlikely to have been exposed to the original content of the studied vessel using the same methods employed in treating the vessel itself to enable the monitoring of vessel contamination from its burial environment (Guasch-Jané et al. 2004; Drieu et al. 2020); (5) Preparing and analyzing blank samples of the solvents used in the extraction procedure to monitor potential contamination from the solvents and laboratory work (Whelton et al. 2021).

Archaeological Background

To interpret pottery as wine receptacles requires supportive archaeological and historical data: deposition environment and context, vessel forms, associated botanical remains, etc. Two kinds of relationships exist between the chemical studies and archaeological/historical data, each reflecting a different balance between the lab results and the archaeological contexts: (1) the analytical results support the well-determined archaeological context; (2) the archaeological evidence is not conclusive, and therefore, a larger weight is placed on the lab results.

In the first case, the archaeological data provides definite evidence or a clear indication of the vessel's content, and the chemical analysis is preformed to confirm this conclusion. In many such cases, the researchers relied on very few molecules to identify the wine, while the archaeological context highly supported this identification. For example, Egyptian wine vessels labeled with inscriptions indicating that they contained wine were chemically identified based solely on tartaric acid or tartaric and syringic acids (Guasch-Jané et al. 2004; McGovern et al. 2009). These molecules were well-preserved due to the arid environment in Egypt. The presence of wine in jars from a cave near Areni, Armenia, was determined based on syringic acid alone. The archaeological context pointed to a wine-production site, supporting this identification—the jars were found near an installation that resembled historical grape-pressing installations. Furthermore, the rich botanic remains found in their vicinity corroborated that the installations were used for grape crushing and pressing, and that the vessels were there to store the produced wine (Barnard et al. 2011).

In the second case, the chemical analysis provides a definite identification of the vessel's content, which is further supported by its archaeological context. For example, the study of amphorae from Oplontis Villa B in Torre Annunziata, Italy, identified a set of compounds indicative of either wine or vinegar. The researchers interpreted the amphorae as intended for wine storage based on their archaeological context and vessel form, as such vessel types are known to have been used for wine transportation, rather than for vinegar (Pecci et al. 2017).

Very few studies have been published concerning the application of organic residue analysis to identify wine in pottery excavated in archaeological sites in Israel. Of note are the wine cellar at Tel Kabri (Koh, Yasur-Landau and Cline 2014), the storage jars (rosette-stamped) from the City of David Area U and the Giv'ati Parking Lot excavations in Jerusalem (Amir et al. 2022), and the wine vessels from intramural tombs at Megiddo (Amir et al. 2023). These studies comply with the second category, demonstrating an interpretation based on integrating chemical results with the archaeological context.

The residue analysis results from Tel Kabri included tartaric acid in all 32 tested jars, and syringic acid in all but three. Most jars also contained compounds that could be interpreted as wine additive residues (cinnamic and oleanoic acids, methyl syringate, cineole, cedrol), which alone cannot be taken as definitive evidence for a wine's presence, but as mentioned above, reinforce this conclusion in association with the tartaric and syringic acids. In this case, the archaeological and historical contexts reinforce interpreting these vessels as wine storage

Fig. 3. *In situ* storage jars at Tel Kabri (see Koh et al. 2014: Fig. 1).

containers. The jars were similar, and their typology attested that they held liquids, considering their narrow necks. Their location in a closed and sealed storage room within a palace is similar to other contemporary storerooms found in palaces throughout the Mediterranean basin and Mesopotamia. This, as well as the room's and the jars' good state of preservation raise the potential of good molecular preservation (Fig. 3). Palatial wine storage rooms dated to the Bronze Age are known from textual sources both in the Mediterranean and Mesopotamia. Of particular note are the Mari archives (Chambon 2009), which are contemporary with the Kabri palace. Egyptian sources and archaeological finds from Tel Naḥf, adjacent to Tel Kabri, attest to wine production in the region (Koh et al. 2014). Therefore, indicative chemical results, even when not definitive on their own, combined with such a clear archaeological context, of both the site and the vessel typology, may allow a confident identification of the vessels as wine storage jars and the room, as a wine cellar.

Two jar assemblages were found in storerooms of public buildings in Jerusalem—in the Giv'ati Parking Lot excavations and in Area U—each containing a set of biomarkers (tartaric, citric and malic acid derivatives, fumaric, glutaric, succinic, maleic and malonic acids), which as a set attested to the presence of wine. A pithos found in the same locus as the storage jars in the Giv'ati Parking Lot excavations, and sediments from both assemblages' vicinity, were also sampled and analyzed as controls; their chromatograms

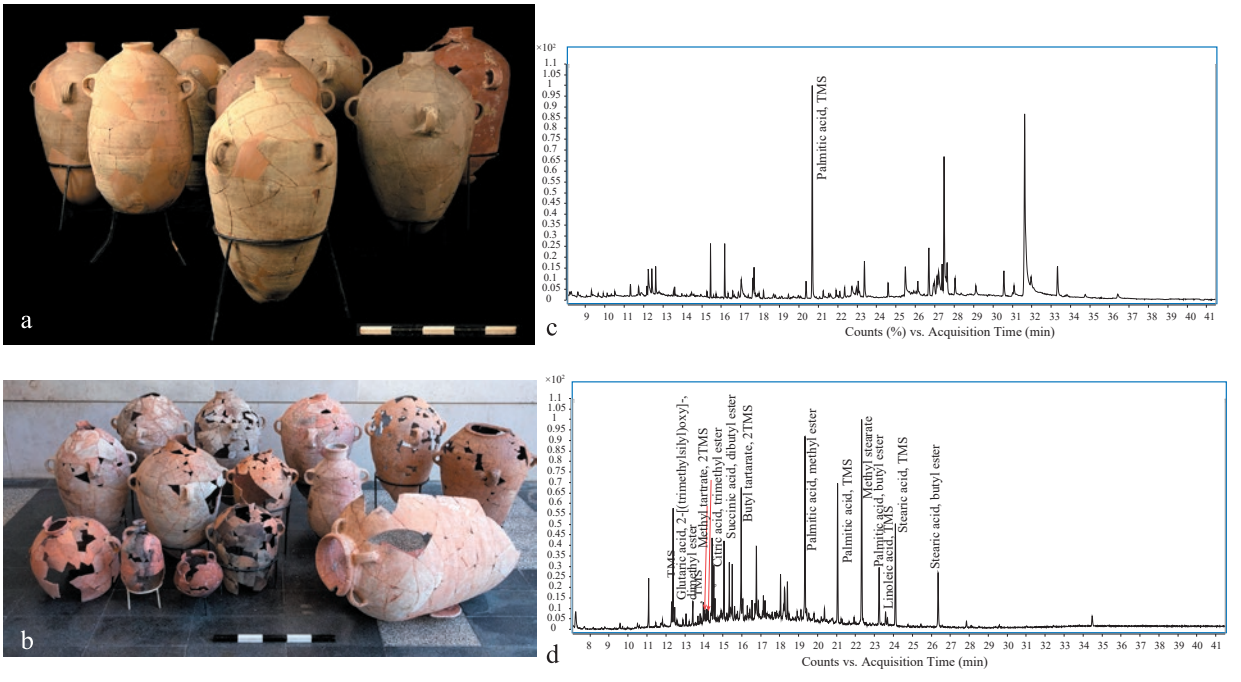

Fig. 4. Storage jars from Jerusalem and representative chromatograms: (a) Area U assemblage (photography: D. Gazit); (b) Giv‘ati Parking Lot excavation assemblage (photography: S. Flit); (c) Pithos chromatogram; (d) chromatogram of a jar from Area U, identified as a wine storage jar (see Amir et al. 2022: Figs. 2; 3).

were completely different and lacking wine-markers (Fig. 4; Amir et al. 2022). These results rule out environmental contamination as a source of the wine-markers in the vessels, and thus strengthen the reliability of correctly interpreting the residues within the jars. The archaeological and historical data also reinforce the conclusion that the jars were used for wine storage. The rosette-stamped jars were part of the Judahite Kingdom administrative system, in which liquid commodities were collected as tax, whose proceeds were then paid to Assyria and also used for supporting the palace and Temple in Jerusalem. Wine and olive oil were the two typical products of the Judahite Kingdom during the Iron Age (Finkelstein et al. 2021; Lipschits 2021).

Lastly, the case of Tel Megiddo emphasizes the important contribution of archaeological data to the reliability of the interpretation and identification of vessels as wine containers. Storage vessels and carinated bowls, which are assumed to have served as drinking cups (Yasur-Landau 2005), indeed contained similar “wine profiles” with tartaric, fumaric, malic, citric, malonic, maleic, glutaric, succinic, oxalic, glycolic and lactic acids. The finding of two bowls and two jugs that contained tartaric acid but no other wine markers was puzzling, as their forms and archaeological contexts led to different conclusions regarding their use and the origin of the tartaric acid. The bowls seem to have been contaminated by the spilling of wine from adjacent wine vessels. The jugs indeed contained wine, but saw only short-term

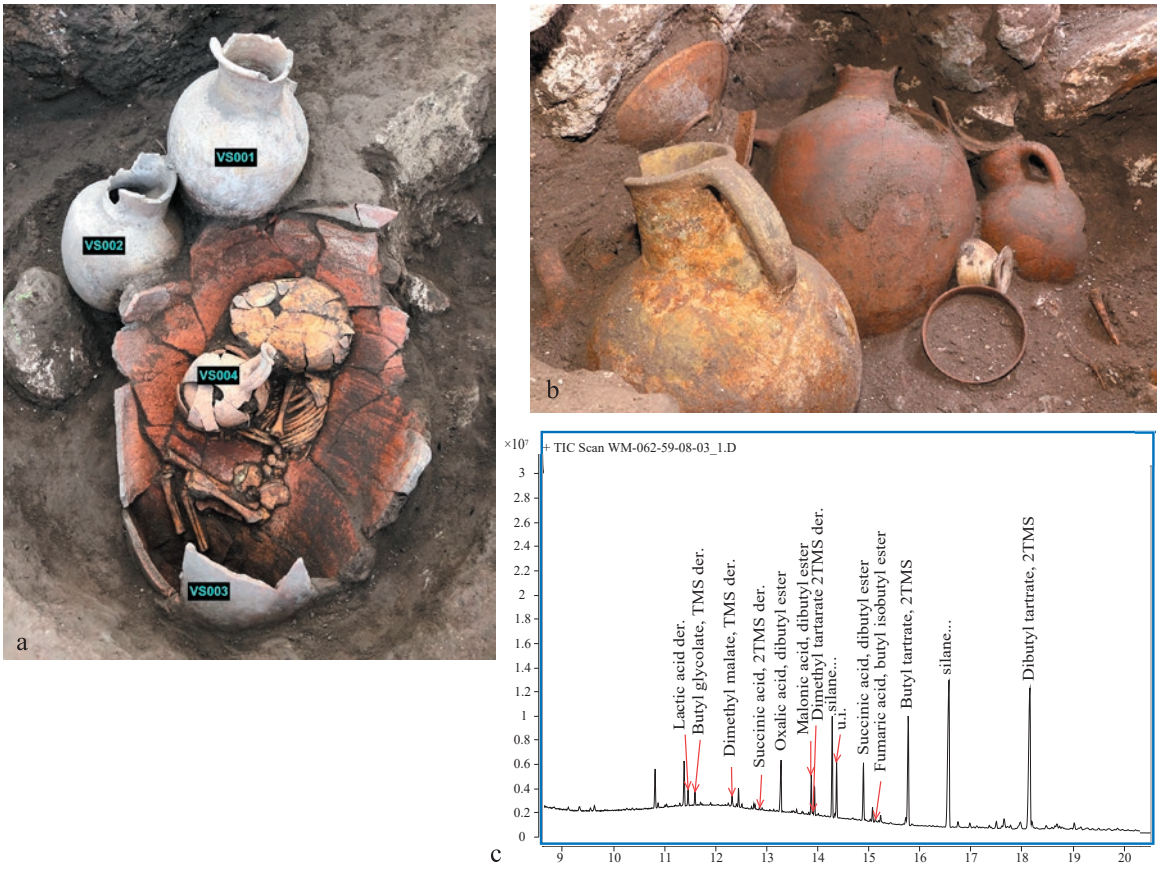

Fig. 5. Findings from intramural tombs at Megiddo: (a) vertical libation jars (VS001, VS002) near a jar burial; (b) vessels from Tomb 50; (c) representative chromatographic wine profile, see Amir et al. 2023: Figs. 2c; 4c).

use as libation vessels during a burial ceremony that did not allow for the preservation of the molecules, as opposed to long-term wine storage. Thus, tartaric acid could still be detected, while other wine-markers, which are present in smaller amounts, were not preserved in detectable concentrations (Fig. 5; Amir et al. 2023). This study also sampled and analyzed other vessels from the very same graves, as well as the surrounding sediments, none of which contained wine-markers. These results rule out burial environment contamination as the origin of the tartaric acid found in the bowls and jugs, and thus strengthen the credibility of the results identifying the jar residue as wine. The geographical proximity, and similar date of Tel Kabri's wine cellar and the MB II–III Megiddo tombs, further reinforce the wine identification in the Megiddo vessels. The overall picture points to a widespread regional culture of wine consumption.

Conclusions

Wine played an important role in many aspects of life and death throughout the history of the Mediterranean basin and Near Eastern societies. Its significance in the Southern Levant is evident from both archaeological and textual data. Therefore, its identification in the archaeological context is of great importance for understanding this region's broader archaeological and historical picture. Until recently, archaeological evidence for wine production and consumption mainly included wine pressing installations and botanical remains, but physical evidence for the wine itself was missing.

Organic residue analysis of ceramic vessels bridges this gap. Properly conducted, by applying a suitable treatment and a correct detection method, it is now possible to reliably identify wine-markers in archaeological artifacts. Organic compounds of biological origin are distributed in many plants. Therefore, when evidence is available primarily from chemical testing, the identification of wine should rely on the detection of the "wine profile"—a set of compounds typical of wine, i.e., wine-markers. In some cases, when the archaeological context clearly points to the presence of wine, its identification can rely on just a few organic compounds. A review of the various methods showed that an acidic treatment is best for extracting tartaric acid and additional diacids and hydroxy acids, and an alkaline test is best for extracting syringic acid. The preferred detection method is LC-MS/MS or GC-MS.

It may now be safely concluded that organic residue analysis (ORA) provides important and reliable information regarding wine production, consumption, trade, culture, economy, etc. The historical researcher and archaeologist alike should consult ORA results and integrate them into their respective considerations and interpretations. It should be emphasized that although this chemical analysis method is a powerful tool to identify wine-markers and may attest to a wine's presence in ancient ceramics, one should always corroborate the laboratory data with the site's archaeological and historical context.

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