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Prehistoric wine-making at Dikili Tash (Northern Greece): Integrating residue analysis and archaeobotany

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ABSTRACT

A new two-step analytical protocol has permitted the reliable structural identification of red wine thanks to the presence of dark grape (tartaric, malic, syringic acids) and fermentation markers (succinic and pyruvic acids) in a smashed, large, coarse jar and a jug excavated inside a Neolithic house destroyed by fire around 4300 BCE at the site of Dikili Tash in northern Greece. This new method, which has also been tested successfully on other vessels, exploits the chemical break-down of the clay and the simultaneous liberation and derivatization of biomarkers. Since aldaric acids are not extracted by a simple solvent extraction, but only when submitted to the second acido-catalyzed extraction, their detection in the second extract indicates organic residues are more deeply impregnated and bound to the clay structure than previously thought. Chromatography coupled with mass spectrometry leads to the very sensitive detection (<10 ng/g sherd for tartaric acid, *i.e.* $< 10^{-6}$ mL of wine/g sherd) and reliable identification of fermented grape biomarkers. Their identification in a Neolithic jar from Dikili Tash corroborates the finding of pressed grapes consisting of loose pips, skins, and pips still enclosed by skin in association with this jar. Our results demonstrate Neolithic wine-making in the northern Aegean, and provide the earliest solid evidence for the Eastern Mediterranean and Europe. This new method could be more widely used for detecting wine traces in all sorts of archaeological artefacts or structures. It constitutes an essential tool for a better understanding of wine-making and of contexts of consumption in ancient civilizations.

1. Introduction

The origins of viticulture and winemaking have been at the core of discussions concerning the emergence of social stratification among prehistoric societies of the Old World (McGovern et al., 1997). Viticulture has been considered as a means to exploit soils less suited for crop cultivation thus contributing to the emergence of redistributive societies and Late Bronze Age Aegean palaces (Renfrew, 1972a) or a means to tie people to their land due to the large labour investment and late return of cultivating grapevines (Gilman, 1981). Wine in turn holds a key role concerning the emergence of Bronze Age elites, among the Sumerians, Egyptians, Minoans and Mycenaeans (Renfrew, 1995; Gorny, 1995). Textual evidence underlines the luxury nature of wine, its consumption in relation to rituals, funerary customs and feasting and its appropriation by Bronze Age elites (Sumerian, Assyrian and Hittite states: Powell, 1995; Mycenaean Greece: Palmer, 1995).

The origins of viticulture and winemaking could lie anywhere in the wider area of the distribution of the wild grapevine, *Vitis vinifera* sbsp. *sylvestris* (Gmelin) Hegi, which spreads from western Europe to the *Trans*-Caucasian zone and a large part of the Mediterranean basin (Levadoux, 1956; Arnold et al., 1998) and might have occurred several times rather than once (McGovern, 2003). The available archaeobotanical evidence suggests that grape vine has been exploited and probably also cultivated in a wide region ranging from the Aegean to the eastern fringes of western Asia, including the Caucasus, since at least the 5th millennium BC. Despite this, a widespread scholarly position proposes that viticulture and/or grape domestication originated in a specific region from which it spread, e.g. Transcaucasia (McGovern, 2003; Olmo, 1995; Terrall et al., 2010), the Levant (Zohary, 1995; Zohary et al.,

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2012) or the eastern end of western Asia (Miller, 2008). While for western Asia various lines of evidence may support this suggestion, the evidence from Transcaucasia is extremely limited (McGovern, 2003) or only partly published (Barnard et al., 2011). For the Aegean, Renfrew (1995), in a comprehensive review of the grape finds from the region, suggests that the cultivation of the grape vine is indicated by the 5th millennium BC with wild and domesticated forms being both present.

It is important to emphasize, however, that the means of detecting domestication in the archaeobotanical record are problematic for two main reasons: (*i*) a great overlap in pip measurements between wild and domesticated grapes, aggravated by distortion caused by charring (Stummer, 1911; Smith and Jones, 1991), and (*ii*) early cultivated and domesticated grapevines need not have necessarily developed seeds morphologically domesticated as the pips of modern domesticated grape vines as Pinot noir and Clairette clearly demonstrate (cf Rivera-Nunez and Walker, 1989; Terral et al., 2010): the wide variation and overlap of pip morphology depends on how well developed this domestication trait is in the material examined (Zohary, 1995 p. 27, Renfrew, 1995; Valamoti et al., 2007). Early tending of wild grapevines within the wider area where the plant grows naturally could have gradually developed towards the cultivation of wild grape vines and later to the vegetative reproduction of desired traits such as sweetness, colour, and size of grapes (McGovern, 2003; Olmo, 1995; Renfrew, 1995). Finds of *Vitis* charcoal, apparently from pruning used as fuel at various 3rd millennium sites in Western Asia, are also considered as indications for grapevine domestication, *i.e*. cultivation through vegetative propagation, probably selecting individual hermaphrodite plants with sweet bearing fruits and pruning them to increase yield (Miller, 2008).

Winemaking, on the other hand, does not necessarily require viticulture as a prerequisite: wine can be fermented from either wild or cultivated grapes. Wild grape wine may have been the first important alcoholic beverage due to the simple process required for fermentation: a rich harvest of wild grapes can result in fermentation and winemaking by simply crushing the fruit and allowing contact of the juice with the yeasts present on the skin (Singleton, 1995; McGovern, 2003; Miller, 2008; Valamoti et al., 2007). Various lines of evidence have been used to detect early winemaking in the archaeological record, including pottery typology, chemical residue analysis and archaeobotanical remains.

Pressed grapes found in archaeobotanical deposits are eloquent indicators of juice extraction from pressed fruits, which would ferment to different degrees, depending on the duration of the process. Charred finds of early grape pressings are reported from 5th millennium BC Dikili Tash in northern Greece (Valamoti et al., 2007), 3rd millennium BC Kurban Höyük in Turkey (Miller, 2008), 3rd millennium BC Myrtos (Renfrew J.M.1972) and 2nd millennium BC Monastiraki on Crete (Sarpaki, 2012). Although these finds *per se* are not conclusive as to the specific use of the grape juice, wine is a very likely candidate, though alternative uses such as the preparation of grape syrup or vinegar cannot be ruled out.

Concerning residue analyses, tartaric acid has been recognized as a relevant biomarker of grape (Ribéreau-Gayon et al., 2006 p. 4; Ehling and Cole, 2011). However, tartrates, formed during winemaking and ageing, although highly insoluble and therefore well preserved (Michel et al., 1993), present a real technical challenge for extraction and identification by chemical structural analysis. Several analytical approaches have been developed in the past decades. The first one, spot tests, has been intensively used (McGovern and Michel, 1995) but it is now recognized that these tests are conducive to numerous false positives (Feigl, 1966; Stern et al., 2008). Infrared (IR) spectrometry has been implemented for the characterisation of crushed sherds or residues adhering to ceramics (McGovern et al., 1996). However, identifying a chemical function of a molecule by IR-spectrometry or by UV-spectroscopy (McGovern, 1997) does not permit its total and sure identification. Results obtained by this method have often been over-interpreted for detecting tartaric acid and then wine. Confronted with the frequent use of such methods, many analysts underline "a need of more rigour in the analysis of wine residues" (Boulton and Heron, 2000). Over the last twenty years, structural methods such as gas or liquid chromatography coupled with mass spectrometry (GC-MS, LC-MS) have progressively become the reference methodology for wine or grape identification (Guash-Jané et al., 2004; Barnard et al., 2011). Drastic conditions are needed in order to extract grape markers, aldaric acids or polyphenols, because of their high insolubility (Singleton, 1995; Barnard et al., 2011) and/or high degree of polymerization (Cheynier et al., 2006; Garnier et al., 2003). Alkaline fusion of well-preserved residues coming from sealed amphorae found in the tomb of Tutankhamun allowed the rapid wine detection, using tandem LC-MS/MS, by the simultaneous identification of traces of both tartaric and syringic acids (Guash-Jané et al., 2004, 2006a,b). The latter is released from malvidin, a specific marker of dark and '*teinturier*' grapes (Singleton, 1995) although it can also be found in other organic materials such as pomegranates (*Punica granatum* L.) or high mallow (*Malva sylvestris* L.) (Barnard et al., 2011) which may have been used during ancient times. Furthermore, syringic acid can be found in the free acid form in several natural products: cereals e.g. barley, oat, rice (Sosulski et al., 1982), wheat bran (Kim et al., 2005), fruits such as grapes and red and rosé wines (Pozo-Bayón et al., 2003), vegetables such as swiss chard and pumpkin (Li and Beta, 2013). In order to rule out these alternative sources and differentiate free syringic acid from that released by malvidin, a two-step extraction has to be implemented (Barnard et al., 2011).

Thus, the challenge for chemists is the extraction and dissolution of tartrates whose high insolubility prevents extraction by common organic solvents and consequently analysis by liquid chromatography (McGovern et al., 2013), the latter permitting only the elution and separation of soluble molecules. Indeed, direct chromatography of highly insoluble tartrate salts is not possible and requires their preliminary solubilisation by displacement of solubility equilibrium under acidic or alkaline conditions. Recently, specific protocols have been developed in order to enhance the extraction step of tartaric acid from insoluble tartrates present as deposits in ceramics or as absorbed residues in floors or vats from wine-producing installations, both modern and ancient (Pecci et al., 2013a, 2013b). However, the drastic conditions of extraction do not guarantee a good preservation of the other lipids present. In order to identify the wider range of preserved biomarkers, soluble lipids and insoluble fruit and fermentation markers, and to determine the original content(s) of vessels, a sequence of two extractions implemented in parallel or as a series and an analysis of both extracts are needed.

Here, we attempt for the first time an integrated approach towards the detection of prehistoric wine that combines archaeobotanical remains of pressed grapes and a new two-step protocol for the detection of grape biomarkers, applied to an exceptional archaeological context from Neolithic Dikili Tash in northern Greece.

2. Materials

All organic analytical grade solvents and reagents were purchased from Acros Organics (Geel, Belgium) and from Sigma (St-Quentin Fallavier, France).

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