BEESWAX PRESERVED IN A LATE CHALCOLITHIC BEVELLED-RIM BOWL FROM THE TEHRAN PLAIN, IRAN

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Abstract
This paper presents the observation of lipid residue, identified as beeswax, preserved in the ceramic matrix of a Late Chalcolithic (c. 3700–3000 BC) bevelled-rim bowl (BRB) from the site of Tepe Sofalin on the Tehran Plain. Gas chromatography-mass spectrometry (GC-MS) was used to separate and identify the lipid constituents preserved in the matrix of a BRB sherd. Lipid biomarkers were recovered including long-chain n-alkanes, n-alkenes, palmitic wax monoesters, fatty acids and n-alcohols characteristic of beeswax. In addition to two disaccharides, cholesterol and β-sitosterol as contaminants were retrieved by solvent soluble extraction from a number of different locations from the ceramic matrix of the analysed sherd.

Keywords
Residue analysis; GC-MS; ceramics; beeswax; bevelled-rim bowl; Tepe Chougali, Iran.

I. INTRODUCTION

I.1. The Tehran Plain and its ceramics

The Tehran Plain occupies part of the northern rim of the central plateau of Iran (Fig. 1) and prehistoric sites of different chronological periods have been known in the region since 1912.1 Systematic excavations and surveys between 1997 and 2003 have greatly enhanced our knowledge of the spatial and temporal distribution patterns of prehistoric sites within the plain.2 These sites are largely defined by their ceramic assemblages, which are themselves characterised by different vessel forms, fabrics and decoration related to each chronological period.3 The most significant periods are the Late Neolithic (c. 6200–5300 BC); Transitional Chalcolithic (c. 5300–4300 BC); Early Chalcolithic (c. 4300–4000 BC); Middle Chalcolithic (c. 4000–3700 BC) and Late Chalcolithic (c. 3700–3000 BC).4 Whilst some of these periods are known for their intricately decorated fine wares, such as the so-called “Cheshmeh Ali Ware” of the Transitional Chalcolithic, the Late Chalcolithic period is characterised by the appearance of coarse-ware ceramics at a number of sites, including bevelled-rim bowls (BRBs).5 This vessel type has been found at Maymonabad and Tepe Sofalin on the Tehran Plain as well as at Ghbris-tan on the adjoining Qazvin Plain. Such bowls have also been recovered at Tepe Mamorin near Imam Khomeini International Airport, Vavan, south of Tehran and Maral Tepe, west of Tehran.6 The BRB is typically an unslipped and undecorated buff vessel, which is roughly made (Fig. 2). It has also been noted that BRBs from Uruk period settlements in Mesopotamia and as far east as Tepe Yahya in south-east Iran, were all made from coarse clay containing grit, pebbles, chaff or stray seeds, which is fired at high temperature, has a porous fabric, and an internal surface that is often smoother than the external one.7

It has been suggested that the limited distribution of BRBs at Late Chalcolithic settlements on the Tehran Plain reflects the existence of some form of

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1 E.g. Schmidt 1935; Fazeli 2001: 9, 15, 17; Coningham et al. 2004.
2 Coningham et al. 2004; 2006; Gillmore et al. 2011.
4 As defined by Fazeli et al. 2004 and Coningham et al. 2006.
6 Potts 2009; see also Abdi 1999: 84; Majidzadeh 2000; 2001a; 2001b.
“administrative exchange system”. The presence of BRBs and other lowland vessel forms has been used as evidence of the ability of Late Uruk Mesopotamian populations to access raw materials in highland Iran, but it has also been suggested that the relationship that is implied may have been with settlements in other areas, such as Susa. It is also clear that BRBs continued to be used during the Proto-Elamite period across much of Iran.

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**I.2. The archaeological contexts and distribution areas of bevelled-rim bowls**

BRBs have a widespread distribution in the Near East during the fourth millennium BC, especially in Mesopotamia, Iran and Anatolia and have been considered to be the crudest vessels used in ancient Mesopotamia. It has, however, been claimed that “bevelled rim bowls are one of the most important features of the early urban civilisations in Iran and Mesopotamia” and they are characteristic artefacts of the Late Uruk and Jemdet Nasr periods (c. 3300–2900 BC).

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8 E.g. Fazeli 2001: 46, 235; Coningham et al. 2006.
10 Weiss and Young 1975.
11 Potts 2009.
numbers of BRBs have been found at Uruk period settlements in Iraq, often being recovered from temples and administrative structures.\textsuperscript{14}

BRBs have been found in most parts of the Iranian plateau, including examples from Miri Qalat in Pakistani Baluchistan.\textsuperscript{15} As many as a quarter of a million BRB sherds were found at Chogha Mish in Khuzestan, including examples found in association with kilns.\textsuperscript{16} BRBs have been found with ashes in the kitchen areas at Godin Tepe, and with other distinctive materials such as numerical or proto-Elamite tablets, seals, seal impressions and large biconical storage jars with lugs at settlements including Godin Tepe, Tappeh Siialk and Tepe Yahya.\textsuperscript{17} At Arisman, BRBs have been found in association with a large slag-heap and furnaces.\textsuperscript{18}

BRBs typically have dimensions of around 10 cm in height and 18 cm in diameter at the mouth, gradually decreasing to about half of that diameter at the base, although at sites in south-west Iran they are known to vary from 18 to 28 cm in diameter and appear to have no fixed height.\textsuperscript{19} It has been suggested that BRBs were made to a standard size of around 0.8 l, which implies that they may have contained a standard volume.\textsuperscript{20} Beale has noted that the

\textsuperscript{14} Buccellati 1990.
\textsuperscript{15} Potts 2009.
\textsuperscript{16} Delougaz and Kantor 1996; Alizadeh 2008.
\textsuperscript{17} Millard 1988; Potts 2009.
\textsuperscript{19} Millard 1988; Helwing 2005.
\textsuperscript{20} Brown 2000.
BRBs from Tepe Yahya in south-east Iran are identical in manufacture method to their counterparts in Mesopotamia and Khuzistan, but proposed that their limited capacity and lack of standardisation suggests that they were not used as ration bowls in any of these areas. Beale also suggested that the conservatism in the shape and method of production of the BRB over several centuries of use and across a wide geographical area implies that these characteristics may have had a symbolic significance.

I.3. Functions of bevelled-rim bowls

The precise function of the BRB is unclear, and a number of different functions have been proposed, including: (1) votive bowl; (2) mass-produced and disposable container; (3) feeding bowl; (4) ration container; (5) strainer and container for products of milk; (6) bread mould; and (7) container for salt production. These proposals have been based on various practical and theoretical observations such as the use of a BRB shaped vessel as a proto-literate Sumerian sign, and the shape, size, fabric, frequency, distribution, and archaeological context of the vessel (i.e. intact or broken, dispersed or in clusters, its appearance with other symbolic or administrative artefacts). One inorganic chemical analysis on ceramics similar to briquetage has been carried out. However, despite the contrasting theories about their function, no chemical study pertaining to the analysis of organic residues that might have been preserved in BRBs has been undertaken prior to the present study.

I.4. Organic residues preserved in ceramics

Ceramics were exploited in the past for different purposes, such as cooking, preparing and processing, storage, transport and consumption of foodstuffs and other natural products, as well as in beekeeping. All of these uses can result in organic residues being present on the interior or exterior surfaces of the ceramic or absorbed into the ceramic matrix of an unglazed vessel. The extent of absorption, however, depends on the porosity of the ceramic fabric, the nature of the material used, anthropogenic effects during use such as heating, and frequency of use, as well as degradation and contamination during use and burial. In this study, a BRB sherd was tested for the preservation of organic residues on its surfaces using gas chromatography-mass spectrometry (GC-MS), in order to assess whether residue is preserved in the bowl and determine the possible use of the bowl.

II. MATERIALS AND METHODS

The BRB sherd analysed in this study (Fig. 2) is a surface find collected from the Late Chalcolithic site of Tepe Sofalin (Fig. 1). It was part of a set of 83 other sherds from the Tehran Plain, Iran that were analysed for the presence of residues. Of these, only two were BRBs, and the other BRB yielded no lipid material. The analysed vessel is of low quality, and is characterised by its coarse and porous fabric, undecorated surfaces, and the thickness of its wall and base. It has most likely been produced in a mould rather than wheel-thrown as the heavy temper needed for BRBs would make wheel-throwing more difficult. The preliminary results of an elemental chemical provenance

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21 Beale 1978.
22 Beale 1978.
23 Thompson and Hutchinson 1931; Beale 1978.
29 Buccellati 1990.
32 Eerkens 2002; Reber and Evershed 2004a; 2004b.
35 Mayyas 2007: 368.
36 Goulder 2010.
of the ceramic suggest that this sherd was manufactured from local raw materials.\textsuperscript{37}

\textbf{II.1. Extraction of lipids by conventional solvent extraction}

Powdered samples were taken from four different locations on the internal and external surfaces of the sherd, and in a similar manner two samples from both surfaces were also taken from a modern potsherd, which was used to assess the contamination that could have occurred during handling and laboratory preparations. The first 2 mm layer, with an area of approximately $1 \times 1$ cm, from the interior and exterior surfaces of the sherd, were separately obtained as a powder using an electrical modelling drill fitted with a tungsten abrasive bit.

The abrasive bit was washed with DCM (dichloromethane) after each step of drilling, while aluminum foil was used to collect the drilled powders, with 0.1 g of each powder extracted three times. Each time, 1 mL mixture of dichloromethane/methanol solution (2:1 v/v) was added to the powder, underwent ultrasonic treatment for 5 minutes and was centrifuged (2000 rpm $\times$ 5 min), and then the supernatant liquid was transferred into another glass vial. The three supernatant liquids from the successive extractions were combined in a small glass vial, and the solvent was evaporated under a stream of nitrogen gas and mild heat.

\textbf{II.2. Derivatisation of lipids and preparing the extracts for analysis}

Derivatisation of lipid constituents was carried out using 3 to 5 drops of $N,O$-bis(trimethylsilyl)trifluoroacetamide (BSTFA) present in 1% v/v trimethylchlorosilane (TMCS). After 24 hours, a few drops of DCM were added, and all the DCM and the unreacted BSTFA were evaporated under stream of nitrogen and mild heat. To each derivatised extract, 20 $\mu$L DCM and a measured amount of approximately 1 $\mu$L of the internal standard (IS; tetratriacontane; $C_{34}H_{70}$; with concentration at 1 mg/mL DCM) were added. The solution was gently mixed, and approximately 1 $\mu$L of the sample solution was taken and analysed using GC-MS.

\textbf{II.3. The analytical technique (GC-MS)}

Combined gas chromatography-mass spectrometry (GC-MS) was carried out using a Hewlett Packard 5890 series II GC connected to a 5972 series mass-selective detector. The GC was equipped with a split/splitless injector. Splitless mode was used. Helium was the carrier gas, with a constant head pressure of 1 psi and a flow rate of 1 mL/min at 50°C. The injector of the GC and the interface of MS were maintained at 300°C and 340°C, respectively. The temperature of the oven was programmed from 50°C (2 min isothermal) to 340°C (12 min isothermal) at a rate of 10°C/min. The column was directly inserted into the ion source. Electron impact (EI) spectra were obtained at 70 eV, with full scan from $m/z$ 50 to 700.

\textbf{III. DISCUSSION}

\textbf{III.1. Results}

Four organic extracts of four powdered samples obtained from the bevelled-rim bowl sherd (locations shown in Fig. 2; details presented in Table 1) were tested in terms of the preservation of organic residues in the sherd using GC-MS. One of these extracts, labelled LC-CG-IW, showed a significant preservation of lipid constituents that have originated from beeswax and sugars. The other three extracts showed lipid profiles of unknown origin.

The total concentration of the lipid constituents in the solvent extract from the bevelled-rim bowl sherd was less than 50 $\mu$g lipid per gram of sherd powder. Three diagnostic classes of preserved biomarkers (Fig. 3) were detected in this lipid extract and identified as shown in Table 1. Monoenes were characterised by two fragment ions at $m/z$ 257 and 239 and the fragment ion $[M + 1]^+$ at $m/z$ 593, 621, 649, 677 for each wax monoester, respectively, except for $C_{48}^+$ ($m/z$ 705), which was not detected because this molecular ion has greater mass unit than the upper mass limit (700 amu) of the mass spectrometer. Free $C_{16}$ fatty acid was detected with characteristic fragment ions at $m/z$ 73, 117, 132 and 145 and $[M-15]^+$ ions at $m/z$ 313, in addition to the molecular ion $[M]^+$ at $m/z$ 328. Other constituents (alcohols, monounsaturated n-alkene and disaccharides) were also detected in the same extract.

\textsuperscript{37} Mayyas 2007: 368.
Table 1. Four powdered samples taken from the ceramic sherd, their locations on the sherd, organic constituents detected in their extracts and the total amount of the lipid in each extract.

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Organic extract</th>
<th>Location on the sherd (see Fig. 2)</th>
<th>Organic constituents preserved</th>
<th>Total amount of the extracted lipid (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LC-CG-IW</td>
<td>Internal surface of the wall of the sherd</td>
<td>Three diagnostic classes of preserved biomarkers: 1. long-chain monoesters of even carbon numbers (C_{40}–C_{48}) each with C_{16:0} fatty acid moiety. 2. n-alkanes (C_{23}–C_{36}) with odd-over-even carbon number dominance. 3. C_{16:0} fatty acid. Other preserved biomarkers: – even-numbered long-chain n-alcohols (C_{30} and C_{32}). – short chain n-alcohols (C_{16} and C_{18}). – monounsaturated n-alkene (C_{33:1}). – sugars, possibly disaccharides.</td>
<td>48</td>
</tr>
<tr>
<td>2</td>
<td>LC-CG-RIW</td>
<td>On the internal surface at the lower part of the wall of the sherd</td>
<td>– two disaccharides (sucrose and trehalose).  – fatty acids (C_{14:0} and C_{16:0}).  – n-alkanes (C_{19} to C_{24} and C_{29}).  – n-alcohols (C_{16}, C_{18}, C_{28} and C_{30}).  – β-sitosterol and cholesterol.</td>
<td>28</td>
</tr>
<tr>
<td>3</td>
<td>LC-CG-IB</td>
<td>Internal surface at the base of the sherd</td>
<td>No lipid components</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>LC-CG-EB</td>
<td>External surface at the base of the sherd</td>
<td>– saturated C_{14:0}, C_{15:0}, C_{16:0}, C_{17:0} and C_{18:0} fatty acids.  – unsaturated C_{16:1}, C_{18:1} and C_{18:2} fatty acids.</td>
<td>23</td>
</tr>
</tbody>
</table>

Beeswax can be identified by three classes of preserved biomarkers: (1) even-numbered long-chain wax monoesters (C_{40}–C_{52}); (2) odd-numbered n-alkanes (C_{21}–C_{33}); and (3) even-numbered free fatty acids (C_{22:0}–C_{34:0}). Other chemical components such as hydroxyl esters and n-alkenes are also present in authentic beeswax, but are often of low abundance. The identification of the biomarkers mentioned above and shown in Figure 3 in a combination in the LC-CG-IW sample is therefore characteristic of beeswax.\[^{39}\]

\[^{38}\] Kolattukudy 1976; Regert et al. 2001; 2003a; 2003b.

Saturated monoesters from beeswax consist of long-chain alcohols (C_{24}–C_{36}) esterified with palmitic (C_{16:0}) fatty acid.\[^{40}\] However, identification of lipid components, such as those of beeswax, is inevitably complicated by degradative processes that occur during vessel use and burial.\[^{41}\] Since the recovered wax monoesters contain an even number of carbon atoms (even-numbered n-alcohols, C_{24}–C_{32}, esterified with the C_{16:0} fatty acid), the presence of very low abun-

\[^{40}\] Patel et al. 2001.
\[^{41}\] Evershed et al. 1995; Dudd et al. 1998; 1999; Evershed et al. 2001: 331–32.
dances of both \( C_{16:0} \) fatty acid and two free even-numbered \( n \)-alcohols \( (C_{30} \) and \( C_{32} \)) in the same extract is distinctive of the partial degradation of the beeswax, which is known to occur due to the hydrolysis of the wax esters.\(^{42}\) The two alcohols \( (C_{30} \) and \( C_{32} \)) were also produced by hydrolysis of wax esters \( (C_{46} \) and \( C_{48} \) respectively).\(^{43}\) The presence of \( n \)-alcohols \( (C_{16} \) and \( C_{18} \)) at trace levels is not associated with the beeswax, but they are ubiquitous lipids and could have formed biosynthetically from \( C_{16:0} \) and \( C_{18:0} \) fatty acids by microbial sources.\(^{44}\)

Monounsaturated \( n \)-alkenes are also one of the components present in modern beeswax with the \( C_{33:1} \) monounsaturated \( n \)-alkene being the major constituent.\(^{45}\) This constituent \( (C_{33:1}) \) was the only \( n \)-alkene detected in this lipid extract. Possible reasons for the lack of other \( n \)-alkenes include the oxidation of the unsaturated double bond(s) of \( n \)-alkenes, either under atmospheric conditions (with exposure to heat, oxygen and moisture/rainfall, in addition to the catalytic effect of the clay minerals) and/or due to degradative microbial oxidation during burial.\(^{46}\) If so, then the oxidation products (most probably hydroxy or dicarboxylic acids) were lost by leaching with water during burial or by washing by rain on the surface of the site or after recovery, as these products are more water-soluble than their precursors.\(^{47}\) The degradation of beeswax components in the extract LC-CG-IW can also be concluded from the absence of the hydroxy monoesters (one of the components of beeswax), which could be attributed to leaching with ground water,\(^{48}\) rainfall or washing the sherd.

Neither phytosterol markers, such as the major plant sterol, \( \beta \)-sitosterol,\(^{49}\) nor animal biomarkers, such as cholesterol, were detected. This potentially indicates

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\(^{43}\) Reber and Evershed 2004b.

\(^{44}\) Killops and Killops 1993: 154; Feng et al. 2005.

that the beeswax was not mixed with any other natural material of botanical or animal origin. Concerning contamination from handling, no common contaminants, such as squalene with cholesterol, were detected.

The three peaks within the retention time 19.1 to 19.4 min (Fig. 3) are probably related to sugars, possibly disaccharides, as they have characteristic fragment ions at m/z 435, 361, 217, 204, 191 and 147.

The second lipid extract LC-CG-RIW from this sherd is shown in Figure 4. This is from a small buff-coloured visible encrustation on the internal surface of the lower part of the wall of the same sherd (location shown in Fig. 2). Organic constituents detected in this extract are shown in Table 1. n-Alkanes and n-alcohols are two components of the epicuticular waxes of higher plants, while β-sitosterol is a major plant biomarker, it is present at low concentration in this extract. However, the presence of C_{16,0} and n-alcohols (C_{28} and C_{30}) in this extract may suggest degraded beeswax on the internal surface of the sherd, but their origin could also be from the soil. Unfortunately, cholesterol was also recovered from the internal surface of the modern potsherd used for comparison and, therefore, it is most likely that the cholesterol in the archaeological sample originated from handling during sample preparation.

The third lipid extract from this sherd is LC-CG-IB from the interior surface at the base of the sherd (GC-MS chromatogram is not shown herein). No lipid components were recovered from this extract.

The fourth lipid extract from this sherd is extract LC-CG-EB (GC-MS chromatogram is not shown herein) from the external surface of the base of the sherd, which consists of saturated and unsaturated free fatty acids (Table 1). However, with the absence of other biomarkers, it is not possible to provide a clear identification of the exact source of the lipid based on the fatty acid distribution alone, since more than one source of lipid, including plant, animal, marine and/or microbe, can be suggested.
III.2 Beeswax properties, uses and its significance in this case study.

Beeswax is one of the most important natural products. It is characterised by its hydrophobicity, plasticity and healing properties; therefore, it could have been used in the past for a wide range of functions. According to the results of chemical analysis of ceramic vessels from western and Mediterranean Europe, beeswax had been exploited from the Neolithic to the Roman periods for a variety of purposes. These include: body care (preparation of cosmetics or medical commodities), symbolic and artistic roles (in paintings, sculptures, rituals and embalming) and technological purposes (adhesive, sealing agent, shipbuilding, writing ground, lost wax casting and corrosion protection), in addition to its use as illuminant.

In this case study, and with the absence of markers of heat, such as soot, burnt surface, cracking, or thermal degradative products of lipids, it is likely that the presence of beeswax in a specific location in the upper part of the internal surface of the wall of this porous sherd (Fig. 2) is related to four possibilities.

(1) The internal surface of the bowl might have been sealed with beeswax as a waterproofing agent or sealant, then the beeswax has since completely degraded except at one location on its internal surface. Beeswax has been previously reported by different researchers as a sealant and a waterproofing material for archaeological vessels from different periods back to the Neolithic.

(2) It is also possible that the bowl was used to retain a commodity, which was possibly covered with beeswax. This would expose only part of the interior wall of the vessel to the beeswax.

(3) The beeswax might have been heated with a liquid (possibly water) inside the bowl, which floated the beeswax over the surface of the liquid forming a molten disc of beeswax, some of which was absorbed into the ceramic fabric at that height of the internal surface of the bowl. This possibility, however, is of low probability because of the porosity of the ceramic fabric of this bowl.

(4) Although the size of this bowl makes it seem unlikely, we cannot exclude the possibility that the bowl was accidentally colonised by bees or intentionally used to collect honey and beeswax in antiquity. The occurrence of beeswax together with the sucrose and trehalose disaccharides in this coarse bowl (i.e. with a rough surface that may have encouraged bees to attach their honeycomb to the internal surface of the wall) may support this possibility. There is evidence of similar examples of the use of ceramic vessels to collect honey and beeswax dating back to the fourth century BC. Such beehives were important from an economic point of view, because honey could be used as a sweetening agent, a component in medicines and fixing scents, and a divine offering, whilst beeswax was used for writing, sealing items, lighting and lining vessels.

IV. CONCLUSION

For the first time, residue analysis has been applied to a Late Chalcolithic BRB. Lipids were extracted from three of the four sampling locations on the same vessel. From the interior wall, lipids were characterised as originating from beeswax, with some indications of partial degradation via hydrolysis. Other lipid and sugar constituents were also recovered from the bowl, but they cannot be so clearly attributed to a
source as they could possibly originate from contaminants such as soil or are not diagnostic of a single source. No significant anthropogenic effects, such as heating, were observed on the surfaces of this bowl or concluded from the results of the lipid analysis applied to this bowl. One explanation for the occurrence of beeswax in the internal surface of this coarse and undecorated vessel is that the beeswax was used as a waterproofing agent or as a material to retain a commodity inside the bowl, offering a further clue as to the potential functions of this vessel type. This interpretation does, however, need to be treated with caution due to the issues of variable lipid preservation from this single sherd.

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Bibliography


the Western Central Iranian Plateau: the First Five Years of Work (Archaeology in Iran und Turan 9), Philipp von Zabern, Mainz: 196–253.


Acid Concentration Gradients Across Potsherds: a Case Study Using Late Bronze Age Canaanite Amphorae”, *Archaeometry* 42: 399–414.


