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Lipid preservation in ceramics and bones from the Iranian Plateau: Implications for dietary and chronological reconstructions

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ABSTRACT

This paper presents the first investigations of lipid preserved in bones and ceramics, from sites of various ages and depositional environments in Iran with the aim of using them in palaeodietary and chronological reconstructions. We also characterized modern dairy products from the region using stable carbon isotope values to provide a local reference range for dairy lipid identification in archaeological ceramics. The dataset recorded on pottery suggests lipids are recovered in appreciable concentrations regardless of the environment or the age of settlements, but rather the vessel function may play an important role. The results showed exploitation of ruminant adipose and dairy products in pottery vessels from the Late Neolithic and Chalcolithic periods. Our dataset suggests lipids in pottery are viable materials for palaeodietary and chronological reconstructions for the Iranian region. Lipid preservation in bones, similar to collagen, is more variable. Lipid and collagen preservation in bones are not directly related and one of the bones studied showed diagnostic lipids in a context of low collagen preservation. This suggests lipids have limited potential to be used for chronological reconstructions as an alternative to collagen when the latter does not survive.

1. Introduction

Marking the Eastern wing of the Fertile-Crescent with the Zagros Mountains, Iran is a key region for the early emergence of agriculture and pastoralism. This high plateau, limited in the North and the West by two imposing chains of mountains, presents a large basin with deserts known as Dasht-e Kavir and Dasht-e Lut. The country also possesses two coastal areas, the Caspian Sea in the North and the Persian Gulf in the South, with several large rivers system, highly threatened today by a growing drought and dam constructions. Due to its geography and vast

area, Iran experiences diverse climates, ranging from continental, humid, subtropical, semi-arid and arid with very large temperature amplitude including extreme winter conditions in the West and North-west of Iran and extreme heat in the Lut desert (Lyons et al., 2020).

Biomolecular archaeology, which studies organic molecules surviving in archaeological matrices (Brown and Brown, 2011), is increasing substantially in terms of methodological development and applications to various materials and regions. The study of bones, ceramics, seeds or coprolites with molecular and isotopic approaches allows information to be gained on prehistoric population behaviour, economy and

Abbreviations: FA, fatty acid; FAME, fatty acid methyl ester; GC, Gas chromatography; GC-MS, Gas chromatography mass spectrometry; GC-C-IRMS, Gas chromatography - combustion - isotope ratio mass spectrometry; TLE, total lipid extract.

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Table 1

Archaeological sites studied, relative age, environment and number of material studied per site.

Site name	Province	Relative age	Climate/ environment	Pottery	Bones
Kholāh Kouh	Qazvin	Transition from Neolithic to Chalcolithic (5200–4600 BCE)	semi-arid/steppe	Plain buff ware $n = 3$ Painted buff ware $n = 3$ Crusted buff ware $n = 5$	Human $n = 1$ Caprine $n = 2$ Small ruminant $n = 1$ Large mammal $n = 1$
Shahrān	Gilan	Late Neolithic/Early Chalcolithic (5000–4000 BCE) Iron Age (800–550 BCE)	Humid/forest	Plain red ware $n = 6$	Human $n = 1$ Small ruminant $n = 3$
Qazānchi	Kermanshah	Pre-Pottery Neolithic (8600–8200 BCE) Late Chalcolithic (4600–4200 BCE) Middle Bronze Age (2600–2200 BCE)	Mediterranean (subtropics)/forest	Plain plum ware $n = 6$	Human $n = 1$ Caprine $n = 2$ Small ruminant $n = 1$ Large mammal $n = 2$
Ālou	Qazvin	Late Chalcolithic (3700–3400 BCE)	semi-arid/steppe	Plain plum ware $n = 1$ Plain buff ware $n = 5$ Plain red ware $n = 1$	Caprine $n = 2$ Cattle $n = 2$
Zāgheh Ābyek	Qazvin	Early Chalcolithic (4300–3900 BCE) Transition from Neolithic to Chalcolithic (5000–4300 BCE)	semi-arid/steppe	Plain plum ware $n = 3$ Plain buff ware $n = 3$ Crusted buff ware $n = 3$	Caprine $n = 4$ Small ruminant $n = 1$
Tepe Sohz	Behbahan plain	Early Chalcolithic (5000–4000 BCE)	Semi-arid	–	Caprine $n = 2$
Wezmeh	Kermanshah	Late Neolithic/Chalcolithic	Mediterranean/ continental	–	Cattle $n = 1$ Deer $n = 1$
Nishāpur	Khorasan Razavi	Sassanian-Islamic period (since 4th century CE)	semi-arid/steppe	Fuqā'a' $n = 3$ Plain buff ware $n = 2$	–

interactions with the environment. The exploration of some aspects of biomolecular archaeology, i.e. lipids, is nonetheless scarce for the Iranian region and potential exists of further investigation. The various climatic environments existing in Iran can likely have effects on the lipid preservation in archaeological artefacts across the country.

Developed about 30 years ago organic residue analysis of lipids preserved in pottery vessels has established itself as a key method to study ancient diet, agriculture and culinary practices (Evershed, 2008; Evershed et al., 1990). Lipids in pottery entrapped in the clay matrix remain protected from contaminants in the burial environment (Evershed, 2008; Evershed et al., 1990). Based on molecular and isotopic analyses at the molecular level, this method allows identification of specific food and other natural products likely to have been processed in pottery vessels. Commonly recovered in pottery vessels, degraded animal fats are identified by the presence of $C_{16:0}$ and $C_{18:0}$ fatty acids (FAs) with their source being determined using the $\delta^{13}C$ values on individual FAs. The use of local $\delta^{13}C_{16:0}$ and $\delta^{13}C_{18:0}$ values on FAs from reference animals, fed on controlled diet comparable to prehistoric ones are used, as the $\delta^{13}C$ values are influenced by the diet and environment (Copley et al., 2003; Roffet-Salque et al., 2017). The use of $\Delta^{13}C$ ($= \delta^{13}C_{18:0} - \delta^{13}C_{16:0}$) values removes the environmental effect and is known as the universal proxy. This proxy shows nonetheless some limits for the identification of the mixing of foods within the same vessel. For this reason, recording local ranges for a particular region would be beneficial for better interpreting the results, especially if some mixing of commodities existed. There are to date, no modern references from Iran and the Near East. Based on reference fats available from more arid environments such as Libya (Dunne et al., 2012) and Kazakhstan (Outram et al., 2009), $\delta^{13}C$ values higher than the modern references of adipose and dairy products from UK ruminants fed on a pure C_3 diet are expected for Iran.

Lipid residue analyses in pottery vessels are widespread across Europe, Africa and western Asia particularly for the Neolithic period (e.g. Casanova et al., 2020a; Chakraborty et al., 2020; Courel et al., 2021; Cramp et al., 2019; Debono Spiteri et al., 2016; Dunne et al., 2012; Evershed et al., 2008; Grillo et al., 2020; Lucquin et al., 2016; Oras et al., 2017; Outram et al., 2011; Stojanovski et al., 2020). With the exception of Turkey, Syria and Israel for which substantial pottery assemblages have been studied (Evershed et al., 2008; Nieuwenhuys et al., 2015), the Near East and Southwest Asia have been less studied than other regions. So far studies of lipid from Iran are limited to one Chalcolithic potsherd from Tepe Sofalin where beeswax lipids were identified

(Mayyas et al., 2012) and an assemblage of 20 pottery vessels from the Iron Age site of Kani-Zirin (Qanbari-Taheri et al., 2020) where plant and animal lipids were identified; the study does not provide stable carbon isotope analyses to distinguish the sources of animal fats. The scarcity of analyses from this region means Iran and surrounding areas remain an unexplored ground for lipid residue analyses in ceramics and their use in palaeodietary reconstructions.

The analysis of organic residues in bones relies mainly on collagen for radiocarbon dating or palaeodietary reconstructions. Previous studies of archaeological bones from Iran showed collagen preservation is not affected by age but rather climate (Bocherens et al., 2000). The dry and cold environments are favourable for collagen preservation compared to the warmer regions of the country (Bocherens et al., 2000). Aridity therefore affects the preservation of collagen and impacts palaeodietary and dating programs based on collagen in the most arid areas of the country. While the mechanisms of degradation of bone collagen are understood, the degradation of other biomolecules in bones such as lipids still require investigation (Collins et al., 2002). The study of lipids preserved in bones is uncommon compared to collagen. Early studies showed cholesterol and fatty acids to be the main lipids preserved in human and other animal bones thus they are considered diagnostic lipids the palaeodietary determinations (Evershed et al., 1995; Stott and Evershed, 1996; Stott et al., 1999). Stable carbon isotope analyses of cholesterol showed that values are representative of the last year of diet compared to collagen or apatite that reflect the last 10–25 years of the adult life (Stenhouse and Baxter, 1979; Hedges et al., 2007). More recent attention to lipid extraction and contamination removal protocol for the study of archaeological bone lipids have been undertaken (Colonese et al., 2015). These studies on human and animal bones from the UK, Scandinavia, Spain and Brazil highlighted the potential of bone lipids to be used as a palaeodietary indicator complementary to bone collagen analysis (Evershed et al., 1995; Stott and Evershed, 1996; Stott et al., 1999; Colonese et al., 2015; Jim et al., 2004). The use of bone lipids biomarker in palaeodietary reconstructions remains overall scarce and not a common practice.

Bone collagen is one of the most targeted fractions for radiocarbon dating of archaeological sites. The aridity of the Iranian region affecting bone collagen preservation means other materials or carbon fraction need to be targeted (Bocherens et al., 2000). In such cases bone bioapatite fraction can be used as an alternative to date archaeological bones without collagen, returning a minimum age determination (Zazzo and Saliège 2011). Other compounds such as charcoal or charred

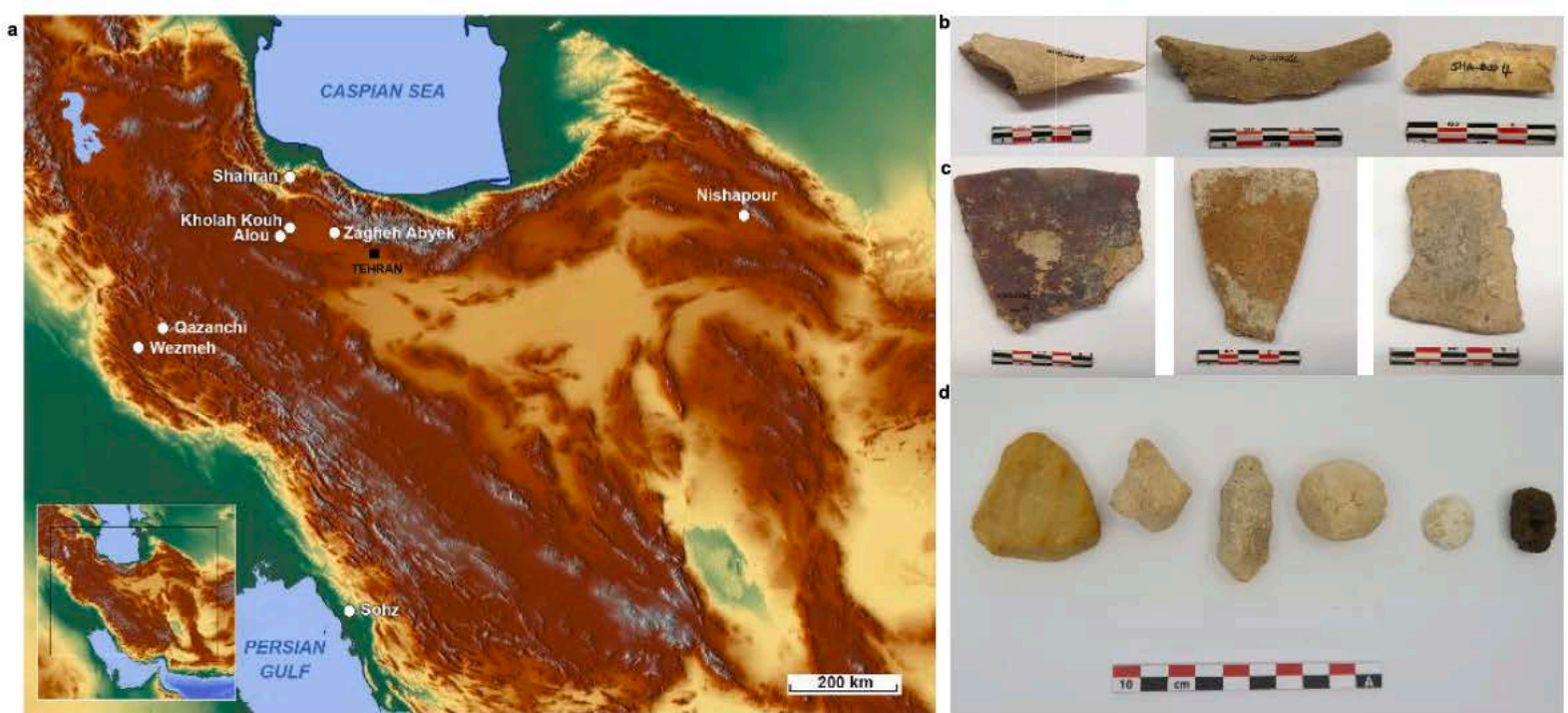


Fig. 1. (a) Map of Iran with studied sites (b) selection of bones showing lipids in appreciable concentration, (c) selection of analysed potsherds showing animal fat residues in appreciable concentration, (d) *Kashk* (five cream/white dried paste on the left side) and *Qara qurut*, (one black paste on the right side).

remains are also used as alternatives. Recent developments for the direct radiocarbon dating of lipids preserved in pottery vessels offers a new alternative to date archaeological sites lacking conventional materials for ^{14}C dating, to date directly the period of use of pottery vessels and to date specific food commodities (Casanova et al., 2020c). This method targets the most abundant and most widely recovered compounds preserved in the lipid extracts, the palmitic and stearic acids. Pottery vessels from arid areas, such as Libya and Turkey were radiocarbon dated by this method (Casanova et al., 2020c) demonstrating the possibility to ^{14}C date well preserved pottery lipids from arid areas. The potential of lipids from bone as a viable source of carbon for chronological studies using the radiocarbon method has nonetheless not yet been addressed. As mentioned previously the aridity affects bone collagen preservation in Iran. Exploring other options to obtain radiocarbon measurements from pottery and bone lipid fraction can provide suitable new alternatives for chronological studies in the Iranian plateau.

This paper aims at a first view of lipid preservation in ceramics and bones in various environments from Iranian context and assesses their potential for use in palaeodietary and chronological investigations. For bone lipid preservation we particularly focus on the chronological aspect. An additional aim of the study was to start establishing a baseline with local modern references.

2. Materials and methods

2.1. Sites and materials

Archaeological materials including 41 potsherds and 28 bones from eight sites were selected to evaluate the lipid preservation in various burial environments of the Iranian Plateau (Table 1, Fig. 1a, Supplementary Information). Three sites, Tappeh Kholāh Kouh (transition from Neolithic to Chalcolithic), Ālou (Late Chalcolithic) and Tappeh Zāgheh Ābyek (transition from Neolithic to and Early Chalcolithic), are located in the semi-arid/steppe environment of the Qazvin Plain (Davoudi et al., 2021, 2019a, 2019b; Moghimi and Davoudi, 2020). The site of Qazānchi (Pre-Pottery, Late Chalcolithic and Middle Bronze Age) and Wezmeh (Late Neolithic/Chalcolithic) are located in a Mediterranean/sub-tropical forest environment of Kermanshah (Mashkour et al., 2020, in press), the site of Shahrān (Late Neolithic/Early Chalcolithic and Iron Age) in the humid forested environment of Gilan and the site of Nishāpur

(Islamic period) is from the semi-arid/steppe environment of the Khorasan Razavi region (Laleh and Lorzadeh, 2019; Fig. 1a). The site of Tepe Sohz (Chalcolithic) is located in the semi-arid environment of the Behbahan plain. All the sites phases were assigned relatively by pottery typology.

The bones from these sites correspond to human ($n = 4$), caprine ($n = 12$), cattle ($n = 3$), deer ($n = 1$), small ruminant ($n = 6$) and large mammals ($n = 3$; Fig. 1b). The bones from Tepe Sohz and Wezmeh showed poor collagen preservation for ^{14}C dating in previous programs (thus remained unpublished). All potsherds derived from common plain coarse or painted vessels (Fig. 1c), except for three vessels from Nishāpur that correspond to specialised vessels named “*Fuqā’a*” (grenade shape).

Modern goat dairy products of *Kashk* ($n = 5$), a dried casein paste, and *Qara qurut* ($n = 1$; a black residue from *Kashk* production used as condiment; Fig. 1d) produced by contemporaneous mobile pastoralists of the Zagros Mountains were collected in the Zagros mountains region by MM during several visits to Bakhtiari nomads and analysed for their lipid molecular and isotopic signatures. The aim of these analyses is to provide some local references for Iran and gain some information on these typical products of the Near East (easily transportable as they are dried) which could have a long tradition (Digard, 1981). Some studies previously focused on the characterization of the volatile, chemical and protein component (Iranmanesh et al., 2018; Mashak et al., 2014; Pourjoula et al., 2020) but not the stable carbon isotope signatures.

2.2. FTIR analysis

FTIR analyses on modern dairy products were performed to identify the presence of lipids in these dehydrated casein pastes. FTIR spectra were recorded on a Perkin Elmer Spectrum 100 spectrometer. Less than a 1 mg of sample was pressed using an ATR sampling accessory and the data were processed using Spectrum IR software.

Collagen preservation was evaluated using ATR-FTIR by quantifying collagen using a peak area ratio approach on the amide I and phosphate bands following the method presented in Lebon et al., (2016). Spectra were recorded on a Vertex 70 (Bruker) spectrometer. <1 mg of bone powder was pressed against diamond crystal using a single reflection diamond ATR accessory (Extended Range Diamond Quest accessory, Specac). ATR-spectra were acquired with a spectral resolution of 4 cm^{-1}

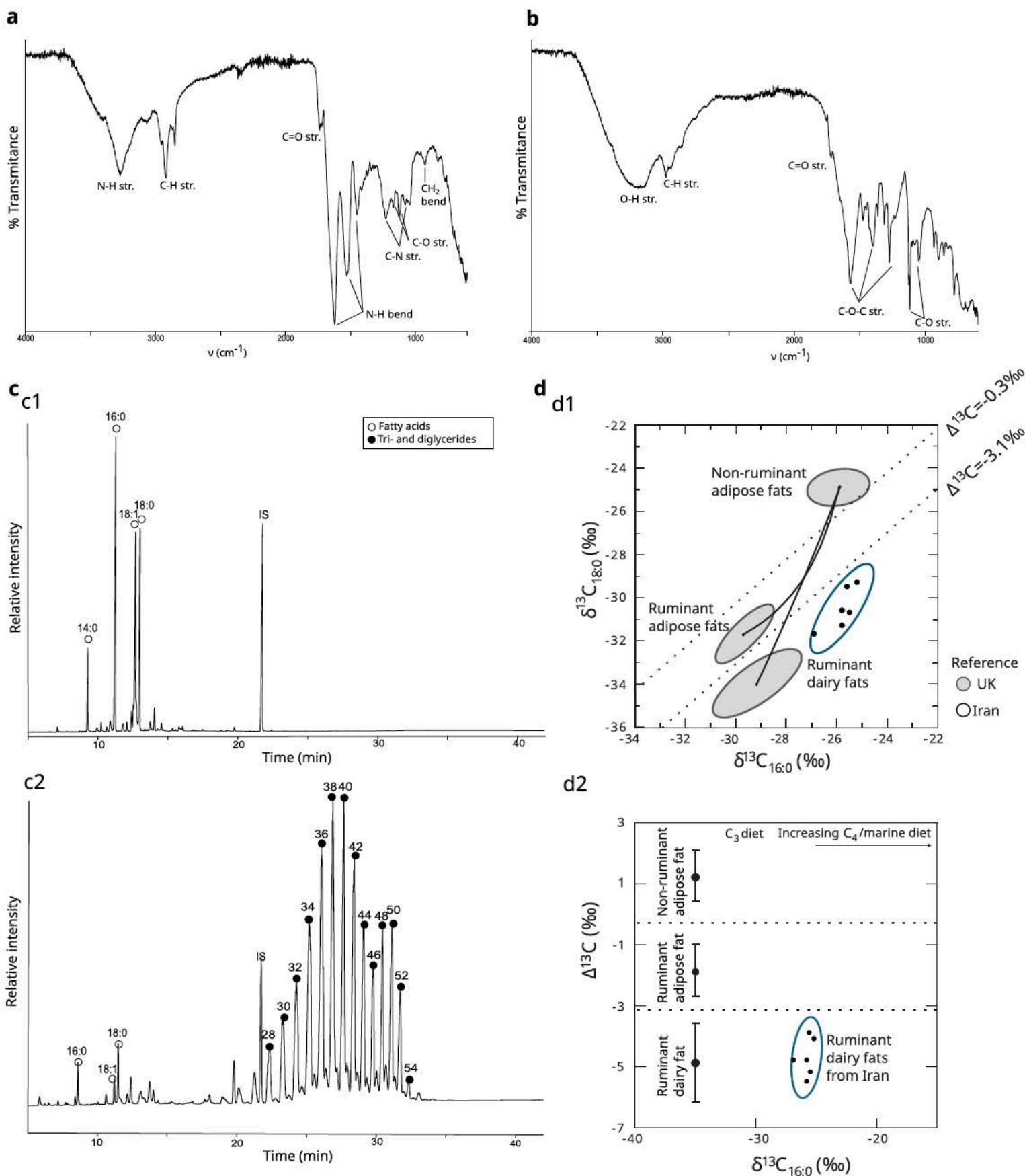


Fig. 2. a, b. FTIR spectra of *Kashk* and *Qara qurut*, respectively. (c) c1. Partial gas chromatogram of *Kashk* (sample K-3) lipid extract using the methanolic/sulphuric acid extraction, c2. Partial gas chromatogram of the same *Kashk* using the solvent extraction. (d) d1. $\delta^{13}\text{C}_{18:0}$ values plotted against the $\delta^{13}\text{C}_{16:0}$ values of fatty acids for the modern Iranian dairy products. Numbers represent the carbon chain length, IS the internal standard. Ellipses denote the 68 % confidence intervals recorded on reference animal fed on a pure C₃ diet from the UK and the ones obtained on Iranian dairy products. d2 $\Delta^{13}\text{C}$ ($= \delta^{13}\text{C}_{18:0} - \delta^{13}\text{C}_{16:0}$) values plotted against the $\delta^{13}\text{C}_{16:0}$ for the same modern dairy products.

and the accumulation of 32 scans in the range of 4000–400 cm^{-1} . Upon comparison with modern bone, collagen is considered datable when the percentage of collagen preservation is above 4 % (Lebon et al., 2016).

2.3. Lipid residue analyses

A piece of about 10 mg from modern dairy products was carefully removed with a scalpel for analysis. The surface of ceramics and bones were cleaned using a modelling drill (~1 g for ceramics and ~ 0.5 g for

Table 2

Lipid concentration and stable carbon isotopes values on individual fatty acids (corrected for the Suess effect) from *Kashk* and *Qara qurut*.

Sample	Solvent extraction		Acid extraction (mean of 3 replicates)				
	Lipid concentration ($\mu\text{g}\cdot\text{mg}^{-1}$)	% lipids	Lipid concentration ($\mu\text{g}\cdot\text{mg}^{-1}$)	% lipids	$\delta^{13}\text{C}_{16:0}$ (‰)	$\delta^{13}\text{C}_{18:0}$ (‰)	$\Delta^{13}\text{C}$ (‰)
Quara Qurut	5.3	0.5	9.0	0.9	-25.8	-31.3	-5.5
Kashk 1	189.8	19.0	76.9	7.7	-25.2	-29.3	-4.1
Kashk 2	148.4	14.8	69.3	6.9	-26.9	-31.7	-4.8
Kashk 3	101.3	10.1	59.3	5.9	-25.6	-29.5	-3.9
Kashk 4	13.1	1.3	9.0	0.9	-25.8	-30.6	-4.8
Kashk 5	37.6	3.8	42.0	4.2	-25.5	-30.7	-5.2

bones) then ground to fine powder in a mortar for their analyses. Ceramic powders were prepared at the Bioarchaeological Laboratory at the University of Teheran before their export for extraction and analysis at the University of Bristol. The whole procedure for bones and modern dairy products was performed at the University of Bristol. The powdered samples were transferred to a culture tube to which a known amount of an internal standard (*n*-tetratriacontane) was added for lipid quantification.

Lipids were extracted from modern dairy products (both on the surface and the middle), ceramic powder and bone powder using an acidified solution of methanol ($\text{H}_2\text{SO}_4/\text{MeOH}$ 4 % v/v, 5 mL, 70 °C, 1 h) followed by a liquid/liquid extraction (*n*-hexane, 4 × 3 mL) and concentrated under a nitrogen stream, based on published procedures (Correa-Ascencio and Evershed, 2014). The same protocol developed for extraction of lipids in pottery vessels (Correa-Ascencio and Evershed, 2014) was used for archaeological bones, as it was shown that the use of acid solution is also relevant for bone lipids extraction (Colonese et al., 2015). During the extraction fatty acids (FAs) are simultaneously derivatised into fatty acids methyl esters (FAMES). An aliquot of the TLE (total lipid extract) was treated with BSTFA (*N,O*-bis(trimethylsilyl)trifluoroacetamide, 20 μL) for derivatisation, then dissolved in an appropriate amount of solvent for their analyses by gas chromatography (GC), GC mass spectrometer (MS) and GC-combustion-isotope ratio MS (GC-C-IRMS).

TLEs (1 μL) were injected in an Agilent Technology 7890A GC-FID (flame ionisation detector) instrument for lipid quantification. Analyses were performed on a non-polar column (15 m × 0.32 mm i.d., DB1 stationary phase, 0.17 μm film thickness). The GC oven temperature was held for 2 min at 50 °C (40 °C for the GC-C-IRMS) and increased to 300 °C at 10 °C $\cdot\text{min}^{-1}$ and held for 10 min. Quantification of archaeological lipids was performed using the peak areas of the internal standard of known amount and concentration, while removing the contribution of any modern contamination (e.g. plasticizer) detected in the TLEs.

Another aliquot of the TLEs was injected into a Trace GC coupled to a Finnigan Trace mass spectrometer (MS) instrument and into an Agilent Technologies 7890A, coupled to an Isoprime GC5 combustion interface and an Isoprime 100 MS (TLEs from ceramics only) for lipid structure and source identification. Columns and temperature program are the same as mentioned above with a starting temperature program at 40 °C for the GC-C-IRMS analyses. GC-C-IRMS measurements accuracy were controlled using an in-house standard solution of FAMES in between two samples, and calibrated against the Pee Dee Vee international standard. The $\delta^{13}\text{C}$ measurements of FAMES were then corrected for the derivative carbon using a mass balance calculation to obtain the $\delta^{13}\text{C}$ values of the FAs (Rieley 1994). The mean of $\delta^{13}\text{C}$ FAs values agreeing within 0.5‰ (two for pottery lipids, three for dairy products) corresponds to the final values reported. The modern dairy products were additionally extracted using a chloroform/methanol solution (2 × 10 mL, sonication 20 min), following a published procedure (Evershed et al., 1990), to identify the full TAGs (triacylglycerols), DAGs (diacylglycerols) and MAGs (monoacylglycerols) distributions in high temperature (HT) GC and HTGC-MS analyses. The column and temperature program for HTGC and HTGC-

MS are the same as described above excepted that final temperature was set at 350 °C and held for 15 min.

3. Results

3.1. Modern references of dairy products produced in the Zagros mountains

FTIR analyses were performed on *Kashk* and *Qara qurut* as a first investigation of whether a lipid fraction remains through the *chaîne opératoire* of producing these dehydrated and proteinaceous dairy products.

FTIR analyses show all *Kashk* samples (Fig. 2a) display a large band of medium intensity centered at 3274 cm^{-1} (N—H and/or O—H stretching), two medium bands at 2922 cm^{-1} and 2853 cm^{-1} (C—H asymmetric and symmetric stretching), intense peaks at 1621 cm^{-1} (N—H stretching of amides) and 1527 cm^{-1} (N—H bending and C—N stretching), a medium intensity band at 1450 cm^{-1} (N—H bending) and medium intensity bands at 1228 cm^{-1} and 1078 cm^{-1} (C—N stretching) and a band of low intensity at 924 cm^{-1} (C—H₂ rocking). These bands are all consistent with amides, carboxylic and aliphatic carbon functions found in proteinaceous compounds such as casein. A lipidic component is also visible to a lesser extent by the presence of a sharp band of medium intensity at 1740 cm^{-1} (C=O of stretching carboxylic acids) and medium intensity bands at 1170 cm^{-1} and 1110 cm^{-1} (C—O stretching).

The *Qara qurut* FTIR spectrum (Fig. 2b) differs significantly from the *Kashk* spectrum. This was expected as this product is a residue from the *Kashk* production. The intense bands at 1621 cm^{-1} , 1527 cm^{-1} and 1450 cm^{-1} characteristics of amides functions are missing, but a large band centred on 1574 cm^{-1} is visible and suggest C—O stretching bonds of carbohydrates rather than the C—N stretching of amides. The FTIR spectrum also displays an intense band centred at 3200 cm^{-1} (O—H stretching), at 1400 cm^{-1} and 1274 cm^{-1} (C—O stretching of ether), at 1047 cm^{-1} (C—O stretching of alcohols) are coherent with the presence of ether functions found in lactose. The bands at 2978 and 2926 cm^{-1} (C—H stretching), at 1720 cm^{-1} (C=O of stretching carboxylic acids) and medium intensity bands at 1117 cm^{-1} (C—O stretching) suggest a lipid component remains in the *Qara qurut* composition.

Kashk and *Qara qurut* were further analysed for their lipid contents by GC, GC-MS and GC-C-IRMS using two extraction methods, a solvent method that preserves all lipid classes and an acidic method that hydrolyzes the esters and makes them amenable directly for GC-MS and GC-C-IRMS analyses (Table 2). The TLEs, after acid treatment, display a lipid distribution dominated by the C_{16:0}, C_{18:1} and C_{18:0} FAs for all of the products (Fig. 2c1). The total lipid extracts (TLEs) obtained through the solvent extraction showed intact triglycerides with T₂₈ to T₅₄ carbon chain length (Fig. 2c2). These components were of relatively low intensity in the *Qara qurut* compared to the *Kashk* samples.

The lipids correspond on average to 9.3 ± 7 % of the mass, with extreme values of 1 to 19 %, (solvent method) or 4.4 ± 4 % of the mass (acidic method; two samples taken from the outer and the middle part), with extreme values of 0.9 to 8 %. The *Qara qurut* contained, however, 1 % of lipids (values obtained using both methods). The results support a

Table 3

Summary of pottery vessels (uncontaminated) with appreciable lipid preservation, their lipid concentrations, stable carbon isotope values and source assignment. FA = Fatty acids (*n*-alkanoic acids), OH = *n*-alkanols, A = *n*-alkanes.

Site name	Sample number	Concentration ($\mu\text{g. g}^{-1}$)	Lipid detected	$\delta^{13}\text{C}_{16:0}$ (‰)	$\delta^{13}\text{C}_{18:0}$ (‰)	$\Delta^{13}\text{C}$ (‰)	Assignment
Kholāh Kouh	KHO-C-0001	10	C _{14:0} , C _{16:0} -C _{18:0} FA	-26.6	-29.5	-3.0	Ruminant adipose fats
	KHO-C-0003	20	-	-	-	-	nd
	KHO-C-0004	76	C _{14:0} , C _{16:0} -C _{18:0} FA	-26.3	-30.9	-4.6	Ruminant dairy fats
	KHO-C-0005	53	C _{14:0} , C _{16:0} -C _{18:0} FA	-27.0	-28.8	-1.7	Ruminant adipose fats
	KHO-C-0008	65	-	-	-	-	nd
KHO-C-0009	273	C _{14:0} , C _{16:0} -C _{18:0} FA	-28.1	-31.2	-3.1	Ruminant adipose fats	
Shahrān	SHA-C-0011	6	-	-	-	-	nd
	SHA-C-0012	11	-	-	-	-	nd
	SHA-C-0013	24	-	-	-	-	nd
Qazānchi	SHA-C-0015	514	C _{14:0} -C _{18:0} FA	-27.8	-32.2	-4.4	Ruminant dairy fats
	QAZ-C-0016	83	C _{14:0} -C _{18:0} FA	-26.1	-29.5	-3.4	Ruminant dairy fats
	QAZ-C-0017	104	C _{14:0} -C _{18:0} FA	-25.9	-29.5	-3.6	Ruminant dairy fats
	QAZ-C-0018	12	-	-	-	-	nd
	QAZ-C-0020	45	C _{14:0} -C _{18:0} FA	-28.7	-28.1	0.7	Non-ruminant/aquatic fats
Ālou	ALO-C-0022	246	C _{14:0} -C _{18:0} FA	-25.6	-26.2	-0.5	Ruminant adipose fats
	ALO-C-0026	93	C _{14:0} , C _{16:0} -C _{18:0} FA	-25.0	-27.6	-2.6	Ruminant adipose fats
	ALO-C-0027	10	-	-	-	-	nd
Zāgheh Abyek	ZAG-C-0029	152	C _{14:0} , C _{16:0} -C _{18:0} FA	-27.9	-28.9	-1.0	Ruminant adipose fats
	ZAG-C-0030	53	C _{14:0} , C _{16:0} -C _{18:0} FA	-25.4	-29.4	-4.0	Ruminant dairy fats
	ZAG-C-0034	474	C _{14:0} , C _{16:0} -C _{18:0} FA	-22.9	-26.6	-3.7	Ruminant dairy fats
	ZAG-C-0035	32	-	-	-	-	nd
	ZAG-C-0036	53	C _{14:0} -C _{18:0} FA	-26.5	-27.4	-0.9	Ruminant adipose fats
Nishāpur	NIS-C-0037	39	C _{14:0} -C _{18:0} FA	nd	nd	nd	Animal fats
	NIS-C-0038	23	-	-	-	-	nd
	NIS-C-0039	19	-	-	-	-	nd
	NIS-C-0040	9	-	-	-	-	nd
	NIS-C-0041	284	C _{14:0} -C _{18:0} FA, C ₁₄ -C ₂₀ OH, C ₂₃ -C ₂₈ A	-	-	-	Plant oil

relatively low lipid content, as was expected from these dehydrated dairy products. It also indicates that only a small portion of lipids goes into the *Qara qurut* formation during *Kashk* production.

The $\delta^{13}\text{C}_{16:0}$ and $\delta^{13}\text{C}_{18:0}$ values were recorded on the TLEs from the acid treatment. The $\delta^{13}\text{C}_{16:0}$ and $\delta^{13}\text{C}_{18:0}$ values (mean of values obtained from 3 replicates of the same sample) were corrected for the post-industrial revolution effects of fossil fuel burning (Copley et al. 2003). They are enriched compared to modern reference ellipses of ruminant dairy products from UK animal raised on a pure C₃ diet (Table2; Fig. 2d1). They remain coherent with the universal dairy products range with $\Delta^{13}\text{C}$ (= $\delta^{13}\text{C}_{18:0}$ - $\delta^{13}\text{C}_{16:0}$) values below -3.1 ‰ (Fig. 2d2). This dataset gives a first range of the regional $\delta^{13}\text{C}$ values of dairy products

and the shift from the $\delta^{13}\text{C}$ values from the UK C₃ raised animals reference database, is coherent with $\delta^{13}\text{C}$ reference values from other arid countries, such as Libya or Kazakhstan (Dunne et al., 2012; Outram et al., 2009).

3.2. Lipid preservation in archaeological ceramics

A total of 14 TLEs were shown to be contaminated with modern fats, likely originating from post-excavation cleaning treatments. This was evident from the C_{18:1} fatty acid being present in abundances comparable to the C_{16:0} and C_{18:0} fatty acids. This unsaturated fatty acid usually degrades in an archaeological context, so its recovery in high

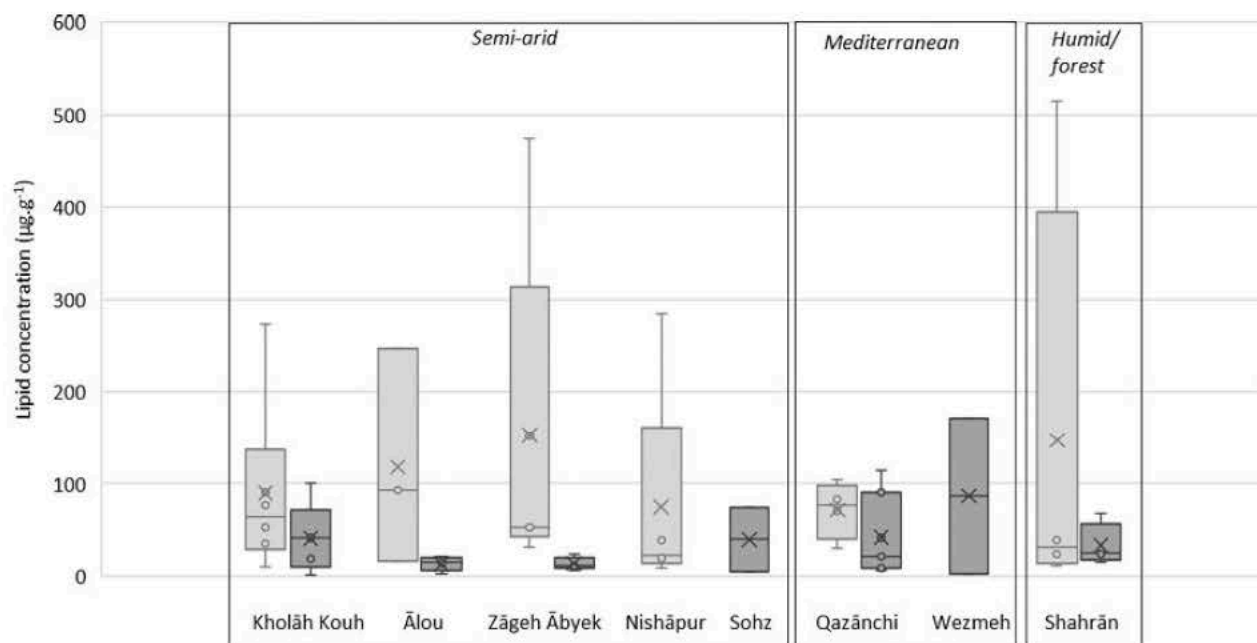


Fig. 3. Box and Whisker plot of lipid concentrations for archaeological pottery (in light grey) and bones (in dark grey) for each site and environment.

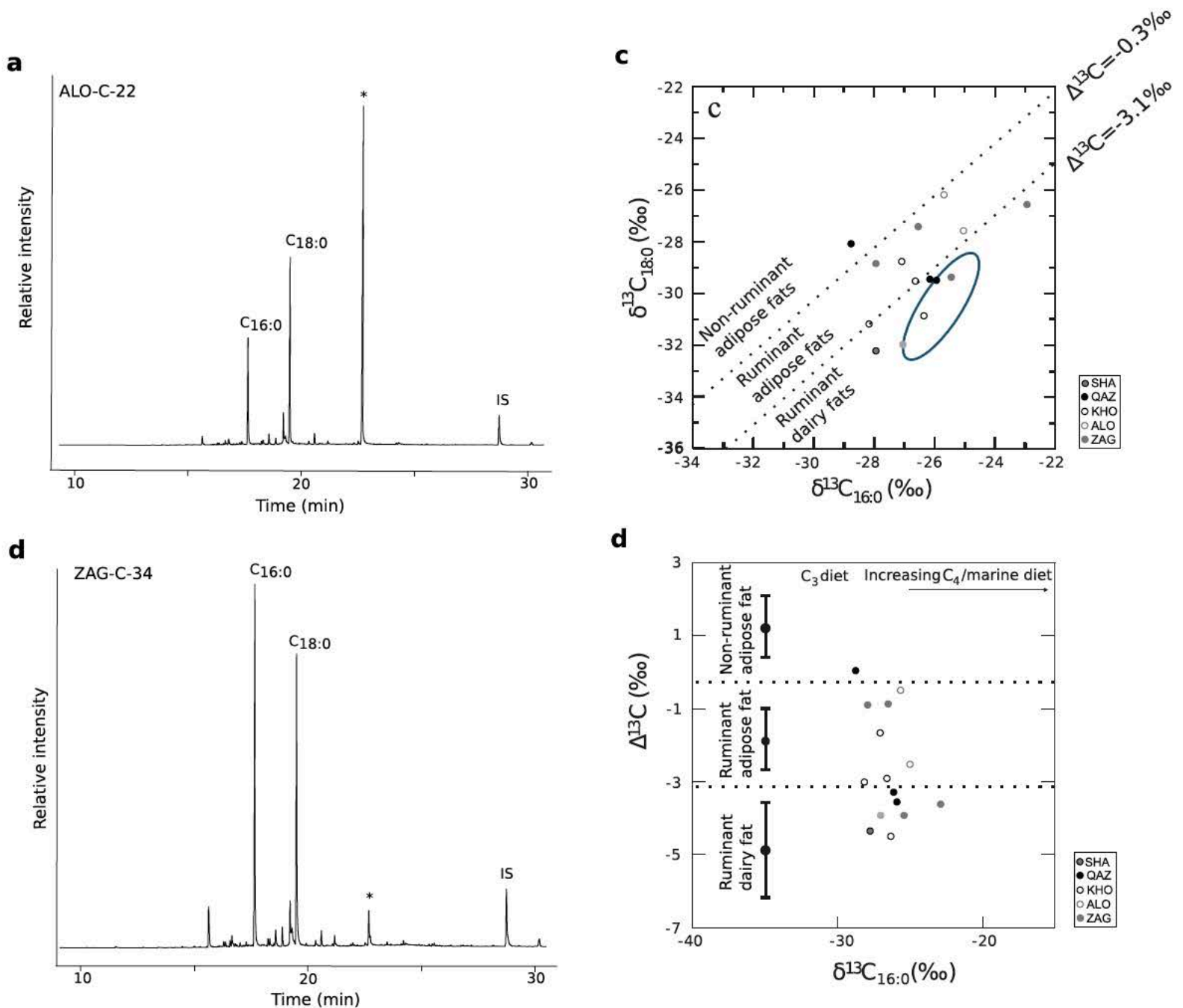


Fig. 4. Partial gas chromatograms of TLEs from pottery vessels from the site of (a) Ālou and (b) Zāgheh Ābyek. *denotes modern plasticizer contamination. (c) $\delta^{13}\text{C}_{18:0}$ values plotted against the $\delta^{13}\text{C}_{16:0}$ values of fatty acids for archaeological potsherds containing animal fats. The ellipse denotes the 68 % confidence intervals recorded on reference dairy products from Iran. (d) $\Delta^{13}\text{C}$ ($= \delta^{13}\text{C}_{18:0} - \delta^{13}\text{C}_{16:0}$) values plotted against the $\delta^{13}\text{C}_{16:0}$ for the same archaeological potsherds containing animal fats. Ranges denotes reference values for animal products across the world (Copley et al., 2003; Dunne et al., 2012; Outram et al., 2009). KHO = Kholāh Kouh, SHA = Shahrān, QAZ = Qazānchi, ALO = Ālou, ZAG = Zāgheh Ābyek, NIS = Nishāpur.

abundances is unlikely. In addition, these TLEs present a cluster of peaks eluting before the internal standard (IS) that corresponds to modern contamination. These TLEs were likely contaminated by an industrial detergent used that contained triglycerides despite them not being listed in the chemical list; it was removed from the cleaning procedure during preparation of the other potsherds. These TLEs were disregarded in the remainder of the analyses as the archaeological origin of $\text{C}_{16:0}$ and $\text{C}_{18:0}$ fatty acids are dubious in this case. Therefore, the results discussed hereafter are on the 27 remaining potsherds. Most TLEs also showed some modern plasticizer contamination, likely arising from their storage in plastic bags.

The remaining potsherds ($n = 26$ out of 27) displayed lipid concentrations in appreciable abundances ($>5 \mu\text{g}\cdot\text{g}^{-1}$ of sherd) with an average lipid concentration of $108 \mu\text{g}\cdot\text{g}^{-1}$ of clay with the maximum concentration reaching $514 \mu\text{g}\cdot\text{g}^{-1}$ (Table 3, Fig. 3). A total of 15 TLEs (or 55 % of the uncontaminated sherds) were dominated by the $\text{C}_{16:0}$ and $\text{C}_{18:0}$ fatty acids characteristic of degraded animal fats (Fig. 3a,b). These

included $n = 4$ of the TLEs from Kholāh Kouh, $n = 2$ of the TLEs from Ālou, $n = 4$ from Zāgheh Ābyek, $n = 3$ from Qazānchi, $n = 1$ from Nishāpur and $n = 1$ from Shahrān with a maximum lipid concentration of 273, 246, 474, 104, 38 and $514 \mu\text{g}\cdot\text{g}^{-1}$ of clay, respectively. At the site of Nishāpur one of the two undecorated vessels showed appreciable lipids with an even numbered distribution of fatty acids ($\text{C}_{14:0}$ - $\text{C}_{18:0}$) dominated by the $\text{C}_{16:0}$ FAs, unsaturated $\text{C}_{16:1}$ and $\text{C}_{18:1}$ FAs, a suite of even numbered n -alkanols (C_{14} - C_{20}), and a suite of n -alkanes (C_{20} - C_{28}). The presence of unsaturated FAs is not questionable in this case as this ceramic is from a relatively recent period (Islamic), where survival of such compounds in elevated concentration remains possible. This lipid distribution suggests plant oil processing in this particular vessel (Gunstone, 2004; Dunne et al., 2016). One of the *Fuqā'a* showed a weak preservation of animal fats, the other two, however, showed low lipid recoveries that could not be traced to specific products and would need further investigation with high resolution GC-MS.

The $\delta^{13}\text{C}$ values of individual fatty acids were recorded for all the

Table 4

Summary collagen (prediction) and lipid preservation in archaeological bones. The mass required for ^{14}C lipid dating was calculated based on the peak area of the main compound. D = datable, ND = non-datable, FA = fatty acids, C = cholesterol, nd = non-determined, * = non-diagnostic lipids likely soil contamination.

Site name	Sample number	Collagen preservation (%)	Datable by ^{14}C on collagen	Lipid concentration ($\mu\text{g. g}^{-1}$)	Lipids identification	Mass required for ^{14}C lipid dating (g)
Kholah Kouh	KHO-B-0001	7.18	D	19	C	35
	KHO-B-0002	17.29	D	41	C ₁₆ -C ₁₈ FA	14
	KHO-B-0003	13.23	D	42	C	19
	KHO-B-0004	5.09	D	0	–	–
	KHO-B-0005	14.31	D	100	C ₁₆ -C ₁₈ FA, C	9
Shahrān	SHA-B-0006	4.79	D	67	C	26
	SHA-B-0007	4.95	D	26	C ₁₆ -C ₁₈ FA, C	50
	SHA-B-0008	3.97	D	15	nd	–
	SHA-B-0009	9.95	D	24	C	32
Qazānchi	QAZ-B-0010	7.47	D	6	nd	–
	QAZ-B-0011	18.07	D	114	C ₁₆ -C ₁₈ FA, C	7
	QAZ-B-0012	12.05	D	91	C	6
	QAZ-B-0013	2.99	ND	5	–	–
	QAZ-B-0014	3.36	ND	7	nd	–
	QAZ-B-0015	3.49	ND	41	nd	–
	QAZ-B-0016	2.71	ND	22	nd	–
Ālou	ALO-B-0017	4.8	D	3	–	–
	ALO-B-0018	10.05	D	16	C ₁₆ -C ₁₈ FA, C	40
	ALO-B-0019	6.17	D	14	C	41
	ALO-B-0020	5.2	D	21	C ₁₆ -C ₁₈ FA	19
Zāgheh	ZAG-B-0021	9.13	D	11	nd	–
Abyek	ZAG-B-0022	7.22	D	15	nd	–
	ZAG-B-0023	5.96	D	6	nd	–
	ZAG-B-0024	8.74	D	24	nd	–
	ZAG-B-0025	5.87	D	3	–	–
	Tepe Sohz	2-Muse234	3.31	ND	74	nd
	3-Muse228	3.78	ND	5	–	–
Wezmeh	6-Muse16158	2.77	ND	2	–	–
	7-Muse16160	14.65	D	171	C ₁₆ -C ₁₈ FA, C	6

sites (except Nishāpur where the concentration was too weak to be determined) and displayed values characteristic of non-ruminant ($n = 1$) ruminant adipose ($n = 7$) and ruminant dairy fats ($n = 7$; Fig. 4c, d). None of the data plots in the modern reference range of ruminant animal products raised in a pure C₃ diet and display elevated $\delta^{13}\text{C}_{16:0}$ values typical of arid environments (Copley et al., 2003; Dunne et al., 2012; Outram et al., 2009). Half of $\delta^{13}\text{C}_{16:0}$ and $\delta^{13}\text{C}_{18:0}$ values assigned to ruminant dairy fats fits within the local range of $\delta^{13}\text{C}_{16:0}$ and $\delta^{13}\text{C}_{18:0}$ values from modern *Kashk* and *Qara qurut*. The remainder display enrichment in ^{13}C compared to the UK reference values. TLEs from the site of Zāgheh Ābyek originates from both ruminant adipose ($n = 2$) and ruminant dairy ($n = 2$), and TLEs from the site of Ālou are of ruminant adipose origin ($n = 2$). Both sites showed higher $\delta^{13}\text{C}$ values than for the site of Kholāh Kouh where ruminant adipose ($n = 3$) and ruminant dairy ($n = 1$) fats were identified. These three sites are in the same local area suggesting access of their livestock animals to various pastures containing mixtures of C₃ and C₄ plants. The $\delta^{13}\text{C}$ values on individual FAs from the site of Qazānchi are characteristic of ruminant dairy fat ($n = 2$) and non-ruminant adipose fats ($n = 1$). The latter show lower $\delta^{13}\text{C}$ values than reference range of porcine adipose fats (Fig. 3c, Fig. 4c) which suggests, if not an outlier, an aquatic contribution. Finally, the $\delta^{13}\text{C}$ values of individual FAs at Shahrān are characteristic of ruminant dairy fats ($n = 1$).

Overall, the lipid distribution and concentration are similar for all the environments (Table 3, Fig. 3) of ceramics in the region. The site of Nishāpur is the only one with a specific pottery type, the *Fuqā'a*, which showed a low lipid content likely related to the vessel function although one of them showed low content of animal fats. Some dairy products were processed in pottery vessels at four of the study sites, suggesting these would have been available as early as the Late Neolithic (Shahrān, Qazānchi) and pursued in the Chalcolithic period. It should, nonetheless, be noted that this pilot study is based on a low number of sherds only, so a proper picture of the use of pottery for specific food products processing in prehistoric Iran is not possible from this dataset.

3.3. Lipid and collagen preservation in archaeological bones

The lipid recoveries from the 28 bones analysed averaged $34.6 \mu\text{g. g}^{-1}$ of bone and ranged from 0.5 to $171.4 \mu\text{g. g}^{-1}$ (Table 4, Fig. 3). A total of thirteen bones (46 %) were dominated by diagnostic lipids (i.e. cholesterol and/or fatty acids; Fig. 5a,b; Evershed et al., 1995). Other lipid compounds in some bones could likely come from the soil, thus of exogenous origin. Diagnostic lipids were identified, $n = 3$ from Shahrān, $n = 3$ from Ālou, in $n = 4$ from Kholāh Kouh, in $n = 2$ from Qazānchi, and in $n = 1$ from Wezmeh. The sites of Tappeh Zāgheh Ābyek and Tepe Sohz showed the poorest preservation with no diagnostic lipids identified. The lipid concentration of bones with diagnostic lipids is quite variable. It is rather low with a maximum of $21 \mu\text{g. g}^{-1}$ at Ālou but reaches over $100 \mu\text{g. g}^{-1}$ at Kholāh Kouh, Qazānchi and Wezmeh. The sites of Qazānchi and Wezmeh showed the highest lipid concentration of bones with diagnostic lipids compared to the other sites (Fig. 3). This difference could be related to their depositional environment (here Mediterranean (subtropical)/forest), the sample size is nonetheless too small to draw a pattern based on the burial environment or ages of the sites.

For the purpose of investigating the potential of bone lipid as an alternative fraction in case of poor collagen preservation for radiocarbon dating, we assessed as well collagen preservation in the studied bones. FTIR analyses on bones for collagen preservation prediction (Lebon et al., 2016) suggest that collagen is well preserved (i.e. $> 4\%$) for all the bones from the sites of Kholāh Kouh, Shahrān, Ālou and Zāgheh Ābyek and $n = 3$ of bones from Qazānchi (Table 4, Fig. 5c,d). Four bones from Qazānchi had a low collagen content, as did the two bones from Tepe Sohz and one from Wezmeh ($< 4\%$), from which no collagen could be extracted.

Fig. 6 represents the lipid concentration in bones with diagnostic lipids identified plotted against the percentage of collagen preserved in bones. The linear trendline set to intercept with the origin gives a correlation coefficient of 0.7415 which supports no direct correlation of lipid and collagen preservation for the studied bones. The sites of Kholāh Kouh, Qazānchi and Wezmeh showed the highest preservation of

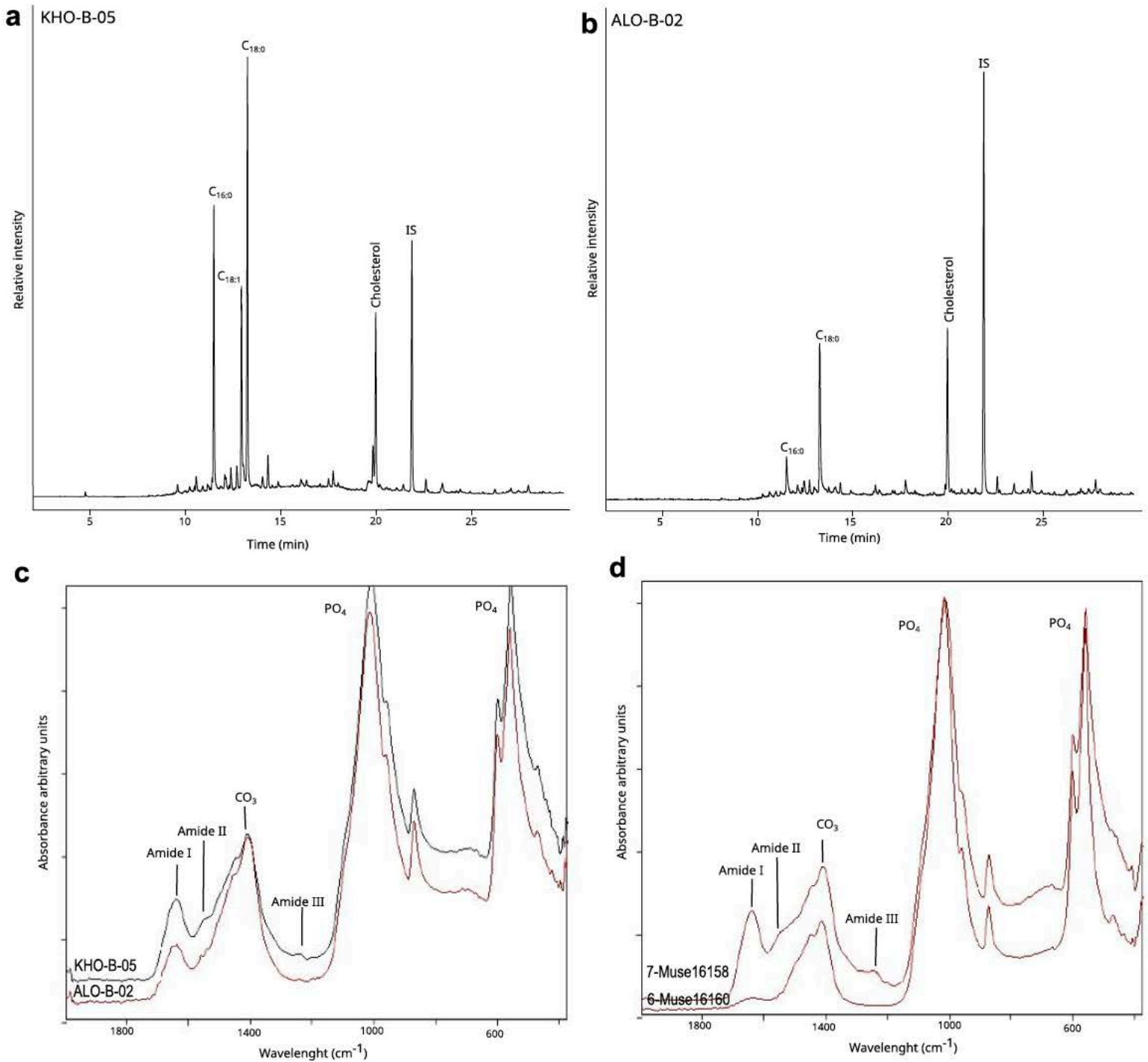


Fig. 5. Partial gas chromatogram of lipid extracted from bones at (a) Kholāh Kouh and (b) Ālou. (c) FTIR spectra of the same bones, showing appreciable collagen preservation. (d) FTIR spectra of the two Wezmeh bones, showing poor collagen preservation for one (6-Muse16160) and appreciable collagen (7-Muse16158), although none could be extracted for ¹⁴C dating.

collagen with bones containing over 10 % of collagen. These bones also showed diagnostic lipids in appreciable concentrations. Interestingly two bones from Qazānchi show good collagen and lipid preservation, one good collagen preservation without the survival of lipids and three both poor lipid and collagen preservation. Preservation of the organic matter in bones at this site is highly variable and raises questions about these contrasting patterns of preservation. The bones from the sites Shahrān and Ālou showed that sufficient collagen was preserved (>4 %) with some relatively low preservation of diagnostic lipids. The preservation of both lipids and collagen in appreciable concentrations suggests palaeodietary studies on bone lipid and collagen at these sites are possible. Palaeodietary reconstruction at Tappeh Zāgheh Ābyek is likely limited to bone collagen, which appears well preserved compare to bone lipids, and seems not possible at Tepe Sohz which showed no collagen or diagnostic lipid preservation in the bones. Again, it should be noted this dataset is too small to draw definitive conclusions. One of the bones

from Wezmeh showed diagnostic lipids (fatty acids and cholesterol) in relatively elevated concentrations (171 μg.g⁻¹ of bone). Collagen preservation prediction from this bone suggested it to be datable, however, no collagen could be extracted using conventional extraction methods, denoting poor collagen preservation. This highlights the possibility of lipid survival in cases of poor collagen preservation which could be used as alternative for dietary and chronological reconstructions.

This difference of bone lipid and collagen preservation between these sites could be related to the depositional environment, as previous studies showed it affects collagen preservation in the region of Iran (Bocherens et al., 2000). Study of a larger dataset of bones would be required to confirm this hypothesis.

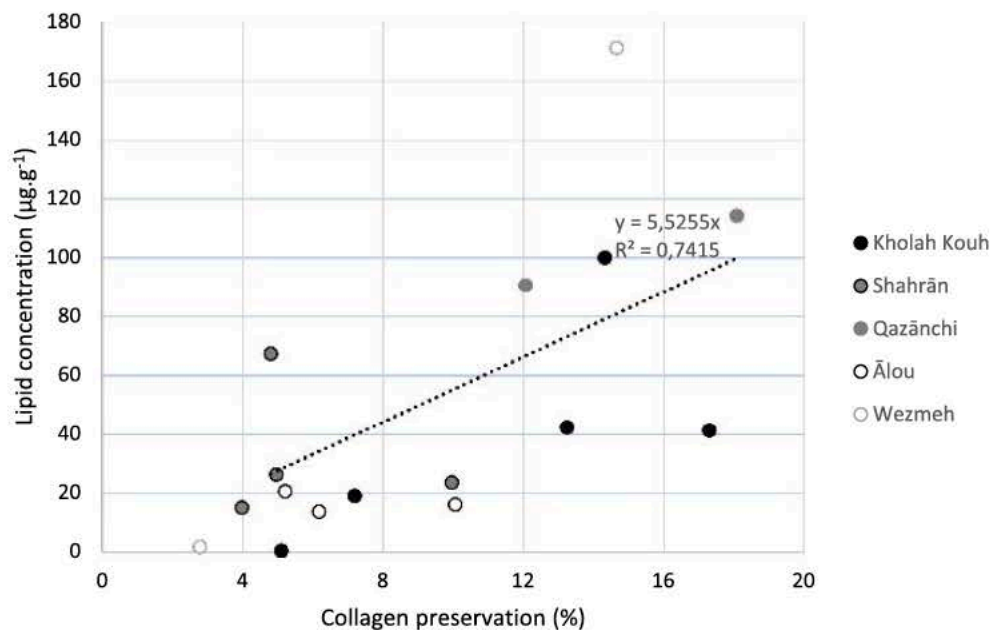


Fig. 6. Lipid concentration preserved in archaeological bones with diagnostic lipids identified against the collagen preservation in the same bones. The dotted line is the linear regression line, y the equation and R^2 the correlation coefficient.

4. Discussion

4.1. Implications for dietary studies

The dataset presented on lipid preservation in ceramics from Iran suggests lipid residue analysis programs have potential for the regional dietary reconstruction and that the climatic variation in deposition conditions in the region is unlikely to cause lipid preservation problems for this country (Fig. 3). Not unexpectedly, the $\delta^{13}\text{C}$ values for individual fatty acids from ruminant products are affected by contributions of C_4 plants in the forages of the grazing animals. The locally sourced modern *Kashk* dairy products provide useful reference materials for interpreting the use of the dairy products in antiquity. Due to the presence of both C_3 and C_4 plants in the environment the universal proxy ($\Delta^{13}\text{C}$ values; Evershed et al., 1999, 2008) is better suited to qualitatively identify the source of animal fats for the region. To perform quantitative analyses and evaluate mixtures of commodities based on $\delta^{13}\text{C}$ values and mixing plots (Mukherjee et al., 2007; Casanova et al., 2020b), recording more local references on conventional dairy products (milk, butter), ruminant adipose fats but also non-ruminant adipose fats would be needed. In Iran, in addition to ruminant animals, numerous Neolithic sites showed also the exploitation of wild local animals such as hemionies or turtles (e. g. Mashkour et al. 2006) which were part of the diet and could complement the modern reference database.

About 46 % of the bones investigated here contained diagnostic lipids (cholesterol and fatty acids; Fig. 3). This suggests the palaeodietary information available from human and animal remains using $\delta^{13}\text{C}$ values on cholesterol may be scarcer than for other regions worldwide that showed better preservation (Evershed et al., 1995; Stott and Evershed, 1996, 1999; Colonese et al. 2015). The dietary record from bone lipids does not seem to provide as much information as the one that can be obtained from collagen for the Iran region, as two sites showed collagen preservation in absence of lipid preservation. Nonetheless, where present, cholesterol offers a complement to bone collagen for palaeodietary reconstruction, as it reflects different macronutrients in the diet (Jim et al., 2004). The sample size is also not representative enough to draw definitive conclusions.

4.2. Implications for chronological studies

In terms of chronology established using radiocarbon dating, studies

involving the direct dating of lipid residues preserved in pottery using the compound-specific radiocarbon analysis (CSRA) approach usually require a lipid concentration of 500 μg per g of clay or more (Casanova et al., 2020c). The maximum lipid concentration recovered in this study reached 510 $\mu\text{g.g}^{-1}$ which is at the lower limit of what is required to yield 200 μg of C for each FAs. At a concentration of 250 $\mu\text{g.g}^{-1}$ about 10 g of pottery would be required for a ^{14}C date. This is usually the maximum amount of clay we can sample from the same potsherd to minimise its destruction. This means smaller samples sizes (<200 μg) may need to be considered to radiocarbon date ceramics in the region, which would involve some methodological adjustments to handle lower amounts of carbon.

The possibility to directly date bone lipids was explored following the same approach as for pottery lipid dating, which is targeting ~ 200 μg of C per single lipid (e.g. cholesterol, fatty acid) for ^{14}C dating. The results obtained here are highly variable for the sites. At Qazānchi and Wezmeh 6 to 7 g of bones would be required for a ^{14}C date, whereas at Kholāh Kouh 8 to 18 g of bones are required and about 20 to 50 g of bones are required at Ālou and Shahrān. Lipid preservation in bones suggests that lipid concentration above 100 μg per g of bone is required to minimise the amount of bone used for a radiocarbon date (<10 g). Only one bone (at Wezmeh) showed diagnostic lipids in sufficient concentration to attempt dating bones in instances of low collagen preservation. This dataset highlights the potential for lipids in bones as being an alternative to collagen for radiocarbon dating in the absence of collagen preservation. Nonetheless, the number of specimens needing pre-screening to find a bone with the potential to be dated seems rather high and may not be considered a suitable alternative based on the extra amount of analysis required. More bone lipid analyses from Iran and other surrounding regions need to be studied to determine the full potential and limits of this alternative.

5. Conclusions

This study shows lipids are recovered in appreciable concentrations in ceramics despite the extreme environmental conditions found in Iran. The $\delta^{13}\text{C}$ values from locally sourced modern dried dairy products showed the Iran region to be influenced by aridity effects, and this should be taken in consideration when interpreting an archaeological dataset. The lipids preserved in ceramics indicate dairy products were already exploited during the late Neolithic period in Iran. Dietary

reconstruction and chronological studies involving the analysis of lipids preserved in ceramics have therefore good potential for this area.

Lipid preservation in bones is very variable and the number of bones studied here does not allow us to draw definitive conclusions on the conditions of lipid survival. The dataset suggests lipid preservation could offer some alternatives for dietary and chronological studies in cases of poor collagen preservation but would not be possible at all the sites from the region.

Overall, lipids from ceramics have a better potential for dietary and chronological study due to their wider recovery in higher concentrations than bone lipids. Pottery vessels used to process food accumulate lipids from their usage and can reach extremely rich lipid concentrations in the clay matrix (e.g., Dunne et al., 2012). Lipid concentrations in archaeological bones are likely limited to their natural abundances in bones, which do not exceed 50 $\mu\text{g}\cdot\text{g}^{-1}$ in the mineral phase and about 900 $\mu\text{g}\cdot\text{g}^{-1}$ in the bone marrow cavity (During et al., 2015).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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