Earliest direct evidence of plant processing in prehistoric Saharan pottery

Julie Dunne\textsuperscript{a}, Anna Maria Mercuri\textsuperscript{b}, Richard P. Evershed\textsuperscript{a}, Silvia Bruni\textsuperscript{c} and Savino di Lernia\textsuperscript{d,e}

\textsuperscript{a}Organic Geochemistry Unit, School of Chemistry, University of Bristol, Cantock’s Close, Bristol BS8 1TS, UK

\textsuperscript{b}Laboratorio di Palinologia e Paleobotanica, Dipartimento di Scienze della Vita, Università degli Studi di Modena e Reggio Emilia, Viale Caduti in Guerra 127, 41121 Modena, Italy

\textsuperscript{c} Dipartimento di Chimica, Università degli Studi di Milano, Via C. Golgi 19, 20133 Milano, Italy

\textsuperscript{d} Dipartimento di Scienze dell’Antichità, Sapienza, Università di Roma, Via dei Volsci, 122 - 00185 Roma, Italy

\textsuperscript{e}School of Geography, Archaeology & Environmental Sciences, University of the Witwatersrand, Johannesburg, Private Bag 3, Wits 2050, South Africa

The invention of thermally resistant ceramic cooking vessels around 15,000 years ago was a major advance in human diet and nutrition\textsuperscript{1-3}, opening up new food groups and preparation techniques. Previous investigations of lipid biomarkers contained in food residues have routinely demonstrated the importance of prehistoric cooking pots for the processing of animal products across the world\textsuperscript{4}. Remarkably, however, direct evidence for plant processing in prehistoric pottery has not been forthcoming, despite the potential to cook otherwise unpalatable or even toxic plants\textsuperscript{2,5}. In north Africa, archaeobotanical evidence of charred and desiccated plant organs denotes Early Holocene hunter gatherers routinely exploited a wide range of plant resources\textsuperscript{6}. Here, we reveal the earliest direct evidence for plant processing in pottery globally, from the sites of Takarkori and Uan Afuda in the Libyan Sahara, dated to 8200-6400 calBC. Characteristic carbon number distributions and $\delta^{13}$C values for plant wax-derived $n$-alkanes and alkanoic acids indicate sustained and systematic processing of C$_3$/C$_4$ grasses
and aquatic plants, gathered from the savannahs and lakes in the Early to Middle Holocene green Sahara.

Diet is a driving force in human evolution, linked with the development of physiology together with ecological, social, and cultural change within the hominin lineage\textsuperscript{1-3}. The processing of foodstuffs was a major innovation, with the cooking of plants a crucial step as this would have increased the availability of starch as an energy source and rendered otherwise toxic and/or inedible plants palatable and digestible\textsuperscript{2,5}. The need for increased processing likely arose with the expansion in dietary plant diversity suggested by the increased complexity of plant palaeobotanical assemblages recovered from Pleistocene and Early Holocene hunter-gatherer sites across the world\textsuperscript{7}. Specialisation in particular plants, notably cereals and pulses, is regarded as one of the characteristics of the Neolithic domestic agricultural “package” in the Near East and Europe, although the sequence and nature of plant and animal domestication varied markedly geographically.

This is particularly manifest in North Africa where the early Holocene green Sahara\textsuperscript{8} comprised a mosaic of humid savannah with extensive herds of large fauna, interspersed with networks of rivers and lakes supporting aquatic plants and animals. The richness of the environment provided significant food procurement opportunities, initially for the semi-sedentary pottery-using hunter-gatherers of the region and then for the first pastoralists who exploited domesticated livestock, such as cattle, sheep and goat\textsuperscript{9}.

North Africa is one of the two known centres worldwide for the invention of pottery (c. 10,000 calBC), with East Asia (c. 14,000 calBC) being the other\textsuperscript{10,11}. Crucially, pottery from two well-dated Libyan Saharan archaeological sites allows the investigation of plant processing as a dietary strategy over this period. Uan Afuda cave\textsuperscript{12} was occupied by hunter-
gatherers during the period dated 8200-6700 BC and the Takarkori rock shelter is one of the few Saharan sites which records the transition from hunter-gathering (8200-6400 BC) to food production (6400-3000 BC), with nearly 5000 years of human occupation\textsuperscript{13} (Supplementary information Figs. 1, 2 & 3; Map of Tadrart Acacus Mountains, Libya; Uan Afuda cave and Takarkori rock shelter). Both sites yielded sedimentary deposits extraordinarily rich in pollen and plant macrofossils, suggesting exploitation for human consumption\textsuperscript{14,15}. At Takarkori, these included exceptionally well-preserved organs from plants such as Typha, Ficus, Cupressus, Tragus, Cassia and Balanites aegyptica (Fig. 1) together with Panicoideae fruits (e.g. Echinochloa, Panicum and Setaria). Significantly, pottery was also introduced around this time\textsuperscript{10,11} presenting the unique possibility to explore plant exploitation and processing amongst these Holocene hunter-gatherer people through organic residues preserved in some of the regions earliest cooking vessels.

A total of 110 potsherds from Early to Middle Holocene contexts at Takarkori and Uan Afuda (Supplementary information Figs. 4 & 5) were solvent extracted using established protocols and analysed using GC, GC-MS and GC-C-IRMS\textsuperscript{4,9}. Of the 81 sherds analysed from Takarkori, $n = 29$ displayed distributions typical of an animal fat origin\textsuperscript{9}, $n = 38$ displayed distributions strongly indicative of a plant origin (Late Acacus, $n = 4$; Early Pastoral, $n = 2$ and Middle Pastoral, $n = 32$; Supplementary Tables 1 and 2) with the remainder likely reflecting either the processing of both plant and animal products in the vessels or the multi-use of vessels. Potsherd samples from the Uan Afuda cave, Libya, all from Late Acacus stratigraphic contexts dated by multiple radiocarbon measures, totalled $n = 29$, of which $n = 22$ yielded appreciable lipid concentrations (76\%). Of these, $n = 18$ of the TLEs yielded lipid profiles indicative of a plant origin (82\%).

The lipid profiles from both sites are characterised by unusually complex mixtures of aliphatic compounds including short, medium and long-chain fatty acids, diacids, $\alpha,\omega-$
hydroxyacids and n-alkanes (Fig. 2). The exceptional preservation of lipids in the desert environment presented opportunities to use a range of diagnostic criteria and proxies to explore the nature of the lipid distributions in the pottery: palmitic/stearic acid ratios (P/S ratio), average chain length\(^1\) (ACL), carbon preference index\(^2\) (CPI), \(P_{aq}\) proxy ratio\(^3\) and compound-specific \(\delta^{13}C\) values are summarised in Table 1 (see also Supplementary Information Tables 1 and 2).

The saturated fatty acids seen in all gas chromatograms (Fig. 2a-c) are common degradation products of acyl lipids. Fresh fatty acids of plants are dominated by unsaturated components (such as C\(_{18:1}\) and C\(_{18:2}\)) but these are either absent or greatly reduced in abundance in aged fats and oils due to oxidation. Well-known plant degradation products are evident in the gas chromatograms as short-chain fatty acids, such as n-nonanoic acid and diacids, e.g. azelaic acid. Strong evidence for plant lipids dominating the extracts comes from the high abundance of palmitic versus stearic acid expressed by high P/S ratios (>4), a pattern never seen in animal fats, especially those of archaeological origin\(^4\). The high abundance of lauric (C\(_{12:0}\)) and myristic (C\(_{14:0}\)) acids is very unusual as these compounds exist only at very low abundance in most plant lipids (Fig. 2c). They occur in high abundance in palm kernel oil\(^5\) but the date palm was not thought to have been present in the Sahara at that time, its natural range in prehistory being restricted to Southwest Asia. Seed oil chain lengths can range from 8 to 24 carbons, with degrees of unsaturation ranging from 0 to 4\(^6\). Likely candidates for seed oil processing in the vessels might be both C\(_3\) and C\(_4\) wild grasses, ubiquitous in the archaeological deposits at both sites. The high P/S ratios of these residues also suggest that oil was processed in the pots\(^7\), and interestingly, some vessels with high P/S ratios do not include n-alkanes, denoting the presence of plant waxes, suggesting the dedicated processing of plant fruits and seeds rather than leafy plants or stems.
However, the presence of long-chain fatty acids up to C\textsubscript{30} is strongly indicative of an origin in leaf or stem epicuticular waxes, although such compounds are also found in suberin\textsuperscript{24}, an aliphatic polyester found in all plants. Overall, the different distributions of fatty acids points to extensive processing of a range of different plant types and organs, such as grains/seeds and leafy plants and stems, in the pottery.

The abundant \textit{n}-alkanes also derive from plant epicuticular waxes, with two main signatures dominating the extracts: i.e. either medium chain-length \textit{n}-alkanes, C\textsubscript{25} or C\textsubscript{27}, or longer chain \textit{n}-alkanes, namely the C\textsubscript{31} \textit{n}-alkane (Fig. 2a,b). Comparison to the archaeobotanical record from the sites, and known affiliations, suggests the lipid profiles dominated by C\textsubscript{31} \textit{n}-alkanes likely originate from C\textsubscript{3} or C\textsubscript{4} wild grasses or lake-margin plants, such as sedges\textsuperscript{25-27}. However, lipid profiles with typical \textit{n}-alkane distributions maximising at C\textsubscript{25} are highly unusual (Fig. 2a) and more diagnostic to plant type. A predominance of C\textsubscript{23} and C\textsubscript{25} \textit{n}-alkanes is known to be characteristic of submerged and floating aquatic plants\textsuperscript{18,27}, such as \textit{Potamogeton}\textsuperscript{28}, also found in the pollen records in the region\textsuperscript{29}. Calculation of the previously proposed $P_{aq}$ proxy ratio further confirmed the lipid profiles with C\textsubscript{25} \textit{n}-alkane maxima likely originating from aquatic plants (Table 1 and Supplementary information Table 1), with $P_{aq}$ ratio values between 0.4-1.0 indicative of submerged or floating macrophytes at both sites. It is especially significant that continuity is evident in the processing of aquatic plants in pottery spanning the Early to Middle Holocene, which includes the transition from hunter-gathering to pastoralism.

The extremely broad range of $\delta^{13}$C values for both the alkanoic acids and \textit{n}-alkanes confirms mixtures of C\textsubscript{3} and C\textsubscript{4} plants were being processed in the vessels (Fig. 3a,b and Supplementary Information Table 1). The individual $\delta^{13}$C values for the leaf wax \textit{n}-alkanes from both sites range from -30.0 to -17.7 ‰ for the C\textsubscript{25} \textit{n}-alkane, from -32.6 to -23.1 ‰ for the C\textsubscript{31} \textit{n}-alkane and from -27.4 to -13.8 ‰ for the C\textsubscript{16:0} fatty acid. These ranges reflect the
known δ¹³C values for both bulk plant lipids (from -32 to -20 ‰ for C₃ plants and from -17 to -9 ‰ for C₄ plants) and for leaf wax lipids, which are more depleted in ¹³C than the biomass (between -39 and -29 ‰ in C₃ plants and -26 and -14 ‰ in C₄ plants). These ranges also encompass the carbon isotope values of freshwater aquatic plants, which commonly display a C₄-like signature but, as discussed above, are separable based on their respective n-alkane distributions.

Hence, the biomarker and stable isotope evidence from the pottery are entirely consistent with the archaeobotanical record, which comprises plants commonly found in the savannah and freshwater habitats present in the Holocene green Sahara (Supplementary Information Fig. 6). What is especially significant is that this is the first evidence that these plants were being processed in pottery vessels at least 10,000 years ago, with a prevalence of plant over animal lipid residues (54% of the total residues recovered from the vessels have a predominantly plant source, with the remainder comprising animal fats or mixtures of plant and animal products) in the pottery assemblages, emphasising the importance of a wide variety of plants, including grains/seeds, leafy and aquatic plants, in these prehistoric people’s diet. Significantly, although the archaeobotanical record across North African sites suggests the consumption of plantstuffs such as cereals (seeds) and sedges, confirmed by these data, the role of aquatic plants in the diets of these prehistoric groups was not previously known. This exploitation of such a variety of plants highlights the sophistication of these early hunter-gatherer groups. Specific examples of where the pottery lipid and archaeobotanical records converge include: (i) evidence for different parts of Typha or cattail, found at Takarkori (Fig. 1a) and Uan Afuda, including rhizomes, peeled stems, flower spikes and pollen, which are known to have been exploited as a food source across the world, and (ii) consumption of leaves, stems and starchy edible rhizomes of some Potamogeton. Processing of this type of emergent flora has a long history of use in north Africa, based on
finds of carbonised rhizomes of several sedges (*Cyperus rotundus, Scirpus maritimus* and *S. tuberosus*) at Wadi Kubbaniya, Egypt, c. 17,000 to 15,000 BC. Grindstones, ubiquitous in north African archaeological deposits, and abundant in the archaeological layers at Uan Afuda and Takarkori, would have facilitated the processing of these wild plants.

In summary, these findings provide unequivocal evidence for extensive early processing of plant products in pottery vessels, likely invented in this region for this purpose\textsuperscript{10,36}. The higher frequency of plant product processing compared to animal products is unique in prehistoric pottery assemblages. From a temporal perspective the results indicate prolonged processing of a broad range of plant material within vessels, dating from the Early Holocene. This is contemporaneous with the introduction of pottery in the region and continues for over 4,000 years. Viewed together, this highlights the sophistication of both food procurement strategies and processing techniques of early Holocene north African foragers, having important implications for dietary security in the changing environments of the green Sahara. Ultimately, the adoption of these broad resource economies, together with a ‘package’ of ceramic containers, stone tools, grinding equipment, and storage facilities, were the cultural prerequisites for the rapid adoption of domesticated animals in North Africa. Interestingly, these data demonstrate that plant processing maintains its importance in the subsistence strategies of these prehistoric groups, occurring both contemporaneously with, and following, the adoption of domesticates and the exploitation of secondary products\textsuperscript{9}.

Significantly, African plant domestication did not occur until much later, around 2500BC, likely in part because the mid-Holocene savannah provided sufficient wild-growing grains and other plants to meet the people’s dietary needs. Finally, adoption of these new plant processing techniques, using thermally resistant ceramic cooking vessels, would also have had far-reaching implications for improvements in human nutrition, health and energy gain. Critically, significant evolutionary advantages would have accrued through the provision of
cooked foods, soft enough to be palatable for infants, potentially leading to earlier weaning and shorter interbirth intervals, thereby enhancing the fertility of women in early pastoral communities.

1944 words

Method section

Lipid analysis and interpretations were performed using established protocols described in detail in earlier publications\textsuperscript{4,9}. All solvents used were HPLC grade (Rathburn) and the reagents were analytical grade (typically > 98\% of purity). Briefly, \textasciitilde 2 g of potsherd were sampled and surfaces cleaned with a modelling drill to remove any exogenous lipids. The sherds were then ground to a powder, an internal standard added to enable quantification of the lipid extract (\textit{n}-tetratriacontane, typically 40 \( \mu \)g) and solvent extracted by ultrasonication (chloroform/methanol, 2:1 \( v/v \), 2 \( \times \) 10 ml). The solvent was evaporated under a gentle stream of nitrogen to obtain the total lipid extract (TLE). Aliquots of the TLE were trimethylsilylated (\( N,O \)-bis(trimethylsilyl)trifluoroacetamide, Sigma Aldrich, 80 \( \mu \)l, 70 °C, 1 hr) and then analysed by high-temperature gas chromatography (HTGC) and gas chromatography-mass spectrometry (GC/MS) to identify the major compounds present. All TLEs were initially screened in a Agilent Industries 7890A GC system equipped with a fused-silica capillary column (15 m \( \times \) 0.32 mm) coated with dimethyl polysiloxane stationary phase (DB-1HT; film thickness, 0.1 \( \mu \)m; Agilent Technologies). Derivatized extracts (1.0 \( \mu \)l) were injected on-column using a cool on-column inlet in track oven mode. The temperature was held isothermally for 2 min at 50 °C and then increased at a rate of 10 °C min\(^{-1}\) and held at 350 °C for 5 minutes. The flame ionization detector (FID) was set at a temperature of 350 °C.
Helium was used as a carrier gas, set to a constant flow (4.6 ml min⁻¹). Data acquisition and processing were carried out using the HP Chemstation software (Rev. B.03.02 (341), Agilent Technologies).

GC/MS analyses of trimethylsilylated aliquots were performed using a ThermoFinnigan TraceMS operating at 70 eV with a scanning range of 60-600 Daltons. Samples were introduced by on-column injection. The analytical column (15 m x 0.32 mm) was coated with dimethyl polysiloxane (ZB-1; film thickness, 0.12 µm). The temperature programming was from 50 to 300 °C at 10°C min⁻¹, following a 2 min isothermal hold at 50 °C. At the end of the temperature programming, the GC oven was kept at 300 °C for 10 minutes. Helium was used as the carrier gas. Data acquisition and processing were carried out using XCalibur software (version 2.0.6). Peaks were identified on the basis of their mass spectra and gas chromatography (GC) retention times, by comparison with the NIST mass spectral library (version 2.0).

Further aliquots of the TLE were treated with NaOH/H₂O (9:1 w/v) in methanol (5% w/v, 70 °C, 1 h). Following neutralization, lipids were extracted into chloroform and the excess solvent evaporated under a gentle stream of nitrogen. Fatty acid methyl esters (FAMEs) were prepared by reaction with BF₃-methanol (14% w/v, Sigma Aldrich, 70 °C, 1 hr). The FAMEs were extracted with chloroform and the solvent removed under nitrogen. The FAMEs were re-dissolved into hexane for analysis by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS).

The majority of carbon isotope analyses were carried out by GC-C-IRMS using an Agilent 6890 GC gas chromatograph, with a CTC A200S autosampler, coupled to a Finnegan MAT DeltaPlus XL mass spectrometer via a Finnegan MAT GCCIII interface. Samples were injected via a PTV injector in splitless mode, with a temperature increasing from 70 °C to 300 °C. The
GC was fitted with a Varian fused silica capillary column (CP-Sil5CB, 100% dimethylpolysiloxane with 0.12 µm film thickness, 50 m x 0.32 i.d.). Helium was used as the carrier gas at a flow rate set at 2 mL min⁻¹. Cu, Ni, Pt (0.1 mm) were used in the alumina combustion reactor (0.5 mm i.d.). The combustion reactor temperature was maintained at 950 °C. The temperature programme comprised of a 2 min isothermal period at 50 °C increasing to 250 °C at a rate of 10 °C min⁻¹, followed by an isothermal period of 15 min at 250 °C. Faraday cups were used to select ions of m/z 44 (¹²C¹⁶O₂), m/z 45 (¹³C¹⁶O₂ and ¹²C¹⁷O¹⁶O) and m/z 46 (¹²C¹⁸O¹⁶O).

References

1. Wrangham, R. W., Holland Jones, J., Laden, G., Pilbeam, D. and Conklin-Brittain, N. (1999). The Raw and the Stolen: Cooking and the Ecology of Human Origins. *Curr. Anth.* **40**(5): 567-594.

2. Carmody, R. N. and Wrangham, R. W. (2009). The energetic significance of cooking. *J. Hum. Evol.* **57**(4): 379-391.

3. Sponheimer, M. et al. (2013). Isotopic evidence of early hominin diets. *Proc. Natl Acad. Sci. USA* **110**(26): 10513-10518.

4. Evershed, R. P. et al. (2008). Earliest date for milk use in the Near East and southeastern Europe linked to cattle herding. *Nature* **455**(7212): 528-531.

5. Wandsnider, L. (1997). The roasted and the boiled: food composition and heat treatment with special emphasis on pit-hearth cooking. *J. Anth. Archaeol.* **16**(1): 1-48.

6. Mercuri, A. M. (2008). Plant exploitation and ethnopalynological evidence from the Wadi Teshuinat area (Tadrart Acacus, Libyan Sahara). *J. Archaeol. Sci.* **35**(6): 1619-1642.
7. **Zeder, M. A.** (2012). The broad spectrum revolution at 40: resource diversity, intensification, and an alternative to optimal foraging explanations. *J. Anth. Archaeol.* **31**(3): 241-264.

8. **deMenocal, P. et al.** (2000). Abrupt onset and termination of the African Humid Period: rapid climate responses to gradual insolation forcing. *Quat. Sci. Rev.* **19**(1-5): 347-361.

9. **Dunne, J. et al.** (2012). First dairying in green Saharan Africa in the fifth millennium BC. *Nature* **486**(7403): 390-394.

10. **Huysecom, E. et al.** (2009). The emergence of pottery in Africa during the tenth millennium cal BC: new evidence from Ounjougou (Mali). *Antiquity* **83**(322): 905-917.

11. **Jordan, P. et al.** (2016). Modelling the diffusion of pottery technologies across Afro-Eurasia: emerging insights and future research. *Antiquity* **90**(351): 590-603.

12. **di Lernia, S.** Ed. (1999). *The Uan Afuda Cave: Hunter-Gatherers Societies of Central Sahara*. AZA Monographs. Firenze: All'Insegna del Giglio.

13. **Biagetti, S. and di Lernia, S.** (2013). Holocene deposits of Saharan rock shelters: the case of Takarkori and other sites from the Tadrart Acacus Mts. (southwest Libya). *Afric. Archaeol. Rev.* **30**(3): 305-328.

14. **Castelletti, L., Castiglioni, E., Cottini, M. and Rottoli, M.** (1999). Archaeobotanical evidence at Uan Afuda: charcoal, wood and seeds from the early Holocene sequence. In *The Uan Afuda Cave: Hunter-gatherer Societies of Central Sahara. Arid Zone Archaeology, Monographs 1*, ed. S. di Lernia, Firenze: All’Insegna del Giglio. 131-148.
15. **Olmi, L. et al.** (2011). Morphological and genetic analyses of Early and Mid Holocene wild cereals from the Takarkori Rockshelter (Central Sahara, Libya) In *Windows on the African Past: Current approaches to African archaeobotany*, eds. A. Fahmy, S. Kahlheber and A. C. D'Andrea, Kiel: Breitschuh & Kock.

16. **Eglinton, G.** and **Hamilton, R. J.** (1967). Leaf epicuticular waxes. *Science* 156(3780): 1322-1335.

17. **Diefendorf, A. F., Freeman, K. H., Wing, S. L. and Graham, H. V.** (2011). Production of n-alkyl lipids in living plants and implications for the geologic past. *Geochim. et Cosmochim. Acta* 75(23): 7472-7485.

18. **Ficken, K. J., Li, B., Swain, D. L. and Eglinton, G.** (2000). An n-alkane proxy for the sedimentary input of submerged/floating freshwater aquatic macrophytes. *Org. Geochem.* 31(7–8): 745-749.

19. **Mills, J. S. and White, R.** (1994). *The Organic Chemistry of Museum Objects*. London: Butterworth and Co. Ltd.

20. **Eckey, E. W.** (1954). *Vegetable fats and oils*. New York: Reinhold.

21. **Hilditch, T. P. and Williams, P. N.** (1964). *The chemical constitution of natural fats*. New York: Wiley.

22. **Gurr, M. I.** (1980). The biosynthesis of triacylglycerols. In *Lipids: structure and function*, ed. P. K. Stumpf, New York: Academic Press. 205-248

23. **Romanus, K. et al.** (2007). An evaluation of analytical and interpretative methodologies for the extraction and identification of lipids associated with pottery sherds from the site of Sagalassos, Turkey *Archaeometry* 49(4): 729-747

24. **Walton, T. J.** (1990). Waxes, cutin and suberin. In *Methods in Plant Biochemistry, vol. 4.*, eds. J. L. Harwood and J. R. Bowyer, London: Academic Press. 105-158
25. **Maffei, M.** (1996). Chemotaxonomic significance of leaf wax alkanes in the Gramineae. *Biochem. System. and Ecol.* **24**(1): 53-64.

26. **Rommerskirchen, F., Plader, A., Eglinton, G., Chikaraishi, Y. and Rullkötter, J.** (2006). Chemotaxonomic significance of distribution and stable carbon isotopic composition of long-chain alkanes and alkan-1-ols in C₄ grass waxes. *Org. Geochem.* **37**(10): 1303-1332.

27. **Kolattukudy, P. E., Croteau, R. and Buckner, J. S.** (1976). Biochemistry of plant waxes. In *Chemistry and biochemistry of natural waxes*, ed. P. E. Kolattukudy, Amsterdam: Elsevier. 289-347

28. **Tuo, J., Wu, C., Zhang, M. and Chen, R.** (2011). Distribution and carbon isotope composition of lipid biomarkers in Lake Erhai and Lake Gahai sediments on the Tibetan Plateau. *J. Grt Lakes Res.* **37**(3): 447-455.

29. **Cremaschi, M. et al.** (2014). Takarkori rock shelter (SW Libya): an archive of Holocene climate and environmental changes in the central Sahara. *Quat. Sci. Rev.* **101**(0): 36-60.

30. **Boutton, T. W.** (1991). Stable carbon isotope ratios of natural materials II: atmospheric, terrestrial, marine and freshwater environments. In *Carbon Isotope Techniques* eds. D. C. Coleman and B. Fry, New York: Academic Press.

31. **Bi, X., Sheng, G., Liu, X., Li, C. and Fu, J.** (2005). Molecular and carbon and hydrogen isotopic composition of *n*-alkanes in plant leaf waxes. *Org. Geochem.* **36**(10): 1405-1417.

32. **Keeley, J. E. and Sandquist, D. R.** (1992). Carbon: freshwater plants. *Plant, Cell & Environ.* **15**(9): 1021-1035.
33. Gott, B. (1999). Cumbungi, *Typha* species: a staple Aboriginal food in Southern Australia. *Austr. Aboriginal Stud.*: 33-50.

34. Vizgirdas, R. and Rey-Vizgirdas, E. (2006). *Wild Plants of the Sierra Nevada*. Reno: University of Nevada Press.

35. Hillman, G. C. (1989). Late Palaeolithic plant foods from Wadi Kubbaniya in Upper Egypt: dietary diversity infant weaning, and seasonality in a riverine environment. In *Foraging and Farming: the Evolution of Plant Exploitation*, eds. D. R. Harris and G. C. Hillman, London: Unwin Hyman. 207-239

36. Haaland, R. (2007). Porridge and pot, bread and oven: food ways and symbolism in Africa and the Near East from the Neolithic to the present. *Cambridge Arch Jnl* 17(02): 165-182.
End notes

Correspondance and requests for material should be addressed to Professor Richard P. Evershed r.p.evershed@bristol.ac.uk

Acknowledgements We thank the UK Natural Environment Research Council for the Life Science Mass Spectrometry Facility (contract no. R8/H10/63; http://www.lsmsf.co.uk) and a PhD studentship to J. D (NE/1528242/1). We also thank Helen Grant of the NERC Life Sciences Mass Spectrometry Facility (Lancaster node) for stable isotopic characterisation of reference standards and derivatizing agents. Sapienza University of Rome (Grandi Scavi di Ateneo) and Italian Minister of Foreign Affairs (DGSP) are thanked for funding for the Italian Archaeological Mission in the Sahara to S.D.L. Libyan colleagues of the Department of Archaeology in Tripoli and Ghat, in particular the former President Dr Salah Agab, Tripoli, are also thanked. Two PhD students, Linda Olmi and Rita Fornaciari, who studied the wild cereal archaeobotanical record are also thanked.

Author Contributions R.P.E. and S.D.L. conceived and planned the project. J.D., R.P.E., S.D.L. and A.M.M. wrote the paper. J.D. performed analytical work and data analysis. S.D.L. designed and directed the excavations and field sampling; A.M.M. studied the archaeobotanical materials and S.B. performed analytical work. All authors read and approved the final manuscript.
Table 1. P/S ratios, CPI, ACL, weighted mean, $P_{aq}$ values and classifications of trimethylsilylated total lipid extracts from Takarkori rock shelter and Uan Afuda cave.

| Sample no. | Archaeological phase | P/S ratio | CPI $C_{23}$-$C_{33}$ | ACL $C_{23}$-$C_{33}$ | Weighted mean $\delta^{13}C$ | $P_{aq}$ | Classification               |
|------------|----------------------|-----------|------------------------|------------------------|-----------------------------|--------|-----------------------------|
| TAK4       | Late Acacus          | 3.1       | 2.1                    | 27.7                   | -23.2                       | 0.55   | Aquatic plant               |
| TAK14      | Middle Pastoral      | 2.0       | n/d                    | 28.9                   | -29.2                       | n/d    | C$_3$ grass                 |
| TAK23      | Late Acacus          | 2.6       | 5.7                    | 28.7                   | -21.4                       | n/d    | C$_4$ grass or aquatic       |
| TAK24      | Early Pastoral       | 5.4       | n/d                    | 28.5                   | -22.4                       | n/d    | C$_4$ grass or aquatic       |
| TAK82      | Middle Pastoral      | 2.8       | 8.1                    | 28.5                   | -21.2                       | n/d    | C$_4$ grass or aquatic       |
| TAK135     | Middle Pastoral      | 5.0       | n/d                    | 28.2                   | -23.3                       | 0.55   | Aquatic plant               |
| TAK159     | Middle Pastoral      | 4.2       | n/d                    | 27.4                   | -20.7                       | 0.61   | Aquatic plant               |
| TAK479     | Middle Pastoral      | 4.6       | 5.2                    | 27.7                   | -27.2                       | 0.58   | Aquatic plant               |
| TAK709     | Middle Pastoral      | 2.5       | 3.9                    | 29.4                   | n/d                         | n/d    | C$_4$ grass                 |
| TAK730     | Middle Pastoral      | 4.8       | 5.1                    | 28.9                   | -25.0                       | n/d    | C$_4$ grass                 |
| TAK766     | Middle Pastoral      | 2.9       | 8.1                    | 26.3                   | -19.5                       | 0.80   | Aquatic plant               |
| TAK860     | Middle Pastoral      | 4.0       | n/d                    | 28.6                   | -21.4                       | n/d    | C$_4$ grass or aquatic       |
| TAK873     | Middle Pastoral      | 2.0       | 4.6                    | 29.5                   | -22.4                       | n/d    | C$_4$ grass/sedge            |
| TAK953     | Middle Pastoral      | 2.1       | 7.6                    | 28.9                   | -24.0                       | 0.35   | C$_4$ grass/sedge            |
| TAK1008    | Middle Pastoral      | 2.5       | n/d                    | 27.9                   | -26.3                       | n/d    | Aquatic plant?               |
| TAK1054    | Middle Pastoral      | 3.1       | n/d                    | 28.4                   | -23.9                       | 0.58   | Aquatic plant               |
| TAK1072    | Middle Pastoral      | 2.9       | n/d                    | 28.1                   | -20.7                       | 0.57   | Aquatic plant               |
| TAK1531    | Middle Pastoral      | 4.0       | n/d                    | 28.4                   | -24.9                       | n/d    | Aquatic plant?               |
| UAF A1     | Late Acacus          | 3.7       | n/d                    | 29.2                   | -24.9                       | n/d    | C$_4$ plant                 |
| UAF A3     | Late Acacus          | 3.7       | n/d                    | 27.3                   | n/d                         | 0.89   | Aquatic plant               |
| UAF A20    | Late Acacus          | 1.5       | n/d                    | 28.7                   | -21.5                       | n/d    | C$_3$ plant                 |
| UAF A45    | Late Acacus          | 4.7       | n/d                    | 26.0                   | n/d                         | 0.75   | Aquatic plant               |
| UAF A46    | Late Acacus          | 4.7       | n/d                    | 27.7                   | -26.4                       | n/d    | Aquatic plant?               |
| UAF A50    | Late Acacus          | 4.2       | n/d                    | 26.3                   | n/d                         | n/d    | Aquatic plant?               |
| UAF A84    | Late Acacus          | 14.3      | n/d                    | 26.9                   | n/d                         | n/d    | Aquatic plant?               |

n/d - not determined, signal intensity too low

Late Acacus period 8900-7400 years uncalibrated years BP, 8300-6100 calBC
Early Pastoral 7400-6400 years uncalibrated years BP, 6300-5300 calBC
Middle Pastoral 6100-5000 years uncalibrated years BP, 5200-3900 calBC

P/S ratio - relative abundance ratio $C_{16:0}/C_{18:0}$ fatty acids values greater than 4 indicate a plant origin
CPI - measures the relative abundance of odd over even carbon chain lengths, e.g. CPI values for all plant species have strong odd-chain preferences, with CPI values varying between 1.6 and 82.1
ACL - weight-averaged number of carbon atoms of the higher plant $C_{25} - C_{33}$ n-alkanes

$P_{aq}$ - emergent and non-emergent aquatic macrophyte input, $P_{aq} < 0.1$ corresponds to a terrestrial plant input, $P_{aq}$ 0.1-0.4 to emergent macrophytes and $P_{aq}$ 0.4-1.0 to submerged or floating macrophytes.
Figure 1 Exceptionally preserved archaeobotanical remains from Takarkori rock shelter (Tadrart Acacus, SW Libya), dating approximately from c. 7500 to 4200 calBC (a) Inflorescence of *Typha* (Late Acacus 3 - c. 6800 calBC), (b) Syconium of *Ficus* sp., and
details (Late Acacus 2 - c. 7500 calBC), (c) Galbulus of *Cupressus* (Middle Pastoral 2), (d) spikelet of *Tragus* (Middle Pastoral 2 - c. 4200 calBC), (e) legumes of *Cassia* (Early Pastoral 1 - c. 6350 calBC), (f) fruit of *Balanites aegyptica* (Late Acacus 3 - c. 6800 calBC), (g) spikelet of *Dactyloctenium aegyptium* and details of grain (Middle Pastoral 2 - c. 4200 calBC). (© The Archaeological Mission in the Sahara, Sapienza University of Rome)
Figure 2 Partial gas chromatograms of trimethylsilylated total lipid extracts (TLEs) from potsherds excavated from Takarkori rock shelter. a-c, Chromatographic peak identities denoted by filled triangles comprise \( n \)-alkanes in the carbon change range \( C_{25:0} \) to \( C_{33:0} \) and filled circles indicate straight-chain fatty acids in the carbon chain range \( C_{9:0} \) to \( C_{30:0} \), maximising at \( C_{16:0} \). a-c, the distributions show leaf wax \( n \)-alkanes and plant fatty acids (a) \( n \)-alkanes maximising at \( C_{25} \) characteristic of an aquatic plant origin, (b) \( n \)-alkanes maximising at \( C_{31} \) originate from \( C_3 \) or \( C_4 \) wild grasses or lake-margin plants, such as sedges, and (c) plant fatty acid profile showing the predominance of the \( C_{16:0} \) over the \( C_{18:0} \) fatty acid and high abundance of \( C_{12:0} \) and \( C_{14:0} \) fatty acids, characteristic of plant seed oil lipids. IS, internal standard, \( C_{34} \) \( n \)-tetracontane.
Figure 3 Plot showing range of δ^{13}C values for the alkanoic acids and n-alkane lipids derived from absorbed residues preserved in pottery from the Uan Afuda cave and Takarkori rockshelter, Libyan Sahara. These δ^{13}C values confirm a combination of C₃ and C₄ plants were being processed in the vessels. The ranges reflect the known δ^{13}C values for both bulk plant lipids (from -32 to -20 ‰ and from -17 to -9 ‰ for C₄ plants^{30}) and for leaf wax lipids which are more depleted in ^{13}C than the biomass (between -39 and -29 ‰ in C₃ plants and -26 and -14 ‰ in C₄ plants^{31})