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Earliest direct evidence of plant processing in prehistoric Saharan pottery

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The invention of thermally resistant ceramic cooking vessels around 15,000 years ago was a major advance in human diet and nutrition[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[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[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[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 8,200–6,400 calBC. Characteristic carbon number distributions and δ^{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[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[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[7]. Specialization 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[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 goats[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[10,11]. Crucially, pottery from two well-dated Libyan Saharan archaeological sites allows the investigation of plant processing as a dietary strategy throughout this period. Uan Afuda cave[12] was occupied by hunter-gatherers during the period 8,200–6,700 BC, and the Takarkori rock shelter is one of the few Saharan sites which records the transition from hunter-gathering (8,200–6,400 BC) to food production (6,400–3,000 BC), with nearly 5,000 years of human occupation[13] (Supplementary information Figs 1–3; 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[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 (for example, *Echinochloa*, *Panicum* and *Setaria*). Significantly, pottery was also introduced around this time[10,11] presenting the unique possibility to explore plant exploitation and processing among 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 and 5) were solvent extracted using established protocols and analysed using gas chromatography (GC), gas chromatography mass spectrometry (GCMS) and gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS)[4,9]. Of the 81 sherds analysed from Takarkori, 29 displayed distributions typical of an animal fat origin[9] and 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 to reflect either the processing of both plant and animal products in 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 29, of which 22 yielded appreciable lipid concentrations (76%). Of these, 18 of the total lipid extracts (TLEs) yielded lipid profiles indicative of a plant origin (82%).

The lipid profiles from both sites are characterized by unusually complex mixtures of aliphatic compounds, including short-, medium- and long-chain fatty acids, diacids, α,ω-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[16] (ACL), carbon preference index[17] (CPI), P_{aq} proxy ratio[18] and compound-specific δ^{13}C values are summarized 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

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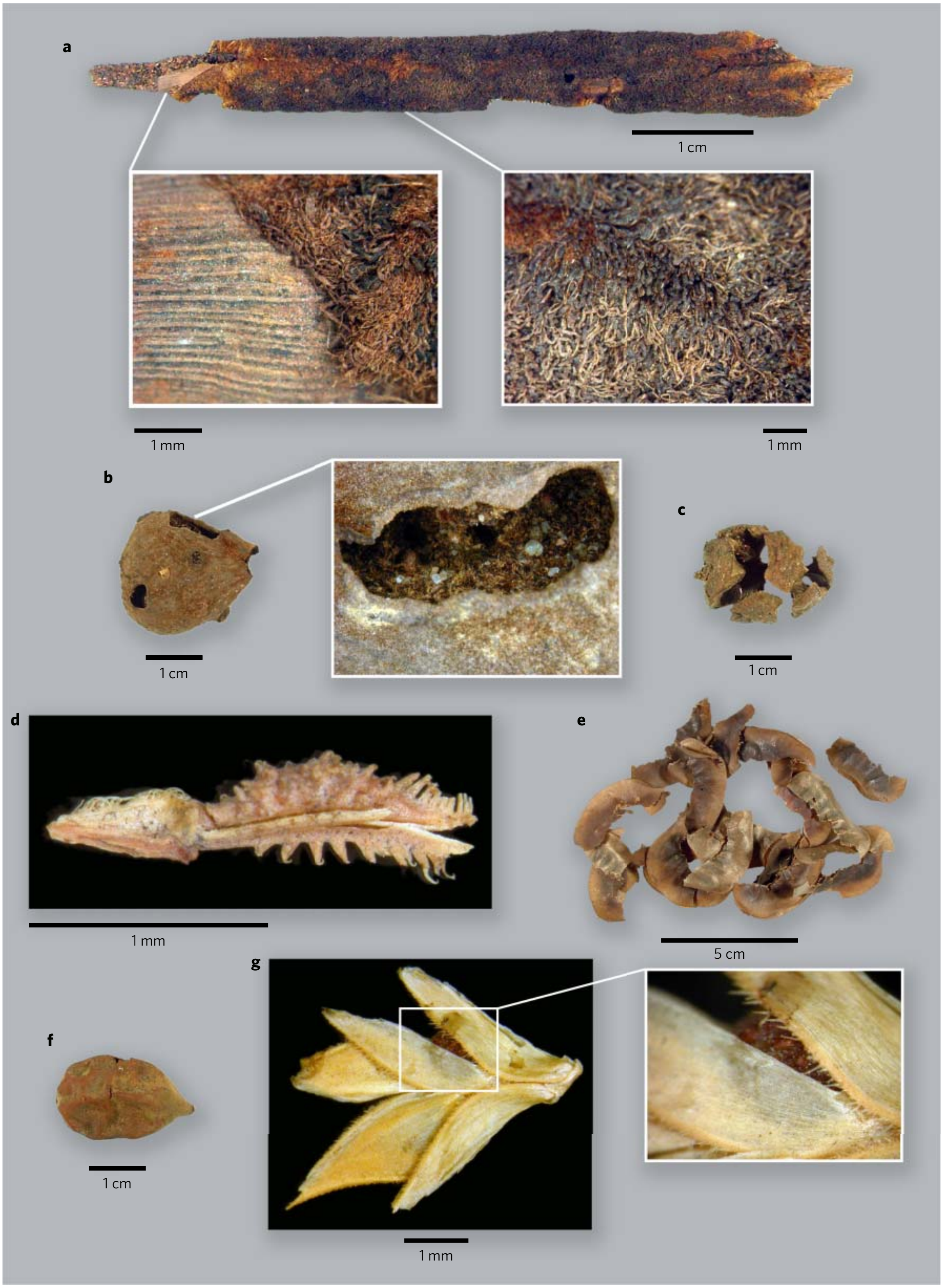

Figure 1 | Exceptionally preserved archaeobotanical remains from Takarkori rock shelter (Tadrart Acacus, SW Libya), dating approximately from *c*. 7,500 to 4,200 calBC. a, Inflorescence of *Typha* (Late Acacus 3 to *c*. 6,800 calBC). **b**, Syconium of *Ficus* sp., and details (Late Acacus 2 to *c*. 7,500 calBC). **c**, Galbulus of *Cupressus* (Middle Pastoral 2). **d**, spikelet of *Tragus* (Middle Pastoral 2 to *c*. 4,200 calBC). **e**, legumes of *Cassia* (Early Pastoral 1 to *c*. 6,350 calBC). **f**, Fruit of *Balanites aegyptica* (Late Acacus 3 to *c*. 6,800 calBC). **g**, Spikelet of *Dactyloctenium aegyptium* and details of grain (Middle Pastoral 2 to *c*. 4,200 calBC). (© The Archaeological Mission in the Sahara, Sapienza University of Rome).

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 because of oxidation. Well-known plant degradation products are evident in the gas chromatograms as short-chain fatty acids, such as *n*-nonanoic acid and diacids, for example azelaic acid. Strong evidence for plant lipids dominating

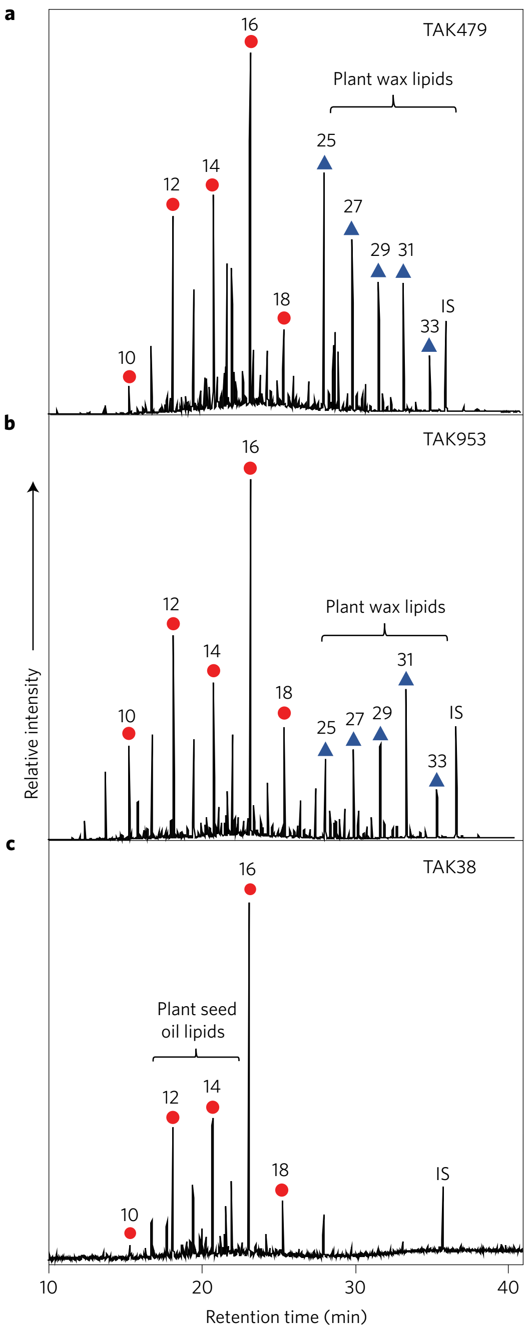

Figure 2 | Partial gas chromatograms of trimethylsilylated TLEs from potsherds excavated from Takarkori rock shelter. a–c, Chromatographic peak identities denoted by filled triangles comprise *n*-alkanes in the carbon change range $C_{25:0}$–$C_{33:0}$ and filled circles indicate straight-chain fatty acids in the carbon chain range $C_{9:0}$–$C_{30:0}$, maximizing at $C_{16:0}$. **a–c**, The distributions show leaf wax *n*-alkanes and plant fatty acids *n*-alkanes maximizing at C_{25} characteristic of an aquatic plant origin (**a**), *n*-alkanes maximizing at C_{31} originate from C_3 or C_4 wild grasses or lake-margin plants, such as sedges, (**b**) and 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 (**c**). IS, internal standard, C_{34} *n*-tetratriacontane.

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[19]. 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[20–21] 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[20–22]. 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[23], 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_{30} is strongly indicative of origin in leaf or stem epicuticular waxes, although such compounds are also found in suberin[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 *n*-alkanes also derive from plant epicuticular waxes, with two main signatures dominating the extracts: either medium chain length *n*-alkanes, C_{25} or C_{27}, or longer chain *n*-alkanes, namely the C_{31} *n*-alkane (Fig. 2a,b). Comparison with the archaeobotanical record from the sites, and known affiliations, suggests the lipid profiles dominated by C_{31} *n*-alkanes are likely to originate from C_3 or C_4 wild grasses or lake-margin plants, such as sedges[25–27]. However, lipid profiles with typical *n*-alkane distributions maximizing at C_{25} are highly unusual (Fig. 2a) and more diagnostic to plant type. A predominance of C_{23} and C_{25} *n*-alkanes is known to be characteristic of submerged and floating aquatic plants[18,27], such as *Potamogeton*[28], also found in the pollen records in the region[29]. Calculation of the previously proposed P_{aq} proxy ratio further confirmed the lipid profiles with C_{25} *n*-alkane maxima likely to originate from aquatic plants (Table 1 and Supplementary information Table 1), with P_{aq} ratio values between 0.4 and 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 *n*-alkanes confirms mixtures of C_3 and C_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 *n*-alkanes from both sites range from −30.0 to −17.7‰ for the C_{25} *n*-alkane, from −32.6 to −23.1‰ for the C_{31} *n*-alkane and from −27.4 to −13.8‰ for the $C_{16:0}$ fatty acid. These ranges reflect the known $\delta^{13}C$ values for both bulk plant lipids (from −32 to −20‰ for C_3 plants and from −17 to −9‰ for C_4 plants[30]) and for leaf wax lipids, which are more depleted in ^{13}C than the biomass (between −39 and −29‰ in C_3 plants and −26 and −14‰ in C_4 plants[31]). These ranges also encompass the carbon isotope values of freshwater aquatic plants, which commonly display a C_4-like signature[32] 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, emphasizing the importance of a wide

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 δ^{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_3 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_3 plant
UAFA3	Late Acacus	3.7	n/d	27.3	n/d	0.89	Aquatic plant
UAF20	Late Acacus	1.5	n/d	28.7	−21.5	n/d	C_3 plant
UAF45	Late Acacus	4.7	n/d	26.0	n/d	0.75	Aquatic plant
UAF46	Late Acacus	4.7	n/d	27.7	−26.4	n/d	Aquatic plant?
UAF50	Late Acacus	4.2	n/d	26.3	n/d	n/d	Aquatic plant?
UAF84	Late Acacus	14.3	n/d	26.9	n/d	n/d	Aquatic plant?

Late Acacus period 8,900–7,400 years uncalibrated years BP, 8,300–6,100 calBC. Early Pastoral 7,400–6,400 years uncalibrated years BP, 6,300–5,300 calBC. Middle Pastoral 6,100–5,000 years uncalibrated years BP, 5,200–3,900 calBC[12,13]. n/d, not determined, signal intensity too low; 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, for example CPI values for all plant species have strong odd-chain preferences, with CPI values varying between 1.6 and 82.1[17]. ACL, weight-averaged number of carbon atoms of the higher plant C_{25}–C_{33} *n*-alkanes[16]; 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[18].

variety of plants, including grains/seeds, leafy and aquatic plants in the diet of these prehistoric people. 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 (1) 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[6,33], and (2) consumption of leaves, stems and starchy edible rhizomes of some *Potamogeton*[34]. Processing of this type of emergent flora has a long history of use in North Africa[35], based on finds of carbonized rhizomes of several sedges (*Cyperus rotundus*, *Scirpus maritimus* and *S. tuberosus*) at Wadi Kubbaniya, Egypt, *c.* 17,000–15,000 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.

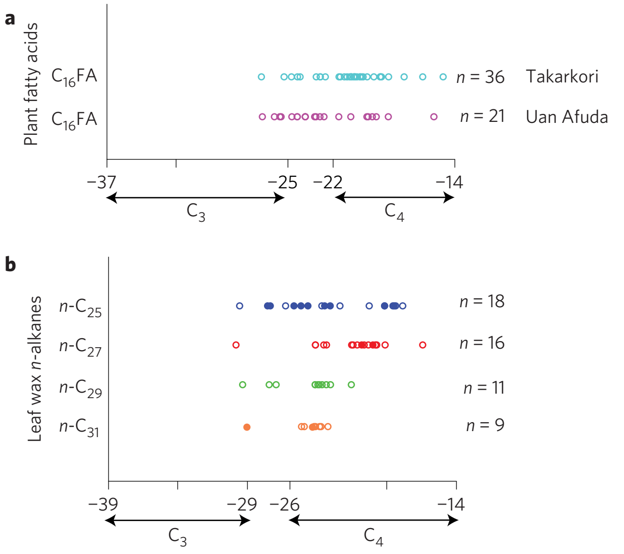

Figure 3 | Plot showing range of δ^{13}C values for the alkanoic acids and *n*-alkane lipids derived from absorbed residues preserved in pottery from the Uan Afuda cave and Takarkori rockshelter, Libyan Sahara. These δ^{13}C values confirm a combination of C_3 and C_4 plants were being processed in the 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 C_4 plants[30]) and for leaf wax lipids that are more depleted in ^{13}C than the biomass (between −39 and −29‰ in C_3 plants and −26 and −14‰ in C_4 plants[31]). FA, fatty acid.

In summary, these findings provide unequivocal evidence for extensive early processing of plant products in pottery vessels, likely to have been invented in this region for this purpose[10,36]. The higher frequency of plant product processing than 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 continued for more than 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[9].

Significantly, African plant domestication did not occur until much later, around 2,500 BC, likely to be 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.

Methods

Lipid analysis and interpretations were performed using established protocols described in detail in earlier publications[4,9]. All solvents used were HPLC grade (Rathburn) and the reagents were analytical grade (typically >98% of purity). Briefly, ~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 (*n*-tetratriacontane, typically 40 µg) and solvent extracted by ultrasonication (chloroform/methanol, 2:1 *v/v*, 2 × 10 ml). The solvent was evaporated under a gentle stream of nitrogen to obtain the TLE. Aliquots of the TLE were trimethylsilylated (*N*,*O*-bis(trimethylsilyl) trifluoroacetamide, Sigma Aldrich, 80 µl, 70 °C, 1 h) and then analysed by high-temperature gas chromatography (HTGC) and GCMS to identify the major compounds present. All TLEs were initially screened in a Agilent Industries 7,890A GC system equipped with a fused-silica capillary column (15 m × 0.32 mm) coated with dimethyl polysiloxane stationary phase (DB-1HT; film thickness, 0.1 µm; Agilent Technologies). Derivatized extracts (1.0 µl) 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 min. 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).

GCMS 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 × 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 min. 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 GC retention times, by comparison with the NIST mass spectral library (version 2.0).

Further aliquots of the TLE were treated with NaOH/H_2O (9:1 *w/v*) in methanol (5% *v/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 BF_3-methanol (14% *w/v*, Sigma Aldrich, 70 °C, 1 h). The FAMEs were extracted with chloroform and the solvent removed under nitrogen. The FAMEs were redissolved into hexane for analysis by GC-C-IRMS.

The majority of carbon isotope analyses were carried out by GC-C-IRMS using an Agilent 6,890 GC gas chromatograph, with a CTC A200S autosampler, coupled to a Finnegan MAT Delta$^{\text{plus}}$ XL mass spectrometer using a Finnigan MAT GCCIII interface. Samples were injected by means of a PTV injector in splitless mode, with a temperature increasing from 70 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 × 0.32 i.d.). Helium was used as the carrier gas at a flow rate set at 2 ml min^{-1}. Copper, nickel and platinum (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 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 ($^{12}C^{16}O_2$), m/z 45 ($^{13}C^{16}O_2$ and $^{12}C^{17}O^{16}O$) and m/z 46 ($^{12}C^{18}O^{16}O$).

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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.

Additional information

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Competing interests

The authors declare no competing financial interests.

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