Molecular and isotopic investigations of pottery and 'charred remains' from Sannai Maruyama and Sannai Maruyama No. 9, Aomori Prefecture, Japan

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ABSTRACT

This paper presents a preliminary study of the analysis of organic residues of Early and Middle Jomon pottery and 'charred remains.' Samples are taken from the Sannai Maruyama site and the Sannai Maruyama No. 9 site in Aomori City, Aomori Prefecture in northern Japan. The following questions are addressed in this study: (i) Do organic residues survive in association with pottery vessels and charred remains? (ii) Can the residues be identified based on molecular and isotopic criteria applied in other investigations? (iii) Are the residues associated with the charred remains common to the residues associated with the pottery vessels? (iv) How do these residues contribute to our understanding of food processing and consumption? Results of our analysis indicate that the lipid composition of the pottery extracts is remarkably similar although some of the sherds exhibited better preservation and a wider range of molecules were detected albeit in lower abundance. There is a marked contrast with the composition of the lipid extracts of the 'charred remains.' The lipid compositions of sample sets from Sannai Maruyama and Sannai Maruyama No. 9 suggest aquatic resources in the pottery but with a plant contribution. The 'charred remains' from Sannai Maruyama contain plant tissues most likely with a high starch composition such as nuts. Lipids were recovered from the majority of the samples.

KEYWORDS: lipid, molecular analysis, isotopic analysis, Jomon pottery, Sannai Maruyama

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1. Introduction

As an example of early complex hunter-gatherer cultures, the Jomon culture (*ca.* 14,000–500 BC) on the Japanese archipelago has attracted the attention of many prehistoric archaeologists (Habu 2004; Imamura 1996; Kobayashi 2004). Studies of the Jomon culture can provide us with a unique opportunity to examine the causal relationships between subsistence intensification, population size, climate change and vulnerability of socioeconomic systems. In particular, the Middle Jomon (*ca.* 5300–4400 cal. BP) of northeastern Japan is known for an abundance of large settlements and the elaboration of pottery decoration. The subsistence base for these large settlements has been a topic of debate. In this context, a key question is the importance of marine food in Jomon diet and its changes through time in relation to changes in settlement size and residential mobility. This question is also tied to a more theoretical question of whether large, sedentary hunter-gatherer settlements in the temperate zone reflect the use of a wide variety of food, or whether their subsistence activities have focused on a specific type of food resource (e.g., Habu 2014). Carbon and nitrogen stable isotope analysis of Jomon skeletal remains have helped us address these questions in terms of identifying the importance of marine *vs.* terrestrial protein sources (e.g., Minagawa 2001; Minagawa & Akazawa 1992; Yoneda 2010; Yoneda *et al.* 2011), but this method is applicable only when skeletal remains are well preserved. In the case of Japan where the soil is extremely acidic, this means primarily at shell midden sites only.

This paper presents a preliminary study to approach this issue through the analysis of organic residues of Early and Middle Jomon pottery. Samples are taken from the Sannai Maruyama site (*ca.* 5900–4400 cal. BP) and the Sannai Maruyama No. 9 site (*ca.* 5300–4400 cal. BP) in Aomori City, Aomori Prefecture in northern Japan. Eighteen pottery vessel sherds and eight 'charred remains' were investigated. The charred remains are isolated finds and not associated with, or adhering to, pottery vessel sherds. Nevertheless the curvature on these finds does indicate that they were once in contact with a container although this may or may not be ceramic in nature. Sannai Maruyama is the largest Jomon settlement site associated with more than 600 Early and Middle Jomon pit-dwellings (Habu 2004: 108–132; Okada 2003). Sannai Maruyama No. 9 is another Middle Jomon site in Aomori City (Aomori Archaeological Center 2007, 2008, 2010). Approximately 500 m away from the Sannai Maruyama site, it is associated with waterlogged middens. Figure 1 shows the locations of these sites.

The pottery chronology indicates that Sannai Maruyama was occupied from the middle of the Early Jomon period (*ca.* 5900 cal. BP) to the end of the Middle Jomon period (*ca.* 4400 cal. BP). The occupational span can be divided into 12 pottery phases.

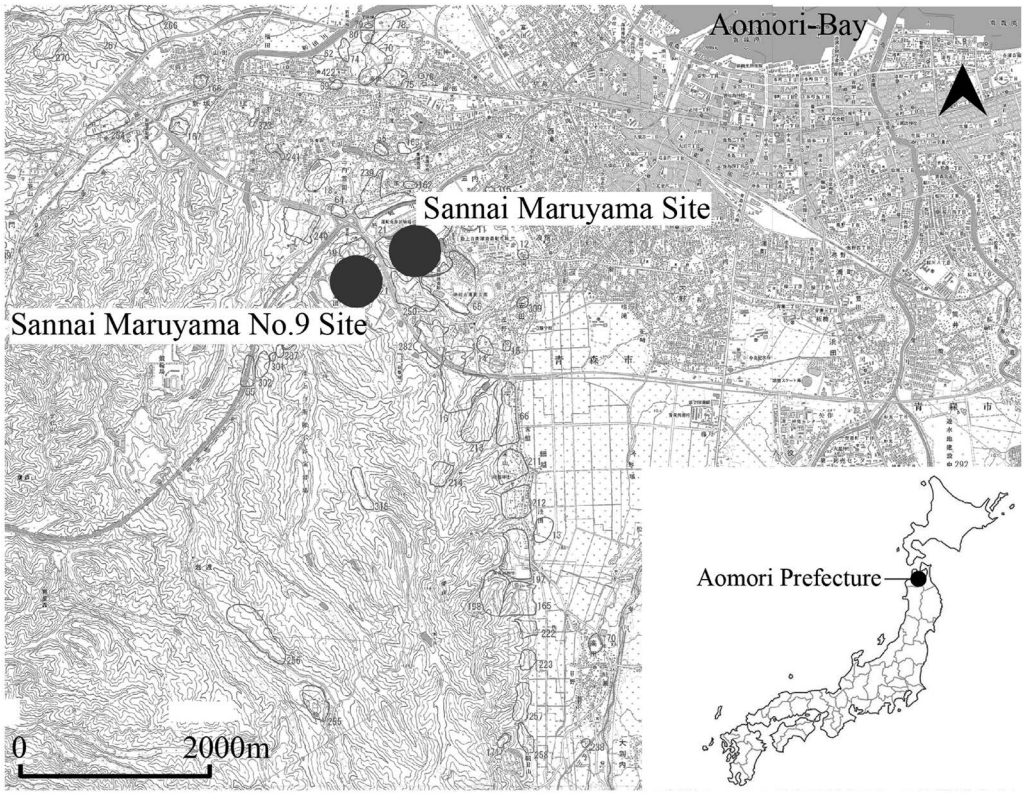

Figure 1. Site locations

They are: Early Jomon Lower Ento-a through d, Middle Jomon Upper Ento-a through e, Enokibayashi, Saibana, and Daigi 10 phases from the oldest to the latest.

Subsistence strategies of the Sannai Maruyama and Sannai Maruyama No. 9 sites have been a topic of debate. Many scholars have suggested the possibility that chestnut (*Castanea Crenata*) was the staple food of the Sannai Maruyama residents from the Lower-Ento-b to Upper Ento e-phases, although an abundance of chestnut pollen is more evident in the Early Jomon layers. Due to uneven preservation conditions within the site, reports of faunal remains from the Sannai Maruyama site are primarily from the first two phases of the site occupation: Lower-Ento-a and b phases (c. 5900–5600 cal. BP) (see Habu 2004). An abundance of grinding stones, presumably plant food processing tools, is particularly noticeable from the Upper-Ento-b to e phases (*ca.* 5200–4900 cal. BP) (Habu 2004, 2008). The Sannai Maruyama No. 9 site, which is dated to the Middle Jomon period, is known for an abundance of buckeye (*Aesculus turbinata*; also known as horse chestnut) remains from its waterlogged midden. Chestnut remains are also

recovered from the same midden, and the relative frequency of these two taxa changed through time. No faunal remains have been reported from this site. Given these lines of evidence, we expect that the foodways of the residents of these sites may have changed significantly through time.

The potential for the analysis of organic residues from pottery vessels of the Jomon culture has been demonstrated in a number of recent publications (e.g., Craig *et al.* 2013; Horiuchi *et al.* 2015; Lucquin *et al.* 2016). The following questions are addressed in this study: (i) Do organic residues survive in association with pottery vessels and charred remains? (ii) Can the residues be identified based on molecular and isotopic criteria applied in other investigations? (iii) Are the residues associated with the charred remains common to the residues absorbed in the pottery sherds? (iv) How do these residues contribute to our understanding of food processing and consumption at the sites?

2. The samples

Sannai Maruyama and Sannai Maruyama No. 9: Potsherds (Figure 2)

Two potsherds excavated from the Sannai Maruyama No. 9 site (J1−J2) and sixteen pottery sherds excavated from the Sannai Maruyama site (J3−J18) were selected for this analysis.

J1 and J2 are from the Sannai Maruyama No. 9 site. Both of them were collected in summer 2007 from the block soil sample that had been collected in 2006 from the waterlogged midden at the site by Aomori Archaeological Center (Aomori Archaeological Center 2008). The midden layers from which these sherds were excavated are dated to the first half of the Middle Jomon period, from the Upper-Ento-b phase to the Upper-Ento-e phase. AMS dates obtained from these midden layers range from *ca.* 5200 to 5000 cal. BP (Ito & Habu 2015).

The 16 potsherds excavated from the Sannai Maruyama (J3−J18) come from three different contexts. J3−J13 were obtained in Summer 1997 from the 6th Excavation Area of the site, a waterlogged midden at the northwestern edge of the site (Cultural Affairs Section of the Agency of Education of Aomori Prefecture 1998b). J3 is dated to the Middle Jomon Upper-Ento-b phase (*ca.* 5200 cal. BP). J4−J13 are dated to the Lower-Ento-d phase, the last pottery phase of the Early Jomon period dated to *ca.* 5500–5300 cal. BP. J14-17 are Middle Jomon potsherds, and were excavated at the time of the 30th Excavation in Summer 2006 (Cultural Affairs Section of the Agency of Education of Aomori Prefecture 2008). This was a re-excavation of the 6th Excavation area. These potsherds were recovered at the time of the removal of the backfill of the 1997 excavation. Because Middle Jomon large postholes are reported from this area, it is most likely that these potsherds were originally associated with a Middle Jomon feature

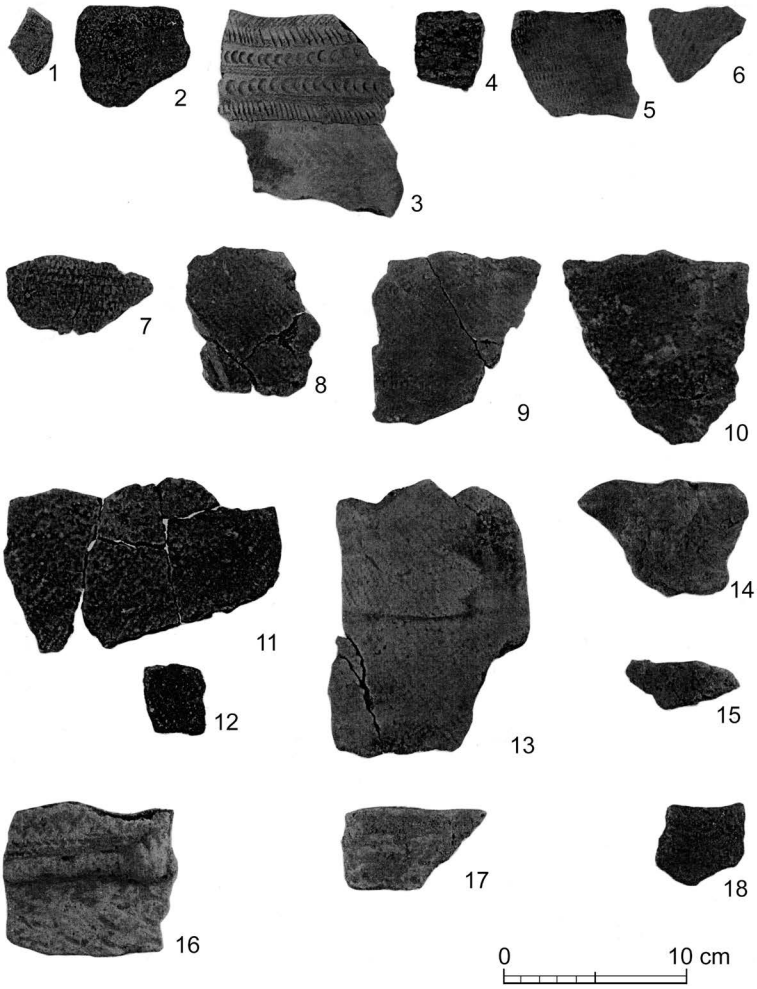

Figure 2. Photos of potsherd samples (J1−J18)

dug into the Early Jomon layers. J18 was found from the test excavation area of the Sannai Maruyama site in summer 2007. The sherd came from a small-pit, most likely dated to the latter half of the Middle Jomon period. Among all the samples, this was the only one that comes from a non-waterlogged depositional context.

Sannai Maruyama: 'Charred remains' (Figures 3 and 4)

At Sannai Maruyama, charred remains that look like 'foodcrusts' (the charred deposits occasionally observed on pottery vessel surfaces) were recovered through wet-sieving of soil samples obtained from waterlogged middens. These were recovered in isolation and

Table 1. Pottery sherds from Sannai Maruyama No. 9 (J1−J2) and Sannai Maruyama (J3−J18)

Sample No. I=interior; E=exterior	Context & original sample No.	Collection date	Phase	Jomon Sub-period	Depositional context	Sherd Condition	Sample description
J1I	Sannai Maruyama No. 9, SX-10, Block 2, Layer 2a	8/4/2007	Upper-Ento?	Middle Jomon	Waterlogged	Potsherd, unwashed and stored in *Ziploc* after recovery	Interior surface drilling
J2I	Sannai Maruyama No. 9, SX-10, Block 2, Layer 2d-3	8/4/2007	Upper-Ento?	Middle Jomon	Waterlogged	Potsherd, unwashed in aluminium foil, then *Ziploc*	Interior surface drilling
J3I	Sannai Maruyama, 6th Excavation Area, North Wall	7/29/1997	Upper-Ento-b	Middle Jomon	Waterlogged	Potsherd, washed then stored in *Ziploc*	Interior surface drilling
J4I	Sannai Maruyama, 6th Excavation Area, North Wall, Cut 40-Sherd a	7/25/1997	Lower-Ento-d	Early Jomon	Waterlogged	Potsherd, washed then stored in *Ziploc*	Interior surface drilling
J4E	Sannai Maruyama, 6th Excavation Area, North Wall, Cut 40-Sherd a	7/25/1997	Lower-Ento-d	Early Jomon	Waterlogged	Potsherd, washed then stored in *Ziploc*	Exterior surface drilling
J5I	Sannai Maruyama, 6th Excavation Area, North Wall, Cut 40-Sherd b	7/25/1997	Lower-Ento-d	Early Jomon	Waterlogged	Potsherd, washed then stored in *Ziploc*	Interior surface drilling
J6I	Sannnai Maruyama, 6th Excavation Area, North Wall, Cut 40-Sherd c	7/25/1997	Lower-Ento-d	Early Jomon	Waterlogged	Potsherd, washed then stored in *Ziploc*	Interior surface drilling
J7I	Sannai Maruyama, 6th Excavation Area, North Wall, Cut 41	7/25/1997	Lower-Ento-d	Early Jomon	Waterlogged	Potsherd, washed then stored in *Ziploc*	Interior surface drilling
J8I	Sannai Maruyama, 6th Excavation Area, North Wall, Cut 47-Sherd a	7/28/1997	Lower-Ento-d	Early Jomon	Waterlogged	Potsherd, washed then stored in *Ziploc*	Interior surface drilling
J9I	Sannai Maruyama, 6th Excavation Area, North Wall, Cut 47-Sherd b	7/28/1997	Lower-Ento-d	Early Jomon	Waterlogged	Potsherd, washed then stored in *Ziploc*	Interior surface drilling
J10I	Sannai Maruyama, 6th Excavation Area, North Wall, Cut 47-Sherd c	7/28/1997	Lower-Ento-d	Early Jomon	Waterlogged	Potsherd, washed then stored in *Ziploc*	Interior surface drilling
J11I	Sannai Maruyama, 6th Excavation Area, North Wall, Cut 47-Sherd d	7/28/1997	Lower-Ento-d	Early Jomon	Waterlogged	Potsherd, washed then stored in *Ziploc*	Interior surface drilling

Table 1. Continued

Sample No. I=interior; E=exterior	Context & original sample No.	Collection date	Phase	Jomon Sub-period	Depositional context	Sherd Condition	Sample description
J12I	Sannai Maruyama, 6th Excavation Area, North Wall, Cut 47-Sherd e	7/28/1997	Lower-Ento-d	Early Jomon	Waterlogged	Potsherd, washed then stored in *Ziploc*	Interior surface drilling
J13I	Sannai Maruyama, 6th Excavation Area, North Wall, Cut 47-Sherd f	7/28/1997	Lower-Ento-d	Early Jomon	Waterlogged	Potsherd, washed then stored in *Ziploc*	Interior surface drilling
J14I	Sannai Maruyama, 30th Excavation, backfill-Sherd a	7/2006	Upper-Ento	Middle Jomon	Waterlogged	Unwashed, stored in *Ziploc* after recovery	Interior surface drilling
J15I	Sannai Maruyama, 30th Excavation, backfill-Sherd b	7/2006	Upper Ento	Middle Jomon	Waterlogged	Unwashed, stored in *Ziploc* after recovery	Interior surface drilling
J16I	Sannai Maruyama, 30th Excavation, backfill-Sherd c	7/2006	Upper-Ento	Middle Jomon	Waterlogged	Unwashed, stored in *Ziploc* after recovery	Interior surface drilling
J17I	Sannai Maruyama, 30th Excavation, backfill-Sherd d	7/2006	Upper-Ento	Middle Jomon	Waterlogged	Unwashed, stored in *Ziploc* after recovery	Interior surface drilling
J17E	Sannai Maruyama, 30th Excavation, backfill-Sherd d	7/2006	Upper-Ento	Middle Jomon	Waterlogged	Unwashed, stored in *Ziploc* after recovery	Exterior surface drilling
J18I	Sannai Maruyama, Test excavation, Test Trench 6, 'post hole?' top	8/8/2007	Un-known	Middle Jomon	Dry	Unwashed, wrapped in aluminium foil, then in a paper envelope placed in plastic box	Interior surface drilling

not adhering to pottery or other vessel surfaces. That noted some displayed smooth and curved surfaces suggesting the possibility that they had originally been in contact with some form of container. Impressions in some of these charred remains suggest some form of basketry. Eight samples of these charred remains were selected for analysis with the aim of determining if the lipid residues from the pottery share a common origin with the lipids extracted from these charred remains. Photos and drawings of these samples are shown in Figures 3 and 4. All samples were first water-screened, then dried and stored in *Ziploc* plastic bags. Given their unique form, we assume that these are different from so-called 'Jomon cookies' and other solid forms of charred food remains that have been reported from Jomon sites in central Japan.

All of these samples were found by site technicians at the time of sorting organic remains within the water-screened samples, and were provided by the Preservation Office

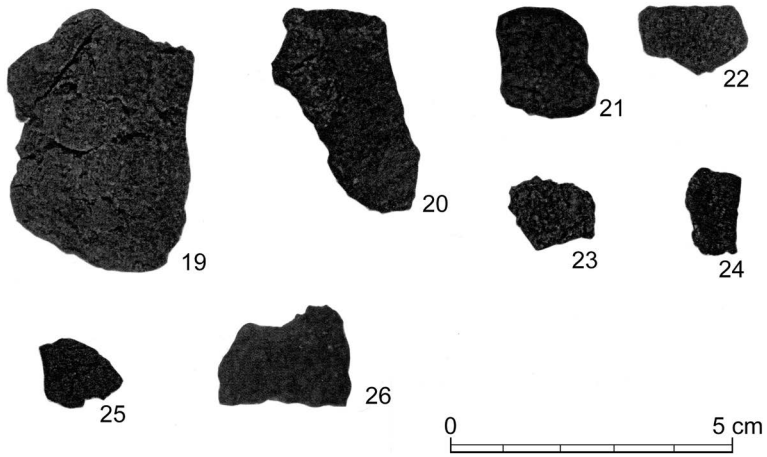

Figure 3. Photos of samples of the charred remains (J19–J26)

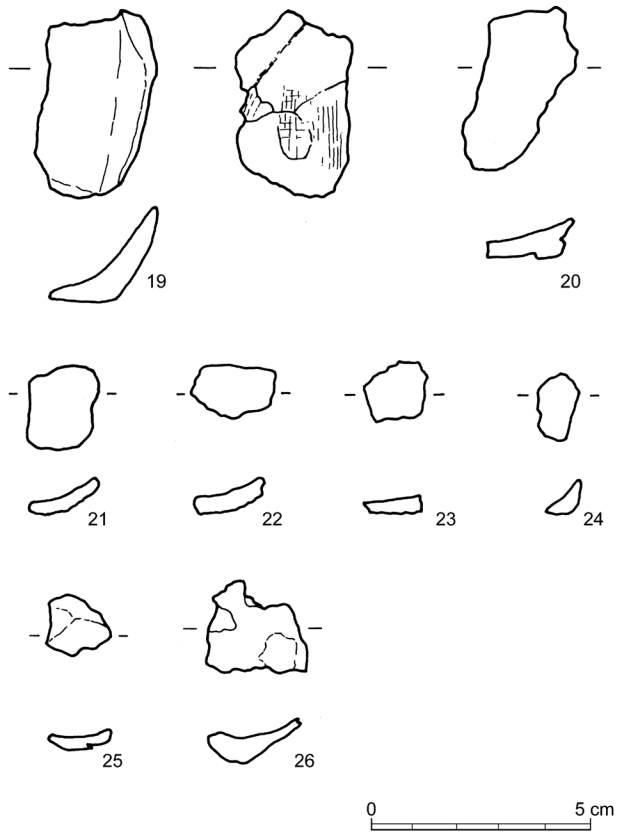

Figure 4. Drawings of samples of the charred remains (J19–J26)

Table 2. 'Charred remains' from Sannai Maruyama

Sample	Context and original sample No.	Phase	Jomon Sub-period	Depositional context
J19	Sannai Maruyama, T-tower, VII F-74-4, Layer VIa, Specimen "a"	Lower- Ento-a	Early Jomon	Waterlogged
J20	Sannai Maruyama, T-tower, VII F-74-4, Layer VIa, Specimen "b"	Lower- Ento-a	Early Jomon	Waterlogged
J21	Sannai Maruyama, T-tower, VII F-74-4, Layer VIa, Specimen "c"	Lower- Ento-a	Early Jomon	Waterlogged
J22	Sannai Maruyama, T-tower, VII F-74-4, Layer VIa, Specimen "d"	Lower- Ento-a	Early Jomon	Waterlogged
J23	Sannai Maruyama, T-tower, VII F-74-4, Layer VIa, Specimen "e"	Lower- Ento-a	Early Jomon	Waterlogged
J24	Sannai Maruyama, T-tower, VII F-74-4, Layer VIa, Specimen "f"	Lower- Ento-a	Early Jomon	Waterlogged
J25	Sannai Maruyama, T-tower, VII F-74-4, Layer VIa, Specimen "g"	Lower- Ento-a	Early Jomon	Waterlogged
J26	Sannai Maruyama, Northern Valley, F-30274, 4 mm	Lower-Ento-a or b	Early Jomon	Waterlogged

of the Sannai Maruyama site to the East Asian Archaeology Laboratory of the University of California for further analysis. Samples J19−J25 were originally excavated from Layer VIa of the Transmission Tower (Cultural Affairs Section of the Agency of Education of Aomori Prefecture 1998a), a waterlogged midden layer dated to the Lower-Ento-a phase of the Early Jomon period (*ca.* 5900–5650 cal BP). J26 is from a waterlogged midden called "the Northern Valley" (Cultural Affairs Section of the Agency of Education of Aomori Prefecture 2014), which is dated to the Lower-Ento-a to Lower-Ento-b phase of the Early Jomon period (*ca.* 5900–5500 cal BP).

3. Materials and methods

Prior to GC-MS (gas chromatography-mass spectrometry), drilled pottery samples (2 g) or powdered charred remains (10–20 mg) were weighed into scintillation vials and covered with 10 mL of *AnalaR* grade dichloromethane/methanol (2/1 v/v ratio), ultrasonicated for 2×15 minutes, and then centrifuged for 5 minutes at 2000 rpm. The supernatant was decanted using a disposable Pasteur pipette, and placed in a Hach tube. The extraction process was repeated three times to ensure maximum lipid extraction. The solvent was

then evaporated under a gentle stream of nitrogen and mild heating to retain the lipid extract. The extracts were either methylated or silylated. Methylation was achieved using 100 μL boron trifluoride in methanol (14% w/v), and heating the mixture for 20 minutes at 70°C. The reaction was quenched using two drops of deionised water and extracted using 3×1 mL aliquots of hexane. The samples were then dried under nitrogen and redissolved in dichloromethane. Silylation was achieved by adding three drops of BSTFA (*N*,*O*-bis(trimethylsilyl)trifluoroacetamide) with 1% TMCS (trimethylchlorosilane) to each of the dried lipid extracts and heated at 60°C for 15 minutes. The samples were once again evaporated to dryness under a gentle stream of nitrogen and redissolved in dichloromethane.

Prior to GC-C-IRMS (gas chromatography-combustion-isotope ratio mass spectrometry), lipids were extracted from the sherds and charred remains in one-step with acidified methanol. Methanol (1 and 4 mL respectively) was added to homogenized charred remains (10–30 mg) or sherds (1 g) and the mixture ultrasonicated for 15 min. The solvent was then acidified with concentrated sulphuric acid (200 or 800 mL respectively). The acidified suspension was heated in sealed tubes for 4 h at 70°C and then cooled, and lipids were extracted with *n*-hexane (3x). Dissolved sulphur was removed by treatment with activated copper turnings. The samples were analysed directly by GC-C-IRMS as described below.

Bulk isotope analysis

Bulk carbon and nitrogen isotope analysis was undertaken on the charred remains (1–2 mg). The samples were dried and weighed into tin capsules. C and N isotope analyses were performed on a Europa 20-20 mass spectrometer fitted with a Roboprep combustion unit. All samples were determined in duplicate.

Gas Chromatography-Mass Spectrometry (GC-MS)

Analysis was carried out on an Agilent 7890A Series GC connected to a 5975C Inert XL mass selective detector. The splitless injector and interface were set at 300°C and 325°C respectively, and helium was used as the carrier gas at constant inlet pressure. The oven temperature was initially kept at 50°C for 2 minutes, then ramped to 325°C at 10°C min^{-1} and held for 10 minutes. The GC column, a 30 m×0.25 mm, 0.25 μm HP-5MS 5% Phenyl, 95% dimethylpolysiloxane phase fused silica column, was directly inserted into the ion source. Electron impact (EI) spectra were obtained at 70 eV with full scan from *m*/*z* 50 to 800.

Gas Chromatography-combustion—Isotope Ratio Mass Spectrometry (GC-C-IRMS)

The stable carbon isotopic compositions of individual lipids were determined in

duplicate using a Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher, Bremen, Germany) linked to a Trace Ultra gas chromatograph (Thermo Fisher) with a ConFlo IV interface (Cu/Ni combustion reactor held at 1000°C; Thermo Fisher). Samples were diluted in hexane and 1 mL was injected onto a DB-5MS fused silica column (30 m×0.25 mm×0.25 mm; J&W Scientific). The temperature programme was 1 min at 45°C min^{-1} to 295°C and 15 min at 295°C. Ultra-high purity grade helium was used as the carrier gas (at a flow rate of 1.4 mL min^{-1}). The ion intensities of *m*/*z* 44, 45, and 46 were monitored and the $^{13}C/^{12}C$ and $^{18}O/^{16}O$ ratios of each sample peak were automatically computed (Isodat version 3.0; Thermo Fisher) by comparison with a standard reference CO_2 gas of known isotopic composition, which was repeatedly measured with each sample. All results are reported in per mil (‰) relative to VPDB international standard. Replicate measurements of each sample and a mixture of FAMEs with $\delta^{13}C$ values traceable to international standards (Indiana F8 standard; obtained from Arndt Schimmelmann, Indiana University, Bloomington, IN, USA) were used to determine instrument precision (<0.3‰) and accuracy (<0.5‰). The values of unknown samples were corrected for methylation by comparison with standard $C_{18:0}$ and $C_{16:0}$ fatty acids of known isotopic composition, which were methylated with each batch of samples using identical reagents and procedures.

Starch granules and phytoliths

To investigate the presence of plant remains, starch granules, phytoliths (silica bodies) and plant tissues were extracted from samples taken from six charred remains. The extraction procedures followed established protocols by Saul *et al.* (2012). Charred remains ranging from 4.79 mg to 9.12 mg were first treated with 3% H_2O_2; 10 mL for 15–30 min and then manually disaggregated. The samples were then centrifuged (1000x; 3 min). Then the supernatant was reduced to 2 mL, and the residues washed three times with UltraPure water. Residues were then made up to 1 mL suspensions. The supernatant was mounted on microscope slides and left to dry at room temperature. Finally a drop of glycerol was placed at top of the dried residues and a glass cover slip placed above it to allow any starch granules or phytoliths present in the mixture to be observed in rotated planes. The mounted slides were examined using an inverted polarising microscope fitted with a digital camera. All silica bodies and starches were counted by scanning the mounted specimen in a grid pattern at a magnification of 630x.

4. Results

Bulk isotopic analysis

The results are shown in Table 3. The %nitrogen in the charred remains is consistently

Table 3. Bulk carbon isotope determinations undertaken in duplicate on the charred remains

Sample	%C	δ^{13}C (‰)	%N	δ^{15}N (‰)	C/N ratio
J19	51.1	−24.7	1.3	−1.8	47.3
J20	54.5	−24.8	1.3	−1.1	50.4
J21	53.7	−25.5	1.5	0.6	40.7
J22	49.7	−24.8	1.2	0.2	48.1
J23	53.7	−25.1	1.2	−1.6	52.1
J24	61.7	−25.9	1.3	−1.7	57.1
J25	53.4	−25.0	1.5	−1.5	40.5
J26	53.8	−25.2	1.1	1.1	56.7

low (1.1–1.5%) with much higher %carbon (49.7–61.7%). The δ^{13}C values range from −24.7 to −25.9‰. Such values could indicate terrestrial C3 plants or animals consuming C3 plants. The very high (>40) C/N atomic ratios compare well with the C/N ratios of charred modern starchy foods cooked in pottery vessels including acorns and chestnuts (Yoshida *et al.* 2013, Figure 4). This study also showed that cooking acorn, chestnut and horse chestnut in pottery vessels resulted in relatively small shifts in δ^{13}C values within ±2‰. The C/N ratios for terrestrial animal tissues and marine foods were all below 22. Interestingly the δ^{13}C values given by the starchy foods in this study are between −22 to −30‰, which is consistent with the values reported in here. Values for modern C3 nuts, corrected according to the Suess effect, have been reported with δ^{13}C values of −25.4±1.6‰ and δ^{15}N values of 1.2±2.4‰ (n=16; Shimojo reported in Yoneda *et al.* 2004, 103).

Figure 5 plots δ^{15}N values against atomic C:N ratio for the charred remains from Sannai Maruyama. These data are compared with charred deposits (n=70) recovered from pottery vessel surfaces from Torihama (Fukui Prefecture, Incipient, Initial and Early Jomon contexts) and examples of charred plants dating to the Incipient Jomon (Kudo 2014). The Torihama samples have been shown, by GC-MS and GC-C-IRMS analysis, to be dominated by lipids consistent with aquatic sources, both marine and freshwater (Lucquin *et al.* 2016). In contrast, the Sannai Maruyama remains, with low δ^{15}N values and high C:N ratios, are consistent with plant tissues as evidenced by the comparison with examples of charred plant remains from Incipient Jomon contexts and by comparison with literature values.

Gas Chromatography-Mass Spectrometry (GC-MS)

It has been demonstrated, largely by experimental means, that both quantitative and qualitative changes in lipids occur during pottery use and burial (Evershed 2008a). These data suggest an overall depletion in lipids over time. Qualitatively, loss of triacylglycerols

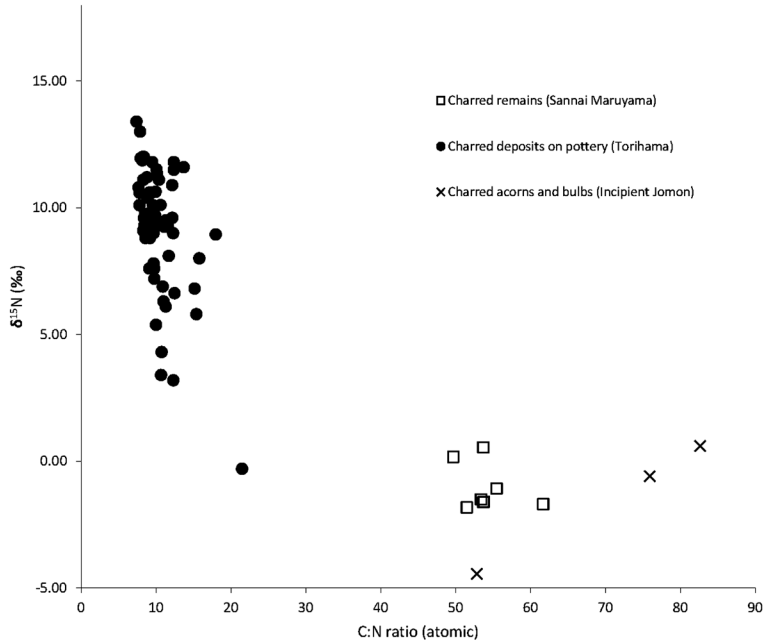

Figure 5. Plot of $\delta^{15}N$ values vs. atomic C:N ratio showing the charred remains from Sannai Maruyama compared with 'foodcrusts' (n=70) recovered from pottery vessel surfaces from Torihama (Fukui Prefecture; Incipient, Initial and Early Jomon contexts) and examples of charred plants dating to the Incipient Jomon

by hydrolysis and unsaturated *n*-alkenoic acids by oxidation are commonly observed although the rate of change is dependent on the burial environment. Clear differences are observed between degraded food lipids absorbed in pottery vessel sherds and lipids extracted from the associated sedimentary matrix (e.g., Heron *et al.* 1991). Most lipid compounds in foods and sedimentary matrices are hydrophobic and immobile although a minor contribution of soil lipid to the extracts from the pottery sherds and the charred remains cannot be ruled out entirely. The range of lipid marker compounds detected in archaeological residues must be evaluated carefully to identify the most likely origin. The presence of molecules derived from thermal alteration of lipids (*ω*-(*o*-alkylphenyl)-alkanoic acids) and sugars (levoglucusan) reinforces the view that archaeological residues are consistent with food processing, such as cooking. Further evaluation of the food source is obtained by compound specific carbon isotope analysis of the most abundant *n*-alkanoic acids in the residues.

Sannai Maruyama 'charred remains'—methylated extracts

Gas chromatography-mass spectrometry was undertaken on methylated solvent extracts

Table 4. Lipid composition of residues extracted from the charred remains (methylated extracts)

Sample	*n*-alkanoic acids	*n*-alkenoic acids	Dioic acids	ω-(*o*-alkylphenyl)alkanoic acids
J19	$C_{14:0}$–$C_{28:0}$	$C_{18:1}$	$C_{16:0}$–$C_{22:0}$	C18
J20	$C_{14:0}$–$C_{22:0}$	$C_{18:1}$	$C_{16:0}$–$C_{22:0}$	C18
J21	$C_{14:0}$–$C_{26:0}$	$C_{18:1}$	nd	C18
J22	$C_{14:0}$–$C_{22:0}$	$C_{18:1}$	nd	C18
J23	$C_{14:0}$–$C_{20:0}$	$C_{18:1}$	trace	C18
J24	$C_{14:0}$–$C_{24:0}$	$C_{18:1}$	nd	C18
J25	$C_{14:0}$–$C_{24:0}$	$C_{18:1}$	nd	C18
J26	$C_{14:0}$–$C_{28:0}$	$C_{18:1}$	nd	C18

nd—not detected

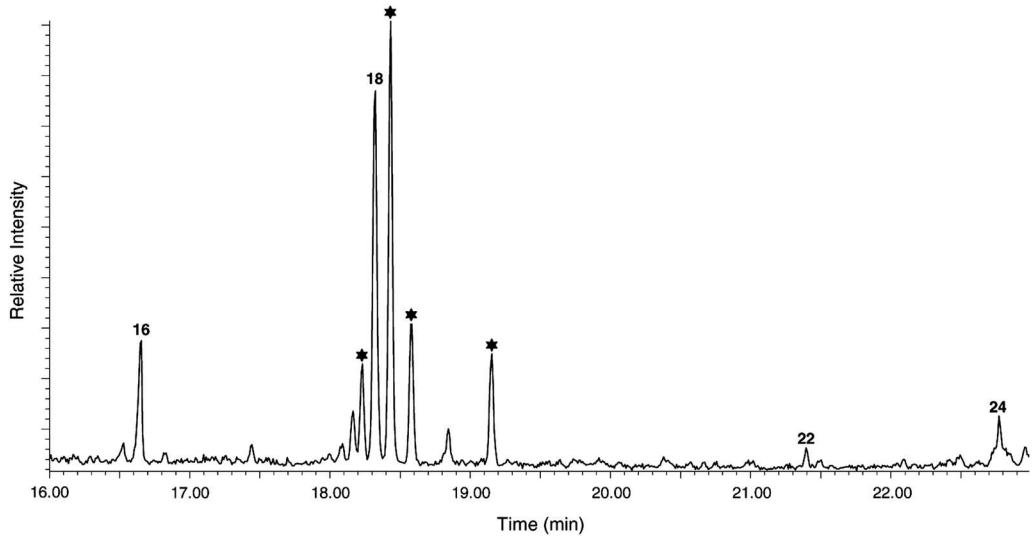

*Figure 6. Extracted ion (m/z 105) chromatogram highlighting C18 ω-(o-alkylphenyl)alkanoic acid isomers (peaks labelled *) in sample J20 (charred remains). The peaks labelled 16, 18, 20 and 22 correspond to n-alkanoic acids with n carbon atoms respectively*

of the charred remains (Table 4). The major components in each sample are saturated *n*-alkanoic acids. Although dominated by hexadecanoic ($C_{16:0}$; palmitic) and octadecanoic ($C_{18:0}$; stearic) acids, a wider range of *n*-alkanoic acids is present from tetradecanoic acid ($C_{14:0}$) up to octacosanoic acid ($C_{28:0}$) in two cases. Unsaturated *n*-alkenoic acids are restricted to a trace of octadecenoic acid ($C_{18:1}$). Long-chain dioic (dicarboxylic) acids were detected in three samples. Sterols are absent as are isoprenoid acids such as phytanic

acid. No *n*-alkanols or dihydroxyalkanoic acids were detected. The methylated extracts of all samples also show clear evidence for the presence C18 *ω*-(*o*-alkylphenyl)alkanoic acid isomers (Figure 6). The mechanism underlying the formation of these compounds as a result of thermal action on *n*-alkenoic acids has been addressed in detail in Evershed *et al.* (2008). A wider range (from C_{16}–C_{22}) of these molecules has previously been reported as constituents of heated marine and freshwater organisms in pottery vessels of archaeological date and form from unsaturated fatty acids present in the fresh tissue lipid (Evershed 2008b; Heron *et al.* 2010; Craig *et al.* 2011, 2013). Only C18 *ω*-(*o*-alkylphenyl)-alkanoic acid isomers were detected in the charred remains and, together with the absence of any isoprenoid acids, it suggests that the charred remains do not comprise a marine or freshwater contribution. The detection of C18 *ω*-(*o*-alkylphenyl)alkanoic acid isomers only is likely to derive from a source dominated by C18 alkenoic acids ($C_{18:1}$, $C_{18:2}$ and $C_{18:3}$). This supports a plant source and is consistent with the bulk isotope data.

Sannai Maruyama 'charred remains'—trimethylsilylated extracts

Lipid extracts of two samples (J19 and J21) were subjected to trimethylsilylation prior to GC-MS and the summary table is shown in Table 5. These extracts are characterised by a wider range of short-chain alkanoic acids than was detected in the methylated extracts. A plant sterol (sitosterol) was detected in J19 but not in J21. Levoglucosan and other unidentified sugars were detected in the trimethylsilylated extracts. Levoglucusan is a monosaccharide pyrolysis product of cellulose and hemicellulose (Simoneit *et al.* 1999), although it is also produced by combustion of lignite or brown coal (Fabbri *et al.* 2009). This lends further support to the plant origin of the charred remains. In addition, butanedioic acid was identified along with several substituted benzoic acid derivatives in low abundance.

Sannai Maruyama pottery sherds—methylated extracts

The lipid composition of the methylated extracts is shown in Table 6. The absence of a wider range of compound classes in these extracts, such as sterols, *n*-alkanols and so on, is explained by the fact that since many of them have free –OH groups they will not be methylated and thus are unlikely to pass through the GC column. No *ω*-(*o*-alkylphenyl)-alkanoic acids were identified. Most samples comprise low levels of a relatively narrow range of *n*-alkanoic acids. However, several extracts comprise a well-preserved lipid fraction with a range of *n*-alkenoic acids present. The isoprenoid, phytanic acid, was also detected in these samples.

Sannai Maruyama pottery sherds—trimethylsilylated extracts

Three of the well-preserved lipid residues (J10, J11 and J13) were re-extracted and

Table 5. Lipid composition of residues extracted from the charred remains and trimethylsilylated prior to analysis

Sample	*n*-alkanoic acids	*n*-alkenoic acids	Sitosterol	Sugars
J19	$C_{6:0}$–$C_{26:0}$	$C_{18:1}$	Yes	Yes
J21	$C_{6:0}$–$C_{26:0}$	$C_{18:1}$	nd	Yes

nd—not detected

Table 6. Lipid composition of methylated residues extracted from Sannai Maruyama pottery sherds

Sample	*n*-alkanoic acids	*n*-alkenoic acids	Phytanic acid
J1	nd	nd	No
J2	$C_{14:0}$–$C_{30:0}$	$C_{18:1}$–$C_{24:1}$	Yes
J3	$C_{14:0}$–$C_{18:0}$	$C_{18:1}$	nd
J4I	$C_{16:0}$–$C_{18:0}$	$C_{18:1}$	nd
J4E	$C_{16:0}$	nd	nd
J5	$C_{16:0}$–$C_{18:0}$	nd	nd
J6	$C_{16:0}$–$C_{18:0}$	$C_{18:1}$	nd
J7	$C_{16:0}$–$C_{28:0}$	$C_{18:1}$	nd
J8	$C_{16:0}$	nd	nd
J9	$C_{14:0}$–$C_{18:0}$	nd	nd
J10	$C_{14:0}$–$C_{30:0}$	$C_{18:1}$–$C_{22:1}$	Yes
J11	$C_{14:0}$–$C_{26:0}$	$C_{18:1}$–$C_{22:1}$	Yes
J12	$C_{16:0}$	nd	nd
J13	$C_{14:0}$–$C_{30:0}$	$C_{18:1}$–$C_{22:1}$	Yes
J14	$C_{16:0}$–$C_{18:0}$	nd	nd
J15	$C_{16:0}$–$C_{18:0}$	nd	nd
J16	$C_{16:0}$–$C_{18:0}$	$C_{18:1}$	nd
J17I	$C_{16:0}$–$C_{18:0}$	$C_{18:1}$	nd
J17E	nd	nd	nd
J18	nd	nd	nd

nd—not detected

trimethylsilylated prior to GC-MS. The results are presented in Table 7. The lipid composition of these residues is remarkably similar. Figure 7 shows the lipid extract of sherd J10. The main constituents are $C_{16:0}$ and $C_{18:0}$. A wider range of *n*-alkanoic acids was detected in lower abundance from $C_{6:0}$ to $C_{30:0}$. As expected, even-carbon number acids dominate over odd although traces of $C_{11:0}$ to $C_{17:0}$ are clearly seen. Unsaturated (alkenoic) acids are detected in very low abundance. In addition to the detection of octadecenoic ($C_{18:1}$) acid, eicosenoic ($C_{20:1}$), docosenoic ($C_{22:1}$) and tetracosenoic ($C_{24:1}$) were identified. Traces of hexadecenoic ($C_{16:1}$) and hexacosenoic ($C_{26:1}$) acids were also

Table 7. Lipid composition of residues extracted from Sannai Maruyama pottery sherds (trimethylsilylated extracts)

Sample	*n*-alkanoic acids	*n*-alkenoic acids	Phytanic acid	Cholesterol	Sitosterol	*n*-alkanols	Dihydroxyalkanoic acids
J10	$C_{6:0}$–$C_{30:0}$	$C_{16:1}$–$C_{26:1}$	Yes	Yes	Yes	C_{22}–C_{32}	$C_{16:0}$–$C_{22:0}$
J11	$C_{6:0}$–$C_{30:0}$	$C_{16:1}$–$C_{26:1}$	Yes	Yes	Yes	C_{22}–C_{32}	$C_{16:0}$–$C_{22:0}$
J13	$C_{6:0}$–$C_{30:0}$	$C_{16:1}$–$C_{26:1}$	Yes	Yes	Yes	C_{22}–C_{32}	$C_{16:0}$–$C_{22:0}$

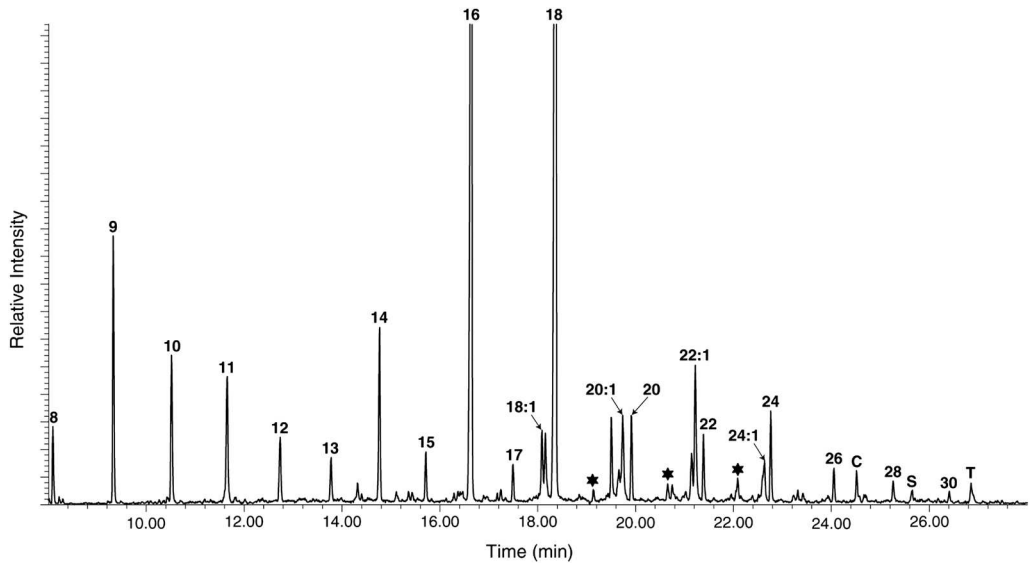

*Figure 7. Extracted ion current (m/z 129) chromatogram of the lipid extract of sample J10. The peaks labelled 8 to 30 are saturated n-alkanoic acids with n carbon atoms. $C_{16:0}$ and $C_{18:0}$ are plotted off the scale to highlight the wide distribution of acids in low abundance. Peaks labelled n:1 are n-alkenoic acids with n carbon atoms and one double bond. Peaks labelled * are dihydroxyalkanoic acids with 18, 20 and 22 carbon atoms respectively in order of elution. C—cholesterol, S—sitosterol, T—unassigned triterpenoid (base peak, m/z 189)*

identified in very low abundance. Longer chain *n*-alkenoic acids are not commonly reported in lipid residues of archaeological date. These molecules can be found in plant tissues, although their occurrence is restricted, and in the tissues of aquatic organisms. One isoprenoid acid (phytanic) is present. Both cholesterol and sitosterol are present and suggest both animal and plant products associated with the vessels. Acyl lipid is highly depleted in all samples. In J10 a trace of monopalmitin survives suggesting that hydrolysis has impacted significantly on the lipid residue. A series of *n*-alkanols (C22–C32) was also detected by scanning for the characteristic *m*/*z* 103 ion. This also supports

a plant contribution.

Although *ω*-(*o*-alkylphenyl)alkanoic and dioic acids are absent, a suite of dihydroxyalkanoic ($C_{16:0}$–$C_{22:0}$) is present in all three extracts. Although molecular ions are absent, M-15 ions are seen at *m/z* 489, 517, 545 and 573 respectively in mass spectra of the $C_{16:0}$, $C_{18:0}$, $C_{20:0}$ and $C_{22:0}$ acids. Dihydroxyalkanoic acids are degradation products of monounsaturated alkenoic acids formed *via* secondary reactions with hydroperoxides (Hansel & Evershed 2009). This study demonstrated that the positions of the hydroxyl groups correspond to the position of the double bond in the precursor fatty acid. This biomarker can help to distinguish aquatic from terrestrial resources (Hansel & Evershed 2009; Heron *et al.* 2010) and can be helpful where vessel contents may not have been subject to protracted heating leading to the formation of *ω*-(*o*-alkylphenyl)alkanoic acids (Hansel *et al.* 2011).

The presence of dihydroxyalkanoic acids in the residues adds significantly to the interpretative potential of the lipid biomarker evidence since the positions of the hydroxyl groups identify the position of the double bond in the precursor alkenoic acid. Whereas 9,10-dihydroxyoctadecanoic acid is indicative of 9-octadecenoic acid—a ubiquitous constituent of most fats and oils, 11,12-dihydroxydocosanoic acid derives from 11-docosenoic acid ($C_{22:1}$; cetoleic acid)—the most abundant $C_{22:1}$ *n*-alkenoic acid in marine organisms (Morris & Culkin 1989, 149; Heron *et al.* 2010). In contrast 13,14-dihydroxydocosanoic acid is formed from 13-docosenoic acid (erucic acid) and is the most common $C_{22:1}$ *n*-alkenoic acid found in terrestrial plants. Indeed 13,14-dihydroxydocosanoic acid has been reported in residues of probable plant (Brassicaceae) oils in pottery vessels of archaeological date (Colombini *et al.* 2005; Copley *et al.* 2005). The predominance of the 11,12-dihydroxydocosanoic acid isomer in sample J10 suggests the presence of lipid derived from aquatic organisms—either marine or freshwater. The survival of long chain *n*-alkenoic acids ($C_{20:1}$, $C_{22:1}$, $C_{24:1}$, and $C_{26:1}$) provides strong corroborative evidence of this conclusion.

Further data in support of an aquatic contribution to these residues is the presence of phytanic (3,7,11,15-tetramethylhexadecanoic) acid. Plant tissues do not contain phytanic acid. Nevertheless it is a constituent of ruminant animals (both in the adipose tissues and milk) as well as marine and freshwater organisms and is produced when phytol, liberated from chlorophyll in the gut, is converted to phytanic acid and incorporated into the tissues of the organism. This molecule was detected in both the methylated and trimethylsilylated extracts of J10, J11 and J13. Aquatic tissues comprise relatively high concentrations of isoprenoid acids, particularly phytanic acid, pristanic (2,6,10,14-tetramethylpentadecanoic) acid and 4,8,12-TMTD (4,8,12-trimethyltridecanoic acid). These molecules have also been identified in pottery vessels associated with the processing of marine and freshwater resources (Hansel *et al.* 2004; Craig *et al.* 2007; Craig *et al.* 2011; Craig *et al.* 2013).

Compound specific carbon isotope analysis

Analysis of the carbon isotope composition of the $C_{16:0}$ and $C_{18:0}$ *n*-alkanoic acids from both pottery residues and the charred remains show distinctive profiles (Figure 8). Two of the charred remains plot in the range identified for chestnuts from analysis of modern Japanese reference materials, while the lipids extracted from the pottery are indicative of terrestrial animals, salmonids and, in one instance, marine resources. It is also worthy of note, however, that some of the intermediate values for these two *n*-alkanoic acids could be the result of two-member mixing between plant and marine resources as suggested by the lipid biomarker data. The differences between the charred remains and the pottery lipids suggest that the two types of samples found at the site represent distinct food processing activities.

Microscopic analysis of 'charred remains'

The analysis yielded very low counts of starch granules and phytoliths, identified in only two samples. However all samples were found to have a variable amount of poorly diagnostic plant tissues, mostly very small in size, suggesting plant processing (for storage) was occurring. Among these are the remains of epidermis tissues consistent with the pericarp and spermoderm of chestnuts, buckeyes and acorns. Although unambiguous identification cannot be achieved from these data alone, in part due to the fragmentary nature of the remains, the presence of plant tissues in the charred remains supports the

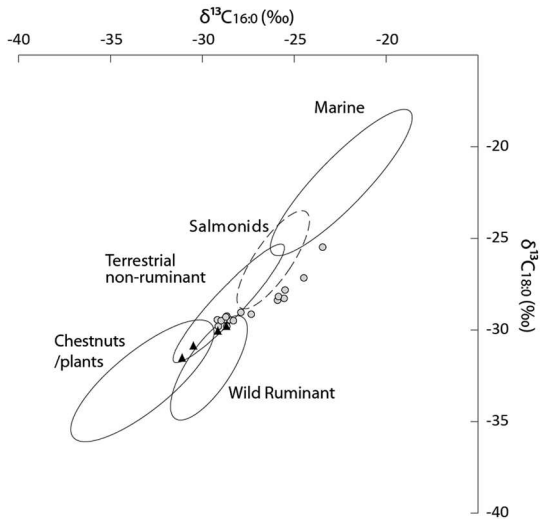

Figure 8. $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ n-alkanoic acids extracted from pottery (grey circles) and charred remains (black triangles). The data are compared with reference ranges for authentic reference lipids from modern tissues and archaeological bone (66.7% confidence)

molecular and isotope data for the plant origin of the charred remains.

5. Conclusions

The lipid composition of the pottery extracts is remarkably similar although some of the sherds exhibited better preservation and a wider range of molecules were detected albeit in lower abundance. There is a marked contrast with the composition of the lipid extracts of the charred remains. Table 8 summarises the findings. The lipid compositions of these two sample sets suggest aquatic resources in the pottery but with a plant contribution. The charred remains contain plant tissues most likely with a high starch composition such as nuts.

Lipids were recovered from the majority of the samples. Preservation varies although *n*-alkenoic acids and acyl lipids are considerably depleted compared with modern fats and oils. This is in accordance with published data on lipids extracted from pottery vessels and charred remains from other parts of the world. Methylation provided evidence of the range of fatty acids present together with the presence of *ω*-(*o*-alkylphenyl)alkanoic acids. Trimethylsilylation enabled a wider range of compound classes to be detected especially those containing free –OH groups.

The presence of long-chain *n*-alkenoic acids, dihydroxyalkanoic acids (especially the dominance of the precursor molecule cetoleic acid over other $C_{22:1}$ isomers) and phytanic acid suggests that at least some of the pottery vessels were used for the processing of aquatic tissues. The absence of *ω*-(*o*-alkylphenyl)alkanoic acids tends to suggest that either they are below the limits of detection (although C_{18} acids were found in the methylated extracts of the charred remains) or the temperatures in the vessels were not high enough to allow for their formation. Based on a series of experiments, Evershed *et al.* (2008) conclude that *ω*-(*o*-alkylphenyl)alkanoic acids form when *n*-alkenoic acids are

Table 8. Differences in molecular composition between the absorbed pottery residues and 'charred remains'

Pottery residues	Charred remains
Low $C_{16:0}/C_{18:0}$ ratios	High $C_{16:0}/C_{18:0}$ ratios
$C_{16:1}$–$C_{26:1}$	$C_{18:1}$
Isoprenoid acid (phytanic acid)	nd
Dihydroxyalkanoic acids	nd
nd	C18 *ω*-(*o*-alkylphenyl)alkanoic acids
Cholesterol dominates over trace of plant sterol	Plant sterol (sitosterol) in all samples; cholesterol absent
nd	Levoglucosan and other sugars

nd—not detected

subjected to prolonged heating above 270°C with a ceramic matrix. The charred remains not directly associated with pottery may derive from basketry which no longer survives. This serves as a reminder that food processing takes place in many ways and in many different contexts than may be represented by surviving pottery vessels alone.

In conclusion, molecular and isotope investigations can be a powerful tool for advancing our understanding of the foodways of prehistoric people. The results of our analyses indicate that organic residues do survive in association with pottery vessels and charred remains excavated from Jomon sites. Lipids were recovered from the majority of the samples, although the preservation varied: given that our samples were obtained primarily from waterlogged middens, where the preservation is generally better than in dry sites, preservation condition will be an important factor to be considered when conducting this type of research in the future. The separation between the lipid extracts of pottery with marine or aquatic signature, and those of the charred remains with indications of plant origins is particularly worth noting. The evidence may point to specialised culinary practices involving distinct cooking technologies. It is particularly interesting that pottery seems to be largely dedicated for processing aquatic foods, as observed in other Jomon contexts (Craig et al., 2013; Lucquin *et al*. 2016; Horiuchi *et al*. 2015), whilst other aceramic technologies were presumably used to cook plant foods. Since our sample size is still too small to infer temporal and spatial variability, further analysis is needed to understand changes through time in Jomon foodways as well as variability between sites or locations.

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