Rapid, Cost-effective Lipid Analysis of Small Samples of Archaeological Ceramic by Pyrolysis GC-MS

Shinya SHODA[1, 2], Kazuko MATSUI[3] and Chuichi WATANABE[3]

1 Nara National Research Institute for Cultural Properties, Nara, Japan
2 BioArCh, University of York, York, United Kingdom
3 Frontier Laboratories Ltd., Koriyama, Japan

Ⅰ Introduction

Lipid residue analysis of archaeological ceramics is frequently applied in many parts of the world with the number of applications continuing to broaden both chronologically and regionally (Admiraal et al., 2020; Courel et al., 2020; Craig et al., 2013; Evershed, 2008; Roffet-Salque et al., 2017; Shoda et al., 2020). This technique requires the destruction of pottery, to obtain a powdered sample of 1–2 g, often preventing its application to rare and valuable objects on display in museum collections. Furthermore, sample collection, preparation, and lipid extraction methodologies are laborious, requiring 8–9 hours input before instrument analysis. As such, this technique is often applied to

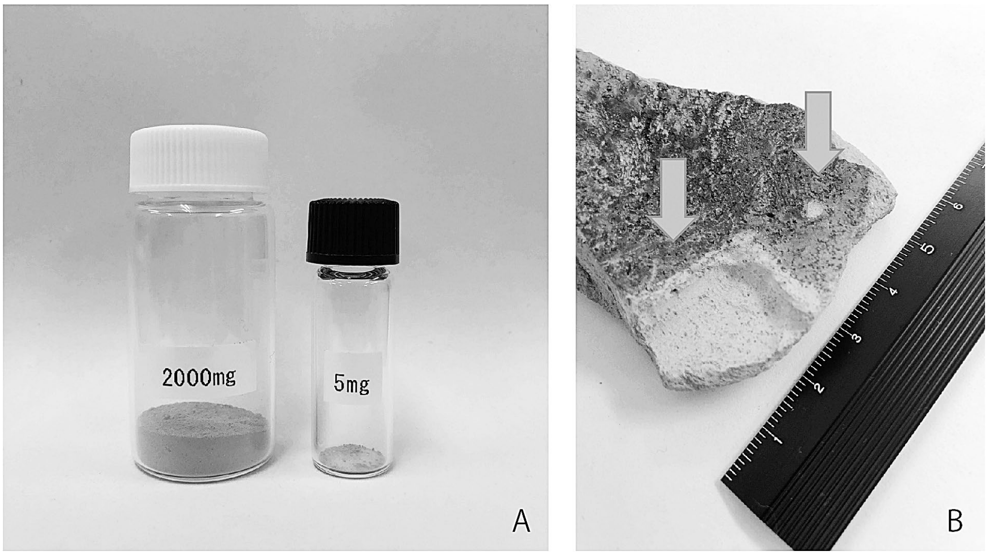

Figure 1. Photographs comparing the sample mass (**A**) and sampling area (**B**) of conventional acid/solvent extraction and THM (presented in this study). **A** conventional acid/solvent (2000 mg, left) and THM (5 mg, right) sample mass. **B** representative conventional acid/solvent (bottom left) and THM (top right) sample area.

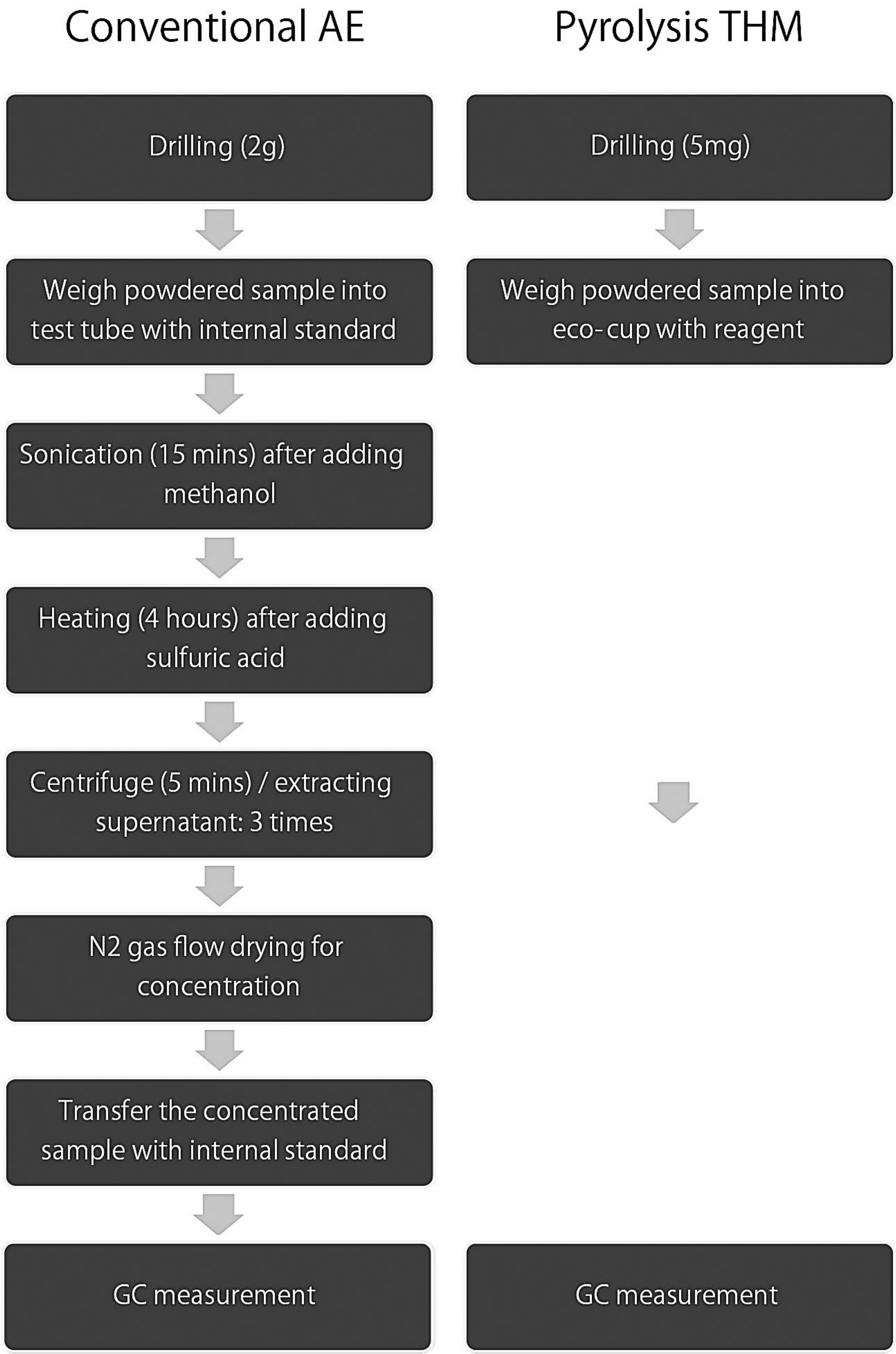

Figure 2. Differences between the analytical procedures of conventional AE methods and THM method proposed in this study.

only a small number of vessels, from larger assemblages, raising questions of representivity.

To address these issues, we present a new approach for lipid residue analysis, from just 5 mg of pottery (Figure 1), by thermally assisted hydrolysis and methylation (THM)-GC/MS, using tetramethylammonium hydroxide (TMAH) reagent with a multi-shot pyrolyzer (Frontier Laboratories Ltd., model EGA/PY-3030D). This approach involves direct pyrolysis of organic residues in the ceramic matrix, negating the need for conventional extraction. Accordingly, the lengthy procedures for sample preparation can be skipped (Figure 2) and no glassware consumables, such as test tubes, vials and pipettes, as well as laboratory instruments, such as block heater, centrifuge, and concentrator are required.

Using a pyrolyzer for the analysis of archaeological material itself is not new. Charred remains attached to both the interior and exterior wall of pottery vessels (20-30μg) were analyzed using curie-point pyrolysis (Oudemans & Boon, 1991), demonstrating the differences of chemical compositions among different archaeological samples. Also, pottery matrices were anlayzed by pyrolysis-GC/MS combined with principal component analysis to evaluate the organic temper in archaeological ceramic samples (Kaal et al., 2014). Possible "wine residues" were presented by Py-GC/MS approach combined with FTIR and LIBS spectroscopy and thermoliminescence analysis (Legnaioli et al., 2013). However, any archaeo-biochemical fingerprints directly related to the ingredients of pottery cooking had not been highlighted by using pyrolysis approach although the recognition of its excellence as a screening method. Therefore, this paper examines how to make use of this rapid and cost-effective approach to identify archaeological biomarkers related to specific groups of food resources, as well as its limitation in the archaeological application and interpretation.

Ⅱ Material and method

Two archaeological pottery powder samples that have been analyzed in previous studies (Heron et al., 2016; Shoda et al., 2017) were used for the analysis. In the former study, by using conventional acid extraction method, a series of aquatic biomarkers (Evershed et al., 2008; Hansel et al., 2004) were identified in the Korean Neolithic pottery from the Jukbyeon-ri site (JBR38, 7.9-6.9ka cal BP), while in the latter, miliacin (olean-18-en-3β-ol methyl ether), a biomarker of broomcorn millet (Heron et al., 2016), has been identified in the Korean Bronze Age pottery form the Majeon-ri site (MJR10, 2.8–2.5 ka cal BP) by both acid and solvent extractions. The total lipid concentration shown in these studies are 434 μg g^{-1} for JBR38, 123 μg g^{-1} for MJR10, respectively.

Ceramic powder (5.064 mg for JBR38, 4.830 mg for MJR10) was embedded in a sample cup to which 10 μl of methyl derivatization reagent (Tetramethylammonium hydroxide (TMAH) 25 wt% in methanol) was added. The sample was analyzed by Thermally assisted hydrolysis and methylation (THM)-GC/MS, using multi-shot pyrolyzer (EGA/PY-3030D, Frontier Laboratories Ltd) connected with GC (Agilent 7890, Agilent technologies) and MS (JMS-Q1500GC, JEOL Ltd.). The sample cup was released in the pyrolyzer, which was set to 350 °C, then introduced to GC at 300 °C by splitless mode. The Ultra ALLOY-5 (30 m – 0.25 mm – 0.25 μm (Frontier Laboratories Ltd) column was used at the flow rate of 1.0 mL/min of helium gas and anlyzed at the

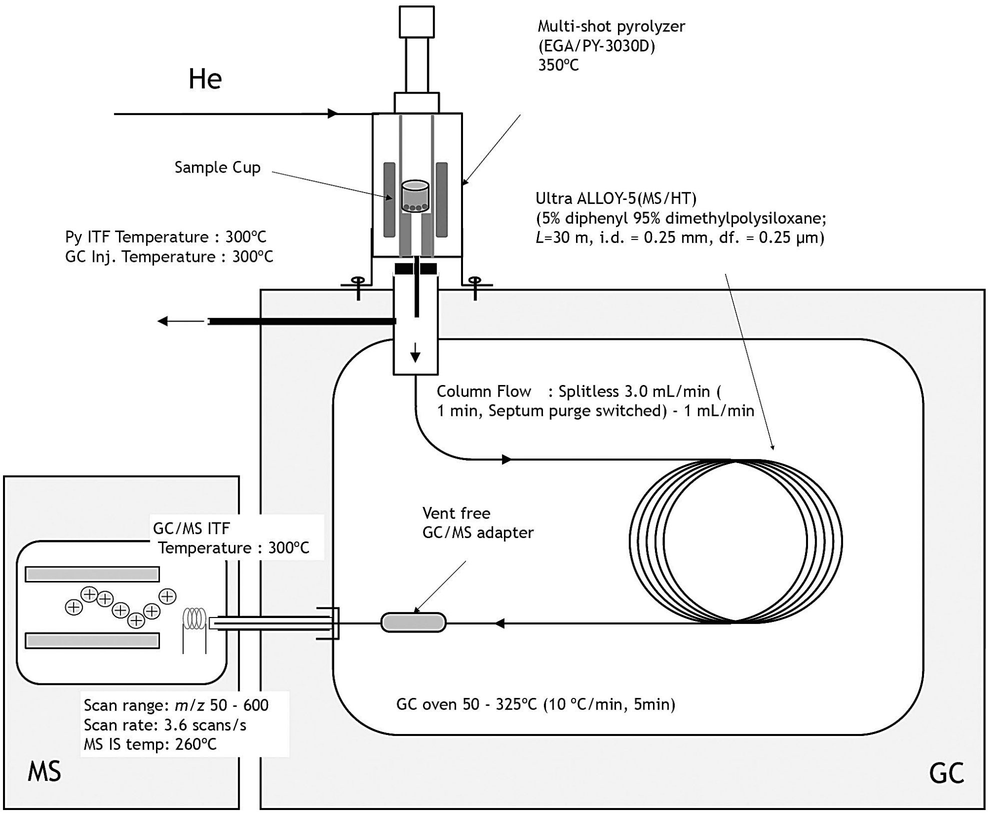

Figure 3. A schematic illustration of THM-GC/MS Analysis used in this study (Frontier Laboratories Ltd., Koriyama, Japan).

split ratio 1/20. For scanning the temperature was set at 50 °C then raised by 10 °C/min until it reached 325 °C where it was held for 5 minutes. The ionization energy of the MS was 70 eV and spectra was obtained between m/z 50 and 600. The temperature at the interface was kept at 300 °C. Details of the Py-GC/MS instrumental settings in each section are shown in Figure 3.

Ⅲ Results and discussion

Clear separation of the peaks are confirmed in both samples that lead to robust identifications of some well-known biomarkers. From a Korean Neolithic potsherd JBR38, ω-(o-alkylphenyl)alkanoic acids (APAAs) with carbon length 18, 20 and 22, as well as isoprenoid fatty acids, such as phytanic and pristanic acids that characterize oils from aquatic organisms, were reliably identified (Figure 4). Also, the pentacyclic triterpene methyl ether, miliacin, a diagnostic compound for broomcorn millet was identified in a Korean Bronze Age sample MJR10 (Figure 5). The presence of these compounds was identified by conventional lipid analysis (acidified methanol/solvent extraction followed by GC-MS measurements) but required 400 times the amount of ceramic material.

In short, we successfully show that THM-GC/MS method can be used for rapid identification of various kinds of biomarkers that are archaeologically important. It can be used for screening the aquatic or millet biomarkers in a much shorter time, with a much smaller sample size, than the conventional method. This method is especially useful if one has large numbers of pottery sherds (i.e. 100–1,000 sherds) and aims to understand the tendency of either aquatic or millet consumption in that group.

Of course, with advantages always come disadvantages. As this method consumes the whole powder sample, it is not possible to re-analyze in other devices, such as GC-c-IRMS, that sometimes play crucial roles in identifying the origin of lipid residues preserved in pottery. Also, as there are no “extracts” remained, it is difficult to analyze the exact same sample with different methods and settings to further examine the detected compounds.

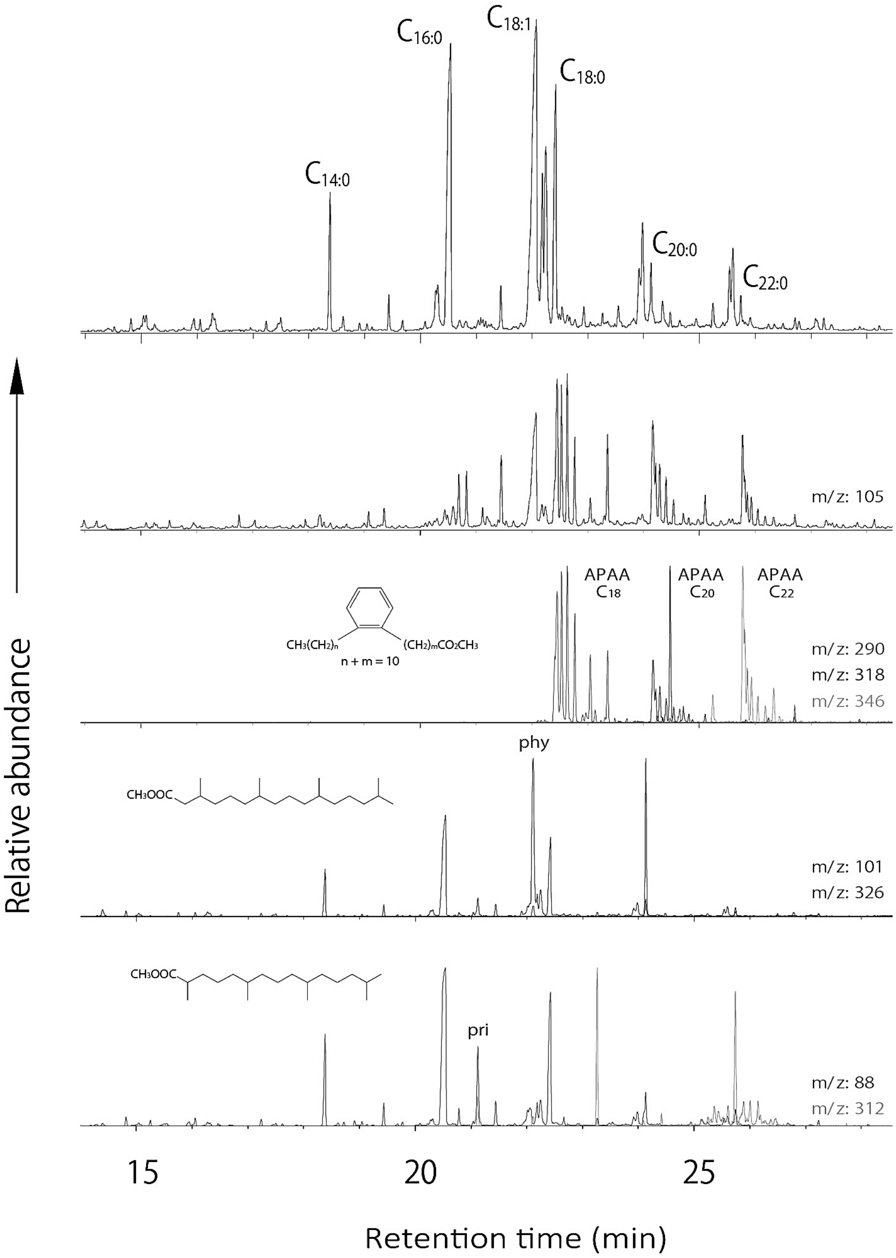

Figure 4. Results of THM-GC/MS analysis A: Partial total and ion chromatograms of extracts of a Korean Neolithic pottery (JBR38) with ω-(o-alkylphenyl)alkanoic acids (APAAs) and isoprenoid fatty acids (phy: phytanic acid, pri: pristanic acid) which supports the origin from aquatic organism (Hansel et al. 2004: Evershed et al. 2008). Cx:y denotes saturated fatty acids with x carbon length and number of unsaturations y.

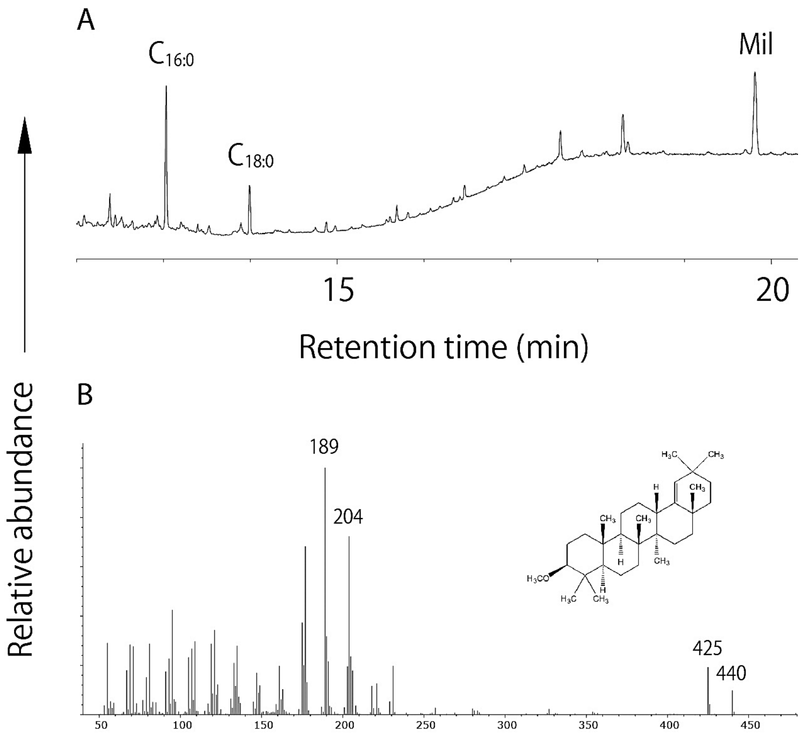

Figure 5. Results of THM-GC/MS analysis B: A Partial total ion chromatogram of extracts from a Korean Bronze Age pottery (MJR10) containing miliacin, a biomarker for broomcorn millet (Heron et al. 2016), and B the mass spectrum and chemical structure of miliacin. Cx:y denotes saturated fatty acids with x carbon length and number of unsaturations y. Mil: miliacin.

Ⅳ Conclusion

THM-GC/MS allows a ten to twenty fold reduction in sample preparation time and requires only 1/200 to 1/400 of the amount of sample compared to the conventional protocols. Although Pyrolysis GC-MS had been used for archaeological organic residue analysis previously, this study shows the direct identification of the biomarkers that are useful for archaeologically reconstructing ancient diet and culinary practices for the first time.

The extremely small sample size used enables us to examine other types of archaeological findings. For example, dental calculus is frequently used for reconstructing diets in the past (Warinner et al., 2014) but it is difficult to conduct conventional lipid residue analysis on them, due to the limited sample size available. In addition, Py-GC/MS approach may be used for assessing the quality of protein and DNA preservation in the fossil remains (Poinar & Stankiewicz, 1999). Utilisation of these screening methods can greatly contribute to reducing the consumption of precious archaeological and anthropological samples. In addition, Py-CSIA (compound specific stable isotopic analysis) approach (González-Pérez et al., 2015) which connect pyrolyzer to GC-FID and GC-c-IRMS (Py-GC-(FID)-C\TC-IRMS), might be worth introducing to analyses of archaeological samples to obtain carbon stable isotope values of palmitic ($\delta^{13}C_{16:0}$) and stearic ($\delta^{13}C_{18:0}$) acids, although no such studies have been conducted so far.

Advanced pyrolysis techniques, such as Py-CSIA (compound-specific stable isotope analysis, González-Pérez et al., 2015) have not yet been applied to archaeological materials. However, great potential exists to combine the practical efficiency of pyrolysis, with the compound separation capabilities of GC/MS, and specific compound isotope measurements of GC-c-IRMS, in order to obtain isotopic measurements of palmitic ($\delta^{13}C_{16:0}$) and stearic ($\delta^{13}C_{18:0}$) acids from milligrams worth of samples.

To conclude, the pyrolysis approach to archaeological ceramic samples, such as THM-GC/MS, is quite useful for screening not only lipid preservation but also detecting some key biomarkers in archaeological discussion. Further studies are required to enlarge the potential of this method for minimizing the time and cost for the analysis, while maximizing the quality of data that will be obtained.

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