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Towards the detection of dietary cereal processing through absorbed lipid biomarkers in archaeological pottery

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ABSTRACT

The uptake of cereal agriculture in the Neolithic is one of the most important processes in later human prehistory. However, in many parts of Europe, early evidence from pollen or macrofossils is scarce or inconclusive, and there are considerable ambiguities about timing, intensity and the mode of transition to agriculture in these regions.

An alternative approach is organic residue analysis, a technique that targets lipids preserved in the walls of unglazed ceramic pots used for storage and processing of foodstuffs. By analysing the molecular and isotopic composition of absorbed lipid residues, many different food items and processing techniques can be detected and distinguished. However, this approach is by-and-large limited to animal-based food sources, and despite their importance, many plant-based food items including cereals are currently not accessible with this approach.

For a better understanding of the behaviour of cereal lipids, cooking experiments were conducted and the uptake of cereal-specific compounds such as alkylresorcinols and plant sterols into the ceramic matrix was investigated using a new sensitive method based on GC-Q-ToF-MS. Furthermore, changes in the lipid composition through post-burial degradation was assessed by incubation of potsherds dosed with cereal lipids at 35 °C in compost. The cooking experiments showed that only small quantities of cereal lipids are liberated, but additional lipid sources (meat) can increase the transfer of cereal biomarkers into the ceramic matrix. Anoxic degradation conditions allowed for twentyfold higher levels of alkylresorcinols and twofold higher levels of plant sterols after 20 weeks compared to oxic conditions. Therefore, samples from anoxic burial environments should be targeted and high sensitivity methods are a necessity to detect the trace amounts of cereal-specific biomarkers. To test the applicability of these biomarkers for archaeological pottery, organic residues from ten coarse ware vessels from an anoxic burial context at Vindolanda were analysed. Plant sterols and stanols were detected in three sherds, and two of the sherds also contained traces of alkylresorcinols.

1. Introduction

Cereal is a collective term for grasses with grains used for nutritional purposes. Today they are a staple food all over the world with annual production exceeding 2.5 billion tons (Food and Agriculture Organisation of the United Nations, 2017). The domestication of wild plants and the start of agriculture is regarded as one of the greatest and most important achievements in later human prehistory (Brown et al., 2009). The domestication of wild cereal ancestors was initiated in multiple parts of the world over 10 000 years ago, including the so-called “Fertile Crescent” of the Near East (Brown et al., 2009; Lougas et al., 2007). From there, agriculture spread westwards, reaching Europe about 6000 BCE, the British Isles and Northern Europe around 4000 and 3000 BCE, respectively (Lougas et al., 2007). However, there is still a significant debate about the timing and mode of uptake of cereal processing in certain regions (Rowley-Conwy, 2004; Lahtinen and Rowley-Conwy, 2013; Behre, 2006; Price, 2000).

The most common lines of evidence to follow and detect cereal usage and agricultural technology in prehistory are based on pollen analysis, macrofossils such as charred grains or agricultural artefacts such as sickles. All palaeodietary proxies, however, carry inherent biases which have created controversies regarding the role of cereal agriculture in certain regions (Behre, 2006; Brown,

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2007; Lahtinen and Rowley-Conwy, 2013; Stevens and Fuller, 2012). An alternative approach allowing the direct evidence of a commodity as an exploited resource is lipid residue analysis of archaeological potsherds (Evershed, 2008a, 2008b). This approach is based on the premise that storage and preparation of food items in unglazed ceramic pottery facilitates the transfer of constituents from the food into the ceramic matrix. Of these constituents, mainly DNA, proteins, carbohydrates and lipids, the latter have the highest chance of survival over archaeological timescales (Evershed, 2008b). However, usually not the original lipid compound is recovered but a characteristic degradation product (for example ω-(*o*-alkylphenyl)-alkanoic acids formed from heated polyunsaturated fatty acid in fish lipids), which can be used as a proxy for certain lipid sources (Evershed, 2008a, 2008b). By analysing the lipids, their characteristic degradation products and their isotopic signatures, information about the original composition and the lipid source can be gathered. This can then be used to reconstruct dietary and cultural habits as well as food processing technologies of past communities (Roffet-Salque et al., 2015; Salque et al., 2013; Cramp and Evershed, 2014; Cramp et al., 2015; Roffet-Salque et al., 2016).

To this day this approach is by-and-large limited to the analysis of animal-based food items. Despite their importance cereals and other plant-based food items are hard to detect in the archaeological record using organic residue analysis. The lipid content of plant-based food generally is at least tenfold lower, and the contribution will be likely masked by concomitant animal fat and therefore almost invisible (Colonese et al., 2017). Furthermore, only for very few plant-based food items (members of brassica family, maize kernels) have robust biomarkers been proposed and tested. Recently, Heron et al. showed the applicability of the triterpenoid miliacin as a biomarker for broomcorn millet processing in bronze age pottery (Heron et al., 2016). Furthermore, Colonese et al. identified wheat and rye kernels in an amorphous residue found in a well-preserved Bronze Age wooden container (Colonese et al., 2017). In addition to macrobotanical and proteomics data, they were able to detect alkylresorcinols and plant sterols, which are both well-known minor compounds of cereals (Ross et al., 2003; Piironen et al., 2002).

While this demonstrates that these compounds can be preserved under favourable conditions it raises the question why molecular evidence of cereals in archaeological pottery has been elusive so far. One explanation could be that the compounds are not liberated from the grains during cooking and thus are not absorbed into the ceramic matrix in appreciable quantities in the first place. Another explanation could lie in a high susceptibility to microbial degradation during millennia in the soil.

To shed more light on these questions, cereals were cooked multiple times in replica Neolithic pottery and absorption of cereal lipids into the ceramic matrix was quantified using a new and sensitive approach based on gas chromatography coupled to quadrupole-time-of-flight mass spectrometry (GC-Q-ToF-MS). Furthermore, potsherds containing cereal lipids were incubated at elevated temperatures in the laboratory to simulate accelerated microbial decay in the soil. With these experiments, we wished to evaluate how and under which conditions cereal lipids could be detected in archaeological pottery. The new method was then applied for the analysis of ten potsherds from a Roman cavalry barrack excavated at Vindolanda (105–120 CE).

2. Material and methods

2.1. Chemicals, standards and samples

Dichloromethane (DCM), *n*-hexane, methanol and chloroform (all HPLC grade) were from Rathburn Chemicals (Walkerburn, UK). Pyridine (>99%), the silylating agent consisting of N,O-Bis(trifluoroacetamide)/trimethylchlorosilane (BSTFA/TMCS) 99:1 (v/v), tetratriacontane (C34, >98%), and methyl heptadecanoate (17:0-ME, >99%) were from Sigma-Aldrich. An authentic standard of 5-*n*-docosoylresorcinol (AR-22, >99%) was from ReseaChem (Burgdorf, Switzerland).

Commercial compost, milk and pork was obtained in Bristol, UK and cereal samples (organically produced grains of spelt, rye and pot barley) were bought online. Cooking experiments were performed using modern replica pots made up from a clay/sand mixture (3:1) which were fired under 1000 °C (The Pot Shop, Lincoln/UK). After firing care was taken to avoid contamination from skin lipids. The pots (10 cm high, 11 cm diameter, 1 cm wall thickness) had a volume of about 500 mL. Ten coarse ware vessels (including one mortarium) from excavations at Vindolanda/UK were analysed. Sherds were selected from the rim part of the vessels to maximise lipid recovery. The vessels were from a drain between three rooms of a period IV cavalry barrack (ca. 105–120 CE). The conditions of this context were anaerobic, and as such, remarkable preservation of organic remains such as wooden writing tablets and textiles was observed.

2.2. Cooking experiments

About 80 g of cereals (equal parts of spelt, rye and barley) was placed together with 250 mL of (in-lab produced) ultrapure water into a replica pot and heated on a laboratory mantle heater. The pot was left to simmer for about 1 h until the kernels were soft. The mixture was stirred every 10–15 min and water lost from evaporation was replaced. Afterwards, the pot was emptied and cleaned with water and re-used for cooking until ten repeated cooking steps were performed. The same experiment was performed with two additional pots (Samples W1-3).

In two further experiments, 80 g of cereals, that had been ground in a coffee grinder (W4) and 80 g of cereals, which had been soaked in water for 12 h to soften them (W5), were simmered in a pot using 250 mL water for 1 h (five repeated steps).

Analogous to the first experiment 80 g of cereals were cooked in one pot in 250 mL of milk (3.5% fat) for ten repeated steps (Sample M).

Finally, one pot was used to cook cereals (80 g) together with pork shoulder (100 g, 10% fat) in 250 mL of water for ten repeated steps (Sample P).

Portions of the pots from the rim area (waterline) were removed using a hammer and a cleaned chisel, cleaned using a modelling drill and extracted as described below.

To assess the extractability of cereal lipids by water 20 g of cereals were refluxed in 200 mL of water for 22 h. After cooling, the lipids were extracted from the aqueous phase using 3 × 50 mL of DCM. The DCM phase was dried over sodium sulphate, the solvent was evaporated, and an aliquot of the re-dissolved sample was trimethylsilylated and analysed by GC/MS.

2.3. Dosing of potsherds with cereal lipids

A total of 12 g of the cereal mixture used for cooking experiments was extracted in 6 subsamples using 2 × 10 mL chloroform/methanol 2:1 (v/v) under sonication (20 min). After centrifugation the organic phases were combined and evaporated using a gentle stream of nitrogen, then re-diluted in 5 mL chloroform/methanol 2:1 (v/v). All total lipid extracts (TLEs) were combined and made up to 100 mL with chloroform/methanol 2:1 (v/v) and an aliquot of the solution was analysed by GC and GC-MS. The concentration of the TLE was about 1 mg/mL (GC-FID).