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Immunological detection of denatured proteins as a method for rapid identification of food residues on archaeological pottery

Jaroslav Pavelka [a], Ladislav Smejda [b, c, *], Radovan Hynek [d], Stepanka Hrdlickova Kuckova [d]

[a] Centre of Biology, Geosciences and Environmental Education, University of West Bohemia, Univerzitni 8, 30614, Plzen, Czech Republic
[b] Department of Archaeology, University of West Bohemia, Univerzitni 8, 30614, Plzen, Czech Republic
[c] Department of Ecology, Czech University of Life Sciences Prague, Kamycka 961/129, 165 00, Praha 6-Suchdol, Czech Republic
[d] Department of Biochemistry and Microbiology, University of Chemistry and Technology, Prague, Technicka 3, 166 28, Prague 6, Czech Republic

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ABSTRACT

Our understanding of human diet in different periods of history can be enhanced by investigating direct evidence represented by accidentally preserved food remains found on pottery. So far, this task has been accomplished by the application of gas chromatography/mass spectrometry, often in combination with stable isotope analysis. These methods require specialised laboratories and their cost prevents wider penetration into the daily practice of archaeology and related disciplines. We have tested commercially available immunochromatographic kits for this task, which are designed to detect contaminants and allergens in the modern food industry. Unlike the previously published studies on archaeological material, we focus specifically on the identification of damaged and denatured proteins, which correspond better to the state of preservation of proteins in desiccated and carbonised organic residues that have survived from antiquity. We report the first successful qualitative detection of bird eggs, animal meat, milk (and species of origin), and to some extent also the presence of plant food, especially cereals and hazelnuts. The immunoassay is a methodology that is well suited for use in the field and resource-poor environments, so it is ideal for most archaeological excavations and museums. With necessary caution, the results can be used as a proxy for human diet in the past and reconstructions of anthropogenically modified environments.

1. Introduction

It is occasionally possible to find ancient food residues attached to archaeological pottery, usually in the form of accidentally desiccated or charred organic material. Analysis of such remains is highly desirable, as the results may suggest the final purpose of a particular vessel, before it went out of use and was discarded (Pollard et al., 2007:22–23). The significance of this type of research is twofold. It may reveal a cultural association between certain types of pots and meals that were cooked in them and possible variations in the use of ceramic inventories of households. Secondly, it provides data on human diet in specific cultural and social contexts, if these can be recognised from the archaeological record. Alternatively – and complementarily – approaches that help study subsistence on the basis of archaeological finds include the compositional analysis of animal bones and finds of preserved botanical macrofossils. Well preserved bones and plant remains are not ubiquitous on ancient sites, depending heavily on local taphonomic conditions (Dincauze, 2000; van der Veen, 2007; Wright, 2003). Indirect approaches, which can detect only broad types of diet, include complex dental analysis and the analysis of stable isotopes in human bones (Day, 2013; Forshaw, 2014; Klippel, 2001; Landon, 2005; Reitsema et al., 2010).

For a detailed analysis of carbonised food residues usually found as attachments on the surface of cooking vessels, there are currently two dominant approaches available, each with its own pros and cons. The more widespread method is the analysis of lipids through the identification of fatty acids by gas chromatography-mass spectrometry (GC-MS), sometimes coupled with the analysis of carbon stable isotopes, which allows the distinction between ruminant and non-ruminant fat in remains of animal origin (Cramp et al., 2014; Dudd et al., 1999; Regert, 2011; Salvini et al., 2008). A possible advantage of the study of lipids

* Corresponding author. Department of Archaeology, University of West Bohemia, Univerzitni 8, 30614, Plzen, Czech Republic.
E-mail address: smejda@kar.zcu.cz (L. Smejda).

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over proteins is seen in the fact that lipids should be less susceptible to leaching and diagenetic degradation than proteins (Craig et al., 2005).

The second major approach aims to identify source-specific proteins in the preserved organic residues. Today this task can be performed either by mass spectrometry or by more traditional immunological methods. Recently, mass spectrometry made significant advances in this direction and has been applied to analyses of protein components in historic art works (Hynek et al., 2004; Kuckova et al., 2013) and in historic mortars (Kuckova et al., 2009). The approach to food residue analysis by peptide mapping is very analogous (Kučková et al., 2010). MS proteomics currently uses several instrumentation variants based on liquid chromatography (LC) combined with matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF-MS). Contrary to lipid analysis, this technique should be able to detect distinct differences in the amino acid loci of alpha S1 casein characteristic for individual species raised for milk (Buckley et al., 2013; Hong et al., 2012). A relatively high cost of peptide mapping and its high sensitivity to preservation of target protein structures versus contamination (e.g. by human agents or microorganisms) presents barriers to its routine use in archaeology.

In this paper we explore current prospects in the field of protein identification in ancient food residues by the immunochromatographic method. Although this technique has been known for a long time, we pay attention to certain recent trends in this field, which are promising for the large-scale investigation of organic residues on pottery vessels.

2. Immunological analysis of food residues

Immunological detection of proteins is a well-known and routinely used technique in many disciplines. Pavelka et al. (2011) used this method for taxonomic determination of animal bones from archaeological sites to enhance the rate of successful identification of species by standard osteological approach. Examples of its application to ancient food residues have already been published and verified by an independent method (Craig et al., 2005). However, immunological tests can be prepared in many particular configurations requiring somewhat different laboratory protocols. Their performance and sensitivity mainly depend on the state of protein preservation in largely variable diagenetic conditions of recovery contexts. The methods used for analysis should therefore specifically target types of proteins that are likely to be present in the samples.

The same analytical approaches can be applied to either organic remains visibly adhering to the surface of pots or to the detection of organic residues soaked into the porous mass of ancient ceramics. However, proteinaceous crusts of organic material attached to the surface of cooking pots can be analysed more readily, while proteins adsorbed to the ceramic matrix and siliceous minerals are generally more difficult to extract (Craig and Collins, 2000).

In our study, we mainly focused on the analysis of carbonised attachments to the pottery surface, opened by recent advances in commercial immunological tests used in the food industry (Dzantiev et al., 2014). This novel approach opens broad possibilities of application in the study of past diet patterns.

Although organic materials can be in principle tested for the presence of DNA, the origin of which could subsequently be assessed, this approach seems to be rather unpromising for the investigation of archaeological finds of food remains. In the existing archaeological samples of food remains, which are typically small and degraded by heat and other diagenetic factors, the preservation of ancient DNA is likely to be poor. Proteins are more resistant than DNA and only very small regions of protein molecules, known as epitopes, are needed for binding of specific antibodies (Hofreiter et al., 2012). The immunological detection therefore usually has a higher chance of being successful compared to ancient DNA extraction and identification. This is especially true when denatured or thermostable forms of proteins are targeted, which can be expected to be preserved in food remains attached to cooking vessels.

We used relatively simple tests based on the immunological antigen-antibody interaction. The most innovative part of our approach is aimed specifically at those types and forms of proteins, which can be expected to be the most characteristic for archaeological samples in the studied region of temperate Europe. A number of earlier attempts using antibody tests on archaeological material did not yield consistent results and were consequently viewed rather critically. The problem of cross-reactions was mentioned in Child and Pollard (1992) and studied experimentally by Collins et al. (1992). Dongoske et al. (2000) repeated the warning that the degradation of proteins may result in antigen binding that accounts for nonspecific reactions of ELISA tests. Problems linked with immunological techniques applied to old samples were elaborated in a more detailed study by Brandt et al. (2002), who investigated the effects of diagenesis and contamination on the detection success of non-collagenous proteins from human and other mammal samples of modern and ancient origin.

It must be understood that types of antibody tests used in past studies were mostly developed for use with modern biological samples. This factor indeed limits their applicability in studies working with archaeological material. However, it is possible to use antibody tests targeted specifically at denatured/degraded proteins, which can make a profound difference in the quality of obtained results. A wide range of detection tests based on immunological reaction are commercially available today, which were developed for the identification of various components in heat-treated foods. This category of commercial test is purposefully designed to work with degraded traces of biological tissues in the food industry and they are undergoing rigorous evaluation of their ability to detect the ingredients in processed food correctly (Bjorklund et al., 2001).

Many food components can be identified on the basis of the presence of some typical proteins which have a well-known chemical structure; the producers of commercial kits make use of these properties for their reliable detection. Currently used food-safety tests are constantly improving to reduce the possibility of cross-reactions e.g. with bacteria (this risk is described in Brandt et al., 2002). The kits we have chosen for testing archaeological material are highly sensitive for the detection of allergens in the food industry. This means that they are expected to identify even trace amounts of specific proteins that may present unwanted contamination in food. Manufacturers of these kits indicate that the detection limit for the target content can be at single-digit ppm, although this may vary depending on sample type and extraction efficiency. We evaluate their performance on archaeologically retrieved food remains, as it can be anticipated that these modern, specifically targeted assays could be more apt for such a complex task than traditional tests applied in archaeological studies in previous decades.

All the kits used in this study were based on commercially available antibodies (Table 1). The market of immunochromatographic tests has become very dynamic in the last decade.

The production of different kits for food-safety testing is growing globally every year (Dzantiev et al., 2014), and the competition among companies is strong. This implies that the names of producers and their marketed kits may be subject to rapid change. However, alternative tests should be available and improved technologies will lead to more varied products tailored to the specific needs of individual groups of users. Due to differences