UNIVERSITY *of* York

This is a repository copy of *Detection of dairy products from multiple taxa in Late Neolithic pottery from Poland: an integrated biomolecular approach*.

White Rose Research Online URL for this paper:
https://eprints.whiterose.ac.uk/197430/

Version: Published Version

Article:

White Rose
university consortium
Universities of Leeds, Sheffield & York

eprints@whiterose.ac.uk
https://eprints.whiterose.ac.uk/

Author for correspondence:
Miranda Evans
e-mail: mae52@cam.ac.uk

THE ROYAL SOCIETY PUBLISHING

Detection of dairy products from multiple taxa in Late Neolithic pottery from Poland: an integrated biomolecular approach

Miranda Evans[1,2], Jasmine Lundy[1], Alexandre Lucquin[1], Richard Hagan[1], Łukasz Kowalski[3], Jarosław Wilczyński[4], Penny Bickle[1], Kamil Adamczak[3], Oliver E. Craig[1], Harry K. Robson[1] and Jessica Hendy[1]

[1]Department of Archaeology, University of York, Heslington YO10 5DD, UK
[2]Department of Archaeology, The University of Cambridge, Cambridge CB2 3DZ, UK
[3]Institute of Archaeology, Centre for Applied Archaeology, Nicolaus Copernicus University in Toruń, Szosa Bydgoska 44/48, 87-100 Toruń, Poland
[4]Department of Vertebrate Zoology, Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Sławkowska 17, 31-016 Kraków, Poland

ME, 0000-0002-9284-9268; JL, 0000-0001-8250-7697; AL, 0000-0003-4892-6323; RH, 0000-0003-1270-100X; ŁK, 0000-0003-0647-826X; JW, 0000-0002-9786-0693; PB, 0000-0003-2482-0268; KA, 0000-0002-8847-5670; OEC, 0000-0002-4296-8402; HKR, 0000-0002-4850-692X; JH, 0000-0002-3718-1058

The detection of dairy processing is pivotal to our understanding of ancient subsistence strategies. This culinary process is linked to key arguments surrounding the evolution of lactase persistence in prehistory. Despite extensive evidence indicating the presence of dairy products in ceramics in the European Neolithic, questions remain about the nature and extent of milk (and lactose) processing and consumption. In order to investigate past patterns of dairy processing, here we analyse ancient proteins identified from Late Neolithic Funnel Beaker ceramics, scrutinizing the principle that curd and whey proteins partition during the production of dairy foods from milk. Our results indicate the presence of casein-rich dairy products in these vessels suggesting the creation of curd-enriched products from raw milk. Moreover, this analysis reveals the use of multiple species for their dairy products in the Late Neolithic, adding to a growing body of evidence for the period.

Alongside palaeoproteomic analysis, we applied well-established lipid residue analysis. Differential interpretations between these two approaches show that palaeoproteomics is especially useful where the effects from isotope mixing may underestimate the frequency of dairy products in archaeological ceramics, highlighting the potential utility of a multi-stranded approach to understand life histories of vessel use.

1. Introduction

Cheese making is a unique and important culinary invention, enabling milk to be preserved, stored and transported while offering considerable nutritional value, containing protein, sugars and fats. The detection of cheese making in the past has substantial implications for understanding choices surrounding the use of primary and secondary products in daily life, for personal preferences and cultural identities [1–3]. Moreover, identifying the species used for dairy production informs us about environmental niches and pastoral strategies, e.g. [4,5]. The link between dairy consumption, processing and lactase persistence (LP) evolution has also received extensive attention and debate, and it has been proposed that cheese making may have been used as a strategy for reducing the lactose content of milk products, thus rendering foods rich in protein and fat digestible to lactase non-persistence individuals [6]. While the use of dairy products has been extensively shown throughout Neolithic Europe through faunal mortality patterns, lipid residue analysis and ancient dental calculus [7–13], recent research suggests that the presence of dairying does not necessarily correspond with LP allele frequency trajectories [9], and other research has demonstrated the consumption of dairy foods in individuals likely to be non-lactase persistent, e.g. [13,14]. Therefore, questions remain about the extent and nature of dairy processing in relation to lactose content.

1.1. Dairying in the Funnel Beaker culture

A growing body of archaeological evidence demonstrates that milk was consumed from the beginning of the Neolithic in Central Europe. The presence of dairy lipids in pottery from Hungary and Poland dating to the sixth millennium BCE supports the idea that dairying was part of the cultural package travelling with early farmers from the Near East to Europe. There is little evidence, however, that milk formed a significant component of the diet during the earliest Neolithic, but was rather consumed occasionally as a product of a broad mixed economy [6,12,15–17]. Many studies have suggested that this pattern of milk consumption changed profoundly during the mid-fourth millennium BCE, when a focus on secondary animal products becomes visible in mortality profiles and sex ratios of herds [12]. There are strong archaeological indications to suggest that the later generations of the Funnel Beaker (*Trichterbecherkultur*, hereafter TRB) farmers of north-central Europe saw this shift towards a more diversified exploitation of domestic cattle and ovicaprinae, which is often summed up as a Secondary Products Revolution [18–21].

In Poland, the traditional standpoint has been that the subsistence economy of the TRB was focused on domestic livestock exploitation for primary (meat, hide and bone) and secondary products (milk, wool and animal traction), and only occasionally did their economic repertoire also include hunting and gathering [19]. Although the presence of ceramic strainers that could be used to separate curds and whey seem to point to dairying in the TRB, the respective age-at-death profiles have generally proven scarce [22,23]. However, the positive indication for intense milk production in the region of modern Poland can be identified through the sex ratios of cattle herds, showing that in the later stages of the Neolithic, the number of cows is twice the number of bulls compared with the herding structures at the beginning of the Neolithic [24]. Perhaps the most direct evidence for the use of dairy comes from the lipid residue analysis of TRB ceramics from Kopydłowo, central Poland [25]. It is clear that a considerable portion of ceramic beakers recovered from the site were the prime containers for milk, and we now know that a small-scale dairy economy was present from the onset of the TRB in some regions, such as the western Baltic and Southern Scandinavia [7,10,26,27].

1.2. Organic residue analysis and dairying

The analysis of lipids extracted from ancient pottery has been instrumental in providing direct evidence for dairying in the archaeological record. Previous research has revealed the association between dairy

2

fats and ceramic vessels in Southern Europe and the Near East [28,29], sub-Saharan Africa [30] and Northern Europe [6]. Salque et al. [6] argue that the presence of dairy lipid residues present in ceramic sieves demonstrates their specialized use in cheese production. Differences in fatty acid isotope values have been reported between fermented and unfermented dairy products [31], although these are more likely explained by variation in modern farming practices, including maize silage in animal feed [32]. Currently there are no reliable molecular or isotopic criteria to distinguish lipid residues derived from raw milk from those derived from fermented dairy products such as cheese, yoghurt or kefir.

The analysis of milk proteins has also emerged as a powerful tool for understanding ancient dairying and dairy consumption, with ceramic vessels and their residues, and ancient dental calculus often targeted. For example in ceramics, immunological methods revealed dairy proteins from the Early Iron Age Outer Hebrides [16], and more recently liquid chromatography tandem mass spectrometry (LC-MS/MS)-based proteomics was applied to ceramic limescale deposits from the sites of Çatalhöyük West [33], Baḥrija [34] and two sites in the Campania Region [35]. With results from Çatalhöyük West revealing that mixing occurred between sheep and goat dairy products and a range of plant foods [33]. Specifically, this also revealed cow dairy and milk whey processing occurred in specific vessels, potentially indicating specialized food preparation processes [33]. Proteomic analyses of ancient dental calculus have frequently produced taxonomically specific information on dairy consumption, as opposed to processing, with the whey protein beta-lactoglobulin (BLG) being commonly preserved [36]. This approach has now been applied to a range of contexts, revealing the consumption of dairy foods in victims of the Great Irish Famine [37], Neolithic Britain [13], Neolithic-Meroitic Eastern Africa [38] and the East Eurasian Steppe [14].

1.3. Milk processing and the milk proteome

The processing of milk into dairy foods results in characteristic changes to protein abundance and content (figure 1). For example, in rennet-based cheese making, casein micelles (spherical aggregates of four different casein proteins) coagulate when kappa-casein in the micelle reacts with rennet (composed primarily of chymosin, itself a protein). This results in the separation of these coagulated casein proteins from water-soluble proteins found in the whey, such as BLG, alpha-lactalbumin and lactotransferrin, which are left in the whey solution together with much of the milk sugar (lactose) and fats. With the growing applicability of palaeoproteomics to studies of ancient dairying, there is an opportunity to explore to what extent these biochemical processes inherent in dairy processing can be identified in archaeological substrates. This would enable a better understanding of the nature and extent of ancient dairying processes and culinary practices associated with this key food. Moreover, identification of processed dairy products also informs us more precisely about the degree of lactose consumption of ancient populations and its relationship to LP selection.

Some initial attempts exploring how specific milk proteins may be related to dairy processing in archaeological contexts have been undertaken, but the results remain inconclusive. Warinner et al. [36] note that the presence of the whey protein, BLG, may act as a proxy for lactose consumption, given that BLG partitions with lactose during cheese coagulation. Charlton et al. [13], Jeong et al. [14] and Bleasdale et al. [38] expanded this approach to British, Central Asian and East African contexts, respectively, where BLG was detected in individuals who were, or were likely to be lactase non-persistent, suggesting the consumption of lactose by individuals with no evidence for LP alleles. Hendy et al. [33], analysing ceramic vessels, observed the presence of whey proteins in a Neolithic ceramic jar which may indicate the presence of this liquid fraction, as opposed to raw milk. Examining well-preserved whole dairy products from Early Bronze Age China, Yang et al. [41] studied the cleavage patterns of kappa-casein, a protein integral in casein micelle aggregation. Detecting a lack of bond cleavage due to rennet, and the presence of Lactobacillus kefiranofaciens protein, they suggest that these products were created by a kefir-based production. Although this body of literature has revealed that palaeoproteomics is a useful approach for detecting ancient dairying, it has also revealed that there are preservation biases that may impact our ability to accurately interpret dairy processing. For example, Hendy et al. [42] observed a high abundance of the milk whey protein BLG compared with other dietary proteins in ancient dental calculus, despite the fact that casein proteins make up 80–85% of milk protein content [40]. When casein proteins are detected, it is often in well-preserved contexts, such as the arid conditions of Xinjiang, China [41,43], and the cold conditions of Mongolia [5].

In this study, we examined archaeological and modern replica ceramics to explore the degree to which the presence and abundance of milk proteins indicate the production of processed dairy curds. We

(a)

(b)   coagulation and straining   ageing, fermentation
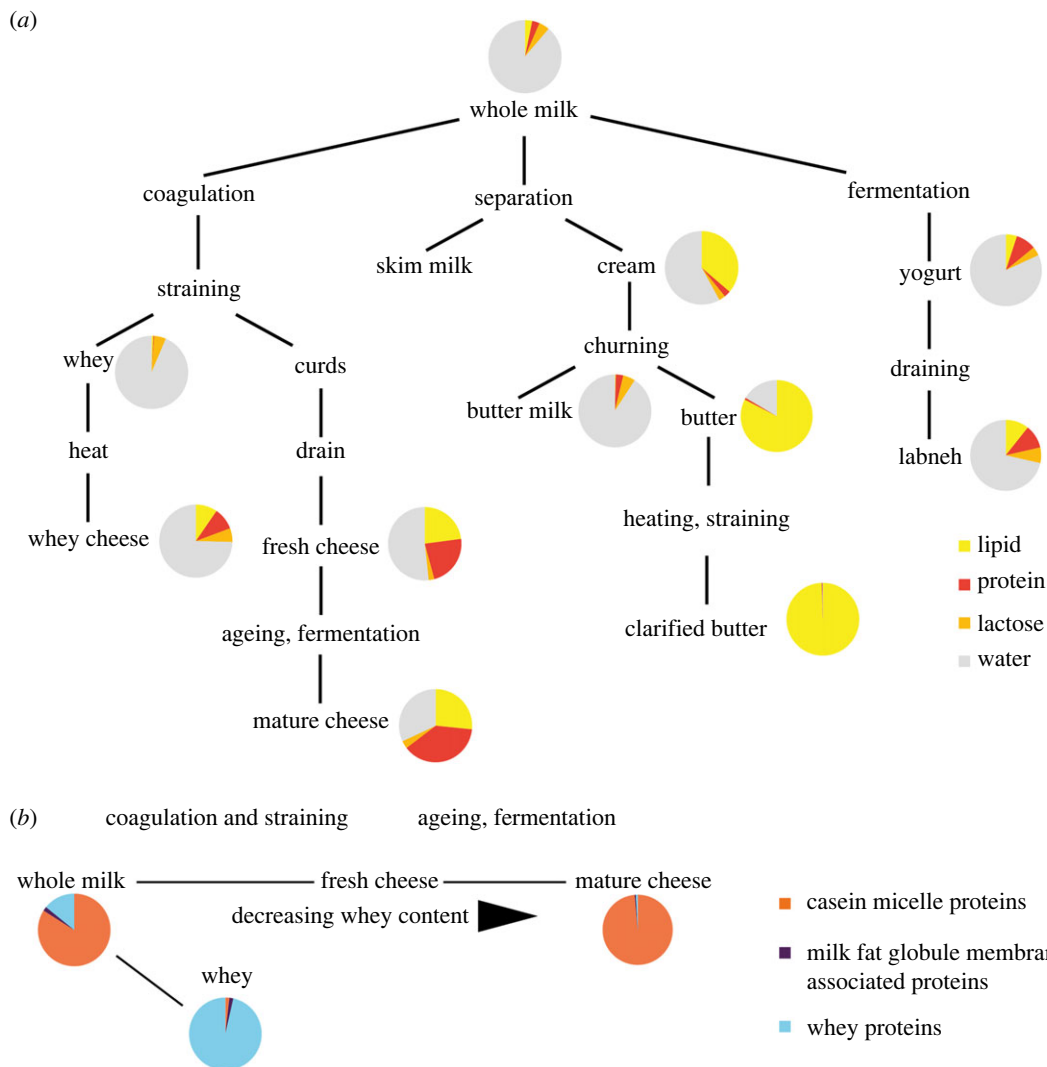
Figure 1. (a) Simplified model of a range of dairy processing methods showing the change in the proportion of lipid, protein, lactose and water content (after figure 39 in [39] using nutritional data (electronic supplementary material, table S1)). (b) Simplified model of change in the proportion of different dairy protein fractions (casein micelle proteins, milk fat globule membrane-associated proteins and whey proteins) during the process of cheese making (data from [40]).

undertake proteomic analysis of four TRB vessels from the site of Sławęcinek in central Poland [44] and four modern replica analogues of cheese bowls and strainers, where curdled milk products have been created and strained, respectively. In the vessels from Sławęcinek, we also compare the palaeoproteomic results with the analysis of extracted lipids.

2. Methods

The site of Sławęcinek is located near Inowrocław in central Poland (figure 2) and was discovered during rescue excavations undertaken in 2016. Late Neolithic strata (*ca* 3650–3100 cal BCE) were uncovered, revealing a TRB settlement with four houses, water wells, burials and evidence for both domestic and ritual events at the site [44]. Accelerator mass spectrometry (AMS) radiocarbon dating was undertaken on bone material from the pits, returning the following ages: (i) SLA_3 and 4: 4680 ± 35 BP; 3517–3375 cal BCE at 68.2%; (ii) SLA_8: 4700 ± 30 BP; 3522–3378 cal BCE at 68.2%; and (iii) SLA_10: 4660 ± 40 BP; 3511–3371 cal BCE at 68.2%; all dates presented in this paper were modelled in OxCal v. 4.3.2, using the IntCal20 calibration curve [45,46].

Among the many potsherds unearthed from the site, a small proportion of fragments of collared flasks contained white mineralized deposits adhering to their interior walls (0.2% of the total assemblage).
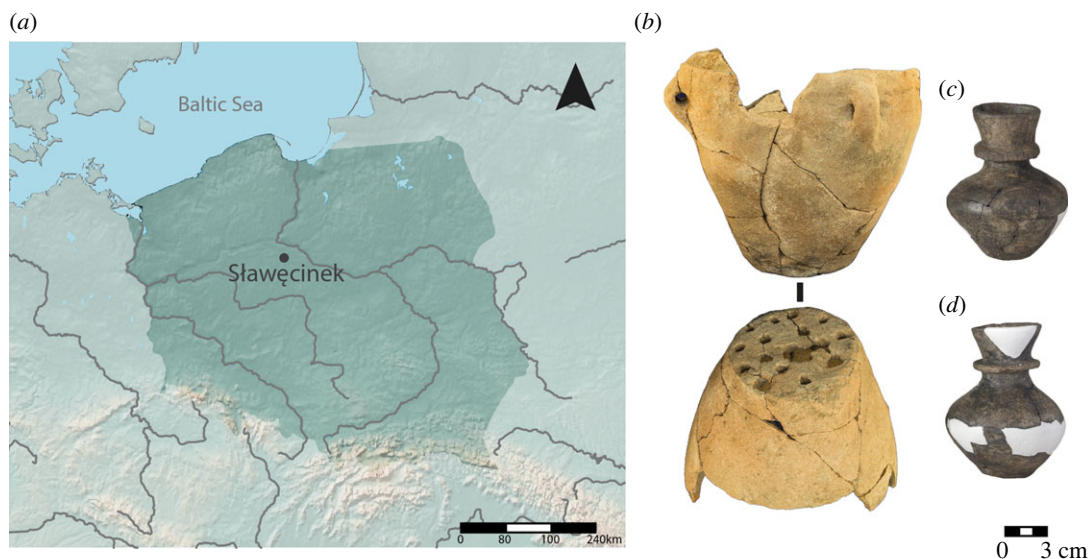
Figure 2. (*a*) Map showing the location of Sławęcinek. (*b*) Ceramic strainer SLA_10 (adapted from [47]). (*c*) Collared flask SLA_1. (*d*) Collared flask SLA_2.

Scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDX) analysis confirmed that the residues are composed of calcium and oxygen (full methodological details on SEM-EDX analysis can be found in the electronic supplementary material, file S1).

Proteomic analysis on the residues adhering to the vessel walls was undertaken on three collared flasks, SLA_3, SLA_4 and SLA_8, and a ceramic strainer, SLA_10 (figure 2*b*; electronic supplementary material, figure S1). Then, lipid residue analysis was undertaken on the same vessels on the ceramic itself, supplemented by a further four collared flasks and two flasks from the assemblage (electronic supplementary material, table S2).

For comparison, proteins were also analysed from residues adhering to four modern vessels where milk had either been warmed and coagulated, or coagulated and strained. The modern comparative assemblage comprises residues from four modern vessels, including two strainers and two bowls (electronic supplementary material, table S2 and figure S2), which were used to make cows' milk cheese using both microbial rennet and acid precipitation for a public outreach archaeology project [48]. In the bowls, milk was warmed to approximately 37°C and coagulated via microbial rennet and acid precipitation, while the strainers were used to strain the curds from the whey, with the coagulation taking place in a separate vessel. Repeated use of these modern replica vessels left visible white residues on their interior surfaces (electronic supplementary material, figure S2), which were visually similar to those on the archaeological samples (electronic supplementary material, figure S1).

2.1. Protein extraction and identification

Proteins were extracted separately from the archaeological and modern replica vessels and analysed by LC-MS/MS. Four archaeological vessels were sampled for proteomic analysis, including three collared flasks (SLA_3, SLA_4 and SLA_8) and a strainer (SLA_10). The extraction of all ancient and modern samples was undertaken using a version of the filter-aided sample preparation protocol modified for ancient samples [18] followed by desalting using C18 100 µl pipette tips. Ancient samples were analysed in a laboratory dedicated to ancient samples. Full details of the methodology used for protein extraction, identification and data analysis can be found in the electronic supplementary material, file S1. All samples were analysed by LC-MS/MS at the Centre of Excellence in Mass Spectrometry at the University of York on an Orbitrap Fusion.

To interrogate the data, two data analysis approaches were performed. First, data from the ancient samples were searched using Mascot against all of Swiss-Prot (release: 2020_02) to assess a wide range of potential dietary and non-dietary proteins present (electronic supplementary material, table S3). Then, all ancient and modern samples were searched using MaxQuant v. 1.6.17.0 against a database comprising all annotated dairy protein sequences from any taxa (electronic supplementary material, file S2) and a database of common contaminants (electronic supplementary material, file S3), given the fact that the only putative dietary proteins identified were milk proteins. In MaxQuant,

peptides were searched allowing for semi-tryptic cleavage, minimum length of seven, with both a protein and peptide target false discovery rate of 1%. This MaxQuant data forms the basis of the dairy interpretations presented in the study (electronic supplementary material, tables S4 and S5).

Peptides from putative dietary sources were then BLAST searched (NCBI protein–protein BLAST) to establish the lowest common taxonomic ancestor of each identified peptide. Many of the dairy peptides matched both to a milk protein reference and a *Jeotgalicoccus* sequence (a facultatively anaerobic, and halotolerant group, first reported in fermented seafood). Wilkin *et al.* [49] recently noted that the *Jeotgalicoccus* sequence WP_188349304.1 is likely to have high levels of milk contamination from laboratory analysis; as such, *Jeotgalicoccus* was not considered a valid result in taxonomic attribution. While deamidation patterns have been used to investigate the preservation quality of ancient dietary proteins [50], recently the high variability of deamidation patterns in dairy proteins has been demonstrated, rendering their application to samples with low peptide recovery challenging [51]. As such, although deamidation of asparagine and glutamine were detected in this data analysis, this line of enquiry was not used.

2.2. Lipid extraction and identification

Absorbed lipids were extracted using an acidified methanol extraction (AE) and characterized using gas chromatography-mass spectrometry (GC-MS) techniques. Full details of the methodology used for lipid extraction, identification and data analysis can be found in the electronic supplementary material, file S1. First, an AE was applied [52,53]. To further understand the origin of these lipids, the extracts were analysed by gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS) to determine the stable carbon isotope values of the major fatty acids ($C_{16:0}$ and $C_{18:0}$). This approach has shown to be useful for distinguishing ruminant adipose (i.e. carcass fats), ruminant dairy fats, non-ruminant adipose, as well as marine and freshwater resources based on comparison of the δ^{13}C values with modern reference values [53–56]. The equipment and instrument parameters are as previously described [57] and are outlined in the electronic supplementary material, file S1.

3. Results and discussion

## 3.1. Vessel contents at Sławęcinek

Proteins were recovered from all four TRB vessels, with the majority of protein groups matched to common laboratory and handling contaminants, such as human keratin and other skin-associated proteins which have been previously observed in proteomic analyses of ceramic residues [33,34] (electronic supplementary material, table S3). Proteins detected in extraction blanks used to monitor laboratory contamination included only keratin proteins, trypsin and streptavidin, a protein commonly used in molecular biology laboratories. A positive control of porcine gelatine yielded a high abundance of collagen peptides, indicating the efficacy of the extraction protocol.

Putative dietary proteins identified in the assemblage were exclusively dairy (figure 3*a*). No meat, plant or yeast proteins were identified.

The proteomic results reveal dairy taxonomy specific to the subfamily level, with evidence of both Caprinae (probably sheep and/or goat) and Bovinae (probably cow) milk. Collared flask SLA_3 bears evidence for Bovinae. However, samples SLA_4, SLA_8 (collared flasks) and SLA_10 (ceramic strainer) all bear evidence specific to the Bovinae subfamily as well as separate evidence for Caprinae (electronic supplementary material, figures S3–S6). This demonstrates that, at least at Sławęcinek, a mixed dairy species economy was being practised during the TRB.

This taxonomic evidence generated by proteomic analysis is congruent with the faunal assemblage recovered from the site, revealing a diversified dairy strategy which used both Bovinae and Caprinae. The number of identified specimens (NISP) and minimum number of individuals (MNI) established for the site strongly support this interpretation. Cattle (*Bos taurus*; NISP = 1404/64.4%, MNI = 33/45.2%), followed by sheep/goat (*Ovis aries/Capra hircus*; NISP = 302/14.1%, MNI = 12/16.4%) and pig (*Sus scrofa f. domestica*; NISP = 283/13.2%, MNI = 19/26%) predominated the assemblage [44].

Further support for milk production at Sławęcinek comes from mandibular mortality profiles of 17 cattle, which shift towards older animals, showing that four of them were immature (*ca* six months of age), five individuals were subadults (*ca* 24 months), and the remaining eight mandibles belonged to adult individuals (greater than 2.5 years). A mixed dairy species strategy at the site is further evidenced by the age-at-death
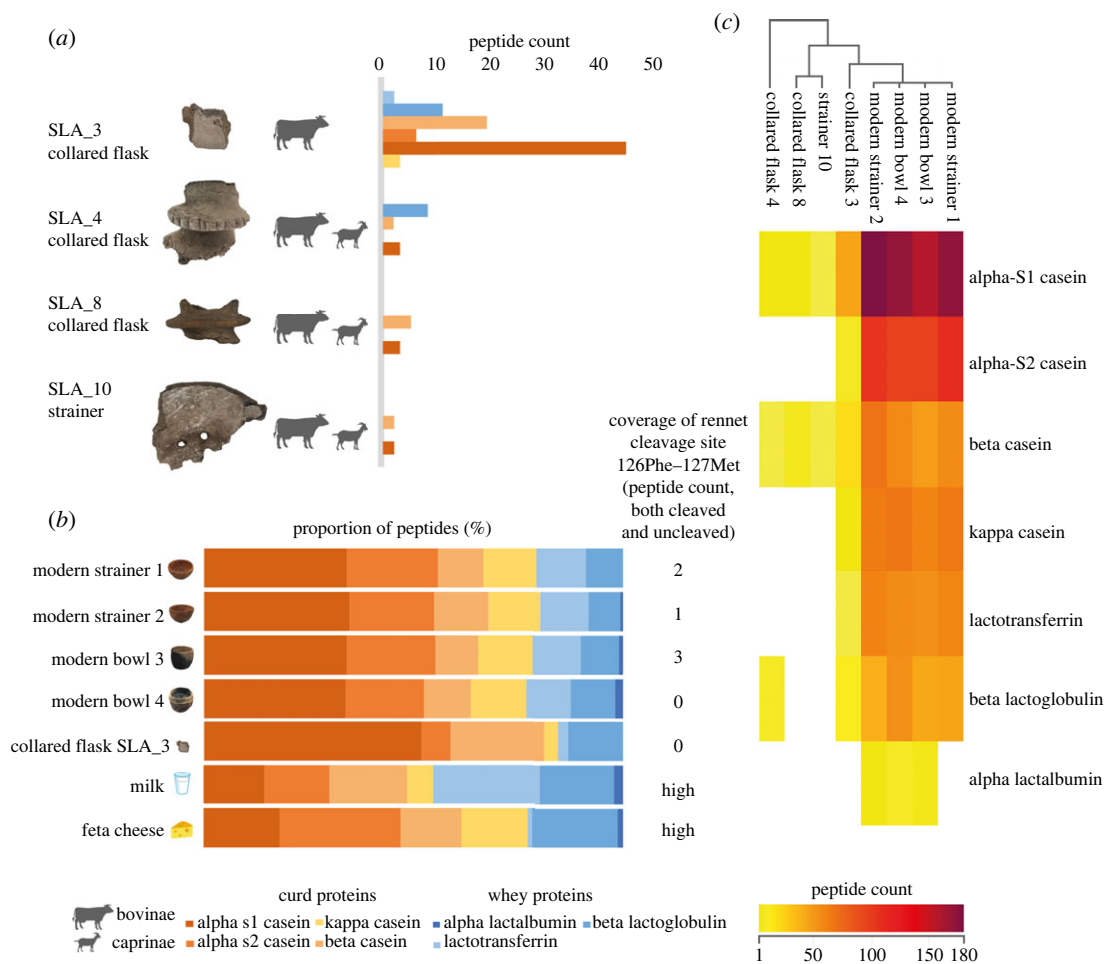
Figure 3. (a) Dairy proteins detected in the four TRB vessels from Sławęcinek, indicating the presence of a range of Bovinae and Caprinae milk proteins. (b) Proportion of peptides by protein type in the modern cheese-making strainers and pots, ancient sample SLA_3, and modern milk and feta cheese (data from [41]). (c) Cluster analysis of the proportion of peptides by protein type present in the modern cheese-making vessels and ancient samples from Sławęcinek (Pearson correlation, Perseus v. 1.6.14.0 [58]), noting the similarity between the two modern strainers and two bowls analysed in this study.

rates for ovicaprinae, with most individuals culled when older; contrary to the exploitation pattern observed for pigs, which points to a focus on a meat-oriented strategy. Overall, the domestic cattle and ovicaprinae management at Sławęcinek signals the intensification in milk production, and it is interesting to note that the nearby TRB site of Kopydłowo produced similar evidence for the incorporation of dairy foods reduced in lactose into the subsistence economy of the region in the mid-fourth millennium BCE [25].

3.2. Indicators of dairy processing

A range of dairy proteins was identified in the collared flasks and ceramic strainer analysed in this study, including peptides matching to alpha-S1 and alpha-S2 casein, beta-casein, kappa-casein, BLG and lactotransferrin. Caseins were the most frequently identified class of protein, in particular beta-casein and alpha-S1 casein, both by peptide count and peptide intensities from label-free quantification. Compared with ceramic residues published in Hendy *et al.* [33], the ratio of BLG to casein peptide identification was low.

The dominance of caseins in this assemblage could suggest that the residues formed on these vessels are the result of the presence of casein-rich curd products, rather than milk or whey products. Cheese is composed primarily of curd proteins while the whey proteins and the majority of the lactose remain in the whey portion when the curds coagulate (figure 1). Identification of dietary proteins was especially abundant in collared flask sample SLA_3 compared with the other ancient samples, and here the relative proportion of abundant curd proteins present in sample SLA_3 resembles that seen in experimental soft-

curd cheese-making vessels (figure 3b,c). We note the contrast in the ancient milk proteome detected in this study compared with other studies identifying milk proteins in ceramic residues [33] and ancient dental calculus [13,38,42], which are dominated by the presence of BLG, a whey protein. We also note that only four vessels were examined, and there is variation in the number of milk peptides detected between vessels. This variability in protein identification has been noted elsewhere [13] and may be due to differences in preservation, pot use, frequency of use or depositional environment.

To further explore whether the proportion of caseins and whey proteins might reflect dairy processing in the vessels, we analysed a set of replica modern vessels used in cheese making. Analysis of the extracted proteins demonstrated that the profiles were not distinctive between the modern bowls and strainers, with the proportion of curd (casein) peptides to whey peptides being similar in the strainers and bowls (figure 3b). In contrast with our hypothesis, this indicates that, at least in the modern vessels examined here, there is no discernible difference in the proteins detected (using both peptide count and label-free quantification as metrics for abundance) in vessels used for straining curds versus those where milk has been warmed and coagulated. This could be owing to the mechanisms of protein entrapment in these residues, with further controlled work needed to explore the preservation of milk proteins from a diversity of dairy products in ceramics and ceramic residues. There was, however, a notable difference between the dairy peptide proportion identified in the experimental pottery compared with that of raw milk (data from [41]). The heightened curd protein content of all experimental cheese-making vessels closely resembles that of cheese, differing markedly from the lower curd peptide proportion observed in raw milk (figure 3b,c), indicating that the identification of particular proteins and peptides in visible residues may be associated with their preservation in milk scale, rather than reflecting the method of processing. Moreover, sample SLA_3, which bears the most abundant peptide evidence of the archaeological samples, similarly resembles the high proportion of curd peptides to whey peptides observed in the modern cheese-making vessels, and in fresh cheese.

Rennet (a set of enzymes derived from ruminant stomachs, primarily the protein chymosin) is commonly used in cheese making to coagulate raw milk into curds. Chymosin causes the cleavage of kappa-casein at [126]Phe–[127]Met, and therefore, the presence of this cleavage may indicate the use of this curdling technique. The presence of the intact peptide spanning this cleavage site was used to infer that cheese curds preserved from Bronze Age Xiaohe were not generated by ruminant rennet [41]. In the Neolithic samples from Sławęcinek, the relevant portion of the kappa-casein sequence was not present, so here we cannot use this marker to indicate the use of rennet.

However, to examine the efficacy of this approach to detect rennet use in vessel residues, we also examined kappa-casein in the modern cheese-making vessels. We note the very low number of instances of peptides covering the region [126]Phe–[127]Met, even in the modern cheese-making residues (figure 3b,c), which together with their absence in the archaeological samples of this study suggests that it is unlikely that rennet detection will be possible in poorly preserved ancient pottery residue samples which have experienced further taphonomic degradation.

3.3. Lipid residue analysis

To further enrich our understanding of vessel use, we performed lipid residue analysis on the ceramic powder obtained from the same vessels used to identify proteins and six additional vessels that did not have adhering limescale deposits. All samples yielded interpretable quantities of lipids ranging from 31 to 3057 µg g^{-1} (electronic supplementary material, table S6). Through analysis by GC-MS, the majority of samples yielded profiles typical of degraded animal fats, characterized by an approximately equal dominance of palmitic ($C_{16:0}$) and stearic acids ($C_{18:0}$) in addition to relatively low concentrations of unsaturated fatty acids ($C_{18:1}$). Furthermore, the presence of odd-chain branched fatty acids ($C_{15:0}$ and $C_{17:0}$) in all samples analysed is consistent with the presence of ruminant fats (adipose or dairy) [59]. In addition to degraded animal fats, diterpenoids (e.g. abietic acid) and their oxidation by-products were identified in SLA_10, indicating the presence of *Pinaceae* resin, potentially used as an adhesive or as a waterproofing agent for the vessel [60–62]. A distribution of long odd *n*-chain alkanes with a dominance of *n*-alkane C_{27} and C_{29} (δ^{13}C −30.0‰ and −34.3‰, respectively) was also identified in SLA_10, indicative of the presence of leafy plants mixed with animal fats and pine products (electronic supplementary material, table S6) [63].

The lipid profiles of samples SLA_1 and SLA_7 are instead indicative of plant oil based on a relatively high oleic to stearic acid ratio ($C_{18:1}/C_{18:0}$ greater than 2) and palmitic to stearic acid ratio $C_{16:0}/$
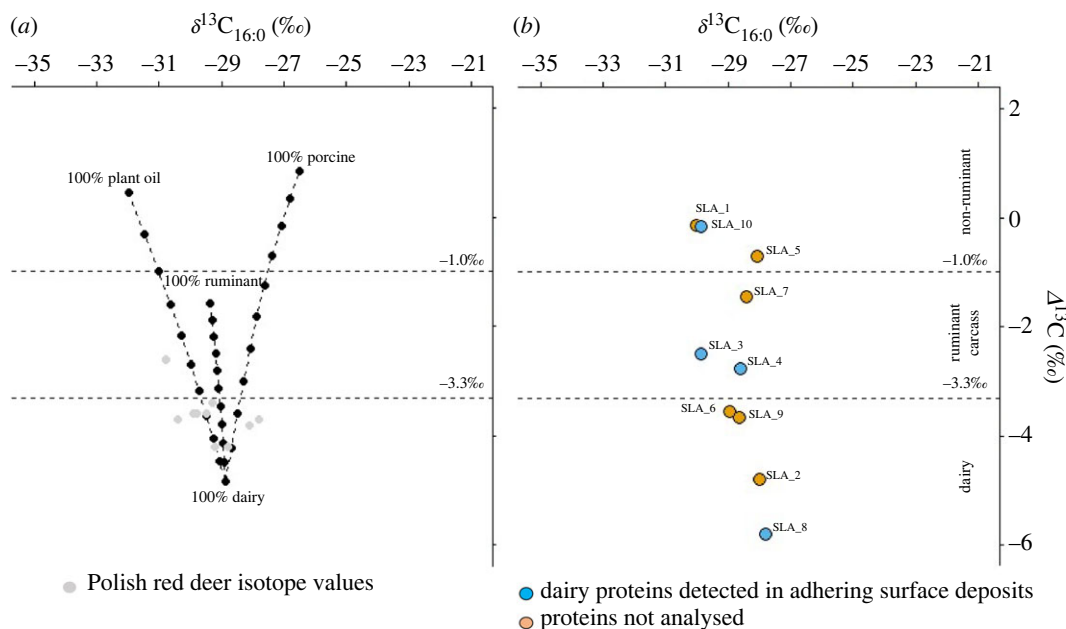
Figure 4. (a) Theoretical mixing curves of $\delta^{13}C_{16:0}$ and $\Delta^{13}C$ values resulting from mixing modern dairy with modern plant oils, porcine fat and ruminant carcass fat, where filled circles are representative of percentage contributions in 10% increments between the mean values. The $\delta^{13}C$ values of modern products used in the mixing model are previously published and reported in [8] or electronic supplementary material, file S4. Grey circles show the $\delta^{13}C_{16:0}$ and $\Delta^{13}C$ values of modern red deer from Poland [55]. (b) Difference in the $\delta^{13}C$ isotope values ($\Delta^{13}C$) of the individual mid-chain length fatty acids ($C_{16:0}$ and $C_{18:0}$) obtained from all samples analysed from Sławęcinek. Blue dots indicate samples where dairy proteins were identified in the calcite surface deposits. $\Delta^{13}C$ values less than −3.3‰ are typically associated with ruminant fats, between −3.3‰ and −1.0‰ are associated with ruminant carcass fats, and above −1.0‰ are associated with non-ruminant fats based on modern reference values [55].

$C_{18:0}$ greater than 3. However, rapid degradation of plant oils in the burial environment and through the use of the pot can make it difficult to determine their origin [64].

To further understand the origin of the extracted lipids, carbon stable isotope ($\delta^{13}C$) values of palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acid were measured and compared with modern reference values (figure 4b). Fatty acid stable isotope values from different tissues reflect differences in their biosynthesis. This approach has been particularly useful for identifying ruminant dairy, carcass and non-ruminant lipids in archaeological pottery [65]. Here, two samples (SLA_2 and SLA_8) had $\Delta^{13}C$ values (defined as $\delta^{13}C_{18:0} - \delta^{13}C_{16:0}$) of less than −4.3‰ and can be said to have been used unequivocally to process/store ruminant dairy products (figure 4b). Lipids from a further two samples (SLA_6 and SLA_9) meet the criteria used by Copley et al. [65] for dairy products (i.e. less than −3.3‰) but also are within the range of carcass fats from wild ruminants such as red deer [55], which were present in the faunal assemblage at Sławęcinek. Three samples (SLA_3, SLA_4 and SLA_7) have $\Delta^{13}C$ values outside the dairy range and within the range of modern ruminant adipose fats, i.e. $\Delta^{13}C$ values greater than −3.3‰ and less than −1.0‰, and the remaining three samples (SLA_1, SLA_5 and SLA_10) are within the range of modern non-ruminant fats (e.g. porcine), i.e. $\Delta^{13}C$ values greater than −1.0‰. Mixing of fatty acids derived from different products further complicates the interpretation and is illustrated in figure 4a.

3.4. Comparison between proteins and lipids

This study provided a valuable opportunity to compare lipid and protein approaches for identifying vessel contents, albeit only four vessels were available for comparison using both methods. We note that although four vessels had adhering surface deposits containing dairy proteins, only one (SLA_8) had a lipid $\Delta^{13}C$ value less than −4.3‰ with conclusive evidence of dairy products [55]. SLA_3 and SLA_4 had $\Delta^{13}C$ values of −2.5‰ and −2.8‰, respectively, indicating ruminant carcass fats, yet both of these vessels had deposits adhered to their surfaces containing dairy proteins, which were particularly abundant in the case of SLA_3. Contrasting with the protein results, the $\Delta^{13}C$ value of the ceramic strainer (SLA_10, approx. −0.2‰) is not congruent with ruminant dairy fats and is instead within the range of non-ruminant animal fats or plant oils, with additional lipids suggestive of plant

resin and oil. Despite this, we note that the number of dairy peptides in this sample is relatively low compared with the other vessels, and that other potential dietary proteins were not detected.

Interestingly, the results from both the lipid and protein analyses from the vessels do not necessarily align, for example vessel SLA_10 yielded markedly different interpretations from the two approaches. From the lipid results alone, three of the four pots would not have been interpreted as containing dairy, yet milk proteins were detected. A likely explanation for the discrepancy is that the lipids absorbed in the pottery are derived from a mixture of different sources, as has been suggested previously [33,66]. To illustrate, mixing lines between the mean isotope values for dairy and other products are shown in figure 4*a* with 10% increments. From this it is clear that Δ^{13}C values outside the dairy range are achievable when substantial amounts of dairy fats are mixed with other fats and oils.

Other explanations for the discrepancy in these two approaches could be that non-dairy proteins may have indeed been part of the vessel's use-life, but that entrapment, decay and extraction have impacted the observed protein extractome. Moreover, in this study, protein analysis was performed on the adhering residue of the vessel, while the lipid extractions were performed on the ceramic itself and therefore these differing results could also be indicative of different periods of the vessel's use-life (as has been shown in foodcrusts [67]). For example, the presence of *Pinaceae* resin in the ceramic of SLA_10 could indicate the use of a sealant, while the presence of milk protein in the adhering residue reflects the vessel's contents.

Nevertheless, the discrepancy between protein- and lipid-based approaches has implications for the interpretation of ancient vessel use which extends beyond this study. For example, using Δ^{13}C values less than or equal to −3.1‰ as a proxy for milk fats, Evershed *et al.* [9] built up a detailed picture of dairying across prehistoric Europe through the analysis of over 6500 δ^{13}C values. Although this proxy is undoubtedly heavily influenced by the presence of dairy fats, the actual frequencies obtained through time and space are almost certainly an underestimation of the actual frequency of milk use in prehistoric pottery as, unlike the protein evidence presented here, Δ^{13}C values are strongly influenced by mixing of different food products (figure 4*a*). Significantly, in regions where the majority of pots have Δ^{13}C values greater than −3.1‰, such as Northern Greece, seemingly an outlier in the context of Neolithic Europe, the data might reflect differences in culinary practice, such as a greater propensity for mixing of food products in pottery, rather than fundamental differences in the economy. This does not diminish the utility of Δ^{13}C values for inferring dairying, although in some cases, as here, wild ruminant carcass fats must be considered as an alternative source [55], and it is not disputed here that this practice was widespread across Neolithic Europe, only that it is difficult to measure dairying intensity without considering the issue of mixing [8].

Both lipid- and protein-based approaches have methodological strengths. One methodological advantage of palaeoproteomics is that it is possible to identify a degree of taxonomic specificity, owing to key single amino acid polymorphism in the milk proteins of different taxa. Here, as in other studies [5], this characteristic facilitates the identification of dairy from multiple different species. Second, a potential strength of palaeoproteomics is its ability to detect multiple different proteins present in dairy, a property explored in this study to investigate the possibility that an abundance of curd proteins relative to whey proteins may indicate the production of curd-rich dairy products such as cheese. However, other studies have noted the bias towards the detection of dairy over other food-derived proteins in many instances [42], and we remain ignorant of the taphonomic impacts on food proteins in archaeological samples. Moreover, substantial challenges persist in the identification of proteins from ceramics (e.g. [68]), hence the need to focus on mineralized deposits and the limited number of samples used in this study, while the high survivability and extractability of absorbed lipids in the archaeological record has enabled the creation of a vast temporal and geographical corpus of lipid results. This study highlights the advantage of comparing protein results with those gained from lipid residue analysis and should provide a useful example to future studies. Further analyses using a larger dataset would be of benefit to understand both the method and the archaeological context. Moreover, additional experimental studies using larger sample sizes of modern vessels with known protein inputs will be instrumental in uncovering the biases associated with food protein entrapment, extraction and identification.

3.5. The significance of dairying multiple species

Previously, it has been proposed that the earliest dairy processing in the Near East was linked to cattle herding, based on correspondence between faunal and lipid data [28]. Here we show that this does not seem to be true for the Northern European Neolithic, nor previously at Çatalhöyük on the Anatolian plain [33], where, in both cases, dairy products from multiple taxa were processed, sometimes in the

same vessel. Proteomic analysis of dental calculus also supports the exploitation of multiple taxa for dairy during the Neolithic, rather than a single species. Interestingly, evidence from Britain [13] and East Africa [38] indicates that this practice occurred as soon as domesticated animals were introduced. In Funnel Beaker contexts, the data are less forthcoming, however, Gron et al. [26] suggest that cattle were exploited for both meat and dairy through a complex multi-season birthing system, from the onset of the Neolithic, a practice probably brought about by incoming farmers into the region. Others too have also highlighted the close relationship between the Funnel Beaker culture, subsequent Globular Amphora culture and cattle [11], but this does not necessarily preclude dairying of other species. Cattle might have been revered more for their value 'on the hoof' as live animals rather than the products they produce, which were seemingly treated in the same way as those from other species. Indeed, some of the earliest evidence for wheeled transport in Europe derives from the TRB [69,70], including the depiction of a wagon on a TRB vessel from the Polish site of Bronocice [71]. Overall, we suggest that dairying at least initially encompassed a broader range of domesticated ruminants than previously thought, but further work is required to see if more specialized dairy economies developed later in prehistory.

4. Conclusion

In this study, evidence of dairy processing was identified at the TRB site of Sławęcinek in modern-day Poland, demonstrating the presence of Caprinae and Bovinae dairy products and therefore utilization of both of these taxonomies for their dairy products. The milk proteome identified in this study provided an opportunity to explore patterns of milk processing, beyond simply detecting the presence of dairy foods, and we note the dominance of caseins involved in curd-formation. This evidence suggests that TRB individuals may have been consuming dairy foods reduced in lactose, which has implications for our understanding of LP selection during this period. This study also yielded a valuable opportunity to compare protein and lipid-based approaches in organic residue analysis, where we significantly note that using $\Delta^{13}C$ values less than or equal to $-3.1‰$ as a proxy for dairy fat may be underestimating the frequency of milk use in prehistoric pottery. We note that further experimental research is vital to identify key methodological biases between these two approaches in organic residue analysis.

bowls, Helen Talbot (University of York) for the maintenance of GC-MS instruments at BioArCh and Manon Bondetti (University of York) for supplying the modern reference values of plants.

References

1. Twiss KC. 2003 Archaeology of food and identity. *Anthropol. News* **44**, 17. (doi:10.1111/an.2003.44.8.17)

2. Smith ML. 2006 The archaeology of food preference. *Am. Anthropol.* **108**, 480–493. (doi:10.1525/aa.2006.108.3.480)

3. Hastorf CA. 2016 *The social archaeology of food: thinking about eating from prehistory to the present*. Cambridge, UK: Cambridge University Press.

4. McClure SB. 2015 The pastoral effect: niche construction, domestic animals, and the spread of farming in Europe. *Curr. Anthropol.* **56**, 901–910. (doi:10.1086/684102)

5. Wilkin S *et al.* 2020 Dairy pastoralism sustained eastern Eurasian steppe populations for 5,000 years. *Nat. Ecol. Evol.* **4**, 346–355. (doi:10.1038/s41559-020-1120-y)

6. Salque M, Bogucki PI, Pyzel J, Sobkowiak-Tabaka I, Grygiel R, Szmyt M, Evershed RP. 2013 Earliest evidence for cheese making in the sixth millennium BC in northern Europe. *Nature* **493**, 522–525. (doi:10.1038/nature11698)

7. Craig OE *et al.* 2011 Ancient lipids reveal continuity in culinary practices across the transition to agriculture in Northern Europe. *Proc. Natl Acad. Sci. USA* **108**, 17 910–17 915. (doi:10.1073/pnas.1107202108)

8. Cubas M *et al.* 2020 Latitudinal gradient in dairy production with the introduction of farming in Atlantic Europe. *Nat. Commun.* **11**, 1–9. (doi:10.1038/s41467-020-15907-4)

9. Evershed RP *et al.* 2022 Dairying, diseases and the evolution of lactase persistence in Europe. *Nature* **608**, 336–345. (doi:10.1038/s41586-022-05010-7)

10. Robson HK, Saul H, Steele VJ, Meadows J, Nielsen PO, Fischer A, Heron CP, Craig OE. 2021 Organic residue analysis of Early Neolithic 'bog pots' from Denmark demonstrates the processing of wild and domestic foodstuffs. *J. Archaeol. Sci. Rep.* **36**, 102829. (doi:10.1016/j.jasrep.2021.102829)

11. Weber J, Brozio JP, Müller J, Schwark L. 2020 Grave gifts manifest the ritual status of cattle in Neolithic societies of Northern Germany. *J. Archaeol. Sci.* **117**, 105122. (doi:10.1016/j.jas.2020.105122)

12. Greenfield HJ, Arnold ER. 2015 'Go(a)t milk?' New perspectives on the zooarchaeological evidence for the earliest intensification of dairying in south eastern Europe. *World Archaeol.* **47**, 792–818. (doi:10.1080/00438243.2015.1029076)

13. Charlton S, Ramsøe A, Collins M, Craig OE, Fischer R, Alexander M, Speller CF. 2019 New insights into Neolithic milk consumption through proteomic analysis of dental calculus. *Archaeol. Anthropol. Sci.* **11**, 6183–6196. (doi:10.1007/s12520-019-00911-7)

14. Jeong C *et al.* 2018 Bronze Age population dynamics and the rise of dairy pastoralism on the eastern Eurasian steppe. *Proc. Natl Acad. Sci. USA* **115**, E11 248–E11 255. (doi:10.1073/pnas.1813608115)

15. Craig OE, Chapman J, Heron C, Willis LH, Bartosiewicz L, Taylor G, Whittle A, Collins M. 2005 Did the first farmers of central and eastern Europe produce dairy foods? *Antiquity* **79**, 882–894. (doi:10.1017/S0003598X00115017)

16. Craig OE, Mulville J, Pearson MP, Sokol R, Gelsthorpe K, Stacey R, Collins M. 2000 Detecting milk proteins in ancient pots. *Nature* **408**, 312. (doi:10.1038/35042684)

17. Greenfield HJ. 2010 The Secondary Products Revolution: the past, the present and the future. *World Archaeol.* **42**, 29–54. (doi:10.1080/00438240903429722)

18. Midgley M. 1992 *TRB culture: the first farmers of the North European plain*. Edinburgh, UK: Edinburgh University Press.

19. Nowak M. 2009 *Drugi etap neolityzacji ziem polskich*. Kraków, Poland: Księgarnia Akademicka.

20. Sherratt A. 1981 Plough and pastoralism: aspects of the Secondary Products Revolution. In *Pattern of the past: studies in the honour of David Clarke* (eds I Hodder, G Isaac, N Hammond), pp. 261–305. Cambridge, UK: Cambridge University Press.

21. Sherratt A. 1983 The secondary exploitation of animals in the Old World. *World Archaeol.* **15**, 90–104. (doi:10.1080/00438243.1983.9979887)

22. Marciniak A. 2004 Mikrospołeczny wymiar pasterstwa i nomadyzmu i tafonomiczne podstawy ich identyfikacji w materiałach faunistycznych. In *Nomadyzm a pastoralizm w międzyrzeczu Wisły i Dniepru (neolit, eneolit, epoka brązu)* (eds A Kośko, M Szmyt), pp. 35–43. Poznań, Poland: Wydawnictwo Poznańskie.

23. Przybył A. 2017 From South to North: Baden culture people and their neighbours. In *The past societies: Polish lands from the first evidence of human presence to the Early Middle Ages, vol. 2: 5500–2000 BC* (ed. P Włodarczak), pp. 171–210. Warszawa, Poland: Institute of Archaeology and Ethnology of the Polish Academy of Sciences.

24. Lasota-Moskalewska A. 2005 *Zwierzęta udomowione w dziejach ludzkości*. Warszawa, Poland: Wydawnictwa Uniwersytetu Warszawskiego.

25. Roffet-Salque M, Evershed R. 2015 Shifting pottery use and animal management at Kopydłowo (Poland) traced through lipid residue analyses of pottery vessels. In *Kopydłowo, stanowisko 6. Osady neolityczne z pogranicza Kujaw i Wielkopolski*, pp. 133–142. Poznań, Poland: Uniwersytet im.

Adama MIckiewicza, Wydawnictwo Profil-Archeo.

26. Gron KJ, Montgomery J, Rowley-Conwy P. 2015 Cattle management for dairying in Scandinavia's Earliest Neolithic. *PLoS ONE* **10**, e0131267. (doi:10.1371/journal.pone.0131267)

27. Gron KJ, Montgomery J, Nielsen PO, Nowell GM, Peterkin JL, Sørensen L, Rowley-Conwy P. 2016 Strontium isotope evidence of early Funnel Beaker Culture movement of cattle. *J. Archaeol. Sci. Rep.* **6**, 248–251. (doi:10.1016/j.jasrep.2016.02.015)

28. Evershed RP *et al.* 2008 Earliest date for milk use in the Near East and southeastern Europe linked to cattle herding. *Nature* **455**, 528–531. (doi:10.1038/nature07180)

29. Debono Spiteri C *et al.* 2016 Regional asynchronicity in dairy production and processing in early farming communities of the northern Mediterranean. *Proc. Natl Acad. Sci. USA* **113**, 13 594–13 599. (doi:10.1073/pnas.1607810113)

30. Dunne J, Evershed RP, Salque M, Cramp L, Bruni S, Ryan K, Biagetti S, di Lernia S. 2012 First dairying in green Saharan Africa in the fifth millennium BC. *Nature* **486**, 390–394. (doi:10.1038/nature11186)

31. McClure SB, Magill C, Podrug E, Moore AMT, Harper TK, Culleton BJ, Kennett DJ, Freeman KH. 2018 Fatty acid specific δ13C values reveal earliest Mediterranean cheese production 7,200 years ago. *PLoS ONE* **13**, e0202807. (doi:10.1371/journal.pone.0202807)

32. Roffet-Salque M, Lee MR, Timpson A, Evershed RP. 2017 Impact of modern cattle feeding practices on milk fatty acid stable carbon isotope compositions emphasise the need for caution in selecting reference animal tissues and products for archaeological investigations. *Archaeol. Anthropol. Sci.* **9**, 1343–1348. (doi:10.1007/s12520-016-0357-5)

33. Hendy J *et al.* 2018 Ancient proteins from ceramic vessels at Çatalhöyük West reveal the hidden cuisine of early farmers. *Nat. Commun.* **9**, 1–10. (doi:10.1038/s41467-018-06335-6)

34. Tanasi D, Cucina A, Cunsolo V, Saletti R, Di Francesco A, Greco E, Foti S. 2021 Paleoproteomic profiling of organic residues on prehistoric pottery from Malta. *Amino Acids* **53**, 295–312. (doi:10.1007/s00726-021-02946-4)

35. Siano F, Picariello G, Caruso T, Esposito S, Rescigno C, Addeo F, Vasca E. 2022 Proteomics and integrated techniques to characterize organic residues in funerary findings from Italic populations of the First Millennium BC. *J. Proteome Res.* **21**, 1330–1339. (doi:10.1021/acs.jproteome.2c00093)

36. Warinner C *et al.* 2014 Direct evidence of milk consumption from ancient human dental

calculus. *Sci. Rep.* **4**, 7104. (doi:10.1038/srep07104)

37. Geber J, Tromp M, Scott A, Bouwman A, Nanni P, Grossmann J, Hendy J, Warinner C. 2019 Relief food subsistence revealed by microparticle and proteomic analyses of dental calculus from victims of the Great Irish Famine. *Proc. Natl Acad. Sci. USA* **116**, 19 380–19 385. (doi:10.1073/pnas.1908839116)

38. Bleasdale M *et al.* 2021 Ancient proteins provide evidence of dairy consumption in eastern Africa. *Nat. Commun.* **12**, 1–11. (doi:10.1038/s41467-020-20682-3)

39. Cappers R. 2018 *Digital atlas of traditional food made from cereals and milk*. Groningen, The Netherlands: Barkhuis and Groningen University Library.

40. Bär C, Mathis D, Neuhaus P, Dürr D, Bisig W, Egger L, Portmann R. 2019 Protein profile of dairy products: simultaneous quantification of twenty bovine milk proteins. *Int. Dairy J.* **97**, 167–175. (doi:10.1016/j.idairyj.2019.01.001)

41. Yang Y, Shevchenko A, Knaust A, Abuduresule I, Li W, Hu X, Wang C, Shevchenko A. 2014 Proteomics evidence for kefir dairy in Early Bronze Age China. *J. Archaeol. Sci.* **45**, 178–186. (doi:10.1016/j.jas.2014.02.005)

42. Hendy J *et al.* 2018 Proteomic evidence of dietary sources in ancient dental calculus. *Proc. R. Soc. B* **285**, 20180977. (doi:10.1098/rspb.2018.0977)

43. Xie M, Shevchenko A, Wang B, Shevchenko A, Wang C, Yang Y. 2016 Identification of a dairy product in the grass woven basket from Gumugou Cemetery (3800 BP, northwestern China). *Quat. Int.* **426**, 158–165. (doi:10.1016/j.quaint.2016.04.015)

44. Adamczak K *et al.* 2021 New evidence for deer valorisation by the TRB farmers from Poland using ZooMS and micro-CT scanning. *J. Archaeol. Sci. Rep.* **40**, 103230. (doi:10.1016/j.jasrep.2021.103230)

45. Ramsey CB. 2009 Bayesian analysis of radiocarbon dates. *Radiocarbon* **51**, 337–360. (doi:10.1017/S0033822200033865)

46. Reimer PJ *et al.* 2020 The IntCal20 Northern Hemisphere radiocarbon age calibration curve (0–55 cal kBP). *Radiocarbon* **62**, 725–757. (doi:10.1017/RDC.2020.41)

47. Śledzińska M, Błaszczyk K, Nierychlewska A, Zawilski P. 2017 Opracowanie wyników ratowniczych badań archeologicznych przeprowadzonych na stanowisku nr 13 w Sławęcinku. (gm. Inowrocław, pow. inowrocławski, woj. kujawsko-pomorskie AZP 44-40/18). Łódź, Poland: Firma Archeologiczna ARTEFAKT 2017.

48. Hendy J, Bickle P, Copper M, Charlton S. 2016 Neolithic cheese making: experimental archaeology and public engagement with replica vessels. *PAST: The Newsletter of the Prehistoric Society* **84**, 12–14.

49. Wilkin S *et al.* 2021 Dairying enabled Early Bronze Age Yamnaya steppe expansions. *Nature* **598**, 629–633. (doi:10.1038/s41586-021-03798-4)

50. Ramsøe A, van Heekeren V, Ponce P, Fischer R, Barnes I, Speller C, Collins MJ. 2020 DeamiDATE 1.0: site-specific deamidation as a tool to assess authenticity of members of ancient proteomes. *J. Archaeol. Sci.* **115**, 105080. (doi:10.1016/j.jas.2020.105080)

51. Ramsøe A, Crispin M, Mackie M, McGrath K, Fischer R, Demarchi B, Collins MJ, Hendy J, Speller C. 2021 Assessing the degradation of ancient milk proteins through site-specific deamidation patterns. *Sci. Rep.* **11**, 7795. (doi:10.1038/s41598-021-87125-x)

52. Correa-Ascencio M, Evershed RP. 2014 High throughput screening of organic residues in archaeological potsherds using direct acidified methanol extraction. *Anal. Methods* **6**, 1330–1340. (doi:10.1039/C3AY41678J)

53. Craig OE *et al.* 2013 Earliest evidence for the use of pottery. *Nature* **496**, 351–354. (doi:10.1038/nature12109)

54. Copley MS, Bland HA, Rose P, Horton M, Evershed RP. 2005 Gas chromatographic, mass spectrometric and stable carbon isotopic investigations of organic residues of plant oils and animal fats employed as illuminants in archaeological lamps from Egypt. *Analyst* **130**, 860–871. (doi:10.1039/b500403a)

55. Craig OE, Allen RB, Thompson A, Stevens RE, Steele VJ, Heron C. 2012 Distinguishing wild ruminant lipids by gas chromatography/combustion/isotope ratio mass spectrometry. *Rapid Commun. Mass Spectrom.* **26**, 2359–2364. (doi:10.1002/rcm.6349)

56. Dudd SN, Evershed RP. 1998 Direct demonstration of milk as an element of archaeological economies. *Science* **282**, 1478–1481. (doi:10.1126/science.282.5393.1478)

57. Lundy J *et al.* 2021 New insights into early medieval Islamic cuisine: organic residue analysis of pottery from rural and urban Sicily. *PLoS ONE* **16**, e0252225. (doi:10.1371/journal.pone.0252225)

58. Tyanova S, Temu T, Sinitcyn P, Carlson A, Hein MY, Geiger T, Mann M, Cox J. 2016 The Perseus computational platform for comprehensive analysis of (prote)omics data. *Nat. Methods* **13**, 731–740. (doi:10.1038/nmeth.3901)

59. Christie WW. 1978 The composition, structure and function of lipids in the tissues of ruminant animals. *Prog. Lipid Res.* **17**, 111–205. (doi:10.1016/0079-6832(78)90007-1)

60. Regert M. 2004 Investigating the history of prehistoric glues by gas chromatography-mass spectrometry. *J. Sep. Sci.* **27**, 244–254. (doi:10.1002/jssc.200301608)

61. Mitkidou S, Dimitrakoudi E, Urem-Kotsou D, Papadopoulou D, Kotsakis K, Stratis JA, Stephanidou-Stephanatou I. 2008 Organic residue analysis of Neolithic pottery from North Greece. *Microchim. Acta* **160**, 493–498. (doi:10.1007/s00604-007-0811-2)

62. Bondetti M *et al.* 2021 Neolithic farmers or Neolithic foragers? Organic residue analysis of early pottery from Rakushechny Yar on the Lower Don (Russia). *Archaeol. Anthropol. Sci.* **13**, 141. (doi:10.1007/s12520-021-01412-2)

63. Eglinton G, Hamilton RJ. 1967 Leaf epicuticular waxes. *Science* **156**, 1322–1335. (doi:10.1126/science.156.3780.1322)

64. Dudd SN, Regert M, Evershed RP. 1998 Assessing microbial lipid contributions during laboratory degradations of fats and oils and pure triacylglycerols absorbed in ceramic potsherds. *Org. Geochem.* **29**, 1345–1354. (doi:10.1016/s0146-6380(98)00093-x)

65. Copley MS, Berstan R, Dudd SN, Docherty G, Mukherjee AJ, Straker V, Payne S, Evershed RP. 2003 Direct chemical evidence for widespread dairying in prehistoric Britain. *Proc. Natl Acad. Sci. USA* **100**, 1524–1529. (doi:10.1073/pnas.0335955100)

66. Fernandes R, Eley Y, Brabec M, Lucquin A, Millard A, Craig OE. 2018 Reconstruction of prehistoric pottery use from fatty acid carbon isotope signatures using Bayesian inference. *Org. Geochem.* **117**, 31–42. (doi:10.1016/j.orggeochem.2017.11.014)

67. Miller MJ *et al.* 2020 Interpreting ancient food practices: stable isotope and molecular analyses of visible and absorbed residues from a year-long cooking experiment. *Sci. Rep.* **10**, 13704. (doi:10.1038/s41598-020-70109-8)

68. Barker A, Dombrosky J, Venables B, Wolverton S. 2018 Taphonomy and negative results: an integrated approach to ceramic-bound protein residue analysis. *J. Archaeol. Sci.* **94**, 32–43. (doi:10.1016/j.jas.2018.03.004)

69. Bakker JA, Kruk J, Lanting AE, Milisauskas S. 1999 The earliest evidence of wheeled vehicles in Europe and the Near East. *Antiquity* **73**, 778–790. (doi:10.1017/S0003598X00065522)

70. Mischka D. 2011 The Neolithic burial sequence at Flintbek LA 3, north Germany, and its cart tracks: a precise chronology. *Antiquity* **85**, 742–758. (doi:10.1017/S0003598X00068289)

71. Milisauskas S, Kruk J, Hudson K. 2019 Bronocice Funnel Beaker vessel with wagon motif: different narratives. *Archaeol. Polona* **57**, 233–240. (doi:10.23858/APa57.2019.016)

72. Evans M *et al.* 2023 Data from: Detection of dairy products from multiple taxa in Late Neolithic pottery from Poland: an integrated biomolecular approach. Dryad Digital Repository. (doi:10.5061/dryad.905qfttpt)

73. Evans M *et al.* 2023 Detection of dairy products from multiple taxa in Late Neolithic pottery from Poland: an integrated biomolecular approach. Figshare. (doi:10.6084/m9.figshare.c.6452213)