International Biodeterioration & Biodegradation 80 (2013) 48–59

Contents lists available at SciVerse ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Proteomic evaluation of the biodegradation of wool fabrics in experimental burials

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ARTICLE INFO

Article history:
Received 10 September 2012
Received in revised form
6 November 2012
Accepted 7 November 2012
Available online

Keywords:
Wool
Keratin and keratin-associated proteins
Bogs
Sphagnum moss
Madder
Weld
Indigo
Proteomics
Hydrolysis
Cross-linking

ABSTRACT

Woollen textiles recovered from archaeological excavations are usually fragile, discoloured, mineralised, or highly biodeteriorated fragmentary remains. The nature and extent of preservation is highly dependent on the site of burial and factors such as soil composition, pH, temperature, oxygen content, and contact with a wood coffin or metals. Understanding the particular biodegradation in archaeological sites is important for biomolecular studies of textiles, and to assist in the conservation of these finds. Wool fabrics dyed and buried for up to 8 yr in bog-type soils in Denmark (Lejre) and Norway (Rørmyra), and in marine sediments in Sweden (Marstrand) were evaluated by proteomics analysis. Wool degradation was found to occur through a range of differing mechanisms, mainly due to the complex nature of wool itself with its many families of proteins (keratin and keratin-associated proteins) and structures. Microbial activity was a large contributory factor to the physical deterioration of the wool fabrics at Lejre and Marstrand, and might result in faster loss of keratin-associated proteins over keratins. Additional hydrolysis took place at Marstrand, influenced by the environmental conditions of the sediment, and in particular the alkaline pH, contributing to the degradation of keratins. However, cross-linking was associated with the long-term preservation of the fabrics at Rørmyra, where pH, temperature, and vegetative composition of the bog prevented microbial activity, and sphagnum moss might preserve wool by binding with keratins.

1. Introduction

Archaeological hair and wool are becoming of increasing interest in biomolecular studies. In recent years, DNA has been extracted from mammoth (Gilbert et al., 2007) and human hair (Rasmussen et al., 2010) that was preserved in permafrost for thousands of years. Isotopic analysis of human hair has been used to reveal the diet of ancient populations (Macko et al., 1999; Sharp et al., 2003; Wilson et al., 2007b). Similar studies on wool have recently been published to identify the source of textiles, through DNA analysis (Brandt et al., 2011), protein characterisation (Solazzo et al., 2011; Hollemeyer et al., 2008, 2012), and isotopic analysis of strontium and light isotopes (Hedges et al., 2005; Frei et al., 2009a,b, von Holstein, 2011). The protein composition of wool and silk has also been investigated to date textiles by means of amino acid racemisation and deamidation evaluation (Araki and Moini, 2011; Moini et al., 2011). These recent innovations are opening up exciting new areas within the field of ancient textile research. However, little is known with respect to fibre degradation at the molecular level that could potentially influence the feasibility and interpretation of such biomolecular studies, in particular for textiles excavated from archaeological sites.

Deterioration of archaeological textiles is dependent on burial conditions (soil composition, pH, temperature, oxygen content, contact with wood coffins, metals, etc.), and on how these environmental factors regulate microbial activity. In wool, a high cysteine content confers high resistance to chemical degradation by forming extensive cross-linking (disulphide bridges). Keratins are therefore primarily degraded in soil via specialised keratinolytic

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http://dx.doi.org/10.1016/j.ibiod.2012.11.013

microorganisms, as they produce enzymes capable of degrading keratins. At least 300 reported fungi use keratins as a source of nutrients (C, N, and S) (Błyskal, 2009). Fibre degradation is initiated by denaturation of the constituent proteins and breaking of the disulphide bridges, necessary steps for proteolytic enzymes to access and attack keratin. Surface erosion and radial penetration are the two known mechanical modes for microorganisms to attack wool fibres (Filipello Marchisio, 2000; Kornikowicz-Kowalska and Bohacz, 2011). The former results in progressive degradation from the cuticle to the cortex, while in the latter holes in the fibre are caused by perforating organs or hyphae. Wilson et al. (2007a) assessed microorganism activity on human hair buried in soil and observed fungi deterioration in the form of tunnelling. Cases of both surface erosion and radial penetration were observed, showing either fibrillation (loss of cuticle and separation of cuticle cells), or collapse of the fibre by loss of the cortex (degradation of the cortex with intact cuticle) (Wilson et al., 2010). Studies on human hair have also demonstrated that the structures that make up the cuticle and cortex are gradually degraded by fungi. In both cuticle and cortex, the cell membranes and cytoplasmic residues are attacked first. This is followed by invasion of the endocuticle, and of the intermacrofibrillar matrix and microfibrils in the cortex. The exocuticle, a-layer, and intermicrofibrillar matrix are the most resistant to enzymatic digestion (Filipello Marchisio, 2000).

The physical degradation of wool in different burial contexts has been reported from actual archaeological finds and experimentally buried fabrics and include loss of cuticular scale, fibrillation, pitting, discolouration, and staining (Peacock, 1996a,b). Wool, however, survives well in cold wet and hot arid environments, waterlogged soils, peat bogs and salt-saturated soils, when bacterial and fungal activity is reduced. We analysed wool fabrics from experimental burials to evaluate chemical deterioration and biodegradation in an archaeological context. The samples were derived from two separate experiments conducted between 1998 and 2009 (Table 1). The first experiment took place from 1998 to 2006, during which time wool fabrics were buried at Lejre in Denmark in a lowland bog, and at Rørmyra in Norway in a raised bog (Peacock, 2004; Turner-Walker and Peacock, 2008). The second, ongoing experiment began in 2002 and similar samples were buried in marine sediments in the harbour at Marstrand in Sweden for retrieval after periods of up to 48 yr. These samples are part of a project investigating reburial as a method for preserving excavated archaeological materials (Bergstrand et al., 2002; Bergstrand and Godfrey, 2007; Godfrey et al., 2009). In this experiment, one series of samples was exposed to the sediments, while a second series was enclosed in geotextile fabric. The fabrics were left undyed or dyed using madder, weld, and indigo. Samples included in this study were buried for up to 7 yr at Marstrand and 8 yr at Lejre and Rørmyra.

The physical deterioration of the samples was compared to the proteins identified after analysis by nanoLC-ESI-MS/MS. For each dye and burial site, we assessed the type and speed of protein degradation, compared to the controls kept in storage. The results indicating the occurrence of both hydrolysis and protein–protein cross-linking were highly variable with respect to dyeing treatment and site of burial.

2. Materials and methods

2.1. Materials

2.1.1. Samples

Samples included in the study were of a burial-degraded modern textile fabric. The fabric was a highly fulled twill (vadmel) woven in modern natural pigmented white wool fibre by Røros Tweed a/s (Røros, Norway) in 1997. Pieces of the fabric were dyed red, yellow, and blue, respectively, at the Textile Workshop at "Land of Legends Lejre" (formerly Historical-Archaeological Research Centre Lejre, Denmark). A piece of

Table 1
Burial sites characteristics.

	Control (1998)	Lejre (1998–2006)	Marstrand (2002–2009)	Rørmyra (1998–2006)
Location	NTNU Museum, Trondheim, Norway	Land of Legends Lejre (formerly Lejre Historical Archaeological Research Centre), Denmark	Marstrand Harbour, Sweden	Rørmyra Nature Reserve, Bymarka, Sør-Trondelag County, Norway
Coordinates	NA	55° 36′ 30″ N; 11° 56′ 20″ E	57° 54′ 13″ N; 11° 32′ 43″ E	63° 21′ 30″ N; 10° 17′ 50″ E
Elevation	NA	58 m	0 m	175 m
Environment	Museum	Fenland bog, humid oceanic climate	Harbour	Raised bog, subarctic climate
Topography	NA	Low rolling hills	Sandy	Below wooded upland
Geology	NA	Clay	Marine sediments	Glacial till
Soil water pH	NA	5.6	7.2–7.5	5.0
Dissolved O_2	NA	0.5%	0%	1.6%
Annual air temperature (average)	18 °C, 50% RH	9.2 °C	7.4 °C	3.3 °C
Annual temperature 1 m (average)	NA	8.6 °C	NA	4.2 °C
Potential redox	NA	NA	−150 to −200 mV strongly reducing (sulphate reducing)	NA
Nature of the sediments	NA	"Sediments at Lejre were much darker and more silty, with less obvious organic matter and a strong smell of sulphur" "Closer to both urban areas and farmland" "the sub-soil to the bog is a silty clay and the lower levels of the peat contain considerable silt"	Fine sand. Analysis showed a near neutral pH, a reducing environment below 50 cm and low water content. Organic matter content 5–7%	"Almost pure sphagnum peat, with visibly well-preserved vegetable matter including small twigs and leaves" "Spectacular preserving qualities of sphagnum moss" due to sphagnan "Inhibits microbial action by deactivating proteolytic enzymes and binding free amino groups, thus denying micro-organisms access to nutrition"

fabric was left undyed. Dyeing protocols followed procedures established at Lejre that replicate methods used in antiquity. The red colour was obtained by the addition of dry madder (*Rubia tinctorum* L.) root; whereas yellow was achieved with dry and fresh weld (*Reseda luteola* L.). Both madder- and weld-dyed pieces were mordanted with alum ($AlK(SO_4)_2 \cdot 12H_2O$). The blue colour was produced by the addition of dry natural indigo (indigotin) to fermented urine.

2.1.2. Burial sites

Control samples were kept in darkness in climate-control stores at the NTNU Museum, Norwegian University of Science and Technology in Trondheim, Norway. Two series of burial experiments were conducted. The first series of experiments was conducted from 1998 to 2006 and samples were retrieved after 1, 2, 4, and 8 yr. Samples were placed together with excavated soil into perforated PVC plastic pipes (16 mm diameter) (Peacock, 2004) and the modules were buried in hand-drilled boreholes 1 m deep. The modules were buried in bogs at "Land of Legends Lejre" (Denmark) and Rørmyra, Sør-Trondelag County (Norway) (Turner-Walker and Peacock, 2008). The characteristics of each site are presented in Table 1.

The second series of experiments was initiated in 2002 at Marstrand harbour on the coast of West Sweden. Samples were sewn into an open mesh nylon envelope (uncovered samples). A duplicate mesh envelope of samples was enclosed in an additional envelope constructed of a non-woven geotextile fabric (covered samples). One envelope of each was laced side-by-side with nylon cord in the bottom of a perforated plastic tray. One tray was prepared per burial period. Sample trays were buried in the harbour sediment at a depth of 50 cm; samples included in this study were retrieved after 1, 2, 3, and 7 yr (Bergstrand et al., 2002). (The remaining samples will be excavated after an additional 5, 17, and 41 yr).

2.1.3. Chemicals

Urea, tris(2-carboxyethyl)phosphine (TCEP), and iodoacetic acid (IAA) were provided by Sigma–Aldrich™ (USA), and ammonium bicarbonate (AB) by BDH AnalaR® (UK). Acetonitrile (ACN) and water (H_2O) were provided by Fisher Scientific (Fair Lawn, NJ, USA), methanol (MeOH) was from Scharlau (Spain), trifluoroacetic acid (TFA) was provided by Fisher Scientific (USA), and formic acid by Ajax Finechem Pty Ltd (Univar® analytical reagents), Thermo Fisher Scientific (NZ). Quantitation of protein was determined with the 2-D Quant kit from GE Healthcare (G-Biosciences, USA) using a copper solution.

2.2. Proteomics analysis

2.2.1. Sample preparation

Up to 10 mg of wool was ground in liquid nitrogen unless it was too fragile, and washed with deionised water to remove as many soil particles as possible. The samples were extracted by overnight shaking with 0.5 ml of a solution of 8 M urea, 50 mM Tris, and 50 mM TCEP at pH 8.3. An aliquot of 200 μl of supernatant was alkylated with 150 mM IAA and vortexed for 4 h in the dark. This was followed by 24 h of dialysis with 0.1 M AB (two changes) on 3000-Da units. Protein concentration was measured on 10 and 20 μl of the dialysed extracts. The standard curve was obtained from standards of bovine serum albumin and sample concentration was obtained from reading absorbance at 480 nm using an Implen NanoPhotometer P-Class (Implen GmBH, Germany). About 25 μg of the samples were digested with 0.5 μg of trypsin, overnight at 37 °C. Samples were then dried down and re-solubilised in 10 μl of 0.1% TFA. Due to time limitations, each sample was digested and evaluated once by MS/MS analysis.

2.2.2. Protein analysis by nanoLC-ESI-MS/MS

Protein separation was carried out on an Ultimate nanoflow nanoLC equipped with a Famos autosampler and Switchos column switching module (LC-Packings, The Netherlands). A 10-μl sample was loaded on a C18 precolumn (Varian Microsorb, 300-μm ID, 5-μm particles, 300-Å pore size) at a flow rate of 8 μl min^{-1}. The precolumn was then switched in line with the analytical column (Microsorb C18, 20 cm, 75-μm ID, 5-μm particles, 300-Å pore size), and eluted at a flow rate of 150 nl min^{-1}, with a gradient from 2% to 55% B in 50 min. Solvent A was HPLC-grade H_2O (Fisher Scientific, USA) with 0.2% formic acid; solvent B was LCMS-grade ACN with 0.2% formic acid. Using a stainless steel nanospray needle (Proxeon, Denmark), the column outlet was directly connected to a QSTAR Pulsar i mass spectrometer (Applied Biosystems, USA) that was programmed to acquire MS/MS traces of 1+, 2+, 3+, 4+, and 5+ peptides in three simultaneous MS/MS.

2.2.3. Bioinformatics analysis

Mascot Daemon (Matrix Science, UK) was used to extract peak lists from the LC–MS/MS data files. The peak lists from all m/z segments of each sample were concatenated and imported in Protein-Scape v2.1 (Bruker Daltonics). Subsequently, Mascot was used to search for matches with known *Ovis aries* sequences, using an in-house database compiled and curated by AgResearch, NZ. Parameters were set as follows: no enzyme with two missed cleavages, peptide mass tolerance (MS) of 150 ppm, fragment mass tolerance (MS/MS) of 0.4 Da, carboxymethylation as a fixed modification and acetyl (N-term), carbamyl (N-term), deamidated (NQ), Gln- > pyro-Glu (N-term Q), methyl (DE), and oxidation (M) as variable modifications. For oxidative modifications, single, double, and triple oxidation of cysteine were allowed, as well as single and double oxidation of H, W, F and Y, kynurenine and hydroxykynurenine, quinone and hydroxyquinone.

3. Results

3.1. Burial-induced deterioration

Burial-induced deterioration varied as a result of burial environment and duration (Fig. 1 and Figs. S1–S4 in Supplementary Data). Overall, preservation at Lejre was poor; there was a reduction in both surface fibre density and fabric areal density after one year, which increased thereafter (Table S1). With increasing burial time, samples became thinner and compacted and eventually broke down. Colours faded, becoming duller overall, and the fabrics were covered with adhering soil particles. Microscopy showed loss of internal structure, evidence of pitting and erosion, lifting of the cuticle, and exposed cortex (Peacock, 2004). Samples were highly degraded following year 4 except for the madder-dyed sample, which was less faded and remained in better condition. The other samples were fragile and discoloured from year 4, and especially fragmented and brittle from year 8.

Visually, Marstrand samples exhibited loss of surface fibres and fabric integrity, reduced fabric areal density, and the fading of colour. Samples enclosed in the geotextile envelope degraded more slowly than those directly exposed to the surrounding harbour sediment (Peacock, 2003, 2005). The undyed and indigo-dyed samples degraded quickly after year 1 for the uncovered samples and after year 2 for the covered ones. At year 7 the uncovered samples were fragmented and brittle and the covered ones were highly deteriorated. The madder-dyed samples retained their strong red colour and were in good condition up to year 3.

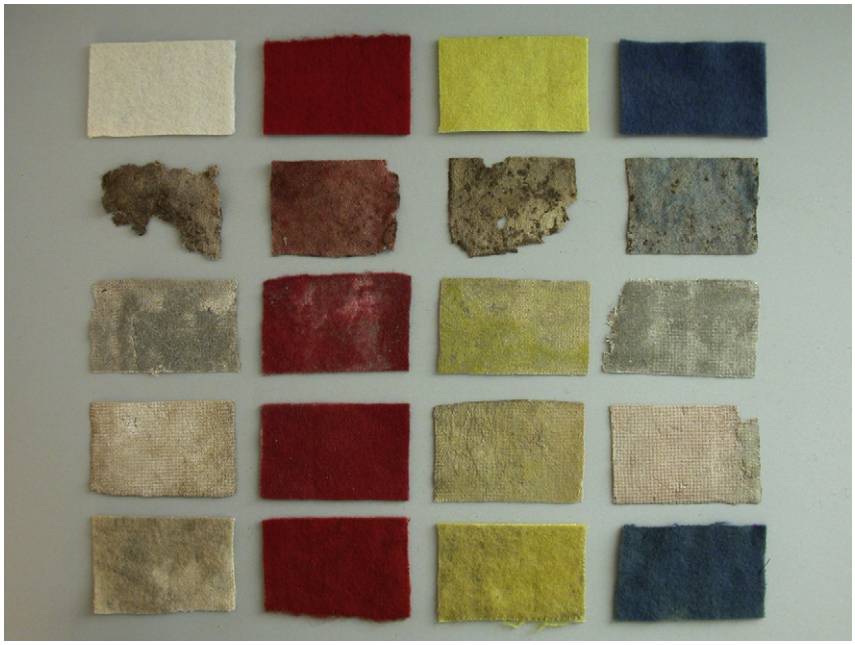

Fig. 1. Pictures of samples – from left to right: undyed, madder-dyed, weld-dyed, and indigo-dyed; and from top to bottom: control (not buried), 4-yr Lejre, 3-yr Marstrand uncovered, 3-yr Marstrand covered, and 4-yr Rørmyra. (Photo: Elizabeth E Peacock, ©NTNU University Museum).

Discolouration and fabric degradation were rapid from year 1 and year 2 for the uncovered and covered weld-dyed samples, respectively. The 7-year indigo-dyed sample was completely degraded in the uncovered set, as no sample was found.

On the other hand, preservation was very good at Rørmyra, where fabrics retained strong colours, surface fibre density, and fabric areal density. They retained their scale pattern after 2 yr (Peacock, 2004). The undyed samples remained in good condition for up to 8 yr with only a slight change in colour to beige, while the madder-dyed samples retained a deep red. The weld sample appeared more affected at year 8. The colour rapidly changed from a bright yellow (year 1) to dull yellow (year 8). The indigo-dyed samples remained in good condition up to 8 yr, but the colour changed to a blue-green following year 8.

3.2. Proteomics analysis

The complex structure of wool has been well illustrated in recent publications (Plowman, 2003, 2007; Clerens et al., 2010; Koehn et al., 2010). Wool fibres are composed of a cortex wrapped into a layer of cuticular cells. In the cortex, proteins are arranged in macrofibrils where the keratins (or intermediate filaments proteins [IFPs]), made of alpha-helical domains, are embedded in a matrix of keratin associated proteins (KAPs). The IFPs are classified into two families: type I (K31 to K40) and type II (K81 to K87) proteins. The cysteines in the IFPs (about 6% of all amino acids, mainly located in the head and tail domains of the proteins) are bound to the KAPs by disulfide bridges. The KAPs, which are also the prime component of the cuticle, are small amorphous proteins classified in 27 families, some with a very high content of sulphur from cysteine.

3.2.1. Protein extraction and digestion

A standard protein extraction for wool analysis was applied here, using a urea-based solution for solubilising the fibres (Clerens et al., 2010). Whenever possible, an average of 10 mg of sample was used in 1 ml of extraction solution. Table 2 shows the quantity of proteins obtained in micrograms per 10 μl of extract. Differences in extraction yields appear with the dye used, as well as the sites and time of burial (where the introduction of soil matter in the buried fabrics might misrepresent the real weight of the samples). The quantity of proteins extracted in the weld control is less than half that of the undyed and madder-dyed samples, and the quantity in the indigo sample is very low, at less than 3 μg. Indigo particles are probably keeping the denaturant from efficiently unfolding and solubilising the protein chains. At this stage however, the dye was not removed, to avoid any interaction from additional chemicals. The amount of extract was adjusted for enzymatic digestion with trypsin in order to digest a similar amount of protein in each sample (about 25 μg). Trypsin cleaves proteins at the carboxyl side (C-terminus) of arginine and lysine, two basic amino acids regularly found in keratins, but less often in the keratin-associated proteins.

3.2.2. Protein identification before burial

The type and number of peptides identified vary between undyed and dyed samples. The individual graphs in Fig. 2 show the total number of peptides identified in each sample, with the IFPs in black and the KAPs in white. The highest numbers of identified peptides are found in the undyed control U-0 (146 IFPs and 10 KAPs, Fig. 2a), and the weld-dyed control W-0 (139 IFPs, 37 KAPs, Fig. 2c), followed by the madder-dyed control M-0 (117 IFPs, 11 KAPs, Fig. 2b), and the indigo-dyed control I-0 (54 IFPs, 55 KAPs, Fig. 2d). It is interesting to note that, while extraction and/or digestion of the IFPs appear less efficient in the indigo-dyed control, it does not seem to be the case for the KAPs. KAPs are typically difficult proteins to identify in a single LC-MS/MS run as they are present in lower quantity and have fewer basic residues available for trypsin digestion. This usually results in the over-representation of the IFPs over the KAPs.

3.2.3. Protein identification after burial

The total number of peptides (IFPs + KAPs) identified at Lejre tends to decrease with time for the undyed, madder-, and weld-dyed samples (Fig. 2a–c) compared to the control samples, while it generally remains high or increases at Marstrand (see 3.2.4). At Rørmyra, fewer peptides are observed compared to the control samples but their number remains constant from 1 to 8 yr of burial.

Table 2
Controls and buried fabrics from each experimental site are described by treatment (no dye/dye), years of burial (1–8), sample abbreviation, mass (in milligrams) used for analysis, and protein quantitation obtained after fabric solubilisation. For each fabric, two samples of the protein extract were measured for quantitation and the average quantity of protein (with standard deviation) is indicated in micrograms for 10 μl. NR stands for no result.

Dyeing	Controls				Lejre			Marstrand uncovered			Marstrand covered			Rørmyra		
	Y.	Abbr.	M. (mg)	Quant. (μg/10 μl)	Abbr.	M. (mg)	Quant. (μg/10 μl)	Abbr.	M. (mg)	Quant. (μg/10 μl)	Abbr.	M. (mg)	Quant. (μg/10 μl)	Abbr.	M. (mg)	Quant. (μg/10 μl)
Undyed	0	U-0	9	47.96 (13.11)												
	1				U-L1	10	54.06 (19.43)	U-Mu1	6	33.49 (3.46)	U-Mc1	7	30.39 (2.81)	U-R1	7	36.96 (4.63)
	2				U-L2	8	40.81 (5.43)	U-Mu2	4	4.35 (0.84)	U-Mc2	5	10.96 (0.83)	U-R2	7	34.64 (5.28)
	3/4				U-L4	5	3.81 (0.83)	U-Mu3	4	6.49 (2.06)	U-Mc3	7	22.42 (0.54)	U-R4	7	32.03 (4.53)
	7/8				U-L8	7	4.53 (1.23)	U-Mu7	10	4.37 (1.25)	U-Mc7	10	NR	U-R8	7	37.60 (4.93)
Madder	0	M-0	9	47.28 (12.86)												
	1				M-L1	9	43.92 (8.72)	M-Mu1	5	8.17 (4.57)	M-Mc1	9	10.96 (2.44)	M-R1	10	17.99 (0.68)
	2				M-L2	4	10.06 (1.45)	M-Mu2	7	10.96 (1.64)	M-Mc2	4	5.92 (0.98)	M-R2	10	18.96 (1.64)
	3/4				M-L4	8	17.39 (2.81)	M-Mu3	7	5.28 (0.47)	M-Mc3	7	8.96 (1.43)	M-R4	10	20.85 (2.15)
	7/8				M-L8	2	5.28 (0.27)	M-Mu7	12	NR	M-Mc7	8	NR	M-R8	10	15.89 (1.54)
Weld	0	W-0	10	20.18 (0.46)												
	1				W-L1	11	4.35 (3.96)	W-Mu1	8	8.03 (0.19)	W-Mc1	11	13.38 (0.53)	W-R1	10	13.38 (2.37)
	2				W-L2	5	5.12 (3.08)	W-Mu2	9	10.13 (2.37)	W-Mc2	10	6.95 (1.48)	W-R2	10	8.95 (0.85)
	3/4				W-L4	6	1.03 (0.50)	W-Mu3	7	2.56 (2.41)	W-Mc3	11	6.52 (2.10)	W-R4	10	9.12 (1.67)
	7/8				W-L8	10	0.95 (1.35)	W-Mu7	9	NR	W-Mc7	8	NR	W-R8	10	12.95 (2.76)
Indigo	0	I-0	10	2.53 (0.28)												
	1				I-L1	10	6.34 (1.07)	I-Mu1	7	6.09 (0.16)	I-Mc1	10	6.94 (0.08)	I-R1	11	3.24 (1.48)
	2				I-L2	10	7.45 (0.99)	I-Mu2	7	2.99 (1.29)	I-Mc2	10	4.58 (0.08)	I-R2	11	5.47 (2.59)
	3/4				I-L4	10	6.18 (0.10)	I-Mu3	7	2.59 (0.92)	I-Mc3	10	4.94 (1.29)	I-R4	11	5.23 (0.60)
	7/8				I-L8	7	0.58 (0.82)	I-Mu7	No sample		I-Mc7	7	NR	I-R8	11	4.15 (1.48)

At Rørmyra the number of observed IFPs is also much lower for the undyed and madder-dyed samples, but this is compensated for by a high number of KAPs (the numbers, on average, are 34% and 57%, respectively). It is, however, significantly higher for the weld-dyed samples, where the number of KAP peptides identified comprises on average 18% of all peptides, a number that is comparable to the Lejre and Marstrand extracts.

For the indigo-dyed samples, more peptides are identified in the Lejre and Marstrand sites than in the control sample, in spite of the samples getting physically degraded. Better fibre solubilisation (characterised by an increase in protein quantity in the first years of burial, Table 2) and trypsin digestion are probably facilitated by the loss of indigo. In the Rørmyra samples, where the samples are the least discoloured, the number of extracted peptides remains low. We again notice that a large proportion of the total number of peptides originates from KAPs (61% on average).

Tables 3 and 4 show commonly observed tryptic IFP peptides identified by nanoLC-ESI-MS/MS in the control samples and in the buried samples for all dyes and all years of burial. The peptide frequency is due to two key factors: their susceptibility to ionise and their relative commonality among the keratins (such as peptides that are found in more than one protein). Generally, common IFP-derived peptides remain detectable in the Lejre and Marstrand sites up to 8 and 7 yr, independent of the dye, while many become absent in the Rørmyra samples.

3.2.4. Protein chain hydrolysis

Protein chain hydrolysis has been observed as an effect of ageing in archaeological and historical fibres (Wyeth, 2004). The consequence of protein hydrolysis on the proteomics analysis of wool fibres would be the creation of shorter protein fragments and random cleavage sites introduced prior to trypsin digestion. A small proportion of semi-tryptic peptides, resulting from cleavages at non-basic residues (usually at the N-terminus position), is usually observed after standard overnight trypsin digestion (18 h at 37 °C) (Strader et al., 2005; Picotti et al., 2007). If these peptides are present in low abundance, they increase the total number of peptides in a protein digest. The proportion in semi-tryptic peptides, however, only increases if the quantity of trypsin is increased but is not influenced by any preparation steps or contaminants (Picotti et al., 2007). In this study, the proportion trypsin/sample (in micrograms) was kept in the order of 1:50 for all samples. The importance of these cleavage sites was assessed by allowing the search for semi- (one non-tryptic cleavage) and non-tryptic peptides (two non-tryptic cleavages) in the Mascot search parameters. Semi-tryptic peptides (ST) were observed during the digestion of unaged wool, but non-tryptic peptides (NT) were not. Fig. 3 shows the quantities of ST and NT formed from the IFPs in the controls and at each site. Compared to the controls, the number of ST peptides increases in the Lejre samples (where they are mainly observed in the 4- and 8-yr indigo-dyed samples) and especially in the Marstrand samples, where it is significantly higher. Furthermore, NT peptides are observed only in the Marstrand site. Of the 32 peptides created, one is present in the 1-yr samples, two in the 2-yr samples, ten in the 3-yr samples, and 29 in the 7-yr samples, indicating that more extensive hydrolysis is taking place with time. In addition, 26 are found in the indigo-dyed samples, showing that they are more susceptible to this type of hydrolysis; generally, in all sites as well as in the control, the indigo-dyed samples have a higher ratio of semi and non-tryptic IFPs peptides.

3.2.5. Markers of oxidation

Degradation of wool textiles in museums has been associated with the release of sulphur compounds originating from cysteine degradation (Brimblecombe et al., 1992). Amino acids profiles of historical textiles and artificially aged modern samples have demonstrated a loss of some amino acids such as tyrosine and cysteine while showing an increase in acidic amino acids and cysteic acid (resulting from the oxidation of cysteine) (Vanden Berghe and Wouters, 2005; Odlyha et al., 2007; Vanden Berghe, 2012). Cysteine oxidation is a chemical modification that contributes to

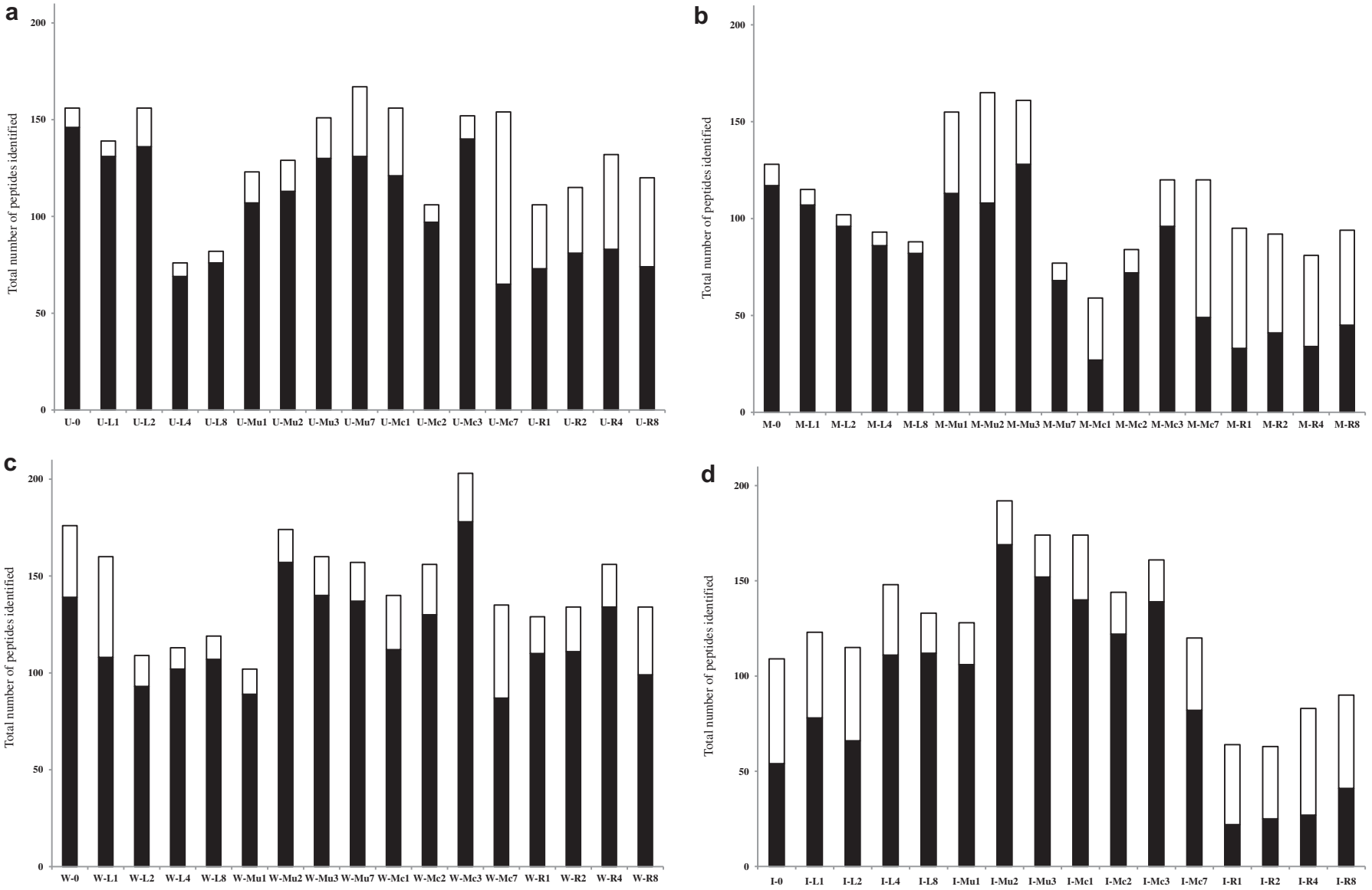

Fig. 2. Total number of peptides identified in the samples for: a-undyed samples, b-madder-dyed samples, c-weld-dyed samples, and d-indigo-dyed samples. The lower part filled in black indicates the number of IFPs (keratins) and the upper part indicates the KAPs (keratin-associated proteins); see Table 2 for samples' names.

Table 3
Identified tryptic type I peptides in controls and buried samples with protein modifications, and presence in undyed (u) and dyed samples (m, w, i). For oxidative modifications, *x* is indicated as follows: Oxidation (YWHC) + O → (YWHC)1, (YWHC) + 2O → (YWHC)2, C + 3O → C3. Proteins in which peptides are found are indicated as follows: [1]K31, [2]K32, [3]K33a, [4]K33b, [5]K34, [6]K35, [7]K38.

Type I peptides	Controls	Lejre	M uncov	M cov	Rormyra
SYNFCLPNLSFR.S[4]	u, m, w, i	u, m, w, i	m, w, i	u, m, w, i	u, m, w, i
SFNFCLPNLSFR.S[1,3]	u, m, w, i	u, m, w, i^{F2}	m, w, i	u, m, w, i	u, m, w^{F1}, i
R.LASYLEK.V[1,3,4,5,6,7]	m, w, i	u, m, w, i	u, w, i	u, m, w, i	u, w, i
R.SQQQEPLVCPNYQSYFR.T[1,3,4]	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i	u, w, i
R.SQQQEPLLCPNYQSYFR.T[5]	m, w	u, w, i	u, m, w, i	u, m, w, i	u, w
R.TIEELQQK.I[1,2,3,4,5]	u, m, w	u, m, w, i	u, m, w, i	u, m, w, i	w
R.LVVQIDNAK.L[1,3,4,6]	u, m, w	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i
R.LVIQIDNAK.L[5]	u, m, w	u, m, w, i	u, m, w, i	u, w	u, m, w
K.LAADDFR.T[1,2,4,6,7]	u, m, w	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w
K.LASDDFR.T[3,5]	u	u, m, w	u, m, w	u, m, w	w
K.YQTELGLR.Q[4]	u, m, w	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w
R.TKYETELGLR.Q[1]	u, m, w	u, m, w, i	u, m, w, i	u, m, w, i	u, w
R.TKYETEVSLR.Q[3]	u, m	u, m, w, i	u, m, w, i	u, m, w, i	u, w
R.QLVESDINGLRR.I[1,4]	u, m	u, m, w	u, w, i	u, w	w
R.QLVESDINGLR.R[1,4]	u, w, i	u, m, w, i	u, m, w, i	u, m, w, i	u, w, i
R.QLVESDINSLR.R[5]	w	u, m, w, i	u, m, w, i	u, m, w, i	u, w
R.QLVEADLNGLR.R[3]	w	w, i	u, m, w, i	u, m, w, i	w
R.ILDELTLCK.S[1,2,3,4,5]	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w
K.SDLEAQVESLKEELICLK.S[1,4]	u, m, w, i	u, m, w, i	u, m, w^{C3}, i	u, m, w, i	u, w
K.QNHEQEVNTLR.S[3,4]	u, w	u, m, w, i	u^{H1}, m, w, i	u, m, w^{H1}, i	u, w
K.SNHEEEVNTLR.S[1]	u, w	u, m, w, i	u, m, w, i	u, m, w, i	w
R.LNVEVDAAPTVDLNR.V[1,4,5]	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i
R.LNVEVDAAPTVDLNHVLNETR.A[3]	u, m, w	u, m, w, i	w, i	u, m, w, i	u, w
R.AQYEALVETNRR.D[1,3,4,5]	u, m	u, m, w, i	u, m, w, i	u, m, w, i	u, w, i
R.AQYEALVETNR.R[1,3,4,5]	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i
R.DVEEWYIR.Q[1,3,4,5]	u, m, w, i	u, m, w, i	u, m, w, i	u^{W2}, m, w, i	u, m, w
K.QVVSSSEQLQSYQAEIIELR.R[4,5,6]	u, w	u, m, w, i	u, m, w, i	w, i	w
R.TVNALEVELQAQHNLR.D[1,3,4,5]	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i
R.DSLENTLTETEAR.Y[1,2,3,4,5]	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i
R.YSCQLSQVQSLIVNVESQLAEIR.S[4]	u, m, i	u, m^{C2}, w, i	i	w, i	w
R.YSCQLSQVQSLIVSVESQLAEIR.S[5]	u, m	u, m	–	–	w
R.YSCQLNQVQSLISNVESQLAEIR.G[1]	u, m, i	u, m, w, i	w, i	–	w, i
R.QNQEYQVLLDVR.A[1,3,4,6]	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i
R.QNQEYQVLLDVK.A[5,7]	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i
R.LEAEINTYR.G[2]	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i
R.LESEINTYR.G[4,5]	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i
R.LECEINTYR.G[1,3]	u, w	u, w, i	u, m, w^{C3}, i	u, m, w^{C3}, i	u, m, w

the physical-mechanical degradation of fibres, while the oxidation of residues with aromatic side-chains (tyrosine, tryptophan, phenylalanine, and histidine) has been related to photo-oxidation (yellowing, discolouration, and eventually fibre breakage) (Dyer et al., 2010; Solazzo et al., 2012). However, these studies relate to thermal and photo-oxidative damage of wool and silk in museum environments and are unlikely to correlate well to degradation of samples during burial.

Oxidation of a specific phenylalanine (within the type II IFP-derived peptide FAAFIDK, Table 4) was found in the undyed and weld-dyed controls, and regularly in the buried samples for all but madder-dyed samples. More instances of oxidation were observed on cysteine, usually in the form of trioxidation, which is the complete oxidation from cysteine to cysteic acid. Cysteine oxidation was observed mainly in the IFPs for the undyed (nine peptides) and madder-dyed controls (seven peptides) and mainly in the KAPs for the weld- and indigo-dyed controls (five and four peptides). In all three sites, there is no significant increase of oxidised peptides, independent of the dye. In the sites of burial, oxygen supplies are limited; they are at a minimum at Marstrand, where less oxidised peptides are observed (on average three), and at a maximum at Rørmyra, where most oxidised peptides are observed (on average eight). Oxidative damage would be a more likely factor of degradation in museum environments than in the burial sites. It is noteworthy that oxidation in the control samples (in storage for 13 yr prior to analysis) is not null, reflecting a baseline level of oxidation that is independent of burial.

4. Discussion

4.1. Influence of the dye

In all sites, the samples dyed with weld and indigo, as well as the undyed samples, degraded and faded at a similar rate. The physical degradation of the samples dyed with madder, which is known to be the most washfast and lightfast of the natural dyes tested here, is slower than for the other samples, and in all three sites of burial these samples retain a distinctive red tone. Furthermore, madder has some antimicrobial activity that reduces bacterial growth on dyed wool and therefore helps in the preservation of the fabric (Gupta et al., 2004; Kalyoncu et al., 2006; Çalış et al., 2009). The most efficient extraction of proteins, however, is observed in the weld-dyed samples, resulting mainly in good sequence coverage of IFPs.

Both madder and weld dyes are mordant dyes; they bind to the protein chains in wool through an intermediate metallic complex, which is most often an aluminium ion in ancient recipes. The dyes bind to the aluminium complex via co-ordination to their hydroxyl and carbonyl groups and the complex in turn binds to the proteins primarily through association with the carboxylate groups of aspartic and glutamic acid side-chains. Fig. 4 shows the alizarin molecule, the main component of madder, and luteolin, the main component of weld. The presence of two extra hydroxyl groups in luteolin, the bulkiness of the molecule, and the ability to rotate around a C–C axis (as opposed to the planar structure of alizarin) would facilitate water circulation and make binding of weld less

Table 4
Identified tryptic type II peptides in controls and buried samples with protein modifications, and presence in undyed (u) and dyed samples (m, w, i). For oxidative modifications, *x* is indicated as follows: Oxidation (YWHC) + O → (YWHC)1, (YWHC) + 2O → (YWHC)2. Proteins in which peptides are found are indicated as follows: [1]K81, [2]K82, [3]K83, [4]K85, [5]K86, [6]K87.

Type II peptides	Controls	Lejre	M uncov	M cov	Rormyra
R.ISPGYSVTR.T[4,5]	u, m, i	u, m, w, i	u, m, w, i	u, m, w	u, m, w
R.TFSSCSAVAPK.T[4,5]	u, w	u, w	u, m, w	u, m, w, i	u, m, w
R.CCITAAPYR.G[1,3]	m, w, i	u, m, w, i	u, m, w	u, m, w, i	u, m, w, i
R.GLTGGFGSR.S[1,3]	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w
R.FAAFIDK.V[1,3,4,5,6]	u[F1], m, w[F1], i	u[F1], m, w[F1], i	u[F1], m, w[F1], i[F1]	u, m, w[F1]	u, m, w[F1], i
R.QCCESNLEPLFSGYIETLR.R[1,5]	u, m, w	u, w, i	m, w, i	u, m, w, i	w
K.LQFFQNR.Q[3]	u, m, w	u, m[F1], w	u, m, w, i	u, m, w, i	u, m, w
R.LSSELNSLQEVLEGYKK.R[1,5]	u, m, w	u, m, w, i	u, m, w, i	u, w, i	u, w, i
R.LSSELNSLQEVLEGYK.K[1,5]	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i
R.LSSELNHVQEVLEGYKK.K[3]	u, m	u, m, w	u, w, i	u, w, i	u, w
R.LSSELNHVQEVLEGYK.K[3]	u, m, w	u, m, w, i	u, m, w, i	u, m, w, i	u, w
R.LASELNHVQEVLEGYK.K[6]	u, m, w	u, m, w, i	u, m, w, i	u, m, w, i	u, w
K.KYEEEVALR.A[4]	u, m, w	u, m, w, i	u, m, w, i	u, m, w, i	u, w
R.YEEEVALR.A[1,4,5]	u, w	u, m, w, i	u, m, w, i	u, m, w, i	u, w
K.RYEEEIALR.A[5]	w, i	u, m, w, i	u, m, w, i	u, m, w, i	u, w, i
R.YEEEIALR.A[5]	u, m	u, m, w, i	u, m, w, i	u, w, i	u, w
R.ATAENEFVVLK.K[4]	u, w	u, w, i	u, m, w, i	u, m, w, i	u, w
R.ATAENEFVALKK.D[1,3,5]	u, m, w	u, m, w	u, m, w, i	u, w, i	u, w
R.ATAENEFVALK.K[1,3,5]	u, w, i	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i
K.DVDCAYLR.K[1,4,5]	u, m, w	u, m, w	u, m, w	u, m, w	w
R.KSDLEANVEALVEESNFLK.R[4]	u, w	u, m, w, i	m, w, i	u, m, w, i	u, w
R.KSDLEANSEALIQEIDFLR.R[3]	u, m, w	u, m, w, i	w, i	u, m, w, i	w
R.LYEEEIR.V[1,5]	u, m, w	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w
R.LYDEEIQILNAHISDTSVIVK.M[4]	u, m	u, m, w, i	m, w, i	u, w, i	u, w, i
R.VLQAHISDTSVIVK.M[1,5]	u, m	u, m, w, i	u, m, w, i	u, m, w, i	u, w
R.VLQANISDTSVIVK.M[3]	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i	w, i
R.DLNMDNIVAEIK.A[1,5]	u, m, w	u, m, w, i	u, w, i	u, m, w, i	w
K.AQYDDIASR.S[1,3,4]	u, m	u, m, w	u, m, w, i	u, m, w, i	u, m, w
R.AEAESWYR.S[1,3,4,5,6]	u, w	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w
R.TKEEINELNR.V[1,3,4,5,6]	u, m, w	u, m, w, i	u, m, w, i	u, m, w, i	u, w, i
K.LEAAVTQAEQQGEVALNDAR.C[3]	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i
K.LEAAVTQAEQQGEAALADAK.R[1,5]	u, w, i	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i
R.TKLEAAVAEAEQQGEAALNDAR.S[4]	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i
K.LEAAVAEAEQQGEAALNDAR.S[4]	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i
K.LAGLEEALQK.A[1,2,3,4,5]	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i
K.LGLDIEIATYR.R[1,2,3,4,5,6]	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i
R.LLEGEEQR.L[3,4,5,6,]	u, w, i	u, m, w, i	u, m, w, i	u, m, w, i	u, m, w, i
R.LCEGVGAVNVCVSSSR.G[3,5]	m, w, i	w	u, m, i	m, i	–

efficient. Madder, on the other hand, can also be used without mordant, indicating that some compounds also bind directly to the amino side groups of basic residues (lysine and arginine), and this is likely to lower trypsin efficiency.

Indigo is a vat dye and insoluble in its solid form. To extract the dye, indigotin needs to be reduced to its leuco-indigo form (white indigo). The leuco form is ionised in the alkaline dyebath, and under oxidation reverts to the insoluble coloured form (Fig. 4) (Božič and Vanja, 2008; Blackburn et al., 2009). The dye is trapped as a pigment within the fibre and its insolubility provides the high washfastness of indigo-dyed wool. At Marstrand, where the environment is reducing and the pH alkaline, indigo is more readily reduced to its soluble form and washed away. This is consistent with the observation that the Marstrand indigo-dyed samples discolour faster than at Lejre and Rørmyra. At Rørmyra, on the other hand, where rapid washing of the dye is prevented by the acidic pH of the environment, the samples take a greenish colour due to staining by the bog moss. Whenever indigo is still present in the samples (at Rørmyra but also in the control and 1- and 2-yr Lejre samples), fewer peptides, particularly IFPs, are observed, possibly suggesting a higher level of interaction of the dye with the keratins rather than with the keratin-associated proteins.

4.2. Influence of the site

At Lejre, degradation of samples is well advanced after 4 yr of burial, except for the madder-dyed fabric. A progressive loss of peptides is observed in the undyed and madder-dyed samples while the peptide profile of the weld samples remains unchanged and is characterised by a high number of peptides, in spite of the obvious deterioration of the samples. With the indigo-dyed samples, as mentioned earlier, the more degraded the samples get, the more peptides are identified. With a pH of 5.6 and a temperate climate, conditions at Lejre are conducive to biodegradation by microorganisms. Most keratinases are serine and metal proteinases, and work at neutral or alkaline pH (Korniłłowicz-Kowalska and Bohacz, 2011), optimally in the pH 6 to 9 range (Kunert, 2000). The process of keratin degradation involves the denaturation of the protein by sulphitolysis, in which the disulfide bridges are broken in the presence of sulphite produced by the fungi, creating cysteine and sulphocysteine. It is subsequently followed by proteolysis from the fungi's proteases (Kunert, 2000; Korniłłowicz-Kowalska and Bohacz, 2011). Sulphitolysis would probably degrade cysteine-rich peptides faster and might contribute to the low recovery of KAPs proteins at Lejre. The observed loss of cuticle would also indicate greater loss of the KAPs since the cuticle is made mainly of these proteins.

At Marstrand, degradation is also fast, with samples in poor condition after 7 yr. Degradation is faster for the uncovered samples as they are directly in contact with the sediments. The madder-dyed samples fare better and remain in better condition. The environmental conditions in Marstrand have been carefully monitored over the years of burial (Bergstrand and Godfrey, 2007; Godfrey et al., 2009) and are characterised by a neutral to alkaline

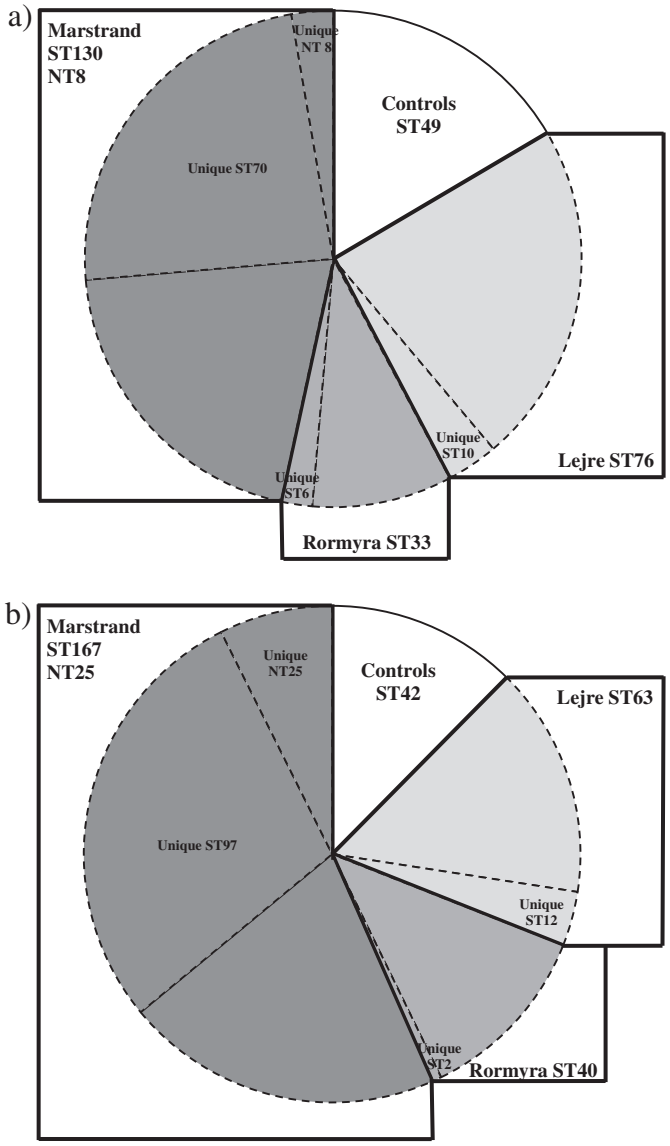

Fig. 3. Number of semi-tryptic (ST) and non-tryptic (NT) IFP peptides identified in the controls and in each site. The total number of peptides is indicated for Lejre (light grey), Rørmyra (grey), and Marstrand (dark grey), and the proportions of unique ST and NT peptides (peptides found only in one site) are indicated in the subdivisions. a-Type I peptides, and b-Type II peptides.

pH (pH 7.2–7.5) and reducing conditions, which are favourable for keratinolytic activity. Sulphate reduction was observed at Marstrand, leading to the increase of sulphites in the sediments as depth increases, and therefore indicating intense microbial activity. However, additional protein hydrolysis is evidenced by the increase with burial time of shorter peptidic fragments, characterised by protein-chain cleavage at non-tryptic sites, likely independent from experimental digestion with trypsin. Hydrolysis is manifested by the formation of peptides losing one residue after the other, starting from either the C- or N-terminus, or both. For example, peptide R.ILDELTLCK.S is hydrolysed on the C-terminus as R.ILDELTLC.K, and R.ILDELTL.C, on the N-terminus, as I.LDELTLCK.S and L.DELTLCK.S, and on both as I.LDELTLC.K. Cleavages at the C-terminus in particular are unlikely to originate from trypsin digestion. This type of hydrolysis is even more evident in the covered samples that are not in direct contact with the sediments and therefore more protected from microorganisms; in the 7-yr samples, semi- and non-tryptic peptides make up on average 63% of all IFPs in the covered samples but only 35% in the uncovered samples. The creation of non-enzymatic peptides is influenced by high temperatures, presence of water, and pH. During alkaline hydrolysis, a process practiced in industry at high temperatures for the disposal of animal waste, some amino acids, such as arginine, asparagine, glutamine, and serine, are destroyed (Thacker, 2004), and proteins are hydrolysed into free amino acids or small peptides of a few residues (Gousterova et al., 2005). Alkaline hydrolysis also occurs naturally in soil of neutral or alkaline pH to assist the decomposition of animal remains by microorganisms (Kaye et al., 2004). The extra formation of hydrolytic peptides in the indigo-dyed samples is interesting considering that the pH of the indigo vat has to be alkaline (pH 9) for indigo to solubilise. These results suggest that this process might influence weakening of the peptidic bonds and denaturation of the protein chains, and facilitate further degradation.

Such chemical hydrolysis could contribute to the degradation of proteins in a more selective way, with some amino acids being more readily lost. Interestingly, all covered samples see a large increase of KAPs in the 7-yr samples, maybe indicating that, in this case, better isolation from microorganisms results in better survival of KAPs (on average 45% of peptides identified as KAPs in the covered samples against 15% in the uncovered ones) as opposed to IFPs. This is potentially significant for archaeological textiles buried in a context that would protect them from microbial degradation.

At Rørmyra the fabrics remain in very good condition after 8 yr of burial in the acidic peat; they discolour with time, and lose their surface fibres but retain their physical integrity (shape and size). Rørmyra is a raised bog, characterised by a cold and acidic type of soil (pH = 5.0). It is rich in sphagnum moss, believed to be

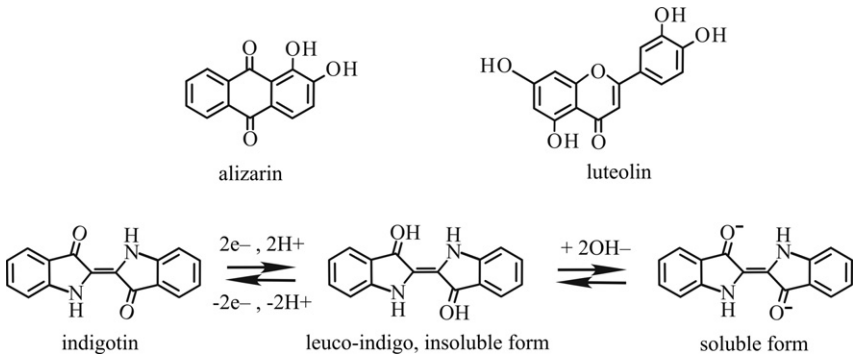

Fig. 4. Structure of alizarin (madder), luteolin (weld), and reduction of indigotin (indigo) into the soluble form of leuco-indigo.

responsible for the exceptional preservation of bog bodies through antibacterial and tanning properties (Stankiewicz et al., 1997; Turner-Walker and Peacock, 2008). The antibacterial effect of sphagnum moss has been attributed to its high cation exchange capacity, which limits the supply of cations for microorganisms (Hájek and Adamec, 2009). A high content of galacturonic acid in pectin (10–30% of the weight of sphagnum) was recently shown to inhibit microbial growth by lowering the pH (Ballance et al., 2012). Studies on bacterial populations have shown that the populations of *Firmicutes* and *Bacteroidetes*, two major bacterial groups responsible for degradation of biopolymers, were highly depleted in acidic sphagnum peat bog (Dedysh et al., 2006), and that most bacterial strains were inactivated below pH 4.6 (Stalheim et al., 2009). Furthermore, pectin is capable of chelating heavy metals, in particular zinc, needed by enzymes such as metalloproteases to be effective (Dronnet et al., 1996; Ho et al., 1996). The good physical condition of the Rørmyra samples is a consequence of the lack of biodegradation. However, fewer peptides are identified in comparison to the control samples. The proteomics results therefore suggest that some form of cross-linking might also contribute to the preservation of the samples. Painter described the process of protein tanning in sphagnum bogs as a Maillard reaction between carbonyl groups present in the moss and amino groups present either in the side-chains of basic amino acids lysine and arginine, or in amido groups of glutamine and asparagine (Painter, 1991, 1998). If this type of cross-linking is present, they would limit digestion of proteins at the lysine and arginine, affecting the IFPs more than the KAPs (composed mainly of cysteine, serine, glycine, threonine, and tyrosine). In the undyed, madder-, and indigo-dyed samples, numbers of IFPs are indeed low compared to KAPs, but the opposite is observed in the weld-dyed samples. Only at year 8 does the number of IFPs decrease while the percentage of KAPs increases.

5. Conclusions

Textile preservation in burial contexts is variable and both environmental condition and fibre processing significantly influence protein recovery. Binding of dyes on fibres alters protein solubilisation and digestibility, and removal of the dye prior to protein analysis might be considered, in particular with indigo-dyed fabrics. In ancient buried textiles, however, the dye is usually faded and less strongly bound to the fibres, as observed at Lejre and Marstrand, where protein identification is good in spite of the quantitative loss of proteins with time. Dyes such as madder help preserve wool longer but, ultimately, the environmental conditions of burial determine the way protein degradation occurs.

Microbial activity at sites such as Lejre and Marstrand leads to rapid fibre degradation and eventually complete destruction of the textiles through hydrolysis of proteins. However, at Marstrand, two mechanisms of protein hydrolysis (enzymatic and chemical) seem to compete, resulting in a unique set of peptides getting shorter and shorter. Enzymatic hydrolysis is more active in the uncovered samples, resulting for example in the complete destruction of the indigo-dyed sample after 7 yr, while chemical hydrolysis is more evident in the covered samples that might benefit from a better protection from microbial activity. Chemical hydrolysis in the covered samples is particularly evident after 7 yr when most of the non-tryptic peptides are identified. In these samples, less biodeterioration also seems to contribute to the survival of KAPs. At Rørmyra, the pH and vegetation composition of the bog prevent or reduce microbial activity. Reaction of sphagnum moss with amino acids has often been invoked to explain the preservation of bog bodies. The difficulty in getting good peptidic profiles indirectly proves the presence of cross-linking and our data suggest that this generally affects IFPs more than KAPs. Furthermore, the presence of dyes seems to influence further the interaction between fibre and moss, in particular for samples dyed with madder and indigo. Analysis after longer burial times would be particularly useful at Rørmyra to better understand the preservation mechanisms in place and the interaction between the keratins and their environment.

The proteomics evaluation strategy adopted here favours the identification of IFPs, and other studies on archaeological wool have shown the predominance of IFPs in the protein extract (unpublished data). This derives either from the excess of IFPs over KAPs, the complete loss of KAPs, or the poor solubilisation and digestion of the matrix and cuticle compared to the microfibrils. The KAPs are short and amorphous proteins and would be expected to degrade more readily than the IFPs under normal keratinolytic activity. However, KAPs are found in abundance in the covered 7-yr samples from Marstrand, showing that they can survive for long periods of burial in certain conditions, and are less sensitive than IFPs to chemical hydrolysis. KAPs also become predominant in situations where digestion of IFPs is poor, and microbial degradation is minimal. KAPs can therefore still be identified in buried samples if appropriate analytical strategies are adopted to select these proteins, and they could be useful biomarkers. They could, for instance, be used for differentiating between sheep breeds, as 2-D gel patterns have shown that KAPs present more variety in number of proteins, types and sequences and are less conserved than IFPs (Flanagan et al., 2002). In addition, KAPs are rich in cysteine and tyrosine and could also be used to assess degradation by oxidation. Amino-acid side-chain modifications were not significant during the short burial time, but archaeological woollen textiles show additional chemical changes occurring at the molecular level with long-term burial, in particular oxidation and deamidation (unpublished data).

To conclude, wool degradation can occur through a range of differing mechanisms, mainly due to the complex nature of wool itself with its many families of proteins and structures. Here we have shown that the specific burial conditions of the fibre play a major role in directing this degradation, and in determining which molecular degradation pathways predominate. These findings have significant implications in strategies for the evaluation of historical and archaeological textiles.

Acknowledgements

This research was supported by a Marie Curie International Outgoing Fellowship (FP7-PEOPLE-IOF-GA-2009-236425, THREADs) between the University of York, UK, and AgResearch, NZ. We wish to thank Dr. Santanu Deb-Choudhury and Matt Paulik from AgResearch, as well as Isabella von Holstein from BioArCh, for their technical help and advice.

Appendix ASupplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ibiod.2012.11.013.

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