NATURALLY DYED WOOL AND SILK AND THEIR ATOMIC C:N RATIO FOR QUALITY CONTROL OF ^{14}C SAMPLE TREATMENT

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ABSTRACT. Quality control of sample material (e.g. charcoal, collagen) is receiving considerable attention in the effort to obtain more reliable ^{14}C dates. The atomic carbon to nitrogen (C:N) ratio is a useful indicator of contamination and/or degradation of bone collagen. Wool and silk are also composed of proteinaceous material such as bone collagen, and the C:N ratio may also be a useful quality indicator for archaeological wool and silk. Analyses of modern undyed, mordanted, non-mordanted, and naturally dyed silk and wool were done in order to determine a C:N range that indicates the sample quality. The C:N range can be different for every material as the amino acid composition of wool, silk, and bone collagen are distinct. The measured minimum and maximum C:N values were used to set up a C:N range of uncontamined and undegraded wool and silk. Then, the C:N ratio and ^{14}C were analyzed of archaeological wool and silk samples. The applicability of the C:N ratio as a quality indicator for archaeological silk and wool was shown by the good agreement of the ^{14}C dates with the presumed historical dates for the uncontaminated samples and the disagreement of the ^{14}C dates with the presumed historical dates for contaminated samples.

KEYWORDS: C:N ratio, wool, silk, quality control.

INTRODUCTION

Wool and silk, and textiles in general, are gaining more attention as suitable radiocarbon dating material due to their short lifespan, potentially presenting the true age of an object made of these materials (Rageth 2004; Van Strydonck et al. 2004; van der Plicht et al. 2004; Kim et al. 2008; Mannering et al. 2010; Kuzmin et al. 2012; Van Strydonck and Grömer 2013; Vedeler and Bender Jørgensen 2013; Hajdas et al. 2014).

The quality control of sample material (e.g. charcoal, collagen) is receiving considerable attention in order to obtain more reliable ^{14}C dates (DeNiro 1985; Alon et al. 2002; Van Strydonck et al. 2005; Boudin et al. 2011). Boudin et al. (2011) demonstrated that humic substances are a major contaminant in archaeological wool and silk samples by nondestructive fluorescence spectroscopy analyses. However, degraded wool and silk may contain other contaminants such as mold, fungus, dirt, or other carbon-containing materials (Kim et al. 2008). Therefore, the atomic carbon to nitrogen (C:N) ratio could serve as a quality control indicator of archaeological wool and silk as it does for bone collagen to indicate contamination and/or degradation (DeNiro 1985; Schoeninger et al. 1989; Ambrose 1990). The C:N range can be different for every protein-containing material as the amino acid composition of wool, silk, and collagen are distinct. Wool consists mainly of the protein keratin, which is a α-helix structure with cystine, leucine, glutamic acid, arginine, and serine as the most abundant amino acids (Sibley and Jakes 1984). However, wool proteins are composed mainly of 21 amino acids, with variations in amino acid content due to breed of sheep, diet, climate, and other external influences (Gillespie et al. 1969; Maclaren and Milligan 1981; Leeder and Marshall 1982; Jones et al. 1998). Silk protein (fibroin) is a polypeptide polymer comprising 15 amino acids; the largest percentage (80%) in the fiber consists of the amino acids glycine, alanine, and serine in an approximate 3:2:1 ratio. These substances are small in size, with no large side groups. Therefore, the polypeptide chains can pack together closely as β-sheets (Sibley and Jakes 1984; Becker et al. 1997). For archaeological human and animal hair isotopic analysis, the C:N ratio can be used to indicate if the hair keratin is contaminated or not. Analysis of modern human hair defines a C:N range for uncontaminated archaeological hair between 2.9 and 3.8 (O'Connell

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and Hedges 1999a, 1999b; O'Connell et al. 2001). Taylor et al. (1995) conducted ^{14}C, C:N, and amino acid composition analyses on hair. The glycine/glutamine and glycine/aspartic acid ratios indicate well-preserved chemical structures of the hair. The C:N ratios of the archaeological hair fall within the range of 2.9 and 3.8, determined by modern human hair analyses. The ^{14}C dates of the hair were also in agreement with the archaeological expectations. This suggests that the C:N ratio may be a useful indicator for quality ^{14}C control of human hair and undyed wool (animal hair). However, mordanting and/or dyeing may alter the C:N ratio. Boudin et al. (2013) proposed a C:N range for modern undyed, mordanted, non-mordanted, and naturally dyed silk (all *Bombyx mori*). Based on their analyses of a small data set, the value was found be between 2.9 and 3.4.

Dyeing is an ancient art, which predates written records. It was practiced during the Bronze Age in Europe. Primitive dyeing techniques included sticking plants to fabric or rubbing crushed pigments into cloth. The methods became more sophisticated with time and techniques were developed using natural dyes from crushed fruits, berries, and other plants, which were boiled into the fabric and gave light and water fastness (resistance). Until the mid-19th century all dyestuffs were made from natural material, mainly vegetable and animal matter. Synthetic organic dyes were introduced in the mid-19th century, with mauveïne as the first synthetic organic dye produced in AD 1856 (Herbst and Hunger 1997).

Few natural dyes are color-fast with fibers. Mordants are substances used to fix a dye to the fibers (IUPAC 2006). Different types of mordants are available, including metals in the form of salts (chrome, cupper, tin, iron, aluminium), tannins, cream of tartar, baking soda, and vinegar. Different mordants will give different color with the same dye (Aspland 1997).

The classification of natural dyestuffs can be determined on the basis of the following:

- Chemical constitution of the dyestuff molecule. This can be based on the chromophore structure, as with anthraquinones (e.g. madder) or flavonoids (e.g. weld) or the derivatives of one dye (Dedhia 1998; Hofenk de Graaff 2004).
- The application method of the dyestuff. In the case of natural dyestuffs, only three groups are of importance (Gulrajani and Gupta 1992; Hofenk de Graaff 2004):

1. Dyestuffs that are soluble in water but form metal complexes on the fiber. They can only be applied with the help of water-soluble metal salts (mordants) and are therefore called *mordant dyes*.
2. Dyestuffs that are insoluble in water and therefore have to be first converted to their water-soluble form before application to the fibers. These are *vat dyestuffs*.
3. Dyestuffs that dissolve in water and can be applied as such. These are *direct dyestuffs*.

Mordanting and dyeing of wool and silk may alter the C:N ratio because most dyes and some mordants contain carbon and/or nitrogen atoms in their molecular structure, which are added to the textile fiber or fabric. Therefore, in this study, the C:N ratio of modern undyed, mordanted, non-mordanted, and naturally dyed silk (*Bombyx mori*) and wool were measured to determine a C:N range of uncontaminated and undegraded wool and silk. The viability of the established C:N range as a quality indicator for archaeological silk and wool was tested by comparing ^{14}C analysis of archaeological wool and silk with the presumed historical dates.

MATERIALS AND METHODS

Sample Selection

Different modern wool and silk fibers were dyed in the Textile Laboratory of the Royal Institute of Cultural Heritage (RICH), Brussels, Belgium. Some of the most frequently applied dyestuffs as listed in Table 1 (kermes, cochineal, madder, brazilwood, red sandalwood, Persian berries, barberry root, safflower, turmeric, dyer's broom, woad, indigo, orchil, galls) for wool and silk were used, but also less common dyestuffs were applied in this study as listed in Table 2.

Archaeological silk and wool samples from different geographical origins and with different color were selected to study (see Tables 4 and 5).

Design

First, C:N analyses of modern undyed, mordanted, non-mordanted, and naturally dyed silk and wool were conducted in order to determine their C:N range, by using the minimum and maximum value, for uncontamined and undegraded wool and silk. This study design is based on the papers of DeNiro (1985) and O'Connell and Hedges (1999a) wherein modern bone collagen and hair were analyzed to define a C:N range for uncontaminated bone collagen and hair.

Secondly, the C:N ratio, dye, and ^{14}C analyses were conducted of archaeological wool and silk samples. The viability of the C:N ratio as a quality indicator for archaeological silk and wool was tested by comparing the ^{14}C dates with the presumed historical dates. If the C:N ratio of the bulk did not fall within the proposed C:N range in this study and indicated contamination, cross-flow nanofiltration on the bulk samples was performed to remove contaminants and improve the sample quality by observing a C:N decrease (bulk samples that underwent cross-flow nanofiltration are referred to as permeate). The efficiency of cross-flow nanofiltration to obtain better sample quality and the used protocol are described in Boudin et al. (2013, 2014).

METHODS

Sample Pretreatment

Preliminary tests with the standard pretreatment protocol for archaeological wool and silk were performed on modern wool and silk. No C:N alteration of modern wool and silk was observed. Therefore, the modern undyed and dyed silk and wool samples were not pretreated prior to analysis. The archaeological wool and silk samples (also referred to as bulk samples) were pretreated with hexane, acetone, ethanol, Milli-Q™ water (Merck Millipore, Belgium), 1% NaOH, and 1% HCl for bulk ^{14}C and C:N analyses as described in detail in Boudin et al. (2011).

Atomic C:N Ratio

Analyses were performed in duplicate on a ThermoFlash EA/HT elemental analyzer. The standard used was acetanilide (0.5–1 mg was used).

[14]C Dating

[14]C dates were measured on the AMS at the Leibniz Labor für Altersbestimmung und Isotopenforschung in Kiel, Germany (lab code KIA) (Nadeau et al. 1998) or at the Royal

Table 1 The most frequently used natural dyestuffs for wool and silk, the timescale of practice, and its practice location (Hofenk de Graaff 2004).

	Timescale of dye practice	Dye practice location
Red dyestuffs		
Alkanna	Antiquity	Egypt, Mesopotamia, Romans
Henna	Antiquity	Palestine, Syria, Egypt
Coccid dyestuffs		
Kermes	Since antiquity	Europe, Near East, Asia
Polish cochineal	Since antiquity	Europe and Mediterranean countries
Armenian cochineal	Since antiquity	Europe, Near East, Far East
Cochineal	Since AD 1518 in Europe Since ancient times in Mexico Since antiquity in India and Japan Since AD 1790 in Europe	
Madder-type dyestuffs		
Madder	Since antiquity	Worldwide
Wild madder	Since antiquity	Mediterranean countries
Munjeet	Since antiquity in Far East Since 13th century in Europe	
Chay root	Since antiquity	India
Galium species	Since ancient times	Worldwide
Relbunium species	Pre-Columbian	South and Middle America
Indian mulberry	Since antiquity	India, Indonesia, and Polynesian Islands
Redwoods		
Brazilwood	Since ancient times in Orient and Far East Since Early Middle Ages in Europe	
Red sandalwood	Since ancient times in India Since middle of 16th century AD in Europe	
Safflower	Since antiquity in the Orient and southern Asia 2nd century BC in China and Japan 6th century BC in Persia Since 18th century in Europe	
Yellow dyestuffs		
Annatto	Pre-Columbian in Peru Since 16th century in Europe	
Barberry root	Before AD 1200	China North and South America Tibet
Yellowwoods		
Young fustic	Since antiquity	
Fustic	Since 16th century	Europe, Brazil, Argentina, Middle America
Quercitron	Since 16th century AD	Europe
Persian berries	Since antiquity	Europe, Near East
Saffron	Since antiquity	Far and Near East, and on a lesser scale in Europe
Turmeric (curcuma)	Since classical times	Polynesia, Micronesia, Melanesia, East Indies, Mesopotamia, Rome, and Greece
Weld	Before AD 1492	Europe
Dyer's broom	Since prehistoric times and early Middle Ages	Europe
Sawwort	Medieval times	Europe

Blue dyestuffs

Logwood	Since 16th century AD	Europe
Indigoid dyestuffs		
Woad	Since antiquity	Europe, Egypt, Mesopotamia, Syria and Palestine
Indigo	Sine antiquity in India Since early 17th century AD in Europe	
Indigo carmine	Since AD 1740	Europe

Purple dyestuff

Tyrian purple	Since antiquity, but production declined after AD 700 in Greece, Persia, and ancient Rome Dyeing continued until AD 1200 in Byzantium and Sicily Produced from *Murex* shells: provokes reservoir effect
Orchil	Antiquity but its use seems to have been lost in Middle Ages. In AD 1300, orchil was again imported into Europe by a Florentine merchant and more often used in Europe in the 16th century

Black dyestuffs

Gallotannin dyestuffs		
Galls	Ancient times: worldwide for silk Medieval times western Europe for wool	
Alder bark	Since antiquity	worldwide
Sumac	Since antiquity	worldwide
Catechu	Since antiquity	Far East
Black walnut	Since ancient times in the Near East Medieval times in Europe and America	

Institute for Cultural Heritage, Brussels (lab code RICH) (Boudin et al. 2015). CO_2 was obtained by sample combustion in the presence of CuO and Ag. Graphitization was done with H_2 over a Fe catalyst. Targets were prepared at the Royal Institute for Cultural Heritage in Brussels (Van Strydonck and Van der Borg 1990–1991). [14]C calibrations were performed using OxCal 3 (Bronk Ramsey 1995, 2001) and IntCal13 calibration curve data (Reimer et al. 2013).

Dye Analysis

Each dye analysis requires a sample between 0.2 and 0.5 mg. After pre-examination with a binocular microscope to avoid visible contamination, the dyes were extracted from the yarn using the following extraction method. To the sample of yarn, 250 μL water/methanol/37% hydrochloric acid (1/1/2, v/v/v) is added. The mixture is heated for 10 min at 105°C in open Pyrex™ tubes in a heating block. After cooling, it is filtered through a porous polyethylene frit. The filtrate is dried in an evacuated desiccator over NaOH pellets. The dry residues are taken up in 50 mL of methanol/water (1/1, v/v) and 20 mL of this solution is injected for analysis. The high-performance liquid chromatography (HPLC) equipment consists of a high-pressure pump (Model M615, Waters, USA), a photodiode array detector (Model 996, Waters, USA), and a system for data storage, manipulation, and retrieval (Empower, Waters, USA).

Table 2 Name (species) of dye source and its dye color of uncommon natural dyes used in this study.

Name (species) of dye source	Dye color
Grass (*Miscanthus tinctorius*)	yellow to light brown
Pagoda tree (*Sophora japonica*)	yellow to olive green
Red cedar (*Juniperus* berries)	brown
Pot marygold (*Calendula officinalis*)	yellow
Yellow chamomile (*Anthemis tinctoria*)	yellow
Birch (*Betula*)	yellow (leaves)-green (leaves)-reddish brown (bark)- yellowish brown (lichen)
Chamomile (*Chamaemelum nobile*)	yellow
Elder (*Sambucus nigra*)	green
Black mangrove (*Bruguiera gymnorrhiza*)	red-brown

Chromatographic conditions were applied using a temperature-controlled (20–22°C) Lichrosorb RP-18 column (4.0 × 125 mm, 5 mm particle size, VWR, Belgium). Three solvents are used: water, methanol, and 5% (w/v) phosphoric acid in water. The elution program is 60A/30B/10C for 3 min, followed by a linear gradient to 10A/80B/10C for 26 min. A flow rate of 1.2 mL/min was used.

Identification of the dye components was done by comparison of the spectral data with the reference spectra in the RICH database (130 references) at the maximum absorbance wavelength of each peak (Wouters 1985; Van den Berghe et al. 2009).

RESULTS AND DISCUSSION

C:N Analyses of Modern Wool and Silk

Comparing the C:N ratios of untreated textiles with mordanted and/or dyed textiles indicates that mordanting and/or dyeing may slightly change the C:N ratio (see Table 3). However, almost all mordanted and/or dyed textiles fall into the C:N ranges (see Table 3):

1. For hair/wool, proposed by O'Connell and colleagues (O'Connell and Hedges 1999a, 1999b; O'Connell et al. 2001): between 2.9 and 3.8;

2. For silk, proposed by Boudin et al. (2013): between 2.9 and 3.4.

This means that the C:N ratio may be a good indicator to control the sample quality of naturally dyed (until AD 1856) archaeological wool and silk for ^{14}C dating. The minimum value for wool in this study is 3.4 and is in perfect agreement with the theoretical C:N value of keratine. This allows us to refine the C:N range proposed by O'Connell and colleagues (O'Connell and Hedges 1999a, 1999b; O'Connell et al. 2001). The C:N range for uncontaminated wool should be between 3.4 and 3.8. The C:N range proposed by Boudin et al. (2013) for silk is valid for dyed and/or mordanted wool or silk, based on this data set, and can thus serve as an indication if a sample is contaminated or not.

Table 3 Atomic C:N ratio of undyed/dyed/mordanted modern wool and silk. The typical values are 3.0–3.2 for silk and 3.4–3.7 for wool.

Sample material	Dye and/or mordant	Atomic C:N
SILK		
Silk yarn I		
	Barberry root: mordanted and unmordanted	3.2
	Indigo: different dyeing duration	3.2
	Grass (*Miscanthus tinctorius*): alum mordant	**3.8**
	Pagoda tree (*Sophora japonica*): alum mordant	**3.3**
	Madder	3.1
Silk yarn J		
	Untreated	3.1
	Indigo	3.0
	Brazilwood: alum mordant	3.0
Silk yarn K		
	Cochineal: alum mordant	3.0
	Indigo: unmordanted	3.0
	Mordanted (alum + cream of tartar)	3.0
	Dyer's broom: alum mordant (with potash)	3.1
WOOL		
Rowan purelife		
British sheep breeds dk		
	Untreated	3.4
	Mordanted, undyed	3.5
	Indigo	3.5
	Persian berrie (*Rhamnus catharticus*): mordant: 8% alum + 7% cream of tartar	3.6
	Kermes: alum mordant	3.5
	Woad	3.5
	Red cedar (*Juniperus* berries): mordant: 8% alum + 7% cream of tartar	3.6
	Pot marygold (*Calendula officinalis*): mordant: 8% alum + 7% cream of tartar	3.6
	Safflower: mordant: 8% alum + 7% cream of tartar	3.6
	Red sandalwood: mordant: 8% alum + 7% cream of tartar	3.6
	Indian lac: mordant: 8% alum + 7% cream of tartar	3.6
	Turmeric: mordant: 8% alum + 7% cream of tartar	3.6
	Anatto: mordant: 8% alum + 7% cream of tartar	3.6
	Yellow chamomile (*Anthemis tinctoria*): mordant: 8% alum + 7% cream of tartar	3.7
	Birch (*Betula* sp.): mordant: 8% alum + 7% cream of tartar	3.7
	Chamomile (*Chamaemelum nobile*): mordant: 8% alum + 7% cream of tartar	3.7
	Dyer's broom: mordant: 8% alum + 7% cream of tartar	3.6
	Elder (*Sambucus nigra*): mordant: 8% alum + 7% cream of tartar	3.8
	Galls	3.7
	Madder	3.6

Table 3 (*Continued*)

Sample material	Dye and/or mordant	Atomic C:N
	Brazilwood	3.6
	Orchil	3.6
	Alum mordant medieval dyeing	3.6
	Caesalpinia; mordant: 25% alum, method extraction vinager	3.6
	Rubia tinctoria; mordant: 25% alum or 100% clubmoss	3.6
	Cudbear powder, unmordanted	3.5
	Bruguiera gymnorrhiza, mordanted alum or unmordanted	3.5
	Miscanthus tinctorius, mordanted alum or unmordanted	**4.2**
	Sophora japonica, mordanted alum or unmordanted	**4.3**
Wool A		
	Untreated	3.4
	Washed	3.4
	Mordanted	3.5
	Green weed no potassy (alum mordanted wool)	3.5
	Dyer's green weed with potassium	3.5
	Indigo on wool	3.5
	Indigo on wool (recipe I) (no mordanted wool)	3.4
	Safflower yellow	3.5
	Safflower (red) on wool	3.6
	Sappanwood on wool (mordanted)	3.5
	Cochineal, alum mordanted	3.4
	Gallic acid, mordanted with FeSO$_4$ and/or CuSO$_4$	**4.1**
	Rubia peregrina, dyes extracted from different plants	3.6
	Cocheneal, unmordanted, and mordanted with CuSO$_4$ or ZnCl$_2$	3.6
Wool B		
	Ochrolechia tartarea (lichen, moss)	3.6
	Umbilicaria pustulata (lichen, moss)	3.6
	Parmelia omphalodes (lichen, moss)	3.7
	Parmelia saxatilis (lichen, moss)	3.6
	Xanthoria parietina (lichen, moss)	3.7

The only exceptions (marked bold in Table 3) are the textiles dyed with *Miscanthus tinctorius*, *Sophora japonica*, and gallic acid. *Miscanthus tinctorius* and *Sophora japonica* were only used in Eastern Asian textiles (Bechtold and Mussak 2009). *Miscanthus tinctorius* ranges in color from yellow to light brown depending on the mordant, while *Sophora japonica* is yellow to olive green depending on the mordant. Gallic acid was used globally. Although gallic acid was often applied as a mordant, it can also be used as a dye, but very rarely (like in this study). When applied as a dye, it is more concentrated and consequently increases the C:N more than when used as a mordant. The obtained color of the dye gallic acid varies from yellowish brown to dark brown depending on the mordant.

Table 4 Archaeological site, sample color (used dye shown in parentheses if dye analysis was conducted), laboratory code, ^{14}C ages (BP), calibrated ages (2σ), presumed historical date, and atomic C:N ratio of analyzed archaeological uncontaminated wool samples.

Sample name	Archaeological site	Sample color	Atomic C:N	Lab code	^{14}C age (BP)	Calibrated age (2σ)	Presumed historical date
WOOL							
E29479	Egypt	ochre yellow	3.7	KIA-42363	1555±30	AD 420 (95.4%) 580	AD 400–700
E31963	Egypt	red	3.7	KIA-42370	1560±35	AD 410 (95.4%) 580	AD 400–700
N1027	Egypt	yellow-beige	3.7	KIA-42369	890±30	AD 1040 (95.4%) 1220	?
E29477	Egypt	yellow-beige	3.7	KIA-42372	1530±30	AD 430 (95.4%) 600	AD 400–700
KTN 2298	Egypt	beige	3.7	KIA-47945	1510±25	AD 430 (10.3%) 490 AD 530 (85.1%) 620	AD 500–700
Colutus	Egypt	blue (indigoid)	3.8	RICH-20058	1663±31	AD 250 (7.8%) 300 AD 310 (85.2%) 440 AD 490 (2.4%) 530	AD 400–500
Bourrelet	Egypt	red	3.7	KIA-39433	1675±30	AD 250 (11.8%) 300 AD 320 (83.6%) 430	?
E32155	Egypt	yellow	3.8	RICH-20309	1520±30	AD 430 (95.4%) 610	1st millennium AD
E31971	Egypt	orange	3.8	RICH-20305	1318±30	AD 650 (95.4%) 780	1st millennium AD
E31972	Egypt	red	3.8	RICH-20308	1515±30	AD 430 (95.4%) 620	1st millennium AD
E29477 bourrelet	Egypt	yellow	3.7	KIA-42372	1530±30	AD 420 (95.4%) 580	?
E31963 bourrelet	Egypt	red	3.7	KIA-42370	1560±30	AD 410 (95.4%) 580	?
KTN 2187	Egypt	yellow-brown	3.7	RICH-20382	1562±32	AD 430 (95.4%) 610	AD 600–800
KTN 857-03	Egypt	red-orange	3.7	RICH-20377	1315±28	AD 650 (95.4%) 780	AD 400–700
KTN 614	Egypt	green-red-yellow	3.7	RICH-20380	1321±29	AD 650 (95.4%) 780	AD 600–800
Hallstat 79-448	Austria	yellow-beige	3.6	KIA-42753	2485±30	780 (92.1%) 490 BC 470 (3.3%) 410 BC	800–400 BC
Hallstatt 07-065 C	Austria	red	3.8	KIA-42749	2980±30	1370 (2.1%) 1340 BC 1320 (93.3%) 1110 BC	1350 BC
Hallstatt 78-551	Austria	red-brown	3.8	KIA-42754	2445±30	760 (23.7%) 680 BC 670 (10.5%) 610 BC 600 (61.2%) 400 BC	800–400 BC
Hallstatt 79-429	Austria	yellow-brown	3.6	KIA-42752	2525±30	800 (29.5%) 720 BC 700 (65.9%) 530 BC	800–400 BC
Hallstatt 79-442	Austria	blue-black	3.6	KIA-42750	2555±30	810 (53.5%) 740 BC 690 (16.4%) 660 BC 650 (25.5%) 550 BC	800–400 BC
Hallstatt 78-552	Austria	light brown	3.5	KIA-42755	2380±30	720 (2.8%) 690 BC 540 (92.6%) 390 BC	800–400 BC

Table 4 (*Continued*)

Sample name	Archaeological site	Sample color	Atomic C:N	Lab code	^{14}C age (BP)	Calibrated age (2σ)	Presumed historical date
SILK							
Zandaniji Maaseik	South Iran	yellow-green (*Reseda luteola* + Morus type)	3.1	KIA-40826	1325±25	AD 650 (77.5%) 720	AD 700–900
Davidszijde Tx 606	? Iraq	red (Indian lac) red-orange-green	3.2 3.1	KIA-40827 KIA-48819	1285±25 1245±35	AD 660 (95.4%) 780 AD 670 (95.4%) 880	AD 700–800 AD 744–750 (Marwan II)
Tx 372	East Iran or Central Asia	yellowish brown (ellagic acid) - dark blue (red wood) - beige (indigoid)	3.1	KIA-48816	1190±35	AD 710 (5.1%) 750 AD 760 (83.2%) 900	AD 700–900
Tx 373 Tx 609	Byzantium Byzantium	brown dark brown-dark green-dark red (mainly tannic acids, kermes, madder, indigoid)	3.3 3.1	KIA-48815 KIA-48818	1255±30 1085±30	AD 670 (95.4%) 870 AD 890 (95.4%) 1020	AD 700–900 AD 700–900
Tx 371	Byzantium	orange (kermes, madder, traces of tannic acids)-green-white	3.0	KIA-48817	1195±30	AD 710 (4.3%) 750 AD 760 (88.8%) 900 AD 920 (2.2%) 940	AD 700–900
KTN 2400	Central Asia	brown	3.2	RICH-20059	1338±31	AD 640 (80.9%) 720 AD 740 (14.5%) 770	AD 600–800
KTN 2480 DM1923-01	Central Asia Egypt	brown beige	3.1 3.1	RICH-20059 KIA-42113	1288±28 1220±35	AD 660 (95.4%) 780 AD 680 (95.4%) 890	AD 600–800 AD 600–900

Table 5 Archaeological site, sample color, laboratory code, ^{14}C ages (**BP**), calibrated ages (2σ), presumed historical date, and atomic C:N ratio of analyzed archaeological contaminated wool and silk samples before (bulk) and after (permeate) cross-flow nanofiltration.

Sample name	Archaeological site	Sample color	Atomic C:N ratio	Lab code	^{14}C age (BP)	Calibrated age (2σ)	Presumed historical date
WOOL							
Beerlegem	Belgium						
bulk		brown	3.9	KIA-42365	1705 ± 30	AD 250 (95.4%) 410	AD 587
permeate			3.4	KIA-46083	1510 ± 25	AD 430 (10.3%) 490	
						AD 530 (85.1%) 620	
Mainz 2	Germany						
bulk		black	4.0	KIA-41535	2075 ± 25	180 (95.4%) 30 BC	5 BC
permeate			3.5	KIA-46193	2005 ± 20	50 BC (95.4%) AD 55	
Mainz 5	Germany						
bulk		black	4.0	KIA-37616	2080 ± 30	200 (94.3%) 20 BC	5 BC
						10 BC (1.1%) AD	
permeate			3.5	KIA-47417	1915 ± 35	AD (92.8%) 180	
						AD 190 (2.6%) 220	
SILK							
Baldwin 46	Belgium						
bulk		dark brown	3.6	KIA-47415	1260 ± 30	AD 660 (91.9%) 830	AD 1068
						AD 840 (3.5%) 870	
permeate			2.9	KIA-47418	1175 ± 30	AD 770 (83.1%) 900	
						AD 910 (12.3%) 970	
Baldwin 43	Belgium						
bulk		dark brown	3.7	KIA-47414	1210 ± 30	AD 690 (12.2%) 750	AD 1068
						AD 760 (83.2%) 900	
permeate			2.9	KIA-47811	1180 ± 30	AD 770 (86.0%) 900	
						AD 910 (9.4%) 970	

C:N Ratio, Dye, and [14]C Analyses of Archaeological Wool and Silk

Table 4 lists all the archaeological wool and silk samples from which the C:N ratio falls within the proposed C:N range of 3.4–3.8 for wool and 2.9–3.4 for silk, indicating uncontaminated samples. The ^{14}C dates of all the samples are in perfect agreement with the presumed historical date, suggesting the samples are not contaminated.

The sample color was visually determined prior to analysis and dye analyses were conducted on some samples. The detected dyes are shown in parentheses next to the determined sample color in Table 4. The analyzed archaeological yellowish and brown silk and wool samples from Egypt, Austria, south Iran, Byzantium, and Central Asia have a C:N ratio lower than the upper boundary, respectively, 3.8 for wool and 3.4 for silk. This excludes exogenous contamination or the use of gallic acid as a dye (see Table 4).

The C:N ratios of the bulk samples (wool and silk) fall outside the proposed C:N ranges, indicating contamination (see Table 5). Moreover, the ^{14}C ages of samples with untypical C:N ratios do not show a good agreement with the presumed historical date. Cross-flow nanofiltration decreased the C:N ratio and thus improved the sample quality. The obtained C:N ratio of the permeates falls within the proposed C:N ranges of uncontaminated wool and silk, indicating the absence of contaminants in the permeates. The ^{14}C dates of the cross-flow nanofiltrated wool samples (permeate) are in perfect agreement with the presumed historical dates, suggesting uncontaminated wool samples after cross-flow nanofiltration. The ^{14}C dates of the permeate of the silk samples (Baldwin 46 and Baldwin 43) are not in agreement with the presumed historical date, but this disagreement is probably due to the reuse of older (parts of) textiles as suggested by Van Strydonck and Bénazeth (2014).

The brown color of the contaminated samples Beerlegem, Baldwin 46, and Baldwin 43 are likely due to the presence of humic substances, as shown by nondestructive spectrofluorescence analyses in Boudin et al. (2011, 2014), and also demonstrated by the decrease of C:N ratio of the permeate after cross-flow nanofiltration as well as the good agreement of the ^{14}C date with the presumed historical date.

CONCLUSION

The C:N ratio is a good indicator for sample quality (contamination) of naturally dyed (until AD 1856) archaeological wool and silk for ^{14}C dating. The C:N range indicating good (uncontaminated) sample quality is between 2.9 and 3.4 for silk and between 3.4 and 3.8 for wool. If the C:N ratio of the archaeological sample is greater than the upper boundary of the C:N range of wool and silk, respectively, 3.8 and 3.4, then the following may be true:

1. The sample may be dyed with *Miscanthus tinctorius* or with *Sophora japonica* in the case of Eastern Asian textiles or with gallic acid (used worldwide). A dye analysis can reveal if one of these three dyes was applied on the textile. If the dye analysis is positive, the sample is not contaminated and can undergo ^{14}C analysis.
2. However, if the dye analysis is negative (none of the aforementioned dyes detected), the sample is contaminated and should be rejected for ^{14}C dating, with the too-high C:N ratio indicating contamination. An option to avoid dye analysis is taking samples for ^{14}C dating with a different color than the color obtained by applying these three dyes.

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