Radiocarbon

An International Journal of Cosmogenic Isotope Research

VOLUME 56 / NUMBER 2 / 2014

14 & ARCHAEOLOGY

7th International Symposium 8-12 April 2013, Ghent, Belgium

Guest Editors Mark Van Strydonck, Philippe Crombé, and Guy De Mulder

Radiocarbon, Vol 56, Nr 2, 2014, p 603–617 DOI: 10.2458/56.16951

AN ARCHAEOLOGICAL MYSTERY REVEALED BY RADIOCARBON DATING OF CROSS-FLOW NANOFILTRATED AMINO ACIDS DERIVED FROM BONE COLLAGEN, SILK, AND HAIR: CASE STUDY OF THE BISHOPS BALDWIN I AND RADBOT II FROM NOYON-TOURNAI

Mathieu Boudin[1,2] • Pascal Boeckx[2] • Peter Vandenabeele[3] • Mark Van Strydonck[1]

ABSTRACT. Excavations in the cathedral of Tournai revealed two sepultures, which were identified by the excavators as those of bishops because of their special location in the cathedral. One burial was assigned to Baldwin I, who died in AD 1068, because (1) a ring with the inscription "BAL" was found and (2) a funeral stone with text was present on top of the grave mentioning the name Baldewinus. The second burial probably belongs to Radbot II, who was the successor of Baldwin I, and died in AD 1098. Both burials contained textiles (silk), the skeleton, a wooden pastoral staff, and human hair was still present on the skull of what was presumed to be Radbot II. All the protein-containing materials were degraded and/or contaminated. Standard sample pretreatment methods were not able to remove all the contaminants. Single and double cross-flow nanofiltration of the hydrolyzed protein-containing materials were performed. The sample quality for radiocarbon dating was improved and ^{14}C data revealed interesting and surprising results. The ^{14}C dates of the wooden pastoral staff and permeate femur confirm that the skeleton and tomb belong to bishop Baldwin I. The ^{14}C dates of hair and permeate skull indicate that the skeleton may indeed belong to bishop Radbot II. The younger ^{14}C dates of the wooden pastoral staff and silk samples indicate a postburial disturbance of the site burial during the 12th–13th century.

INTRODUCTION

Tournai Cathedral is one of Belgium's heritage sites and was listed in 2000 as a UNESCO World Heritage site. A team from CRAN (Centre de recherche d'archéologie nationale), Université Catholique de Louvain (Belgium), excavated the cathedral and its surroundings between 1996 and 2010 in order to understand the configuration of the monuments that preceded the present cathedral (Brulet 2012a). The 12th century cathedral was preceded by a fairly large 11th century cathedral, a smaller Carolingian church, and by a basilica from the beginning of the early Middle Ages.

The complete plan of the 11th century cathedral could be reconstructed; it was the first cathedral whose existence was attested in the written sources. In that church, a flight of stairs gave access to the split-level choir. At the foot of the second flight of steps, the intact tombs of two 11th century bishops were excavated: Baldwin I (year of death: AD 1068) and probably Radbot II (year of death: AD 1098) (Brulet 2012a,b). This was quite surprising because normally the bishops of Noyon-Tournai were buried in Noyon (France) before the autonomy of the diocese of Tournai in AD 1146. Both tombs contained textiles (silk), the skeleton, a wooden pastoral staff, and human hair was still present on the skull of Radbot II. The wooden pastoral staff is a symbol of the pastoral care of the bishop for the faithful and was added to the tomb of bishops during the Middle Ages (den Hartog 2012).

The aim of this research was to verify whether the skeletons could be assigned to Baldwin I and Radbot II. This study presents the results of the analysis of protein-containing samples after standard pretreatment as well as after hydrolysis into amino acids and cross-flow nanofiltration. Cross-flow nanofiltration of the hydrolyzed protein leads to more accurate ^{14}C results, as described in Boudin et al. (2013).

1. Royal Institute for Cultural Heritage, Jubelpark 1, B-1000 Brussels, Belgium. Corresponding author. Email: Mathieu.boudin@kikirpa.be; mathieu.boudin@ugent.be.
2. Ghent University, Isotope Bioscience Laboratory – ISOFYS, Faculty of Bioscience Engineering, Coupure Links 653, B-9000 Ghent, Belgium.
3. Ghent University, Department of Archaeology, Sint-Pietersnieuwstraat 35, B-9000 Ghent, Belgium.

Proceedings of the Radiocarbon and Archaeology 7th International Symposium
Ghent, Belgium, April 2013 | Edited by Mark Van Strydonck, Philippe Crombé, and Guy De Mulder

MATERIALS AND METHODS

Sample Selection

Table 1 lists the bone, hair, and silk samples for analyses derived from the burials of Baldwin I and Radbot II, chosen to represent different preservation states. The table also refers to our sampling of the wooden pastoral staff, probably a branch or young tree, which excludes an old-wood effect.

Table 1 Samples derived from the burials of Baldwin I and Radbot II, chosen to represent different preservation states, in order to determine if the skeletons can belong to Baldwin I and Radbot II.

	Sample name	
Analyzed material	Baldwin (AD 1068)	Radbot II (AD 1098)
Hair (keratin)		Hair
Bone collagen	Skull Femur	Skull fragment A Skull fragment B Bone 80 tibia
Silk	43A 43B 46	20 22 49
Wood	Wooden pastoral staff, species *Prunus*	Wooden pastoral staff, species probably *Ligustrum vulgare*

Sample Preparation

Bulk Analyses

Collagen was extracted from the bones following the Longin (1971) method. A 1% NaOH wash was introduced between the demineralization and hydrolization steps for 15 min. The hair sample was pretreated with hexane, acetone, ethanol, Milli-Q water (Merck Millipore, Belgium), 1% NaOH, and 1% HCl as described in detail in Boudin et al. (2011) for bulk ^{14}C and stable isotope analysis. All silk samples could only be pretreated with hexane, acetone, ethanol, and Milli-Q water as they dissolved completely during the NaOH step.

Extracted bone collagen, pretreated hair, and silk samples will be referred to as *bulk* in this paper. The wood samples underwent AAA (acid-alkali-acid) treatment by using 1% HCl and 1% NaOH in 1-hr washes at 90°C.

Treatment for Cross-Flow Nanofiltrated Amino Acid Analyses

Nanofiltration is a pressure-driven membrane process and is characterized by a membrane pore size between 0.5 and 2 nm and operating pressures between 5 and 40 Bar. During cross-flow filtration, the feed flow travels tangentially across the surface of the filter. The main advantage of this filtration is that the filter cake or clogging layer (which can block the filter) is substantially washed away during the filtration process, thereby increasing operation time of a filter unit (Koros et al. 1996).

The in-house-developed filtration installation and the protocol used is described by Boudin et al. (2013). A ceramic filter with a cutoff of 200 Dalton (Da) was used in order to selectively collect amino acids in the permeate of hydrolyzed protein samples (molecular weight of amino acids varies between 75.07 and 204.23 Da) and the humic substances (HSs) (molecular weight of HSs varies be-

tween ~1000 and 300,000 Da) in the retentate (Stevenson 1982). This filter can theoretically retain 90% of 200 Da and greater molecules by a single cross-flow nanofiltration. Performing a second cross-flow nanofiltration of permeate (double cross-flow nanofiltration) should increase the retention of contaminants. Consequently, a better C:N ratio should be obtained and thus a more accurate ^{14}C date.

For cross-flow nanofiltrated amino acid analyses, the pretreated bulk samples underwent the following steps:

1. Hydrolysis of ~15–50 mg material using 2 mL 6M HCl in a sealed tube under N_2 atmosphere at 110°C for 24 hr;
2. The hydrolyzate was filtrated through a 0.7-μm glass fiber filter (Millipore APFF03700);
3. The filtrate was diluted to 100 mL with Milli-Q water;
4. Diluted filtrate was treated by cross-flow nanofiltration;
5. The permeate, which comprises the cross-flown nanofiltrated amino acids, was freeze-dried;
6. The freeze-dried permeate was dissolved in 1 mL Milli-Q water;
7. 50 μL of solution was transferred in a tin cup and dried at 50°C for stable isotope and C:N analyses; and
8. The remaining solution was transferred into a quartz tube with CuO and Ag and dried in a desiccator for graphite production and subsequent ^{14}C analysis.

Sample Quality Assessment

Stable Isotopes ($\delta^{13}C$ and $\delta^{15}N$), %C, %N, and C:N Ratio

Carbon and nitrogen stable isotope compositions were measured as the ratios of the heavy isotope to the light isotopes ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) and are reported in delta (δ) notation as parts per thousand (‰), where $\delta^{13}C$ or $\delta^{15}N = ([R_{sample}/R_{standard}] - 1) \times 1000$, and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$, relative to internationally defined standards for carbon (Vienna Pee Dee Belemnite, VPDB) and nitrogen (ambient inhalable reservoir, AIR).

Analyses were performed in duplicate on a Thermo Flash EA/HT elemental analyzer, coupled to a Thermo DeltaV Advantage Isotope Ratio Mass Spectrometer via a ConfloIV interface (ThermoFisher Scientific, Bremen, Germany). Standards used were IAEA-N1, IAEA-C6, and internally calibrated acetanilide.

Analytical precision was 0.25‰ for both $\delta^{13}C$ and $\delta^{15}N$ based on multiple measurements of the standard acetanilide. Stable isotope ($\delta^{13}C$ and $\delta^{15}N$) C:N ratio analyses were done on the bulk samples and the permeate of the hydrolyzed protein sample. %C and %N were determined from the pretreated bulk samples.

Fluorescence Spectroscopy

Nondestructive fluorescence spectroscopy (Cary Eclipse, Varian, Belgium) analyses of the textile samples were carried out using a fiberoptic probe. Spectra were acquired in the excitation wavelength range of 340–475 nm and 509 nm as the emission wavelength. The fluorescence intensity measured between 460 and 475 nm originates from humic substance (HS) presence (Simpson et al. 1997). Therefore, a slope was calculated using a linear fit (least squares) to the curve between 465 and 475 nm. The fluorescence slope can be used as a qualitative indicator for HS presence:

1. Negative slopes indicate HS absence and consequently an uncontaminated sample; and
2. Positive slopes indicate HS presence, indicating sample contamination.

The method is described in detail in Boudin et al. (2011).

Classification of Uncontaminated vs. Contaminated

Spectrofluorescence analyses and C:N ratio determinations were obtained on pretreated bulk silk and hair samples to classify the samples as uncontaminated or contaminated. The C:N ratio of the bone collagen samples was used to classify the collagen samples as uncontaminated or contaminated. The applied criteria to define uncontaminated archaeological samples were as follows:

1. Collagen: C:N ratio between 2.9 and 3.6 (DeNiro 1985; Ambrose 1990);
2. Wool and hair: C:N between 2.9 and 3.8 and a negative fluorescence slope (O'Connell and Hedges 1999a,b; O'Connell et al. 2001; Boudin et al. 2011); and
3. Silk: C:N between 2.9 and 3.4 and a negative fluorescence slope (Boudin et al. 2011, 2013).

Samples not fulfilling these conditions were defined as contaminated. A higher C:N is the result of introduction of exogenous carbon-containing compounds (i.e. contamination).

Classification of Well-Preserved vs. Poorly Preserved (Degraded)

The amount of carbon and nitrogen present in the bulk sample (bone gelatin, hair, or silk) in relation to the bulk weight was determined and will be referred to as carbon and nitrogen weight proportion in percent (%C and %N). These two quality indicators provide information on protein degradation. A higher %C than the average %C of modern bulk may indicate contamination.

The %C and %N of modern undyed, mordanted, non-mordanted, and naturally dyed silk (n = 14, all *Bombyx mori*) were determined, as there were no data available in the literature; the values were 44.3 ± 2.6 and 16.7 ± 0.7, respectively. Ambrose (1990) cites a collagen weight %C and %N range for modern bone and tooth between 15.3 to 47% and 5.5 to 17.3% for C and N, respectively. Hair protein contains ~45%C and 15%N (Benfer et al. 1978).

The amount of extracted collagen (gelatin) in relation to the weight of the whole bone sample will be referred to as collagen weight proportion in percent (% collagen). The % collagen is a quality indicator for collagen preservation. The threshold used in our laboratory is 2%; bones containing less than 2% collagen are considered poorly preserved.

^{14}C Dating

Dried samples were transferred into quartz tubes with CuO and Ag and combusted to CO_2. Graphitization of CO_2 was carried out using H_2 over a Fe catalyst. Targets were prepared at the Royal Institute for Cultural Heritage in Brussels (Belgium) (Van Strydonck and van der Borg 1990–1991) and ^{14}C concentrations were measured with accelerator mass spectrometry (AMS) at the Leibniz Labor für Altersbestimmung und Isotopenforschung in Kiel (Germany) (Nadeau et al. 1998). ^{14}C results are expressed in pMC (percentage modern carbon) and indicate the percent of modern (1950) carbon corrected for fractionation using the δ^{13}C measurement. Calibrations of ^{14}C dates and χ^2 test (weighted mean) calculations were performed using OxCal v 3.10 and the IntCal09 calibration curve data (Shennan 1988; Bronk Ramsey 1995, 2001; Reimer et al. 2009).

RESULTS AND DISCUSSION

Baldwin I

Table 2 lists the results of the analyses of the samples from the burial of Baldwin I.

Bone Collagen

The %C, %N, and % collagen of the bulk (collagen) samples indicate degradation (diagenetic alteration or breakdown), while introduction of exogenous carbon-containing compounds (contamination) is observed from their C:N ratio (Table 2).

The permeate C:N ratio of sample Skull was not within the C:N boundaries of uncontaminated samples; hence, an unreliable ^{14}C date is obtained (Table 2). On the other hand, the permeate C:N of Femur falls with the desired C:N range and results in a more accurate ^{14}C date (Table 2).

However, the calibrated ^{14}C date (AD 890 (95.4%) 1020) of sample Femur is too old compared to the presumed historical date of AD 1068 (Brulet 2012b). Stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) indicate that freshwater fish was part of the diet, which provokes a reservoir effect on the ^{14}C date, explaining the too-old ^{14}C date (Table 2):

1. Elevated $\delta^{15}N$ indicates aquatic (marine or freshwater) food consumption but cannot distinguish marine from freshwater;

2. The $\delta^{13}C$ indicates freshwater fish consumption mixed with terrestrial food, while marine foods have a more positive $\delta^{13}C$ and thus can be excluded here (Lanting and van der Plicht 1996, 1998; Cook et al. 2001, 2002).

Stable isotope comparisons of femur permeate with the average values of Belgian Medieval human bones indicates a diet enriched above that of individuals who likely ate a mainly vegetable and terrestrial animal diet. In Figure 1, the average $\delta^{13}C$ and $\delta^{15}N$ for animal bones and human bones from a Belgian Medieval context (Ervynck et al., these proceedings) provide an indication of human diet unaffected by fish. We therefore conclude that freshwater fish was a part of Baldwin I's diet (Figure 1).

It is also striking that the stable isotopes of permeate of Baldwin I's femur are almost equal to Waldetrudis' stable isotopes (Table 3 and Figure 1). Waldetrudis and her husband Vincentius are known as 7th century AD promoters of the Christian faith and became saints shortly after their death (Deveseleer 1999, 2001). The published ^{14}C date for Vincentius coincides with the historical context for this saint (Deveseleer 1999, 2001; Van Strydonck et al. 2009) and his stable isotope values indicate a mixed diet of terrestrial plant and animal products (Müldner 2009), while the ^{14}C date for Waldetrudis, who is known to have died around the same date as her husband, was significantly older than expected (5th–6th century AD) (Van Strydonck et al. 2009) but can be explained by a freshwater reservoir effect suggested by the stable isotope values (Table 3 and Figure 1). At first sight, the difference in diet between wife and husband might seem surprising, but historical records indicate that both persons ended their lives staying in separate monasteries (Deveseleer 1999, 2001), in which food habits or rules could well have been rather different (Van Strydonck et al. 2009).

Tournai is located next to the River Scheldt and Soignies, the city of Vincentius and Waldetrudis, is situated on the River Senne. The Senne is an affluent of the River Dyle, while the Dyle is an affluent of the Scheldt. It is therefore highly probable that a similar reservoir effect was present in these

Table 2 Laboratory codes, ^{14}C ages (BP), calibrated ages (2σ), stable isotopes ($\delta^{13}C$ and $\delta^{15}N$), C:N before (bulk) and after cross-flow nanofiltration (permeate), %C, %N, % collagen and fluorescence slope values of samples derived from Baldwin I's burial (presumed historical date: AD 1068).

Sample name	Lab code	^{14}C age (BP)	Calibrated age (2σ)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Atomic C:N	%C	%N	% collagen	Fluorescence slope
Bone collagen										
Skull										
bulk	KIA-47420	1185 ± 30	AD 720 (1.9%) 740 AD 770 (87.7%) 900 AD 910 (5.8%) 950	−19.5	16.6	4.8	14.0	3.4	0.55	
permeate	KIA-47422	930 ± 30	AD 1020 (95.4%) 1170	−19.2	16.4	3.9				
Femur										
bulk	KIA-47421	1510 ± 30	AD 430 (14.9%) 490 AD 500 (80.5%) 630	−19.7	15.0	3.8	15.2	4.7	0.71	
permeate	KIA-47423	1080 ± 30	AD 890 (95.4%) 1020	−20.4	15.0	3.4				
Silk										
43A										
bulk		n.a.		−25.9	7.5	3.8	45.2	13.9		2.33
permeate	KIA-47419	1305 ± 30	AD 650 (95.4%) 780	−24.9	6.6	3.0				
43B										
bulk	KIA-47414	1210 ± 30	AD 690 (12.2%) 750 AD 760 (83.2%) 900	−24.5	6.8	3.7	45.6	14.4		2.07
permeate	KIA-47811	1180 ± 30	AD 770 (86.0%) 900 AD 910 (9.4%) 970	−23.5	6.2	2.9				
46										
bulk	KIA-47415	1260 ± 30	AD 660 (91.9%) 830 AD 840 (3.5%) 870	−24.9	6.3	3.6	42.4	13.9		2.62
permeate	KIA-47418	1175 ± 30	AD 770 (83.1%) 900 AD 910 (12.3%) 970	−24.1	6.1	2.9				
Wood										
Wooden pastoral staff	KIA-47416	1010 ± 30	AD 960 (95.4%) 1160							

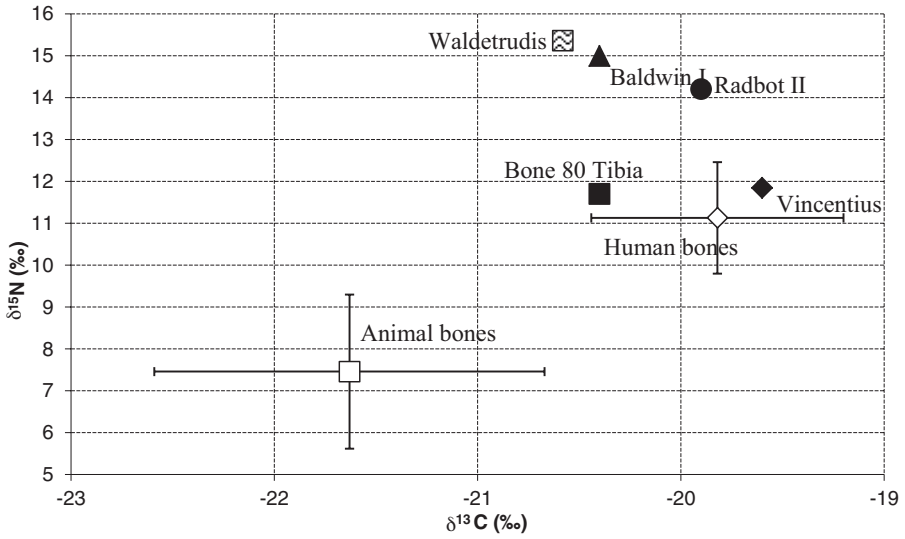

Figure 1 Stable isotope data ($\delta^{13}C$ and $\delta^{15}N$) from Radbot II, Baldwin I, Bone 80 tibia derived from Radbot II's burial, Vincentius, and Waldetrudis compared with measurements from Medieval animal (n = 107) and human (n = 234) bones (Ervynck et al., these proceedings).

two rivers. A reservoir correction was done on the ^{14}C date of the permeate of Baldwin I's femur applying a simple linear relationship between $\delta^{13}C$ or $\delta^{15}N$ and the calculated age offset between the ^{14}C dates of Vincentius and Waldetridus (Table 3) (Cook et al. 2001). For the permeate from Baldwin I's femur, the corrected ^{14}C age determined with $\delta^{13}C$ is in accord with the corrected ^{14}C age determined with $\delta^{15}N$, and their calibrated ages correspond with the presumed historical date (AD 1068) (Table 3).

Silk

Three archaeological silk samples were analyzed (Table 2). The %C and %N of the archaeological bulk silk samples correspond to the values of modern analyzed silk samples. This indicates good preservation of the archaeological silk samples. However, the C:N ratio and positive fluorescence slope indicate contaminated bulk samples and the fluorescence slope suggests HS contamination. The bulk ^{14}C dates of these samples are therefore considered unreliable.

The permeate ^{14}C dates of the silk samples are considered reliable because the C:N of the permeate falls within the specified C:N boundaries for uncontaminated silk. However, the bulk and permeate ^{14}C date of 43B are not statistically different, applying the χ^2 test (df = 1, T = 0.5(5% 3.8)). The possible HS contamination can be explained by *in situ* humification. The permeate ^{14}C dates of the silk samples 43A, 43B, and 46 are too old compared to the presumed historical date (AD 1068) for Baldwin I's death (Brulet 2012b). However, previous research has shown that in several cases, (parts of) textiles were reused and this may explain the older ^{14}C dates of the permeate compared to the presumed historical date (Bénazeth and Van Strydonck 2006).

Wood

The ^{14}C date of the wooden pastoral staff, probably a branch or young tree that excludes an old-wood effect, is in agreement with the presumed historical date (AD 1068).

Table 3 Laboratory codes, uncorrected and freshwater reservoir-corrected ^{14}C ages of bone samples from Baldwin I and Radbot II (BP), calibrated ages (2σ), stable isotopes ($\delta^{13}C$ and $\delta^{15}N$), C:N, age offset (BP) in bone from Vincentius and Waldetrudis.

Sample name	Lab code	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Atomic C:N	^{14}C age (BP)	Age offset (BP)	Corrected ^{14}C age (BP) with $\delta^{15}N$	Calibrated age (2σ)	Corrected ^{14}C age (BP) with $\delta^{13}C$	Calibrated age (2 σ)
Vincentius	KIA-10575	−19.6	11.9	3.3	1385 ± 35					
Waldetrudis	UtC-9694	−20.6	15.4	n.a.	1530 ± 40	145 ± 53				
Radbot II skull fragment A permeate	KIA-46957	−19.9	14.2	3.4	1025 ± 25		891 ± 59	AD 1020 (95.4%) 1260	885 ± 59	AD 1020 (95.4%) 1260
Radbot II skull fragment B bulk	KIA-47968	−20.4	14.5	3.6	995 ± 25		858 ± 59	AD 1030 (95.4%) 1280	851 ± 59	AD 1020 (95.4%) 1260
Baldwin I femur permeate	KIA-47423	−20.4	15	3.4	1080 ± 30		938 ± 61	AD 980 (95.4%) 1220	936 ± 61	AD 990 (95.4%) 1230

Conclusion for Baldwin I

^{14}C dates of the wooden pastoral staff and permeate of femur indicate that the burial can be assigned to Baldwin I, which is supported by the associated grave goods. The latter were a ring with the inscription "BAL" and a funeral stone with text was present on top of the grave. The name Baldewinus and his burial date, 28 April 1068, are mentioned (Brulet 2012b). An average ^{14}C date of the wooden pastoral staff (KIA-47416: 1010 ± 30 BP, Table 2) and corrected ^{14}C date of Femur permeate (938 ± 61 or 936 ± 61 BP, Table 4) can be calculated: Baldwin I: 996 ± 27 BP (95.4% probability: AD 970–1170; χ^2 test: *df* = 1, T = 1.1(5% 3.8)) is in perfect agreement with the presumed historical date (AD 1068).

Radbot II

Table 4 summarizes the analysis results of samples from the burial of Radbot II.

Hair

The %C and %N of the archaeological hair sample correspond with the percentages of well-preserved hair protein, 45%C and 15%N (Benfer et al. 1978). The bulk C:N of hair (3.8) indicates an uncontaminated sample, while the positive fluorescence slope indicates HS contamination. The ^{14}C dates of the bulk and permeate hair samples match and an average can be calculated (Table 4). Possible HS contamination can be explained by *in situ* humification where hair organic matter (predominantly keratin) generates HS through the Maillard reaction (Maillard 1913; Gillespie and Hedges 1983). However, the average ^{14}C date is too old compared to the historical date (AD 1098). Stable isotope analysis suggests that freshwater fish was an important part of this individual's diet (Figure 1). A reservoir effect for freshwater organisms could thus explain the anomaly in the ^{14}C date and will be discussed further using the results of bone collagen, extracted from the skull of the Radbot II sample (Table 4).

Bone Collagen

The %C, %N, and % collagen of the collagen of Skull fragment A indicate degradation, while introduction of exogenous carbon-containing compounds (contamination) is observed from its C:N ratio (Table 4). The collagen of Skull fragment B is quite well-preserved, shown by %C and %N and uncontaminated shown by its C:N, which is at the top of the acceptable C:N range for uncontaminated collagen. The collagen of Bone 80 tibia is very well-preserved, as indicated by %C, %N, and % collagen, and uncontaminated, proven by its C:N.

Cross-flow nanofiltration of the hydrolyzate of Skull fragment A resulted in a C:N decrease (Table 4) and a more accurate ^{14}C date should be obtained. A good agreement between the ^{14}C date of the permeate of the contaminated collagen of Skull fragment A and the bulk ^{14}C date of the uncontaminated collagen of reference sample Skull fragment B is observed and a weighted mean can be calculated: 1010 ± 18 BP, χ^2 test: *df* = 1, *T* = 0.7(5% 3.8). However, the calibrated ^{14}C date (AD 985 (95.4%) 1035, 95.4% probability) is too old compared to the presumed historical date (AD 1098) (Brulet 2012b). The stable isotope values indicate that freshwater fish was part of the diet, which provokes a reservoir effect on the ^{14}C date, explaining the too-old ^{14}C date (Figure 1 and Table 4). The same reservoir correction is applied on permeate of Skull fragment A and on the bulk of Skull fragment B as with Baldwin I (Table 3). The corrected ^{14}C ages for both samples determined with δ^{13}C and δ^{15}N are now in accord with each other, and the calibrated ages correspond with the presumed historical date of AD 1098 (Table 3).

Table 4 Laboratory codes, ^{14}C ages (BP), calibrated ages (2σ), stable isotopes ($\delta^{13}C$ and $\delta^{15}N$), C:N before (bulk) and after cross-flow nanofiltration (permeate), %C, %N, % collagen, and fluorescence slope values of samples derived from Radbot II his burial (presumed historical date: AD 1098).

Sample name	Lab code	^{14}C age (BP)	Calibrated age (2σ)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Atomic C:N	%C	%N	% collagen	Fluorescence slope
Hair (keratin)										
Hair										
bulk	KIA-46953	1025 ± 25	AD 970 (95.4%) 1040	−21.6	12.8	3.8	40.8	12.8		0.25
permeate	KIA-46955	1040 ± 20	AD 970 (95.4%) 1025	−20.3	12.8	3.4				
Average (weighted)		1034 ± 16	AD 985 (95.4%) 1025							
Bone collagen										
Skull fragment A										
bulk	KIA-46954	1165 ± 25	AD 770 (77.4%) 900 AD 910 (18.0%) 970	−20.4	13.7	3.7	11.3	3.5	1.70	
permeate	KIA-46957	1025 ± 25	AD 970 (95.4%) 1040	−19.9	14.2	3.4				
Skull fragment B										
bulk	KIA-47968	995 ± 25	AD 980 (70.4%) 1050 AD 1080 (25.0%) 1160	−20.4	14.5	3.6	33.5	10.6	1.23	
Average (weighted) of KIA-46957 and KIA-47968		1010 ± 18	AD 985 (95.4%) 1035							
Bone 80 tibia										
bulk	KIA-46959	980 ± 25	AD 990 (48.6%) 1060 AD 1070 (46.8%) 1160	−20.4	11.7	3.1	41.4	15.6	8	
Silk										
20										
bulk	KIA-46960	900 ± 25	AD 1040 (95.4%) 1220	−20.7	8.1	4.4	41.1	10.9		2.07
permeate single filtration	KIA-46979	845 ± 25	AD 1150 (95.4%) 1260	−19.5	7.2	3.8				
permeate double filtration	KIA-47574	775 ± 25	AD 1215 (95.4%) 1280	−19.4	7.3	3.6				

Table 4 (*Continued*)

Sample name	Lab code	^{14}C age (BP)	Calibrated age (2σ)	δ^{13}C (‰)	δ^{15}N (‰)	Atomic C:N	%C	%N	% collagen	Fluorescence slope
22										
bulk	KIA-46958	890 ± 35	AD 1030 (95.4%) 1220	−20.6	8.1	4.1	41.9	11.8		1.49
permeate single filtration	KIA-46970	875 ± 25	AD 1040 (17.8%) 1100 AD 1120 (77.6%) 1230	−20	7.8	3.6				
49										
bulk	KIA-46952	910 ± 25	AD 1030 (95.4%) 1210	−20.6	7.5	4.5	41.7	10.8		1.95
permeate single filtration	KIA-46956	815 ± 25	AD 1175 (95.4%) 1270	−20.4	7	3.6				
Wood										
Wooden pastoral staff	KIA-46574	815 ± 25	AD 1175 (95.4%) 1270	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	KIA-47969	850 ± 35	AD 1040 (8.8%) 1090 AD 1120 (2.6%) 1140 AD 1150 (84.0%) 1270	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Weighted mean		827 ± 20	AD 1170 (95.4%) 1260							

The calibrated age of Bone 80 tibia is also in agreement with the presumed historical date (AD 1098) (Table 4). However, this bone comes from another individual, demonstrated by:

1. Aberrant stable isotopes compared to permeate of Skull fragment A. Stable isotope values of Bone 80 tibia fall into the cluster of Belgian Medieval humans indicating a mixed diet of terrestrial plant and animal products and no fish consumption (Figure 1 and Table 4).
2. Collagen yield (8%) is much higher than from the skull (1.23–1.70%) (Table 4).
3. Bone 80 tibia was visually very different than all the other bones from Radbot II.
4. Bone 80 was very hard (only possible to cut it with a microdrill) and all the other bones were very fragile (breakable manually).

We therefore speculate that Bone 80 tibia was added to the burial and this practice was not uncommon in relic shrines (Van Strydonck et al. 2006, 2009).

Silk

Three archaeological silk samples were analyzed (Table 4). The %C and %N of the bulk archaeological silk samples correspond with the values of the modern analyzed silk samples. This corroborates the good preservation of the archaeological silk samples. However, the C:N ratio and positive fluorescence slope indicate contaminated bulk samples, and the fluorescence slope suggests HS contamination. The bulk ^{14}C dates of these samples are therefore considered unreliable. The permeate C:N ratio of all the silk samples was still not within the C:N boundaries of uncontaminated samples, even after double cross-flow nanofiltration for sample 20 (Table 4). Thus, all the ^{14}C dates should be treated with extreme caution.

Increasing the number of cross-flow nanofiltrations results in a lower C:N for sample 20, and thus improved sample quality for ^{14}C dating (Table 4). The C:N decrease between bulk, permeate of single cross-flow nanofiltration, and permeate of double cross-flow nanofiltration corresponds with a decreasing ^{14}C date. The ^{14}C date of the permeate after double cross-flow filtration cannot be considered reliable due to its too high C:N, unless this C:N value is caused by *in situ* humification. If *in situ* humification is not the case, introduction of exogenous carbon contaminants could explain this C:N value. In the latter case, the ^{14}C date of the permeate after double cross-flow filtration should be considered a *terminus post quem* date. The bulk and permeate ^{14}C dates of sample 20 are statistically not different when applying the χ^2 test ($df = 1$, $T = 0.1(5\%\ 3.8)$), despite the C:N decrease and thus better ^{14}C sample quality after filtration. The removed contamination is therefore possibly HSs, which were formed by *in situ* humification.

For sample 49, the permeate ^{14}C date is younger than the bulk ^{14}C date, which indicates contamination by older exogenous carbon. Therefore, the less reliable ^{14}C date of the permeate due to their C:N can be considered a *terminus post quem* date, Unless the C:N values, out of the C:N range for uncontaminated silk, are provoked by *in situ* humification.

None of the silk dates are in agreement with the presumed historical date of AD 1098.

Wood

The calibrated ^{14}C date from the weighted mean of the wooden pastoral staff was AD 1170–1260 (95.4% probability; Table 4), not in agreement with the presumed historical date of Radbot II's death (AD 1098). The wooden pastoral staff was a small branch or young tree, which excludes an old-wood effect.

Conclusion for Radbot II

The ^{14}C dates of the bulk and permeate of hair, the ^{14}C date of permeate Skull fragment A, and the ^{14}C date of bulk Skull fragment B indicate that the skeleton can be assigned to Radbot II. If we assume that the C:N ratios of the silk samples are caused by *in situ* humification, then we observe a good agreement between wood ^{14}C date and two of the three silk ^{14}C dates (Figure 2). But if the C:N ratios of the silk samples, out of the expected range for uncontaminated samples, are caused by younger exogenous carbon, then younger ^{14}C dates for the silk samples are expected. The wood ^{14}C date and the silk ^{14}C dates indicate that perhaps a "disturbance" occurred between AD 1170 and 1260 (which is the calibrated ^{14}C date for the wooden pastoral staff). The discrepancy leads to two questions: was Radbot II reburied there in the 13th century, or were the clothes and the wooden pastoral staff added to the burial in the 13th century?

It is, though, an interesting coincidence that the ^{14}C dates correspond well with the construction of the new choir, which began in AD 1242 and ended in 1255, shown by the black vertical line in Figure 2), and the following arguments support the possibility of a 13th century activity in the burial site:

- Bishop Gautier de Marvis (AD 1219–1252) had the original Romanesque choir demolished in the 13th century in order to replace it with a Gothic choir of larger dimensions, inspired by the likes of Amiens Cathedral (ICOMOS 2000).
- Both episcopal burials were explored in the two ancient choirs of the new cathedral and were situated in the 11th cathedral, in the narthex and at the bottom of the stairs that lead to the choir of the current cathedral (Brulet 2012b).
- The two burials were placed in the center of the building. Two blue stone plates without inscription marked the location of the tombs. The concrete floor that enclosed the tombs was restored after the insertion of these funerary stone plates (Brulet 2012b).

This 13th century activity in the grave was really unexpected as the cathedral, constructed during the 12th century, is located 2.5 m higher than the 11th century cathedral. The ancient structures were completely buried by a layer of rubble and also the episcopal tombs. But the concrete floor of the Roman church was not conserved at this location, which may indicate the presence of an element that localized these episcopal tombs (Brulet 2012b).

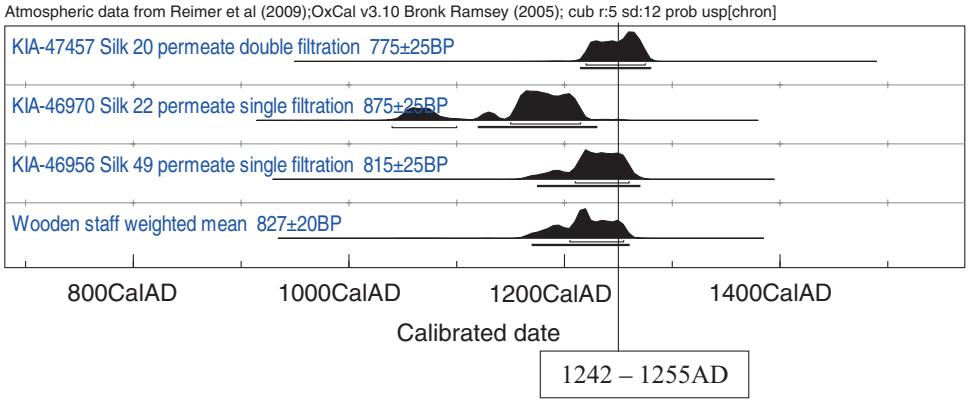

Figure 2 Calibrated ^{14}C ages of the weighted mean of wooden pastoral staff and of permeate silk samples derived from the burial of Radbot II in the case of *in situ* humification.

CONCLUSION

The degraded bone collagen and silk material derived from Baldwin I and Radbot II were contaminated. Carbon, nitrogen, and fluorescence spectroscopy on pretreated *bulk* samples indicated that standard sample pretreatment methods were insufficient to remove all the contamination for ^{14}C dating. Similar results from analyses of permeate samples treated by cross-flow nanofiltration of hydrolyzed contaminated protein-containing samples indicate that it is a viable technique to improve sample quality for ^{14}C dating. However, single cross-flow nanofiltration may not be sufficient to obtain a C:N ratio within the boundaries of the uncontaminated sample. Double cross-flow nanofiltration may be a solution, as shown by silk sample 20 of Radbot II, but requires further investigation.

Regarding Baldwin I, the ^{14}C date of the wooden pastoral staff, the freshwater reservoir-corrected ^{14}C date of permeate femur, the excavated ring with the inscription BAL, and the funeral stone with the inscription Baldewinus together indicate that the skeleton and tomb belong to bishop Baldwin I. The older permeate ^{14}C dates of the silk samples indicate reuse.

For Radbot II, the freshwater reservoir-corrected ^{14}C dates of the hair and permeate skull indicate that the skeleton may belong to bishop Radbot II. The younger ^{14}C dates of the wooden pastoral staff and silk samples indicate a postburial disturbance of the site during the 12th–13th century, which is supported by several historical arguments. For example, a bone was also added to this burial, which was a practice not uncommon in relic shrines.

ACKNOWLEDGMENTS

The authors wish to thank the European Project "Clothing and Identities. New perspectives on textiles in the Roman Empire (DressID)" (Contract Nr. 2007 – 1765 / 001 – 001 CTU COOPMU) for financial support for ^{14}C dates; Anita Buekenhoudt, Fanny Van Cleven, and Michelle De Brueker for their help; and in particular Professor Raymond Brulet who provided samples for this study. This research (WI/39/J05) was also funded by the Belgian Science Policy.

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