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RETREATMENT OF BONE MATERIAL IN THE GLIWICE RADIOCARBON LABORATORY USING ULTRAFILTRATION

Fatima Pawełczyk[1]* • Magdalena Niedziałkowska[2] • Sławomira Pawełczyk[1] • Natalia Piotrowska[1] • Maciej Sykut[2]

[1]Institute of Physics – CSE, Silesian University of Technology, Konarskiego 22B, 44-100 Gliwice, Poland
[2]Mammal Research Institute Polish Academy of Sciences, Stoczek 1, 17-230 Białowieża, Poland

ABSTRACT. Preparation of bones for radiocarbon (^{14}C) dating is still quite a challenge for researchers. The methods are being tested and improved, to increase reliability of dating results and to verify the previous ones. In this work, a set of gelatine samples, extracted from *Cervus elaphus* and *Cervus canadensis* bones from various sites in Europe and a set of human bones from archaeological sites in Poland were subjected to retreatment using ultrafiltration in Gliwice Radiocarbon Laboratory. The tested samples represent a wide range of ages, from older than 40,000 ^{14}C years BP to modern. The prepared material was subjected to the measurement of C/N atomic ratios and ^{14}C dating using the accelerator mass spectrometry (AMS) technique. Also, the stable isotopes (δ^{13}C and δ^{15}N) values were determined. In a few cases ultrafiltration allows to improve gelatine quality for long-stored samples, by increasing the %C and %N as well as decreasing C/N_{at} ratios. Nevertheless, this effect was not observed for majority of the samples. Remeasurements of long-term stored samples give mostly the same ^{14}C ages for ultrafiltered ones and for those without ultrafiltration.

KEYWORDS: AMS dating, bones, ^{14}C, ultrafiltration.

INTRODUCTION

Radiocarbon (^{14}C) dating of bone material has been a challenge for researchers since the first attempts, giving results contradicting archaeological context. The age offset can be caused by contamination with exogenous carbon. In a natural burial environment these are mostly humic acids reacting with the collagen triple helix (Van Klinken and Hedges 1995). The geochemistry of the environment in which the bone was deposited is important for the collagen degradation and contamination. Another source of contamination with exogenous carbon, in case of archaeological collections, can be preservation agents, like wax, consolidants, adhesives, varnishes, glue or resin (Fedi et al. 2014; Brock et al. 2018; Crann and Grant 2019). These substances cause the effect of altering the ^{14}C date, with the magnitude depending on the age of the bones, their preservation level, and the age of exogenous carbon. Because of that, the results obtained on bone samples in the past were often rejected (Taylor 1992).

Today, ^{14}C dating is done on the extracted collagen or its fractions (not bulk bone) and even small samples, below 100 mg (Fewlass et al. 2019), or very old samples, near the limit of the ^{14}C method (Hajdas et al. 2009) were dated successfully. Most of the methods are based on the classical Longin method of collagen isolation (Longin 1971), supplemented by the step of ultrafiltration (Brown et al. 1988), which removes low-molecular weight material. The ultrafiltration method has been a subject of wide research since it was first used. Different preparation improvements have been tested to solve the encountered problems, like different acid strength and decalcification time (Shammas 2009; Talamo and Richards 2011), gelatinization temperature (Beaumont et al. 2010) or different ultrafilters (Talamo et al. 2021). It was also noticed that the type of vessels and time of heating (as well as heating apparatus used) may affect the gelatinization step (Brock et al. 2013a). Some of the researchers underline that there is a risk of sample contamination at the ultrafiltration step (e.g., Bronk Ramsey et al. 2004; Hüls et al. 2007, 2009; Beaumont et al. 2010; Brock et al. 2013b), so the improvements of ultrafiltration protocols covered also increased intensity of the ultrafilters precleaning (Bronk Ramsey et al. 2004; Brock et al. 2007).

*Corresponding author. Email: fatima.pawelczyk@polsl.pl

Besides ultrafiltration, other methods have also been used. It was proved that chromatographic isolation of collagen-specific peptides by HPLC improves ^{14}C dating of contaminated bones (Van Klinken and Hedges 1992; Van Klinken et al. 1994). Hydroxyproline is supposed to be a bone-specific biomarker, since it constitutes about 10% of bone collagen (Marom et al. 2012, 2013; Marom-Rotem 2012). According to this, a more specific approach is, for example, the chromatographic separation of hydroxyproline or purification of amino acids with XAD-2 resin (e.g., Stafford et al. 1982; Gillespie et al. 1984; Stafford et al. 1988; McCullagh et al. 2010; Marom et al. 2013; Deviese et al. 2018; Minami and Nakamura 2000; Yuan et al. 2000). Another preparation method is the reaction of ninhydrin with amino acids, performed by Nelson (1991), who proved the applicability of this method to any protein, as it allowed to obtain a well-purified sample. Additionally, some experiments to date the bioapatite fraction have been proved successful (e.g., Cherkinsky 2009). For an overview of methods used for preparation bones for ^{14}C dating see Herrando-Pérez (2021).

When ^{14}C dating bones, an assessment of gelatine quality should be performed. A few criteria for that assessment have been adopted. In this study we used the values for %C, %N and C/N_{at} ratios as quality indicator, with range of the values published by Ambrose (1990): 15.3–47% for carbon and 5.5–17.3% for nitrogen, by mass. The C/N ratio for collagen is 3.2 and samples with C/N value between 2.9 and 3.6 are considered acceptable, while higher values suggest contamination with exogenous carbon (DeNiro 1985; Ambrose 1990). Another marker is %N in raw bone, which is an estimation for the amount of the surviving collagen and should be at least 5% for a bone to be considered as useful (DeNiro and Weiner 1988; Van Klinken and Hedges 1992).

In this study, we analyze human and animal bones of different ages that come from many different places in Europe and Western Asia. Among them, there are samples from bones of red deer (*Cervus elaphus*) and wapiti (*Cervus canadensis*). The red deer is a savanna-type deer with a mixed feeding strategy that inhabits mainly Europe and southwestern Asia. Wapiti is a more cold-adapted open-country grazer that inhabits dry, cold, continental regions of East Asia and North America (Geist 1998).

A large set of red deer and wapiti bones was dated between 2015 and 2019 to investigate the spatial distribution of this species in Europe and western Asia since the Late Pleistocene (Doan et al. 2017; Niedziałkowska et al. 2021; Doan et al. 2022). These samples came from various types of sites—caves, river valleys, dunes and sites located in the vicinity of human settlements (for detailed information about the localities, species and type of material used see the supplementary material). Some of the bone samples were very poorly preserved and gave unsatisfactory results of carbon and nitrogen concentrations and carbon to nitrogen atomic ratios. Therefore, we decided to test them again, using a pretreatment extended with ultrafiltration.

Furthermore, we used a set of seven archaeological samples from Poland that came from human bones and deer antler. These samples are numbered 1–7 and have to remain anonymized due to copyright. Finally, 21 samples were chosen for ^{14}C analysis by accelerator mass spectrometry (AMS). Additionally, three reference samples of known age were tested. Our goal was to conduct ultrafiltration tests in the Gliwice Radiocarbon Laboratory to implement this method as a standard method of bone preparation for the AMS ^{14}C dating. We also wanted to investigate the effect of ultrafiltration on long-stored collagen samples.

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MATERIALS AND METHODS

In this work, we prepared two batches of samples, previously dated in our laboratory. Batch 1 comprised seven samples from human bones and deer antler from four archaeological sites located in Poland (see Table 2). The gelatine was extracted previously in our laboratory, following a standard preparation. It included mechanical abrasion of the bones, cleaning in demineralized water in an ultrasonic bath, then drying and grinding in a hand mortar to ~1 mm particles. The gelatine extraction was performed according to the Longin method (Longin 1971), modified by Piotrowska and Goslar (2002), with the use of hydrochloric acid and alkali solution at room temperature. The 0.5 M hydrochloric acid was replaced several times, and the reaction was considered complete when the pH stabilized at < 1 and no bubbles were observed. The insoluble residues were rinsed with demineralized water and treated with 0.1 M sodium hydroxide for 30 min. Then, after rinsing with demineralized water and acid, the residues were subjected to gelatinisation in acid (pH=3) for at least 12 hours in 80°C and dried.

In the present study, these gelatine samples were redissolved and filtered through precleaned 9 mL Ezee Filter™ separators (Elkay). These separators are polypropylene tubes with sintered polyethylene filters. Then, every sample was divided volumetrically into two equal subsamples: A and B. Set "A" was freeze dried (Alpha 1-2 LD plus Martin Christ) after filtration, then graphitized and measured. Set "B" was additionally subjected to the ultrafiltration. This step was carried out using Millipore Amicon® Ultra-15 ultrafiltration tubes (Merck), carefully precleaned following the protocol used at the Ion Beam Physics Laboratory at the ETH Zurich, which is based on Bronk Ramsey et al. (2004) and Brock et al. (2007). The ultrafilters are produced from high recovery Ultracel regenerated cellulose membrane of 30 kD molecular weight cut-off. The cleaning protocol included rinsing in demineralized water, then ultrasonic bath for 15 min and triple centrifugation to remove any residual humectant added by the manufacturer. The gelatine samples with an initial volume of 15 mL were then centrifuged at 4400 rpm for 5–20 min to remove the fraction <30 kD and collect the heavy-molecule fraction >30 kD. The obtained supernatant having a volume of at least 1500 µL was then freeze dried, graphitized, and measured in the same way as in the set "A". Additionally, batch 1 included three gelatine samples: SIRI C, VIRI E and VIRI H from international ^{14}C intercomparison programmes (Scott et al. 2010, 2017; see Table 1). They were also divided into two subsets—A and B, as above—and subjected to the same ^{14}C preparation and dating procedures.

Batch 2 consisted of 14 gelatine samples of red deer and wapiti bones from Russia, Italy, Belarus, Ukraine, Armenia, and Austria, prepared in our laboratory and AMS measured in 2015–2019 (see also the supplementary material). The preparation was slightly different—with the exclusion of alkali treatment, to minimize sample loss. The bone samples, after being abraded and cleaned in an ultrasonic bath in demineralized water, were dried and ground in a ball mill (Pulverisette 6, Fritsch). Next, the bone powder was demineralized in hydrochloric acid and the insoluble residues were rinsed with demineralized water and subjected to gelatinisation, same as batch 1. In this study, the dried gelatine samples stored at room temperature were redissolved and then filtered with precleaned Ezee Filters. Next, every sample was subjected to ultrafiltration and then freeze dried, graphitized, and measured in the same way as batch 1, serie B.

The graphitization step was performed using an AGE-3 system with a VarioMicroCube by Elementar elemental analyzer and an automated graphitization unit (Nemec et al. 2010; Wacker et al. 2010). The analyzer was calibrated with the use of aspartic acid and glutamic acid

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Table 1 Results of weight% of C and N, C/N atomic ratios (C/N_{at}) determinations, and ^{14}C measurements for international ^{14}C intercomparison samples (from batch 1); Treatment methods: A = without ultrafiltration, B = with ultrafiltration; Previously obtained ^{14}C age values after Piotrowska et al. (2019) are listed in italics as set C (without the ultrafiltration step); Consensus values after Scott et al. (2010) for VIRI and Scott et al. (2017) for SIRI.

Lab code (GdA-)	Sample name	Treat. meth.	Date of graphitization	%C	%N	C/N_{at}	Age ^{14}C (BP ± 1σ)	Consensus value (BP)
5339	SIRI C	A	Dec 2020	22.2	7.8	3.3	39700 ± 1100	
		B	Dec 2020	21.9	7.8	3.3	41200 ± 1400	>46550
		C	*Jul 2017*	*28.1*	*10.6*	*3.1*	*44980 ± 1740*	
5341	VIRI H	A	Nov 2020	35.6	12.3	3.4	9450 ± 50	
		B	Dec 2020	37.8	13.1	3.4	9430 ± 50	9528 ± 7
		C	*Jul 2017*	*37.3*	*13.5*	*3.2*	*9535 ± 45*	
5342	VIRI E	A	Dec 2020	36.7	12.9	3.3	35030 ± 610	
		B	Nov 2020	42.9	15.1	3.3	36150 ± 690	39305 ± 21
		C	*Jul 2017*	*41.3*	*14.9*	*3.2*	*35600 ± 480*	

Table 2 Results of the% weight of C and N, C/N_{at} and ^{14}C measurements of anonymized archaeological samples—human bones but GdA-6163 (Sample 7)—deer antler (from batch 1). Samples were divided into two sets of subsamples: A, treated without ultrafiltration, and B, treated with ultrafiltration. In addition, the values obtained previously were listed in italics (= Set C, without the ultrafiltration step). Small samples (below 200 mg of raw sample) were marked with an asterisk(*).

Lab code (GdA-)	Sample name	Treat. meth.	Date of graphitization	Gelatine yield (%)	%C	%N	C/N_{at}	Age ^{14}C (BP ± 1σ)
5407	Sample 1	A	Nov 2020	14.65	44.8	15.7	3.3	340 ± 30
		B	Nov 2020	9.45	45.7	16.2	3.3	335 ± 30
		C	*Feb 2018*		*30.4*	*12.4*	*2.9*	*370 ± 20*
5957	Sample 2	A*	Dec 2020	15.51	42.3	14.6	3.4	2410 ± 30
		B*	Nov 2020	6.39	44.4	14.8	3.5	2415 ± 30
		C	*Aug 2019*		*40.8*	*14.5*	*3.3*	*2470 ± 35*
5958	Sample 3	A*	Dec 2020	6.14	37.2	12.8	3.4	2400 ± 30
		B*	Nov 2020	0.77	45.9	11.7	4.6	2495 ± 35
		C	*Aug 2019*		*35.6*	*12.8*	*3.3*	*2425 ± 38*
6097	Sample 4	A	Nov 2020	5.98	41.5	14.4	3.4	830 ± 30
		B	Nov 2020	3.15	45.5	15.6	3.4	880 ± 30
		C	*May 2020*		*51.0*	*12.7*	*4.7*	*929 ± 29*
6098	Sample 5	A	Dec 2020	3.59	20.1	7.1	3.3	815 ± 30
		B	Dec 2020	0.47	42.5	13.9	3.6	865 ± 30
		C	*May 2020*		*38.1*	*11.6*	*3.8*	*847 ± 28*
6099	Sample 6	A*	Dec 2020	11.58	21.2	7.2	3.4	915 ± 30
		B*	Dec 2020	0.69	43.4	11.0	4.6	1020 ± 60
		C	*May 2020*		*60.5*	*12.2*	*5.8*	*922 ± 29*
6163	Sample 7	A	Dec 2020		40.4	13.9	3.4	5350 ± 40
		B	Nov 2020		44.0	14.6	3.5	5250 ± 40
		C	*Oct 2020*		*42.9*	*14.6*	*3.4*	*5301 ± 34*

as reference materials to obtain the weight percent of C and N. C/N_{at} were calculated taking into account the atomic masses of C and N, according to the formula:

$$C/N_{at} = \frac{\%C}{\%N} * \frac{14}{12}$$

The ^{14}C concentrations in all samples, Oxalic Acid II standards, coal and blank samples were measured by the Poznan Radiocarbon Laboratory, Poland (Goslar et al. 2004).

Furthermore, for eight samples, stable carbon, and nitrogen isotopes ($\delta^{13}C$, $\delta^{15}N$) were determined. Freeze-dried gelatine samples were weighed in tin capsules. If the volume of the sample allowed, three subsamples of the gelatine sample were prepared for measurements. The analysis was performed by the Gliwice Mass Spectrometry Laboratory using an IsoPrime EA-CF-IRMS continuous-flow isotope ratio mass spectrometer connected to the EuroVector elemental analyzer. The instrument precision is 0.1‰ for $\delta^{13}C$ and 0.3‰ for $\delta^{15}N$ for one subsample. The obtained carbon and nitrogen isotope measurements were calibrated to the VPDB and AIR standards, respectively (Gonfiantini et al. 1990; Misarti et al. 2009).

RESULTS AND DISCUSSION

The results of the batch 1 measurements are shown in Table 1 for the reference samples (SIRI C, VIRI H, and E) and in Table 2 for the Polish archaeological samples. Table 3 shows the measurement results for the batch 2—a set of red deer and wapiti samples. All results are presented for both treatment ways: without (A) and with (B) the ultrafiltration step, in comparison to the ones obtained previously without ultrafiltration (C). Figure 1 shows the comparison of the C and N concentration measurements of samples treated with and without ultrafiltration. C/N_{at} and the ^{14}C ages of the investigated samples are shown in Figure 2. Figure 3 shows the results of the stable isotope measurements of the investigated samples.

Remeasurements of the reference samples show a good quality of gelatine preservation, with C/N_{at} values 3.3–3.4 (Table 1). The C/N_{at} values do not differ by more than 0.2 in comparison to the previous ones, which is consistent with the values obtained in intercomparison studies (Sealy et al. 2014) and the usual scatter of C/N measurements as shown by Scirè Calabrisotto et al. (2013) and Svyatko et al. (2015).

The results of the ^{14}C remeasurements (A and B) of the VIRI H show perfect consistency. In case of the sample SIRI C, subsamples A and B are consistent with each other within 1 σ and with the C subsample within 2 σ. It has to be underlined that SIRI C is a very old sample and the uncertainties of measurements are relatively high, over 1000 years. Subsamples A and C of the sample VIRI E show consistency within 1 σ with each other and 2 σ with the ultrafiltered one (B). Each of the VIRI E subsamples gave a result younger than the consensus value but the result of the ultrafiltered sample (B) was the closest one.

It should be underlined that the time interval between previous and current graphitizations and measurements (i.e., the storage time of gelatine samples) varied between 2 and 6 years (see Tables 1 and 2). It may be possible that during this time there was some absorption of modern atmospheric CO_2 as well as microbial activity.

In the case of six samples prepared from raw bone material (Samples 1–6), we observed that ultrafiltration resulted in a significantly lower gelatine yield compared to simple filtration with

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Table 3 Results of weight % of C and N, C/N_{at}, stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$) and ^{14}C measurements of red deer and wapiti bone samples from Europe and Western Asia (Doan et al. 2017; 2022; Niedziałkowska et al. 2021); Treatment methods: B = with ultrafiltration (this study), C = without ultrafiltration (previous studies; in italics). When only one sample was available for stable isotope ratio measurement, it was marked with an asterisk (*).

Lab code (GdA-)	Sample name	Treat. meth.	Date of graphitization	%C	%N	C/N_{at}	$\delta^{13}C$, ‰ ± 1σ VPDB	$\delta^{15}N$, ‰ ± 1σ AIR	Age ^{14}C (BP ± 1σ)
4223	Ur1295	B	*Feb 2021*	26.56	8.83	3.5	–21.2 ± 0.09	7.02 ± 0.05	48000 ± 3000
		C	*Sep 2015*	*18.88*	*5.49*	*4.0*	*–21.05 ± 0.17*	*6.66 ± 0.07*	*39390 ± 520*
4225	Ur1	B	Mar 2021	22.87	8.05	3.3			3095 ± 30
		C	*Oct 2015*	*18.45*	*6.37*	*3.4*			*2990 ± 30*
4227	Ur4	B	Feb 2021	8.68	4.00	2.5	–21.63 ± 0.01	7.02 ± 0.02	36300 ± 1000
		C	*Oct 2015*	*6.34*	*2.13*	*3.5*	*–21.67 ± 0.06*	*7.01 ± 0.14*	*42400 ± 680*
4396	I14	B	Feb 2021	13.47	5.00	3.1			1695 ± 30
		C	*Dec 2015*	*7.60*	*1.92*	*4.6*			*1700 ± 30*
4593	JS9	B	Feb 2021	17.60	5.90	3.5	–20.18 ± 0.1	5.42 ± 0.07	41500 ± 1300
		C	*Apr 2016*	*15.18*	*4.10*	*4.3*	*–20.13 ± 0.06*	*3.98 ± 0.05*	*39140 ± 270*
4601	H12	B	Feb 2021	18.88	6.29	3.5	–23.01 ± 0.06	3.65 ± 0.02	4940 ± 40
		C	*May 2016*	*16.75*	*4.16*	*4.7*	*–23.19 ± 0.11*	*2.11 ± 0.29*	*4925 ± 30*
4609	AR22	B	Feb 2021	23.10	7.49	3.6			215 ± 30
		C	*May 2016*	*8.30*	*2.80*	*3.5*			*200 ± 25*
4611	AR69	B	Feb 2021	15.05	5.51	3.2	–19.59 ± 0.01	8.66 ± 0.05	2050 ± 30
		C	*Apr 2016*	*13.77*	*3.59*	*4.5*	*–19.61 ± 0.09*	*7.0 ± 0.04*	*1980 ± 25*
4617	J466	B	Mar 2021	13.38	4.72	3.3	–19.59 ± 0.07	7.78 ± 0.04	38500 ± 1100
		C	*Apr 2016*	*9.95*	*2.53*	*4.6*	*–19.44 ± 0.24*	*6.38 ± 0.1*	*32430 ± 150*
4620	AR70	B	Feb 2021	14.15	4.80	3.4			5580 ± 40
		C	*Apr 2016*	*8.50*	*2.08*	*4.8*			*5650 ± 30*
5882	I62	B*	Feb 2021	13.06	4.13	3.7	–20.41 ± 0.1	6.99 ± 0.3	13390 ± 80
		C	*May 2019*	*4.54*	*1.05*	*5.0*	*–20.36 ± 0.17*	*5.53 ± 0.22*	*13040 ± 45*
5883	I63	B	Feb 2021	16.87	6.16	3.2	–20.35 ± 0.03	6.76 ± 0.07	13210 ± 80
		C	*May 2019*	*12.39*	*3.12*	*4.6*	*–21.12 ± 0.12*	*3.03 ± 0.01*	*8515 ± 35*
5886	I2	B	Feb 2021	42.80	14.60	3.4			2685 ± 30
		C	*Jun 2019*	*5.58*	*1.37*	*4.8*			*2480 ± 30*
5896	BY42	B	Feb 2021	44.35	15.57	3.3			5240 ± 35
		C	*Jun 2019*	*22.53*	*6.06*	*4.3*			*5135 ± 25*

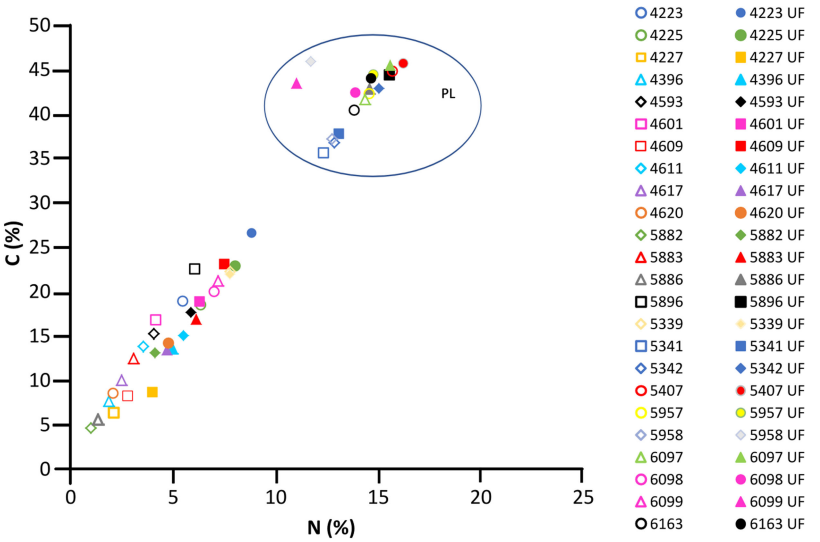

Figure 1 Changes in C and N concentration measured in all investigated samples; preparation without ultrafiltration step—open symbols; preparation with ultrafiltration step—filled symbols. The majority of anonymized Polish archaeological samples marked with an ellipse. Numbers indicate GdA laboratory codes.

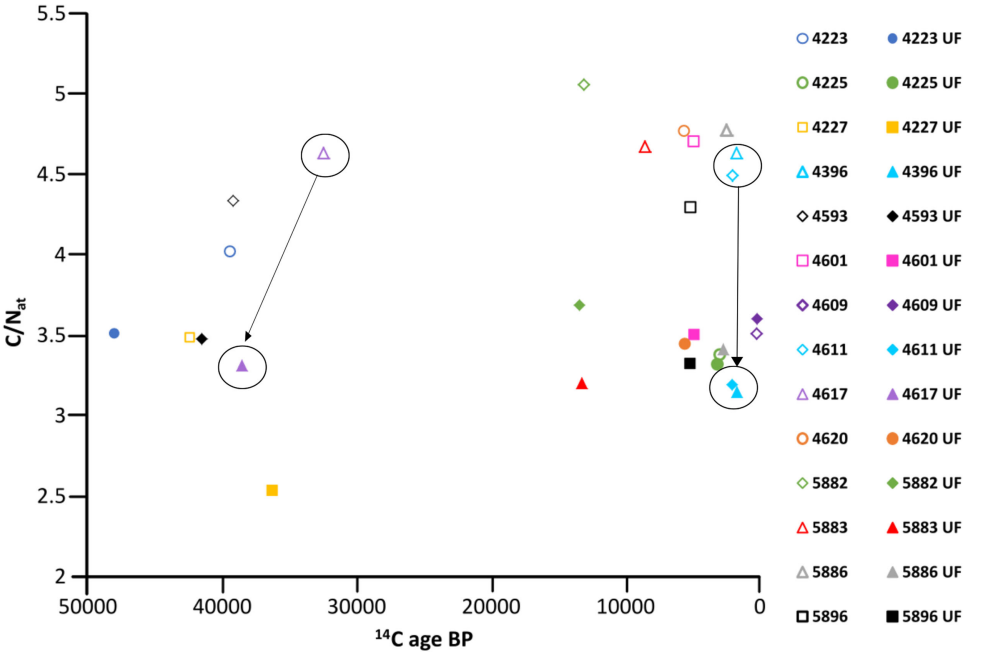

Figure 2 C/N_{at} ratios and ^{14}C ages. Preparation without ultrafiltration step: open symbols; preparation with ultrafiltration step: filled symbols. Numbers indicate GdA laboratory codes.

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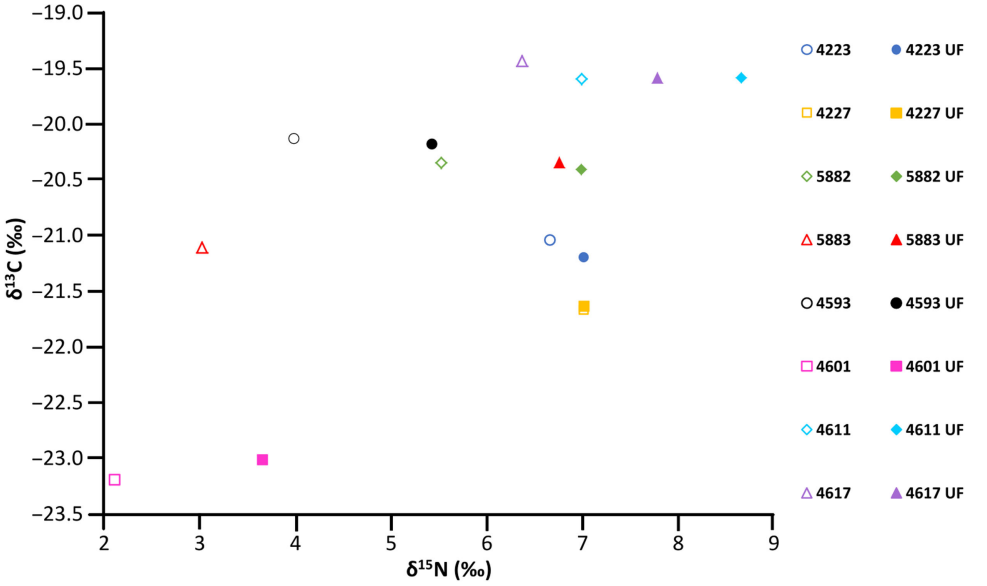

Figure 3 The stable isotope (C and N) composition of the investigated samples. Preparation without ultrafiltration step: open symbols; preparation with ultrafiltration step: filled symbols. Numbers indicate GdA laboratory codes.

Ezee Filters (see Table 2). The gelatine yield for samples treated without ultrafiltration reached between 3.6 and 15.5%, while after ultrafiltration the values decreased and ranged between 0.5 and 9.5%. This fact is not surprising taking into account that ultrafiltration is aimed at discarding the filtrate which contains degraded collagen chains and other unwanted molecules.

As one of results of the ultrafiltration process, we observed changes in the C and N concentration and C/N atomic ratios. The concentration of C and N increased in most of the samples examined (see Figure 1). In addition, in most cases, the C/N_{at} improved to the acceptable value for well-preserved collagen, changing even from 4.6 to 3.2 in the case of the sample GdA-5883 (see Figure 2). However, for two of three small samples GdA-5958 and GdA-6099, such improvement was not observed (see Table 2).

The improvement of carbon and nitrogen parameters was expected as a result of ultrafiltration—apparently there were impurities, some of which added weight, and which were removed during preparation. In general, they caused an increase in the carbon and nitrogen contents. The C/N_{at} indicate these impurities contained excessive carbon atoms, which is characteristic of humic acid contamination. After applied pretreatment the C/N atomic ratio decreased, which suggests that this contamination was reduced.

Humic acids suspected to contaminate bone samples can also be expected to have ages different than a bone sample. In fact, after removal of carbon-bearing contamination with ultrafiltration, older ^{14}C ages were obtained for some of the examined cases (i.e. GdA-4225, GdA-4593, GdA-4617). However, the altering effect is more visible for the set of wapiti and red deer bones than for archaeological samples from Poland. It can be explained by the different conditions in which bones have been deposited over time. Some of the samples of red deer and

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wapiti bones were found in caves, where conditions are unfavourable in terms of bone preservation (Collins et al. 2002).

There is a visible shift in $\delta^{15}N$ values after ultrafiltration in almost every case. Values of $\delta^{15}N$ increased on average by 1.45‰. As for $\delta^{13}C$, no systematic shift in the values was observed (Figure 3).

This situation can be explained by the presence of another component, whose $\delta^{15}N$ was low. As the samples are very different and come from different places, with different environmental conditions, one of the possible explanations for such a systematic shift in $\delta^{15}N$ is the effect of preparation with ultrafilters that screen out short and degraded chains of protein molecules.

Long-term storage of the gelatine samples results in younger ^{14}C ages of the samples. It is visible in the majority of cases samples labeled "C" gave ^{14}C ages older than samples "A" (see e.g., sample GdA-5339 /SIRI C/ in the Table 1 or sample GdA-6097 in the Table 2). It is probably caused by some microbial activity, since samples "C" were measured immediately after preparation and samples "A" after some time, from a few months to a few years. However, this effect can be reduced by applying ultrafiltration (samples labeled "B"). It is visible in both batches of samples, with some exceptions. The results of batch 1 (Tables 1 and 2) show that remeasurements of long-term stored samples give slightly older ^{14}C ages for ultrafiltered ones (set "B") than for those without ultrafiltration (set "A"), however taking into account the uncertainties, the results mostly stay equal. The remeasurements of the red deer and wapiti bones, with the use of ultrafiltration (set "B") gave, in most cases, older ages than set "C" (measured without ultrafiltration). One of the most spectacular cases was sample GdA-5883, for which after ultrafiltration the %C and %N increased, reaching acceptable levels. The C/N_{at} value was lowered from 4.6 to 3.2 and the ^{14}C age increased from 8515 ± 35 to 13210 ± 80 BP. On the other hand, for poorly preserved bone GdA-4227 the ultrafiltration step did not improve %C and %N to an acceptable level, the C/N_{at} was also too low. The age obtained after ultrafiltration was significantly younger than before. Altogether it makes this result unreliable.

CONCLUSIONS

We proved the potential utility of ultrafiltration in contamination removal. Although the ^{14}C ages of bone samples must be evaluated carefully with respect to potential contamination and treatment efficiency, especially for long-stored samples, this method is still highly popular and useful. Ultrafiltration helps to improve the quality of gelatine as evidenced by %C and %N as well as C/N_{at}, by removal of contaminants with the membrane. The cases of poorly preserved bones are still challenging for ^{14}C dating (see the example of GdA-4227), which raises doubts whether the reliable results are possible to achieve. Similarly, the results obtained from small samples of bone, <200 mg, are problematic to interpret in our case. These results might not be reliable due to probable non-linear effects in elemental analysis. The VarioMicroCube Elemental Analyzer, used in our laboratory, gives a greater scatter of results for small samples. This effect cannot be avoided, despite careful calibration.

One of the effects of long-term storage of gelatine samples at room temperature might be some disturbance in the ^{14}C age of the samples obtained. The use of ultrafiltration allows to improve the results for samples stored in such unfavorable conditions, as demonstrated for both very old (>30000 BP) and young samples. Even though, to avoid potential contamination, the time of storage between preparation and ^{14}C measurements should be minimized.

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SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit https://doi.org/10.1017/RDC.2024.32

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