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Thibaut Deviese, Erika Ribechini, Diletta Querci, Thomas Higham. Assessing the efficiency of supercritical fluid extraction for the decontamination of archaeological bones prior to radiocarbon dating. Analyst, 2019, 144 (20), pp.6128-6135. 10.1039/c9an00859d . hal-03207783

HAL Id: hal-03207783

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ARTICLE

Assessing the efficiency of supercritical fluid extraction for the decontamination of archaeological bones prior to radiocarbon dating

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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Bone is one of the main sample types used for building chronologies in archaeology. It is also used in other research areas such as palaeodiet and palaeoenvironmental studies. However, for results to be accurate, samples must be free of exogenous carbon. Contamination can originate from a wide range of sources in the post-depositional environment but may also occur during excavation and post excavation activities (i.e. with the application of conservation materials) or during laboratory handling. Efficient procedures to remove contamination are therefore crucial prior to radiocarbon or stable isotope measurements. This work describes the development of an innovative sample pretreatment for bones, based on using supercritical CO_2, which shows unique solvation properties. The effectiveness of supercritical fluid extraction (SFE) to remove conservation materials was compared with that obtained when applying a routine extraction based on the use of organic solvents (methanol, acetone and chloroform). The chemical composition of the bone samples before and after the two pre-treatments was then investigated using analytical pyrolysis-based techniques: EGA-MS (Evolved Gas Analysis - Mass Spectrometry) and Py-GC/MS (Pyrolysis - Gas Chromatography coupled with Mass Spectrometry). Collagen samples extracted from the same bone, prepared with the two cleaning protocols, were also radiocarbon dated by Accelerator Mass Spectrometry (AMS). The results of this study show that SFE is an efficient alternative method because it was as effective as the established treatment protocol. It removes contaminants such as conservation materials from bone samples with a minimum of handling and can be used routinely in radiocarbon dating laboratories. This work also demonstrates that analytical pyrolysis is not only a very efficient method to identify contaminants in bones but also to assess the effectiveness of the pretreatment prior to the measurement of the samples on the AMS.

Introduction

The development of radiocarbon dating has revolutionized the field of archaeology. Today, this dating technique is routinely applied to a range of artefacts such as wood, charcoal, bones, sediments, textiles, seeds ivory, etc. and contributes significantly to reconstructing human and faunal evolution by providing reliable chronologies [1, 2]. Contamination within the samples must be kept to a minimum level as it can drastically affect the accuracy of radiocarbon measurements. Exogenous carbon can originate from a wide range of sources. Humic substances, derived from decaying vegetation, are present in the post-depositional environment and can penetrate porous materials such as bones. Contamination may also occur during excavation and post excavation activities with the application of conservation materials. Vinyl and acrylic polymers have become popular consolidant agents for preserving archaeologic artefacts. Paraloid B72 ™, Butvar B98 ™ and Polyvinyl Acetate (PVA) are among the most widely used on bones [3]. Such polymers enable the improved preservation of the bone but can interfere significantly with subsequent studies such as stable isotope analyses and radiocarbon dating. Efficient pretreatment procedures designed to isolate autochthonous carbon and remove exogenous carbon from bone are therefore crucial. The ideal purification treatment should have minimal sample handling and the lowest number of steps possible to avoid any laboratory contamination and sample loss. To date, the most reliable pre-treatment for bone samples, derived from the "Longin method" [4], consists of a wash with organic solvents (acetone, methanol and chloroform) to remove possible consolidants or glues followed by a decalcification in acid, a base wash, re-acidification, gelatinisation and ultrafiltration (coded 'AF*' at the Oxford Radiocarbon Accelerator Unit) [5]. This protocol to extract collagen from bones contaminated with conservation materials entails a high number of steps including carbon based solvents and therefore increases the risk of contamination of the samples during laboratory handling.

Different methods have been explored as pre-screening techniques to evaluate the suitability of a bone sample for radiocarbon dating and/or to assess the quality of the collagen extracted prior to its measurement on the AMS [6, 7]. Four diagenetic parameters were proposed for establishing such suitability: histological integrity, porosity, collagen content and crystallinity [8]. Brock et al. demonstrated that the cheapest and most reliable pre-screening technique is to measure the %N content of the whole bone [9]. It was identified that bones with %N lower than 0.76 are mostly not suitable

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for radiocarbon dating because of poor preservation of collagen. Gianfrate et al. developed a quality control protocol for bone samples based on Fourier Transform Infrared (FTIR) spectroscopy to verify the presence of contaminants [10]. Thermal Gravimetric Mass Spectrometry (TGMS) analysis was also applied on archaeological bones exhumed from several Roman sites to determine the proportion of organic material preserved [11]. Most recently, pyrolysis combined with gas chromatography and mass spectrometry has been employed to identify contaminants in bone samples [12, 13] and to study diagenetic alteration of the collagen extracted after pretreatment [14].

This work presents the development of a new method; supercritical fluid extraction (SFE), that could be a more effective routine pre-treatment procedure for the decontamination of archaeological bones prior to radiocarbon dating. Supercritical fluids present extraordinary capabilities for extracting organic compounds from materials. They have been used for a wide range of industrial applications for some time but, to date, there have been very few applications in an archaeological context. These few mostly relate to cleaning, drying, and conservation of delicate historical and archaeological artefacts such as actively eroding iron objects [15], waterlogged corks [16], wooden materials [17, 18], silk textiles [19] and historical records [20]. SFE was also used as a way to extract organic residues from archaeological ceramics [21]. Two other publications have focused on the use of supercritical fluids prior to radiocarbon dating [22, 23]. Rowe et al. used SFE to remove organic materials from cellulosic artefacts (wood/charcoal samples, Egyptian mummy gauzes and Russian textiles) with a minor number of steps. Their results suggest that SFE could have a wide applicability in sample pre-treatment before radiocarbon dating.

In this study, the effectiveness of the SFE cleaning procedure on archaeological bones was tested and compared against the routine pre-treatment commonly used (which includes the use of organic solvents). EGA-MS (Evolved Gas Analysis–Mass Spectrometry) and reactive Py-GC/MS (reactive Pyrolysis-Gas Chromatography coupled with Mass Spectrometry) were used to assess the chemical composition of the samples before and after the pre-treatment protocols (Figure 1). The collagen fractions obtained with the two procedures were also measured by AMS and compared.

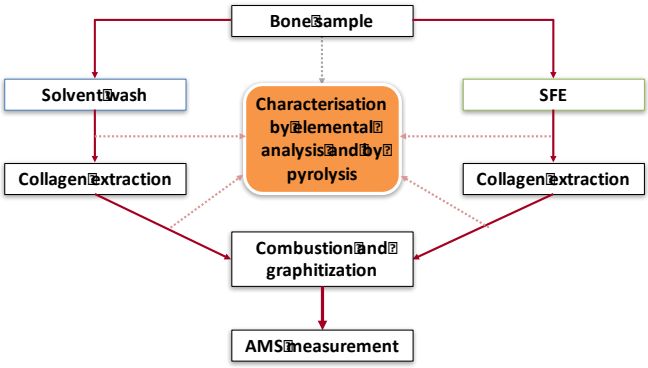


Figure 1: Schema of the analytical strategy showing the two methods of decontamination of the bone samples (solid line arrows) prior to AMS measurement and the steps of sampling for elemental and molecular analyses (dotted line arrows).

Experimental

Solvents and reagents

Cylinders of liquid CO_2 were purchased from BOC, UK. Ethanol (chemical; purity ≥99.8%, gradient grade, for HPLC), used as co-solvent for the SFE, was purchased from Sigma-Aldrich Inc. (USA). Acetone (Distol Pesticide residue grade, Fisher Scientific, UK), methanol (Distol Pesticide residue grade, Fisher Scientific, UK) and chloroform (Chloroform 99+%, for spectroscopy, stabilized with amylene, Fisher Scientific, UK) were used for the "solvent wash step". Hydrochloric acid (HCl ≥ 30% TraceSELECT, Fluka Analytical) and sodium hydroxide (Fisher Scientific, UK) were used for the collagen extraction and purification. Alanine references (Fluka Analytical) were used for quality control during the combustion step.

Reference materials

Butvar B98 ™, manufactured by Acros Organics, was purchased from Fisher (UK), Poly(vinyl acetate) was purchased from Sigma-Aldrich (Milan, Italy) and Paraloid B72 ™ was purchased from Conservation Resources Ltd. (UK). These three synthetic polymers are commonly used as consolidants for preserving archaeological bones and were used as references for the chemical investigations by analytical pyrolysis as well as animal glue, mainly consisting of collagen, purchased from Bresciani srl (Milan, Italy). Data obtained by analytical pyrolysis on these four reference samples are reported in the Electronic Supplementary Information.

Archaeological samples

The archaeological bones used in this study are from the Pleistocene cave site of Zafarraya in Malaga, Spain. The site yielded lithic industries and both faunal and human bones including cut-marked and burned Neanderthal fossils [24]. Previous work showed significant variation in collagen preservation for the bones from this site [25, 26]. A total of 120 samples were screened to check for collagen preservation. From this corpus, 12 samples were selected for this study. Samples selected were large enough (minimum 3 g) to test the 2 different methods of decontamination. Four of them had been preserved with PVA glue (samples P42225-P42228).

Methods and Instrumentation

Measuring %N, %C and C/N All of the samples were mechanically cleaned (sandblasted) and drilled with a tungsten carbide drill to extract 2-5 mg of bone powder, which was then placed and sealed in pre-cleaned cylindrical tin capsules. In addition, alanine standards (2mg) were weighed and sealed in the same capsules. The samples and alanine standards were then measured onto the CF-IR-MS consisting of a CHN Elemental Analyzer (Carlo Erba NA 2000) coupled to a gas source isotope ratio mass spectrometer (Sercon 20/20).

Solvent wash on bones The 12 faunal bone samples were first cleaned with organic solvents prior to collagen extraction. The solvent wash step consists in three sequential extractions of about 1 g of the powdered bone with about 15 mL of acetone (45°C, 45 min), methanol (45°C, 45 min) and chloroform (room temperature, 45 min) before being left to air dry overnight.

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Supercritical Fluid Extraction (SFE) SFE extraction was performed using a Waters® MV-10 ASFE System. About 1g of bone fragment(s) was weighed and placed in a 5 mL extraction vessel. The extraction was achieved using CO_2 mixed with ethanol as an organic modifier. The proportion of modifier was fixed at 50%. The vessel was kept at 50°C and 30 MPa during the extraction. Run time and flow rate were 180 minutes and 2mL/min, respectively.

Collagen extraction Bone collagen was extracted following the routine ORAU procedure outlined by Brock et al. [5]. Bone samples were crushed using a steel pestle and mortar. The samples were then demineralised with three 0.5 M hydrochloric acid treatments at room temperature, the first two for 2 hours and the third one overnight. Following demineralisation, the acid insoluble fraction (mainly made of collagen) was rinsed three times with ultrapure MilliQ™ deionised water. The samples were then treated with 0.1 M sodium hydroxide for 30 min at room temperature and rinsed three times in MilliQ™ deionised water. A final 0.5 M HCl wash was used to eliminate atmospheric carbon dioxide incorporated during the NaOH treatment. Once more, this was followed by three MilliQ™ deionised water rinses. After each acid or base treatment and water rinse, the samples were centrifuged, and the supernatant discarded. The resultant collagen was gelatinised at 75°C for 20 hours in a solution of pH 3 water (10 mL, 1 mM HCl) and filtered using Ezeefilters™ (60–90 µm) and then with ultrafilters (Vivaspin™ 15–30 kDa MWCO). The gelatin was removed from the ultrafilter with ultrapure water and freeze-dried to a final pressure of 0.1 mbar using a VaCo 5 freeze-dryer (Zirbus, Bad Grund, Germany) for approximately 20 hours.

Combustion and graphitization The samples were converted into CO_2 using a Carlo-Erba NA 2000 elemental analyzer. The nitrogen oxide (NO_x) and carbon dioxide (CO_2) gases were passed through a reduction column to reduce NO_x to N_2 then passed through a column containing desiccant to remove the water vapour. N_2 and CO_2 were then separated by gas chromatography. The amount of carbon and nitrogen and their isotopic ratios were measured by passing ~1% of the gas into a mass spectrometer (Sercon Geo 2022). The remaining gas was sent to a purpose-built ORAU collection system with the CO_2 trapped in liquid nitrogen in a reaction rig, loaded with ~2mg of Fe powder (Sigma Aldrich, <10µm, 99,9%) to catalyze the reaction in a side arm and H_2 at 500 mbar. Graphite was then produced in the subsequent reaction for 6 hours in an oven at 560°C. The graphite was then pressed into targets and measured on the AMS at the ORAU.

Evolved gas analysis-mass spectrometry (EGA-MS) The EGA-MS system consists of a micro-furnace Multi-Shot Pyrolyzer EGA/Py-3030D (Frontier Lab) coupled with an Agilent 6890 gas chromatograph (Agilent Technologies, USA) equipped with a deactivated and uncoated stainless steel transfer tube (UADTM-2.5N, 0.15mm i.d. × 2.5 m length, Frontier Lab). The GC was coupled to a 5973 Agilent Mass Selective Detector (Palo Alto, USA) single quadrupole mass. A program temperature was chosen for the micro-furnace chamber: initial temperature 50°C followed by a gradient up to 700°C at 10°C/min. Analyses were performed under 1 mL/min helium flow, with a split ratio 1:20. The micro-furnace interface temperature was kept at 150°C and the inlet temperature was set at 280°C. The chromatographic oven temperature was kept at 300°C. The mass spectrometer was operated in EI positive mode (70 eV, scanning m/z 50-600). The MS transfer line temperature was 300°C. The MS ion source was kept at 230°C and the MS quadrupole at 150°C. The samples, ranging from 800 µg to 1 mg, were placed into a stainless steel cup and inserted into the micro-furnace. The samples underwent thermal decomposition in a helium atmosphere over the chosen heating range, the evolved gaseous compounds were transferred to the mass spectrometer and directly ionized and analysed as a function of time. Thermograms were analysed with the MSD ChemStation D.02.00.275 software (Agilent Technologies). Pyrolysis products were identified by comparison with the literature [27, 28] and mass spectral libraries (Wiley and NIST/EPA/NIH).

Reactive Py-GC/MS (reactive pyrolysis-gas chromatography/mass spectrometry) Reactive pyrolysis is a quite recently developed technique which allows us to perform Py-GC/MS investigations with relatively long pyrolysis times allowing for the analysis of intractable and high molecular weight polymers to be performed. The main instrumental and analytical features of this technique were reported in the literature [29, 30]. Experiments were performed with an EGA/PY-3030D Micro Furnace Pyrolyzer (Frontier Laboratories, Japan) connected to an Agilent 6890 gas chromatograph equipped with a split/splitless injector. The gas chromatograph was coupled with a 5973 Mass Selective Detector (Agilent Technologies, USA). Experiments were performed with a PY1-1050 Micro Reaction Sampler. The analyses were performed with a pyrolysis furnace temperature of 400°C and an interface temperature of 280°C. All the samples were pyrolyzed for 10 min. The GC injector was operated in split mode with a 20:1 ratio at a temperature of 280°C. Chromatographic separation was obtained using an HP-5MS column (30 m x 0.25 mm, film thickness 0.25 µm, Agilent Technologies, USA) coupled with a deactivated silica pre-column (2 m x 0.32 mm, Agilent Technologies, USA) and helium as carrier gas (1 mL/min). The following temperature program was used for the GC oven: 50°C isothermal for 2 min, 15°C/min up to 300°C and 300°C isothermal for 15 min, with no post-run time. The mass spectrometer was operated in EI positive mode (70 eV, m/z range 50-600). The transfer line was kept at 280°C, while the ion source was kept at 230°C and the quadrupole at 150°C. The sample amount was roughly 500 µg for bone samples and 100 µg for collagen samples and synthetic polymers. Pyrograms were analysed with the MSD ChemStation D.02.00.275 software (Agilent Technologies). Pyrolysis products were identified by comparison with the literature [14, 27, 31-33] and mass spectral libraries (Wiley and NIST/EPA/NIH).

Results and discussion

Elemental nitrogen analysis on bone samples

The %N was measured on the 12 bone samples before treatment, after solvent washes (acetone, methanol and chloroform) and after supercritical fluid extraction (CO_2 and ethanol) (Figure 1). The values measured on bone powder after both treatments are similar in most cases and systematically lower than the values measured on the untreated samples (Figure 2). This may be ascribable to the removal of nitrogen-based contaminants such as soil organic materials (e.g. non-collagenous proteins, nitrates, or humics) or to the removal of non-collagenous proteins and/or degraded collagen from the bone samples [9, 34].

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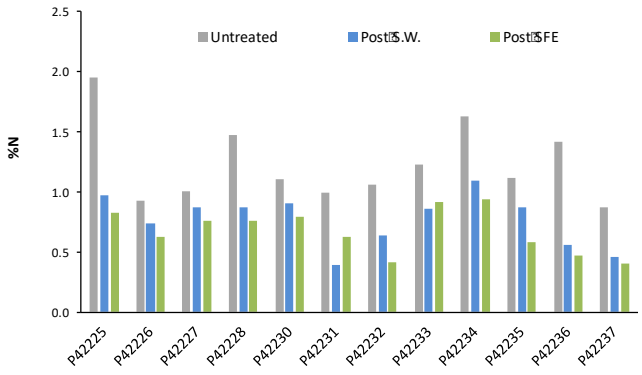


Figure 2: %N measured on bone samples after solvent washes and after supercritical fluid extraction and compared to the values obtained on the untreated samples.

Elemental analysis on collagen samples

Elemental analyses were performed on the 12 bone collagen samples following the two different pretreatments in order to measure their %C, %N and C/N atomic ratio (Table 1). The values for carbon and nitrogen are around 40-45% and 14-15%, respectively. It is also noticeable that most of the values on samples measured after SFE are slightly higher than those obtained after the solvent wash. C/N ratios were very consistent for all samples. They provided values of 3.2 or 3.3 independently of the extraction methods. The C/N atomic ratio is acceptable for collagen if it ranges from 2.9-3.5, but samples contaminated with ~10-15% exogenous carbon will still fall within the range and be passed for dating where measured [35]. Molecular characterization is the best approach to totally rule out the presence of contaminants in samples.

Molecular characterisation

The characterization of several reference materials, including synthetic consolidants and collagen, was performed using both Evolved Gas Analysis Mass Spectrometry (EGA-MS) and Pyrolysis-Gas Chromatography coupled with Mass Spectrometry (Py-GC/MS) in order to identify the pyrolysis products that can be used to assess the effectiveness of the cleaning procedures on archaeological bone samples (data reported in the Electronic Supplementary information). Py-GC/MS was used in reactive mode allowing for less complex pyrograms and an increased sensitivity towards the most stable compounds. Results for these measurements are in total accordance with data already published [27, 28, 31, 32]. Both EGA-MS and reactive Py-GC/MS were then employed to study the 12 bone samples from Zafarraya Cave before and after solvent wash and SFE. In addition, reactive Py-GC/MS was applied to the extracted collagen to monitor the efficiency the whole pre-treatment procedures.

EGA-MS and Reactive pyrolysis-GC/MS on archaeological bone samples The EGA-MS profiles collected on the bone samples not preserved with PVA are similar to each other and show one main peak at approximately 350°C whose mass spectrum can be ascribable to collagen [27]. In contrast, the thermograms obtained for the samples collected from the bones preserved with PVA clearly show two main peaks at approximately 340°C and 440°C, respectively. This indicates a high thermo-complexity of those samples due to the simultaneous presence of collagen and PVA. Figure 3a shows the thermogram of the evolved gas during the thermal degradation of the archaeological sample P42225 before treatment (black), after the solvent washes (blue) and after the SFE treatment (green). The MS spectrum associated with the thermogram (Figure 3b) is dominated by the peak at m/z 60 which is ascribable to acetic acid, one of the major pyrolysis products of polyvinyl acetate (see Electronic Supplementary Information) [28]. Acetic acid is due to the deacetylation reaction that can be achieved quite easily in a temperature range between 300°C and 400°C [36]. The presence of polyvinyl acetate is corroborated by the presence of the peaks at m/z 91, 105, 115, arising from the fragmentation of aromatic compounds such as toluene and styrene, straightforwardly obtained during the pyrolysis of polyvinyl acetate (see Electronic Supplementary Information)[36]. Even though the main peaks in the mass spectrum are due to pyrolysis products from polyvinyl acetate, the presence of collagen can be deduced from the peaks at m/z 67, 80, 94, 107 and 154 due to aromatic and N – containing compounds, such as pyrrole, alkyl-pyrrole and diketopiperazines [27].

The comparison among the Total Ion Thermograms (TITs) of untreated, post solvent wash and post SFE samples (Figure 3a) allows us to observe that the intensity of the thermograms decreases from untreated sample to solvent wash and SFE-treated samples showing that polyvinyl acetate was successfully removed using both treatments. This was confirmed by the extract ion thermogram of m/z 60, the main mass fragment for acetic acid, which is the main pyrolysis product of polyvinyl acetate. Its signal is completely absent in both solvent wash and SFE treated samples (Figure 3c).

Table 1: Elemental analysis of collagen samples after standard pretreatment (coded AF*) and after using the new pretreatment including SFE (coded NRC for non-routine chemistry). %C and % N are the proportions of carbon and nitrogen present in the combusted gelatin samples. C/N is the atomic ratio of carbon to nitrogen.

Samples	Standard pretreatment (AF*)			SFE Pretreatment (NRC)		
	% C	% N	C/N	% C	% N	C/N
P42225	42.3	15.2	3.2	42.9	15.6	3.2
P42226	43.8	15.8	3.2	42.8	15.3	3.3
P42227	42.7	15.4	3.2	43.5	15.7	3.2
P42228	36.3	13.0	3.3	44.5	15.8	3.3
P42230	42.4	15.3	3.2	43.2	15.4	3.3
P42231	40.5	14.5	3.3	44.2	15.9	3.2
P42232	38.0	13.6	3.3	41.1	14.8	3.2
P42233	42.0	15.2	3.2	43.6	15.8	3.2
P42234	40.1	14.4	3.2	44.9	16.2	3.2
P42235	42.4	14.9	3.3	41.0	14.8	3.2
P42236	42.2	15.2	3.2	43.7	15.8	3.2
P42237	40.1	14.1	3.3	42.6	15.4	3.2

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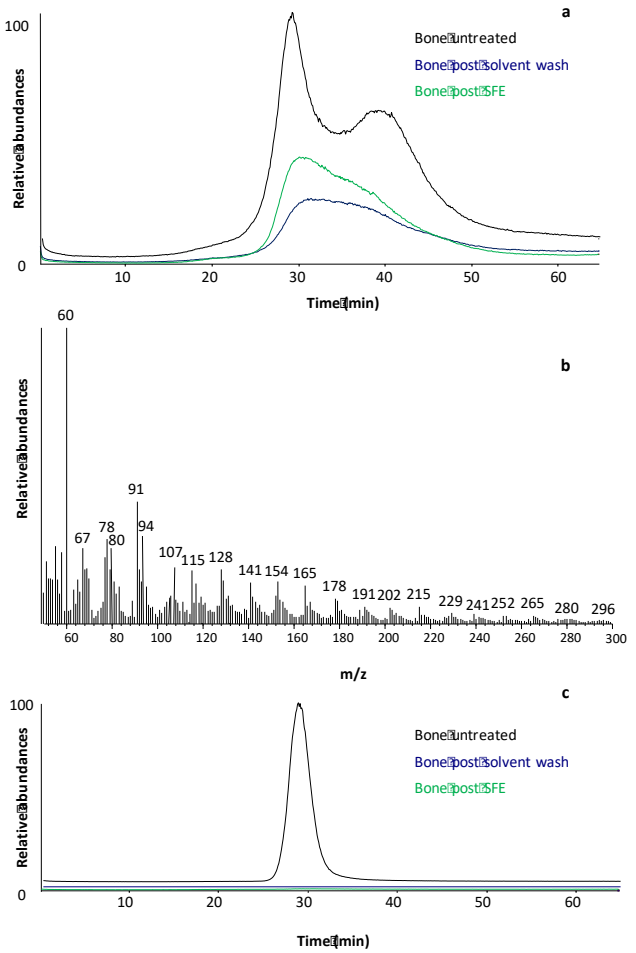


Figure 3: a) Overlay of the Total Ion Thermograms (TITs) of the sample P42225 untreated, post solvent wash and post SFE; b) Mass spectrum associated with the thermogram of the untreated sample; c) Extract ion thermograms of m/z 60 (acetic acid) for the sample P42225 that is consolidated with Polyvinyl acetate.

The reactive pyrolysis experiments gave very similar data to those obtained by EGA-MS allowing us to confirm that SFE is as efficient as a solvent wash in removing contaminants. Figure 4 shows the overlaid Total Ion Chromatograms (TICs) obtained by Py-GC/MS analysis of the sample P42226 untreated, post solvent wash and post SFE. The list of the principal pyrolysis products identified is reported in Table 2. It was possible to detect many of the marker compounds ascribable to the consolidant in the untreated sample and to detect markers related to collagen such as pyrrole and diketopiperazines (DKPs) in the three samples [31]. The presence of aromatic compounds such as toluene in the pyrogram does not imply the presence of residual consolidant in the sample because these compounds are pyrolysis products of both collagen [27] and polyvinyl acetate [28]. Moreover, the intensities of polyvinyl acetate markers are much higher than collagen markers (e.g. the relative abundance of acetic acid (1) compared to pyrrole (4)). Both the solvent wash and SFE extraction efficiently removed the consolidant from the sample and after the first step of the procedures only signals associated with collagen can be detected (Figure 4).

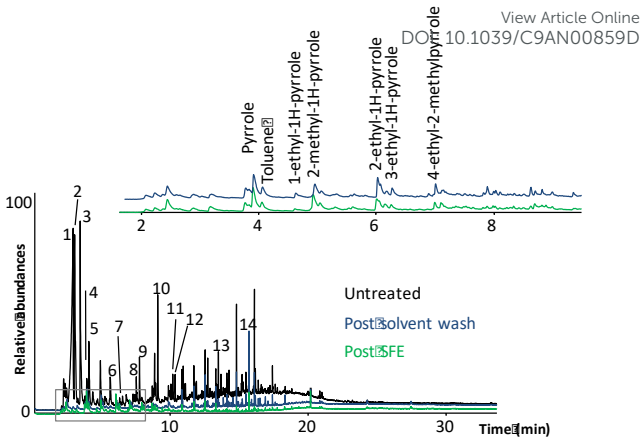


Figure 4: Total Ion pyrograms of untreated, post solvent wash and post SFE for the bone sample P42226. Identification of the pyrolysis products are reported in Table 2.

Table 2: Principal pyrolysis products determined by Py-GC/MS in the untreated sample P42226

	Compound	m/z
1	Acetic Acid	**60**
2	Benzene	**78**, 51
3	Acetic Acid, (trimethylsilyl)-	117, **75**, 60
4	Pyrrole	**67**
5	Toluene	**91**, 65, 51
6	Styrene	**104**, 78, 63, 51
7	Benzaldehyde	**106**, 91, 77, 63, 51
8	Indene	**116**, 89, 63
9	Acetophenone	120, **105**, 94, 77
10	Naphthalene	**128**, 102, 87, 75, 64, 51
11	1-methylnaphtalene	**142**, 115, 102, 89, 71, 63, 51
12	2-methylnaphtalene	**142**, 115, 102, 89, 71, 57, 51
13	Diketodipyrrole	**186**, 130, 93, 65
14	Anthracene	**178**, 152, 89, 76, 63, 51

Reactive pyrolysis-GC/MS on collagen samples Reactive Py-GC/MS was also used to characterise the collagen samples obtained at the end of the pre-treatment procedures for monitoring and comparing their efficiency. The pyrogram profiles associated with the isolated collagen with the two different procedures are the same for all the samples analysed. Figure 5 shows, as an example, the overlaid Total Ion Chromatograms (TICs) of sample P42226, while Table 3 shows the principal pyrolysis products detected in the pyrograms. These data confirm that the two pre-treatment protocols are extremely efficient in isolating the collagen from any contaminants.

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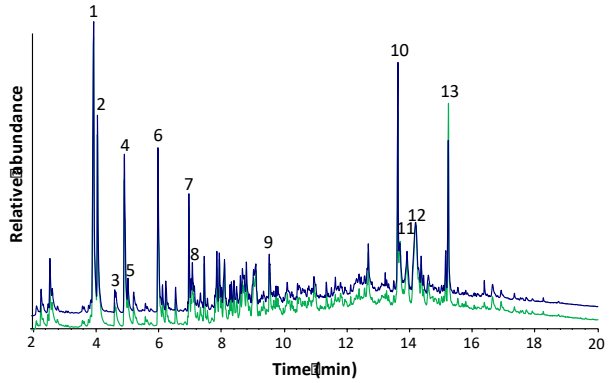


Figure 5: Total Ion pyrograms of collagen obtained following the two procedures from sample P42226 (standard pretreatment in blue and SFE pretreatment in green). Identification of the pyrolysis products are reported in Table 3.

Radiocarbon Dating

The 12 tested samples were dated on the AMS following the two different pretreatments (Figure 1, Table 4). We performed a chi-squared test using the modern carbon fraction (fraction modern, F_m as defined by Reimer et al. [37] and its error to confirm that the 12 pairs of measurements are statistically indistinguishable. The error-weighted-mean in F_m and the t value calculated for each pair of measurements are reported in Table 4. For a chi-squared test with 2 values, the error is significant if t is > 3.84. All the Chi-squared calculated values were lower than the tabulated value for a two-variable test. This shows that each pair of AMS results obtained on the 12 samples, following the two different pretreatments, are statistically identical. It also shows that the CO_2 and ethanol used in the SFE treatment are not introducing modern carbon into the samples. The alternative treatment based on the use of supercritical fluids is therefore as efficient as organic solvents in removing the contamination (soil substances or conservation materials) from samples even if these were heavily contaminated.

Table 3: Principal pyrolysis products determined by Py-GC/MS in the extracted collagen from sample P42226

	Compound	m/z
1	Pyrrole	**67**
2	Toluene	**91,** 65, 51
3	1-ethyl-1H-pyrrole	95, **80**, 67, 53
4	2-methyl-1H-pyrrole	**80**, 53
5	3-ethyl-1H-pyrrole	95, **80**, 67, 53
6	2-ethyl-1H-pyrrole	95, **80**, 53
7	4-ethyl-2-methylpyrrole	109, **94**, 80, 53
8	2,3,4-trimethylpyrrole	**108**, 94, 67
9	Benzenepropanenitrile	**91**, 131
10	Diketodipyrrole	**186**, 130, 93, 65
11	Cyclo (Pro-Ala)	168, 125, 97, **70**
12	Cyclo (Pro-Gly)	154, 111, **83**, 70
13	Cyclo (Pro-Pro)	194, 154, 96, **70**

Table 4: AMS radiocarbon measurements of bone samples. $F^{14}C$ is the fraction of modern ^{14}C .

Sample	Standard Pretreatment		SFE Pretreatment		Pooled mean	error	t
	$F^{14}C$	±	$F^{14}C$	±			
P42225	0.02887	0.00107	0.02716	0.00072	0.02769	0.00060	1.76
P42226	0.01688	0.00100	0.01729	0.00066	0.01717	0.00055	0.12
P42227	0.01607	0.00101	0.01722	0.00067	0.01687	0.00056	0.90
P42228	0.00372	0.00104	0.00317	0.00052	0.00328	0.00047	0.22
P42230	0.00074	0.00096	0.00108	0.00053	0.00100	0.00046	0.10
P42231	0.00174	0.00099	0.00229	0.00053	0.00217	0.00047	0.24
P42232	0.00000	0.00102	0.00062	0.00055	0.00048	0.00048	0.29
P42233	0.00000	0.00096	0.00042	0.00055	0.00032	0.00048	0.14
P42234	0.00037	0.00099	0.00102	0.00054	0.00087	0.00047	0.33
P42235	0.00017	0.00098	0.00110	0.00058	0.00086	0.00050	0.67
P42236	0.00000	0.00097	0.00058	0.00055	0.00044	0.00048	0.27
P42237	0.00128	0.00100	0.00126	0.00053	0.00126	0.00047	0.00

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Conclusions

This experimental work provides interesting new insights into the chemistry of purification methodologies of archaeological bones which is relevant for researchers relying on radiocarbon dating but also scientists from other disciplines working with archaeological bones. The dates obtained on the samples pre-treated with both the routine and the SFE protocol were statistically the same even for those samples contaminated with PVA. These results demonstrate that Supercritical Fluid Extraction is as effective as the standard pretreatment method. It also presents several advantages respect to the routine procedure. It requires less time, it has less preparative steps and it uses no toxic chemicals. More tests are ongoing to optimize further the experimental conditions on bones, such as those at the younger end of the radiocarbon range and other materials but this data already allows us to conclude that SFE could be considered as an alternative to the conventional extraction technique in preparation of bone samples for radiocarbon dating. This work also demonstrates that analytical pyrolysis (EGA-MS and reactive Py-GC/MS) is a very powerful method for assessing the effectiveness of pretreatment procedures on archaeological artefacts prior to radiocarbon dating.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The research leading to these results was supported by the European Research Council [grant agreement 324139] awarded to Tom Higham. It was also supported by a research mobility grant from the Royal Society of Chemistry and a research fellowship from the University of Pisa awarded to Thibaut Devièse. We also would like to acknowledge support from the UK Natural Environment Research Council (NERC) for the Oxford node of the national NERC Radiocarbon facility which provided the core support needed for this research. This work was also performed within the context of the project "Advanced analytical pyrolysis to study polymers in renewable energy, environment, cultural heritage" funded by University of Pisa (PRA_2018_26). We would like to thank Cecilio Barroso and Miguel Caparros for facilitating the access to the archaeological samples from Zafarraya cave and the company Waters for the loan of the SFE system and the technical support. We are also grateful to all staff of the Oxford Radiocarbon Accelerator Unit (ORAU) for their support.

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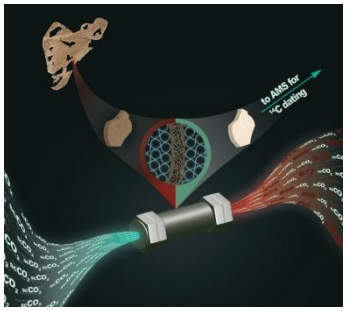
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