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Portable ER-FTIR as a non-destructive method to pre-screen collagen for ZooMS analysis in archaeology

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ABSTRACT

In the last decades, archaeology has witnessed a significant increase in the use of biomolecular analyses to study a variety of materials, including skeletal elements, as they are frequently preserved in archaeological deposits and directly linked to cultural and economic dynamics of ancient human populations. Radiocarbon dating, isotopic studies, and proteomic analyses are particularly useful to explore these questions, while their success is highly dependent on the state of preservation of collagen, the most abundant component of the organic fraction of skeletal elements. Over time collagen degrades, and its preservation is often compromised in very ancient archaeological contexts or when taphonomic processes are particularly severe, which can significantly limit the feasibility of subsequent biomolecular analyses.

The aim of this study is to test whether external reflectance Fourier Transform Infrared Spectroscopy (ER-FTIR) can serve as a rapid, non-destructive pre-screening tool for assessing collagen preservation prior to ZooMS analysis. To evaluate the method's effectiveness, various faunal bone fragments were selected from different archaeological contexts (e.g., rock shelters, pits in dune fields, etc.) located in the Central Sahara (SW Libya), dating to the Middle and Late Holocene (8300–3400 cal BP). The bone fragments were first subjected to ER-FTIR analysis and then to ZooMS (Zooarchaeology by Mass Spectrometry) to compare the results and assess the presence of collagen in the samples.

Our results indicate that collagen was detected in about one-third of the samples, consistently associated with specific spectral features and further validated by ZooMS analyses. The method effectively distinguished well-preserved from poorly preserved samples while avoiding destructive sampling. This pre-screening approach reduces time and financial costs and safeguards the integrity of archaeological bones. Beyond its practical application, it also contributes to bioarchaeology and conservation science by providing a reproducible, non-destructive framework for evaluating biomolecular preservation across different sites and periods.

1. Introduction

Archaeological science increasingly relies on collagen, as it provides the molecular basis for radiocarbon dating, palaeodietary reconstructions, and palaeoproteomic investigations [1,2]. However, collagen rarely survives uniformly across sites and contexts. High temperatures [3–5], soil characteristics, such as extreme acidity or alkalinity [6], and long-term burial all contribute to its degradation, meaning that extensive resources are often devoted to the pre-treatment and analysis of samples that yield no useful results [7,8]. This problem is particularly acute in environments such as deserts, where preservation is generally poor.

In the last decades, several methods have been proposed to reduce the risk of failed analyses by pre-screening bones for collagen preservation. Certainly, FTIR spectroscopy has been at the forefront, used to assess the secondary structure of collagen [9] and its overall organic content [10,11]. ATR-FTIR spectroscopy (Table 1), in particular, has proven to be highly effective as a pre-screening method to assess collagen preservation, requiring only 1–3 mg of powdered sample [12–16].

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Table 1
Pre-screening methods for assessing collagen preservation in bones and tooth dentine, listed in alphabetical order, along with their key references. Their main characteristics listed include: the invasiveness of the analysis (MD: micro-destructive; D: destructive; ND: non-destructive), the "speed" of analysis, which considers both the time required for sample preparation/treatment and data processing (TC: time-consuming, R: rapid), the type of results obtained from the analysis, and the availability of mobile and portable systems.

Methods	Invasiveness	Time investment	Type of results	Portable system	References
C:N atomic weight ratio	MD	TC	Semi-quantitative		Brock et al. 2012
Fluorescence (tooth dentine)	D	TC	Semi-quantitative		Czermak et al. 2019
FT-Raman spectroscopy	ND	R	Semi-quantitative		France et al. 2014
ATR-FTIR spectroscopy	MD	R	Semi-quantitative	X	Weiner et al. 1993; Lebon et al. 2016; Bouchard et al. 2019; Kontopoulos et al. 2020
External Reflectance (ER) FTIR spectroscopy	ND	R	Qualitative	X	this work
MicroCT scanning	ND(?)	TC	Qualitative		Tripp et al. 2010
MicroCT scanning + porosity measurements	D	TC	Quantitative		Tripp et al. 2018
Near Infrared Hyperspectral Chemical Imaging (NIR-HCI)	ND	R	Qualitative		Vincke et al. 2014
Near-Infrared Hyperspectral Imaging (NIR-HSI)	ND	TC	Quantitative		Malegori et al. 2023
NIR spectroscopy	ND	R	Semi-quantitative	X	Sponheimer et al. 2019
Nitrogen content (%N)	MD	TC	Semi-quantitative		Brock et al. 2012
Raman spectroscopy (standard)	ND	R	Semi-quantitative	X	Halcrow et al. 2014; Pestle et al. 2014, 2015
UV-induced fluorescence	D	TC	Semi-quantitative		Hoke et al. 2011
X-rays + thermal neutron radiography (TNR)	ND	TC	Semi-quantitative		Sołtysiak et al. 2018

Other pre-screening approaches include calculating the percentage of nitrogen content (%N) and the atomic C:N ratios in bone powder (5–10 mg) [17,18], as well as fluorescence analysis of bones [19] and dentine in teeth [20].

Pre-screening methods are generally less invasive than subsequent analyses, which may require large quantities of bone (e.g., ≥1 g for radiocarbon dating [21,22]; 300–1000 mg for stable isotopes [23]). An exception is fluorescence analysis [19,20], which exposes the inner bone structure and is therefore destructive. Even micro-destructive techniques, however, still involve removing a few milligrams of bone powder (1–10 mg), and this can compromise the integrity of rare or fragile specimens.

Indeed, the increasing application of biomolecular and isotopic analytical technologies [24] calls for a thorough reflection on sampling strategies and methodologies. Alongside practical limitations, ethical concerns regarding destructive sampling have gained increasing attention. Long discussed in the context of human remains (see [25,26] and references therein), they are now extending to faunal assemblages as well, which are also finite archaeological resources [27].

In this context, non-destructive methods for collagen pre-screening have also been proposed. These include MicroCT scanning [28], X-rays and thermal neutron radiography (TNR) [29], NIR [30], Raman [31–33], and FT-Raman spectroscopy [34], and near-infrared hyperspectral imaging [35,36]. All of them are rapid and relatively cost-effective.

These methods also present limitations. For instance, MicroCT scanning, often considered non-destructive, may still cause chemical degradation and, when combined with porosity measurements, becomes fully destructive, requiring up to 0.5–1.2 g of material [37]. This underlines the need to carefully distinguish between "destructive" and "invasive" approaches, which are not always strictly related to visible physical damage.

While these methods are useful, they are often micro-destructive, time-consuming, or dependent on non-portable laboratory equipment, raising issues of feasibility, cost, and sample export. In some cases, export beyond national borders can be challenging to justify to national authorities, resulting in difficulties in obtaining the necessary permits, particularly when dealing with fragments of bones. Moreover, the continuous transfer of samples between laboratories or their export across continents poses additional risks to their integrity, with the potential loss or damage of packages during long-range shipments.

In this perspective, those techniques, whether micro-invasive or entirely non-destructive, which allow for on-site measurements through mobile and portable systems, are highly desirable. Examples include ATR-FTIR spectroscopy [10,12,38], NIR [30], and Raman spectroscopy [32,33]. As emphasized by several authors [12,38,39], a further key advantage of portable devices is their potential to tailor strategies not only for sampling (in Zooarchaeological or taphonomic studies) but also for excavation within archaeological projects. This allows for significant optimization of both time and financial resources.

Table 1 below summarises the key characteristics of published collagen pre-screening techniques.

Here we present an alternative for an entirely non-destructive, rapid, and cost-effective pre-screening method to evaluate the presence of collagen via external reflectance (ER) FTIR spectroscopy with the use of portable instrumentation. Faunal remains are essential for reconstructing cultural trajectories, subsistence strategies, and dietary practices (e.g., [40–43]), yet species identification is often hindered by fragmentation and the limits of traditional morphometric approaches, especially for taxa with similar morphology (e.g., sheep and goats [44–46]). To overcome these issues, Zooarchaeology by Mass Spectrometry (ZooMS) has become the most widely applied method, using collagen peptides as taxonomic markers [47,48]. Although ZooMS requires only small amounts of material (5–30 mg, up to 150 mg in degraded samples [49]) and is considered minimally invasive [50–53], collagen pre-screening remains crucial to avoid unnecessary destructive analyses. Previous studies have proposed pre-screening collagen in animal bones intended for ZooMS analyses, specifically using ATR-FTIR spectroscopy [12,13,49] or the percentage of nitrogen by weight (%N) [54].

In this study, we tested portable ER-FTIR on prehistoric faunal remains from various contexts in the Sahara Desert (SW Libya) intended for ZooMS analyses, considering the known limitations in the preservation of organic remains at these sites [55,56]. Our assemblage comprises samples from two distinct contexts: a sheltered site and open-air contexts, such as pits in dune fields or stone tumuli placed on a plateau. This study forms part of a broader research project primarily focused on biomolecular ZooMS

analyses. Given the diversity of selected sites and ecological contexts, often featuring poorly preserved bones, this presented an ideal opportunity to experiment with and evaluate ER-FTIR spectroscopy as a non-contact and completely non-destructive collagen screening method.

1.1. External reflectance (ER) FTIR spectroscopy

For a recent overview of external reflection (ER) FTIR spectroscopy applications in archaeology and heritage science, see [57]. In cultural heritage science, it has been applied to the study of pigments [58–60], binders [60,61], surface alteration layers [62], plastics [63,64], varnishes [65], gemstones [66], and related materials. In archaeology, its macroscopic use remains limited, mostly concerning decorated ceramics [67,68] and, more recently, provenance and firing temperature [69]. Beyond ceramics, the technique has been primarily directed towards the analysis of organic residues on lithic industries [70–75], particularly through microspectroscopy, where it has also proven effective in identifying collagen residues (see [76]). Its application to bone fragments remains quite limited, though pioneering and promising studies—again through microspectroscopy (see [77])—have focused particularly on the characterisation of archaeological burnt bones and their state of preservation, as well as very recent experimental approaches integrating portable FTIR spectroscopy in reflectance mode and miniaturised near-infrared (MicroNIR) spectroscopy with multivariate analysis (PCA) [78].

ER-FTIR spectroscopy is an analytical technique particularly suited for collagen pre-screening as it is rapid (the analysis is completed in a few minutes), does not require any sample pretreatment, is completely contactless, non-destructive, and, when used with portable devices, allows measurements on-site and during fieldwork. Infrared (IR) radiation directed on the surface of a sample is reflected by the molecules composing the sample with specific IR wavelengths. The wavelengths correspond to the functional groups and bonds, producing spectra characterized by peaks that are associated with the vibrational frequencies of chemical bonds (e.g., C–H, C=O). The intensity and position of these peaks provide insights into the molecular composition and structure of the analysed sample. IR radiation enables the identification of both organic and inorganic chemical components (see [58,79,80]). The technique thus offers qualitative information regarding the sample's composition, such as the presence or absence of specific molecules, but it is not specific for quantitative analysis.

1.2. ZooMS (Zooarchaeology by Mass Spectrometry)

The analysis of proteins from archaeological remains through palaeoproteomics has quickly become a highly effective tool for species identification and the reconstruction of phylogenetic relationships among both existing and extinct species. Zooarchaeology by Mass Spectrometry (ZooMS) is a rapid and cost-effective proteomics-based analytical technique that uses collagen or other proteins preserved in archaeological and historical artifacts to identify the species from which they originate [47]. Typically, this analysis is applied to morphologically non-diagnostic bone fragments, but it can also be used on a range of other archaeological artifacts and material culture, such as parchment, ivory, eggshells, and leather artifacts [81].

Type I collagen, the most abundant protein in antlers, the dentine of teeth, and bones, is a trimeric protein composed of three polypeptide chains (known as alpha chains) that form a triple helix structure. Variations in collagen chain sequences, identified as differences in the mass of individual peptides, can be used to distinguish species, often at the genus level, even from very small bone fragments. This enables the identification of amino acid sequences unique to specific animal groups through their distinct "peptide fingerprints" in collagen [48].

Utilising collagen peptides as the basic units of analysis, ZooMS overcomes most preservation issues that hinder the extraction and analysis of ancient DNA, as proteins tend to degrade at a significantly slower rate [82]. Several studies have particularly demonstrated the long-term survival of collagen and its excellent preservation [83,84]. Additionally, analyses can be conducted on samples from arid and hot environments because minerals often coat, preserve, and prevent the collapse of collagen fibrils. Collagen, being 'trapped' within this mineral matrix, is more resistant to high temperatures and post-depositional processes [85,86].

2. Research aim

This study aimed to assess the feasibility of using a portable external reflectance (ER) FTIR (Fourier Transform InfraRed) spectrometer to screen bone samples for collagen preservation, with the potential for on-site application during zooarchaeological and taphonomic analyses before conducting ZooMS (Zooarchaeology by Mass Spectrometry) or other collagen-based analyses. To achieve this, we examined animal bone fragments from six archaeological sites in the Central Sahara (southwest of Libya), dating from 8300 to 3400 calibrated years before present (cal BP). These sites, located in a hyper-arid environment, are subjected to numerous and severe syn- and post-depositional processes, both natural and anthropogenic. For such contexts, pre-screening for collagen preservation is crucial to assess the residual presence of collagen in the bones.

The results obtained using reflectance-mode infrared data were compared with those from ZooMS to evaluate its effectiveness as a pre-screening method.

3. Materials and methods

The faunal material was excavated from prehistoric archaeological sites that are geographically located between 24° and 26° north latitude, within the hyper-arid zone of the Sahara Desert, in the southwest of present-day Libya. Today, the region is characterized by a desert climate, with an average annual precipitation of approximately 20 mm and an average annual temperature of around 30 °C [87].

For this study, we examined faunal remains from archaeological contexts with evidence of human frequentation dated between 8300 and 3400 cal BP identified during field activities conducted by The Archaeological Mission in the Sahara of the Libyan Department of Antiquities and Sapienza University of Rome. This chronological span in the central Sahara refers to the historical context of pastoralism that can be subdivided into various cultural phases (see [88]:20–21): Early Pastoral Neolithic (EP: 8300–7200 cal BP), Middle Pastoral Neolithic (MP: 7100–5600 cal BP), Late Pastoral Neolithic (LP: 5900–3400 cal BP).

3.1. Archaeological sites

The sites differ both in terms of the type of site and context, as well as in their location within different ecological settings (Fig. 1), thus potentially subjected to different depositional processes. They are mostly open-air sites, where we expect conservation issues to be particularly significant due to more severe syn- and post-depositional processes.

3.1.1. The Messak Settafet plateau

Sites 301 and 07/39 (investigated through stratigraphic excavation in 2000 and 2007, respectively) are located on the northern

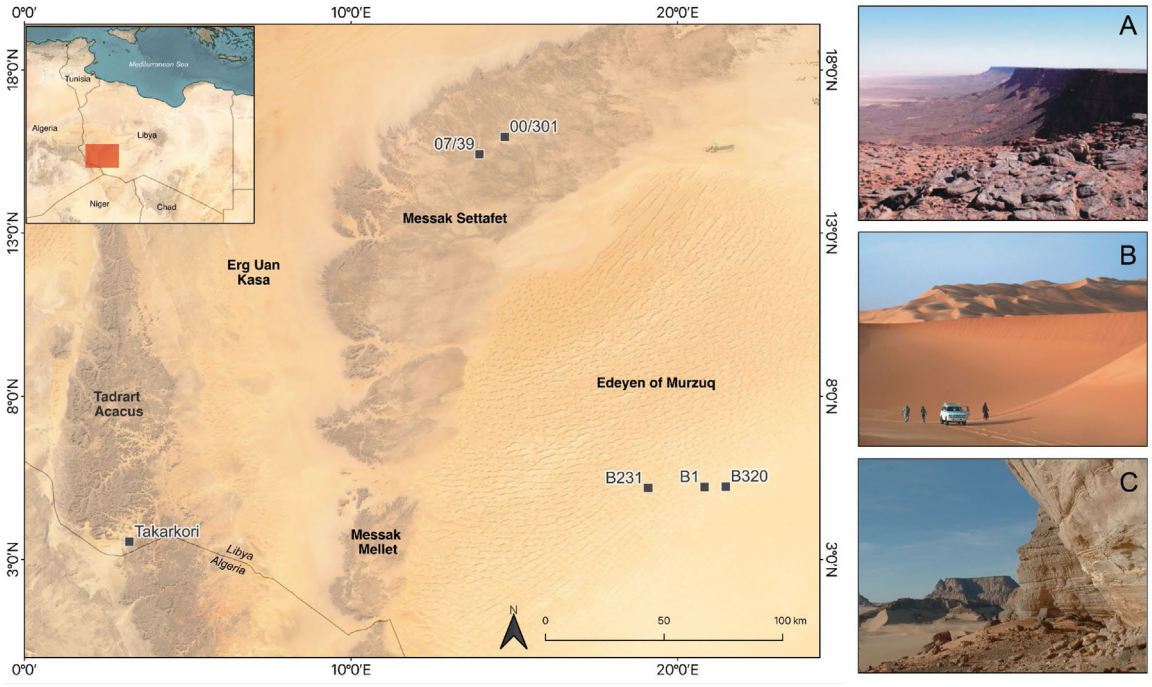


Fig. 1. Map showing the location of the sites studied herein, accompanied by representative images of the different environments in which these contexts are situated: A) the Messak Settafet plateau, B) the dune field of Edeyen of Murzuq, and C) the Tadrart Acacus massif.

part of the Messak Settafet plateau. They are ceremonial stone tumuli containing animal burials dated between 7100–5600 cal BP (Middle Pastoral), part of the archaeological phenomenon known as *cattle cult* (see [56]).

3.1.2. The Edeyen of Murzuq

Three sites (B1, B231, B320) dated to the Middle Pastoral phase, are situated in the Edeyen of Murzuq dune field. These are occupational areas characterized by scattered surface archaeological materials (ceramics, lithics), hearths, and pits dug into the sand containing mostly fragments of faunal remains [89].

3.1.3. The Tadrart Acacus massif

The Takarkori rock shelter is located in the eponymous wadi, in a valley that constitutes the widest passage separating the Tadrart Acacus massif in Libya from the Algerian Tadrart. Extensive stratigraphic excavations conducted between 2003 and 2006 revealed a chronology spanning from approximately 10,200 to 4300 years ago [90].

A total of 86 samples were selected for the experiment, considering approximately 10 samples per site (Table 2). The details of the bone fragments analysed are summarised in SM1. A larger sample was selected for the site of Takarkori due to its multiphase occupation (10 samples for each phase). Bones showing evidence of burning were excluded from sampling, as this process naturally reduces the collagen content in the bones [91,92]. Similarly, any bones displaying signs of natural diagenetic processes—identified through macroscopic examination—that could potentially interfere with the overall state of preservation (e.g., heavily weathered bones) were also avoided.

A three-step analysis protocol was established:

1. Infrared spectra were acquired using a spectrometer (Bruker Alpha R) equipped with a reflectance measurement accessory that does not require preliminary sample treatment, and that enables contactless measurements at near-normal incidence available at the Laboratory of Technological and Functional Analyses of Prehistoric Artefacts (LTFAPA, Department of Ancient World Studies, Sapienza University of Rome). Thanks to its compact size and light weight, the spectrometer is fully portable. The analysed area had a diameter of approximately 3 mm. Spectra were obtained by collecting 50 to 200 scans with a resolution of 4 cm^{-1}.

Unlike other commonly used FTIR modes, such as KBr pellets or ATR, which require destructive sampling and specimen contact, the reflectance mode interacts only with the outermost surface. This means that external and internal measurements may differ, for example, if the bone has undergone heterogeneous taphonomic processes.

To address this limitation, for each sample, one or more measurements were taken, mainly on the outer surface (defined as A: see SM2) and - where possible - on an existing cross-section (in

Table 2
Overview of the archaeological sites examined, indicating the total number of faunal samples analysed from each site.

Site	Area	Chronology	Site type	Context type	N. samples
00/301	Messak Settafet	MP	Open-air	Stone tumulus	10
07/39	Messak Settafet	MP	Open-air	Stone tumulus	14
B1	Edeyen di Murzuq	MP	Open-air	Pits	10
B231	Edeyen di Murzuq	MP	Open-air	Pits	10
B320	Edeyen di Murzuq	MP	Open-air	Pits	12
Takarkori	Tadrart Acacus	EP-MP-LP	Rock shelter	Pluristratified settlement	30

Abbreviations: EP: Early Pastoral; MP: Middle Pastoral; LP: Late Pastoral.

Table 3
Summary table of the main frequencies recorded by ER-FTIR with corresponding assignment and reference bibliography.

Vibration frequency (cm^{-1})	Assignment	References
3573	OH st.	Thompson et al. 2013
3400	OH st.	
3080	N-H amide I	Dal Sasso et al. 2016 (and reference therein)
2851	CH_3 st. sym.	Rey et al 1989; Chadefaux et al. 2009
1655	collagen (C=O st Amide I)	
1540	collagen (ν C-N; bend C-N-H Amide II)	
~1542	$\nu_3(CO_3)$ calcium carbonate A	Dal Sasso et al. 2016 (and reference therein)
~1462	$\nu_3(CO_3)$ calcium carbonate B	
~1465	$\nu_3(CO_3)$ calcium carbonate A	
~1415	$\nu_3(CO_3)$ calcium carbonate B	
1236	collagen (Amide III)	
1030	$\nu_3(PO_4)$ asym st.	
	$\nu_4(PO_4)$	
962	$\nu_1(PO_4)$	
~880	$\nu_2(CO_3)$ calcium carbonate A	
~872	$\nu_2(CO_3)$ calcium carbonate B	
~604	$\nu_4(PO_4)$ bioapatite asym. bend	
~575 sh	$\nu_4(PO_4)$ bioapatite asym. bend	
~565	$\nu_4(PO_4)$ bioapatite	
650–632	OH bend	
712	$\nu_4(CO_3)$ calcite in bioapatite	
472	$\nu_2(PO_4)$ bioapatite sym. bend.	

most cases an ancient fracture: B). In certain specific cases (e.g., fragments of flat bones), the existing cross-section was often too thin to allow for accurate measurements. As a result, we decided to measure the most exposed inner area, specifically the visible portion of the spongy bone (C). Prior to analysis, each spot was preemptively gently cleaned using a scalpel to remove any superficial soil deposits and diagenetic concretions to minimize the possibility of contaminated and/or distorted results.

Vibrational frequencies, as reported by several publications (e.g., [9,93–95]), recorded by the instrument were noted in a database with their respective assignments (Table 3). Notably, absorption peaks mainly observed in the 1655–1540 cm-1 spectral range attributed to C=O stretching (amide I) C–N stretching and N–H bending (amide II) are related to the presence of collagen in bone samples [9,95]. Aiming to detect collagen, three categories were used: presence (Y = yes); absence (N = no); and uncertain (?). These categories were based on the identification and recognisability of collagen-related bands. The 'uncertain' category refers to spectra with a disturbed signal that are difficult to interpret, or to cases where collagen may be present only in minimal quantities and/or in a poor state of preservation, with bands appearing too weak or indistinct to allow a confident assignment.

2. ZooMS analyses.

Samples were selected and processed for ZooMS analysis at Biomolecular Archaeology and Paleoecology laboratory of Department of Prehistory and Institute of Environmental Science and Technology (ICTA-UAB), Universitat Autònoma de Barcelona. We initially employed the standard acid extraction ZooMS protocol (see [47]) on bone fragments. However, due to poor results, we decided to test four modified or alternative extraction protocols (details in SM2). With the exception of two samples that underwent tris-EDTA extraction, all successful samples (those with a ZooMS score greater than 0) were extracted following a modified acid extraction, with the samples first being powdered and then left to demineralize for only two to four hours at room temperature. The remaining procedure was unchanged.

For better data management, the results of the ZooMS analyses were assigned a score (ZooMS Score - ZS, modified from [12] that reflects the level of taxonomic resolution achieved: the lower the score, the broader the taxonomic resolution; Fig. 2).

3. Evaluation of portable ER-FTIR as a pre-screening tool for collagen preservation through comparison with ZooMS results, which inherently confirm the presence or absence of collagen via species identification.

Visualization and statistical analyses were carried out using R version 4.4.2.

4. Results and discussion

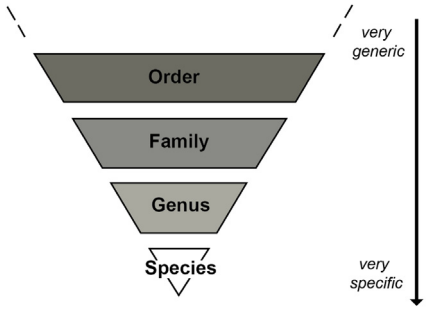
4.1. ER-FTIR

The complete list of ER-FTIR peaks is reported in SM3. The FTIR analysis predominantly identified apatite (calcium hydroxyapatite) in the majority of samples (85 samples, representing 99% of the total), often in combination with various other components, most notably calcium carbonate (31 samples, 36%) (Table 4). In bone, apatite - present in the form of hydroxyapatite - and calcium carbonate - an accessory component within the structure of hydroxyapatite - are both incorporated into the mineral matrix [96].

Collagen, with its typical vibration frequencies, was detected in 34% of the cases (29 samples), only at Takarkori rock shelter (Fig. 3A), with three further samples (Takarkori: TK_157; 07/39: MK_7; B1: MQ_752) defined as uncertain (Fig. 3B). Collagen was always found in association with other components, particularly apatite, and only in two cases associated with CaCO3 (namely MQ_752; MK_7) although its presence was considered to be dubious. All spectra displaying the typical collagen-associated bands, including the three uncertain cases, are provided in SM4.

Except for the samples from Takarkori, where collagen was identified in both the outer surface (A) and cross-section (B) measurements, the two samples with uncertain collagen content (MK_7 and MQ_752) from open-air sites showed collagen only in the cross-section measurements and not on the external surface. Otherwise, no significant differences were observed in the results obtained from the different measured spot locations.

In rare instances, organic residues were found either exclusively with calcium carbonate (1 %, 1 sample) or in combination with apatite and calcium carbonate (10%, 9 samples).



ZooMS Score (ZS)	Taxonomic resolution
0	no ID
1	Family
2	Genus
3	Species

Fig. 2. Simplified schema of the scientific classification of living species with the different systematic categories and the table with the scores assigned to the ZooMS results (ZooMS Score). ZooMS Score 2 includes taxonomic determinations at the genus level, as well as any partial/full spectra that do not allow distinction between two genera that share certain peptide markers.

Table 4
Summary table of results obtained from ER-FTIR measurements. The notation "collagen (?)" indicates an uncertain presence of collagen, i.e. spectra in which collagen-related bands are weak or ambiguous and therefore cannot be confidently assigned. Absolute values (n.) refer to the number of analysed samples, while percentages (n %) indicate the proportion of samples from each site exhibiting the specified spectral association.

Site	apatite		apatite + collagen (?)		apatite + collagen		apatite + $CaCO_3$		apatite + $CaCO_3$ + collagen (?)		apatite + $CaCO_3$ + organic residues		$CaCO_3$ + organic residues	
	n.	n %	n.	n %	n.	n %	n.	n %	n.	n %	n.	n %	n.	n %
00/301	8	9	-	-	-	-	1	1	-	0	1	1	-	-
07/39	-	-	-	-	-	-	9	10	1	1	4	5	-	-
B1	-	-	-	-	-	-	6	7	1	1	2	2	1	1
B231	5	6	-	-	-	-	3	3	-	-	2	2	-	-
B320	11	13	-	-	-	-	1	1	-	-	-	-	-	-
Takarkori	-	-	1	1	29	34	-	-	-	-	-	-	-	-
Total	24	28	1	1	29	34	20	23	2	2	9	10	1	1

4.2. ZooMS

Out of 86 samples, approximately 62% (53 samples) yielded no taxonomic identification (ZooMS Score 0) (Fig. 4). Notably, most of these samples were selected from the open-air sites of Messak and the Murzuq Edeyen, with the exception of one sample from Takarkori (TK_157). In instances where taxonomic identifications could be made from these contexts, they were typically of low resolution (e.g., at the family level) or represented species-level identifications that are unreliable due to incomplete and poorly interpretable spectra. At the sites 00/301 (Messak) and B231 (Murzuq), only ZooMS Scores of 0 were obtained.

In contrast, the Takarkori rock shelter exhibited the greatest variability in ZooMS Scores (Fig. 4), with all scores represented in varying proportions, and provided the highest quality results. Satisfactory taxonomic identifications were achieved in >80% of the Takarkori samples, with species-level identifications (ZooMS Score 3) in 70% and genus-level identifications (ZooMS Score 2) in 13% of the cases.

4.3. Portable ER-FTIR as pre-screening for collagen

In this section, we compare the outcomes of ZooMS and FTIR to evaluate the effectiveness of portable ER-FTIR as a pre-screening tool for collagen preservation. ZooMS is used as a benchmark, as it directly confirms the presence or absence of collagen through taxonomic identification. Where ZooMS failed to provide taxonomic identification (ZooMS Score = 0), ER-FTIR predominantly indicated the absence of collagen. Specifically, 95% of these samples showed no collagen-related bands, while the remaining 5% displayed only weak or uncertain signals. This result indicates a strong association between the lack of taxonomic resolution in ZooMS and the absence of collagen-related peaks in the ER-FTIR spectra, confirmed as statistically significant by Fisher's Exact Test ($p < 2.2e{-}16$).

Interestingly, two out of three samples with uncertain collagen content displayed collagen-related bands only in the cross-section and not on the external surface. This pattern may suggest that the weak bands observed correspond to minimal amounts of surviving collagen, detectable only in the innermost parts of the bone, which are likely better protected from taphonomic agents than the outer surface. It is plausible to assume that both syn- and post-depositional processes played a role in such preservation; however, an in-depth discussion of these factors lies beyond the scope of this study, and the "uncertain" samples considered here are too few to support broader conclusions. Nevertheless, it is noteworthy that, regardless of the specific agent responsible for the weak FTIR signals, each sample derives from a different site with distinct ecological and contextual settings. Even if collagen is indeed present, the quantity appears too limited to provide reliable taxonomic resolution through ZooMS.

For samples with family-level identification (ZooMS Score of 1), ER-FTIR results were variable: collagen was detected in 60% of cases (4 samples) and absent in 40% (3 samples). Despite the limited number of samples under examination (7 samples), it is evident that in this specific context, each case must be evaluated individually, taking into account the archaeological question, the type of samples, and the context. In contexts where samples are limited or irreplaceable, pre-screening becomes particularly important for minimising unnecessary destructive analyses.

In cases where ZooMS provided a taxonomic resolution at the genus or species level- which are, in general, the most useful data for the historical and cultural interpretation of an archaeological context – ER-FTIR consistently detected collagen. These results were obtained exclusively at the Takarkori rock shelter, where the good preservation of collagen in the bones had already been assessed (see [55,97,98]).

It is also noteworthy that the only sample classified with a ZooMS Score of 3, but no collagen detected by FTIR, was, in fact, a sample where only three markers were detected. This is due to

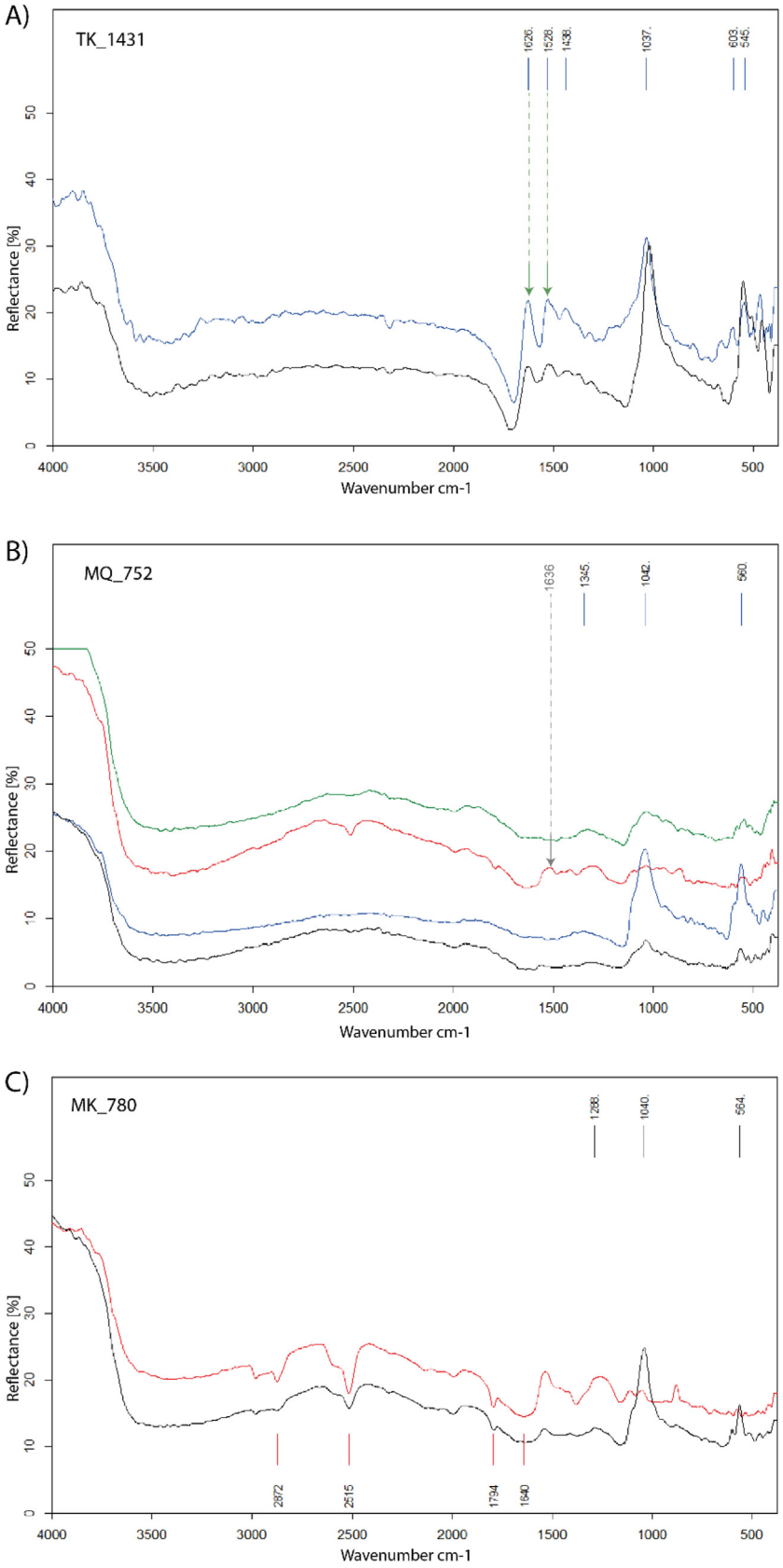


Fig. 3. Examples of spectra resulting from ER-FTIR measurements: A) a sample from the Takarkori site (TK_1431) showing the presence of collagen (green arrows); B) a sample from the B1 site (MQ_752) indicating possible collagen presence (grey arrow); and C) a sample from the 00/301 site (MK_780) in which calcium carbonate, apatite, and organic residues were detected, while no traces of collagen were present.

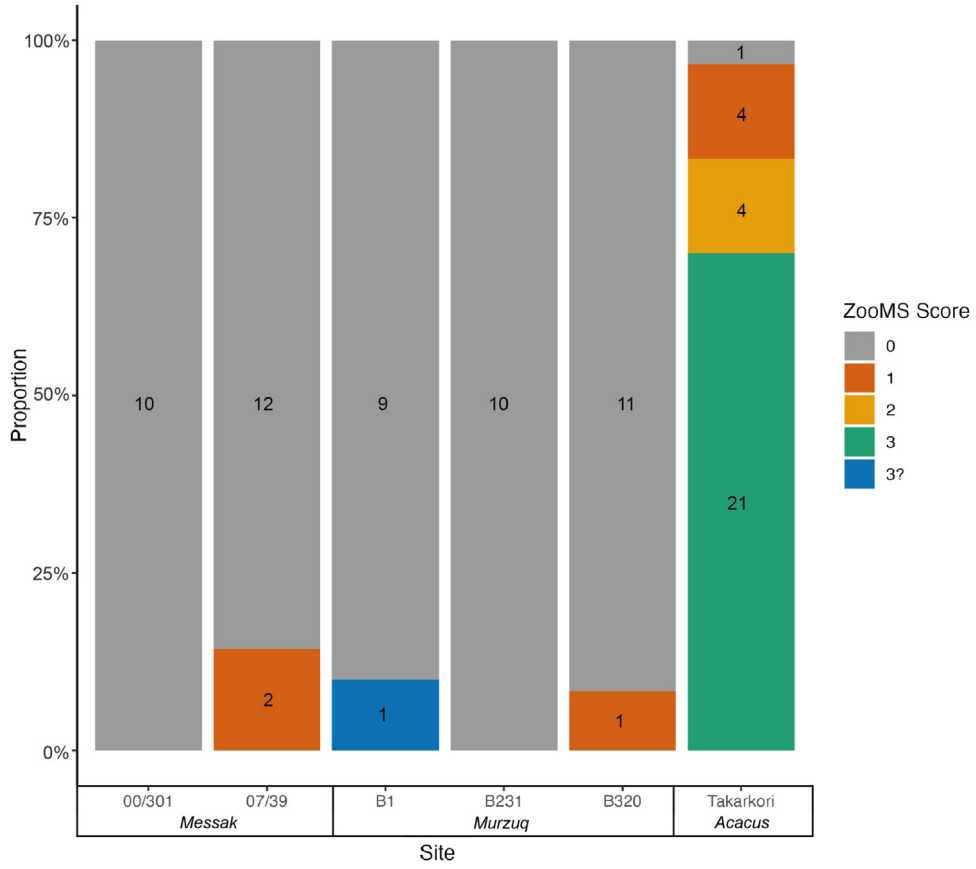


Fig. 4. Summary chart of the results obtained from ZooMS analyses, expressed as ZooMS Scores. Within the bars, the absolute number of samples is indicated.

the fact that partial spectra in ZooMS may occasionally still yield taxonomic insights, though they may require careful interpretation and possible additional corroborative analysis to avoid misclassification (e.g., [99,100]) (Fig. 5).

The results presented here – while acknowledging the limited number of samples, potential site- and sample-specific biases, and the ecological and environmental context under study – demonstrate that portable ER-FTIR can serve as an effective pre-screening method for collagen in bones intended for ZooMS analysis, and other collagen-dependent applications.

Had only ER-FTIR-positive samples been selected for ZooMS, all analysed specimens would have yielded taxonomic identifications, mostly at genus or species level. As previously highlighted, such taxonomic precision can be highly valuable for archaeological inferences, providing insights into the economic, social, and cultural aspects of the human groups under investigation.

The proposed pre-screening method using portable ER-FTIR provides a qualitative assessment of residual collagen in bones, albeit indicating only its probable presence or absence rather than its relative quantity. We deliberately refrained from adopting a protocol that involved further cross-validation with other diagenetic proxies (e.g., %N or C:N) which could have provided quantitative values, to minimise additional sampling beyond that already planned for ZooMS.

Nonetheless, this approach serves as an effective initial test, which, while not excluding the possibility of further targeted analyses, certainly provides a reliable method for ruling out samples with a high likelihood of failure, further limiting unnecessary sampling, destructive analysis, and costs. This analysis also confirms the crucial role that collagen preservation plays in the success of ZooMS for taxonomic identification and suggests that portable ER-FTIR can serve as a complementary tool in assessing the viability of samples for such analyses, especially when the latter comes from difficult contexts.

Pre-screening is beneficial both in contexts with abundant faunal remains, where random sampling may include unsuitable specimens, and in contexts where osteological material is scarce and requires careful conservation. Ethical considerations related to destructive sampling increasingly extend to faunal remains, which also represent a finite archaeological resource (see [27]).

ZooMS, now widely applied as a cornerstone method in archaeozoology, has proven highly effective, particularly due to its minimal sample requirements which circumvent the need for extensive sampling, as is often necessary with techniques like isotopic analyses [26]. While efforts have been made to develop less invasive or minimally destructive sampling methods [50,51,101–106], these are most effective on well-preserved samples. In contrast, traditional destructive methods, like bone chipping, remain more reliable for deteriorated specimens [81]. Thus, a completely non-destructive pre-screening, as we have tested here using portable ER-FTIR, represents the most ethical and efficient approach, especially for assessing collagen preservation in suboptimal samples.

FTIR in external reflectance mode (ER), particularly with instruments such as the Bruker Alpha, offers a fully non-invasive and non-destructive approach, in contrast to ATR-FTIR which nevertheless requires direct contact or micro-sampling (e.g., [12,14]), making it especially suitable for rare or fragile materials and addressing ethical concerns regarding intensive or extensive sampling of archaeological remains. Its portability and rapid execution enable in situ measurements without the need to transfer samples to specialised laboratories, and it allows direct analysis of

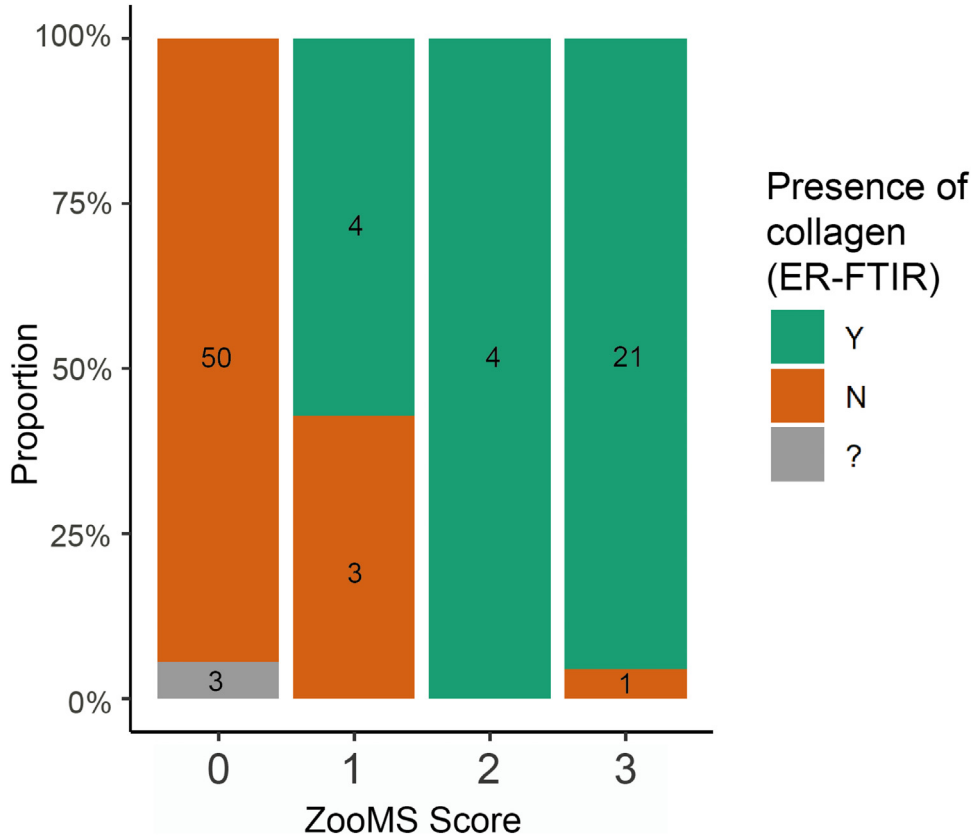


Fig. 5. Proportional relationship between collagen presence as detected by portable ER-FTIR and ZooMS score. The presence of collagen is indicated by "Y" (in green), absence by "N" (in red), and uncertain cases by "?" (in grey). The absolute number of samples is displayed within the bars.

curved or otherwise difficult-to-access surfaces, which is particularly advantageous for bones. While NIR and Raman spectroscopy (e.g., [30,32,35]), which can also be portable, provide valuable information and are increasingly integrated with machine-learning approaches for pattern recognition, their use often requires more specialised equipment, higher costs, or larger reference datasets. In this respect, portable ER-FTIR represents a more practical and accessible solution. Like other spectroscopic techniques (FTIR, NIR, Raman, etc.), it is highly effective in detecting collagen, as collagen-related bands are distinctive and cannot be confused with other substances, thereby reducing the risk of contextual interferences such as synthetic consolidants or soil chemistry. As also demonstrated by previous works (e.g., [76]), ancient collagen, although often degraded and visible in spectra with weaker and broader bands, can still be identified with precision.

Limitations include surface sensitivity, spectral distortions related to texture and optical properties, and reduced reliability in highly heterogeneous samples. Compared to ATR-FTIR, reflectance mode is surface-sensitive and may not always reflect internal composition. As with all portable devices, there is also a greater risk of damage during field deployment than in controlled laboratory settings, particularly under extreme climatic conditions such as high heat or humidity. In addition, the instrument is sensitive to excess carbon dioxide, though this is readily recognisable and can be isolated within the spectrum.

5. Conclusion

Preservation issues are particularly severe in contexts located in arid and hyper-arid environments, where ongoing syn- and post-depositional processes heavily affect the organic component of archaeological assemblages. In the case of bone, collagen pre-screening therefore becomes essential to guide sample selection and to maximise the efficiency of subsequent laboratory analyses.

The increasing use of biomolecular and isotopic approaches requires careful consideration of sampling strategies for both human and animal remains. Our results demonstrate that portable ER-FTIR can reliably distinguish bones retaining collagen from those in which collagen is absent, providing a rapid, non-destructive and cost-effective pre-screening tool. Although the method is qualitative in nature and limited to surface measurements, it nonetheless helps to reduce unnecessary destructive sampling and failed analytical attempts. Its non-contact and portable configuration makes it particularly suitable in situations where laboratory access is limited or the export of samples is restricted.

Beyond its application to ZooMS, the method proposed here can be extended to other collagen-dependent analyses commonly used in archaeology, such as stable isotope studies and radiocarbon dating. Being entirely non-destructive, this approach is also particularly suitable for human remains, which are not only culturally and biologically significant but are often rare and of exceptional value.

From a broader perspective, collagen pre-screening has important implications for environmental sustainability and research logistics. By enabling the targeted selection of samples with a higher likelihood of success, it reduces the number of laboratory analyses required and the consumption of disposable materials and chemical reagents. In regions where geopolitical conditions limit continued fieldwork or access to museum collections, sampling decisions may need to be made rapidly and may involve irreplaceable materials. In such contexts, the availability of a portable, rapid, low-cost and fully non-destructive method capable of assessing collagen preservation in situ is particularly valuable. Portable ER-FTIR effectively addresses these needs by providing immediate and eas-

ily interpretable results that can guide informed sampling strategies while preserving the integrity of archaeological remains.

As biomolecular analyses become increasingly integrated into archaeological research, the ability to identify suitable samples without additional destructive or time-consuming steps will become ever more important. Collagen pre-screening should therefore be regarded as a fundamental stage of research design. Developing a range of complementary methods and instruments will allow researchers to adapt the screening phase to the equipment available in their laboratories, which often represents a key logistical constraint. The aim is not to promote a single technique, but rather to broaden the toolkit available for assessing collagen preservation.

The method explored here uses ER-FTIR to detect collagen in archaeological bone and offers several practical advantages: it is rapid, inexpensive, requires no pre-treatment, and is fully non-destructive and non-contact. These characteristics allow evaluations to be carried out directly in the field, in museums, or in storage facilities where archaeological materials are curated. As a pilot assessment, this study highlights the potential of portable ER-FTIR to significantly reduce analytical time and costs while contributing to the protection and responsible management of archaeological materials. Organic remains, including osteological specimens, form an integral part of cultural heritage and should therefore be treated with the same level of care as any other historical or archaeological artefact.

Declaration of generative AI and AI-assisted technologies in the writing process

During the revision of this work, the authors used ChatGPT 4 to improve the readability and language of the manuscript. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the content of the published article.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.culher.2026.01.009.

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