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# ATR-FTIR pre-screening analyses for determining radiocarbon datable bone samples from the Kings' Valley, Egypt.

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Highlights: (3-5, max 85 characters)

- Issues linked with radiocarbon dating application on human bones from the tomb KV 40
- On-site quantification of bone collagen from Egyptian mummies by ATR-FTIR
- ATR-FTIR prescreening method enabling *in situ* identification of <sup>14</sup>C datable bones
- Validation of the methodology on three ATR-FTIR systems (bench-top and portable)

## Abstract

The tomb KV 40 in the Kings' Valley (Luxor, Egypt) has revealed mummified and highly fragmented remains of 83 adults, children, new-borns and infants, some of whom were, according to inscriptions, 18<sup>th</sup> dynasty king's sons or daughters (~1370 BCE). This tomb was then secondarily reused by priestly families during the 22<sup>nd</sup> to early 25<sup>th</sup> dynasty (~900-700 BCE). Radiocarbon dating of such bone remains would allow the evaluation of the ratio of burials by period and the identification of those from the 18<sup>th</sup> dynasty. As the bones were buried within a shaft tomb under hot and dry climatic conditions and have been partially burnt by fires, the applicability of radiocarbon dating on collagen extracted from them was uncertain and highly challenging. Prior to collecting samples, the state of collagen preservation in the bones had to be evaluated to ensure the feasibility of radiocarbon dating.

Following the method of Lebon et al. (2016), three ATR-FTIR systems (benchtop and portables) were used to determine the effective and appropriate calibration of each instrument thanks to a set of reference bones. From this, we provide methodological recommendations to ensure the reliability of the quantifications obtained whatever the instrument used. As a demonstration of the efficiency of the proposed methodology, portable ATR-FTIR spectroscopy has been tested *in situ* in KV 40, and the N %wt contents thus evaluated have been compared to ATR-FTIR analyses in a laboratory (using a different spectrometer) and to elemental analyses. The results obtained on thirteen KV 40 individuals clearly show quite good consistency on ATR-FTIR analyses between both spectrometers, validating the relevance of this on-site analytical

41 approach with each spectrometer properly calibrated and measurements performed according to a strict  
42 protocol. Regarding the estimation of the nitrogen content, all the KV 40 samples are radiocarbon datable  
43 despite challenging preservation conditions. Discrepancies have been highlighted between some of the  
44 ATR-FTIR results and elemental analyses, the latter systematically providing higher N %wt than the  
45 former. For these samples, the C %wt contents are also very high, showing that these bones are  
46 contaminated by organic materials. This underlines the efficacy of the ATR-FTIR analysis specifically for  
47 the quantification of bone collagen.

48 The developed analytical approach based on the ATR-FTIR method and its mobile instrumentation clearly  
49 operates effectively on site and under challenging conditions, which opens up new perspectives for the  
50 collection of bone remains prior to radiocarbon dating.

51

52

53 *Keywords (3-7): ATR-FTIR, collagen content, radiocarbon dating, Kings' Valley, Egypt, archaeological*  
54 *bone remains*

55

## 56 **Introduction**

57 In the framework of the University of Basel Kings' Valley Project, a series of non-royal tombs has been  
58 excavated in the Kings' Valley in Luxor (Egypt), including the tomb KV 40. The archaeological material  
59 found in this tomb has revealed two occupation phases, with a diachrony of about 500 years between an  
60 original use at the beginning of the 18<sup>th</sup> dynasty (~ca. 1370 BCE), and a second burial phase during the 22<sup>nd</sup>  
61 dynasty to early 25<sup>th</sup> dynasty (~900-700 BCE). This tomb has been looted several times, so all the human  
62 remains have been mixed and partially burnt, and anthropological study reveals at least 83 individuals  
63 belonging to one of the two attested historical periods. Some of them were sons or daughters of 18<sup>th</sup>  
64 dynasty kings, mainly of Amenhotep III.

65 This article focuses on methodological and technical issues linked with the application of radiocarbon  
66 dating to the human bones discovered in tomb KV 40. Attenuated Total Reflectance - Fourier Transform  
67 Infrared Spectroscopy (ATR-FTIR) analyses have been applied as a screening tool to evaluate the state of  
68 collagen preservation of these human remains and to select those which can be <sup>14</sup>C analysed. As  
69 radiocarbon dating is a destructive method and its application results in the destruction of archaeological  
70 materials, it can't be applied unless it is sure to obtain results, in particular when working directly on  
71 remains of ancient human mummies excavated in hot and dry archaeological sites. The development of a  
72 prescreening method enabling on-site identification of datable bones is vital for selecting samples to be  
73 collected, and more broadly, to drastically improve the application of radiocarbon dating to bone samples  
74 in Egypt.

75

76 **I. Archaeological and archaeometrical contexts**

77 • **The tomb KV 40 in the Kings' Valley (Luxor)**

78 Since 2010, the University of Basel, under the direction of Susanne Bickel, has sponsored the *University of*  
79 *Basel Kings' Valley Project* to explore a series of undecorated non-royal tombs from the Kings' Valley in  
80 Luxor, Egypt (Bickel, Paulin-Grothe, 2014; Bickel, 2017). Located along the path leading to the tomb of  
81 Thutmoses III, some of these tombs have been known for several decades without being studied, while one  
82 of them – KV 64 - was discovered by this team in 2012 and remains the most recently discovered tomb in  
83 the Kings' Valley. Adjacent to this previously unknown shaft tomb, KV 40 was known for a long time, but  
84 no archaeological information was collected before 2012. It has revealed carefully mummified remains of  
85 adults, children, new-borns and infants, some of which were, according to inscriptions, 18<sup>th</sup> dynasty king's  
86 sons or daughters (~1370 BCE). This tomb was then secondarily reused by priestly families during the 22<sup>nd</sup>  
87 to early 25<sup>th</sup> dynasty (~900-700 BCE). Ancient pillages are attested between these two occupation phases,  
88 as well as in modern times. The tomb and its contents have also been damaged by violent fires that might  
89 have been linked with those lootings. So all the human remains have been commingled and partially burnt.  
90 The anthropological analysis of the highly fragmented human remains (carried out by a team from the  
91 Institute of Evolutionary Medicine at the University of Zurich) has identified a minimum of 83 individuals  
92 (based on proximal femora) of both sexes and all age groups (Meyer et al., 2020). The classification of  
93 individuals according to the time period of their burial (18<sup>th</sup> or 22<sup>nd</sup> dyn.) has great potential to evaluate the  
94 ratio of burials by period and then to be connected to the anthropological classification of the individuals'  
95 distribution by sex and age. It might also serve later DNA analyses. Revealing royal children's affiliation to  
96 the 18<sup>th</sup> dynasty kings, or family relationships between other individuals, would of course be of crucial  
97 importance for deepening our knowledge of the various social groups that used the Kings' Valley as burial  
98 place.

99 • **Dating Egyptian human mummies: the challenge**

100 Radiocarbon dating is the most efficient tool to date ancient Egyptian mummies. It can be applied to human  
101 bones as well as other organic materials, such as skin, mummies' muscles, or embalming textiles. Egypt's  
102 1983 law on the Protection of Antiquities, which was revised in 2010, bans the exportation of  
103 archaeological samples from Egyptian territory. Thus, all archaeometrical analyses have to be carried out in  
104 Egypt. In this context, the IFAO radiocarbon laboratory was established in Cairo in 2006 to perform  
105 conventional <sup>14</sup>C analyses (Liquid Scintillation Counting, Quiles et al., 2017). Because of the scarcity of the  
106 investigated samples – human mummies – it is of crucial importance to ensure the applicability of the  
107 analytic method prior to collecting samples. Embalming materials used during the funeral ritual processes  
108 can falsify <sup>14</sup>C analyses of textiles and organic materials (Quiles et al., 2014). Further, because of the  
109 history of tomb KV 40 (lootings, fires), the mummies' state of preservations is quite heterogenous, and  
110 some parts only consist of a couple of raw bones, without embalming textiles or any other organic material.  
111 For all these reasons, bone remains seem to be better suited for <sup>14</sup>C analyses.

112 Radiocarbon dating on bone samples is mostly undertaken on extracted collagen (Longin, 1971; van  
113 Klinken, 1999; Hüls et al., 2009, Brock et al., 2010b, 2012; Wood et al., 2010; Dumoulin et al., 2017a, b).  
114 This method has not yet been extensively applied in Egypt, previous studies having been limited by poor  
115 preservation conditions, especially in open-air sites. The question of when the state of preservation of such  
116 samples is good enough to carry out these analyses also arose in this study. Within a shaft tomb partially  
117 burnt by fires and under hot and dry climatic conditions, the applicability of radiocarbon dating to extracted  
118 collagen remained uncertain and highly challenging. In addition, only the conventional radiocarbon method  
119 has been available in Egypt till now, which requires quite significant amounts of material. It was thus  
120 essential to ensure the state-of-conservation of collagen in these human remains before proceeding with  $^{14}\text{C}$   
121 analyses that consume material and time.

122 Various techniques have been employed to pre-screen bones for their levels of collagen preservation prior  
123 to isotopic analysis or  $^{14}\text{C}$  dating. The longest-standing and certainly the most routinely used is the  
124 measurement of nitrogen content by elemental analyses (%N). More recently, less invasive techniques such  
125 as Raman spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), or Near Infrared Spectroscopy  
126 (NIR) have been employed to distinguish altered samples from well preserved ones, and some methods  
127 have been developed to quantify how much collagen remains (Trueman et al., 2004; France et al., 2014;  
128 Vincke et al., 2014; Lebon et al., 2016; Sponheimer et al., 2019).

129 Lebon et al. (2016) and Pothier Bouchard et al. (2019) demonstrated the efficiency of using ATR-FTIR  
130 technique to evaluate the state of preservation of collagen in ancient bones, i.e. not only the quantity of  
131 preserved collagen, but also its quality. This technique provides the triple advantage of presenting limited  
132 invasiveness (since less than a milligram of powder is required, it does not have a significant impact on the  
133 archaeological collection), giving a quick and direct answer about collagen content, and having a low  
134 sensitivity to non-collagenous nitrogen contaminations. FTIR spectroscopy has long been applied to  
135 archaeological sites with a mobile system moving over the excavation site (for the first such application,  
136 see Weiner et al., 1993). Now, as with NIR spectroscopy, portable systems of ATR-FTIR spectroscopy are  
137 available, which offer the opportunity for rapid and low-cost analysis of bone and moreover facilitate on-  
138 site estimation of their collagen content. However, the acquisition and treatment of spectral data with ATR-  
139 FTIR spectroscopy does not make quantification of contents straightforward, even when implementing  
140 several different FTIR instruments. It is therefore necessary to establish analytical control protocols that  
141 make the quantification process reliable by eliminating measurement bias. To that end, analyses of  
142 reference samples were performed using three different ATR-FTIR spectrometers (one benchtop and two  
143 portable ones) to establish and assess the robustness of the procedure for quantifying bone collagen  
144 contents. In addition, this technique makes it possible to detect and identify contaminants that could affect  
145 nitrogen and carbon isotopic analysis and render radiocarbon dating inaccurate.

146 As a proof-of-concept, portable ATR-FTIR spectroscopy has been tested *in situ* in the tomb KV 40 and  
147 under the challenging conditions there. Samples were analysed using two portable ATR-FTIR systems, and  
148 to validate this approach the results were compared to conventional elemental analyses.

149 This study thus describes the development of an efficient and friendly *in-situ* protocol for characterizing  
150 collagen content on bone samples.

151

## 152 **II. Material and Methods**

### 153 • **Samples selection**

154 This study is centred around two sets of samples.

155 For calibration purposes, 28 bone powders already studied in Lebon et al. (2016) were analysed by ATR-  
156 FTIR (Table 1). They come from several archaeological contexts, including samples from the Middle  
157 Palaeolithic to the modern age, and yield varying degrees of collagen preservation.

158 The application was carried out on bones from tomb KV 40 in the King's Valley. It consists of a 6m deep  
159 shaft opening to a corridor that joins a central room with three lateral rooms (Bickel, Paulin-Grothe, 2014).  
160 Bones are currently stored in the left room ("room D"), which has a size of about 6.7 m x 3.1 m. They have  
161 been studied by anthropologists who have identified at least 83 individuals (Meyer et al., 2020). Most of  
162 them show mummification marks using at least two different practices. Linen wrappings are still present on  
163 some of them (Figure 1a); for others, pieces of linen have also been found scattered around. For  
164 preservation concerns, the fragmented mummies have never come out of the tomb. It is strictly prohibited  
165 to take them out of their storing room; thus, any examination and analyses should be performed in this  
166 room under quite challenging conditions in terms of atmosphere and lighting.

167 The archaeological study was carried out on 28 bone powders collected from human bones (femur, pelvis,  
168 rib) of 13 individuals. Depending on each individual's state of preservation, several samples were collected  
169 from the same bone, or from different bones of the same individual. Bone powder was reduced in the  
170 storing room, with the authorisation and supervision of the Egyptian Ministry of Tourism and Antiquities.  
171 Samples were first surface cleaned by abrasion to remove post-mortem pollutions and minimize  
172 contaminations due to the embalming process. Two kinds of contaminants can affect these ancient bones:  
173 - Modern contaminants due to the burial conditions, the nature of soils, and humic and fulvic acids can  
174 influence the diagenesis process of bones (Hedges, 2002; Brock et al., 2010b), and thus affect C and N  
175 contents, as well as FTIR spectra.

176 - Ancient contaminants can also arise from substances used during the embalming process of mummies.  
177 Organic substances used for preparing the deceased body might have been made of materials  
178 contemporaneous with the individual's death, like resins for instance. But they can also be made of  
179 substances older than the date of the individual's death (ancient resin, ancient wax). Or, they can also be  
180 mixed with dead-carbon substances like bitumen. Impacts of such "embalming contaminants" should be  
181 higher at the surface of the bones, but as the individuals were soaked in such solutions for several days, and  
182 due to the bones' porosity, they may have heterogeneously diffused within the bones. Consequently, they  
183 can also modify the C and N contents and thus the FTIR spectra of internal bone samples.

184 In addition, several violent fires have damaged the tomb at different time periods. Some bones have been  
185 partially burnt, while others have been more strongly affected. These events could also have  
186 heterogeneously altered the internal bone structures and their composition, and thus possibly modified  
187 FTIR spectra (Stiner et al., 1995; Lebon et al., 2010; Snoeck et al., 2014).

188 Circular holes of less than 1 mm in diameter were drilled using a diamond drilling tool (Komet) mounted  
189 on a handpiece micromotor (QUBE - Schick Dental) after having ensured that it will not damage the bone  
190 preservation (Figure 1a). The samples were stored in glass vials to be analysed both on site and at the IFAO  
191 laboratory in Cairo.



192  
193 Figure 1: (a) Example of sampling collection for ATR-FTIR analysis on an ancient bone from the tomb KV 40 (sample 8). (b) Set-  
194 up of the Bruker Alpha spectrometer within tomb KV 40.

195

196 • **ATR-FTIR analysis**

197 FTIR analyses were carried out using the Attenuated Total Reflectance (ATR) mode. The ATR mode has  
198 been chosen for its effectiveness in obtaining quality IR absorbance spectra, the small amount of required  
199 material, the limited number of preparation steps, and its easy implementation on site. To assess the  
200 reliability of the proposed protocol, three different spectrometers have been used: a Bruker Vertex 70  
201 benchtop spectrometer equipped with a diamond ATR-Golden Gate (Specac) accessory (Plateau de  
202 spectrométrie infrarouge, MNHN), and two portable FTIR spectrometers, a Bruker Alpha (Monaris lab)  
203 and a Thermo Scientific Nicolet iS5 (IFAO) both equipped with their own diamond-ATR accessories.  
204 Infrared spectra were collected with a spectral resolution of  $4\text{ cm}^{-1}$  and the accumulation of 64 to 128 scans  
205 in the range  $4000\text{-}370\text{ cm}^{-1}$ . At least three spectra were acquired for each sample to test instrumental  
206 repeatability and bone-sample homogeneity. Data acquisition was driven by the OPUS software for Bruker  
207 and the OMNIC software for Nicolet. The amide band area was selected in the range  $1710\text{-}1590\text{ cm}^{-1}$   
208 (linear baseline) and the phosphate band was measured between  $1110$  and  $940\text{ cm}^{-1}$  after a linear baseline  
209 correction between  $1160$  and  $890\text{ cm}^{-1}$ . The limits of quantification were calculated from calibration curves  
210 using the Linest function of Microsoft Excel software according to Feinberg (2012). Standard errors of

211 estimation were calculated using the Steyx function of Microsoft Excel software. Details of calculations are  
212 reported in Lebon et al. (2016).

213

214 The Bruker Vertex 70 (MNHN) instrument was used in a laboratory (France) to establish the method by  
215 analysing calibrate reference bone samples (Lebon et al., 2016).

216 After having been calibrated in the laboratory by measuring reference bones samples, the Bruker Alpha  
217 instrument was used *in-situ* in tomb KV 40 under challenging experimental conditions. The rock tomb is  
218 divided into four subterranean rooms without a ventilation system, except for the natural circulation  
219 through the entrance shaft, whose metal door is open only when the tomb is being accessed. The instrument  
220 was first set up in the bone-storing room to be as close as possible to the sampled bones. The atmosphere  
221 was strongly saturated, and it was not possible to get a background spectrum suitable for the measurements.  
222 To avoid a saturated atmosphere and to be in the most ventilated place, the instrument was finally set up at  
223 the bottom of the shaft, close to the entrance of the tomb (Figure 1b). Background measurements were  
224 regularly performed (~every 2h) to avoid any atmospheric changes that could affect the quality of FTIR  
225 spectra.

226 ATR-FTIR spectra were acquired directly after sampling in the bone-storing room, providing immediate  
227 information about the degree of collagen preservation of the examined bones. A macro on the OPUS  
228 software allows fast extraction of band areas, and the previously established calibration curve on a  
229 Microsoft Excel spreadsheet gives the collagen quantification.

230 Once calibrated using reference bones, the Nicolet iS5 instrument was used in the laboratory (IFAO) to  
231 measure archaeological bone powders transferred to the IFAO lab.

232

233 As recommended by Lebon et al. (2006), in order to enable a reproducible band-intensity ratio, the anvil  
234 pressure on the ATR crystal was adjusted to obtain the same raw absorbance of the  $\nu_3\text{PO}_4$  band around  
235  $1015\text{ cm}^{-1}$  for all spectra (references and unknown samples) acquired with each of the systems. The raw  
236 absorbance chosen for the  $\nu_3\text{PO}_4$  band was 0.5 on the Vertex 70 system, 0.18 on the Bruker Alpha, and  
237 0.21 on the Nicolet iS5. These differences in absorbance values are a consequence of each spectrometer's  
238 specificities and their ATR accessories.

239 KV 40 bone-powder samples were first analysed *in-situ* using the Bruker Alpha instrument, then measured  
240 a second time at the IFAO lab using the Nicolet iS5 instrument. Amide I /  $\text{PO}_4$  area ratios were measured  
241 and collagen contents directly evaluated when preserved.

242 For the Nicolet iS5 and Bruker Alpha instruments, the calibration equations were calculated for this study  
243 using the previously mentioned calibration samples (see III).

244



245           •   **Elemental analyses**

246   Elemental analyses were performed to evaluate the carbon and nitrogen contents of bone powders and to  
247   determine their atomic C:N. The calibration samples were analysed twice at the Service de Spectrométrie  
248   de Masse Isotopique of the MNHN (SSMIM) and at the LIENSs stable isotope facility laboratory (UMR  
249   7266 - CNRS/Université de La Rochelle) on a similar elemental analyser (Thermo Scientific Flash).  
250   Regarding the KV 40 samples, 18 bone powders from the 13 studied mummies were prepared at the IFAO  
251   laboratory and measured using a Thermo Scientific FLASH HT Plus instrument elemental analyser  
252   (Stratochem lab, Cairo).

253

254           **III. Results**

255           •   **ATR-FTIR Calibration curves**

256   Figure 2a presents the mean values and standard deviations of Amide I / PO<sub>4</sub> ratios measured by the three  
257   ATR-FTIR spectrometers compared to N %wt determined by mass spectroscopy for archaeological  
258   samples used for calibration (N=28). These differences between Amide I / PO<sub>4</sub> ratios confirm the inter-  
259   instrument variability of FTIR-ATR measurement observed by Pothier-Bouchard et al. (2019). They  
260   appear to be directly linked to the difference of raw absorbances of the ν<sub>3</sub>PO<sub>4</sub> band around 1015 cm<sup>-1</sup>  
261   adjusted for each spectrometer (at 0.5 on the Vertex 70, at 0.18 on the Bruker Alpha, and at 0.21 on the  
262   Nicolet iS5). With these acquisition conditions, the values obtained from the Bruker Alpha and the Nicolet  
263   iS5 are slightly different (respectively from 0.04 to 0.13 and from 0.04 to 0.14) from those of the Bruker  
264   Vertex 70 spectrometer (from 0.01 to 0.18). However, these differences have little impact on the limit of  
265   quantification (LOQ) for nitrogen since the values are close to 0.5 %wt for the three spectrometers (0.55 on  
266   the Vertex 70, 0.52 on the Bruker Alpha, and 0.53 on the Nicolet iS5). The standard errors of estimations  
267   are also very close, around 0.2 %wt for the three spectrometers (0.21 on the Vertex 70, 0.24 on the Bruker  
268   Alpha system, and 0.25 on the Nicolet iS5).

269   The LOQ seems to be slightly overestimated due to the very good correlation between Amide I / PO<sub>4</sub> ratio  
270   and N %wt in the range of 1 – 4 N %wt. Below and above this N %wt range, the errors are higher and the  
271   estimates less accurate. Given the standard errors of estimates ≈ 0.2 %wt, we consider that it is preferable  
272   not to quantify for samples with a nitrogen content lower than 0.7 %wt.

273

274   As the relationship between Amide I / PO<sub>4</sub> and N %wt is linear between 0.5 and 4 %wt, calibration curves  
275   (Figure 2b) were established only for this range (N=19 samples) in order to allow a calculation of N %wt  
276   by FTIR as close as possible. The calculation of %N may therefore be slightly biased for samples  
277   containing an amount of collagen similar to that of modern bone and obviously for those below the limit of  
278   quantification (0.5 N %wt).

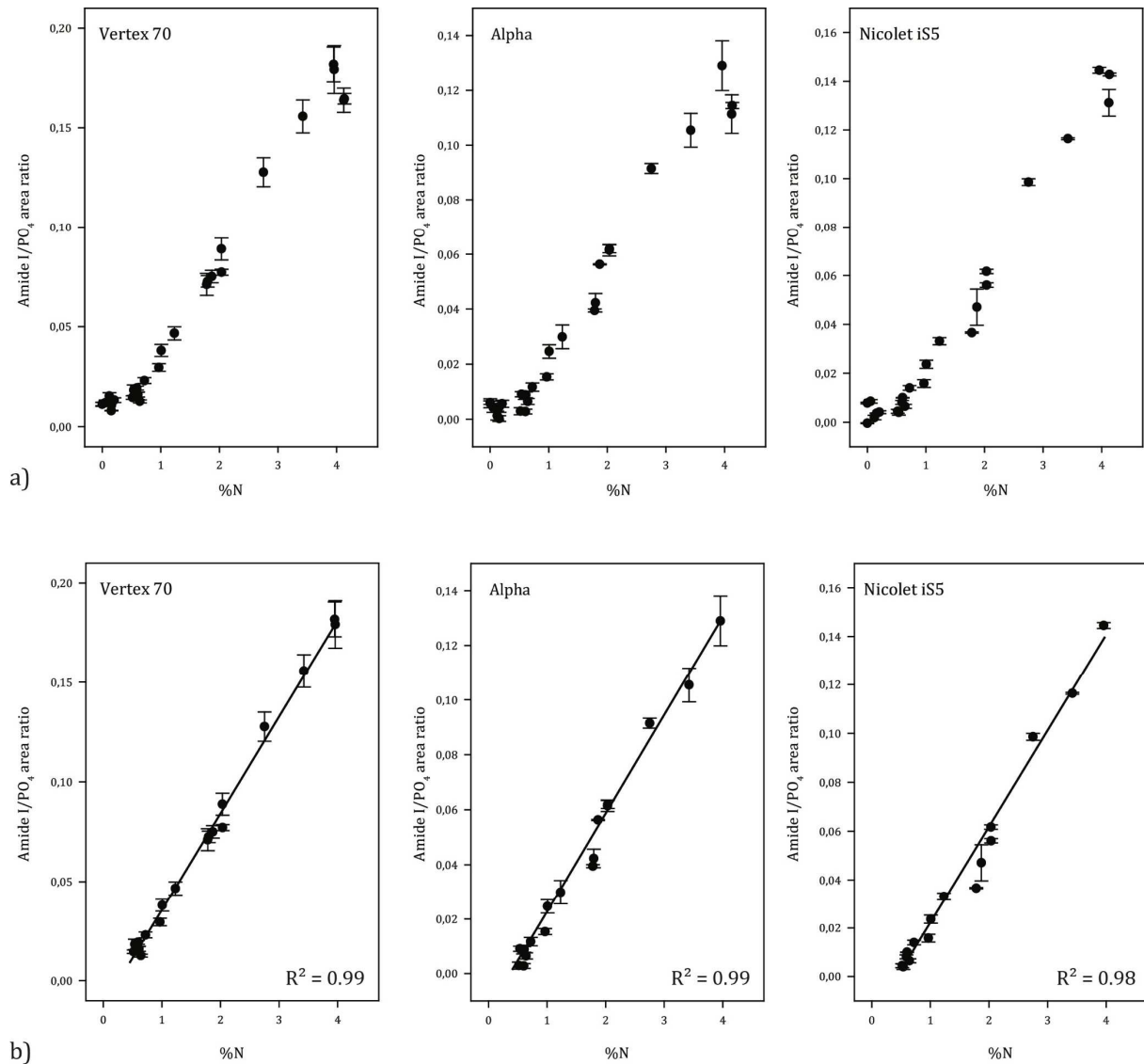
279 The three spectrometers present similar linear correlation, with little scattering of points, even if the values  
280 of the Amide I / PO<sub>4</sub> ratios are different. The calibration equations obtained from the linear regression are  
281  $N \%wt = 27,205 \text{ Amide I} / \text{PO}_4 + 0,4352$  for the Alpha spectrometer and  $N \%wt = 24,738 \text{ Amide I} / \text{PO}_4$   
282  $+ 0,4891$  for the Nicolet spectrometer. For the Bruker Vertex 70 bench-top instrument, the calibration  
283 equation previously established in Lebon et al. (2016) was used:

$$284 \text{ collagen wt\%} = 113.13 \text{ Amide I} / \text{PO}_4 + 1.69$$

285 Collagen content was estimated considering that modern bone contains 22 %wt of collagen corresponding  
286 to  $\approx 4 \%wt$  of nitrogen (Stafford et al. 1988; van Klinken 1999; Collins et al. 2002). Thus, collagen %wt =  
287  $5.5 N \%wt$ .

288 The calculated nitrogen and collagen contents are reported in Table 1 for the three spectrometers. The  
289 calibrations obtained demonstrate that all three spectrometers can be used to obtain an equivalent  
290 estimation of collagen in archaeological bones if measurements are performed following the strict protocol  
291 described.

292



293

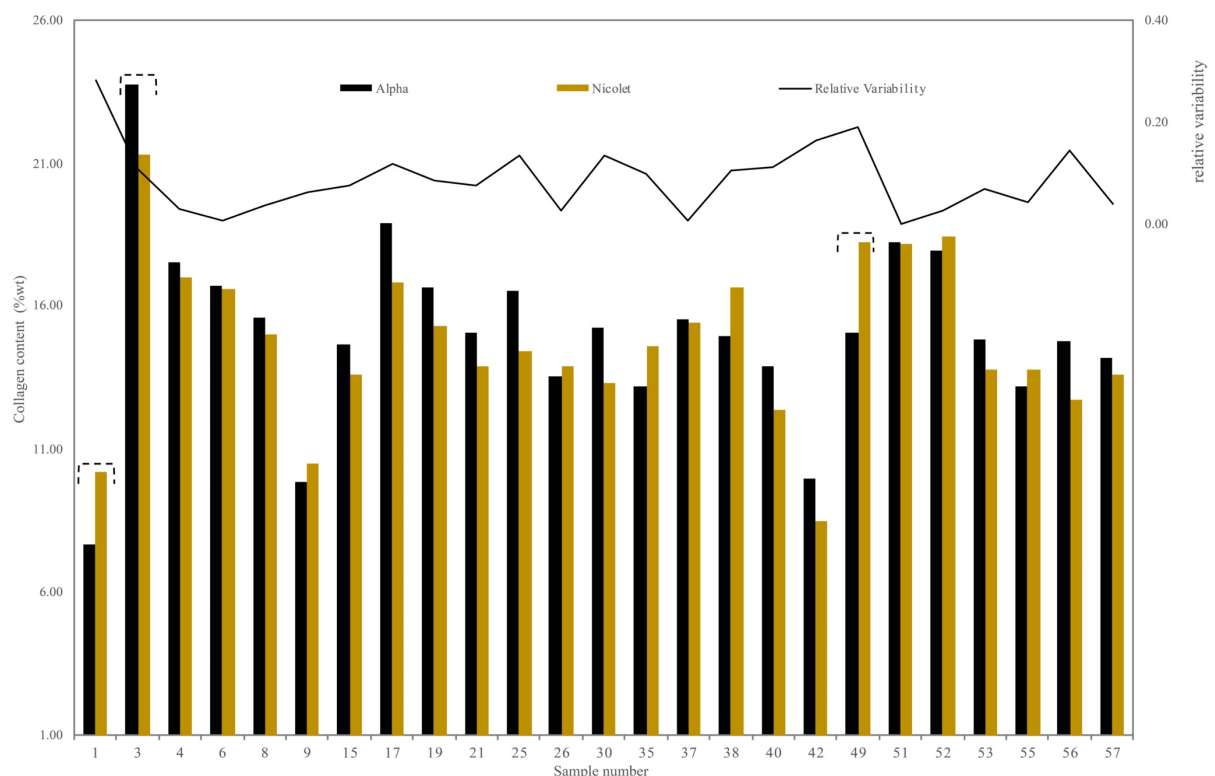
294 Figure 2 : a) Relationship between N content (%wt) and Amide I / PO<sub>4</sub> ratio for 28 calibration samples measured on  
 295 the Bruker Vertex 70, Bruker Alpha and Nicolet iS5 spectrometers. b) Calibration curves using linear regressions  
 296 obtained for these three spectrometers on the range 0.5 and 4 N %wt (corresponding to 19 samples).

297

298 • **ATR-FTIR results of KV 40 samples**

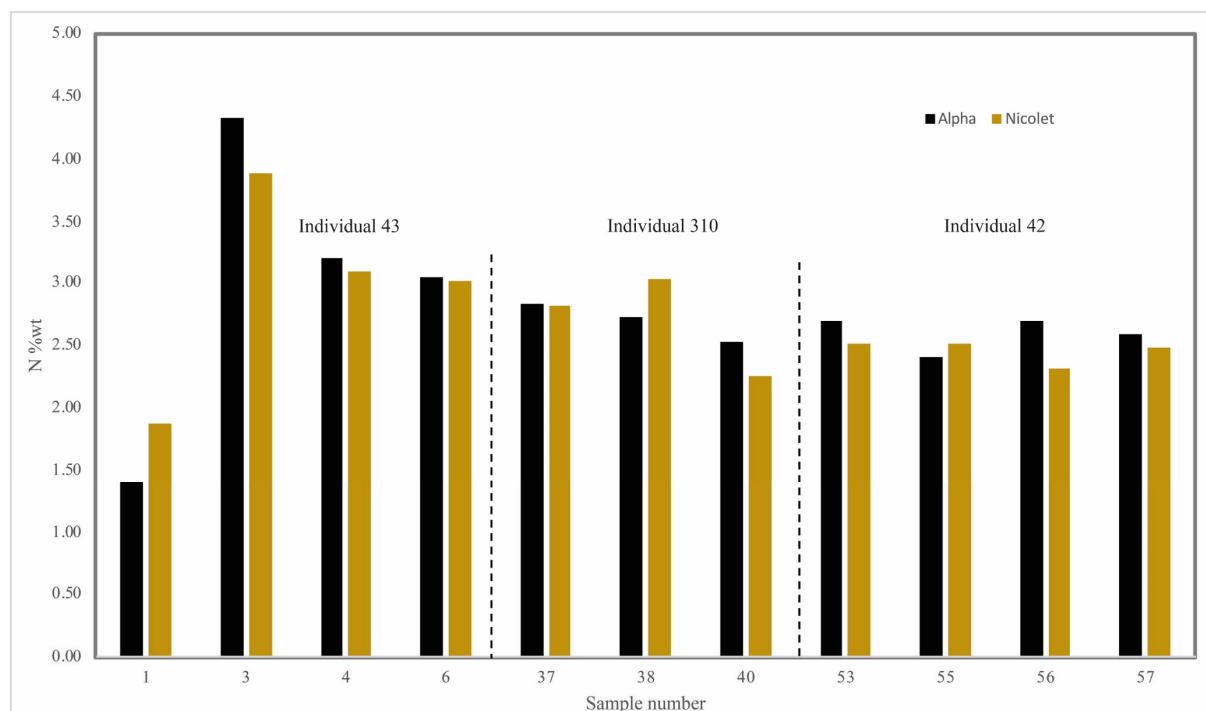
299 The results of N %wt calculated from the ATR-FTIR analyses extend from 1.40 to 4.33 using the Alpha  
 300 Bruker instrument on site, and from 1.55 to 3.88 using the Nicolet iS5 instrument at the lab, which gives  
 301 from 7.72 to 23.79%wt and 8.52 to 21.32 %wt collagen respectively in the whole set of studied samples  
 302 from KV 40 (Table 2). Evaluated collagen content is higher than 17.5%wt for six samples (#3, 4, 17, 49,  
 303 51, 52), whereas only sample 42 is at around 10 %wt (10.04%wt and 8.52%wt respectively). The relative  
 304 difference between collagen contents determined by the two IR spectrometers ranges from 0 to 0.28, with a  
 305 mean at 0.09 (i.e. below 10%). For 25 values, 60% of these differences are below 10% and 88% below

306 15%. The 3 highest differences do not seem to be related to a specific range of collagen content (Figure 3).  
 307 These content variations between the two spectrometers are relatively consistent (Figure 3).  
 308  
 309 Following Brock et al. (2012), a content of N %wt > 0.7 ensures the collagen state of preservation is  
 310 enough to initiate radiocarbon analyses. But it does not necessarily mean that contents slightly lower can't  
 311 produce sufficient collagen for radiocarbon dating, especially because the collagen can be better preserved  
 312 in another area of the bone (Jacob et al., 2018). In any case, all the KV 40 samples reach N %wt far from  
 313 this cut-off value and so are datable regarding the estimation of the nitrogen content by ATR-FTIR  
 314 analyses.



315  
 316 Figure 3: Comparison of collagen content (%wt) measured using the Alpha Bruker spectrometer on site and the Nicolet iS5 at the  
 317 lab (left axis). The three samples having the greatest difference in values between instruments/measures are highlighted with dotted  
 318 brackets, in order to emphasize the maximum variabilities which have been reached. The relative variabilities (right vertical axis)  
 319 have been evaluated using N %wt calculated both from the Alpha and the Nicolet spectrometers.

320 Within individuals, several analyses have been performed on separate bones or on different areas of the  
 321 same bone. Consistency of results is not systematic (Figure 4). The relative variability within one  
 322 individual ranges from 0.02 (individual 316) to 0.93 (individual 43) (Table 2, Figure 4). For three of them,  
 323 from 3 to 4 analyses have been done. They show high heterogeneity of collagen preservation and scattered  
 324 results ranging from 7.7 to 23.8%wt (Alpha Bruker) and from 10.2 to 21.3%wt (Nicolet iS5) for individual  
 325 43, whereas other individuals show more consistent results (individual 310, 42).  
 326

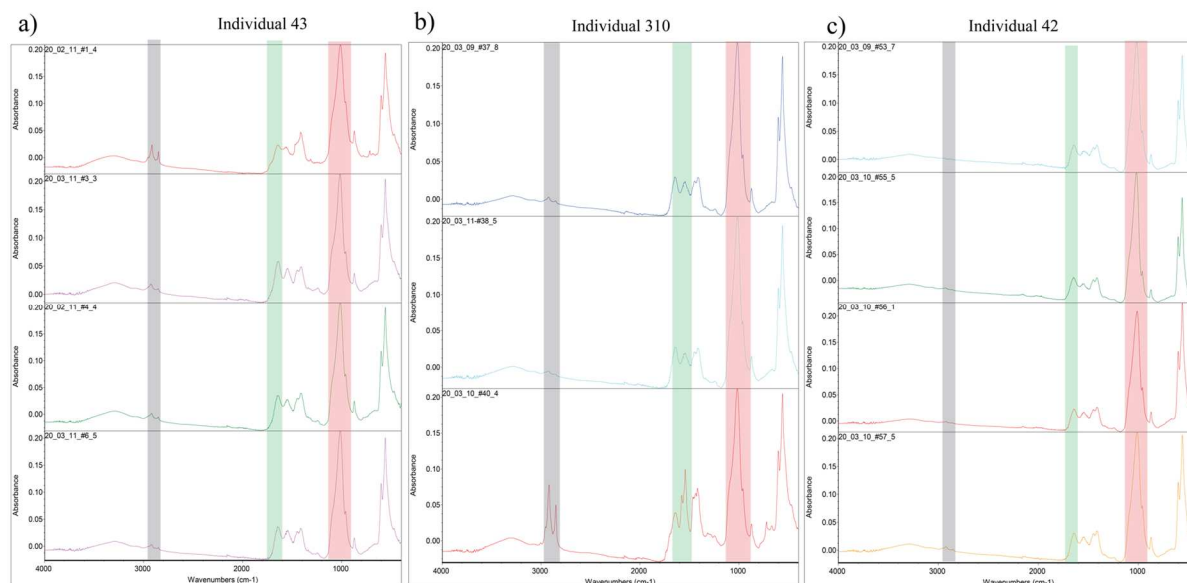


328

329 Figure 4 : Results of N %wt evaluated using ATR-FTIR both *in situ* (Bruker Alpha) and at the lab (Nicolet iS5), regarding  
 330 individuals 43, 310 and 42. Individual 43 shows a high dispersion between the four measured N contents, whereas individuals 310  
 331 and 42 show better reproducibility.

332 This study was administratively authorized to focus on the bone collagen, and the sampling was carried out  
 333 to exclude possible exogenous contamination of the bone (see sample section). However, infrared spectra  
 334 obtained from bone samples sometimes highlight the presence of organic compounds other than those  
 335 associated with bone, although they do not allow a precise and detailed discussion of the identification of  
 336 the nature of these compounds. To address this issue, examinations and analyses should have been focused  
 337 on the embalming products themselves, or the artifacts that may primarily contain them, which was not  
 338 possible in this study.

339 Regarding ATR-FTIR spectra, the 43 individual analyses display quite close spectra (samples 1, 2, 3, 4, 6)  
 340 while they have very disparate N %wt (up to three times more in one of the samples, Figure 4, Figure 5a,  
 341 Table 2). All ATR-FTIR spectra show the C-H bands at  $2920\text{ cm}^{-1}$  and  $2850\text{ cm}^{-1}$  (Figure 5), and  
 342 elemental analyses on two samples provide C %wt of 20 and 29. Sample #1, showing the highest C-H  
 343 bands, has the lowest collagen contents, whereas sample 3 has 24 %wt of collagen and a C %wt of 26.3.  
 344 Regarding individual 310, ATR-FTIR N %wt are consistent with each other (Figure 4, Table 2), while  
 345 sample #40 presents a high C %wt of 29 and its IR spectrum clearly shows the double C-H bands. Samples  
 346 37 and #38 show these double bands less intensively, and the first gets a C %wt of 19.8 (Figure 5b). The  
 347 ATR-FTIR spectra obtained on individual 42 do not strongly display the double C-H bands (Figure 5c),  
 348 and the associated C %wt are of 14.9 to 16.5 (Table 2).



349  
 350 Figure 5 : ATR-FTIR spectra (Nicolet Is5) comparison of the samples analysed from individuals 43 (a), 310 (b) and 42  
 351 (c). On each spectrum, Amide I (1710-1590  $\text{cm}^{-1}$ ) and  $\text{PO}_4$  (1110-940  $\text{cm}^{-1}$ ) bands are highlighted, as well as C-H  
 352 bands (2920 $^{-1}$  and 2850  $\text{cm}^{-1}$ ).

353  
 354  
 355  
 356

- **Elemental analyses**

357 Elemental analyses have been carried out on the whole bone powders. They have provided N %wt  
 358 contents ranging from 3.01 to 9.07 (Table 2). They can vary up to 100% on different powders collected  
 359 from the same individual. For instance, mummy 42 N %wt content extends from 3.01 to 6.15.  
 360 Results of C content are from 14.89 %wt to 33.26 %wt. Those values are less scattered than %N values  
 361 when comparing samples from the same individual, but are really high compared to the C content of a fresh  
 362 bone (Table 1).  
 363 C:N atomic values are from 2.82 to 6.44. As suggested by Brock et al. (2010b), C:N atomic ratios of  
 364 greater than 7 are generally not suitable for dating, and a threshold of 17 gives 71%wt of a chance to extract  
 365 more than 1 %wt of collagen (Brock et al., 2012). It means that all these archaeological bones seem to be  
 366 datable using the  $^{14}\text{C}$  method, according to elemental analyses.

367

#### 368 **IV. Interpretation and discussion**

369  
 370

- **Methodological discussion**

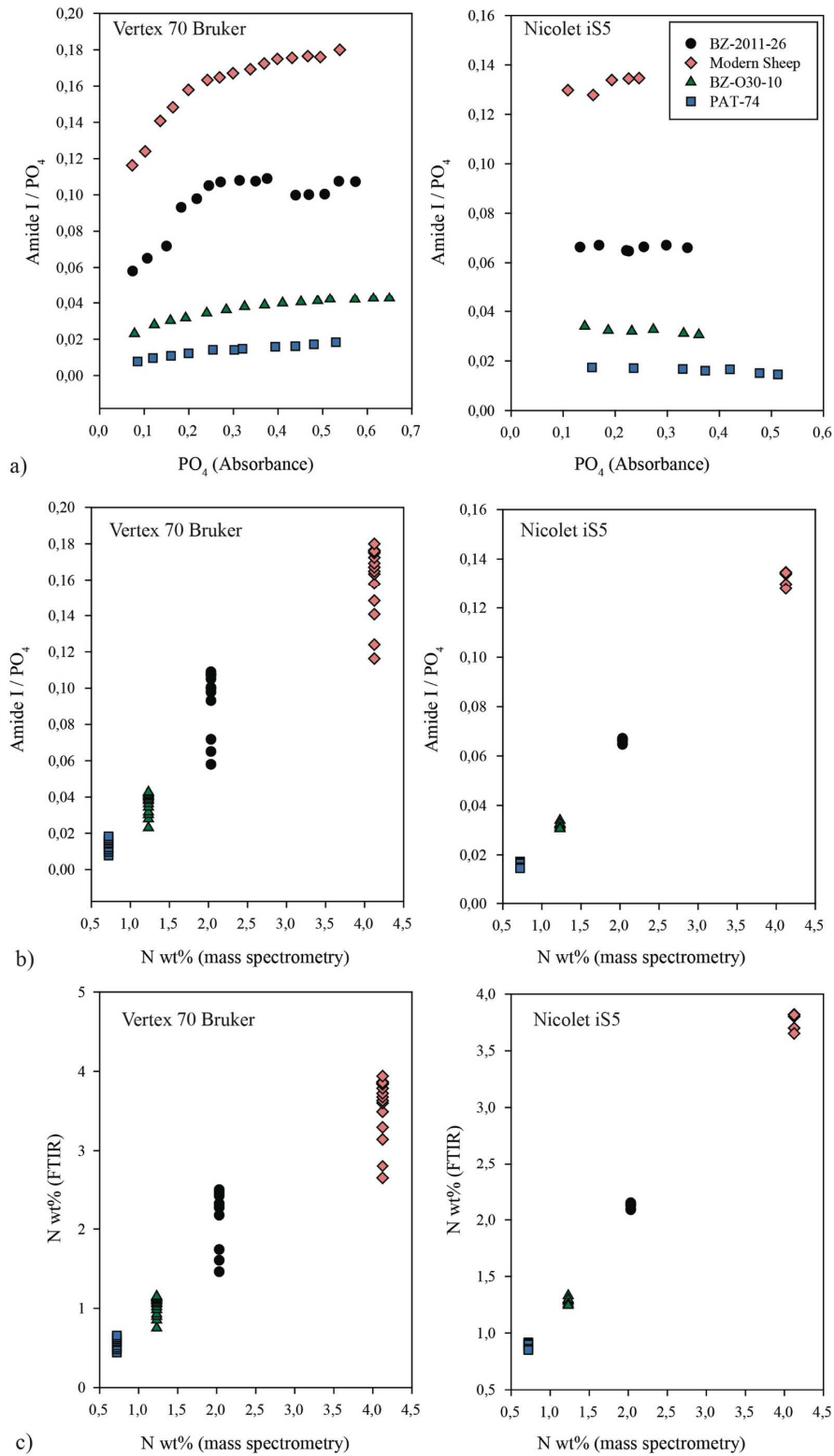
- **Acquisition parameters and ATR-FTIR system calibration**

371 As already pointed out, in order to ensure the measurement reproducibility and the accuracy of each  
372 spectrometer / ATR system, the anvil pressure on the sample is adjusted to obtain a specific intensity for  
373 the  $\nu_3\text{PO}_4$  band around  $1015\text{ cm}^{-1}$ .

374 As each spectrometer has its own “reference value”, this implies obtaining different Amide I /  $\text{PO}_4$  ratios  
375 and then specific calibration curves for N %wt content calculation. In order to evaluate the bias induced by  
376 different acquisition set-ups and by spectra-intensity adjustment during acquisition, four samples selected  
377 from the calibration bone set were measured at increasing intensities on two systems: the laboratory Vertex  
378 70 and the portable Nicolet iS5. The intensity was chosen by adjusting the anvil pressure on the ATR  
379 crystal and monitored by measuring the maximal absorbance of the broad and intense  $\nu_3\text{PO}_4$  band around  
380  $1015\text{ cm}^{-1}$ . Figure 6a shows that the increase of spectra intensity (followed by the absorbance of the  $\nu_3\text{PO}_4$ )  
381 induces an increase of the Amide I /  $\text{PO}_4$  ratio for the Vertex 70 spectrometer while this ratio decreases  
382 slightly for the Nicolet iS5 spectrometer. This is related to the possibility of varying the pressure obtained  
383 with the different ATR anvil systems, so it is an expected specificity. As displayed in Figure 6 b,c, these  
384 variations can induce a large bias on N %wt estimates by FTIR: up to 46 % of variation for the Vertex 70  
385 and 8 % for the Nicolet iS5. The wide variation observed for the Vertex 70 measurements is due to the use  
386 of the Golden Gate ATR accessory (Specac), which allows the application of very significant pressures and  
387 the precise control of the anvil pressure applied to the sample compared to other ATR accessories. Without  
388 special precautions for taking measurements, this leads to a strong dispersion of the quantifications deduced  
389 from the measurements. An increase of spectra intensity by adjusting ATR accessory anvil pressure is not a  
390 simple scale factor but has different effects on the  $\text{PO}_4$  and amide I band. Several studies have highlighted  
391 the difficulty of obtaining accurate estimates of archaeological bone preservation by ATR-FTIR (D’Elia et  
392 al., 2007; Gianfrante et al., 2007). The comparison of experimental and modelled spectra of apatite and  
393 bones with various degrees of preservation have shown that the position, shape and relative intensity of a  
394 strong absorption band are affected by macroscopic electrostatic properties of samples measured by ATR-  
395 FTIR spectroscopy (Aufort et al., 2016, 2018). This is especially the case of the relative fraction of mineral  
396 and organic components, and the solid-to-air fraction in the powder analysed. For a single sample, the  
397 mineral-to-organic fraction depends on collagen preservation and cannot vary according to the anvil  
398 pressure adjustment. However, the increase of pressure could modify powder compaction and thus the  
399 solid-to-air fraction of the sample pressed in contact with the ATR diamond crystal. That could affect the  
400 strong  $\nu_3\text{PO}_4$  absorption band and thus the Amide I /  $\text{PO}_4$  ratio. It could explain the discrepancies observed  
401 between the alteration state of archaeological bone evaluated by ATR-FTIR and by conventional FTIR in  
402 transmission mode (Hollund et al., 2013). These results demonstrate the importance of carefully controlling  
403 the acquisition conditions to achieve a reliable quantification of collagen content by ATR-FTIR  
404 spectroscopy. It involves, first, a stringent standardization of spectral intensity during acquisition, for  
405 example on a strong absorption band (for example  $\nu_3\text{PO}_4$ ); and secondly, a specific calibration established  
406 for each acquisition system.

407 This implies that the formulas presented here or previously published by Lebon et al. (2016), which are  
408 used to estimate the collagen contents from the Amide I / PO<sub>4</sub> ratios, are specific to the instrumental  
409 configurations and measurement protocols used in this study and cannot be used for other systems. If such  
410 a calibration cannot be made, measurements of collagen contents by ATR-FTIR are only semi-quantitative,  
411 and specific sample-selection thresholds have to be chosen, as proposed by Pothier Bouchard et al. (2019)  
412 for pre-screening of zooarchaeology samples by Mass Spectrometry (ZooMS). In any case, a  
413 standardization of spectral intensity during acquisition is recommended to avoid intra-instrument variations  
414 and ensure quantification reliability, especially between samples with low and high collagen content.





415  
 416 Figure 6 : Effects of ATR anvil-pressure variation for the characterisation of four samples of the reference bone set. a)  
 417 Variation of Amide I / PO<sub>4</sub> ratio according to the spectra intensity during acquisition monitored by the intensity of the  
 418 ν<sub>3</sub>PO<sub>4</sub> band. b) Impact of the spectra intensity during acquisition on the Amide I / PO<sub>4</sub> ratio measurement according to  
 419 N %wt measured by elemental analysis. C) Impact of the spectra intensity during acquisition on the estimation of  
 420 measurement of N %wt by FTIR according to N %wt measured by elemental analysis.  
 421

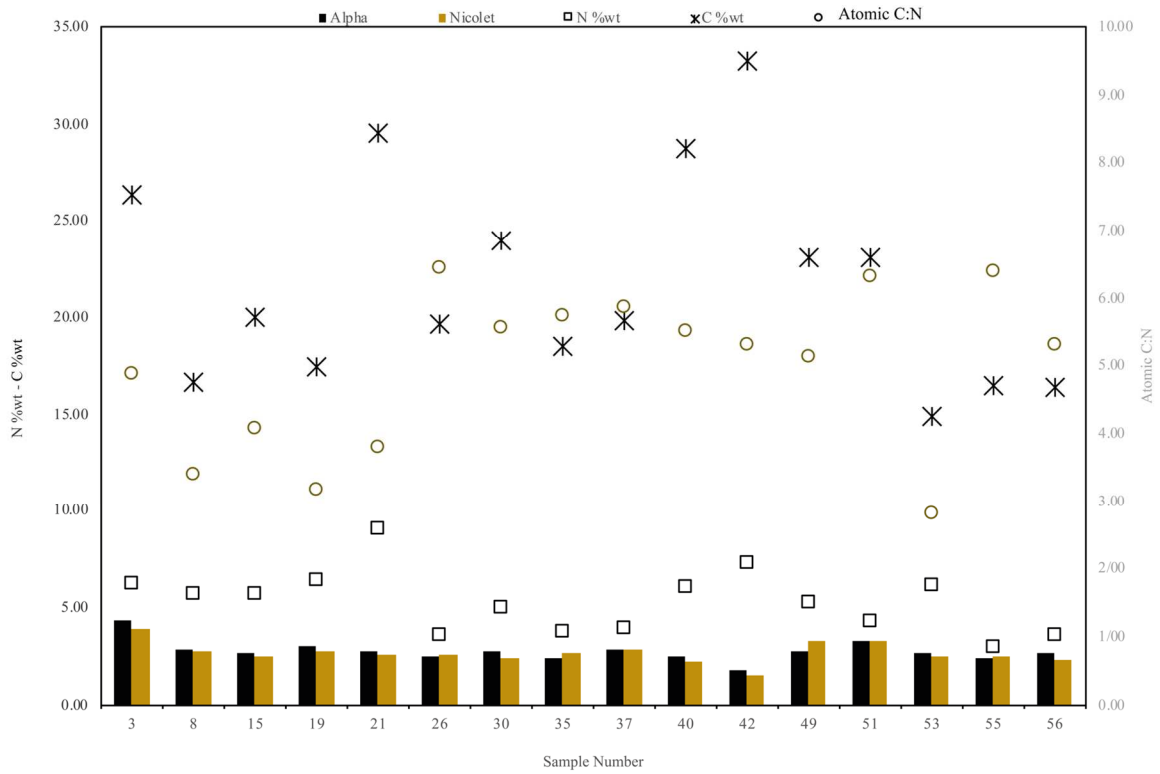
422 - **Elemental analyses versus ATR-FTIR on KV 40 samples**

423 Table 1 shows that the analysed collagen contents of fresh modern bones (Veal, Turkey, Ox, Sheep, Horse)  
424 range from 17.6 to 21.9 % wt, and C from 10.80 to 14.17 %wt. This is consistent with the expected values  
425 found in the literature of 4.3 N %wt, which represents around 22 %wt collagen content, and around 15 C  
426 %wt (van Klinken, 1999; Bocherens et al., 2005).

427 Regarding the KV 40 bone samples, ATR-FTIR analyses carried out on two spectrometers have mainly  
428 provided consistent results of collagen contents (Table 2), but some are slightly higher than the usual  
429 content observed for archaeological bones (measured modern bones are around 17.57 to 21.69 %wt, Table  
430 1). Five of the 28 KV 40 samples provide more than 17.5 %wt of collagen, as if they were fresh bones; and  
431 one of them contains almost 24 %wt of collagen. As previously mentioned, evaluation of collagen content  
432 is not accurate for sample bones with collagen content similar to modern bones. Nitrogen contents  
433 evaluated using ATR-FTIR analyses are systematically lower than the N %wt obtained using elemental  
434 analyses (Table 2, Figure 7), but there is no clear systematic relationship between both (Figure 8a).

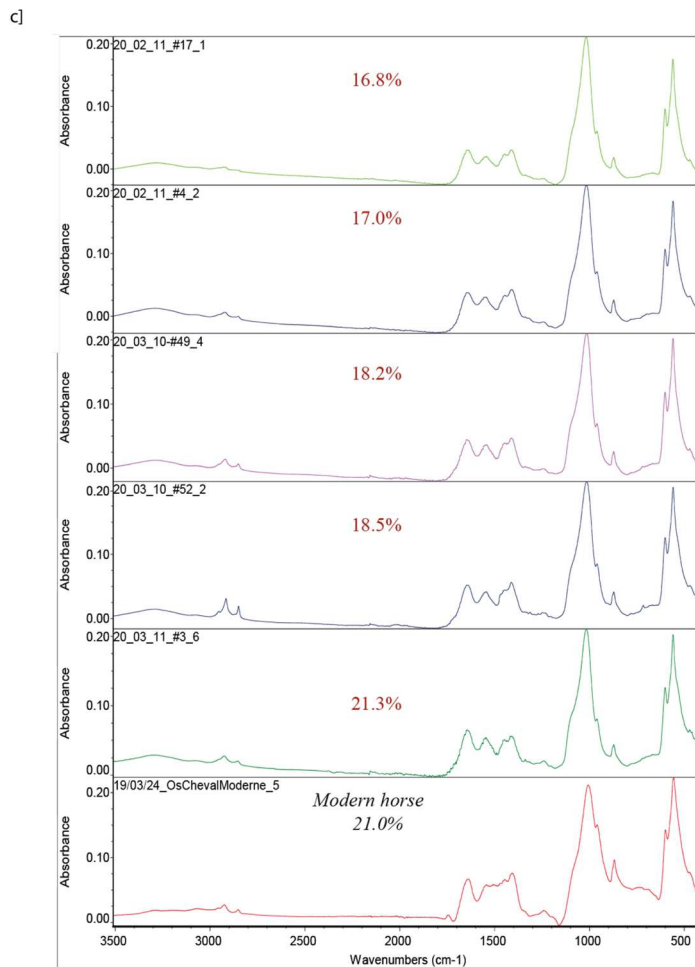
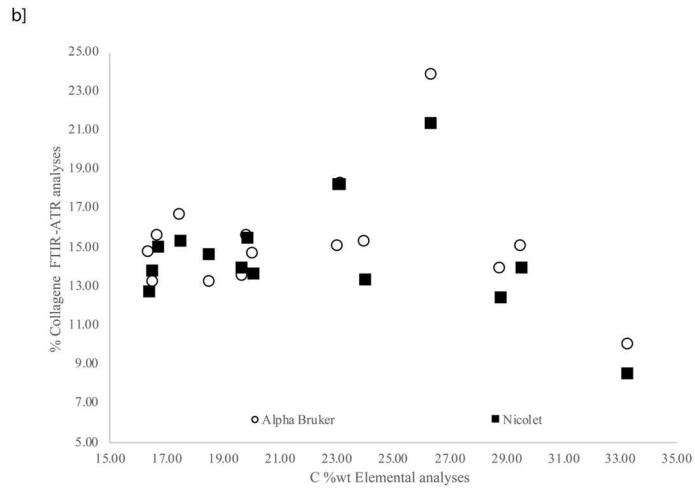
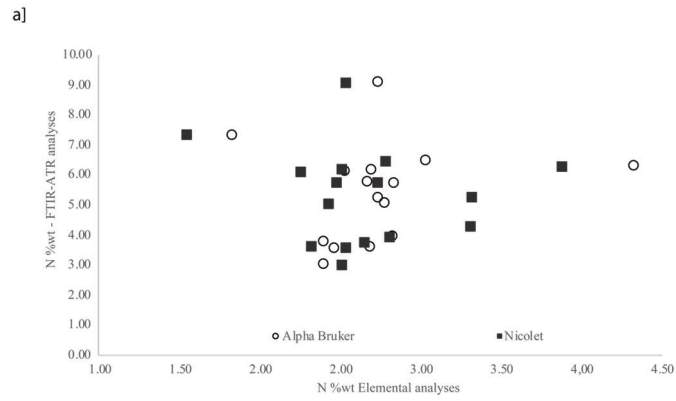
435 In addition, C %wt from elemental analyses are very high, ranging from 14.9 to 33.3, which is higher than  
436 the C %wt of fresh bones (Table 1, Modern bone samples). Moreover, elemental analyses underline that  
437 samples with a high C %wt also get a high N %wt (Figure 7).

438 Regarding collagen content, no relationship is identified between its quantity and C %wt (Figure 8b). For  
439 instance, sample #42, which exhibits the highest C %wt with 33, only got 8.5%wt of collagen, whereas #3,  
440 with the highest collagen contents of 21.3%wt, reaches 26.3 C %wt. Nonetheless, ATR-FTIR spectra of  
441 samples getting more than 17%wt collagen systematically show two C-H bands at 2920 cm<sup>-1</sup> and 2850 cm<sup>-1</sup>  
442 (Figure 8c). This makes clear that not all organic compounds are due to raw bones, because non-  
443 contaminated bones clearly do not have such C-H bands, but are also due to additional organic  
444 contaminants. In addition, and as expected, FTIR spectra of samples with high carbon contents also  
445 systematically show the same C-H double bands at 2920 cm<sup>-1</sup> and 2850 cm<sup>-1</sup>, whereas these bands are  
446 really weak for samples with less than 15% carbon that correspond to the reference value for a modern  
447 bone without contamination (Figure 9). As already mentioned, the measurements performed and  
448 authorized in this study make further analysis of the nature of these contaminants impossible.

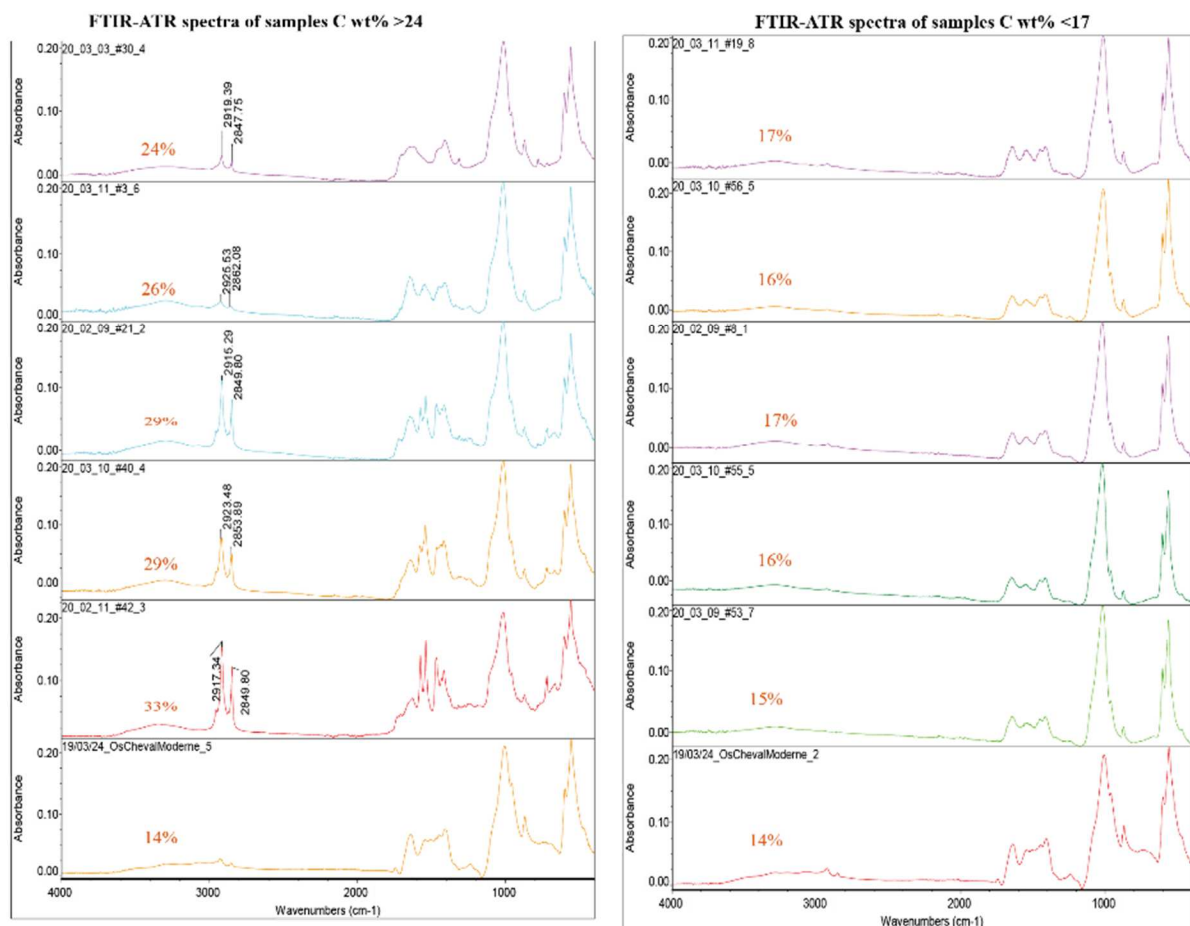


449

450 Figure 7 : Comparison of N %wt evaluated from ATR-FTIR measurements (histogram, Bruker Alpha in brown,  
 451 Nicolet iS5 in gold) and results of elemental analyses (curves, N %wt in square marks, C %wt in cross marks and  
 452 atomic C:N in circle marks).



454 Figure 8 : (a) Comparison of N %wt evaluated from ATR-FTIR (*in situ*, Bruker Alpha in circle marks;  
 455 Lab, Nicolet iS5 in square marks) vs N %wt measured using elemental analyses. (b) Comparison of %wt  
 456 collagen measured using FTIR analyses (*in situ*, Bruker Alpha in black; Lab, Nicolet iS5 in yellow) vs C  
 457 %wt measured by elemental analyses. (c) Nicolet iS5 ATR-FTIR spectra on archaeological bone samples  
 458 with the highest collagen contents ranging from 16.8 to 21.3%wt and comparison with the spectrum of a  
 459 modern horse bone.  
 460



461  
 462 Figure 9 : Comparison of ATR-FTIR spectra (Nicolet iS5) regarding their C %wt, as measured by  
 463 elemental analyses. The C %wt measured by elemental analyses is written in red in each spectrum, those  
 464 with the higher values from 24 to 33%wt are on the left, those with the lowest values from 14 to 17%wt are  
 465 on the right. Both sets of spectra are compared with the spectrum from a modern horse (14%wt).  
 466

467 - **Inhomogeneity of collagen preservation within one individual**

468 As discussed previously, all these archaeological samples are contaminated to varying degrees with  
 469 extraneous organic material that is probably linked with the embalming process. Yet, as detailed in section  
 470 III, N %wt discrepancies within the same individual cannot systematically be explained by extraneous  
 471 contaminations and seem more likely to be linked with the heterogeneity of the internal state of collagen  
 472 preservation. But from the obtained results, it is not possible to evaluate to what extent these

473 contaminations are impacting ATR-FTIR collagen quantification, while the results of elemental analyses  
474 clearly sum values of nitrogen from both collagen and contaminants. In this context, ATR-FTIR  
475 measurements seem to provide more precise results regarding the state of collagen preservation. As the  
476 infrared spectral characteristics are related to structural characteristics of collagen, the elementary  
477 indications derived from them are necessarily related to the corresponding proteins. This is in contrast to  
478 elemental analyses, which sum up the elements present regardless of the molecular structures that contain  
479 them. This could therefore explain why collagen contents measured through infrared spectroscopy are less  
480 sensitive to some organic contaminations.

481  
482 These investigations emphasize the interest of using FTIR analyses as an investigation technique for  
483 obtaining a precise, on-site overview of collagen preservation in archaeological bones, and in particular  
484 embalmed bones. It also highlights the challenge of precisely determining the remaining collagen content  
485 in an ancient bone, in order to evaluate on site the sample quantity needed for a radiocarbon analysis and  
486 thus optimize the chances of successful dating in compliance with the preservation of archaeological  
487 artefacts. The use of the Accelerator Mass Spectrometry (AMS) method seems feasible for the selection of  
488 a bone area where the N %wt is high enough and consistent enough, as this method requires around 1 g of  
489 material (Brock et al., 2010a; Jacob et al., 2018). For the Liquid Scintillation Counting method (LSC,  
490 Quiles et al., 2017), which requires around one thousand times more material than AMS, it remains more  
491 troublesome, as we are only able to evaluate average collagen contents for each bone area. We will have to  
492 proceed by estimating the collagen content from one area to another in order to complete the double  
493 challenge of ensuring a chemical extraction that provides sufficient collagen for dating, while also not  
494 collecting more material than is needed. In the case of well-preserved and clean bones, this is clearly  
495 possible. In this study, it remains quite challenging because of the collagen heterogeneity and extraneous  
496 contaminations. For these reasons the AMS method seems more appropriate in this context.

497         • **Statements regarding radiocarbon dating of KV 40 bones**

498 The set of samples selected for ATR-FTIR analyses clearly still contains enough collagen to be dated using  
499 the radiocarbon method. This result is of utmost importance in regard to the complexity of the tomb's  
500 history (looting, fires). The fire(s) must have been violent but short, which explains that collagen still  
501 remains within bones despite the heat. As we have seen, not all the bones have been burned in the same  
502 way, which can partially explain the heterogeneity of the N %wt within a single bone. Even under  
503 challenging conditions, this study shows that in a protected area like a tomb, and despite hot and dry  
504 climatic conditions, bone samples can be well preserved enough to be dated. This clearly opens up exciting  
505 new perspectives for the study of human and animal remains.

506 Moreover, thanks to the currently available mobile instrumentation, the analytical approach developed here  
507 clearly works on site and under challenging conditions (here within a tomb below a 6-meter deep shaft). It  
508 provides a quick and efficient screening tool leading up to radiocarbon dating, making it possible to

509 determine on site which samples to collect, as well as how much of them will be needed. Thus, the entire  
510 collection process can be immediately done on site.

511 Among the 83 individuals, thirteen have been analysed, and the rest have to be examined as soon as we will  
512 get the work authorisation. As this first set of samples has been selected randomly, the entire lot should  
513 provide a similar answer regarding the state of preservation of their collagen. The KV 40 discovery in the  
514 Kings' Valley, in which more than eighty individuals have been identified, has generated wide interest and  
515 opened up many issues regarding the history of this area during the 18<sup>th</sup> and the 22<sup>nd</sup> dynasties. To go  
516 further in the understanding of this tomb's history, it is crucial to distinguish which of these 83 individuals  
517 belongs to the 18<sup>th</sup> and which to the 22<sup>nd</sup> dynasty. This will enable the comparison of the results with  
518 anthropological observations and the study of mummification techniques, so as to gain new insights into  
519 the evolution of the embalming process during these two periods. It will then allow a better understanding  
520 of how many individuals are linked with the 18<sup>th</sup> dynasty, and could therefore be related with the royal  
521 family. And finally, DNA studies could rely on these results to select individuals from the 18<sup>th</sup> dynasty to  
522 be analysed and to make further progress in their identification.

523

## 524 **Conclusion**

525 For radiocarbon dating purposes, this study aimed at evaluating the state of preservation of the bones of  
526 human mummies from an Egyptian tomb in the Kings' Valley belonging to the 18<sup>th</sup> and the 22<sup>nd</sup> dynasties.  
527 For that purpose, an ATR-FTIR instrument has been tested on site as a screening tool in order to determine  
528 *in situ* the state of collagen preservation of bone remains and thus establish if they are datable or not.  
529 Evaluated N %wt contents have then been compared to ATR-FTIR analyses at the lab (performed using a  
530 different spectrometer), as well as to elemental analyses.

531 The results obtained on thirteen individuals clearly show the ATR-FTIR analyses to be quite consistent  
532 between both spectrometers, which validates the relevance of this analytical approach on site once each  
533 spectrometer has properly been calibrated and when stringent measurement protocols are followed. The  
534 study also emphasizes that this technique requires a deep knowledge of each spectrometer's specificities  
535 regarding, for instance, the behavior of the control of the ATR anvil pressure with respect to each sample.  
536 After establishing the appropriate calibration curves for the quantification of collagen and once the  
537 measurement process has been established by its operator, ATR-FTIR is clearly an efficient, on-site  
538 screening tool.

539 Regarding the characterization of archaeological samples, discrepancies have been highlighted between  
540 ATR-FTIR and the results of elemental analyses, the latter systematically providing higher N %wt than the  
541 former. C %wt contents are also very high, which shows that bones are clearly contaminated by organic  
542 materials, most probably due to the embalming process. ATR-FTIR spectra can highlight such organic  
543 contaminations, but at the same time the calculated collagen contents seem to be less impacted by such  
544 archaeological pollution than elemental analyses (see also discussions in Lebon et al., 2016, on the impacts

545 of contaminations). Thus, infrared spectroscopy provides more reliable estimates of the preservation of  
546 bone collagen.

547 The thirteen KV 40 individuals examined here are well-preserved enough to be dated by radiocarbon,  
548 which shows that in closed contexts like a tomb, the collagen of bones can be preserved despite hot and dry  
549 climatic conditions.

550 The ATR-FTIR method opens up new perspectives for the study of human and animal remains on site,  
551 especially in Egypt, where it is quite often challenging to identify well-preserved bones, in particular in  
552 open-air archaeological sites. This technique is now systematically applied by the IFAO archaeometry  
553 department to screen bone samples on site and to identify before collection if they are datable or not. The  
554 application of ATR-FTIR prescreening for the preservation of bone collagen constitutes an effective  
555 implementation of the process of sample selection, for example before radiocarbon dating, collagen  
556 isotopic analysis, or proteomic studies.



## Tables and Figures

Sample	Site	Treatment	Elemental analyses							ATR-FTIR analyses																
			C %wt			N %wt				C:N	Amide I / PO <sub>4</sub> (Vertex 70)			Amide I / PO <sub>4</sub> (Bruker Alpha)			Amide I / PO <sub>4</sub> (Nicolet iS5)			Vertex 70	Bruker Alpha	Nicolet iS5	Vertex 70	Bruker Alpha	Nicolet iS5	
			Mean	SD	RSD	Mean	SD	RSD	Mean		SD	RSD	Mean	SD	RSD	Mean	SD	RSD	N content % (FTIR)			dispersion	Collagen content % (FTIR)			
PAT-78	Abri Pataud	-	2.10	0.016	0.76	0.06	0.06	0.004	7.08	37.6	0.012	0.001	11.2	0.004	0.002	39.493	0.009	0.001	8.208	0.53	0.55	0.64	0.06	2.89	3.01	3.52
BZ-PJ1-06	Bize-Tournal	-	2.66			0.12	0.12			22.2	0.015	0.002	11.2	0.001	0.002	126.863	0.002	0.001	48.276	0.59	0.47	0.48	0.07	3.26	2.59	2.65
EH2-20	El Harourra	-	4.04			0.16	0.16			26.0	0.008	0.000	0.5	0.000	0.001	404.384				0.45	0.44	0.44	0.00	2.45	2.43	
EH2-25	El Harourra	-	4.27			0.16	0.16			27.0	0.011	0.003	23.6	0.005	0.002	44.697	0.004	0.000	2.681	0.50	0.56	0.52	0.03	2.76	3.07	2.88
EH2-24	El Harourra	-	3.93			0.20	0.20			19.3	0.013	0.001	8.7	0.006	0.001	23.086	0.004	0.001	14.306	0.55	0.59	0.53	0.03	3.04	3.23	2.94
BZ-PJ1-05	Bize-Tournal	-	3.48			0.52	0.52			6.7	0.015	0.001	6.0	0.003	0.001	43.502	0.004	0.001	17.960	0.58	0.51	0.54	0.03	3.19	2.83	2.99
EH2-23	El Harourra	-	4.79			0.54	0.54			8.9	0.018	0.003	14.3	0.009	0.001	10.352	0.004	0.001	27.208	0.65	0.68	0.53	0.08	3.60	3.75	2.93
BZ-O30-18	Bize-Tournal	-	3.78			0.59	0.59			6.4	0.019	0.001	4.9	0.0087	0.0015	17.594	0.008	0.001	9.763	0.68	0.67	0.63	0.03	3.71	3.70	3.46
PAT-85	Abri Pataud	-	4.02			0.60	0.60			6.7	0.015	0.002	11.6	0.003	0.001	31.442	0.010	0.000	0.996	0.60	0.51	0.67	0.08	3.28	2.81	3.71
PAT-87	Abri Pataud	-	3.41			0.61	0.61			5.6	0.016	0.001	7.9	0.009	0.001	11.895				0.61	0.67	0.44	0.12	3.36	3.71	
EH2-26	El Harourra	-	4.82			0.64	0.64			7.5	0.013	0.001	5.8	0.006	0.001	17.273	0.007	0.001	12.769	0.54	0.61	0.59	0.04	2.97	3.35	3.25
PAT-74	Abri Pataud	-	5.13			0.72	0.72			7.1	0.023	0.001	6.3	0.012	0.002	12.930	0.014	0.001	6.909	0.75	0.75	0.77	0.01	4.13	4.14	4.23
MAG	Magura Buduiasca	-	4.91			0.97	0.97			5.1	0.030	0.002	6.3	0.015	0.001	7.134	0.016	0.002	9.941	0.88	0.85	0.81	0.04	4.86	4.69	4.46
BZ-2011-27	Bize-Tournal	-	5.11			1.01	1.01			5.1	0.038	0.003	7.9	0.025	0.002	9.974	0.024	0.002	7.106	1.06	1.10	1.00	0.05	5.81	6.08	5.49
BZ-O30-10	Bize-Tournal	-	5.29	0.044	0.83	1.23	1.23	0.016	1.30	4.30	0.047	0.003	7.1	0.030	0.004	14.413	0.033	0.001	4.378	1.23	1.25	1.22	0.01	6.77	6.87	6.72
BZ-O30-19	Bize-Tournal	-	6.61			1.78	1.78			3.7	0.071	0.006	7.8	0.039	0.001	1.494	0.037	0.000	0.728	1.73	1.51	1.30	0.21	9.50	8.30	7.18
BZ-PJ1-09	Bize-Tournal	-	6.90			1.80	1.80			3.8	0.073	0.003	4.0	0.042	0.003	7.997				1.76	1.58	0.44	0.72	9.67	8.72	
BZ-PJ1-16	Bize-Tournal	-	7.22			1.87	1.87			3.9	0.075	0.003	4.1	0.056	0.000	0.325	0.047	0.007	15.752	1.81	1.97	1.55	0.21	9.94	10.81	8.53
BZ-2011-26	Bize-Tournal	-	7.90			2.03	2.03			3.9	0.089	0.005	6.2	0.061	0.002	3.420	0.062	0.001	1.439	2.09	2.11	1.90	0.12	11.48	11.58	10.44
BZ-2011-02	Bize-Tournal	-	7.49			2.04	2.04			3.7	0.077	0.001	1.9	0.062	0.001	2.381	0.056	0.001	1.564	1.85	2.12	1.77	0.18	10.18	11.66	9.71
HAR	Harsova-tell	-	9.81	3.619	36.89	2.75	2.75	1.188	43.17	3.72	0.128	0.007	5.7	0.091	0.002	2.013	0.099	0.001	1.396	2.88	2.92	2.77	0.08	15.82	16.08	15.25
Modern Veal Bone	Modern	-	10.80			3.42	3.42			3.2	0.156	0.008	5.3	0.105	0.006	5.826	0.116	0.000	0.323	3.45	3.30	3.20	0.13	18.95	18.17	17.57
Modern Turkey Bone	Modern	-	12.66			3.95	3.95			3.2	0.182	0.009	4.8				0.144	0.001	0.828	3.98	0.44	3.86	2.01	21.86		21.23
Modern Ox Bone	Modern	-	12.59			3.96	3.96			3.2	0.179	0.012	6.7	0.129	0.009	7.022				3.92	3.94	0.44	2.02	21.58	21.69	
Modern Sheep Bone	Modern	-	12.95			4.12	4.12			3.1	0.164	0.006	3.7	0.111	0.007	6.343	0.131	0.005	4.183	3.61	3.46	3.54	0.07	19.86	19.05	19.49
Modern Horse Bone	Modern	-	14.17	0.040	0.28	4.13	4.13	0.020	0.49	3.43	0.165	0.003	1.6	0.114	0.001	0.983	0.143	0.001	0.412	3.63	3.55	3.82	0.14	19.94	19.52	21.01
EH2-04	El Harourra	-	2.17			< 0.05	0.00			> 40	0.011	0.001	7.6	0.006	0.001	10.493	0.000	0.000	-41.918							
ORG-3	Orgnac 3	-	2.29			< 0.05	0.00			> 40	0.011	0.001	9.0	0.006	0.002	27.983	0.008	0.000	4.112							

Table 1: Results of elemental analyses and ATR-FTIR analyses carried out on calibration bone samples. Evaluations of N %wt and collagen %wt. For ATR-FTIR analyses, three spectrometers have been used (Vertex 70, Bruker Alpha and Nicolet iS5).

		ATR-FTIR										Elemental Analyses				ATR-FTIR vs Elemental Analyses
		On site (Bruker Alpha)				In lab (Nicolet iS5)				Comparison of spectrometers		Elemental Analyses				
Individual	sample N°	Amide I/ ν <sub>3</sub> PO <sub>4</sub>	N %wt	Collagen %wt	relative variability	Amide I/ ν <sub>3</sub> PO <sub>4</sub>	N %wt	Collagen %wt	relative variability	N %wt average value	Relative collagen variability	Lab code	N %wt	C %wt	Atomic C:N	
43	1	0.036	1.40	7.72		0.056	1.86	10.25		1.63	0.28					
	2	0.100	3.16	17.38												
	3	0.143	4.33	23.79		0.137	3.88	21.32		4.10	0.11	IFEG000025	6.282	26.326	4.889	
	4	0.101	3.19	17.55		0.105	3.09	17.01		3.14	0.03					
	6	0.118	3.64	20.04								IFEG000026	6.837	19.882	3.393	
		0.096	3.04	16.74	0.93	0.102	3.02	16.59	0.68	3.03	0.01					
238	8	0.088	2.84	15.59		0.091	2.73	15.02		2.78	0.04	IFEG000027	5.731	16.684	3.396	
	9	0.050	1.80	9.91	0.45	0.06	1.92	10.56	0.35	1.86	0.06					
572	15	0.082	2.67	14.70		0.080	2.48	13.61		2.57	0.08	IFEG000028	5.733	20.040	4.078	
	17	0.111	3.45	18.95	0.25	0.104	3.06	16.82	0.21	3.25	0.12					
283	19	0.095	3.03	16.68		0.093	2.78	15.30		2.91	0.09	IFEG000029	6.449	17.472	3.161	
228	21	0.085	2.74	15.06		0.08	2.54	13.94		2.64	0.08	IFEG000030	9.065	29.513	3.798	
319	25	0.095	3.01	16.57		0.087	2.63	14.47		2.82	0.14					
	26	0.075	2.46	13.56	0.20	0.083	2.53	13.94	-0.04	2.50	0.03	IFEG000031	3.565	19.666	6.436	
41	30	0.086	2.78	15.29		0.08	2.43	13.35		2.60	0.13	IFEG000032	5.043	24.006	5.554	
	35	0.072	2.40	13.21		0.087	2.65	14.59		2.53	0.10	IFEG000034	3.760	18.495	5.739	
310	37	0.088	2.83	15.56		0.094	2.81	15.44		2.82	0.01	IFEG000035	3.942	19.834	5.870	
	38	0.084	2.72	14.96		0.103	3.03	16.64		2.87	0.11					
	40	0.077	2.53	13.93	0.11	0.071	2.26	12.42	0.28	2.40	0.11	IFEG000036	6.088	28.771	5.513	
284	42	0.051	1.83	10.04		0.043	1.55	8.52		1.69	0.16	IFEG000037	7.321	33.263	5.301	
303	47	0.089	2.87	15.78								IFEG000039	6.987	20.799	3.473	
302	49	0.085	2.74	15.07		0.114	3.32	18.24		3.03	0.19	IFEG000040	5.237	23.055	5.136	
316	51	0.106	3.32	18.25		0.114	3.31	18.20		3.31	0.00	IFEG000041	4.271	23.122	6.316	
	52	0.104	3.26	17.95	0.02	0.116	3.36	18.46	0.01	3.31	0.03					
42	53	0.083	2.70	14.83		0.082	2.51	13.81		2.60	0.07	IFEG000042	6.154	14.890	2.823	
	55	0.072	2.40	13.21		0.082	2.51	13.80		2.46	0.04	IFEG000043	3.007	16.496	6.400	
	56	0.083	2.69	14.78		0.074	2.32	12.75		2.50	0.15	IFEG000044	3.589	16.377	5.324	
	57	0.079	2.58	14.21	0.11	0.081	2.48	13.65	0.08	2.53	0.04					

Table 2: Results of ATR-FTIR and elemental analyses for KV40 samples analysed both in situ and in the lab. Three samples were not analysed by ATR-FTIR in the laboratory as the low amount of sample collected was completely consumed by the elemental analysis. ATR-FTIR results are provided by spectrometer (Amide I / ν<sub>3</sub>PO<sub>4</sub>, N %wt and collagen %wt). Relative variability within one individual has been calculated when several analyses were done on a same individual. Comparison between spectrometers gives the average value and the relative variability between both spectrometers (difference over average value). Elemental analyses provide both N %wt and C %wt measured values, and the deduced atomic C:N. The last column provides the relative variability between both methods. It has been calculated using the difference between the N %wt average FTIR values and the N wt% from CHN value, over the total average value.

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