1The effect of grain size on carbonate contaminant removal from tooth enamel: 2towards an improved pretreatment for radiocarbon dating

3

4Rachel Wood¹, Mathieu Duval^{1,2}, Nguyen Thi Mai Huong³, Nguyen Anh Tuan³, Anne-
5Marie Bacon⁴, Fabrice Demeter⁵, Philippe Düringer⁶, Marc Oxenham⁷, Philip Piper⁷

6

7¹ Research School of Earth Sciences, The Australian National University, Canberra,
82601, Australia

9² Centro Nacional de Investigación sobre la Evolución Humana (CENIEH), Burgos,
10Spain

11³ Anthropological and Palaeoenvironmental Department, The Institute of Archaeology
12of Vietnam, Hanoi, Vietnam

13⁴ UMR 5288 du CNRS, AMIS Anthropologie Moléculaire et Imagerie de Synthèse,
14Faculté de chirurgie dentaire, Montrouge, France.

15⁵ Département Homme Nature Société, UMR 7206 du CNRS, Muséum National
16d'Histoire Naturelle, Musée de l'Homme, Paris, France.

17⁶ Ecole et Observatoire des Sciences de la Terre, Institut de Physique du Globe de
18Strasbourg, UMR 7516 du CNRS, Université de Strasbourg, Strasbourg, France.

19⁷ School of Archaeology and Anthropology, The Australian National University,
20Canberra, 2601, Australia

21

22Corresponding author: rachel.wood@anu.edu.au

23

24Abstract

25

26It is rarely possible to directly radiocarbon date skeletal remains from hot
27environments as collagen rapidly degrades. Although able to survive in the majority
28of burial environments for longer, unburnt biological apatites frequently produce
29inaccurate radiocarbon dates due to contamination from carbonate in the groundwater.
30The location of this contamination within the skeletal material is rarely investigated,
31hampering development of improved methods. This paper focuses on tooth enamel
32and aims to test whether carbonate contaminants are sitting at the crystallite
33boundaries, and from this to test a pretreatment to produce more accurate radiocarbon
34age estimates. Although the porosity of enamel is low, trace elements are known to
35diffuse between enamel prisms and crystallites. Gordon et al. (2015, *Science*, 347
36(6223), 746-750) identified magnesium substituted amorphous calcium phosphate
37between the apatite crystallites. This phase contains the majority of magnesium within
38modern rodent enamel, providing an opportunity to monitor its removal, and thus
39carbonate contaminants sitting between or on the surface of the crystallites. Modern
40*Sus scrofa* enamel and four ancient *Sus scrofa* teeth have been used to demonstrate
41that the more finely ground the enamel, the more magnesium can be removed with an
42acetic acid leach, and the more accurate the radiocarbon dates. After leaching in acetic
43acid, teeth dating to beyond the limit of the radiocarbon method (c.50 ka) produce
44ages of c.20 kBP when hand ground, and c.30 kBP when mechanically ground. This
45suggests that some contaminants are sitting at the crystallite boundaries. However,

46although mechanically grinding substantially increases the amount of carbonate
47contamination removed in an acid leach compared to hand grinding, not all
48contaminants could be removed from the samples examined in this study, and
49radiocarbon dates on tooth apatite should still be regarded as minimum ages.

50

51**Key words**

52Radiocarbon; Dating; Enamel; Teeth; Diagenesis; Stable isotope

53

541. Introduction

55

56Bone, dentine and enamel are made of varying proportions of organic, primarily
57protein, mineral and water phases. The protein fraction is normally targeted when
58radiocarbon dating skeletal remains. A variety of cleaning protocols, or
59‘pretreatments’, that effectively remove contaminants have been developed over
60several decades of intensive research (Brown et al., 1988; Longin, 1971; Marom et al.,
612012; McCullagh et al., 2010; Ramsey et al., 2004; Stafford Jr et al., 1988; Tisnérat-
62Laborde et al., 2003). However, in warm environments collagen rapidly degrades and,
63if water is present, leaches out of bone and dentine (Collins et al., 2002; Hedges,
642002). As a result, in these environments it is rarely possible obtain sufficient collagen
65to accurately radiocarbon date skeletal material beyond a few thousand years (Calo et
66al., 2015; Storm et al., 2013; Wood et al., 2013; Zazzo et al., 2014). Without the
67ability to directly date skeletal remains, it is exceptionally difficult to create high
68quality chronologies for e.g. cemetery sites or the domestication of fauna.

69

70The mineral component of bone and enamel, bioapatite, out-survives collagen in the
71majority of deposition environments. Although it can preserve stable isotopic
72signatures for more than 1 ma (Lee-Thorp, 2002), radiocarbon age estimates are
73normally found to be younger than expected. Within the Holocene, dates on bioapatite
74normally underestimate the expected age of a sample by a few hundred ^{14}C years, but
75in the Pleistocene this can increase to 10,000s ^{14}C years (Grün et al., 1997; Haynes,
761968; Hedges et al., 1995; Zazzo, 2014; Zazzo et al., 2013; Zazzo and Saliège, 2011).

77

78Enamel has long been thought to provide a more reliable material for radiocarbon
79dating than bone apatite (Haynes, 1968) because it has lower porosity and a smaller
80surface area (Hedges et al., 1995; Millard and Hedges, 1996), larger crystallites (26.3
81x 100-1000 nm vs. 5 x 100 nm (Bottero et al., 1992; Cui and Ge, 2007)), and is less
82soluble due to its lower carbonate content (3.5 wt% vs. 6 wt% (Elliott, 2002)). Despite
83these observations, relatively few studies have focused on radiocarbon dating enamel.
84When Zazzo (2014) reviewed the limited data and added several extra case studies he
85found that when bone and enamel apatite were pretreated in a similar manner, they
86underestimated the age of the samples to a similar extent, and he concluded that they
87are equally poor materials for radiocarbon dating.

88

89However, enamel has a complex hierarchical structure which is quite different to that
90of bone. Does this mean that contamination could be more effectively removed from
91enamel if it were pretreated in a different way to bone? This paper aims to consider
92the effect of enamel structure on the potential diagenetic pathways which could add
93exogenous carbonate to tooth enamel, and investigate whether a better understanding
94of one of these pathways may enable more accurate radiocarbon dates to be produced.

95

961.1 The structure of tooth enamel

97Tooth enamel consists of an inorganic mineral phase, the focus of this study, with a

98small proportion of organic material (<1% (Lee-Thorp, 2002)) and water. In
99mammals, the inorganic phase consists of crystallites of bioapatite (human: 26.3 x 68
100x 100-1000 nm (Cui and Ge, 2007)) arranged into micrometer-sized prisms (also
101known as rods) which are woven into various structures through the enamel cross-
102section (known as the schmelzmuster). Between the prisms, crystallites are arranged
103into an interprismatic matrix (terminology follows Koenigswald and Sander (1997),
104figure 1).

105

106The crystallites are formed of hydroxyapatite, also known as bioapatite, with the
107general formula $\text{Ca}_{10}(\text{PO}_4)_6\text{OH}_2$, into which various ions are substituted. Carbonates
108can be located in a number of environments within the apatite phase. Most carbonate
109is found in the B position, substituting for phosphate, with some found in the A
110position, substituting for hydroxyl ions, and the remaining in as yet poorly defined
111environments (Elliott, 1994). Several early studies proposed that non-apatite mineral
112phases may also be present in enamel (Driessens, 1982; Driessens and Verbeeck,
1131982, 1985; Hallsworth et al., 1972), and in 2015 Gordon et al. demonstrated that
114magnesium is concentrated between crystallites in non-pigmented rodent enamel.
115They suggested this was contained in a magnesium substituted amorphous calcium
116phosphate (Mg-ACP). In rodent enamel, the Mg-ACP phase contains carbonate, and
117at crystallite junctions the concentration of carbonate is higher than in the apatite
118(Gordon and Joester, 2015). The chemical location of carbonate within this phase is
119not yet known.

120

121Although enamel porosity is low, pores are present and play an important role in the
122diffusion of water and solutes through the tooth (Shellis and Dibdin, 2000). Pores
123exist both between prisms and within prisms (Shellis and Dibdin, 2000). In agreement
124with this model, desorption-adsorption studies with water suggest pore sizes in human
125molar enamel follow a double-peaked distribution with maxima at 1 nm and 2.5 nm
126(Zahradnik and Moreno, 1975). Porosity also varies throughout a tooth and between
127teeth: enamel density decreases from the outer enamel surface (OES) to the enamel
128dentine junction (EDJ), and tends to be lower in molar than canine enamel due to
129differences in prism packing (Shellis and Dibdin, 2000). Finally, larger pores in the
130form of spindles (the remains of odontoblast processes that become trapped when
131enamel formation begins) and tufts (thin, branching features high in protein) extend
132from the EDJ into the inner enamel.

133

1341.2 Enamel diagenesis and the addition of carbonate

135Carbonate from groundwater is likely to enter the tooth structure in three ways. First
136secondary minerals such as calcite can form (Haynes, 1968), most likely on the
137surface or in the cracks. Second, it is possible for apatite to recrystallize in a mid-high
138pH solution (Larsen and Jensen, 1989). However, it is likely that recrystallized apatite
139will contain less carbonate than the original bioapatite (LeGeros and Tung, 1983), as
140incorporation of carbonate increases solubility of hydroxyapatite (Grøn et al., 1963;
141LeGeros and Tung, 1983) due to the increased strain in the crystal lattice (Blumenthal

et al., 1975). This would reduce the importance of this mechanism for radiocarbon dating.

144

Finally, it is likely carbonate diffuses through enamel. Several models have been built to explain the diffusion of trace elements within the burial environment (Kohn, 2008; Millard and Hedges, 1996), and a diffusion-adsorption mechanism is thought to explain the diffusion of uranium into enamel as employed in U-series dating (Millard and Hedges, 1996). Enamel is an imperfect semi-permeable membrane, which acts as a molecular sieve (Borggreven et al., 1980; Borggreven et al., 1977; Shellis and Dibdin, 2000), and is permeable to water, ions and small organic molecules (Shellis and Dibdin, 2000). Most diffusion occurs at the prism boundaries (Brudevold et al., 1977; Lindén, 1968) where the largest pores are found (Shellis and Dibdin, 2000), but it has also been observed to a more limited extent within the prisms between crystallites in modern teeth (Lindén, 1968). Gordon and Joester (2015) suggest diffusion may also be possible through the hydrated Mg-ACP phase at room temperature and pressure, although further work is needed to clarify this.

158

159 1.3 Radiocarbon dating of enamel

The standard radiocarbon pretreatment applied to both bone and enamel apatite involves leaching in acetic acid under vacuum (Balter et al., 2002; Haynes, 1968; Zazzo and Saliège, 2011). This treatment was designed to remove calcite, which is more soluble than hydroxyapatite in acetic acid (Haynes, 1968), but it is also likely to remove at least some of the Mg-ACP phase (Gordon et al., 2015) and the more soluble apatite high in carbonate (LeGeros and Tung, 1983; Zazzo and Saliège, 2011). Unfortunately the latter is more likely to be original bioapatite than recrystallized material (Sillen and LeGeros, 1991).

168

There is little consistency between laboratories in the conditions under which this leaching occurs. Shorter leaching times (around an hour) are favoured by some researchers (Lee-Thorp and van der Merwe, 1991) as brushite ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) can precipitate below pH4 (Larsen and Jensen, 1989). However, where acid leaching occurs under vacuum (Balter et al., 2002) the formation of brushite is unlikely to affect the radiocarbon age, leading others to advise reaction times of at least 10 hours (Zazzo and Saliège, 2011). Both acetic acid (Haynes, 1968) and hydrochloric acid (Van Strydonck et al., 2009) have been used at varying concentrations to clean bioapatites, and there is little conclusive data to suggest which might be better. In contrast to these continuing conversations, Zazzo (2014) has demonstrated that a grain size of $<160 \mu\text{m}$ produces older age estimates than a grain size of $> 1 \text{ mm}$, although even with the smaller grain size, dates are not accurate.

181

The ability of step-heating to improve age estimates of bone (Haas and Banewicz, 1980) and, of more interest here, enamel (Surovell, 2000) has also been investigated. When an acid leach was used prior to heating, accurate dates

185were obtained. Unfortunately, the temperature required to remove contaminants was
186not consistent between samples, hindering wider application of this technique.

187

188The effect of grain size on age and the ability of preheating to aid contaminant
189removal suggests that that much of the carbonate not removed with the current acid
190leaching protocol in enamel may sit within the enamel structure rather than on the
191surface, and could be related to the diffusion and adsorption of carbonate. The
192experiment reported here aimed to examine whether enamel could be broken into
193individual crystallites so their surface could be removed with an acid leach, and
194subsequently whether age was related to Mg content, and thus presence of the Mg-
195ACP phase. If carbonate contaminants are located at the crystallite boundaries, either
196in the Mg-ACP or on the crystallite surface, Mg should decrease with F14C (the
197fraction ^{14}C in the sample divided by the fraction ^{14}C in the standard, $\delta^{13}\text{C}$ -normalised
198(Reimer et al., 2004)).

199

2002. Materials and methods

201

2022.1 Samples

203This study used enamel from *Sus scrofa* 2nd or 3rd molars to avoid variations resulting
204from differences in enamel structure and chemistry between species and teeth. These
205large teeth have thick enamel, and are abundant in a range of geographic and
206deposition environments. *Sus* 3rd molars are also of considerable archaeological
207interest as they can be readily used to identify not only the species but also provide
208information about the impact and process of domestication (Cucchi et al., 2009; Evin
209et al., 2015).

210

211One modern M3 was used to determine grinding and acid leach conditions, and to
212examine how magnesium content was affected by pretreatment (Table 1). The sample
213was the result of natural death from a piggery near Byron Bay (New South Wales,
214Australia). The maxilla BB1 was left near or on the surface and collected defleshed.

215

216Four ancient samples were collected (Table 1). Three are from the sites of Duoi U’Oi
217(sample DU795) and Lang Trang (samples LT1 and LT4), two caves in tower karsts in
218humid subtropical northern Vietnam, and date to beyond the limit of the radiocarbon
219method. Given their age, these are highly sensitive to young contaminants. One
220sample was recovered from a well-dated Holocene site, Rach Nui, an artificial mound
221in the tropical savanna of southern Vietnam.

222

223An assemblage of faunal remains was recovered from a breccia in Duoi U’Oi (Man
224Duc village, c. 85 km SW Hanoi) in 2003 (Bacon et al., 2008; Bacon et al., 2015). *Sus*
225*scrofa* teeth are common (86 1st, 2nd or 3rd permanent molars) and a speleothem found
226towards the top of the bone bearing breccia has been dated by U-series to 66 ± 3 ka.
227*Sus scrofa* are the most frequent fossil (NISP 1027 (Long et al., 1996)) in Cave 2,
228Breccia 5 at Lang Trang (c.125 km SW Hanoi), excavated in 1989 (Ciochon and

229Olsen, 1991). Although preliminary electron spin resonance dates have been produced
230from Cave 2 (285 ± 24 ka (Ciochon and Olsen, 1991)), finalized results and their
231relationship to the cave stratigraphy have not been published. In contrast, on
232biostratigraphic grounds, the assemblage is thought to be of a similar age to that in
233Duoi U’Oi, dating between 80 – 100 ka (Long et al., 1996). If confirmed by U-series
234to date beyond 50 ka, any ^{14}C in the teeth from Duoi U’Oi and Lang Trang can be
235regarded as a contaminant.

236

237Rach Nui is located in Long An Province, around 50 km south of Ho Chi Minh City
238(Oxenham et al., 2015). It is an artificial mound, resulting from construction and
239reconstruction of at least 14 house platforms, rising 5 – 6 m above the surrounding
240alluvial landscape today. The sampled *Sus scrofa* M2 was excavated in 2012 from an
241exterior surface constructed of silty clay sediments packed with pottery and domestic
242waste such as shell and bone. A radiocarbon chronology based on charcoal has been
243established, suggesting that the mound was constructed in 45 – 210 calibrated years
244(95.4% probability, against IntCal13 (Reimer et al., 2013)). The upper limit of the
245context sampled here is poorly constrained, but the overall sequence of deposits is
246well dated and demonstrates no reworking and little inbuilt age in the charcoal dated.
247A date on charcoal from the unit falls between 3550 – 3360 cal BP (SANU-30823,
2483200 $\pm$ 35 BP, Oxenham et al., 2015).

249

2502.2 U-series dating

251The teeth from Duoi U’Oi and Lang Trang were U-series dated to confirm that they
252fell beyond the limit of radiocarbon dating. Laser ablation U-series analyses were
253carried out at the Research School of Earth Sciences of the Australian National
254University (ANU), using a custom-built laser sampling system interfaced between an
255ArF Excimer laser and a MC-ICP-MS Finnigan Neptune (for details, see Eggins et al.,
2562003; Eggins et al., 2005), following principles and procedures described in Grün et
257al. (2014). Cross sections were cut from each tooth sample and several spots were
258ablated across at least two transects per tooth (figures 2 and 3) to evaluate the spatial
259variability of uranium-series isotopes in both enamel and dentine dental tissues.

260Individual closed system U-series age estimates were calculated for each ablation
261spot, although values for which U-concentration is lower than 0.5 ppm should be
262considered with caution. For each dental tissue, final U-series age results were
263calculated by integrating the data from all the spots of a given tissue.

264

2652.3 Sample pretreatment for radiocarbon

266The dentine and enamel surface, including the surface of cracks, was removed with a
267dremel drill and tungsten carbide drill bit. Teeth were then ground by hand, first by
268crushing in a hardened steel hammer press, and then under MilliQTM water in an agate
269pestle and mortar until smooth to touch (around 15 minutes). Samples were freeze-
270dried, and subdivided.

271

272 Lee-Thorp et al. (1997) have noted that small particle sizes can be generated with a
273 diamond drill. Unfortunately it was not possible to remove the large sample size
274 required in this study with a drill so a McCroneTM Microniser was used to
275 mechanically grind the samples.

276

277 One aliquot of hand ground material was retained, whilst the remaining material was
278 further ground with the microniser. Prior to use and between samples, agate beads and
279 the plastic container were soaked in dilute HCl and then MilliQTM water. To determine
280 the length of grinding required to separate the crystallites, a series of c.1g aliquots of
281 the modern tooth enamel were ground for 5, 15 and 30 minutes in 5 ml MilliQTM
282 water, and freeze-dried. Ancient samples were ground for 30 minutes in 5 ml MilliQTM
283 water.

284

285 Acetic acid leaching followed a protocol similar to that outlined in Zazzo (2011). The
286 sample was weighed into a VacutainerTM and placed under a weak vacuum ($c.1 \times 10^{-2}$
287 Torr) prior to the addition of 1 ml unbuffered 1M acetic acid per 50 mg enamel with a
288 gas tight syringe. After 30 min and 1 hour, evolved gas was removed by freezing the
289 solution in a dry-ice/ ethanol slush, and evacuating until a vacuum of $c. 1 \times 10^{-2}$ Torr
290 was obtained. The modern sample was leached for 15 minutes, 1, 8, 20, 44 and 71
291 hours. Ancient samples were leached for 20 hours. After rinsing five times in
292 MilliQTM, samples were freeze-dried and, to avoid possible adsorption of atmospheric
293 CO₂, stored under a weak vacuum in an ExetainerTM until radiocarbon measurement.
294 To assess yield reproducibility, acid leaching of one sample of ground enamel
295 (BB1_M15_1 hour) was repeated three times giving a standard deviation of $\pm 0.6 \%$.

296

297 2.4 Radiocarbon dating

298 c. 42 mg of enamel was reacted with 5 ml 85% phosphoric acid in a VacutainerTM
299 evacuated to $<3 \times 10^{-3}$ Torr. The resulting carbon dioxide was cryogenically purified
300 and collected before graphitization with hydrogen over an iron catalyst and
301 measurement in an NEC single stage accelerator at the Research School of Earth
302 Sciences at the ANU (Fallon et al., 2010). Dates have been corrected using the $\delta^{13}\text{C}$
303 measured by AMS and calculated according to Stuiver and Polach (1977).

304

305 To examine whether carbon was added during pretreatment and radiocarbon dating, a
306 mixture of geological apatite (purchased from Awaken CrystalsTM) and either marble
307 (IAEA-C1) or a modern oyster shell was used to simulate ancient and modern enamel
308 respectively with 0.7 %C. As the carbonate would completely dissolve during the
309 acetic acid leach, the apatite was ground and pretreated, and the carbonate added
310 immediately prior to dating. No uncontaminated, known age ancient enamel sample
311 suitable for use as a radiocarbon blank standard is known. Therefore, although not a
312 perfect substitute for enamel, this mixture was thought the best substitute possible,
313 having a broadly similar composition and therefore surface area as enamel.

314

315The dates from Rach Nui have been calibrated against IntCal13 (Reimer et al., 2013)
316in OxCal v.4.2 (Ramsey, 2009) for comparison to the dates presented by Oxenham et
317al. (2015).

318

3192.5 Field emission scanning electron microscopy (FESEM)

320Scanning electron microscopy was undertaken to examine grain size. To break the
321aggregates of highly electrostatic nanoparticles, ground samples were suspended by
322ultrasonication in water for 30 min and pipetted onto super smooth silicon mounts
323(Agar Scientific™) prior to analysis without coating. Secondary electron images were
324collected using a Zeiss UltraPlus analytical FESEM housed in the Centre for
325Advanced Microscopy at the ANU. An accelerating voltage of 1-2 kV, a working
326distance of 1.6 – 2.3 mm, and an aperture of 30 µm were used.

327

3282.6 Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES)

32910 mg of enamel was dissolved in 10 ml 2% distilled nitric acid for analysis of
330magnesium content on a Varian Vista AX CCD Simultaneous ICP-AES using
331AccuTrace mixed element standards at the Research School of Earth Sciences at the
332ANU. The detection limit for Mg was 0.05 mg/L, and the standard deviation of the
333three leached aliquots of M15_1 was 0.06 mg/L or 0.003 wt% Mg. Three blanks
334contained 0.00 mg/L.

335

3362.7 Fourier Transform-Infra Red spectroscopy (FTIR)

337FTIR was undertaken to check for brushite formation, which although unlikely to
338affect the radiocarbon age, would affect element ratios and stable isotope analyses. 1-
3391.5 mg of sample was mixed with 200-300 mg dry KBr and pressed into pellets under
340vacuum. The average of 100 transmission spectra were recorded on a Bruker Tensor
341127 at the Research School of Earth Sciences at the ANU between $-400 - -4000\text{cm}^{-1}$, at
342a resolution of 4cm^{-1} . Brushite was identified by a band at -525cm^{-1} resulting from a
343 ν_4 PO bend (Berry and Baddiel, 1967; Petrov et al., 1967). Reproducibility was
344determined through repeat measurement of one ground aliquot (M5) and repeat
345leaching of one sample (M15_1).

346

347It was not possible to use the spectra to investigate changes in the carbonate
348environment. The carbonate ν_2 bands of most interest (Fleet, 2009; Yi et al., 2014)
349overlap with each other and the strong phosphate ν_3 band. In FTIR spectra band width,
350and therefore the amount of overlap between bands, is affected by grain size (Hunt
351and Vincent, 1968). This made comparisons between the hand ground and micronised
352samples sensitive to methods used to subtract the overlapping phosphate band, and
353confident interpretations could not be made.

354

3552.8 Gas Bench-Isotope Ratio Mass Spectrometry (GB-IRMS)

356 $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and %C were measured by GB-IRMS at the Research School of Earth
357Sciences at the ANU. 22 mg enamel was reacted with 3ml 99% H_3PO_4 in an
358Exetainer™ and analysed on a Gas Bench connected to a Sercon 20-22 isotope ratio

mass spectrometer operating in continuous flow mode. An internal carbonate reference and NBS18 was used to scale the data. Reproducibility (1 standard deviation) assessed by repeat measurement of the internal standard, repeat measurement of one ground aliquot (M5) and repeat leaching of one sample (M15_1) was 0.001 ‰ for $\delta^{13}\text{C}$, 0.2 ‰ for $\delta^{18}\text{O}$ and 0.01 for ‰C. $\delta^{13}\text{C}$ reproducibility is typically around 0.1 ‰, and appears unusually low here.

365

3663. Results

367

3683.1 Modern enamel

369

3703.1.1 Effect of grinding protocols on grain structure

As found previously (Frazier, 1970), imaging of ground enamel is hindered by the strongly electrostatic nature of the nano-sized crystallites. Even after ultrasonication the crystallites tended to clump together which, combined with the large range in particle size, made quantification of grain size difficult. However, the images in figure 3752 illustrate how the grinding protocols separated the components of enamel. When ground by hand, enamel prisms were separated. These appear to have remained largely intact with widths around 1 μm (figure 4a-b) although a few crystallites were liberated (figure 4c). Mechanical grinding increased the proportion of free crystallites (figure 3794d). When large clumps were observed, they were composed of agglomerates of individual and broken crystallites (figure 4e). However, after 30 minutes grinding sections of prism around 0.5 μm in width remain (figure 4f). Although significantly better at breaking the prism structure than hand grinding, the McCrone Microniser was not able to separate all crystallites.

384

3853.1.2 Effect of acid leaching on Mg content

Samples leached for longer than 20 hours contained brushite (table 2), and are not discussed further. In the remaining samples there is a clear relationship between grinding time and magnesium content in the acid leached enamel (figure 5a). During the first 15 minutes of leaching there is a rapid decrease in Mg, after which time concentration gradually declines. Concentration of Mg is also dependent on grain size, with the samples ordered in terms of length of micronisation for each acid leach. After 20 hours in acetic acid the hand ground sample contained 0.128 wt% Mg, whilst the sample micronised for 30 minutes contained 0.099 wt% Mg.

394

This pattern is not due to the faster reaction of the smaller grain sizes as the % yield for all leached samples was between 60 – 70 % (table 2). Neither is it due to the formation of large quantities of brushite (table 2). This suggests that the smaller the grain size, the more Mg-ACP is removed. For the conditions examined here, enamel ground for 30 minutes in the microniser and leached for 20 hours is likely to remove the most Mg-ACP.

401

4023.1.3 Effect of acid leaching on $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and ‰C

The majority of change within the IRMS data occurs during the first hour of acetic acid leaching (table 2, figure 6a-c). $\delta^{13}\text{C}$ ranges by just 0.017 ‰, the majority of the range being caused by an unusually high value for M15. Although outside of the reproducibility of the batch, this is a tiny variation in contrast to a typical measurement error of c.0.1 ‰. Therefore although the smaller grain sizes are most depleted during the leaching process, perhaps suggesting that the carbonate in the Mg-ACP phase has a different isotopic value to carbonate within the apatite, further replication is required to confirm the pattern. $\delta^{18}\text{O}$ ranges by 1.6 ‰, increasing in the first hour of leaching, but there is no systematic difference between grain sizes. %C ranges by 0.1 %C, around 15 % of the starting carbon concentration. Overtime the $\delta^{13}\text{C}$ gradually decreases with the exception of a rapid decrease at 15 minutes, followed by increase at 1 hour. Again, there seems no systematic difference between the different grain sizes.

416

417 3.2 Ancient enamel

418

419 3.2.1 U-series dating

Because dental tissues are known to behave as open systems for U-series elements, U-series results should be considered as minimum possible age estimates for the fossil remains (Grün et al., 2014). In the three tooth samples, ablation spots show U-concentration values decreasing from the dentine-enamel junction towards the outer enamel surface (which is virtually free of uranium), suggesting a preferential direction of uranium diffusion into the enamel layer from the dentine. Such a trend is not surprising, as it might simply be related to the density gradient, which increases from the enamel dentine junction to the outer enamel surface (see section 1.1.).

Although U-concentrations values measured in the enamel and dentine of sample DU795 differ by at least one order of magnitude (figure 2), U-series ages are spatially highly consistent, with integrated results of around 100-110 ka for all dental tissues (table 3).

In contrast, data collected for Lang Trang samples (LT1 and LT4, figure 3) are much more scattered. U-concentration values measured in dentine of LT1 are overall spatially constant around 6-7 ppm, while U-series data are close to secular equilibrium, explaining the relatively large age errors. These results indicate that the dentine is older than 350 ka. In comparison, enamel U-series ages are around 210-240 ka, although the very low concentration values induce a large uncertainty for LT1-EN1 (table 3).

In comparison, sample LT4 shows much younger U-series ages. The two dentine domains (DE1 and DE3) show highly consistent results of 115-120 ka, while the enamel domains have more scattered values, mostly because of the very low U-concentration values (<0.5 ppm). Consequently, no integrated age results were obtained for enamel domains EN1 to EN3 (table 3).

444 To summarise, the U-series dating results confirm that the three teeth from Duoi U’Oi
445 and Lang Trang fall beyond the time range covered by the radiocarbon dating method.

446

447 3.2.2 *Effect of acid leaching on Mg content*

448 To examine the effect of the acetic acid leach and grain size, four aliquots of the
449 ancient teeth were examined. Each sample was hand ground and a subsample
450 micronised for 30 minutes. A subsample of both hand ground and micronised aliquots
451 was leached in acetic acid for 20 hours. It was not possible to leach a hand ground
452 aliquot of ISO-6 due to the limited sample size.

453

454 Despite careful washing of the grinding equipment, LT1 must have been contaminated
455 during mechanical grinding as the Mg concentration is higher in the micronised than
456 hand ground aliquot (table 4, figure 5b). Excluding this one data point, the samples
457 follow the same trend as the modern enamel. As with the modern enamel, the hand
458 ground sample contains the most Mg whilst the leached micronised sample contains
459 the least. Although the hand ground Pleistocene samples (0.13-0.16 %Mg) contain
460 less Mg than the modern (0.23 %Mg) and Holocene (0.25 %Mg) enamel, all contain
461 similar concentrations after leaching (0.10 – 0.13 %Mg). Interestingly, the Holocene
462 sample ISO-6 contained much less Mg when mechanically ground (0.15 %Mg) than
463 hand ground (0.25 %Mg), perhaps suggesting leaching of the Mg-ACP phase started
464 during mechanical grinding in water.

465

466 In contrast to the modern enamel, consistently more hand ground material survived
467 the acid leach than the micronised sample (table 4), and a small amount of brushite
468 may be present in LT4 (table 4). Though not as clear as in the modern enamel, these
469 results are suggestive of preferential removal of the Mg-ACP phase.

470

471 3.2.3 *Effect of acid leaching on %C, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$*

472 The differences in %C and carbonate isotopes between the leached hand and leached
473 micronised aliquots seen in the modern tooth are accentuated in the ancient samples
474 (figure 6d-f, table 4), particularly in the micronised samples. For example, in the
475 micronised samples, %C decreases by up to 0.2 %C, $\delta^{18}\text{O}$ by more than 2 ‰ whilst
476 $\delta^{13}\text{C}$ decreases by up to 1.4 ‰ during the acetic acid leach. The large difference in %C
477 is partially related to carbon contamination added to the micronised sample, as
478 discussed further in the radiocarbon section below, although leached micronised
479 samples have around 0.1 less %C than leached hand ground samples. The larger
480 changes occurring within ancient samples compared with the modern tooth is likely to
481 be due to the effect of diagenesis in the ancient teeth, and the removal of exogenous
482 contamination. However, with only one modern tooth examined and no understanding
483 of variability between teeth of the same taxa, further work would be needed to
484 confirm this.

485

486 3.2.4 *Effect of acid leaching on radiocarbon age*

487It appears that carbon contamination was introduced to all samples whilst
488mechanically grinding, as micronised samples have a higher $F_{14}C$ and more carbon
489than hand ground samples (figure 7, table 4). This contamination was identified in the
490mixture of geological apatite and marble used to monitor laboratory backgrounds
491(figure 8). Although mixtures of apatite and modern oyster shell are consistent
492throughout treatment, the mixtures of marble and micronised apatite have higher
493 $F_{14}C$ values than the mixture of marble and hand ground apatite. This suggests that
494around $1.6 \pm 0.05\%$ of the carbon within the micronised sample was a modern
495contaminant, most likely atmospheric carbon adsorbed to the large surface area.
496However, when the apatite was leached in acetic acid for 20 hours, the contamination
497is removed. A larger amount of modern contamination was added to the ancient
498enamel samples (between 3.8 – 5.7%), presumably due to a larger surface area of the
499needle shaped enamel grains compared to the more spherical geological apatite grains.
500However, it is thought likely that the acetic acid removed this contamination, and so
501all ancient enamel samples have had a laboratory blank calculated from the laboratory
502long-term marble average subtracted, as routine practice for carbonates dated at the
503ANU.

504

505All acid leached micronised samples contained less ^{14}C than acid leached hand ground
506samples, and $F_{14}C$ is related to Mg concentration (figure 9). In terms of conventional
507radiocarbon age (figure 7b), untreated hand ground Pleistocene samples had ages of
508around 15 kBP, leached hand ground samples around 20 kBP and leached
509mechanically ground samples around 30 kBP.

510

511Age differences were much smaller in the more recent tooth from Rach Nui. The
512leached mechanically ground aliquot was around 400 years older than the starting
513material. The calibrated range of the mechanically ground and leached sample
514overlaps at 95.4% probability with a fragment of charcoal from the same
515archaeological context and the two conventional ages pass a Chi squared test (X^2 Test:
516 $df = 1$, $T = 2.4$ (5% 3.8)). Unfortunately the sample was too small to radiocarbon date
517a leached hand ground aliquot to assess whether mechanical grinding prior to acetic
518acid leaching improved the age estimate in comparison to grinding the sample by
519hand.

520

5214. Discussion

522

523It has been known for some time that magnesium is preferentially removed during
524acid dissolution of enamel powder (Borggreven et al., 1986) and in early carious
525enamel lesions (Hallsworth et al., 1972). This formed the basis for early hypotheses
526that enamel was not only formed of apatite, but contained a second mineral phase.
527Using modern enamel this study has demonstrated that the amount of magnesium
528removed with acetic acid is dependent upon grain size, as would be expected if the
529majority of magnesium within enamel is located at the crystallite boundaries, either
530in Mg-ACP or on the crystallite surface.

531

532When replicated with ancient enamel, radiocarbon age is found to be related to grain
533size and magnesium content. This suggests that at least some of the carbon
534contamination not removed by the acetic acid leach routinely applied to radiocarbon
535samples appears to be located within the Mg-ACP or at the surface of the crystallites.
536

537This has three implications:

- 538 1. It provides an opportunity to improve radiocarbon age estimates on tooth
539 enamel.
- 540 2. The effect of the acid leach on carbonate stable isotopes can be examined
541 where carbonate contamination is more effectively removed, as judged by
542 improvement in radiocarbon age.
- 543 3. It adds to the body of evidence on how tooth enamel alters in archaeological
544 and palaeontological contexts.

545

5464.1 Implications for radiocarbon dating

547The offset between radiocarbon dates on enamel and the expected age of the tooth are
548compared to published dates on known-age enamel collated by Zazzo (2014) in figure
54910. When the Pleistocene teeth from Duoi U’Oi and Lang Trang were treated as
550routine radiocarbon samples, hand grinding prior to an acid leach, they fall within the
551higher end of the offsets observed for other samples. However, when mechanically
552ground, the underestimation in radiocarbon age is less than all other samples of a
553similar age. Although this improvement in age is promising, the underestimation is
554still uncomfortably large at around 20,000 ^{14}C years.

555

556Although it is clear that Pleistocene enamel is unlikely to generate accurate
557radiocarbon dates using this method, it is useful to consider what effect the level of
558contamination seen in the Pleistocene teeth might have on younger samples. On
559average, the samples from Duoi U’Oi and Lang Trang have a $F_{14\text{C}}$ of 0.022. If we
560assume that this is caused by modern contamination, 2.2% of the carbon in the enamel
561must be a contaminant. In a sample of 3000 years, this level and age of contamination
562would cause the sample to be underestimated by just 70 years, scarcely larger than the
563typical 2 sigma error range ($\pm 50 - 70$ ^{14}C years). The tooth enamel from Rach Nui,
564which is statistically indistinguishable from charcoal from the same context, supports
565the idea that mid-late Holocene samples may not be grossly affected by contamination
566(Zazzo, 2014), if finely ground samples are acid leached prior to dating.

567

568Further work is required to assess how reproducible this age offset is. Very similar age
569underestimates have been obtained from the three sites from two different
570environments. However, given that at least 2% of the carbon in all mechanically
571ground samples is from a contaminant, it is highly likely that age offsets will be
572variable, and dates must be regarded as possible minimum ages. This is particularly
573important to consider where Bayesian chronological modeling is used as this will
574increase precision, exacerbating any minor systematic underestimation in age.

575

576Improvements to the radiocarbon pretreatment protocol will depend on understanding
577where the remaining contamination is located. The mechanical grinding protocol
578employed does not completely break apart the crystallite structure, and it is possible
579that some of the Mg-ACP phase remains. Unfortunately it is not yet possible to use
580the Mg concentration to assess whether all of the Mg-ACP phase was removed during
581the leaching experiments as Mg concentration within apatite crystallite and Mg-ACP
582phase has only been measured in rodent enamel (Gordon et al., 2015), and may differ
583in the enamel of *Sus scrofa*.

584

585It is also likely that other diagenetic mechanisms are occurring, introducing carbon of
586a younger age. For example, it is possible that recrystallisation occurred alongside
587demineralization of areas of the tooth enamel. Further study of enamel diagenesis is
588clearly required to understand and screen for this recrystallization process, alongside
589development of new grinding protocols to more effectively break the crystallites apart
590without adding contamination (for example using SelFrag™).

591

5924.2 Implications for carbonate stable isotope analysis

593Tooth enamel is often washed in acetic acid prior to analysis of carbonate stable
594isotopes (Garvie-Lok et al., 2004; Lee-Thorp, 1989; Lee-Thorp and van der Merwe,
5951991). There has been some debate over whether this pretreatment can affect the final
596result through the formation of secondary minerals or recrystallization of the apatite
597crystallites (Garvie-Lok et al., 2004; Lee-Thorp and van der Merwe, 1991; Pellegrini
598and Snoeck, 2016).

599

600This study adds to the growing body of evidence that an acetic acid leach affects the
601measured stable isotope ratios in modern enamel. For example, like this study,
602Pellegrini and Snoeck (2016) noted that the effect seems minor in terms of $\delta^{13}\text{C}$ (<0.5
603‰), and is unlikely to have a large effect on the interpretation of stable isotopic data
604obtained from archaeological or palaeontological studies. We observed a slightly
605larger change in $\delta^{18}\text{O}$ (>1 ‰) than Pellegrini and Snoeck (2016) which, depending on
606the research question, could have an effect on interpretation. This shift in isotopic
607value may be due to structural alteration induced by the acetic acid, but if so, these
608changes occur within the first hour of treatment and are not visible in the FTIR
609spectra. It is also possible that the isotopic signature of the carbonate within the Mg-
610ACP is different to that in the enamel. To investigate this possibility, further work at
611higher precision is required to assess whether trends in isotopic shift can be observed
612between samples of different grain size.

613

614The effect of acid leaching on stable isotopes in ancient material was larger than in the
615modern tooth. This is probably due to the removal of exogenous contamination, as the
616radiocarbon evidence demonstrates the acetic acid leach reduces contaminant levels
617from at least 15 % (hand ground) to at least 2 % (micronised and leached). An acid
618wash prior to stable isotope analysis of enamel is therefore required.

619

620The effect 2 % contamination on the stable isotopic signature of enamel depends on
621the difference between carbonate in the groundwater and carbonate in the enamel. To
622shift the isotopic signature of the enamel by more than 1 ‰, more than 10 % of the
623carbonate would need to be a contaminant if the two sources of carbonate differed by
62410 ‰. This estimate agrees well with both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in the ancient samples
625examined here, which vary by little more than 1 or 2 ‰ between untreated and
626leached micronised samples (figure 6d,e).

627

6284.3 Implications for enamel diagenesis

629In contrast with the extensive study of bone diagenesis (Collins et al., 2002; Hedges,
6302002), relatively little work has examined how enamel degrades in the burial
631environment. However, it is clear from e.g. radiocarbon analyses (Zazzo, 2014), trace
632element analyses (Hinz and Kohn, 2010; Kohn, 2008; Sponheimer and Lee-Thorp,
6332006; Trueman and Tuross, 2002), U-series dating (Millard and Hedges, 1996; Pike et
634al., 2002), FTIR (Asscher et al., 2011) and microscopy (Hollund et al., 2014), that
635enamel is altered by chemical and possibly microbial processes.

636

637One diagenetic mechanism seems to involve the diffusion of carbonate through the
638enamel matrix, as previously observed with trace elements. The increase in age with
639decreasing grain size, and the relationship between age and Mg concentration
640suggests that the carbonate is not only diffusing along the larger pores at prism
641junctions, but is diffusing between the individual crystallites.

642

643Mg concentration is low in all Pleistocene enamel samples. Whilst this might be part
644of the natural variation in Mg concentration, it may also suggest that the Mg-ACP
645phase dissolves during burial. Nielsen-Marsh and Hedges (2000) have used acetic
646acid leaching to examine the diagenetic processes occurring in bone, arguing that this
647treatment may imitate some of the processes occurring during burial. The acetic acid
648leach reduced Mg concentration in modern enamel, even in the hand ground samples.
649If this process does occur during burial, removal of the Mg-ACP phase may increase
650porosity, facilitating faster diffusion.

651

6525. Conclusions

653

654Mechanically grinding tooth enamel to separate the apatite crystallites can improve
655the ability of the acetic acid wash to remove carbonate contaminants. As a result the
656accuracy of radiocarbon dates on enamel is substantially improved. However, even
657with the grinding protocol and cleaning applied here, at least 2% of the carbonate
658within the treated enamel was a contaminant. Teeth known to be more than 50,000
659years old were dated to c.30 kBP. Within the mid-late Holocene, this level of
660contamination would cause radiocarbon age estimates to be within c.100 ^{14}C years of
661the true sample age. However, only four teeth have been examined, so variation in the

contamination level is unknown. Therefore all radiocarbon dates on enamel must be regarded as potentially inaccurate, and most likely too young.

664

The study highlights the need for further work aiming to understand the diagenetic processes occurring within enamel. Only when we understand where the remaining contaminating carbon is located can we develop effective cleaning protocols to produce accurate radiocarbon dates.

669

670

671 **Acknowledgements**

The research and R. Wood were funded by an Australian Research Council DECRA fellowship (DE150100070). M. Duval's research was funded by the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme (FP7/2007-2013) under REA Grant Agreement n° PIOF-GA-2013-676626474. Jaime Swift (University of Oxford) and Matthew Cupper (University of Melbourne) are thanked for providing the modern pig sample. Rainer Grün, Griffith University, and Les Kinsley, ANU, are thanked for their invaluable support in U-series data acquisition and reduction processes. Stewart Fallon, ANU, is thanked for help with AMS. Frank Brink and Hua Chen at the Centre for Advanced Microscopy, ANU, are thanked for help with SEM. Penny King, ANU, is thanked for discussions about FTIR and carbonates and Linda McMorrow, ANU, for running the ICP-AES samples. Carley Crann (University of Ottawa) is thanked for suggesting SelFrag as an alternative method to mechanical grinding. All errors are, of course, our own.

685

686 **Table headings**

687

Table 1: Samples used in this study. Tooth eruption and wear stages follow (Lemoine et al., 2014) and associated radiocarbon dates have been calibrated against IntCal13 (Reimer et al., 2013) in OxCal v.4.2 (Ramsey, 2009).

691

Table 2: Analytical results of the modern enamel sample BB1 after grinding by hand and micronising for 5, 15 and 30 minutes, and etching in acetic acid for up to 71 hours. All measurements are on the powder remaining after treatment. Where replicates were made, average values are given. Standard deviations are given in the text. N/A refers to samples where analyses were not undertaken due to the presence of brushite.

698

Table 3: U-series results on the three tooth samples from Duoi U'Oi and Lang Trang sites. Values were calculated by integrating the data from all the spots of a given domain with a dental tissue. For all domains, elemental U/Th ratios were found to be above 250, minimizing thus the impact of detrital ^{232}Th contamination on final age results. No age calculations (n.c.) were carried out for U concentrations of ≤ 0.5 ppm. All errors are 2- σ . Key: EN = enamel, DE = dentine.

705

706 Table 4; Analytical results on ancient enamel samples. Micronised samples were
707 milled for 30 minutes. All measurements are on the powder remaining after treatment.
708 Holocene dates have been calibrated against IntCal13 (Reimer et al. 2013) in OxCal
709 v.4.2 (Ramsey 2009) and are given at 95.4% probability.

710

711 Figure headings

712

713 Figure 1: FESEM images of a longitudinal section of a modern *Sus scrofa* M3 after
714 etching in acid (2M HCl, 30 seconds) and coating in Pt, demonstrating the
715 microstructure of tooth enamel. a) Enamel is the pale material to the left and dentine
716 the darker material to the right. Scale bar 20 μm . b) Prisms (white arrows) and
717 interprismatic matrix (black arrows). Scale bar 2 μm . c) Crystallites in prismatic and
718 interprismatic enamel. Scale bar 200 nm.

719

720 Figure 2: U-series closed system age estimates and U concentration of each ablated
721 spot in DU795. For colour, please see online version.

722

723 Figure 3: U-series closed system age estimates and U concentration of each ablated
724 spot from the two teeth from Lang Trang. For colour, please see online version.

725

726 Figure 4: Secondary electron FESEM images of the ground modern BB1 tooth
727 enamel. a-c) hand ground, scale bars 10 μm , 1 μm and 1 μm respectively. d-f)
728 mechanically ground for 30 minutes in a McCrone microniser, scale bars 10 μm , 1 μm
729 and 1 μm respectively.

730

731 Figure 5: Change in Mg concentration in tooth enamel remaining after an acid leach
732 for a) modern and b) palaeontological samples of different grain size. 1 sigma error
733 ranges are smaller than the data points. For colour, please see online version.

734

735 Figure 6: GB-IRMS analysis of $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and %C of a-c) modern and d-f)
736 palaeontological enamel remaining after an acid leach. 1 sigma error ranges are
737 indicated. For colour, please see online version.

738

739 Figure 7: Radiocarbon age of enamel a) F14C and b) conventional radiocarbon age. 1
740 sigma error ranges are within the size of the datapoint. For colour, please see online
741 version.

742

743 Figure 8: Contamination added in the laboratory. F14C is given for mixtures of
744 geological apatite and either marble (IAEA-C1, filled datapoints) and a modern oyster
745 shell (open datapoints, right hand scale). Measurements on carbonate, carbonate
746 mixed with hand ground apatite, carbonate mixed with micronised apatite, and
747 carbonate mixed with micronised and acetic acid leached apatite are given. Errors are

748given at 1σ (smaller than the datapoint size for the marble-apatite mixtures), and
749F14C is presented with no laboratory background subtraction.

750

751Figure 9: Relationship between F14C and Mg concentration. For colour, please see
752online version.

753

754Figure 10; Comparison of results obtained in this study with radiocarbon dates on the
755apatite of bone, dentine and enamel collated by Zazzo (2014). Dates are given against
756the expected age. Only samples Zazzo (2014) ranked highly in terms of security of
757known age are presented.

758

759References

760

- 761Asscher, Y., Regev, L., Weiner, S., Boaretto, E., 2011. Atomic Disorder in Fossil
762Tooth and Bone Mineral: An FTIR Study Using the Grinding Curve Method.
763ArchéoSciences 35.
- 764Bacon, A.M., Demeter, F., Düringer, P., Helm, C., Bano, M., Vu, T.L., Nguyen, T.K.T.,
765Antoine, P.O., Bui, T.M., Nguyen, T.M.H., Dodo, Y., Chabaux, F., Rihs, S., 2008. The
766Late Pleistocene Duoi U'Oï cave in northern Vietnam: palaeontology,
767sedimentology, taphonomy and palaeoenvironments. Quaternary Science
768Reviews 27, 1627-1654.
- 769Bacon, A.M., Westaway, K., Antoine, P.O., Düringer, P., Blin, A., Demeter, F., Ponche,
770J.L., Zhao, J.X., Barnes, L.M., Sayavonkhamdy, T., Nguyen, T.K.T., Vu, T.L., Patole-
771Edoumba, E., Shackelford, L., 2015. Late Pleistocene mammalian assemblages of
772Southeast Asia: New dating, mortality profiles and evolution of the predator-prey
773relationships in an environmental context. Palaeogeography, Palaeoclimatology,
774Palaeoecology 422, 101-127.
- 775Balter, V., Saliège, J.F., Bocherens, H., Person, A., 2002. Evidence of physico-
776chemical and isotopic modifications in archaeological bones during controlled
777acid etching. Archaeometry 44, 329-336.
- 778Berry, E.E., Baddiel, C.B., 1967. The infra-red spectrum of dicalcium phosphate
779dihydrate (brushite). Spectrochimica Acta Part A: Molecular Spectroscopy 23,
7802089-2097.
- 781Blumenthal, N.C., Betts, F., Posner, A.S., 1975. Effect of carbonate and biological
782macromolecules on formation and properties of hydroxyapatite. Calcified Tissue
783Research 18, 81-90.
- 784Borggreven, J.M.P.M., Driessens, F.C.M., van Dijk, J.W.E., 1980. Diffusion through
785bovine tooth enamel as related to the water structure in its pores. Archives of
786Oral Biology 25, 345-348.
- 787Borggreven, J.M.P.M., Driessens, F.C.M., van Dijk, J.W.E., 1986. Dissolution and
788precipitation reactions in human tooth enamel under weak acid conditions.
789Archives of Oral Biology 31, 139-144.
- 790Borggreven, J.M.P.M., van Duk, J.W.E., Driessens, F.C.M., 1977. A quantitative
791radiochemical study of ionic and molecular transport in bovine dental enamel.
792Archives of Oral Biology 22, 467-472.
- 793Bottero, M.J., Yvon, J., Vadot, J., 1992. Multimethod analysis of apatites in sound
794human tooth enamel. Eur. J. Mineral. 4, 1347-1357.

- 795Brown, T.A., Nelson, D.E., Vogel, J.S., Southon, J., 1988. Improved collagen
796extraction by modified Longin method. *Radiocarbon* 30, 171-177.
- 797Brudevold, F., Srinivasan, B.N., Skobe, Z., 1977. Separation of human tooth enamel
798microstructures by density fractionation. *Archives of Oral Biology* 22, 593-597.
- 799Calo, A., Prasetyo, B., Bellwood, P., Lankton, J.W., Gratuze, B., Pryce, T.O., Reinecke,
800A., Leusch, V., Schenk, H., Wood, R., Bawono, R.A., Kompiang Gede, I.D., Citha
801Yuliati, N.L.K., Fenner, J., Reepmeyer, C., Castillo, C., Carter, A.K., 2015. Sembiran
802and Pacung on the north coast of Bali: a strategic crossroads for early trans-
803Asiatic exchange. *Antiquity* 89, 378-396.
- 804Ciochon, R.L., Olsen, J.W., 1991. Paleoanthropological and archaeological
805discoveries from Lang Trang caves: A new Middle Pleistocene hominid site from
806northern Vietnam. *Indo-Pacific Prehistory Association Bulletin* 10, 59-73.
- 807Collins, M.J., Nielsen-Marsh, C.M., Hiller, J., Smith, C.I., Roberts, J.P., Prigodich, R.V.,
808Wess, T.J., Csapò, J., Millard, A.R., Turner-Walker, G., 2002. The survival of organic
809matter in bone: A review. *Archaeometry* 44, 383-394.
- 810Cucchi, T., Fujita, M., Dobney, K., 2009. New insights into pig taxonomy,
811domestication and human dispersal in Island South East Asia: Molar shape
812analysis of *Sus* remains from Niah Caves, Sarawak. *International Journal of*
813*Osteoarchaeology* 19, 508-530.
- 814Cui, F.Z., Ge, J., 2007. New observations of the hierarchical structure of human
815enamel, from nanoscale to microscale. *Journal of Tissue Engineering and*
816*Regenerative Medicine* 1, 185-191.
- 817Driessens, F.C., 1982. Mineral aspects of dentistry. *Monographs in oral science* 10,
8181-215.
- 819Driessens, F.C.M., Verbeeck, R.M.H., 1982. The probable phase composition of the
820mineral in sound enamel and dentine. *Bulletin des Societes Chimiques Belges* 97,
821573-596.
- 822Driessens, F.C.M., Verbeeck, R.M.H., 1985. Dolomite as a possible magnesium-
823containing phase in human tooth enamel. *Calcified Tissue International* 37, 376-
824380.
- 825Eggins, S., Grün, R., Pike, A.W.G., Shelley, M., Taylor, L., 2003. ²³⁸U, ²³²Th
826profiling and U-series isotope analysis of fossil teeth by laser ablation-ICPMS.
827*Quaternary Science Reviews* 22, 1373-1382.
- 828Eggins, S.M., Grün, R., McCulloch, M.T., Pike, A.W.G., Chappell, J., Kinsley, L.,
829Mortimer, G., Shelley, M., Murray-Wallace, C.V., Spötl, C., Taylor, L., 2005. In situ U-
830series dating by laser-ablation multi-collector ICPMS: New prospects for
831Quaternary geochronology. *Quaternary Science Reviews* 24, 2523-2538.
- 832Elliott, J.C., 1994. Structure and chemistry of the apatites and other calcium
833orthophosphates. Elsevier, Amsterdam.
- 834Elliott, J.C., 2002. Calcium phosphate biominerals, *Reviews in Mineralogy and*
835*Geochemistry*.
- 836Evin, A., Dobney, K., Schafberg, R., Owen, J., Strand Vidarsdottir, U., Larson, G.,
837Cucchi, T., 2015. Phenotype and animal domestication: A study of dental variation
838between domestic, wild, captive, hybrid and insular *Sus scrofa*. *BMC Evolutionary*
839*Biology* 15.
- 840Fallon, S.J., Fifield, L.K., Chappell, J.M., 2010. The next chapter in radiocarbon
841dating at the Australian National University: Status report on the single stage
842AMS. *Nuclear Instruments and Methods in Physics Research, Section B: Beam*
843*Interactions with Materials and Atoms* 268, 898-901.

- 844 Fleet, M.E., 2009. Infrared spectra of carbonate apatites: ν_2 -Region bands.
845 *Biomaterials* 30, 1473-1481.
- 846 Frazier, P.G., 1970. II. An electron microscopic study of the effect of grinding.
847 *Calcified Tissue International* 5, 277-287.
- 848 Garvie-Lok, S.J., Varney, T.L., Katzenberg, M.A., 2004. Preparation of bone
849 carbonate for stable isotope analysis: The effects of treatment time and acid
850 concentration. *Journal of Archaeological Science* 31, 763-776.
- 851 Gordon, L.M., Cohen, M.J., MacRenaris, K.W., Pasteris, J.D., Seda, T., Joester, D.,
852 2015. Amorphous intergranular phases control the properties of rodent tooth
853 enamel. *Science* 347, 746-750.
- 854 Gordon, L.M., Joester, D., 2015. Mapping residual organics and carbonate at grain
855 boundaries and the amorphous interphase in mouse incisor enamel. *Frontiers in*
856 *Physiology* 6.
- 857 Grøn, P., Spinelli, M., Trautz, O., Brudevold, F., 1963. The effect of carbonate on the
858 solubility of hydroxylapatite. *Archives of Oral Biology* 8, 251-263.
- 859 Grün, R., Abeyratne, M., Head, J., Tuniz, C., Hedges, R.E.M., 1997. AMS ^{14}C analysis
860 of teeth from archaeological sites showing anomalous ESR dating results.
861 *Quaternary Science Reviews* 16, 437-444.
- 862 Grün, R., Eggins, S., Kinsley, L., Moseley, H., Sambridge, M., 2014. Laser ablation U-
863 series analysis of fossil bones and teeth. *Palaeogeography, Palaeoclimatology,*
864 *Palaeoecology* 416, 150-167.
- 865 Haas, H., Banewicz, J., 1980. Radiocarbon dating of bone apatite using thermal
866 release of CO_2 . *Radiocarbon* 22, 537-544.
- 867 Hallsworth, A.S., Robinson, C., Weatherell, J.A., 1972. Mineral and magnesium
868 distribution within the approximal carious lesion of dental enamel. *Caries*
869 *Research* 6, 156-168.
- 870 Haynes, V., 1968. Radiocarbon: Analysis of inorganic carbon of fossil bone and
871 enamel. *Science* 161, 687-688.
- 872 Hedges, R., Lee-Thorpe, J.A., Tuross, N.C., 1995. Is tooth enamel carbonate a
873 suitable material for radiocarbon dating. *Radiocarbon* 37, 285-290.
- 874 Hedges, R.E.M., 2002. Bone diagenesis: An overview of processes. *Archaeometry*
875 44, 319-328.
- 876 Hinz, E.A., Kohn, M.J., 2010. The effect of tissue structure and soil chemistry on
877 trace element uptake in fossils. *Geochimica et Cosmochimica Acta* 74, 3213-3231.
- 878 Hollund, H.I., Jans, M.M.E., Kars, H., 2014. How are teeth better than bone? An
879 investigation of dental tissue diagenesis and state of preservation at a
880 histological scale (with photo catalogue). *Internet Archaeology*.
- 881 Hunt, G.R., Vincent, R.K., 1968. The behaviour of spectral features in the infrared
882 emission from particulate surfaces of various grain sizes. *Journal of Geophysical*
883 *Research* 73, 6039-6046.
- 884 Koenigswald, W.V., Sander, P.M., 1997. Glossary of terms used for enamel
885 microstructures, in: Koenigswald, W.V., Sander, P.M. (Eds.), *Tooth enamel*
886 *microstructure*. A.A. Balkema, Rotterdam, pp. 267-280.
- 887 Kohn, M.J., 2008. Models of diffusion-limited uptake of trace elements in fossils
888 and rates of fossilization. *Geochimica et Cosmochimica Acta* 72, 3758-3770.
- 889 Larsen, M.J., Jensen, S.J., 1989. Stability and mutual conversion of enamel apatite
890 and brushite at 20 °C as a function of pH of the aqueous phase. *Archives of Oral*
891 *Biology* 34, 963-968.

- 892 Lee-Thorp, J., 2002. Two decades of progress towards understanding fossilization
893 processes and isotopic signals in calcified tissue minerals. *Archaeometry* 44, 435-
894 446.
- 895 Lee-Thorp, J., Manning, L., Sponheimer, M., 1997. Problems and prospects for
896 carbon isotope analysis of very small samples of fossil tooth enamel. *Bulletin de*
897 *la Societe Geologique de France* 168, 767-773.
- 898 Lee-Thorp, J.A., 1989. Stable carbon isotopes in deep time: The diets of fossil
899 fauna and hominids. *Stable Carbon Isotopes in Deep Time: The Diets of Fossil*
900 *Fauna and Hominids*.
- 901 Lee-Thorp, J.A., van der Merwe, N.J., 1991. Aspects of the chemistry of modern
902 and fossil biological apatites. *Journal of Archaeological Science* 18, 343-354.
- 903 LeGeros, R.Z., Tung, M.S., 1983. Chemical stability of carbonate- and fluoride-
904 containing apatites. *Caries Research* 17, 419-429.
- 905 Lemoine, X., Zeder, M.A., Bishop, K.J., Rufolo, S.J., 2014. A new system for
906 computing dentition-based age profiles in *Sus scrofa*. *Journal of Archaeological*
907 *Science* 47, 179-193.
- 908 Lindén, L.A., 1968. Microscopic observations of fluid flow through enamel in
909 vitro. *Odontologisk revy* 19, 349-365.
- 910 Long, V.T., de Vos, J., Ciochon, R.L., 1996. The fossil mammal fauna of the Lang
911 Trang Caves, Vietnam, compared with Southeast Asian fossil and recent mammal
912 faunas: the geographical implications. *Indo-Pacific Prehistory Association*
913 *Bulletin* 14, 101-109.
- 914 Longin, R., 1971. New method of collagen extraction for radiocarbon dating.
915 *Nature* 230, 241-242.
- 916 Marom, A., McCullagh, J.S.O., Higham, T.F.G., Sinitsyn, A.A., Hedges, R.E.M., 2012.
917 Single amino acid radiocarbon dating of Upper Paleolithic modern humans.
918 *Proceedings of the National Academy of Sciences of the United States of America*
919 109, 6878-6881.
- 920 McCullagh, J.S.O., Marom, A., Hedges, R.E.M., 2010. Radiocarbon dating of
921 individual amino acids from archaeological bone collagen. *Radiocarbon* 52, 620-
922 634.
- 923 Millard, A.R., Hedges, R.E.M., 1996. A diffusion-adsorption model of uranium
924 uptake by archaeological bone. *Geochimica et Cosmochimica Acta* 60, 2139-2152.
- 925 Nielsen-Marsh, C.M., Hedges, R.E.M., 2000. Patterns of diagenesis in bone II:
926 Effects of acetic acid treatment and the removal of diagenetic CO₂. *Journal of*
927 *Archaeological Science* 27, 1151-1159.
- 928 Oxenham, M.F., Piper, P.J., Bellwood, P., Bui, C.H., Nguyen, K.T.K., Nguyen, Q.M.,
929 Campos, F., Castillo, C., Wood, R., Sarjeant, C., Amano, N., Willis, A., Ceron, J., 2015.
930 Emergence and Diversification of the Neolithic in Southern Vietnam: Insights
931 From Coastal Rach Nui. *Journal of Island and Coastal Archaeology* 10, 309-338.
- 932 Pellegrini, M., Snoeck, C., 2016. Comparing bioapatite carbonate pre-treatments
933 for isotopic measurements: Part 2 - Impact on carbon and oxygen isotope
934 compositions. *Chemical Geology* 420, 88-96.
- 935 Petrov, I., Šoptrajanov, B., Fuson, N., Lawson, J.R., 1967. Infra-red investigation of
936 dicalcium phosphates. *Spectrochimica Acta Part A: Molecular Spectroscopy* 23,
937 2637-2646.
- 938 Pike, A.W.G., Hedges, R.E.M., Van Calsteren, P., 2002. U-series dating of bone using
939 the diffusion-adsorption model. *Geochimica et Cosmochimica Acta* 66, 4273-
940 4286.

- 941 Ramsey, C.B., 2009. Bayesian analysis of radiocarbon dates. *Radiocarbon* 51, 337-942360.
- 943 Ramsey, C.B., Higham, T., Bowles, A., Hedges, R., 2004. Improvements to the 944 pretreatment of bone at Oxford. *Radiocarbon* 46, 155-163.
- 945 Reimer, P.J., Bard, E., Bayliss, A., Beck, J.W., Blackwell, P.G., Bronk Ramsey, C., Buck, 946 C.E., Cheng, H., Edwards, R.L., Friedrich, M., Grootes, P.M., Guilderson, T.P., 947 Haflidason, H., Hajdas, I., Hatté, C., Heaton, T.J., Hoffmann, D.L., Hogg, A.G., 948 Hughen, K.A., Kaiser, K.F., Kromer, B., Manning, S.W., Niu, M., Reimer, R.W., 949 Richards, D.A., Scott, E.M., Southon, J.R., Staff, R.A., Turney, C.S.M., van der Plicht, 950 J., 2013. IntCal13 and Marine13 radiocarbon age calibration curves 0-50,000 951 years cal BP. *Radiocarbon* 55, 1869-1887.
- 952 Reimer, P.J., Brown, T.A., Reimer, R.W., 2004. Discussion: Reporting and calibration 953 of post-bomb ^{14}C data. *Radiocarbon* 46, 1299-1304.
- 954 Shellis, R.P., Dibdin, G.H., 2000. Enamel microporosity and its functional 955 implications, in: Ferguson, M.W.J., Smith, M.M., Teaford, M.F. (Eds.), *Development, 956 Function and Evolution of Teeth*. Cambridge University Press, West Nyack, New 957 York.
- 958 Sillen, A., LeGeros, R., 1991. Solubility profiles of synthetic apatites and of 959 modern and fossil bones. *Journal of Archaeological Science* 18, 385-397.
- 960 Sponheimer, M., Lee-Thorp, J.A., 2006. Enamel diagenesis at South African 961 Australopithecus sites: Implications for paleoecological reconstruction with trace 962 elements. *Geochimica et Cosmochimica Acta* 70, 1644-1654.
- 963 Stafford Jr, T.W., Brendel, K., Duhamel, R.C., 1988. Radiocarbon, ^{13}C and ^{15}N 964 analysis of fossil bone: Removal of humates with XAD-2 resin. *Geochimica et 965 Cosmochimica Acta* 52, 2257-2267.
- 966 Storm, P., Wood, R., Stringer, C., Bartsiokas, A., De Vos, J., Aubert, M., Kinsley, L., 967 Grün, R., 2013. U-series and radiocarbon analyses of human and faunal remains 968 from Wajak, Indonesia. *Journal of Human Evolution* 64, 356-365.
- 969 Stuiver, M., Polach, H.A., 1977. Reporting of ^{14}C Data. *Radiocarbon* 19, 355-363.
- 970 Surovell, T.A., 2000. Radiocarbon Dating of Bone Apatite by Step Heating. 971 *Geoarchaeology - An International Journal* 15, 591-608.
- 972 Tisnérat-Laborde, N., Valladas, H., Kaltnecker, E., Arnold, M., 2003. AMS 973 radiocarbon dating of bones at LSCE. *Radiocarbon* 45, 409-419.
- 974 Trueman, C.N., Tuross, N., 2002. Trace elements in recent and fossil bone apatite, 975 *Reviews in Mineralogy and Geochemistry*.
- 976 Van Strydonck, M., Boudin, M., De Mulder, G., 2009. ^{14}C dating of cremated bones: 977 The issue of sample contamination. *Radiocarbon* 51, 553-568.
- 978 Wood, R.E., Barroso-Ruiz, C., Caparrós, M., Jordá Pardo, J.F., Santos, B.G., Higham, 979 T.F.G., 2013. Radiocarbon dating casts doubt on the late chronology of the Middle 980 to Upper Palaeolithic transition in southern Iberia. *Proceedings of the National 981 Academy of Sciences of the United States of America* 110, 2781-2786.
- 982 Yi, H., Balan, E., Gervais, C., Ségalen, L., Roche, D., Person, A., Fayon, F., Morin, G., 983 Babonneau, F., 2014. Probing atomic scale transformation of fossil dental enamel 984 using Fourier transform infrared and nuclear magnetic resonance spectroscopy: 985 A case study from the Tugen Hills (Rift Gregory, Kenya). *Acta Biomaterialia* 10, 9863952-3958.
- 987 Zahradnik, R.T., Moreno, E.C., 1975. Structural features of human dental enamel 988 as revealed by isothermal water vapour sorption. *Archives of Oral Biology* 20, 989317-325.

- 990Zazzo, A., 2014. Bone and enamel carbonate diagenesis: A radiocarbon
991prospective. *Palaeogeography, Palaeoclimatology, Palaeoecology* 416, 168-178.
992Zazzo, A., Lebon, M., Chiotti, L., Comby, C., Delqué-Količ, E., Nespoulet, R., Reich, I.,
9932013. Can we use calcined bones for radiocarbon dating the Paleolithic?
994*Radiocarbon* 55, 1409-1421.
995Zazzo, A., Munoz, O., Saliège, J.F., 2014. Diet and mobility in a late neolithic
996population of coastal oman inferred from radiocarbon dating and stable isotope
997analysis. *American Journal of Physical Anthropology* 153, 353-364.
998Zazzo, A., Saliège, J.F., 2011. Radiocarbon dating of biological apatites: A review.
999*Palaeogeography, Palaeoclimatology, Palaeoecology* 310, 52-61.
1000

1001Table 1: Samples used in this study. Tooth eruption and wear stages follow (Lemoine et al., 2014) and associated radiocarbon dates have been
 1002calibrated against IntCal13 (Reimer et al., 2013) in OxCal v.4.2 (Ramsey, 2009) and are given at 95.4% probability.
 1003

Sample ID	BB1	LT1	LT4	DU795	ISO6
Location	Farm, Byron Bay, NSW, Australia	Lang Trang, Vietnam	Lang Trang, Vietnam	Duoi U'Oi, Vietnam	Rach Nui, Vietnam
Context	Surface	Cave 2, Breccia 5	Cave 2, Breccia 5	Breccia	12RN H1L34/1 C1 1034/1
Expected age	Modern	>50 ka	>50ka	>50ka	3550-3360 cal BP (SANU-30823, 3200 ± 35 BP, Oxenham, et al. (2015))
Tooth	M3, lower right	M3, upper right anterior cusp	M3, lower left	M3, lower left	M2
Wear stage	5-6	4-7	4-8	9-10	4-5

1004

Table 2: Analytical results on the modern enamel sample BB1 after grinding by hand and micronizing for 5, 15 and 30 minutes, and etching in acetic acid for up to 71 hours. All measurements are on the powder remaining after treatment.

Length of acetic acid leach	Pretreatment % Yield	ICPAES % Mg	FTIR Brushite present?	$\delta^{13}\text{C}$	IRMS $\delta^{18}\text{O}$	%C
Handground (H)						
0	100	0.227	No	0.702	-4.6	0.61
15 minutes	69.5	0.156	No	0.699	-4.2	0.59
1 hour	67.1	0.142	No	0.699	-4.2	0.60
8 hours	63.9	0.135	No	0.699	-4.3	0.58
20 hours	63.4	0.128	No	0.698	-4.1	0.56
44 hours	66.8	N/A	Yes	N/A	N/A	N/A
71 hours	66.4	N/A	Yes	N/A	N/A	N/A
Micronised_5 min (M_5)						
0	100.0	0.226	No	0.701	-4.8	0.64
15 minutes	67.8	0.137	No	0.697	-4.3	0.61
1 hour	62.9	0.132	No	0.697	-4.3	0.62
8 hours	66.1	0.122	No	0.696	-4.4	0.60
20 hours	65.6	0.119	No	0.697	-4.3	0.59
44 hours	67.8	N/A	Yes	N/A	N/A	N/A
71 hours	69.0	N/A	Yes	N/A	N/A	N/A
Micronised_15 min (M_15)						
0	100.0	0.214	No	0.709	-5.7	0.67
15 minutes	69.3	0.130	No	0.697	-4.6	0.57
1 hour	66.2	0.122	No	0.698	-4.3	0.61
8 hours	65.0	0.117	No	0.696	-4.4	0.57
20 hours	66.0	0.109	No*	0.695	-4.3	0.55
44 hours	65.4	N/A	Yes	N/A	N/A	N/A
71 hours	63.2	N/A	Yes	N/A	N/A	N/A
Micronised 30 mins (M_30)						
0	100.0	0.229	No	0.700	-4.9	0.60
15 minutes	69.7	0.123	No	0.693	-5.3	0.52
1 hour	66.1	0.115	No	0.695	-4.3	0.60
8 hours	68.1	0.108	No	0.695	-4.1	0.58
20 hours	67.0	0.099	No	0.692	-4.6	0.51
44 hours	68.5	N/A	Yes	N/A	N/A	N/A
71 hours	68.5	N/A	Yes	N/A	N/A	N/A

* A small shoulder may be present at around 525 cm^{-1} , suggesting small amounts of brushite may be present.

Table 3: U-series results on the three tooth samples from Duoi U’Oi and Lang Trang sites. No age calculations (n.c.) were carried out for U concentrations of ≤ 0.5 ppm. All errors are 2- σ . Key: EN = enamel, DE = dentine.

1014

Sampl e	Tissue	U(ppm)	Error	R08	R08-error	R48	R48-error	CS age (ka)	CS age error (ka)
DU795	EN1	3.81	2.23	0.781	0.005	1.218	0.005	106.8	1.45
	DE1	82.97	1.02	0.805	0.003	1.220	0.002	112.0	0.79
	EN2	4.38	4.12	0.806	0.012	1.287	0.007	101.8	2.66
	EN3	1.09	0.33	0.803	0.012	1.262	0.009	104.8	2.82
LT1	EN1	0.77	0.44	1.803	0.637	1.818	0.064	235.3	234.07
	DE1	7.16	0.32	2.084	0.010	1.790	0.006	498.1	32.97
	EN2	1.21	0.32	1.758	0.013	1.835	0.008	214.1	4.81
	DE2	5.97	0.29	1.997	0.011	1.795	0.006	360.8	12.47
LT4	EN1	0.27	0.19	0.728	0.024	1.343	0.016	n.c.	
	DE1	1.93	0.58	0.950	0.012	1.372	0.008	118.9	2.95
	EN2	0.06	0.03	1.049	0.194	1.277	0.087	n.c.	
	EN3	0.02	0.00	1.511	0.207	1.248	0.115	n.c.	
	DE3	3.94	0.63	0.909	0.010	1.333	0.007	116.5	2.53

Table 4: Analytical results on ancient enamel samples. Micronised samples were milled for 30 minutes. All measurements are on the powder remaining after treatment. Holocene dates have been calibrated against IntCal13 (Reimer et al. 2013) in OxCal v.4.2 (Ramsey 2009) and are given at 95.4% probability.

Sample		Pre-treatment % Yield	AMS						ICPAES		FTIR	IRMS		
Grinding method	Time in acid (hr)		SANU-	F14C	F14C error	Date (BP)	Date error	Calibrated age (cal BP) from to		% Mg	Brushite present?	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	%C
LT1														
Hand	0	100	44706	0.17123	0.00108	14176	56			0.136	No	-12.962	-7.3	0.77
Hand	20	80.5	44716	0.07939	0.00086	20351	92			0.129	No	-13.614	-6.9	0.72
Micronised	0	100	44711	0.20265	0.00117	12823	51			0.275	No	-13.126	-9.1	0.83
Micronised	20	72.9	44719	0.02788	0.00078	28756	230			0.112	No	-13.876	-6.6	0.65
LT4														
Hand	0	100	44707	0.14092	0.00115	15741	71			0.132	No	-13.514	-8.5	0.73
Hand	20	75.3	44717	0.08022	0.00087	20267	92			0.126	No	-13.988	-8.5	0.77
Micronised	0	100	44712	0.18924	0.00110	13373	52			0.138	No	-13.657	-9.9	0.78
Micronised	20	69.2	44720	0.01796	0.00077	32288	349			0.097	No*	-14.378	-7.6	0.58
DU795														
Hand	0	100	44709	0.12620	0.00099	16627	68			0.157	No	-12.172	-8.2	0.74
Hand	20	78.7	44718	0.07065	0.00093	21288	111			0.135	No	-13.035	-8.1	0.74
Micronised	0	100	44713	0.17632	0.00120	13941	60			0.127	No	-12.275	-9.7	0.80
Micronised	20	68.3	44721	0.02141	0.00078	30878	298			0.098	No	-13.636	-7.5	0.61
ISO-6														
Hand	0	100	44710	0.71781	0.00216	2663	29	2844	2745	0.246	No	-11.760	-5.9	0.77
Micronised	0	100	44714	0.73180	0.00225	2508	30	2738	2489	0.148	No	-11.358	-6.5	0.80
Micronised	20	70.1	44723	0.67728	0.00204	3130	29	3443	3250	0.130	No	-11.793	-4.4	0.63

* A small shoulder may be present at around 525 cm^{-1} , suggesting small amounts of brushite may be present.

● Hand ground ● M_5
● M_15 ● M_30

Grinding conditions_acid leach

+ Bone apatite (Zazzo 2014)

× Enamel apatite (Zazzo 2014)

○ Microniser_acid leach (this study)

▢ Dentine apatite (Zazzo 2014)

● Hand ground_acid leach (this study)