



## Case study

# Can plant material be considered part of cultural heritage? A lesson from *Vitis vinifera* L. remains stored in the Museo Archeologico Nazionale di Napoli, (Italy)



Alessia D'Auria<sup>a,\*</sup>, Fabio Marzaioli<sup>b</sup>, Isabella Passariello<sup>b</sup>, Riccardo Spaccini<sup>c</sup>, Stefano Conti<sup>d</sup>, Giuseppe Melchionna<sup>d</sup>, Maurizio Teobaldelli<sup>a</sup>, Gaetano Di Pasquale<sup>a</sup>

<sup>a</sup> Plant and Wood Anatomy Lab, Department of Agricultural Sciences, University of Naples "Federico II", Via Università 100, 80055, Portici, Naples, Italy

<sup>b</sup> CIRCE INNOVA, Department of Mathematics and Physics of Isotope Ratio Mass Spectrometry Services, University of Campania "Luigi Vanvitelli", Caserta, Naples, Italy

<sup>c</sup> NMR Research Center CERMANU, Department of Agricultural Sciences, University of Naples Federico II, Via Università 100, 80055, Portici, Naples, Italy

<sup>d</sup> Department of Agricultural Sciences, University of Naples "Federico II", Via Università 100, 80055, Portici, Naples, Italy

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## ABSTRACT

Since 1738, archaeological sites in the area around Mount Vesuvius have produced large quantities of botanical remains, constituting the *Collezione dei Commestibili e degli Avanzi organici* (i.e. Collection of Edibles and Organic Remains), currently housed in the *Museo Archeologico Nazionale* in Naples. It is considered as one of the most extensive collections from the classical world and provides in-depth evidence of Roman dietary habits. A significant portion of the material, in fact, can be attributed to food-related taxa, including a distinctive historical find consisting of thousands of uncharred remains of *Vitis vinifera* L. (i.e. grapevine). The carpological assemblage is composed by 6 kilos of grapes, peduncles, stalks and pips attributed to marc remains. A multidisciplinary study (morpho-anatomical and Nuclear Magnetic Resonance analysis, as well as Radiocarbon dating) of these specimens allowed to establish that the collection also includes 'historically false material'. Indeed, the histological structure of the grape seeds and the lignified cell layers appear well preserved. Radiocarbon dating has shown that the material does not date back to the Roman period, but that the age of the *Vitis* remains can be placed between 1782 and 1796, the period corresponding to the first excavations carried out in the Vesuvian area. Accordingly, in order to reconstruct the history of this material, a complementary review of published works, archival records and inventories was carried out, which revealed that the first mention of this material in archival sources appeared in 1885 and has been listed incorrectly ever since.

The information obtained from the survey of archival material and experimental analysis allowed to emphasize the lack of attention, study and inadequate conservation methods of archaeobotanical material.

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## 1. Introduction

The eruption of Vesuvius in 79 AD was one of the greatest natural disasters of Antiquity, an event attested by historical sources and archaeological data, the latter providing a comprehensive picture of the culture and customs of the people living in Campania during the 1st century AD. In particular, the large number of botanical remains from archaeological sites of the Vesuvian area, although consisting mostly of charred ecofacts resulting from the eruption [1], represent an exceptional form of cultural heritage,

shedding light on both the Roman landscape and dietary habits of the early Imperial period. Archaeological excavations were started fortuitously in 1710 by the so-called 'Prince' Emmanuel Maurice d'Elbeuf, followed by brief but systematic activities in 1738 under King Charles of Bourbon. During these excavations, archaeobotanical remains, often consisting of food products (hence edibles) prepared by the local population probably around the time of the eruption, were found in deposits, silos, and small shops, as well as in pots and plates inside the houses of the buried sites [2,3].

The most important nucleus of these materials constitutes the *Collezione dei commestibili e degli avanzi organici* (i.e. Collection of Edibles and Organic Remains) of the *Museo Archeologico Nazionale di Napoli* (MANN). The Collection includes a wide variety of plant remains of both woody and herbaceous species, representing the

\* Corresponding author.

E-mail address: [alessia.dauria@unina.it](mailto:alessia.dauria@unina.it) (A. D'Auria).

largest collection of plant remains dating to the Roman period. In the course of the 18th century, the Collection was stored in the tenth room of Charles of Bourbon's royal palace at Portici, eventually forming the *Museo Ercolanese*. This room, known as the “*Gabinetto dei preziosi*”, was conceived as a “*Wunderkammer*” for the storing and conservation of the most precious findings which included gold and silver works of art, gems, cameos and some plant remains such as fruits, woods and different forms of bread [4,5].

The first document attesting to the transport of findings from archaeological sites to the *Museo Ercolanese* dates back to December 24th, 1760 [6]. In 1805, for reasons of space and security, the Collection was relocated to what is now the *Museo Archeologico Nazionale di Napoli* [3,5]. A new botanical survey of the collection was carried out in 2017 [7]; each element containing plant remains, such as bowls, bags, plastic or wooden crates, whether consisting of a single specimen or thousands of remains, was assigned a record number [7]. A total of 51 taxa were identified, of which 31 could be associated with food [7].

Among the 261 records analysed, remains of *Vitis vinifera* L. were recovered from only two, despite the importance of viticulture in food production during Roman times: one identified as raisins, the other consisting of thousands of remains stored in a modern plastic box. The uniqueness of this archaeobotanical find lies not only in the number and heterogeneity of grapevine remains, but also in their uncharred conditions.

## 2. Research aim

The goal of this work was to analyse the aforementioned *Vitis vinifera* L. remains by combining a historical and scientific approach: A) provide a survey of the historical data of this material; B) verify the presence of other finds previously identified as *V. vinifera* within the collection; C) characterise the material through different methodologies, including Morpho-anatomical, Nuclear Magnetic Resonance analysis (NMR) and, especially, Radiocarbon dating.

## 3. Materials and methods

### 3.1. Archival research

A survey of the inventories and excavation diaries stored in the historical archives of the MANN [8–16] on the presence of *Vitis* remains was carried out, verifying previous reports [17–20] of these finds and the related archaeological documentation. Inventory research proved complex due to the large number of existing copies of these documents and the succession of registers compiled by different museum directors who, over the years, modified their record filing according to different systems.

### 3.2. Experimental analysis

#### 3.2.1. Morpho-anatomical analysis

The morpho-anatomical structure of the ancient grape seeds stored in the MANN was observed at low magnification using a stereo microscope and compared with modern quiescent seeds of *V. vinifera* cv. Aglianico. The latter was chosen as a reference as it constitutes one of the main autochthonous grape cultivars of the region, where it was presumably introduced at the time of the foundation of the Greek colonies as early as the 8th century BC [21]).

A sub-sample of the ancient grape seeds were hand-sectioned with a razor blade along the longitudinal plain as reported by Cadot et al. [22]. In order to preserve their structure, these were not pre-processed prior to sectioning. The modern quiescent seeds used for comparative description were instead sterilized (40 min at

room temperature in 1% sodium hypochlorite) and soaked in water (24 hrs at room temperature in 9 cm diameter Petri dishes over five layers of water-saturated filter paper) in order to soften the hard lignified tissues prior to sectioning.

#### 3.2.2. NMR analysis

Solid-state NMR spectroscopies (<sup>13</sup>C CPMAS NMR) of the grape seeds was acquired on a Bruker AV300 spectrometer equipped with a 4 mm wide bore MAS (magic angle spinning) probe. NMR spectra were obtained with the following technical setup: 13,000 Hz of spin rate; 2 s recycling time; 1 min contact time; 30 min acquisition time; 4000 scans. Powdered samples were packed in 4 mm zirconium rotors with Kel-F caps. The Fourier transform was performed with a data point of 4k and an exponential apodization of 150 Hz line broadening.

For the interpretation of solid-state <sup>13</sup>C NMR spectra, the different signals were conventionally grouped into 6 main chemical shift regions, representative of the functional groups of organic carbon [23]: Alkyl-C: 0–45 ppm; Methoxyl-C or N-alkyl-C: 45–60 ppm; O-Alkyl-C: 60–110 ppm; Aryl-C: 110–140 ppm; Phenolic-C: 140–160 ppm, and Carboxyl-C: 160–190 ppm. The relative amount of each spectral interval was determined by integration (MestreNova 6.2.0 software, Mestre-lab Research, 2010) and expressed as a percentage of the total area.

#### 3.2.3. Radiocarbon dating

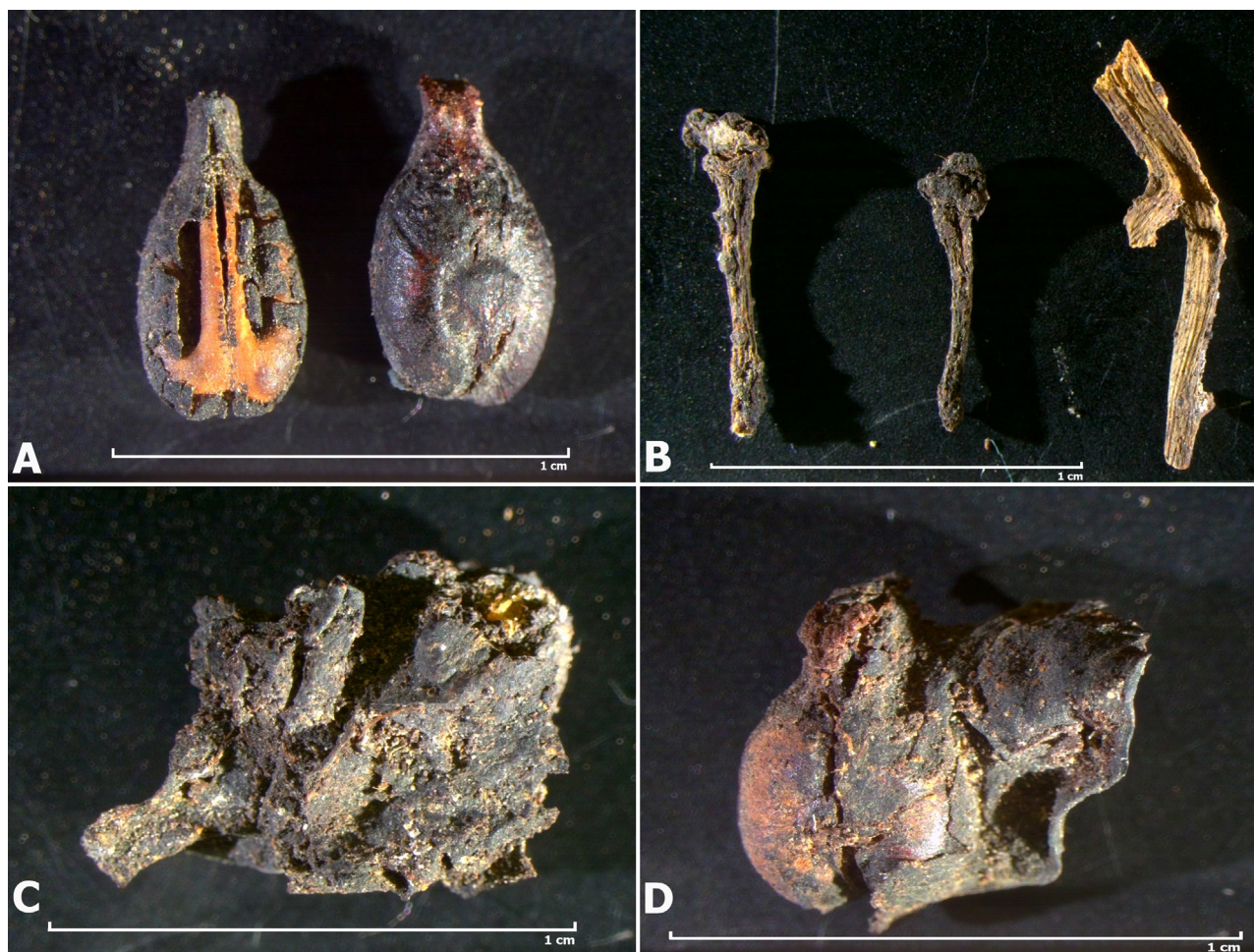
Four seeds were selected for <sup>14</sup>C dating, carried out at the Circe Lab - Department of Mathematics and Physics Research Laboratory Pole – DMF-PoLaR, University of Campania “Luigi Vanvitelli”. Samples were subjected to classical AAA (Acid-Alkali-Acid) chemical attack to remove potential contaminants according to Passariello et al. [24]. Pre-treated samples were combusted under vacuum combustion in a sealed quartz tube at 920 °C for 6.5 h to obtain complete conversion of organic carbon to CO<sub>2</sub>. CO<sub>2</sub> was purified using a cryogenic line and converted to graphite through the sealed tube zinc reduction following Marzaioli et al. [25]. According to CIRCE measurement procedures [26], unknown samples were measured together with machine blanks, procedural blanks, reference materials and quality control checks in an analytical measurement batch to estimate the <sup>14</sup>C/<sup>12</sup>C isotope ratio of the sample and its radiocarbon age [27]. Radiocarbon dates (RC age) were calibrated in calendar age using oxcal [28] and the INTCAL 13 dataset [29]. Results reported always refer to the 1st sigma for both RC and calendar age.

## 4. Results

### 4.1. Archival research

The first mention of *V. vinifera* recorded in archival sources is traditionally attributed to Michele Ruggiero [19], who describes a large number of grapevine remains (“*un Gruppo di uva passa et altro mezzo cofano della stessa*” = “a group of raisins and another half coffer of the same”) found on June the 4th, 1761, at Herculaneum [19]. However, the author refers to raisins (*uva passa*), whereas the present carpological assemblage is composed of grapes, peduncles, stalks and pips (Fig. 1), clearly attributed to marc remains. Despite this inconsistency, Borgogino [3], in his work on the archaeobotanical remains from the 79 AD eruption, argues that this material is the same described by Ruggiero [19] and reports 15 kilos of charred remains [3], whereas a total of 6 kilos of uncharred material has been documented from this study.

It should be noted that Jashemsky in her volume on the natural history of Pompeii [2], dedicated to the study of the landscape, flora and fauna of sites in the Vesuvian district, makes no mention of this find.



**Fig. 1.** Carpological assemblage of *Vitis vinifera* L. remains from the *Collezione dei Commestibili e degli Avanzi organici*. **A.** pips; **B.** left and centre stalks, far right a peduncle; **C.** fully preserved grape; **D.** pip with a portion of still-preserved flesh.



**Fig. 2.** Comparison of longitudinal sections of two ancient *Vitis vinifera* L. seeds (A and B) and a fresh one (C). In specimen A the histological structure appears deteriorated, while in specimen B it is well preserved, allowing all lignified cell layers to be observed.

Historical documentation and inventories indicated the presence, in the storerooms of the MANN, of six records referred to as *Vitis* remains and described as raisins [7].

#### 4.2. Experimental analysis

##### 4.2.1. Morpho-anatomical data

The multi-layered lignified seed coat surrounding the inner endosperm and embryo tissues was observed in longitudinal and transverse sections, corresponding to the well-known anatomical structure reported in literature (see [30]; Fig. 2).

As previously mentioned, to avoid the risk of altering their morpho-anatomical structure, the ancient seeds were sectioned

with no preliminary softening treatment. Although this resulted in the fracture and fragmentation of the samples due to their brittleness, the structure was still identifiable (Fig. 2). A multi-layer seed coating was observed in the structure of the fresh seeds, while the inner tissues of the nucellus and embryo had evolved into an amorphous grainy mass. Light microscopy observation of a fresh seed cross-section revealed the histological structure of the seed coat, with the outer seed cuticle followed by the epidermis, outer, medium and inner integument while the nucellus and embryo occupied the central region of the section, as described by Cadot et al. [22]. The outer histological structure was also well preserved in the ancient seed; here all the lignified cell layers could be observed, from the outer cuticle to the inner integument. Conversely,

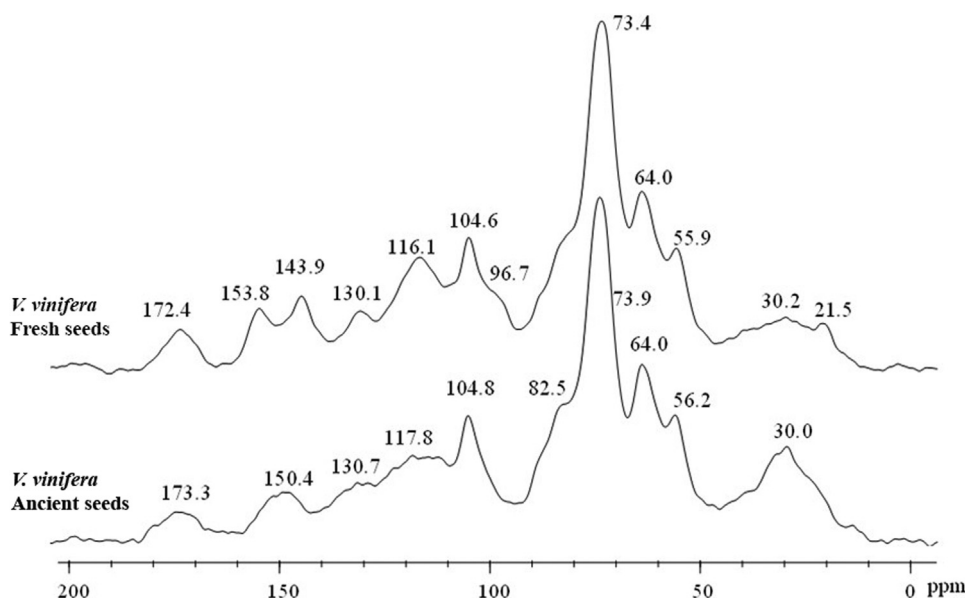


Fig. 3. Solid-state  $^{13}\text{C}$  CPMAS NMR spectra of fresh and ancient grape seeds.

Table 1

Distribution (%) chemical shift regions (ppm) in  $^{13}\text{C}$ -CPMAS-NMR spectra of grape pips.

Notes: <sup>a</sup>Lignin Ratio obtained as (45–60) / (140–160).

	Carboxyl-C (190–160)	O-Aryl-C (160–140)	Aryl-C (140–110)	O-Alkyl-C (110–60)	CH <sub>3</sub> O (60–45)	Alkyl-C (45–0)	LR <sup>a</sup>
Fresh seeds	3.4	8.3	15.3	54.4	8.2	10.4	1.0
Ancient seeds	3.4	3.6	12.2	54.6	9.0	17.2	2.5

the incoherent grainy mass derived from the nucellus - embryo tissues and occupying the central region of the ancient seed section, was lost in the sectioning process.

#### 4.2.2. NMR data

The carbon distribution in the NMR spectra of fresh and ancient grape seeds (Fig. 3) indicates the typical composition found in the  $^{13}\text{C}$  CPMAS analysis of recent and ancient plant tissues [23,31]. The main signals were related to polysaccharides (60–110 ppm) and aromatic (110–160) groups, with overall amounts ranging from 70% to 78% of the total spectral area (Table 1). The resonances of the O-alkyl-C range (60–110 ppm) in cellulose and hemicelluloses chains include the carbon nucleus in position 6 at 64 ppm, followed by the 73 ppm overlap of carbons 2, 3, 5 (Fig. 3), while the shoulder around 84 ppm represents the C4 of monomeric units involved in the  $\beta$  1→4 glycosidic bond with the anomeric C 1 at 105 ppm.

In the aromatic region, the extended bands from 110 to 140 ppm are assigned to the unsubstituted and C-substituted phenyl carbons of lignin monomers, polyphenols lignans and flavonoids. The two sharp signals at 144 and 154 ppm shown by the fresh seeds (Fig. 3) are the O-substituted ring carbons of both lignin and polyphenol derivatives [23,32].

Relatively less abundant Alkyl-C molecules (0–45 ppm) in the NMR spectra include the bulk methylene (CH<sub>2</sub>) building block of alkyl chains of linear and cyclic lipid compounds (i.e. waxes, sterols, etc.). The shoulder at 21 ppm in fresh seeds (Fig. 3) can be associated with the methyl substituents of hemicelluloses and the glucosidic group of flavonoids. The peak at 56 ppm implies the possible overlap of  $\nu$  functional groups due to both methoxyl substituents on the aromatic rings of lignin and lignan components and the C–N bonds of peptidic moieties. Finally, signals in the carbonylic region (160–190 ppm) indicate carboxyl groups of aliphatic

acids, amides in amino acid as well as the acetyl substituents of hemicelluloses and flavonoids.

The main differences between the NMR spectra of fresh and ancient grape seeds are related to the relative increase in Alkyl-C molecules and the decomposition of specific aromatic compounds, underscored by the lower signal intensity in the O-aryl C region (Fig. 3; Table 1).

#### 4.2.3. Radiocarbon dating

Radiocarbon analysis showed unexpected results that may explain the uncharred and exceptional state of preservation of the seed tissues. Calendar ages, in fact, fall in the timeframe known as the Suess Effect region (Fig. 4). Approximately between 1650 and 1950 AD, the  $^{14}\text{C}$  age function shows an averagely flat trend with a sequence of wiggles. This phenomenon is due to anthropogenic fossil fuel emissions which produce a significant bias effect on RC ages lower than 250 years BP, regardless of measurement precision. The RC age indicated two chronological ranges: 1646–1667 AD (probability of 42.6%) and 1782–1796 AD (probability of 25.6%)

## 5. Discussion

In the present case study, all the applied methodologies were chosen to carry out a detailed and adequate characterization of the available materials. One of the former objectives of this work was, in fact, to verify the feasibility of ancient DNA analysis; therefore, morpho-anatomical observations and NMR analysis were carried out first. In order to avoid erroneous assumptions as to the exceptional state of preservation of these alleged archaeological findings, it was necessary to confirm their age. Following this methodological approach, it was possible to point out some inaccurate conclusions from previous works and thus update the current state of

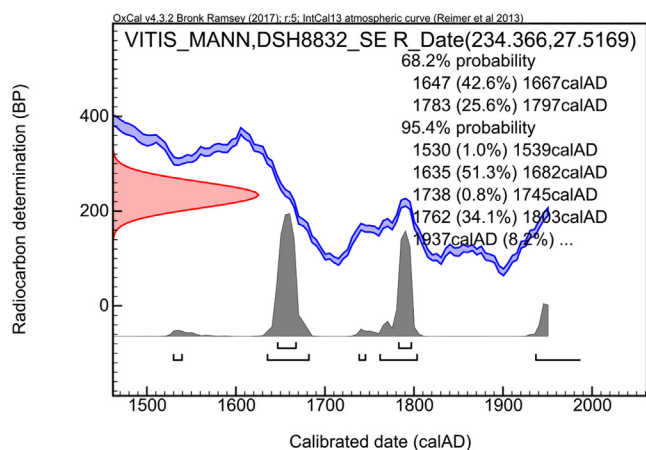


Fig. 4. Calendar age of four ancient *Vitis vinifera* L. seeds fall in the so-called Suess Effect region.

the art of plant remains from the Vesuvian area. In addition, this analytical protocol has been useful in underscoring the lack of attention and conservation of these archaeological materials which instead represent an invaluable historical and cultural heritage.

In the Vesuvian area archaeobotanical remains are found charred, whereas morpho-anatomical analysis have showed that these materials are partially degraded but not carbonized. The multi-layered seed coat was well preserved in most of the ancient seed specimens and this can be ascribed to its histological structure made up of lignified tissue layers. Lignin is an aromatic polymer forming part of the cell wall of woody plant tissues, providing rigidity and resistance to biological attack. Because it is insoluble, chemically complex and lacking in hydrolysable linkages, lignin is a difficult substrate for enzymatic depolymerization [33]. The only organisms known to extensively degrade lignin are fungi [34], which thrive in moist and slightly acidic environments. When these conditions are not met, woody tissues remain unaltered for very long periods of time, thus explaining the excellent conservation status of the external seed coat layers of the ancient grape seeds. Conversely, the inner seed tissues were not as well preserved as the seed coat. In about half of the sub-sample of sectioned seeds, the tissues forming the endosperm and the embryo were still present, although they had evolved into an amorphous grainy mass (Fig. 2B). In the other half, however, no remains of the inner tissues were found in the empty seed coat (Fig. 2A). Since the endosperm and embryo tissues consist of small cells with thin cellulose walls, these tissues are easily subject to biodegradation [35].

NMR analysis showed a relative increase in aliphatic components combined with the cleavage of ether bonds in the lignin structure and condensation of aromatic components, patterns commonly found in progressive transformation processes of various samples of archaeological plant specimens [31,32,36]. These remains are characterized by the selective retention of biochemically recalcitrant hydrophobic alkyl and aromatic derivatives, also favoured by dry environments such as those associated with ancient seed storage [37]. However, as in the case of morpho-anatomical data, this evidence appears inconsistent with both the age and the preservation by charring which should instead characterize these remains.

The radiocarbon age measured for the sample falls within the Suess Effect region, where calendar ages are drastically affected by calibration, leading to a wide chronological range: 1646–1667 AD and 1782–1796 AD with a relative probability of 42.6 % and 25.6 % respectively. The first interval (1646–1667) is unlikely, as it pre-

dates the start of the Bourbon excavation activities. Therefore, the most likely age of the observed plant remains is 1782–1796 AD, a timespan consistent with the period in which the excavations took place.

For all these reasons, the presence of such finds might be explained as a voluntary or accidental contamination of external material during excavation work. Interestingly, in the *Collezione dei Commestibili e degli Avanzi organici* there are eight additional records of uncharred plant remains, one of which, a large number of barley grains (*Hordeum vulgare* L.), comes from Herculaneum [7]. In light of the current study, all of this material will undergo radiocarbon analysis to confirm its authenticity.

Apart from the record discussed in this paper, the MANN collection contains only one confirmed specimen of *V. vinifera* L.; all other records previously inventoried as *Vitis* are today lost or correspond to different species [3,7]. According to the historical documentation and inventories, six records described as raisins, and therefore referred to as *Vitis* remains, are stored in the storerooms of the MANN. Unfortunately, the census carried out in this study revealed the presence of only two records, while the remaining 4 could not be traced. It is worth noting that these 2 records were identified as hazelnut achenes (*Corylus avellana* L.) [7].

## 6. Conclusion

The *Museo Archeologico Nazionale di Napoli* currently houses the largest assortment of archaeobotanical remains pertaining to the classical period, resulting from the eruption of Vesuvius in 79 AD and collected since archaeological excavations in the area first began. Results obtained from archival survey and experimental analyses have been consistent, showing that often in studies previously conducted in the Vesuvian area, plant remains received limited attention, lacking the necessary methods required. The present study therefore represents the first attempt at an analytical protocol aimed at highlighting such errors in the archaeobotanical record, demonstrating that the *Vitis* remains object of this research can be dated to the second half of 18th century.

In conclusion, this work shows the lack of specific archaeobotanical skills (e.g. principles of systematic botany applied to archaeological plant remains, but also criteria for describing the context of archaeological finds) and overall unfamiliarity in approaching this class of material, as well as inadequate conservation methods.

From this point of view, it would be extremely important to bring attention to these unique archaeobotanical collections, which constitute a cornerstone of the cultural heritage [38,39] of the classical world with regard to food culture and ancient landscapes.

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