Research on the existence of the genus *Vitis* in archaeological sites in Romania

(SUMMARY OF PhD THESIS)

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Introduction

The grapevine is one of the classic species cultivated in the Old World (Europe, Asia, Africa) and, together with the olive, fig, pomegranate and palm, is the oldest species cultivated for fruit. Grapes, cereals and olive oil became known as the "Mediterranean triad" for their key role in the diet of the region (SARPAKI, 1992). Grapes contributed significantly to food production, providing sugar-rich fresh fruit, raisins and stum for wine production. At the same time, they constituted a commercial element in the countries that had access to the sea (ZOHARY, 2012).

Before the colonization of North America, viticulture in Europe and Western Asia was restricted to the gene pool present in the *Vitis vinifera* L. complex. However, in modern times, numerous wild species native to the Americas have been used either as additional genetic sources for breeding new grape varieties or as rootstocks to confer resistance against the devastating attack of *Phylloxera* (ZOHARY, 2012).

Some aspects that confirm the cultivation of the grapevine are represented by plant macroresidues (seeds) discovered in archaeological sites. The desiccation characteristic of arid regions favours the preservation of plant remains not usually found in the archaeological record, such as fruits, flowers and leaves (WILKINSON K., 2008). Considering the low frequency with which the biological material found in archaeological sites in Romania is analyzed, the present work presents various contexts in which, in archaeological sites in Europe, the emphasis is on interdisciplinary research of the excavated material.

Another branch of research that confirms the presence of grapevines, especially wine, is that which studies certain chemical markers, which allow the identification of organic residues on ancient ceramic vessels. Such studies have recently been approached in Europe, while in Romania in the XXI century, such analyzes cannot yet be discussed. This can be seen as an opportunity to study such chemical markers to determine whether organic wine residues are present on certain Roman Ceramic vessels from the territory of Roman Dacia.

Analysis of organic residues provides information about trade, technology, diet, medicine, cosmetics, art, trades, agricultural practices, how people organized their homes, and how they prepared their dead for burial (EVERSHED, 2008; EVERSHED, 1999; EVERSHED, 1993; HERON, 1993).

Chemical methods were the only analytical tools available to early analysts of archaeological remains (POLLARD, 2007; ÅSTRÖM, 1969; MATHIASSEN, 1935). Since the 1970s, instrumental methods of analysis have become increasingly available (JEANNE CONDAMIN, 1976). Instrumental chromatography (gas or liquid), more recently combined with mass spectrometry, is the most widely used method. Other methods used to characterize ancient organic residues include various spectroscopies (FTIR, Raman, NMR, UV, etc.), SEM and EDX, XRD, XRF and high magnification light microscopy.

The structure of the PhD thesis

The PhD thesis comprises 113 pages and consists of two parts. The first part is dedicated to the bibliographic study and represents 31% of the thesis (35 pages), and

the second part includes the personal contribution, representing 69% of the thesis (78 pages). The thesis contains 51 figures and 11 tables.

The first part of the thesis includes two chapters:

Chapter 1. *Research on the methods used in the morphometric analysis of grapevine seeds found in archaeological sites* includes a brief history of research carried out worldwide and in Romania to identify grapevine seeds as cultivated or wild.

Chapter 2. *Research on the methods used in the detection of organic residues*, presents a review of research in the field of organic residue analysis, especially wine residues.

The second part of the thesis comprises six chapters:

Chapter 3. *Aim and objectives pursued* include the statement of the thesis's purpose and this research's specific objectives.

Chapter 4. *The provenance of the research material* includes a brief history of the archaeological sites from which the researched material comes and some details related to the modern seeds studied in this work.

Chapter 5. *Material and method* present information about the biological material used in the morphometric analysis of archaeological seeds and about the organic residues analyzed. The chapter also includes information related to the analyses and equipment used.

Chapter 6. *Results and discussion* present the results of research on the analysis of archaeological and modern seeds and the results obtained from the analysis of the organic residues on the amphora fragments.

Chapter 7. *Conclusions and recommendations* include the conclusions drawn from the research, related to the specific objectives. A number of recommendations for future continuation of the research in this paper are offered.

Chapter 8. *Originality and innovative contributions of the thesis* specify the novelty and contribution of the results obtained to the completion of knowledge in the field of the doctoral thesis.

The reference list contains 167 titles, of which 163 are books and articles, most of them recent, and 4 are references to electronic pages.

The aim and objectives pursued

The aim of the research is defined for each individual study. First of all, it was desired to differentiate the archaeological grape seeds from the *Apulum* archaeological site by mathematical methods and compare them with cultivated varieties from the RDSVV Blaj. Secondly, the research aimed to analyze amphora fragments from the *Potaissa* archaeological site, belonging to the Roman Era, using a sensitive and selective methodology, with optimal extraction methods. The methods used allow the identification of markers for food, wine and resins.

A series of research was carried out to achieve the aim with defined specific objectives. The most detailed part of the research was carried out on the residues on three amphora fragments in order to confirm or deny the existence of wine residues. To these is added complementary research aimed at the structure of the samples used.

In order to morphometrically analyze the archaeological grapevine seeds, the following **objectives** were defined:

1. Measurement of LS= length of the stalk, PCH= placement of chalaza, L= total length, B= total breadth for the archaeological seeds;
2. Differentiation of archaeological seeds into wild and cultivated seeds by applying mathematical methods;
3. Choosing current cultivated varieties to compare them with archaeological ones;
4. Measurement of LS= length of the stalk, PCH= placement of chalaza, L= total length, B= total breadth for seeds from RDSVV Blaj;
5. Identification of archaeological seeds belonging to certain varieties.

In order to detect the existing organic residues on amphorae from the Roman Period, the following **objectives** were defined:

1. Identification of amphora deposits on the territory of Roman Dacia;
2. Selection of amphora fragments (lip, neck, handle, body, foot) for analysis;
3. Sampling from the surface of fragments;
4. Selection of samples for the application of the various studied methods;
5. Identification of markers for wine using a more complex methodology for 3 samples;
6. Establishing the work protocol, application of working methods and identification of target compounds.

Material and method

Biological material

In order to carry out the analyses, a sample was taken from a 10-15 cm layer of grape seeds from the *Apulum* archaeological site. The seeds were on a structure supposed to be a winepress. The depth from which the seeds were collected is -1.59 m. The archaeological context was dated on the basis of a coin belonging to the reign of Empress Maria Theresa (1740-1780). The coin was found on the winepress (IULIA-ALEXANDRA FARCAȘ, 2019).

To compare archaeological seeds with those from modern collections, seeds from 6 grape varieties, harvested from RDSVV Blaj in 2016 and stored in a freezer until the time of the study, were used. The studied white wine grape varieties were Fetească albă, Muscat Ottonel, Neuburger, and Selena, and those for red wine were Fetească neagră and Cabernet Sauvignon.

The Amphora deposit within the Roman fort of *Potaissa* was chosen for the procurement of the research material due to its position in a grapevine-growing area near the main Roman road. Amphora fragments such as lip, neck, handle, body, and foot were visually inspected to identify organic residues preserved on their surfaces.

Among the ceramic materials belonging to the Amphora Deposit, 10 fragments were chosen that visually showed organic residues. The amphora fragments were discovered during archaeological excavations from 1980-1984. They have been cleaned with water and stored in crates until now, under conditions specific to archaeological materials.

Research methods

A total of 404 whole seeds were selected from the sample of seeds taken. The seeds were measured using an OPTICA stereomicroscope, to which an Optikam PRO 5

digital camera was attached. The measurements taken on the dorsal side of the seeds were as follows: length of the stalk (LS), placement of chalaza (PCH), total length (L), and total breadth (B) (IULIA-ALEXANDRA FARCAŞ, 2019). The same method was applied to the seeds from RDSVV Blaj. Measurements were made for 100 seeds/variety. The seeds were previously dehydrated.

Using the measured parameters, the LS/L and PCH/L ratios were calculated, as they use fewer simple variables with a reduced contribution from the correlation of the variables (MARIA MANGAFA, 1996). The formulas proposed by MARIA MANGAFA (1996) were used to distinguish between wild and cultivated seeds, originating from the archaeological site.

Boxplots were created to characterize the sample, and Pearson correlation was used to determine the relationship between the measured parameters. PCA was used to determine whether there was a relationship between archaeological seeds and those from modern collections.

Regarding the analysis of organic residues, after analyzing the methods used worldwide, the working protocol was established for 3 samples. The samples were analyzed using mineralogical microscopy in cross-polarized light, XDR, FTIR, SEM microscopy coupled with EDX elemental analysis, and GC-MS. Two of the three samples were further analyzed using HPLC.

Results and discussion

Results and discussion on archaeological seed analysis

Combining the results obtained using the four formulas, all 404 grape seeds were determined to be cultivated (IULIA-ALEXANDRA FARCAŞ, 2019).

Using the second formula proposed by MARIA MANGAFA (1996), it was observed that three grape seeds have a 63.80% probability of being grown (IULIA-ALEXANDRA FARCAŞ, 2019). MARIA MANGAFA (1996) suggests the use of the second and third formulas for the identification of grape seeds from archaeological sites, noting that the overall results are more or less identical.

Considering the measured parameters, the sample can be characterized as heterogeneous. Since the length varies considerably, the seeds were divided into three groups: seeds between 3 and 5 mm long, seeds between 5,001 and 6 mm long, and seeds between 6,001 and 7 mm long (IULIA-ALEXANDRA FARCAŞ, 2019).

A positive correlation could be observed between most parameters, especially between L and PCH, where the correlation is strong (IULIA-ALEXANDRA FARCAŞ, 2019).

Results and discussion on cultivated seed analysis

A strong positive correlation can be observed between L and PCH in the case of seeds from Fetească albă and Fetească neagră varieties. The same positive correlation was observed in the case of archaeological seeds. For seeds from Muscat Ottonel, Neuburger and Selena cultivars, a strong positive correlation between PCH and LS was observed. In the case of Cabernet Sauvignon seeds, a strong positive correlation between L and LS was observed.

By applying PCA to the archaeological seeds and those from the Cabernet Sauvignon variety, one can observe the overlap of 72.53% of the archaeological seeds with the Cabernet Sauvignon seeds. By applying PCA to the archaeological seeds and

those from the Fetească albă variety, it is possible to observe the overlap of only 23.01% of the archaeological seeds with those of Fetească albă, even if a strong positive correlation was found between L and PCH both in the case of archaeological seeds, as well as in the case of Fetească albă. By applying PCA to the archaeological seeds and those from the Fetească neagră variety, it is possible to observe the overlap of 64.85% of the archaeological seeds with those of Fetească neagră. By applying PCA to the archaeological seeds and those from the Muscat Ottonel variety, one can observe the overlap of 66.33% of the archaeological seeds with the Muscat Ottonel seeds. By applying PCA to the archaeological seeds and those from the Neuburger variety, a 63.61% overlap between the archaeological and Neuburger seeds can be observed. By applying PCA to the archaeological seeds and those from the Selena cultivar, it can be observed that 62.21% of the archaeological seeds overlap with those of Selena.

Archaeological seeds with no modern counterpart numbered 79, of which 13 were outside the 95% confidence interval.

Remains of a supposed wine press and grape seeds found in the same context suggest wine production for personal or commercial use. The studied sample did not contain seeds with pulp and/or skin attached to them, but most seeds were covered with a black film. This feature needs to be further investigated to determine whether environmental factors could have affected the colourimetry of grape seeds.

Results and discussion on organic residue analysis

Using mineralogical microscopy in cross-polarized light, it was possible to characterize the crystalline fractions of the samples and confirm the existence of organic material in all 3 analyzed samples.

Amorphous material of organic nature was identified in each sample using XDR analysis. A very good correlation with mineralogical microscopy in cross-polarized light was noted, so sample 2 contains the highest content of organic material, followed by sample 1. Sample 3 has the lowest amorphous material content. Samples 1 and 3 show very small peaks of calcium tartrate, a crystalline compound that is not part of the category of mineral compounds, being relatively close to organic compounds. Calcium tartrate is one of the specific biomarkers of ancient vessels in which wine was stored. The diffractogram of the resting soil shows very well-developed diffraction maxima with a slender allure and relatively high intensities, which confirms the optical mineralogical microscopy observation regarding the high mineral character of this soil. The dominant peak corresponds to quartz, followed by muscovite and calcite. The interaction of the sampled sediment had relatively little contamination from the resting soil, and the mineral input was influenced by particles detached from the amphora wall at the time of sampling.

Following the FTIR analysis, it is noted in the three sediment samples collected from the amphorae that the presence of several chemical bonds associated with different organic residues was discovered in the artefacts of ancient origin. In order to precisely establish the nature of these compounds, high-precision chromatographic investigations were started.

The EDS spectrum related to the SEM image for sample 1 reveals the content of 53.1% carbon and 42.2% oxygen, which illustrates the very high organic character of the highlighted nanostructures. The data from the specialized literature show that the tartaric acid from the wine, when it enters the pores of the amphora, reacts with the

calcium present in the ceramic material and precipitates in its pores (ELIZABETH HOWLAND BRIGGS, 2019; ALESSANDRA PECCI, 2013; STERN, 2008). Calcium tartrate crystallizes in the orthorhombic system, and the shape of these precipitates varies depending on the crystallization conditions, from rhomboid aggregates to prisms. In the present case, they are prisms with a length of 600 – 700 nm and a diameter of 30 – 50 nm. Overall, the scientific results in this paper indicate that the amphora with inventory number 5884 (sample 1) contained wine. The elemental composition of the organic material identified in sample 2, but also its morphology correlates with the aliphatic CH_2 vibration bands highlighted in the FTIR spectrum. Therefore, the organic sediment in sample 2 corresponds to traces of oil crusted over time, reaching the consistency of bitumen. The outward-facing surface of the deposit incorporated fine particles from the resting soil. Therefore, the experimental results from the present research confirm that olive oil was preserved in the amphora with inventory number 5960 (sample 2). The elemental analysis spectrum for sample 3 reveals an extremely high carbon content of 71.1%, which correlated with 24.8% oxygen and 2.8% related to calcium, indicating an organic mass containing calcium tartrate. Very high similarity with the organic nanoparticles from sample 1 is noted. All these aspects tend to correlate with the presence of the tartaric acid - calcium tartrate tandem according to the data from the specialized literature (ELIZABETH HOWLAND BRIGGS, 2019; ALESSANDRA PECCI, 2013; STERN, 2008).

The dominant volatile organic compound identified in all three amphora sediment samples is Squalene, which confirms the origin of the sediments sampled in the ancient contents of these amphorae. Squalene is reported in the literature both in relation to wine residues (LOUISE CHASSOUANT, 2021; ELENI NAZIRI, 2012) and in relation to olive oil (SUSANA GONZÁLEZ-RÁMILA, 2022; SORAYA MOUSAVI, 2022). Comparing the organic compounds identified in all three samples, 1-Hexadecanol and 1-Dodecanol, 2-octyl, as well as the following alkanes: Tridecane, Teradecane and Heptadecane, identified only in samples 1 and 3, are noteworthy. Data from the specialized literature mention these volatile organic compounds in connection with the fermentation process of wine and wine residues, as well as the presence of these alkanes in certain varieties of wine (LUKIĆ, 2022; ASPASIA MASTRALEXI, 2021; NIKOLAOU, 2021; MOHEKAR, 2018). Sample 2 shows a combination of volatile organic compounds, such as Homosalate, Isopropyl palmitate and Octocrylene, which can be correlated with olive oil residues decomposed and solidified over time in the presence of alkaline material induced by the muscovite and calcite content of the resting soil. In sample 2, no volatile organic compounds specific to wine were identified. Therefore, the GC-MS analysis places sample 2 with certainty in the category of amphorae in which olive oil was stored.

The analysis of the HPLC chromatograms obtained for the sediments of amphorae 1 and 3 shows that sediment sample 1 contains 32.81 mg of tartaric acid per 100 grams of raw sample and 10.59 mg of malic acid per 100 grams of raw sample. On the other hand, 11.77 mg of tartaric acid per 100 grams of raw sample and 4.86 mg of malic acid per 100 grams of raw sample were identified in sediment sample 3. The detection of significant amounts of tartaric acid in samples 1 and 3 constitutes strong evidence for the presence of wine in amphorae 5884 and 5960.

Conclusions and recommendations

The morphometric analyzes performed on the seeds of *Vitis vinifera* L. contribute to the acquisition of new knowledge about the history of the grapevine in Transylvania. The history of viticulture in Transylvania is complex, but with the help of morphometric analyzes together with the study of historical resources, the past habits of people can be determined. The incidence with which whole grape seeds are found in archaeological sites in Romania being low, it is opportune to analyze the seeds from a morphometric point of view.

It is recommended to use the method proposed by MARIA MANGAFA (1996), instead of the one proposed by STUMMER (1911) and currently used by Romanian researchers, because it uses more parameters for seed identification, and the results obtained are much more accurate.

The analyzes carried out on the three sediment samples taken from ancient amphora fragments from the Roman *Potaissa* fort confirm that the organic material identified in sample 2 corresponds to the ancient residues of olive oil that was stored in Antiquity and that the organic material identified in samples 1 and 3 corresponds to the ancient wine residues (IULIA-ALEXANDRA FARCAȘ, 2023).

A chemical approach to pottery sherds and resting soil is recommended, including in situations where pottery sherds have been stored for decades following their discovery in archaeological sites. Such an approach contributes significantly to a better understanding of the use of ceramic vessels in the past and provides precise details about their contents.

Originality and innovative contributions of the thesis

Analyzing grape seeds from archaeological sites and comparing them with modern grape seeds can provide valuable information for reconstructing the process of domestication and cultivation of the grapevine. Until now, grape seeds discovered in archaeological sites in Romania have been analyzed using STUMMER's index (1911). As subsequent research has shown, the formulas proposed by MARIA MANGAFA (1996) allow a more accurate characterization of archaeological seeds as wild or cultivated. In the present work, the formulas proposed by MARIA MANGAFA (1996) were used for the characterization of archaeological seeds and they were compared with 6 varieties of seeds from current cultivated varieties. Thus, this can be considered the first complex study on the analysis of archaeological grape seeds.

The analysis of organic residues inside ceramic vessels allows the description of the past contents of the vessel, whether it is about its single use or its reuse. The application of the methods described in the present work made it possible to identify the past contents of 8 fragments of Roman amphora, even though the fragments were washed and stored decades after their discovery. Until now, such analyses have not been carried out in Romania, this can be considered the first complex study regarding the analysis of organic residues on ceramic vessels.

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