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Thermal and microscope analysis as a tool in the characterisation of ancient papyri

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Abstract

Analyses of papyrus plants (*Cyperus papyrus* L.) from the Botanical Garden of the University of Genova and Ciane River (Siracusa) were used as a basis for the detection of histological features in papyrus paper. Both modern and ancient papyrus papers were analysed. Modern papyrus were manufactured at "Museo del Papiro di Siracusa"; two types of ancient papyrus (Egyptian and Greek–Roman, from Cairo Archaeological Museum) were studied. Sections of paper and plant stalks, taken 20 cm from the top, were examined under a scanning electron microscopy (SEM) and optical microscope (OM). The lignificated parts of both the plant and paper were either red (using acid flouroglucine) or blue–green (using Toluidine Blue) and were refracting under polarised light microscopy. Calcium oxalate crystals and starch granules were also detected. It was clear that the ancient Egyptian paper is richer in starch in comparison to the Roman one. It could be presumed that the Egyptian paper contains starch, a material which naturally occurs in the plant as a reserve. It was, in fact, preferably found in the residuals of the vascular bundle sheath. Microscope observations were compared with the results obtained by thermal and calorimetric analyses (TG and DSC). Thermal curves were different depending on which part of the plant was used to manufacture the papyrus and probably depend on the amount of cellulose and lignin present. Moreover, the Egyptian and Greek–Roman were also different in thermal behaviour.
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Keywords: Papyrus; Cellulose; Scanning electron microscopy; Optical microscopy; Thermal analysis

1. Introduction

The papyrus plant, *Cyperus papyrus* L. (*Cyperaceae*) is a triangular stemmed reed, that grows to a height of between 1.5 and 3 m and is topped with a bushy cluster of fine green thread-like strands with small flowers on the end. Papyrus plants, once cultivated and harvested, were one of the most important sources of writing material in the ancient world. The quality of the papyrus sheets depends upon a number of factors, such as the area where papyrus plants grow, the harvesting, and the layer of stem pith used in manufacture. The main aim of our study was to analyse Egyptian and Greek–Roman papyri of archaeological and historical interest from the Egyptian Museum of Cairo, Egypt, using thermal and thermogravimetric analyses combined with optical and electronic microscopy. These techniques have been previously tested on modern papyri manufactured in Cairo and in Siracusa (Italy) [1,2]. Analyses of papyrus plants (*C. papyrus* L.) from the Botanical Garden of the Genova University and the Ciane River, Siracusa, were also used as a basis for the study of histological features, to verify if the anatomical and histological characteristics of the plant are conserved and are detectable in the papyrus paper. Fragments of the paper (modern and ancient) as well as sections of stalks from the sampled plants, were examined and compared using optical microscopy (OM), scanning electron microscopy (SEM), energy dispersive X-ray fluorescence spectroscopy (EDX), differential scanning calorimetry (DSC) and thermogravimetry (TG).

2. Materials and methods

2.1. Anatomical observations

Central stalk portions from plants growing in the Botanical Garden of University of Genova and along the Ciane River, Siracusa, were used. In addition, fragments taken from

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recent and ancient papyrus sheets were analysed. The recent papyrus samples were manufactured at "Museo del Papiro di Siracusa" (Italy), using the ancient manufacturing methods studied in this research centre and treated with controlled quantities of natural additives (milk and/or oxalis obtained from the oxalis plant) [2]. The ancient samples were donated by Cairo Archaeological Museum. They belong to different historical periods and have different provenances. Two samples were chosen, the first one belonging to Greek–Roman age and the second one to the Pharaonic classic age.

For light microscopy, the samples were prepared by selecting and staining them for lignin with acid fluoroglucine. The material was also cut into fragments of 1–2 cm, fixed in FAA (formalin:acetic acid:60% ethanol, v:v:v, 5:5:90) for 24 h, dehydrated in a graded ethanol series, and embedded in JB4 resin (Polysciences Inc., Warrington, PA, USA) in BEEM capsules. 10–20 μm sections were cut with a Reichert Om U2 ultramicrotome equipped with a glass knife. Toluidine Blue O (TBO) at pH 4.4 was used as metachromatic stain [3]. For scanning electron microscopy, after ethanol dehydration the specimens were critical-point dried with liquid CO_2 using a CPD750 Emscope (Bio-Rad), mounted on stubs, coated with gold by AGAR PS3 sputtering (15–20 nm) and viewed with a Philips 515 SEM at an acceleration voltage of 20 kV. Photographs were taken using Kodak Technical PAN film.

2.2. EDX analysis

The material was prepared as for SEM analysis. Composition and energy dispersive spectra were processed using a Link-GEM EDX-spectroscope (Oxford) on a SEM Stereoscan 440 (LEO) with Link ISIS 1.04 software. During microanalysis the accelerating voltage was 20 kV, the specimen temperature was about 20 °C and the column vacuum 6.66×10^{-4} Pa. The elemental composition for the samples was obtained.

2.3. DSC analysis

DSC was performed on a Mettler-Toledo® Instrument DSC 821^e.

Samples (0.5–1.5 mg) were loaded into sealed aluminium pans with lids and heated to 625 °C at a heating rate of 1 °C min^{-1} in oxygen flux (100 ml min^{-1}). The empty aluminium pan was used as reference and the heat flow between the sample and reference pans was recorded. Measurements were repeated at least three times.

2.4. TG analysis

A TG–DTA apparatus STA 409 (Netzsch) was used. Samples between 5 and 10 mg were heated over the temperature

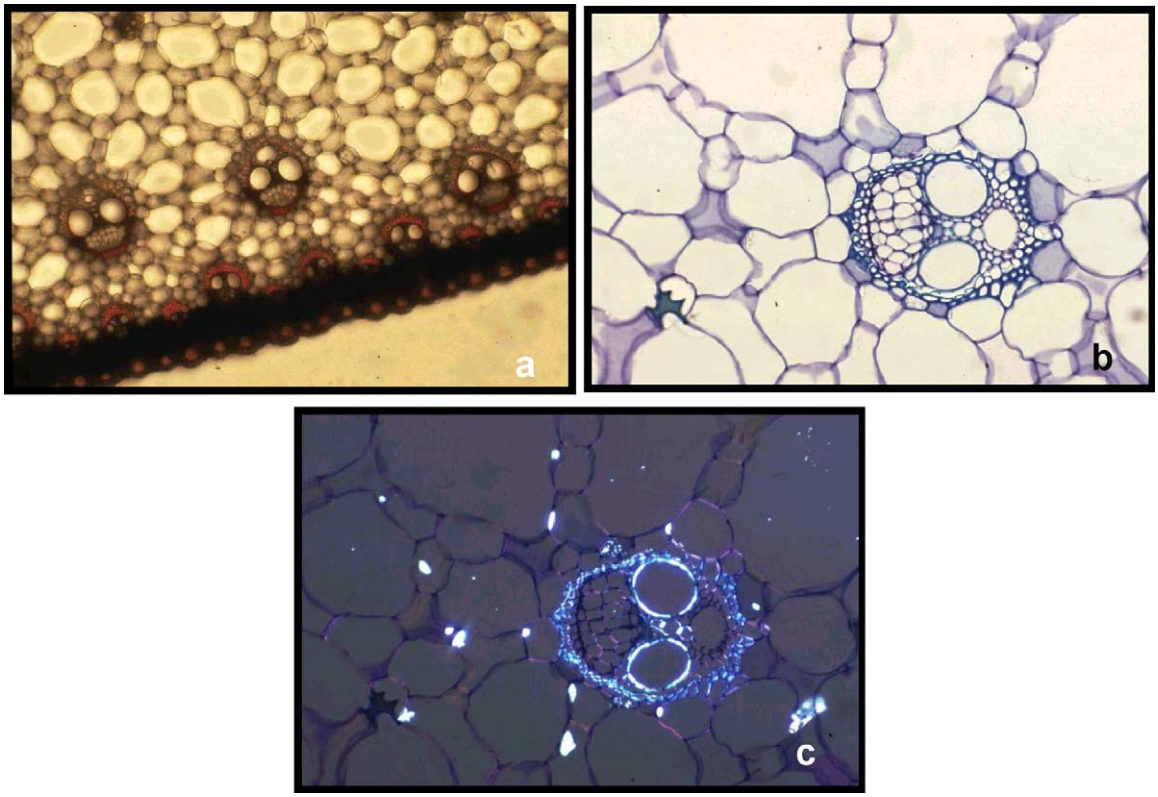

Fig. 1. (a) Handmade section: acid fluoroglucine staining: the lignified tissues (part of the vascular boundless and the epidermis) appear red; (b) semi-thin section (JB4 resin): Toluidine Blue (TBO pH 4.4) staining: the lignified portions of the vascular bundle appear blue–green; (c) semi-thin section (JB4 resin): Toluidine Blue (TBO pH 4.4) staining: the lignified portions of the vascular bundle appear birifringent using polarised light.

range from room temperature to 800 °C at a heating rate of 5 °C min^{-1} in oxygen at a flow rate of 100 ml min^{-1} Al_2O_3 was used as the reference material.

3. Results and discussion

3.1. Microscope investigations

In both the plant material and paper sheets, the lignified parts turned red using acid fluoroglucine (Fig. 1a) or appeared blue–green stained with Toluidine Blue O (Fig. 1b) and were refracting (or birifrangent) under polarised light (Fig. 1c). This showed a dense lignification of tissues in the peripheral zone of the plant stem, commonly discarded in papyrus manufacture. Conversely the pith, which is used for paper production, showed lignification only in the xylematic elements and fibres of the vascular bundles. For a better comparison, the SEM images (Fig. 2) obtained for the central portion of plant coming from Ciane River and from Botanical Garden of Genova are shown.

By using polarised light microscopy, it was also possible to detect calcium oxalate crystals and starch granules within the plant tissues. The same compounds are also detectable in sections of modern and ancient papyri sheets (Fig. 3).

It seems likely that the different characteristics of the papyrus sheets depend on the manufacturing procedures. In fact, several calcium oxalate crystals are detectable on the surface of modern papyrus sheets prepared by treatment with oxalis and milk (Fig. 4a) or oxalis only (Fig. 4b). In Fig. 4c the high signal of calcium, obtained by SEM–EDX analysis performed on these samples, is shown. In contrast, on the surface of the ancient Greek–Roman papyrus clay minerals were observed (Fig. 5), which in this case are also probably related to manufacturing procedures.

In addition, our results showed that the ancient Egyptian sheet examined is richer in starch in comparison to the Greek–Roman one. It is presumable that the starch in the Egyptian paper is the same as that present naturally in the plant, as it was also possible to observe that this starch was contained in residuals of the vascular bundle sheaths (Fig. 5c).

3.2. Thermal investigations

Microscope observations were compared with the thermal and calorimetric results. In modern papyri, DSC curves

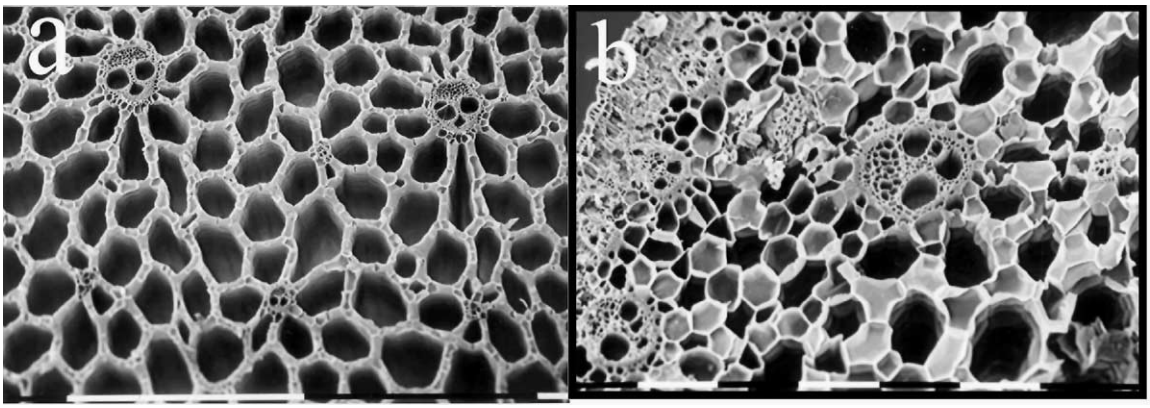

Fig. 2. SEM images obtained for the central portion of plant coming from Ciane River and from the Botanical Garden of the University of Genova.

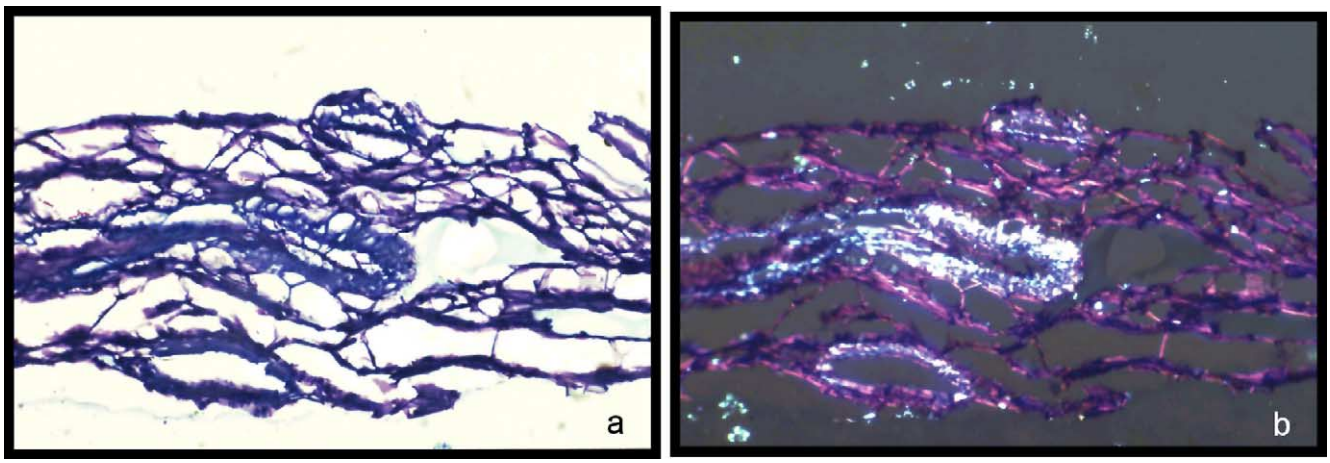

Fig. 3. (a) Section of a papyrus sheet after JB4 embedding and staining with TBO pH 4.4; (b) section of a papyrus sheet after JB4 embedding and staining with TBO pH 4.4 observed in polarised light microscopy.

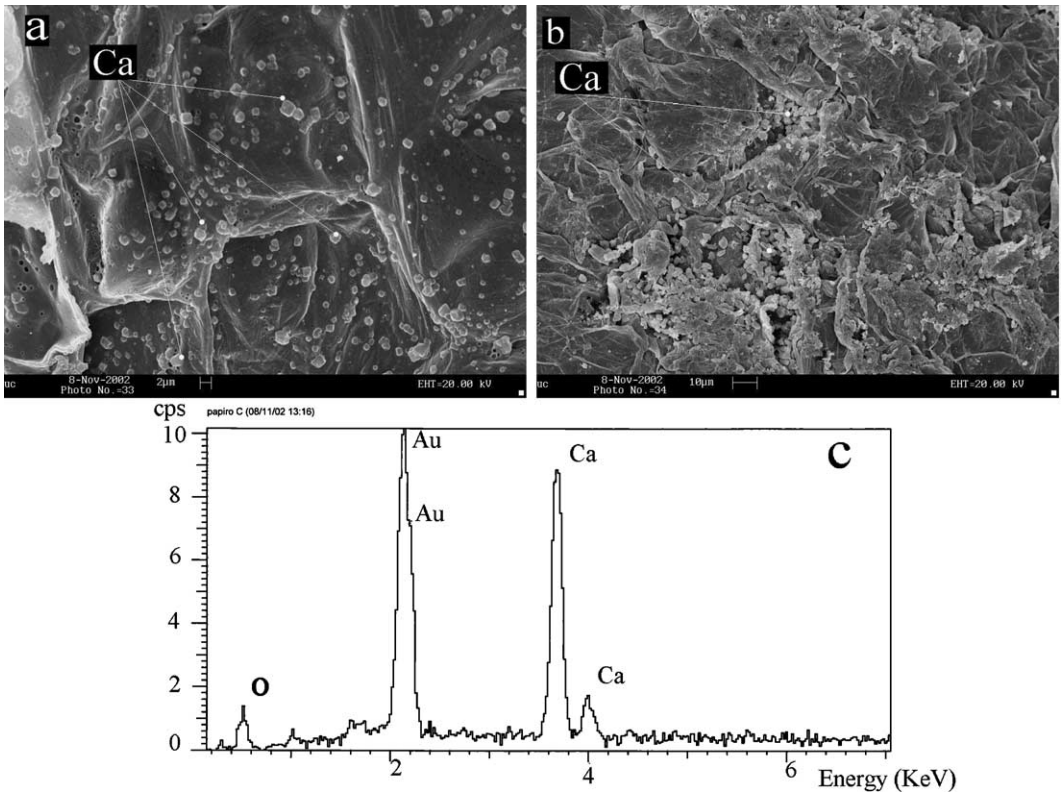

Fig. 4. (a) SEM image showing several calcium oxalate crystals on the surface of the papyri sheets treated with oxalis and milk; (b) SEM image showing several calcium oxalate crystals on the surface of the papyri sheets treated with oxalis; (c) SEM–EDX analysis of a papyrus sheet treated with oxalis showing the high calcium signal (marked spot).

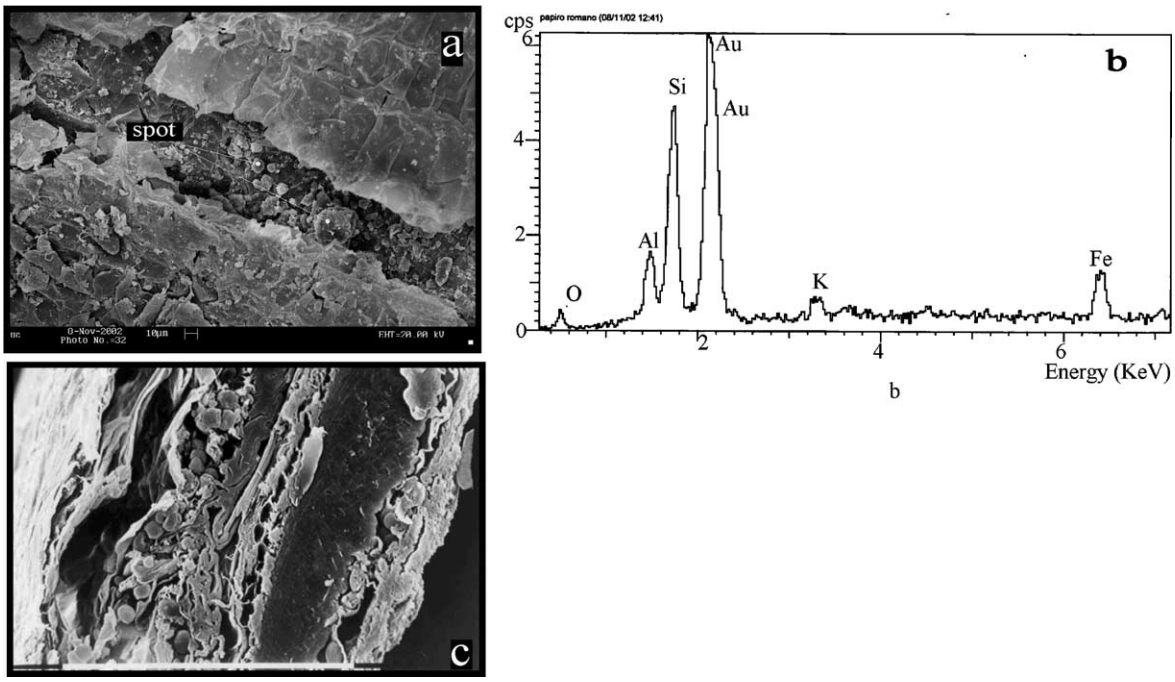

Fig. 5. (a) SEM image of the surface of an ancient papyrus sheet showing the presence of clay; (b) EDX analysis of the clay present in an ancient papyrus sheet; (c) SEM image of the section of an ancient papyrus in which starch is still visible.

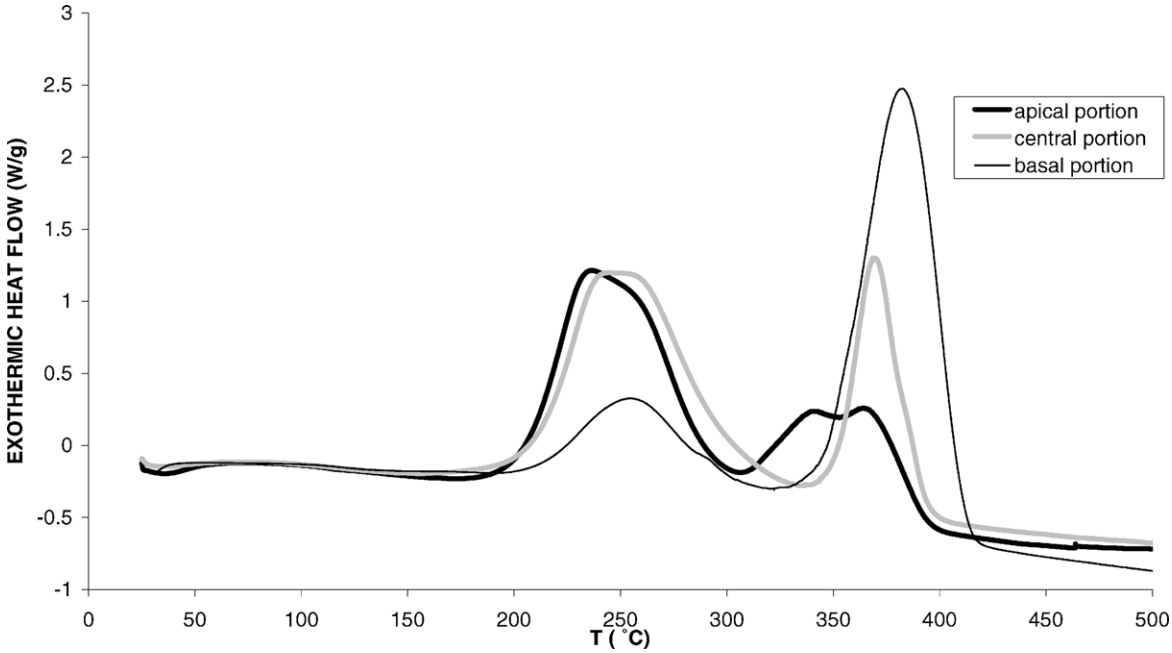

Fig. 6. DSC curves showing different peak shapes in relation to the portion (apical, central and basal) of the plant stem used to prepare papyri sheets.

showed a different behaviour depending on the parts of the plant used to manufacture the sheets (Fig. 6); the difference in the shape of the curves can probably be explained by the different amount of cellulose and lignin present in the different portions of the plant. In every thermogram the first broad peak was assigned to the decomposition and combustion of cellulose and hemicellulose and the second narrow peak was due to the lignin combustion (Fig. 6). A similar behaviour was found in the TG analysis [4], as shown in Fig. 7. This way of behaving is very similar to that found by Wiedemann and co-workers [5,6] and discussed in depth by Basile [1]. A useful comparison for the peak assignment

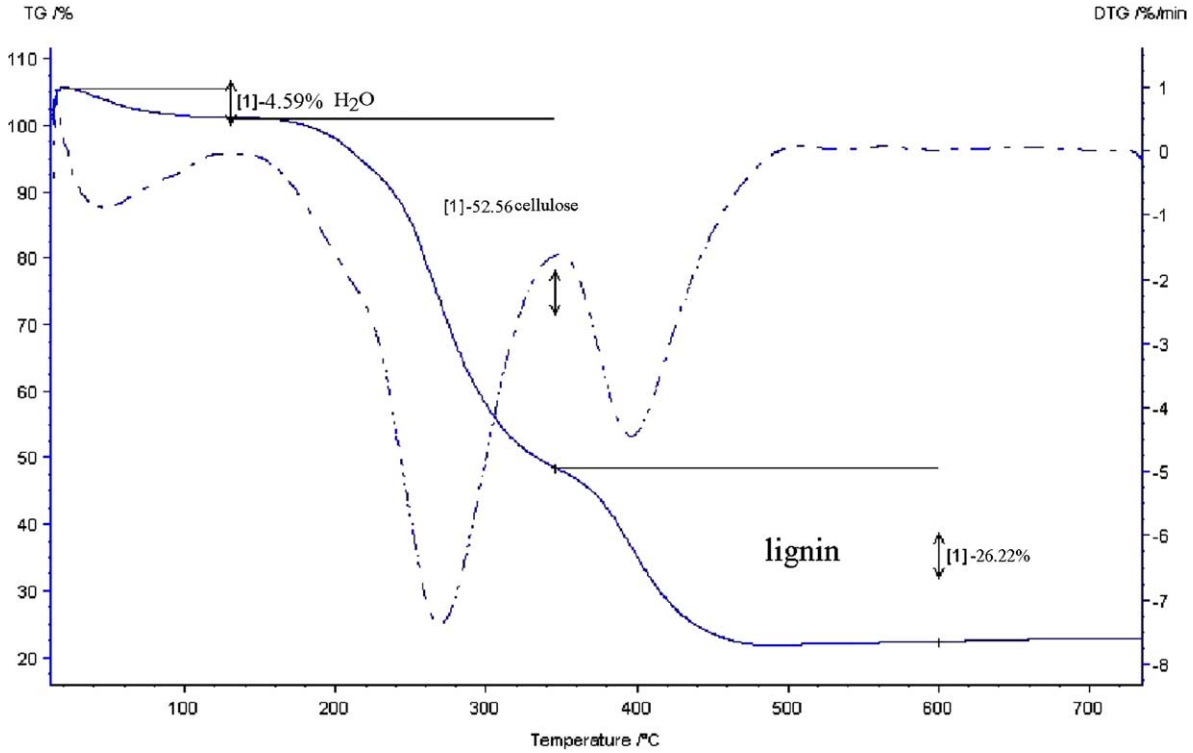

Fig. 7. TG of the papyrus sheet prepared with the central part of the stem.

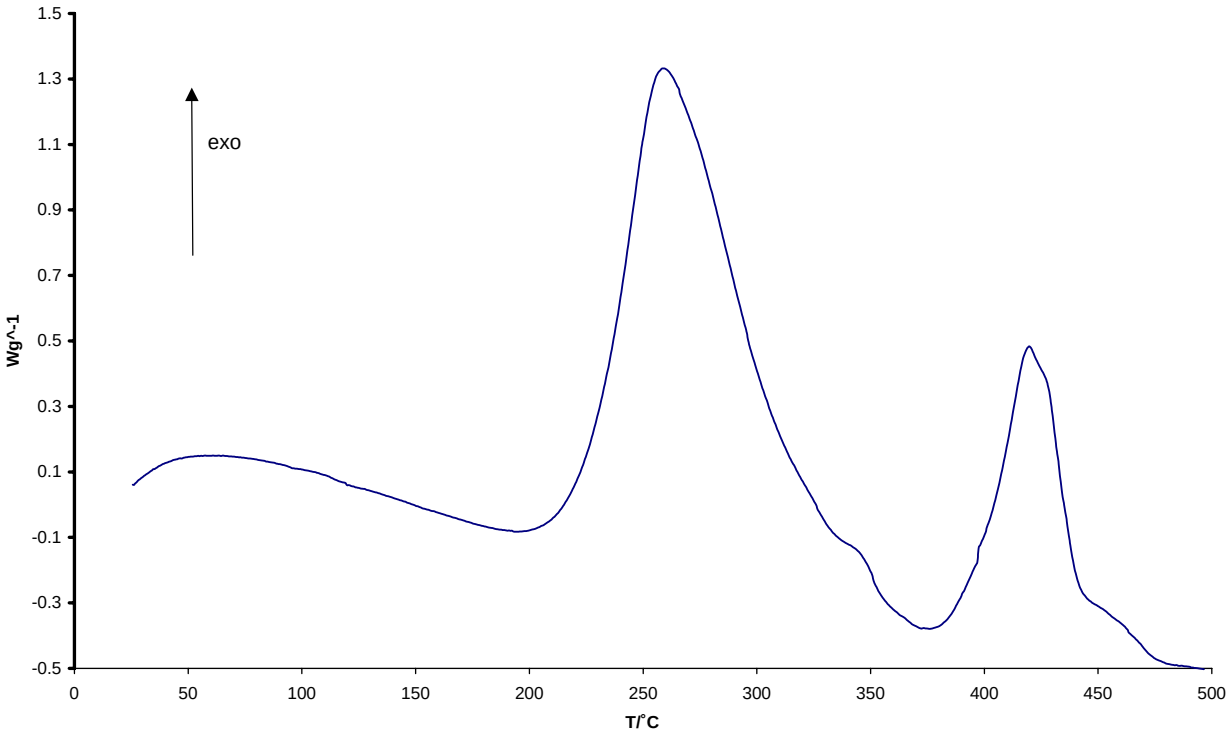

Fig. 8. DSC curve of an ancient Egyptian papyrus sheet of Pharaonic age.

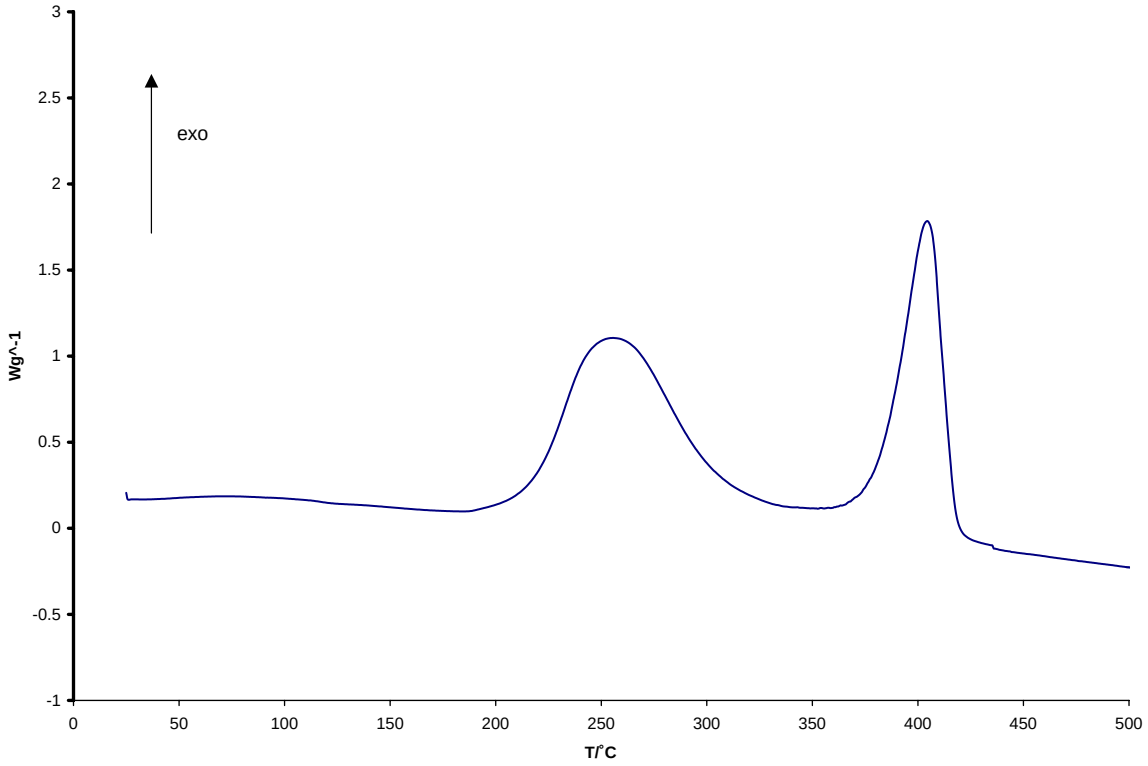

Fig. 9. DSC curve of an ancient Greek–Roman papyrus sheet.

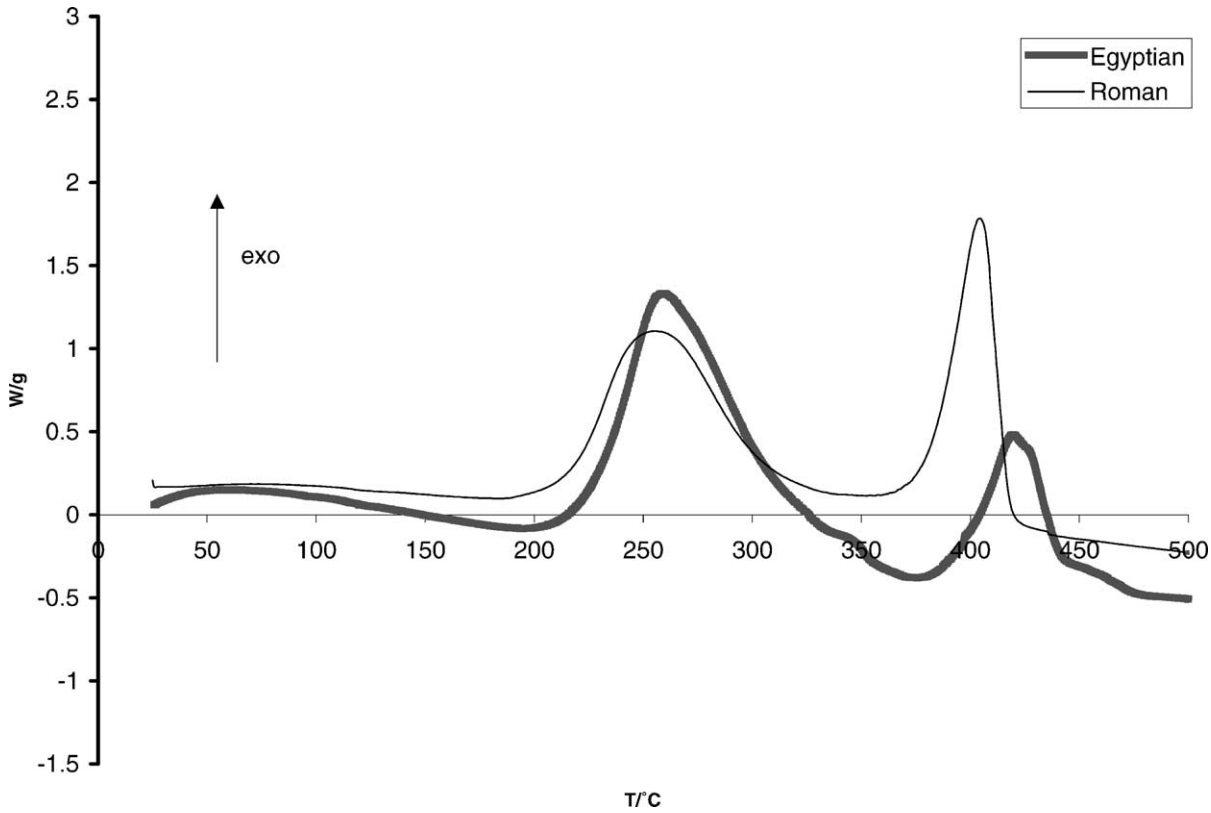

Fig. 10. Comparison between the DSC curves of ancient Egyptian and Roman–Greek papyri sheets.

method can also be found in recent work of Tsujiyama and Miyamori [7], even if the different experimental conditions lead to a shift in the characteristic peak temperatures.

Different thermal behaviour was found between Egyptian (Fig. 8) and Greek–Roman (Fig. 9) papyri. In Fig. 10, the comparison between the two curves is shown and we can observe that:

- the first peak, assigned to the cellulose thermal decomposition, occurs at a slightly different temperature (259.0 and 255.6 °C, respectively), and has some differences in the shape of the curves (the Egyptian one seems to be at least the sum of three effects);
- the second peak, assigned to the lignin thermal decomposition, falls at remarkably different temperatures (421.0 and 405.2 °C, respectively) and has noticeable different areas (i.e. kind and quantity of lignin);
- the small differences in the shapes of the curves are likely to be related also to the different content in starch and clay minerals present.

4. Conclusions

It is remarkable to notice that the morphology features of the papyrus plant are still visible in ancient papyrus sheet, even if they obviously look deformed due to the manufacturing. OM and SEM analyses highlighted different lignin contents in the various portions of the papyrus plant, which agrees with the experimental results obtained by of DSC and TG analyses.

Moreover, SEM and EDX analyses of the modern and ancient papyrus sheets showed that the different manufacturing methods (and also in many cases its origin) cause the curves obtained by thermal and calorimetric analyses to behave differently.

In conclusion, experimental data demonstrated that DSC and TG analyses, combined with electron and optical microscopy may turn out to be useful tools for the study of ancient papyri.

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