



Papyrus production revisited: differences between ancient and modern production modes

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Abstract Papyrus, produced from the white pith of *Cyperus papyrus* L., has been used for millennia as the major writing support by ancient cultures, but there was no continuous papyrus production until modern times. Therefore, papyrus production had to be rediscovered. Modern Egyptian papyrus producers claim that their sheets possess ‘the same physical and chemical properties as ancient papyri’. To study if this is accurate, commercially available papyrus sheets were compared to ancient papyri and papyri produced according to Pliny’s historic description. Material

characterization was performed with a focus on the potentially color-bearing lignin. Two-dimensional nuclear magnetic resonance spectroscopy, derivatization followed by reductive cleavage, and pyrolysis–gas chromatography/mass spectrometry were complemented with microscopy and tests for surface pH and sodium content. The lignin data in the native pith and commercial sheets were compared to 10 ancient samples from the Papyrus Museum Vienna. The analytical data clearly show the involvement of a strong alkaline treatment followed by chlorine bleaching for commercial papyri, as expressed by higher pH values, altered lignin structures, and chlorinated lignin compounds. The inclusion of an alkaline step in ancient papyrus manufacture is discussed but dismissed, since the alkali-treatment causes a huge decrease in lignin content, that was not found for the analysed ancient specimen. We assume that this additional treatment was introduced to obtain yellowish papyrus sheets meeting optical expectations of modern spectators. Linguistic and art historic evidence indicates that such a step would not have made sense in antiquity, since it was desired to produce white papyri.

Florian Bausch and Mario J. Rosado are contributed equally to this work.

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Introduction

Papyrus sheets are produced from the pith of the papyrus (*Cyperus papyrus* L.) sedge, which was common in the shallow water of the Nile Delta in Ancient Egypt. Dating back as far as 5,100 years (Braidwood et al. 1951), papyrus sheets are among the earliest written sources in human history. They have shaped our understanding of Egyptian, ancient Greek, Roman, Coptic and Arabic cultures by conveying concepts, images and other information about ancient literature, philosophy, religion, mathematics, medicine, astronomy and daily life through literary and religious texts, medical and other scientific treatises, personal letters, mercenary contracts, culinary recipes and much more (Černý 1952; Nicholson et al. 2000; Fliedler et al. 2001). This knowledge, as well as its physical carriers, must be preserved for future generations to enjoy and use.

The oldest surviving papyrus, which is blank and approximately 5,100 years old (Braidwood et al. 1951), was found in the Hemaka tomb in Saqqara, Egypt. At about the same time, the hieroglyph representing papyrus is first attested (Petrie 1927; Graefe 2001). This evidence provides an important witness to the appearance of this writing material in history, around the First Dynasty of Ancient Egypt, because the creation of a hieroglyphic representation presupposes the existence and dissemination of the material (Ekschmitt 1964). The first written papyri date

back to the Fifth Dynasty, ca. 2500 BCE (Diringer 1982; Bronk Ramsey et al. 2010). According to current understanding, the term ‘papyrus’ denotes both the papyrus plant and the writing material, and also the ancient Egyptian hieroglyph that depicted a papyrus roll. These three meaning aspects of the word are illustrated in Fig. 1.

Papyrus was one of the principal media for communicating information, including art and transcendent religious ideas, for approximately 4000 years. However, production declined around 1000 CE because of the advent of paper made in Asia (Nielsen 1985). Efforts to recreate this ancient art of papyrus manufacture in modern Europe were anything but straightforward. James Bruce described the results of his own attempts at the end of the eighteenth century: ‘Even the best of it was always thick and heavy, drying very soon, then turning firm and rigid and never white’ (Bruce 1804). The excavations of the famous tombs in the Valley of the Kings and the findings of important papyri as the Papyrus Smith and the Papyrus Ebers led to broad public interest in ancient Egyptian culture in the late nineteenth century, which in turn led to attempts to reproduce papyrus sheets as found, e.g., in the depot of the Technical Museum in Vienna in the form of a demonstration board depicting papyrus production and model papyrus sheets, produced with plant material from Sicily in 1905 (Technisches Museum Wien 2021). Around 1930, papyrus conservator Hugo Ibscher produced white



Fig. 1 Depictions of the papyrus plant, papyrus sheet and roll, and a hieroglyph resembling a papyrus roll **a** papyrus plants (*Cyperus Papyrus* L.); **b** a papyrus sheet (white) and a modern commercial papyrus roll (yellow); **c** the ancient Egyptian hieroglyph for ‘papyrus (roll)’, to the right of the head of Ramses I

(detail of a painting on limestone, Thebes, Valley of the Kings, KV 16, tomb of Ramses I., west wall, 1291–1289 BCE, photo reproduced with kind permission of Sandro Vannini/Laboratorio Tiradritti 2007))

papyrus sheets with plants from the Botanical Garden in Berlin, later attempts were conducted with mixed success by Gunn and Lucas with plant material from Sudan or Gunn's garden in Cairo. The Sudanese raw material had become too dry through transport to produce sheets, the garden's material yielded nearly white sheets with 'numerous light brown specks' (Černý 1952). The detours via various botanical gardens and Sudan were necessary because the *Cyperus papyrus* L. plant had become almost extinct in Egypt (Ekschmitt 1964). Hassan Ragab is credited with reviving commercial papyrus production in Egypt in the 1960s, after six years of unsatisfactory trials (Mann 1989).

All modern attempts to produce papyrus faced the difficulty that no ancient source transmitted an unequivocal description of papyrus production, that allowed for straightforward implementation (Wallert 1989). The most extensive account of papyrus production occurs in the encyclopaedic work by Pliny the Elder (23–79 CE) called *Naturalis Historia*, written ca. 77 CE (Lewis 1974). Since the almost 1,000-year interruption of continuous papyrus production, Pliny's description has remained the most detailed historical source. Pliny's detailed report of ancient papyrus production is schematically summarized in Fig. 2.

Papyrus stalks were cut with a sharp instrument to produce thin broad strips that were wetted and aligned in two orthogonal layers. Pressing the two layers together using rollers or in a press created a two-ply material that was then dried in the sun to produce a papyrus sheet that has become almost synonymous

with the term 'papyrus'. Typically, a series of sheets were glued together, using a flour-based paste containing drops of vinegar, in order to create a papyrus roll. The papyrus was smoothed, uneven wet sections were flattened with a hammer, and the material was subjected to another round of drying.

Linguistic arguments with implications on papyrus manufacture concern the nature of the used instruments (knife, needle for cutting and hammer or rolls for pressing) and the mode of preparing the slices. The 'classical' interpretation speaks of cutting the pith into slices as depicted in Fig. 2 while the 'Groningen' method assumes that the pith was peeled-off similar to veneer production (Nielsen 1985; Wallert 1989; El-Nahawi 2020). Further important 'production parameters' are the rigidity and the color of the sheets, as reported by the pioneers of papyrus reinvention when trying to put Pliny's description into practise (Bruce 1804; Černý 1952).

While Ibscher and Gunn produced 'satisfactory white' papyri (Černý 1952), Bruce's attempts were 'rigid and brown'. In the 1980s, Wallert et al. seemed to have faced similar problems, they describe: 'in water the papyrus strips acquire a slightly brownish and translucent appearance' (Wallert 1989) and 'If necessary, the material was bleached using a hypochlorite solution' (Wallert et al. 1989). Flieder et al. reported on a brown papyrus from Egypt that 'was beaten before dried' (Flieder et al. 2001).

Modern, commercial papyri are neither white nor dark brown, but exhibit a rather yellowish color (Fig. 3). Wallert describes: 'Commercially produced papyrus sheets by Ragab and el Kattan do indeed

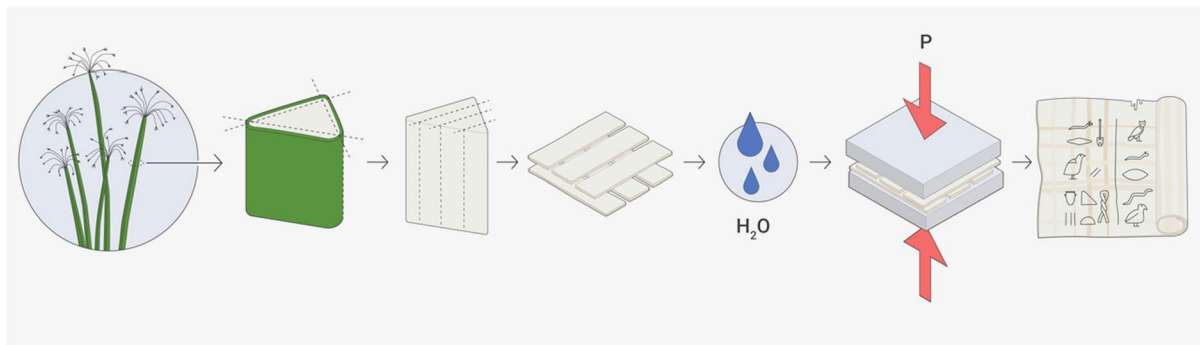


Fig. 2 Schematic of papyrus production according to the report of Pliny the Elder (77 CE). Papyrus stems are cut, the rind is removed, the pith is sliced, the wet slices are aligned

in two layers in rectangular orientation and the layers are pressed to form a sheet

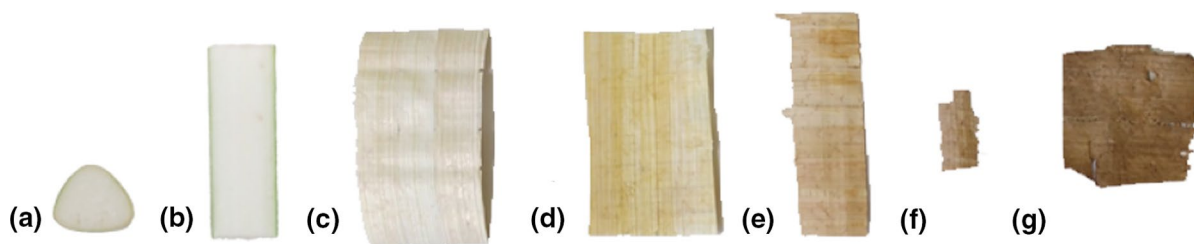


Fig. 3 Comparison of the color of different papyrus samples **a** cross section of papyrus pith; **b** longitudinal section of papyrus pith; **c** white Pliny-type papyrus sheet produced in Tulln, Austria; **d** a modern, yellow, commercial papyrus sheet from

Qaramos, Egypt; **e** a 30-year-old naturally aged papyrus sheet from the Papyrus Museum in Vienna, Austria and **f** and **g** two brownish ancient papyrus samples (ca. 1200 years old) from the Papyrus Museum in Vienna

show some resemblance to antique papyri’ (Wallert 1989), probably referring to the ‘chestnut-like’ color of the best-preserved ancient papyri (Davy 1821).

This color difference of commercial papyri in contrast to the white pith of *Cyperus papyrus* L. interested us in the question, if the historic description by Pliny the Elder was exclusively followed in modern commercial papyrus manufacture, or if an additional step was added to improve or alter their properties. Modern Egyptian papyrus manufacturers have claimed that their products resemble ancient prototypes. They have asserted that the traditional manufacturing process was followed and that the resulting physicochemical properties are the same. If this is not the case, important consequences arise regarding the suitability of commercial papyri to be used as model samples to study the conservation characteristics, the material composition, and the aging behaviour of ancient papyri. Analytical data of ten ancient papyrus samples from the Papyrus Museum in Vienna were included in this study to determine whether deviations of Pliny’s account on papyrus production appeared already in antiquity.

Results

Modern commercial papyri manufactured in Egypt were compared to fresh papyrus pith and papyrus sheets produced in research laboratories in accordance with Pliny’s description. A combination of chemical analytical methods was used to determine the possible effects of a production step that has been forgotten or added since ancient times.

Papyrus pith is white when the stems are freshly cut (see Fig. 3a+b). This is in contrast to the optical impression of modern commercial papyri (see Fig. 3d). In accordance with the historical recipe, a further or auxiliary step (such as the use of bleaching agents) is not needed to produce a white sheet from papyrus stalks (Fig. 3c). Thus, the following questions are posed: How do modern commercial papyri obtain their yellow color? What is the relationship between their physical and chemical properties and the change in color?

The molar mass distribution of cellulose and hemicellulose detected using size exclusion–multi angle light scattering (SEC-MALS) shows mostly an overlay for commercial and Pliny-type papyri (i.e., sheets produced according to Pliny’s description). The alkali-treatment in the production of commercial

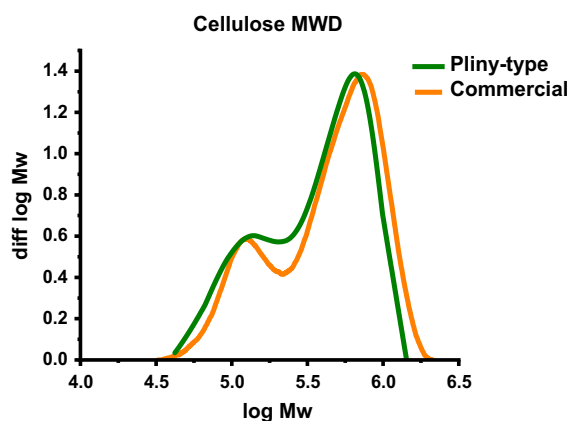


Fig. 4 Pliny-type and commercial papyri: Molecular weight distribution of cellulose and hemicellulose by SEC-MALS

papyri (see below) does not induce significant scissions of the cellulose chains (Fig. 4). A detailed account on the differences of cellulose and hemicelluloses in ancient and modern papyri will be given in an upcoming publication.

The surface pH of commercially produced sheets and Pliny-type papyrus sheets that were produced at different papyrus research and conservation laboratories were analysed, as was a sample of native Egyptian papyrus pith (from Qaramos). All the Pliny-type sheets and the native pith were slightly acidic (pH 5.5–6.0). The commercial papyrus sheets from Egypt were all alkaline (pH 7.9–9.8) and thus distinct from the native Egyptian pith and Pliny-type papyrus sheet samples (Fig. 5a). This pH gap coincides with the color difference between the white Pliny-type sheets and the yellow commercial samples. Clearly, the production of the commercial samples involved some alkalization steps that permanently kept the pH values higher than neutral, in contrast to the values for the Pliny-type sheets.

The research for this study did not reveal the inclusion of potash or natron in any of the ancient papyrus production recipes (more discussion in SI). The sodium content in modern commercial papyri, as measured by inductively coupled plasma optical emission spectroscopy (ICP-OES), was 15–21 g/kg, approximately three times greater than that in papyrus pith and the Pliny-type sheets directly produced from the pith (Fig. 5b). This result was supported by X-ray fluorescence (XRF) and scanning electron

microscopy with energy dispersive X-ray (SEM-EDXS) analysis of papyri (22 fragments from 13 manuscripts from the Tebtunis temple library, Umm el-Breigât, Egypt), which revealed only trace amounts of Na, K and Ca (Christiansen et al. 2017), which were attributed to the inorganic salts (chlorides and/or carbonates) in the cell walls of the original plant stalks. This evidence indicates the inclusion in the commercial manufacturing process of an alkalization step with sodium lye without subsequent thorough washing, as evidenced by both the increased sodium content and the increased surface pH, for which there is no basis in ancient production or Pliny's protocol. Nevertheless, it should be considered that an increased sodium content in ancient papyri can originate from contamination with salts at the excavation site or glass corrosion, if the ancient objects were mounted between glass slides (Nicholson et al. 2000; Graf 2016).

There could be two reasons for the strong alkaline treatment step in modern-day commercial papyrus production. First, it causes an immediate change in color from white to intense yellow. This is illustrated in Fig. 6a in the papyrus strips from Qaramos, Egypt. They were soaked in 4% sodium hydroxide (NaOH) solution for one hour and then dried. Notably, the color change, which occurred immediately upon contact with the alkaline solution, held despite subsequent washing.

Second, for fresh, never-dried pith, this NaOH treatment resulted in a smooth and soft surface. The

Fig. 5 Surface pH and sodium content of commercial and Pliny-type papyrus sheets (large differences between commercial and Pliny-type samples are displayed regarding their surface pH and Na-content) **a** comparison of surface pH for commercial and fresh papyrus sheets produced according to Pliny's description. **b** comparison of sodium content (g/kg) determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) of the papyrus specimen

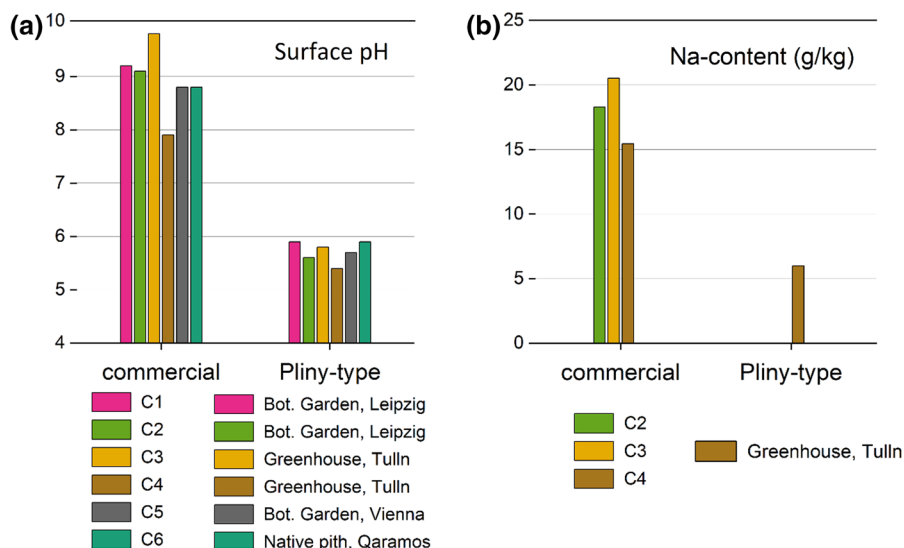




Fig. 6 Color change of papyrus pith after alkaline treatment and lignin staining **a** the effect of an alkaline treatment on dried papyrus pith from Qaramos, Egypt; left, untreated sample; middle, after soaking in 4% NaOH for 1 h; right, after soaking in 4% NaOH and washing until pH-neutral; the color

change stays permanent, independent of the later pH. **b** microscopic image of a *Cyperus papyrus* L. stem in cross section; lignin is stained red with Safranin-O, the cellulosic parts are colored blue/pink with Astra Blue

previously dried material felt like a sponge; however, it was smooth. Thus, a sodium lye treatment could have been incorporated into the papyrus production procedure for both haptic and coloration reasons. This shortcut for obtaining yellow papyri results in a product that might be more in harmony with the optical impression of ancient papyri as they appear today than with the white sheets obtained through Pliny's protocol.

Color changes in lignocellulosic material resulting from pH changes are not unusual. They are generally linked to the polyphenolic lignin constituents. The reversible part of the color change is due to a temporary increase in the electron density in the polyphenolic units because of the formation of phenolate anions. As is well known in lignin and bleaching chemistry, the irreversible part of the color change is linked to oxidative processes involving ambient oxygen and these reactive polyphenolates (Carter 1996; Rosenau et al. 2004; Sixta 2006). It must also be noted that cellulose and hemicellulose, although not contributing to the color changes, can be affected by the lye treatment. Oxidatively damaged celluloses, which bear carbonyl (=oxidized hydroxyl) groups along the polymeric glucan chains, suffer chain scission at the adjacent glycosidic bonds on the basis of a beta-alkoxy elimination mechanism already at a pH slightly above neutral. This has negative implications for long-term mechanical and brightness stability (Potthast et al. 2005; Hosoya et al. 2018; Ahn et al. 2019).

Table 1 Comparison of lignin content of different modern and ancient papyrus specimen

Sample	Lignin content (ABSL, %)	Calibrated age*
Commercial Papyrus	4.8 ± 0.3	2020 CE
Native Pith	16.2 ± 1.8	2020 CE
PM6	20.9 ± 4.0	900 CE
PM19	15.0 ± 1.0	900 CE
PM1	10.3 ± 2.0	800 CE
PM10	10.5 ± 0.5	800 CE
PM20	8.3 ± 1.3	800 CE
PM3	8.8 ± 2.8	700 CE
PM16	15.9 ± 6.5	700 CE
PM13	8.0 ± 0.7	500 CE
PM17	10.2 ± 1.4	500 CE
PM11	13.0 ± 2.5	200 BCE

Lignin contents of ancient Papyri compared to a commercial papyrus sample, papyrus pith and rind, using the ABSL method. *Age calibrated using the IntCal20 curve and the calibration program OxCal v4.4.1. For further information regarding age determination, uncertainties and sample numbers see Table S5 in the supplement

Analysis of the residual lignin of commercial papyrus sheets and comparison with the native lignin from the papyrus pith

The papyrus pith is a lignocellulosic material with approximately 16% lignin (Table 1), despite much higher values (e.g., 47%) incorrectly given in some studies as recently pointed out (Bausch et al. 2021). Lignin is located mainly in the sclerenchyma tissue of the stems (Fig. 6b). It strengthens the vascular

Table 2 Origin of commercial and Pliny-type papyrus sheets and pith used for pH and sodium content determination

Commercial samples	Obtained	Pliny-type	Origin
C1–Papyrus scroll	pgi-versand, Volkach	P1–sheet	Botanical garden + UB Leipzig
C2–Papyrus scroll	pgi-versand, Volkach	P2–sheet	Botanical garden + UB Leipzig
C3–Papyrus sheets	Ebay, online	P3–sheet	Greenhouse, Tulln
C4–Papyrus sheets	Gerstaecker, Eitorf	P4–sheet	Greenhouse, Tulln
C5–Papyrus sheets	Gerstaecker, Eitorf	P5–sheet	Botanical garden, Vienna
C6–Papyrus sheets	Local farmers, Qaramos	P6–Papyrus pith	Local farmers, Qaramos

bundles that constitute the main load-bearing element in papyri. The vascular bundles are observable to the naked eye as the parallel lining of the papyrus sheets (Fig. 3). These areas, which are the densest parts of the papyrus pith, sustain the transport of water and nutrients in the phloem and xylem (Table 2).

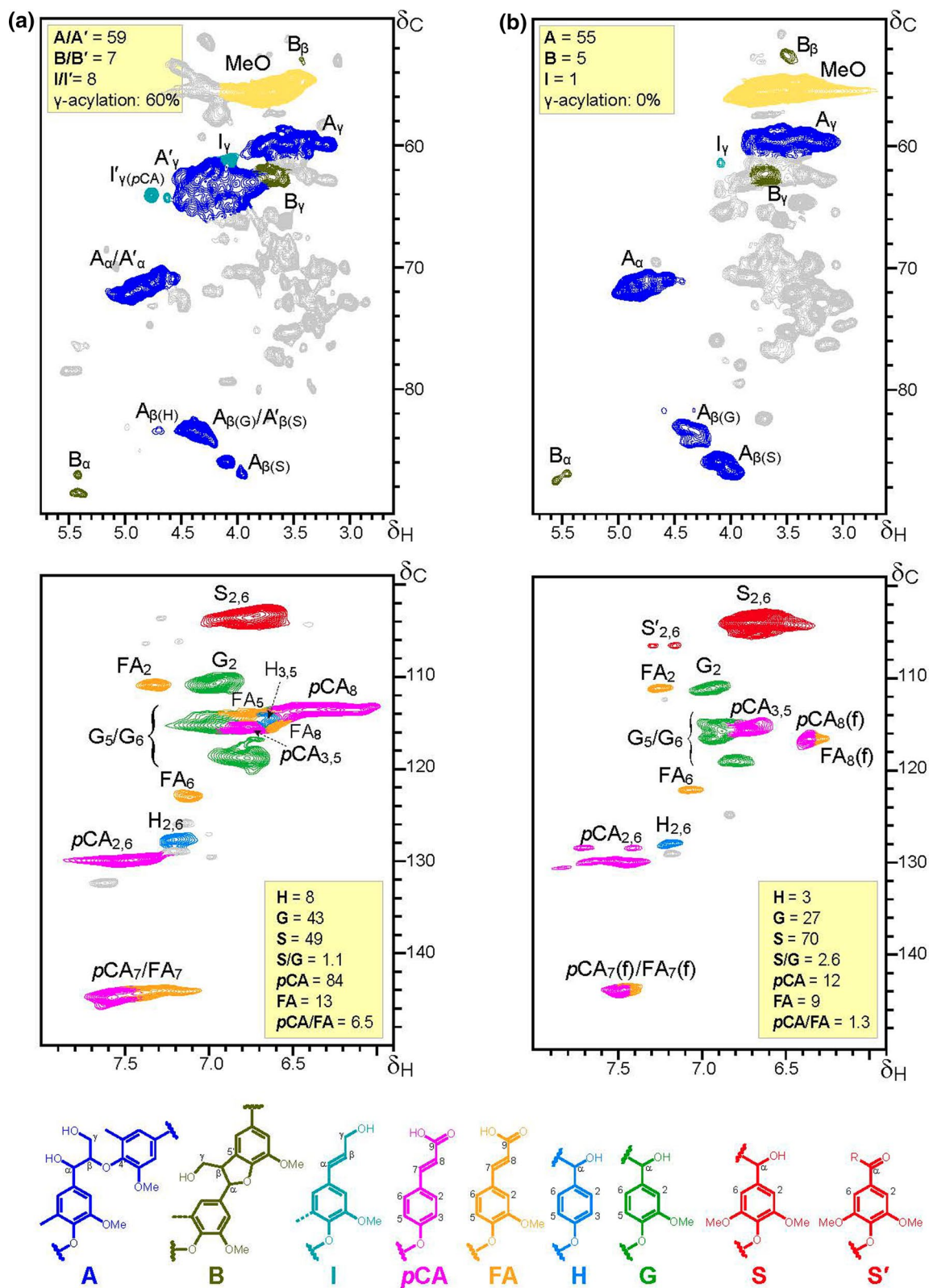
The composition and structure of the lignin from commercial papyri (sample C3) was compared to the lignin of native *Cyperus papyrus* L. pith, elucidated in two previous studies (Rosado et al. 2021; Rencoret et al. 2021) through a combination of two-dimensional NMR spectroscopy, pyrolysis–gas chromatography/mass spectrometry (Py-GC/MS), and the wet-chemical derivatization followed by reductive cleavage (DFRC) method. The relevant sections of the heteronuclear single quantum coherence (HSQC) NMR spectra of the residual lignin isolated from the commercial sheet and the lignin isolated from the native pith, together with the identified main lignin subunits, are shown in Fig. 7. The semiquantitative analysis of the aromatic lignin units, interunit linkages and end groups are illustrated in Fig. 7, based on the assignments given in Table S2 in the supplement.

The main cross-signals in the aromatic region of the HSQC spectra of the lignins that were isolated from the native pith and the commercial papyrus sheet corresponded to the lignin and *p*-hydroxycinnamic acid units. Signals from the *p*-hydroxycinnamyl (H), guaiacyl (G) and syringyl (S) lignin units were observed in the spectra of the isolated lignins. In addition, signals corresponding to the aromatic ring and the olefinic bonds of *p*-coumaric (*p*CA) and ferulic acid (FA) were also observed. The composition of the lignins, as determined by 2D NMR, is presented in Fig. 7 and Table S3 in the supplement. The lignin from the papyrus sheet exhibited a slightly higher S-lignin content (H:G:S 3:27:30; S/G ratio 2.6) than that (H:G:S of 8:43:49; S/G ratio 1.1) of the native

pith. The FA content in both types of lignin was similar (13% and 9% in the lignins from the native pith and the papyrus sheet, respectively, relative to total lignin units). However, the lignin from the native pith contained a significant amount (84%) of *p*CA, which was largely removed from the lignin in the commercial papyrus sheet (only 12% remained).

The NMR spectra also provided important information on the mode of incorporation of the *p*-hydroxycinnamic acids. The HSQC of the lignin from the native pith revealed signals for the lignin structures acylated at the γ -OH of the lignin side chain (A', B', I'). This indicated that this lignin was extensively (60%) acylated, most probably with the *p*-coumarates found in the aromatic region of the spectrum. This commonly occurs in the lignin of grasses and other monocotyledonous plants. Studies have found that *p*CA acylates the γ -OH of the lignin side-chain (Karlen et al. 2018); however, FA is esterified to the arabinose units of the hemicellulose arabinoxylans (Ralph 2010).

In contrast, the HSQC spectrum of the residual lignin from the commercial sheets did not show the presence of any signal from structures acylated at the γ -OH of the lignin side-chain. Furthermore, the detailed analysis of the chemical shifts of the C₇/H₇ and C₈/H₈ correlation signals of the *p*CA and FA indicated that the carboxylic moieties of the *p*-hydroxycinnamic acids in these lignins were not esterified to the lignocellulosic matrix; however, they were present as non-esterified groups. The C₇/H₇ correlation signal for the esterified *p*CA and FA units should appear at δ_C/δ_H 144.4/7.40. However, in the spectrum of the lignin preparation of the commercial sheet, these signals appeared at δ_C/δ_H 143.5/7.50, which corresponded to the *p*CA and FA units bearing a free carboxylic group. Likewise, the C₈/H₈ correlation signal for esterified *p*CA and FA should appear at δ_C/δ_H



◀**Fig. 7** Comparison of 2D-NMR results for lignin in native papyrus pith and commercial papyrus sheets. Side-chain (δ_C/δ_H 50–90/2.6–5.8) and aromatic (δ_C/δ_H 99–150/5.9–8.00) regions in the HSQC spectra of the lignins isolated from **a** the native papyrus pith, and **b** commercial papyrus sheet. The main lignin structures identified are shown at the bottom. Lignin composition represents the abundance of lignin units (H, G, S). The content of *p*-hydroxycinnamic acids (*p*CA and FA), lignin linkages (A/A', B/B'), and end-groups (I/I') are obtained from the integration of their respective signals and are referred to total lignin units (H+G+S=100). A', B', and I' are the γ -acylated A, B, and I structures, which are only present in the lignin from native pith, but not in the modern commercial samples

113.2/6.20. However, in the spectrum of the commercial sheet, this signal appeared at δ_C/δ_H 116.3/6.30, which corresponded to the *p*CA and FA units with a free COOH (Reinoso et al. 2018). Therefore, it is clear that the carboxylic groups of the *p*CA and FA units were not esterified to the lignocellulosic matrix but were present as free carboxylic acid / carboxylate groups. This indicates that the commercial papyrus sheet was treated under conditions that resulted in effective saponification (alkali-induced hydrolysis) of the *p*-hydroxycinnamate ester bonds.

The aliphatic-oxygenated region of the HSQC NMR spectra provided information on the interunit linkages in the lignins of commercial papyrus and the native pith. In this region, the correlation signals from the β -O-4' alkyl-aryl ethers (A), β -5' phenylcoumaran (B) and cinnamyl alcohol end groups (I) were clearly observed. In addition, the signals for the γ -acylated β -O-4' alkyl-aryl ethers (A') and γ -acylated cinnamyl alcohol end groups (I') were clearly present in the spectrum of the native pith lignin but not the spectrum of the residual lignin from the commercial papyrus sheets. This confirmed the complete hydrolysis of the ester bonds. The ratio of interunit linkages in both lignin types (per 100 lignin units) is given in Fig. 7. The most prominent interunit linkages in the residual lignin from papyrus were the β -O-4' alkyl-aryl ethers, which accounted for 55 linkages per 100 lignin units. This was similar to those found in the native pith (59 linkages per 100 lignin units). This indicated the presence of some intact polymeric lignin in the residual lignin of the papyrus sheet despite the delignification process that goes hand in hand with ester hydrolysis.

This hydrolysis of the *p*-hydroxycinnamate ester bonds observed in the 2D NMR spectra of the lignin

in the commercial papyrus sheets was confirmed by DFRC analysis and comparisons with the data from the lignin of the native papyrus pith from Qaramos, Egypt. The expected *p*-coumaroylation of the γ -OH of the lignin side chain was confirmed for the native papyrus (see Fig. 8a). However, it disappeared almost entirely in the lignin of the commercial papyrus sheet (see Fig. 8b). The data indicated that only 1% of the sinapyl (S) units in the lignin from the commercial papyrus sheet were still *p*-coumaroylated; however, 40% of those in the lignin from the native pith were. This was accompanied by a greater decrease in the *p*CA in the lignin of the native pith than in the commercial sample, as indicated by pyrolysis with tetramethylammonium hydroxide (TMAH), see Fig. 9.

Supported by the information on the increased pH value and sodium content of the commercial sheets, these results demonstrate that the modern-day commercial samples have undergone strong alkali treatments. On the one hand, this induces the yellow color and a certain degree of haptic smoothing. On the other hand, it chemically changes the lignin by the ester cleavage of the naturally contained coumarates and the dissolution of those coumarates and, possibly, other parts of the lignin (see below). In addition, as is known from lignin chemistry, free *p*CA is readily oxidized in alkaline media by dehydrogenative polymerization in air. This results in products exhibiting pronounced VIS absorption, which might contribute to the yellowish-brown discoloration induced by the NaOH treatment of the initially white pith material.

The composition of the residual lignin from the papyrus sheet was also analysed by Py-GC/MS and compared with that of the lignin from the native pith (Fig. 9 a + b). The identities and relative molar ratios of the released compounds are listed in Table S4 in the supplement. Free *p*-hydroxycinnamic acids (*p*CA and FA) cannot be analysed by Py-GC/MS because of decarboxylation reactions. Therefore, their presence in the residual lignin of the commercial sheets was addressed by pyrolysis in the presence of a methylating reagent, tetramethylammonium hydroxide (TMAH), which prevents decarboxylation (see Fig. 9 c + d) by in situ conversion to the methyl esters.

Particularly noteworthy was the presence of chlorinated lignin-derived phenolic compounds in the pyrogram of the residual lignin isolated from the commercial papyrus samples (see Fig. 9 and Table S4 in the supplement). This indicated that in

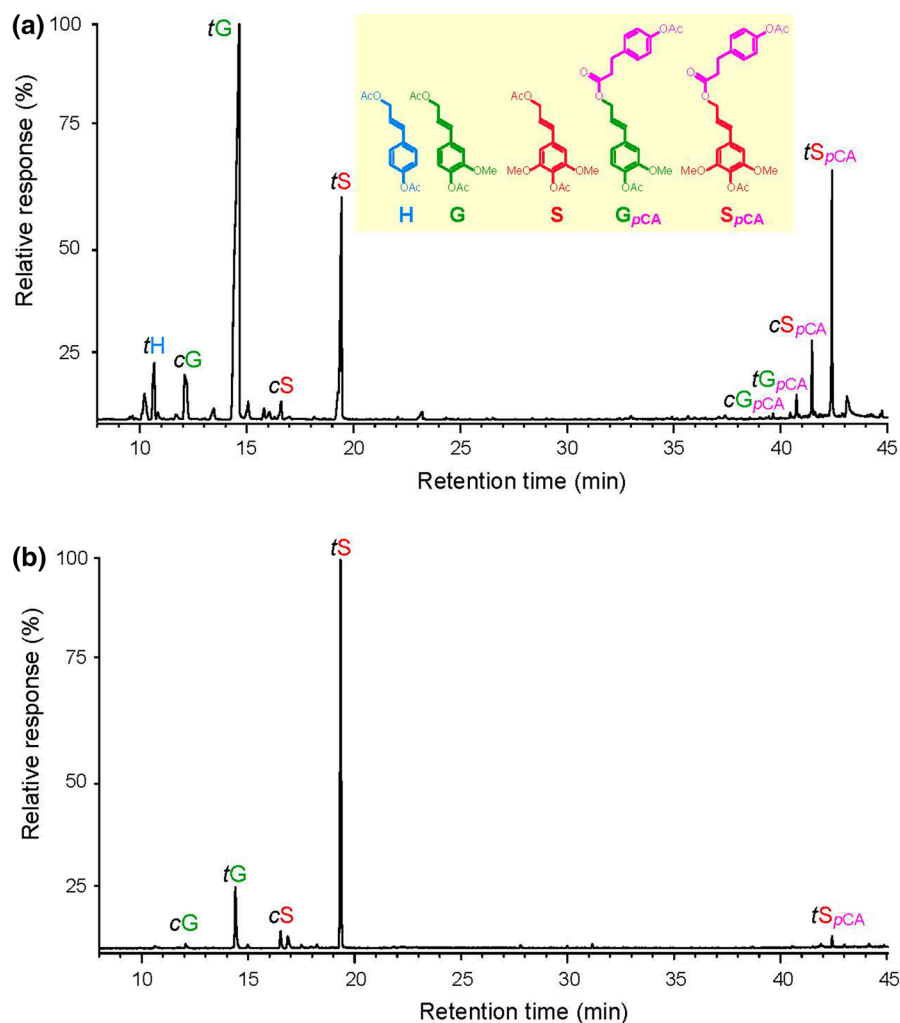


Fig. 8 Differences in acylation of papyrus pith and commercial papyrus sheets lignin analysed using DFRC. Reconstructed ion chromatograms (sum of the ions m/z 192 + 222 + 252 + 370 + 400) of the DFRC degradation products released from the lignins isolated from native papyrus pith (a), and modern commercial papyrus sheets (b). *tH*, *cG*, *tG*, *cS*

and *tS* are the *cis*- and *trans*-4-hydroxyphenyl (H), coniferyl (G) and sinapyl (S) alcohol monomers (as their acetate derivatives); *cGpCA*, *tGpCA*, *cSpCA* and *tSpCA* are the *cis*- and *trans*-coniferyl (G) and sinapyl (S)-dihydro-*p*-coumarates (as their acetate derivatives)

addition to being changed by the sodium hydroxide treatment, the lignin was chlorinated, most likely by chlorine-based bleaching reagents that were used. The Py-GC/MS results confirmed that this chlorination occurred at the aromatic ring as an electrophilic substitution, with the degradation products being chlorinated phenols throughout. Such chlorinated compounds were entirely absent in the lignin from the native papyrus pith and the papyri manufactured in accordance with Pliny. A previous Py-GC/MS

study of a series of ancient papyri also reported the absence of chlorinated compounds (Lucejko et al. 2020). The question of a possible parallel between ancient manufacturing and the NaOH treatment of commercial papyri still needs to be answered (see below). However, it is evident that the process of introducing chlorine into the lignin of modern-day papyri has no ancient equivalent. Chlorine-based bleach was introduced by Humphry Davy in 1811 (Davy 1811; Sixta 2006). Therefore, it is not part of

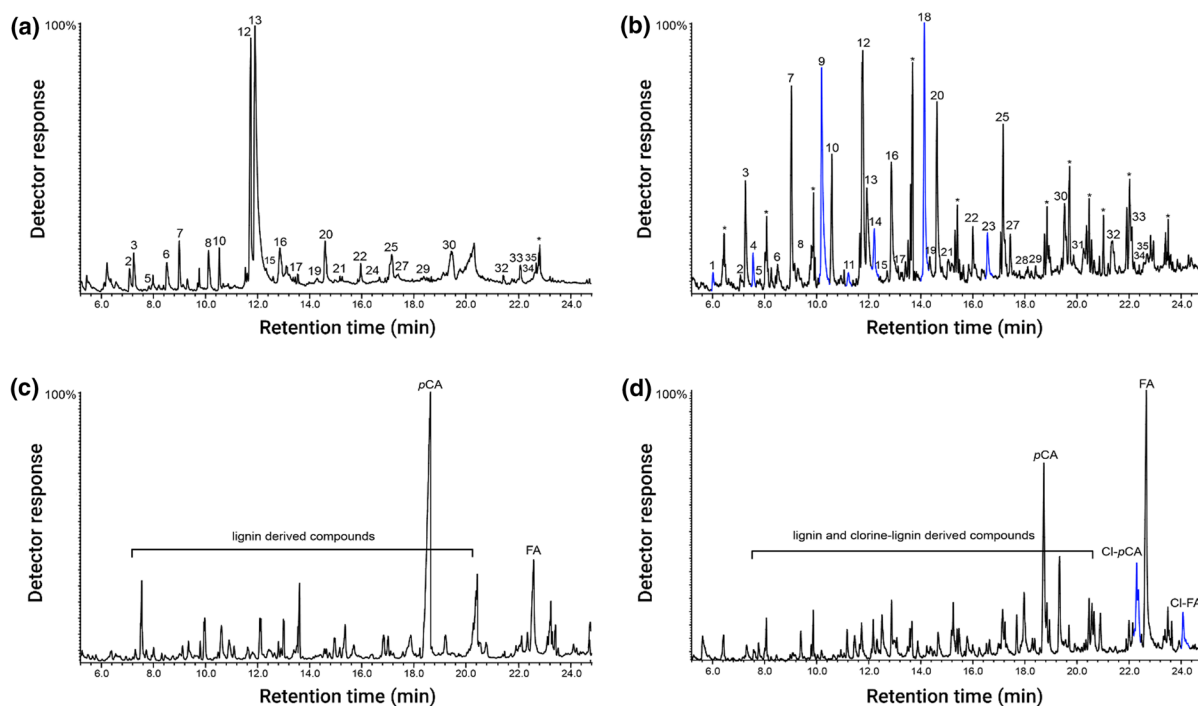


Fig. 9 Py-GC/MS and Py-TMAH-GC/MS chromatograms of papyrus pith and commercial papyrus sheets lignin. The presence of chlorinated compounds is shown for commercial sheets as opposed to the native sample, using Py-GC/MS. The different abundances of pCA and FA are analyzed using Py-TMAH-GC/MS. (Top) Py-GC/MS chromatograms of the lignins isolated from **a** the pith of papyrus stalks, and **b** modern commercial papyrus sheet. (Bottom) Py-TMAH-GC/MS chromatograms of the lignins isolated from **c** the pith of papyrus stalks,

and **d** modern commercial papyrus sheet. The identities and relative abundances of the compounds released upon Py-GC/MS are listed in Tab S4 (supporting information); peaks from chlorinated phenolic compounds are marked in blue color; pCA: *p*-coumaric acid; Cl-pCA: chlorinated *p*-coumaric acid; FA: ferulic acid; Cl-FA: chlorinated ferulic acid (note that the compounds released after Py-TMAH are the per-methylated derivatives). *contaminants

a papyrus protocol from Pharaonic times. It helps to brighten the yellowish-brown color caused by the NaOH treatment of papyrus. Thus, its use in this regard is similar to bleaching in the pulp and paper industry (Suess 2010).

In sum, the results revealed the presence of chlorinated lignin-derived compounds and decoumaroylated lignin in modern yellow-colored commercial papyrus sheets. The white counterparts from the native papyrus pith maintained the high acylation level expected for monocots; they did not contain any such halogenated compounds.

If ancient Egyptians used a strong alkaline natron treatment to adjust the papyrus properties, this should be evidenced by the significantly lower lignin content in ancient papyrus samples. The alkaline treatment cleaves the ester bonds in the lignin structure, as well as some lignin-carbohydrate linkages (Fengel and

Wegener 1984; Sixta 2006), and partly solubilizes the lignin, as was also observed in our studies of fresh papyrus pith. Such a treatment would also remove some hemicellulose. Generally, the processes are analogous to those that occur upon alkali treatments in pulping (Sixta 2006). Therefore, the lignin content of a selection of ancient papyri (200 BCE–800 CE) from the Papyrus Museum in Vienna was evaluated and compared to native papyrus pith and commercial papyrus sheets. The starting values were determined and discussed in detail in a previous publication (Bausch et al. 2021), which found the acetyl bromide soluble lignin assay (ABSL) to be the most suitable method for lignin quantification – due to its excellent agreement with Klason lignin determinations and a significantly lower sample demand (below 5 mg).

The ABSL lignin values were additional confirmation of the adverse effects of alkaline treatment

on papyrus sheets in terms of the lignin quantity and integrity. The lignin content was less than 5% in the commercial sheets and 16% in the native pith. The alkali treatment thus reduced the lignin to approximately one-third. These values can serve as orientation regarding the possible alkaline treatment of ancient papyrus samples. With similar treatment to achieve color, smoothness, haptic effects or other characteristics, the reduced lignin content should remain detectable, even with the superimposition of the effects of natural aging. For further analysis, 1,100–2,200-year-old original papyrus samples were used.

The commercial papyrus sheet contained only one-third of the amount of lignin found in the native pith. However, all the ancient papyri still contained twice or three times the lignin found in the commercial sheet. Some papyri, remarkably even the 2,200-year-old sample, exhibited only slightly less lignin than that in the fresh pith from the field. These results indicate the very good conservation status of lignin in ancient papyri. They also provide strong evidence against the theory of the deliberate use of strong alkaline treatments in ancient Egypt.

Discussion

After providing evidence from the perspective of material science, that contemporary commercial papyri are deliberately yellowed, the question remains why that was introduced. We suspect the root cause lies in the previously mentioned resemblance of ‘commercially produced papyrus sheets to antique papyri’ (Wallert et al. 1989), while the freshly produced Pliny-type papyri present themselves in a white tone, rather similar to rag paper. But which color was expected by the *ancient* observer? The reader may allow us to include some arguments from the fields of linguistics and art history, to shed some light on the question, whether a deliberate yellowing procedure might even have been desired in ancient Egypt.

Linguistic and historical features

Regarding color appearance, one sentence in Pliny’s description addresses ‘quality control’ in papyrus production. In addition to the dimensions of the sheets and scrolls, four parameters were evaluated:

‘*praeterea spectatur in chartis tenuitas, densitas, candor, levor*’ (*Naturalis Historia*, book XIII, chapter 21). The following is a usual translation: ‘The qualities esteemed in paper [i.e. papyrus] are fineness, firmness, brilliance, and smoothness’ (Lewis 1974). Another translation is the following: ‘In addition to the above particulars, paper is esteemed according to its fineness, its stoutness, its whiteness, and its smoothness’ (Riley and Bostock 1855). It appears that the precision of the translation needs to be sharpened by linking the original meaning to the perspectives of modern paper science.

Pliny objectively mentions the assessment of four quality parameters, but without in fact mentioning whether they were appreciated or held in esteem: *spectatur* = ‘it is looked at’ or ‘it is observed’. In the context of modern paper, *tenuitas*, which is more precisely translated as ‘thickness’ rather than ‘fineness’, must also be differentiated from *densitas*, with which it would otherwise overlap. In modern terms, *tenuitas* and *densitas* would refer to paper ‘thickness’ (or ‘caliper’, usually measured in μm) and ‘grammage’ (basis weight, grams per square metre [g/m^2]) on the basis of the International Organization for Standardization (ISO) (ISO 534 2011). Paper ‘density’, expressed in g/cm^3 , is then calculated by dividing the grammage by the caliper. The third quality parameter, *levor*, mentioned by Pliny refers to paper smoothness (surface roughness), which is also a standard parameter in the modern pulp and paper industry (Bekk method) (ISO 5627 1995; TAPPI T479 cm-09 2009).

In the context of color, *candor* is certainly the most relevant of Pliny’s four parameters. It is best translated as ‘whiteness’ and is clearly related to the color white rather than to a generally clear, bright, brilliant, luminous or shiny appearance (Latin *clarus*) or to the figurative conception of these properties (Latin *illustris*). *Candor* was associated with the candidates in ancient Rome when noble Romans exchanged their *togae purpurae* for chalked white togas upon becoming candidates for official, public functions (Merriam Webster 2021). The derivative *candidus* refers specifically to color, originally referring to the white-colored tokens that were used in the Roman senate to cast a ‘yes’ vote, in contrast to the black-colored objection coins. The word gained a wider meaning in Medieval Latin as being clean and free of transgression, in contrast to being dark and sinful. *Candor*, best described as the ‘whiteness parameter’, is also

one of the key qualities in modern paper production. Whiteness is expressed as a percentage of ISO brightness, which is measured against a BaSO_4 or TiO_2 standard (ISO 2470 2016) (the ISO-brightness values for samples shown in Fig. 3 are given in Table S1 in the SI). To the human eye, highly bleached modern papers with ISO brightness greater than 90% are perceived as pure white without a yellowish tint. The general brilliance of the colors in paintings, garments or plumage is referred to as *claritas*. *Candor* is associated only with whiteness. The use of this specific word is a clear linguistic indication that ancient papyrus was expected to be white. That the whiteness of papyrus after production was critically assessed as a quality parameter runs counter to the current notion that fresh papyrus for writing ought to have been yellow.

Pliny, one of the greatest scholars of his time, has left for us a technical account, ‘*praeterea spectatur in chartis tenuitas, densitas, candor, levor*’, of the main writing material of his time. However, there should be no hesitation about reflecting modern paper production principles in the translation of this passage: ‘Among other parameters, calliper, grammage, whiteness and surface roughness of the sheets are evaluated.’

Characteristics of ancient Egyptian art and writing

The ancient Egyptians seem to have perceived the color of papyrus paper as white. The paintings on tomb walls provide a good indication because they are realistic depictions. A mural in ancient Thebes provides a realistic illustration of a wheat harvest in the fourteenth century BCE (see Fig. 10a). Yellow and brown colors are used for cereal, human skin and the writers’ stillages; however, the papyrus rolls and linen cloth are depicted as bright white. This perception seems to have held for a very long time. A mural from Pompeii, created approximately one and a half millennia later, shows a papyrus *rotulus*, that is, a vertical papyrus roll, in at least as bright a color as the toga of the baker Terentius Neo (see Fig. 10b). Jaroslav Černý noted in 1947 that in Egyptian imagery, the papyrus held by writers or readers is always white (Černý 1952). Excavations in Giza have yielded such images with well-preserved colors (Junker 1944), which bleaching or aging effects cannot be assumed to have altered. It seems that the color white was deliberately used to reflect the actual properties of papyrus and linen.

Also the papyrus roll hieroglyph, the sign for the material in both sacred and secular writing, is predominantly white in colored representations (see Fig. 1c). Although the assertion of Ekschmitt (1964) is unequivocal, Černý (1952) noted some exceptions in which a yellow color was used. Comparisons of



Fig. 10 Ancient depictions of papyrus sheets and rolls. **a** taxation of the harvest (painting on plastering, Theban necropolis, Sheikh Abd el-Qurna, tomb of Menna, TT 69, southern part of the east wall, Theban necropolis, fourteenth century BCE (Tiradritti 2007); see also (Matterport 2021) for a virtual

tour; photo reprinted with kind permission from Sandro Vannini/Laboratoriorosso); **b** portrait of Terentius Neo and his wife, Pompeii, 20–30 CE, now at the National Archaeological Museum in Naples (Public domain 2014)

the murals in the tombs in the Valley of the Kings indicate that an overwhelming majority of the occurrences of the papyrus hieroglyph in colored representations are white (Smith 1946; Roveri 1989; Hawass and Vannini 2006). The color of the papyrus *symbol* in paintings, that is, the papyrus hieroglyph as such, might not be as conclusive as the color of the images of the material itself, but given that most figurative hieroglyphs attempted to closely mimic the natural color of the object they represent, the white papyrus hieroglyphs can at least serve as an indication.

To summarize, ancient Egyptian depictions of papyrus sheets and colored papyrus hieroglyphs are mainly white. Yellow is seen only in very rare cases. In Roman times, Pliny the Elder even explicitly mentioned whiteness as a criterion for the quality of papyrus. The native pith of the plant is bright white. Thus, if a modern papyrus is designed to possess the same ‘physical and chemical characteristics’ as an ancient Egyptian papyrus, it seems more accurate to produce a white sheet at first, that is allowed to yellow in the course of time to slowly obtain the resemblance of ancient papyri, as they appear today.

Conclusion and outlook

The obtained results present clear differences between commercial papyri and papyri produced according to Pliny’s historic description. Commercial papyri experience a strong alkaline treatment followed by subsequent chlorine bleaching, possibly to induce a yellow color and smoother feel. While cellulose and hemicelluloses remain largely untouched by this treatment as shown by SEC-MALS results, significant differences arise regarding the contained lignin as confirmed by a detailed analysis using Py–GC/MS, the DFRC method and 2D-NMR. In addition, other parameters, such as the increased pH and sodium content of the commercial sheets, confirm these results. A structurally important consequence was the alkaline hydrolysis of *p*-coumarates in the papyrus lignin and similar ester linkages in the lignin–carbohydrate complexes. This renders the lignin components more soluble and significantly decreases the lignin content in the alkali-treated commercial specimens, despite the remaining lignin coloring the resulting sheets bright yellow through instant formation and oxidation of

phenolates – a color change effect taking decades under natural ageing conditions. The absence of such a lignin loss in ancient papyri allows for the safe conclusion that this type of alkali treatment was not a part of the ancient production process. This indicates congruence with Pliny’s report, which does not mention such a procedure. Despite ample opportunity for lignin degradation during their long lifetimes, the ancient papyri exhibited lignin content values comparable to those of untreated fresh papyrus pith. The systematic treatment of ancient papyri with strong alkaline reagents upon their manufacture can therefore be rejected. The contemporary practice of lye treatment not only deviates from the ancient protocols, but it also causes significant changes, especially in the lignin structure and content. The practice of additional chlorine-based bleach treatments to brighten the initially obtained yellow sheets was confirmed by the presence of chlorinated phenols in the remaining lignin. It is certainly not in accordance with the protocols from ancient times when chlorine bleach was unknown.

Therefore, commercial papyri and Pliny-type papyri have to be regarded as two different materials, with distinct physicochemical properties, despite the opposite claims of modern papyrus traders. The assessment of this finding for the field of conservation science is open to the community. For replicas and facsimiles as the exhibition of the Papyrus Ebers at the University Library in Leipzig, Germany, commercial papyri will remain the logical substrate to print on, since only this material is available in sufficient quantities to produce a 20 m long papyrus roll – the botanical gardens in the area cannot produce enough papyrus pith as would be needed for such an enterprise. It lies beyond our knowledge, whether previous studies differentiated between commercial and Pliny-type papyri, or if they obtained custom-made papyri without previous alkaline treatment. The reported use of a bleaching agent can be helpful, nevertheless, it has to be kept in mind, that an alkaline treatment with subsequent bleaching alters the pH, the lignin structure and the general material composition drastically, rendering this material not representative of its ancient counterpart, especially in the case of studies regarding the conservation behaviour of papyri. Linguistic and art historic arguments indicate that white was the desired color of ancient papyri at the time of production, despite natural yellowing that

caused the chestnut-like color a modern spectator is accustomed to when looking at ancient papyri, rendering any deliberate yellowing treatment obsolete in antiquity. Perhaps Pliny, and possibly the rest of the ancient world, would have asked the same question as present-day chemists involved in pulp and paper production: Why would you *not* want your writing surface to be white?

Materials and methods

Papyrus samples

Papyrus (*Cyperus papyrus* L.) seeds were ordered from Chiltern Seeds, Wallingford, England and grown under controlled conditions in the BOKU greenhouse in Tulln an der Donau, Austria.

Commercial papyrus sheets were bought at pgiversand.de (Volkach, Germany), from Gerstaecker (Eitorf, Germany) and directly from the producers in Qaramos, Sharqia Governorate, Egypt. Fresh, untreated papyrus stem parts were purchased directly from the farmers in Qaramos, Egypt. Further fresh and untreated stem material was obtained from the botanical gardens in Leipzig, Germany and Vienna, Austria. For purchase and collection of the plant material international and national legislation and guidelines were followed properly.

A 30-year-old papyrus sheet, produced according to Pliny's description, was donated by the Papyrus Museum in Vienna, from which 20 unwritten papyrus fragments were also obtained. These samples are from the period of 300 BCE – 800 CE, as confirmed by ^{14}C analysis by VERA-Laboratorium Vienna, following the procedure described by Ramsey et al. (Bronk Ramsey et al. 2010). No further information on the provenience of the samples can be given, as they consist of unwritten fragments of various papyri in the Vienna collection that were separated from the written documents in the course of time and allowed to accumulate gradually, e.g., when the documents were re-mounted between panes of glass. Our laboratory-internal sample description PM1 – PM20 corresponds to the C-14 dating number VERA-7082–VERA-7101.

Papyrus sheet production

The procedure follows Pliny's protocol as discussed above. 10 papyrus stalks of 2–3 cm diameter (thickness) were cut into 10 cm and 20 cm long pieces, thereby determining the dimensions of the resulting papyrus sheet. Rind was removed first, then the pith was cut into slices of 2–4 mm thickness. The slices were submerged in distilled water for 10 min. Successively, the slices were aligned parallel to one another on an acid-free blotting board to form one layer of a sheet, on top of which a second layer of slices was assembled perpendicular to the first. The two layers of slices were then pressed in a mechanical hand press for two days, exchanging the blotting paper regularly to absorb the moisture. The resulting two-ply sheets were dried at room temperature. Starch-glue was prepared using 3 g of wheat flour (Type W700) in 10 ml of distilled water and heating it up to 50 °C. 0.1 ml of food grade acetic acid was added and the mixture heavily stirred, until a gel-like consistency was obtained. The glue was applied with a brush on the surface of one Pliny-type papyrus sheet, to check whether any important differences in optical properties could be observed, as a result of this sizing procedure. Typically, the papyrus sheets were white already without the addition of any other substance.

Sodium content measurement (ICP-OES)

The papyrus pith samples (150 mg) were chopped and dried for one day at 60 °C and milled. The milled samples were dried at 80 °C for 4 h and subsequently left to soak over-night in 3 ml nitric acid. For final digestion, hydrogen peroxide (0.76 ml) was added, and the reaction vessels were sealed tightly. Digestion of the bulk material was achieved in the microwave 'MARS 6 System' (CEM GmbH, Kamp-Lintfort, Germany) at 1000 W; ramp time: 25 min, hold time 20 min at 200 °C. After cooling, 40 ml of distilled water was added, and the mixture thoroughly shaken. The digestates were analysed for their main and trace element contents (Al, B, Ca, Fe, Mg, Mn, Na, P, S and Zn) by ICP-OES (Optima 8300, Perkin Elmer, Waltham, USA).

Surface pH

A drop of water was added onto the papyrus surface and the pH measured after several minutes on a Seven Easy pH-meter with a surface electrode (Mettler-Toledo AG, Schwerzenbach, Switzerland).

Microscopy

Microscopic images of *Cyperus papyrus* L. cross-sections (prepared using a razor blade) were obtained using the Olympus DSX 1000 digital microscope in incident light mode. The cellulosic parts were stained by Astra blue, the lignin parts using Safranin-O.

Chemicals

Sodium hydrogen carbonate (analytical grade), acetic acid (> 99%), sodium acetate trihydrate (> 99%), hydroxylamine hydrochloride (99%) and nitric acid (65%) were purchased from Sigma-Aldrich, Munich, Germany. Sodium carbonate (> 99%), sodium hydroxide (> 99%) and hydrogen peroxide (30%) were purchased from Carl Roth GmbH, Karlsruhe, Germany. Acetyl bromide (98 + %) was purchased from Alfa Aesar GmbH and Co KG, Karlsruhe, Germany.

TMAH (tetramethylammonium hydroxide) (~ 25 wt. % in MeOH), and dimethyl sulfoxide- d_6 (> 99.9%) were purchased from Sigma Aldrich, Munich, Germany. Acetone (> 99.5%), methanol (> 99.9%), and ammonium chloride (analysis grade, > 99.5%) were purchased from Panreac AppliChem (Barcelona, Spain). 1,4-dioxane ($\geq 99.9\%$), *n*-hexane ($\geq 99.9\%$), hydrochloric acid (approx. 36%), acetic acid ($\geq 99\%$), 1,2-dichloroethane ($\geq 99.8\%$), ethanol ($\geq 99.9\%$), diethyl ether ($\geq 99.5\%$), *n*-hexane ($\geq 99\%$), acetic anhydride ($\geq 99\%$), acetyl bromide (> 99.5%) and pyridine ($\geq 99\%$), were purchased from Merck KGaA (Germany).

Samples for lignin analyses

A sample of pith collected from Egyptian *Cyperus papyrus* L. plants and a modern commercial papyrus sheet were chosen for detailed analyses of their lignin components. The air-dried samples were successively extracted with acetone in a Soxhlet apparatus (12 h),

followed by methanol (16 h) and hot water (12 h), prior to the isolation of their lignin constituents.

Isolation of ‘milled-wood lignins’ (MWLs) from the pith of papyrus plant

The ‘Milled-Wood Lignin’ (MWL) preparations were isolated from the pith of the stems of fresh papyrus plants using the standard procedure (Björkman 1956). Briefly, around 70 g of previously extracted samples were finely milled using a Retsch PM100 planetary ball mill (Retsch, Haan, Germany) for 5 h at 400 rpm using a 500 mL agate jar and agate ball bearings (20 × 20 mm). The milled samples were then extracted (3 × 12 h) with dioxane-water (90:10, v/v) (20 mL of solvent per gram of milled sample) and the isolated crude MWLs were subsequently purified as described elsewhere (del Río et al. 2012). The isolated MWLs yield was ~ 20% of the Klason lignin content of the original material.

Lignin isolation and purification of the commercial papyrus sheet

The isolation of the residual lignin from commercial papyrus sheet was performed by mild acidolysis according to a previously published procedure (Gellerstedt et al. 1994; Evtuguin et al. 2001), with some minor modifications. Approx. 100 g of ball-milled extractive-free papyrus sheets were refluxed for 40 min with 1000 mL of 0.1 M HCl in dioxane:water (82:18, v/v) under nitrogen. This extraction process was repeated four times, using fresh solution each time, the last one without adding 0.1 M HCl. After the extractions the papyrus sheet was filtered and washed with dioxane:water (82:18, v/v). The filtrate was evaporated at 40 °C, and then the lignin was precipitated at 4 °C in 1.5 L of cold distilled water under stirring. The precipitated lignin was then centrifuged (25 min, 9000 rpm, 4 °C) and subsequently freeze-dried. The lignin was then submitted to Soxhlet extraction with *n*-hexane for 8 h to remove contaminants. The lignin yield represented around 65% of the Klason lignin content in the analysed papyrus sheet.

Analysis by pyrolysis gas chromatography-mass spectrometry (Py-GC/MS)

The pyrolysis of the lignins isolated from fresh papyrus pith and from commercial papyrus sheets (~0.1 mg) was carried out at 500°C (1 min) in a 3030 micro-furnace pyrolyzer (Frontier Laboratories Ltd., Fukushima, Japan) connected to a GC 7820A and a 5975 mass-selective detector with EI at 70 eV (Agilent Technologies, Inc., Santa Clara, CA). The column used was a 30 m×0.25 mm i.d., 0.25 µm film thickness, DB-1701 (J&W Scientific, Folsom, CA). The oven temperature was programmed from 50 to 100 °C at 20 °C/min and then ramped to 280 °C at a heating rate of 6°C/min and held for 5 min. The carrier gas was helium at 1 mL/min. For the pyrolysis in the presence of tetramethylammonium hydroxide (Py/TMAH), ~0.1 mg of lignin was mixed with 0.5 µL of TMAH (25%, w/w, in methanol), and the pyrolysis was performed under the same conditions as described above. The released phenolic compounds were identified by comparison of their mass spectra with those from our own collection of standards and with those reported in the literature (Ralph and Hatfield 1991). The data from two repetitive analyses were averaged and expressed as percentages.

Analysis by 2D-NMR spectroscopy

Approx. 60 mg of lignin (isolated from fresh papyrus pith or from commercial papyrus sheets) were dissolved in 0.60 mL of DMSO- d_6 . HSQC (heteronuclear single quantum coherence) NMR spectra were recorded at 300 K on a Bruker AVANCE III 500 MHz instrument equipped with a cryogenically-cooled 5 mm TCI gradient probe with inverse geometry, at the NMR facilities of the General Research Services of the University of Seville (SGI-CITIUS). The HSQC experiments were carried out using the Bruker standard pulse program “hsqcetgpsisp2.2” and the following parameters: spectra were acquired from 10 to 0 ppm in F2 (^1H) using 1000 data points for an acquisition time of 100 ms, an interscan delay of 1 s, and from 200 to 0 ppm in F1 (^{13}C) using 256 increments of 32 scan, for a total experiment time of 2 h 34 min. The $^1J_{\text{CH}}$ used was 145 Hz. Processing used typical matched Gaussian apodization in ^1H and a squared cosine bell in ^{13}C . The solvent peak centre was used as an internal reference ($\delta_{\text{C}}/\delta_{\text{H}}$ 39.5/2.49).

2D-NMR cross-signals were assigned by literature comparison (del Río et al. 2012, 2015). A semiquantitative analysis of the volume integrals of the HSQC correlation peaks was performed using Bruker’s Topspin 3.5 processing software. In the aromatic/unsaturated region, the correlation signals of $\text{H}_{2,6}$, G_2 and $\text{S}_{2,6}$ were used to estimate the content of the respective H-, G- and S-lignin units, the signals for $p\text{CA}_{2,6}$ and FA_2 were used to estimate the different *p*-hydroxycinnamates (as signals $\text{H}_{2,6}$, $\text{S}_{2,6}$ and $p\text{CA}_{2,6}$ involve two proton-carbon pairs, their volume integrals were halved). The relative contents of FA and *p*CA were referred to as a percentage of the total lignin units ($\text{H} + \text{S} + \text{G} = 100\%$). The $\text{C}_\alpha/\text{H}_\alpha$ correlation signals of the $\beta\text{-O-4'}$ alkyl aryl ethers (A_α) and phenylcoumarans (B_α) in the aliphatic-oxygenated region of the spectra were used to estimate their relative abundances (per 100 aromatic units), whereas the $\text{C}_\gamma/\text{H}_\gamma$ correlation signal was used for the cinnamyl alcohol end-units (I_γ) analogously (as I_γ involves two proton-carbon pairs, its volume integrals were also halved).

Derivatization followed by reductive cleavage (DFRC)

DFRC degradation was performed according to the original protocol (Lu and Ralph 1997), of which the detailed explanation can be found elsewhere (del Río et al. 2012). Briefly, around 10 mg of lignin isolated from fresh papyrus pith and from commercial papyrus sheet were treated with acetyl bromide in acetic acid (8:92, v/v) at 50°C for 2 h, and then with 50 mg of powdered zinc for 40 min at room temperature. The lignin degradation products were acetylated with an acetic anhydride/pyridine solution (1:1, v/v) and dissolved in dichloromethane for subsequent analysis by GC–MS that was performed in a GCMS-QP2010plus instrument (Shimadzu Co., Kyoto, Japan) using a capillary column (DB-5MS 30 m×0.25 mm I.D., 0.25 µm film thickness). The oven was heated from 140°C (1 min) to 250°C at 3 °C/min, then ramped at 10°C/min to 280°C (1 min) and finally ramped at 20°C/min to 300°C, and held for 18 min at the final temperature. The injector was set at 250°C and the transfer line was kept at 310°C. The carrier gas was Helium at a rate of 1 mL min^{−1}. Characteristic ions for the *cis*- and *trans*-*p*-hydroxyphenyl (m/z 192), coniferyl (m/z 222) and sinapyl (m/z 252) alcohol monomers (as their acetate derivatives), as well as

for the *cis*- and *trans*-coniferyl dihydro-*p*-coumarates (m/z 374), and the *cis*- and *trans*-sinapyl dihydro-*p*-coumarates (m/z 400) (as their acetate derivatives), were collected to produce a reconstructed ion chromatogram.

Lignin content determination by acetyl bromide derivatization and UV-detection

Commercial papyrus sheets, native papyrus pith and rind from Egypt were milled using the Ultra Centrifugal Mill ZM 200 by Retsch, Haan, Germany. Extractives were removed by accelerated solvent extraction (ASE) using an ASE 350 (Dionex, Sunnyvale, USA), based on a literature protocol (Wilför et al. 2006). Conditions were 60 °C, 11 MPa, 15 min static time, 36 ml per cycle. The samples were extracted by two cycles of hexane and 4 to 6 subsequent cycles of acetone/water (95:5) until the filtrate was colorless.

Ancient papyrus samples were gently disintegrated by tweezers and washed with distilled water, shaken overnight in distilled water, and the procedure was repeated using acetone, to remove contaminations on the sample surface.

All samples were dried for several days in a vacuum dryer (Goldbrunn 1450, Berlin, Germany) at 40 °C. The samples (2 – 5 mg in triplicates) were dissolved in a mixture of 25% (v/v) acetyl bromide in glacial acetic acid (0.2 mL), heated to 50 °C and stirred for 2 h, based on (Fukushima and Hatfield 2001). The dissolved samples were added to a mixture of aqueous NaOH (1 ml, 2 M) and hydroxylamine hydrochloride (0.175 ml, 0.5 M) and the volume was adjusted to 10 ml by glacial acetic acid. Absorbance was measured at 280 nm using a PerkinElmer, Waltham, USA Lambda 35 ultraviolet / visible light (UV/Vis) spectrometer. An extinction coefficient of 20 L/g*cm was applied (Karlen et al. 2018) to calculate the acetyl bromide-soluble lignin.

Size exclusion chromatography – multi angle light scattering (SEC-MALS)

30 mg of papyrus samples were disintegrated for 20 s (3–4 times) in distilled water and left to swell in water overnight, while shaken. The samples were washed subsequently in acetone and N,N-dimethylacetamide (DMAc). After that, the samples were resuspended

in 3 ml DMAc. The liquid was filtered off, the solid parts of the sample were transferred to a dry vial and dissolved in 2 ml LiCl/DMAc (9% m/v) for several days. After dissolution, the samples were diluted in DMAc (0.3 ml sample in 0.9 ml DMAc) and filtrated to prepare for SEC-MALS.

The size exclusion chromatography (SEC) system is coupled to multi-angle laser light scattering (MALS) detector, refractive index (RI) and fluorescence detectors with automatic injection and four columns in series. DMAc/LiCl served as eluant in a concentration of 0.9% m/v. The molecular weight distributions and the related parameters were calculated using the corresponding software programs, based on a refractive index increment of 0.140 ml/g for cellulose in DMAc/LiCl (0.9%, m/v). The following SEC-parameters were used: flow: 1.00 mL/min; columns: four PL gel, mixedA, ALS, 20 µm, 7.5×300 mm plus precolumn.

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Data availability Data underlying the current study are available from the corresponding author upon request.

Code availability Not applicable.

Declarations

Conflict of interest All authors have declare that they have no conflict of interest.

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