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ORIGINAL ARTICLE

Evaluation of chemical treatments on dimensional stabilization of archeological waterlogged hardwoods obtained from the Thang Long Imperial Citadel site, Vietnam

Thanh Duc Nguyen[1] · Yohsei Kohdzuma[2] · Rie Endo[3] · Junji Sugiyama[1,4]

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Abstract
In this article, the conservation of seven archeological waterlogged woods (WW) with polyethylene glycol (PEG) 4000, trehalose, and feather keratin was investigated. The results showed that the dimensional stability of WWs significantly improved after the different treatments. The anti-shrink efficiency values of the WWs treated with keratin ranged between 72.5 and 96.2% depending on the species and degree of wood degradation. These values varied from 82.4 to 96.9% for the WWs treated with PEG or trehalose. Microscopic observations showed that the chemically-treated woods maintained their original cell structures, forms, and shapes. It was also revealed that the reinforcement of cell walls by the feather keratin treatment was different from those observed for the PEG or trehalose treatments. It was observed that PEG and trehalose primarily filled the wood voids, while keratin predominantly absorbed on the cell walls and middle lamellae. Based on the improved dimensional stability of wood, shortened impregnation time, removability of chemical, and esthetic results obtained from the treatment, keratin showed a good performance in average as a preservation agent.

Keywords Archeological waterlogged wood · Dimensional stability · Feather keratin · Polyethyleneglycol · Trehalose

Introduction

Archeological waterlogged wood (WW) is often subject to extreme levels of shrinkage and drastic changes in shape upon drying due to its high degree of wood tissue deterioration [1, 2]. The wood structure resembles a sponge, absorbing a large amount of water. The waterlogged wooden objects will retain their shape as long as they remain wet [3–5]. If such a wood is exposed to air, the weakened cell walls are unable to withstand the stresses imposed by the surface tension of receding columns of liquid water, leading to a collapse of the wood structure [6]. Therefore, the first step in WW conservation involves the use of an appropriate consolidation agent to replace the water that fills the WW structure. The conservation treatment will protect wooden objects against shrinkage, collapse, and loss of shape upon drying [3, 4, 6]. Water contained in the WW structure can be replaced only in the lumen of cells, but it is usually necessary to also replace the water in the wood cell walls [1]. The dimensional stabilization of WW depends on the amount of impregnation agent in the treated wood, in particular its penetration in the wood cell walls [7].

Currently, the most widely used consolidation method involves polyethylene glycol (PEG), which is freely soluble in alcohols (ethanol, methanol, and isopropanol) as well as water. Low-molecular weight PEG is appropriate for the conservation of better preserved WW, while high-molecular weight PEG is more suitable for highly decayed wooden objects [3, 8]. The high-molecular weight PEG (starting from 2000 g/mol) can penetrate wood tissues, replace water molecules, and reinforce its structure, thus improving its dimensional stability [9]. PEG-based treatments are commonly used because of the low cost of materials and equipment, and the satisfactory results obtained. Unfortunately,

✉ Junji Sugiyama
sugiyama@rish.kyoto-u.ac.jp

1 Research Institute for Sustainable Humanosphere, Kyoto University, Gokasho, Uji, Kyoto 611-0011, Japan

2 Nara National Research Institute for Cultural Properties, 2-9-1 Niyo-cho, Nara City 630-8577, Japan

3 Toyo Feather Industry Co. Ltd., 2-26-5, Fuchinobe, Chuo Ward, Sagamihara, Kanagawa 252-0206, Japan

4 College of Materials Science and Engineering, Nanjing Forestry University, Nanjing 210037, China

PEG is not a perfect agent for wood conservation due to its disadvantages. The chemical reaction of PEG with other substances can cause further wood degradation. It has been shown that the reactions of PEG with metal and/or the sulfur compounds present in WW (e.g., ships) produce various low-molecular weight organic acids (formic, glycolic, oxalic), causing further wood degradation [10]. Moreover, The PEG impregnation is time consuming; for large objects, the process needs decades [4, 6]. Because of the many adverse effects of PEG, conservators are now exploring additional treatment options to conserve WW. Among them, the use of sugars as a conservation material had been investigated [8, 11–14]. Sugars are organic compounds composed of combinations of saccharides organized in a ring structure. Sugars have many advantages such as being non-toxic, non-corrosive. The conservation of WW with sucrose, mannitol, and recently lactitol and trehalose has been investigated [8, 11, 13, 14]: their impregnation could be accomplished with or without heating because of the small molecular sizes of sucrose, lactitol, and trehalose. The wood thus conserved has a natural color and can be cleaned and glued easily. Sugars can also be easily extracted from treated wood using water, so the conservation treatment can be reversed if necessary [13]. The drawbacks using sugars are the microorganism development during impregnation, and also the cost for some type of sugars [15].

Interest in further seeking green materials (such as keratin [16–19]) for the consolidation of wood has been increasing in recent years [20, 21]. The dimensional stability of the WW treated with keratin was excellent [17, 18], except for the experiment on heavily degraded wood [22]. The treatment involving feather keratin enhanced the mechanical properties of WW [23]. The low-molecular weight of keratin resulted in its fast diffusion into the WW. The color of the keratin-treated wood was quite similar to that of normal wood [23]. Additionally, feather keratin-treated wood had antimicrobial activity. However, there are few reports on the conservation of tropical WWs using keratin.

The purpose of this research was to evaluate the influence of PEG, trehalose, and keratin treatments on the dimensional stability of archeological WW. Finding an effective consolidation agent for WW conservation will facilitate the preservation of such historic resources for future generations.

Materials and methods

Materials

Archeological WW samples were collected from the Thang Long Imperial Citadel excavation site in Hanoi, Vietnam, which is a member of the UNESCO World Heritage list. Based on microscopic observations, the samples were identified as *Albizia* sp., *Afzelia* sp., *Bouea* sp., *Dysoxylum* sp., *Elaeocarpus* sp., *Garcinia* sp., and *Syzygium* sp.. The samples were cut into 2 × 2 × 1 cm (tangential × radial × longitudinal dimensions). Five specimens were used for each treatment.

PEG 4000 (Mw: 3350) and magnesium sulfate heptahydrate ($Mg_2SO_4{\cdot}7H_2O$, Mw: 246.47) were purchased from Wako Pure Chemical Industries, Japan. Trehalose (TREHA®, Mw: 342) was obtained from Hayashibara Co. Ltd., Japan, and feather keratin powder (KERATIDE®, TK-B, Mw: approximately 750) was purchased from Toyo Feather Industry Co. Ltd., Japan. This keratin powder is produced from waterfowl feather via the alkaline hydrolysis dissolution process [24]. All the chemicals were used without further purification.

Methods

Physical properties

Physical parameters such as density and moisture content can be measured to evaluate the state of degradation of WWs [25]. The maximum moisture content (U_{max}) and basic density (BD) were calculated according to the following formulae:

$$U_{max} = \frac{m_w - m_d}{m_d} \times 100 \quad (1)$$

where U_{max}, maximum moisture content (%), m_w, mass of WW saturated with water (g), and m_d, mass of oven-dry sample (g).

$$BD = m_d / V_w, \quad (2)$$

where BD, basic density (g/cm^3), m_d, mass of oven-dry sample (g), and V_w, volume of WW saturated with water (cm^3).

Chemical treatment

The samples were immersed in distilled water under reduced pressure (0.1 MPa) for 40 min and the weight of each specimen was measured. The keratin treatment can reinforce the waterlogged softwoods as reported in the previous paper [18], while the effect was not enough to stabilize waterlogged hardwoods and other fragile materials [22]. The treatment was modified with adding a divalent metal salt process to integrate the keratin [26]. First, the specimens were immersed in a 50% (w/w) aqueous keratin solution at 60 °C for 2 weeks. Then, the WW specimens were removed from the keratin solution and immersed in 4 M magnesium sulfate aqueous solution at 60 °C for 2 weeks. The samples were subsequently removed from the solution and the surplus solution was removed manually from surface of the

samples. Then, the treated samples were dried in ambient temperature.

For the PEG and trehalose impregnations, the blocks were immersed in 20% (w/w) solutions at 60 °C, and the concentration of the PEG or trehalose solution was increased every week in steps of 10%. Finally, the samples were treated with a 70% solution and the surplus solution was removed manually from surface of the samples. The treated samples were dried in air at room temperature for a week.

After drying, all the specimens were seasoned to a constant weight in air at a relative humidity (RH) of about 60% and temperature 20 °C. The equilibrated samples were imaged and the shrinkage or swelling rates were calculated from the sample images obtained using the Image-J software (NIH, USA).

Dimensional stability tests

To evaluate the efficiency of chemical treatment, the dimensional stability of WWs was determined by measuring the shrinkage or swelling rates, anti-shrink efficiency, and increase in the weight of wood after treatment.

The anti-shrink efficiency (ASE) denotes the percentage of shrinkage or swelling of the untreated wood that has been suppressed by a stabilizing treatment. It was calculated from the sample images obtained using the Image-J software according to Eq. (3):

$$\mathrm{ASE} = \frac{\beta_0 - \beta_1}{\beta_0} \times 100, \tag{3}$$

where ASE, anti-shrink efficiency (%), β_0, linear shrinkage or swelling of the untreated wood (%), and β_1, linear shrinkage or swelling of treated wood (%).

Linear wood shrinkage or swelling in the tangential, radial, and longitudinal directions was calculated using Eq. (4):

$$\beta = \frac{l_0 - l_1}{l_0} \times 100, \tag{4}$$

where β, linear wood shrinkage or swelling (%); l_0, initial sample dimension (at the maximum moisture content; waterlogged) (mm); l_1, final sample dimension (after drying and seasoning) (mm).

The weight percent gained (R) was calculated on the basis of an increase in the mass of the treated wood sample relative to the mass of an identical untreated sample according to Eqs. (5) and (6):

$$R = \frac{m_\mathrm{t}}{m_\mathrm{em}} \times 100 = \left(\frac{m_\mathrm{em+i} - m_\mathrm{em}}{m_\mathrm{em}}\right) \times 100, \tag{5}$$

$$m_\mathrm{em} = \frac{m_\mathrm{w}}{U_\mathrm{max} + 1}, \tag{6}$$

where R, weight percent gained; m_t, mass of a treatment medium; m_em, estimated mass of an oven-dried untreated sample; $m_\mathrm{em+i}$, mass of an oven-dried treated sample; m_w, mass of a saturated waterlogged control sample, and U_max, the maximum moisture content of the control sample.

Scanning electron microscopy (SEM)

The untreated and chemically treated specimens were prepared from internal part of samples. They were freeze-dried for 4 days and then coated with platinum using an auto fine coater (JFC-1600, JEOL, Japan) operated at 30 mA for 90 s. A field-emission scanning electron microscope (JSM-7800F prime, JEOL, Japan) was operated at an accelerating voltage of 2 kV.

Results and discussion

Figure 1 presents the properties of all tested WWs. All the samples corresponding to the different species showed the same behavior. A higher water content is associated with the lower basic density of WW. The maximum moisture content in all WWs ranged between 315 and 612%, while the basic density of wood was between 0.14 and 0.25 g/cm^3. The degradation of waterlogged archeological wood results in the loss of wood substances such as the polysaccharides cellulose and hemicellulose. Such losses are immediately compensated by the filling of woods with water. Therefore, the increase of maximum wood moisture content (U_max) and decrease of basic density together indicate increasing wood degradation [3, 4]. Among the samples being researched, two classes of wood degradation can be distinguished, according to U_max limit values of 185 and 400% [4]. The *Elaeocarpus* sp. and *Albizia* sp. samples evaluated according

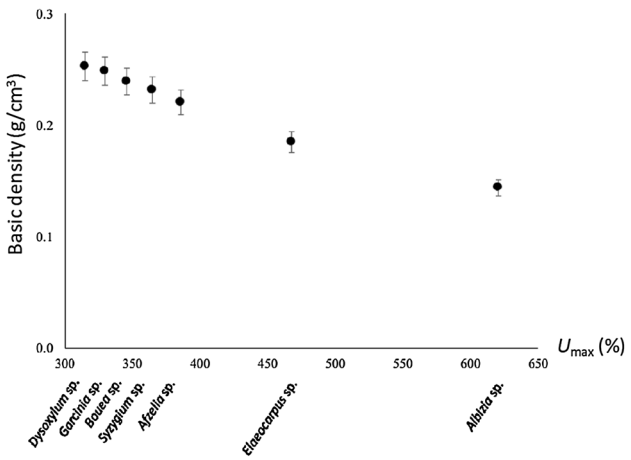

Fig. 1 Relationship between basic density and the maximum moisture content. The bars represent the standard deviations

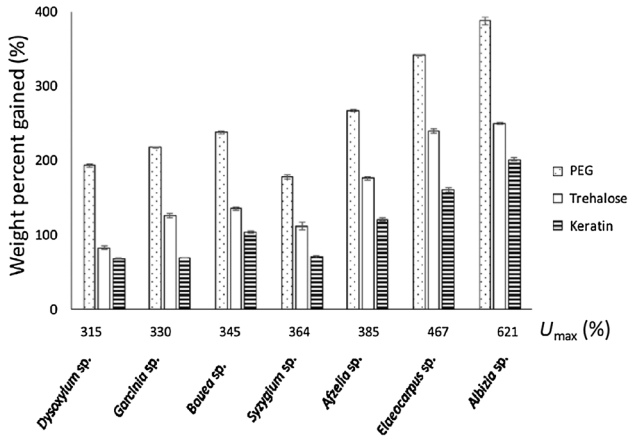

Fig. 2 The weight percent gained after chemical treatments for the different specimens. The bars represent the standard deviations

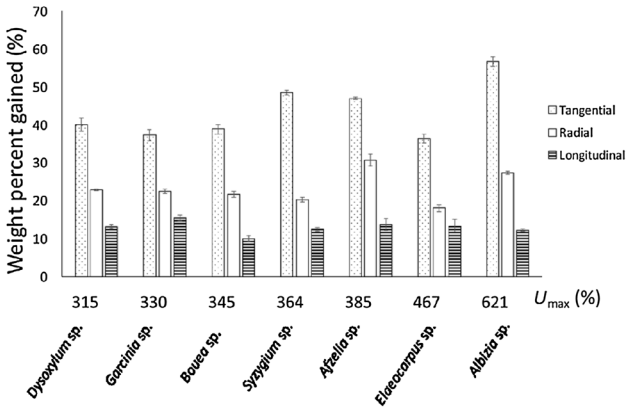

Fig. 3 Shrinkage ratios of the untreated waterlogged woods. The bars represent the standard deviations

to the above criterion can be classified as highly deteriorated (U_{max} from 467 to 621%). The remaining samples were evaluated based on the same criterion, and could be classified as medium-degraded (U_{max} from 315 to 385%).

The uptake of chemical agents after the consolidation treatment is shown in Fig. 2. In general, the amount of chemical uptake increased with increasing U_{max}, except for *Syzygium* sp.. The impregnations of keratin and trehalose into WWs were smaller than that of PEG. The weight percent gained after the keratin and trehalose treatments was about twice lower compared to that after the PEG treatment. The weight of PEG-treated WW becomes significantly different in the case of conservation of large wooden objects such as shipwrecks, which may require special support during exhibition.

Chemically-degraded WWs showed the greatest changes in dimensions due to wood tissue degradation. The measurement of the shrinkage rate of untreated WWs showed extremely high values in all three directions (Fig. 3). Depending on the wood species and its extent of degradation, tangential shrinkage varied between 36.3 and 56.7%, while the radial variation was between 18.1 and 30.6%. The longitudinal shrinkage was between 10 and 15.6%. Among the species evaluated, *Elaeocarpus* sp. was less shrinkage despite the high deterioration. This is due to the anisotropic structure of this species that affects the shrinkage value. The previous report showed little shrinkage values were determined in sound *Elaeocarpus* sp [27].

However, the dimensional stability of WWs was significantly improved after chemical treatment (Fig. 4). The linear shrinkage value for the tangential direction ranged between 1.1 and 13%, while the variation in the radial direction was between 0.7 and 6.4%. The shrinkage in the longitudinal direction was less than 2%. In particular, the three-dimensional characteristic of PEG-stabilized WWs was well preserved. After being treated with PEG and subjected to air drying, the shrinkage value in the tangential direction ranged between 1.3 and 6.2%, while it varied between 0.7 and 3.1% in the radial direction. The smallest shrinkage value (< 1.9%) was measured in the longitudinal direction. Similarly, good dimensional stability was achieved in the WWs treated with trehalose and air-dried. The shrinkage varied between 1.1 and 6.8% in the tangential direction, 0.7 and 3.6% in the radial direction, and was less than 1% in the longitudinal direction. After keratin treatment, the dimensions of the WW samples were well preserved. The shrinkage in the tangential direction ranged from 1.7 to 13%, while the contraction in the radial direction varied between 0.7 and 6.4%. Additionally, the longitudinal shrinkage decreased to about 0.2–1.6%. The shrinkage value of *Syzygium* sp. was slightly high after PEG and trehalose treatments. This could be due to low penetrability of this species after chemical treatments as indicated in Fig. 2. Among the species evaluated, dimensional stabilization of heavily degraded *Albizia* sp. preserved with keratin was not so good. This phenomenon was also found in previous report [22]. This could be due to the interactions between degradation products of lignin in WW and keratin were not well established [18]. Within the WW species analyzed, PEG and trehalose treatment provided quite similar shrinkage values, while large variation of shrinkage rate was observed in keratin treatment. This may result from the different interactions between keratin and tannin-like aromatic compounds, such as the residual lignin degradation products present in the WW, as reported [18].

The ASE of different WW species after pretreatment with the tested impregnations, air drying, and equilibrating at 60% RH and 20 °C is presented in Fig. 5. The obtained results clearly indicate that PEG, trehalose, and keratin stabilized the WWs effectively. For the WWs treated with trehalose, the ASE values were about 86.2–96.4%; the corresponding values for PEG treatment were about 87.1–96.9%.

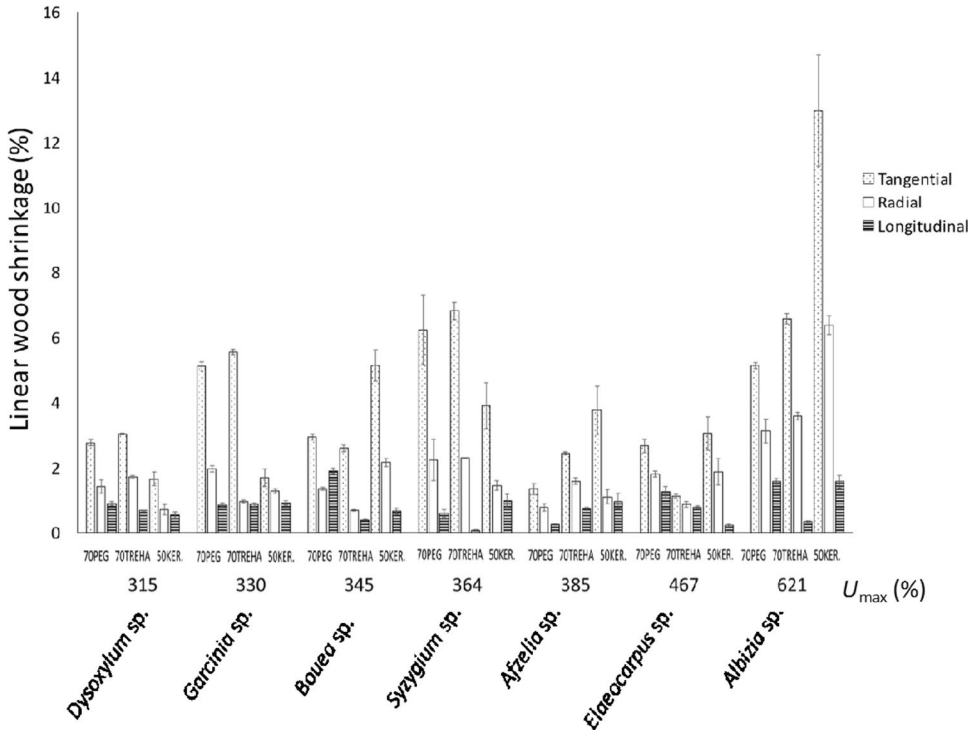

Fig. 4 Shrinkage ratios of treated waterlogged woods. The bars represent the standard deviations

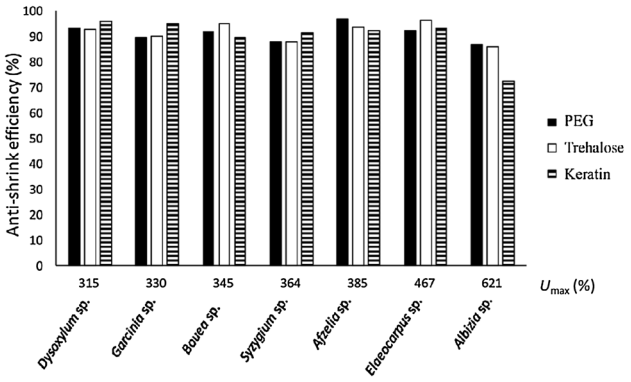

Fig. 5 Anti-shrink efficiencies of treated waterlogged woods

After the treatment with keratin, ASE values increased beyond 89%, except for the case of the heavily degraded WW (U_{max} = 612%, ASE value only 72%). According to previous reports [28], an ASE exceeding 75% is considered as acceptable in conservation science. Therefore, the dimensional stability of highly degraded WWs after keratin treatment was not sufficient enough.

SEM observations

Microscopic examinations of medium-degraded *Dysoxylum* sp. (U_{max} = 315%) and heavily degraded *Albizia* sp. (U_{max} = 621%) samples revealed the significant differences in structures between the untreated and treated wood samples (Fig. 6). The freeze-dried WW without treatment of both species revealed severe degradation of its cells (Fig. 6a_1, 6c_1). Bacterial attack of a WW causes loss of wood strength. Particularly, the S_2 layer of fibers was almost completely destroyed by biological degradation, detached from the middle lamella (ML), and became distorted, as seen in Fig. 6b_1, d_1. The parenchyma cells were, however, still intact (Fig. 6a_1, c_1). The dimensional stability of WWs involved filling the inside of both cell walls and/or cell lumina. The appearances of the woods treated with PEG and trehalose were similar. Most fibers and parenchyma cells were filled by the impregnation agent, while lumen of the vessels remained empty (Fig. 6a_2, a_3, c_2, c_3). However, in the keratin-treated wood, the lumen of cells was almost empty (Fig. 6a_4, c_4). This observation indicated that the stabilizing effect of keratin was different from those of PEG or trehalose. Keratin specifically adsorbed on the lignin-rich ML. Keratin also has an affinity for lignin degradation products, as has been previously reported [18]. The ML of *Dysoxylum* sp. maintained the original structural dimension without the support of fillers such as PEG or trehalose. However, the stabilizing effect of keratin treatment on heavily degraded *Albizia* sp. was not satisfactory. The wood cells became slightly collapsed and shrank after drying process (Fig. 6c_4, d_4). This is probably due to the interactions between keratin and the residual lignin degradation products present in this wood were not effectively established [18].

Although the stabilization effect of chemical treatment on WWs is one of the most important parameters, the

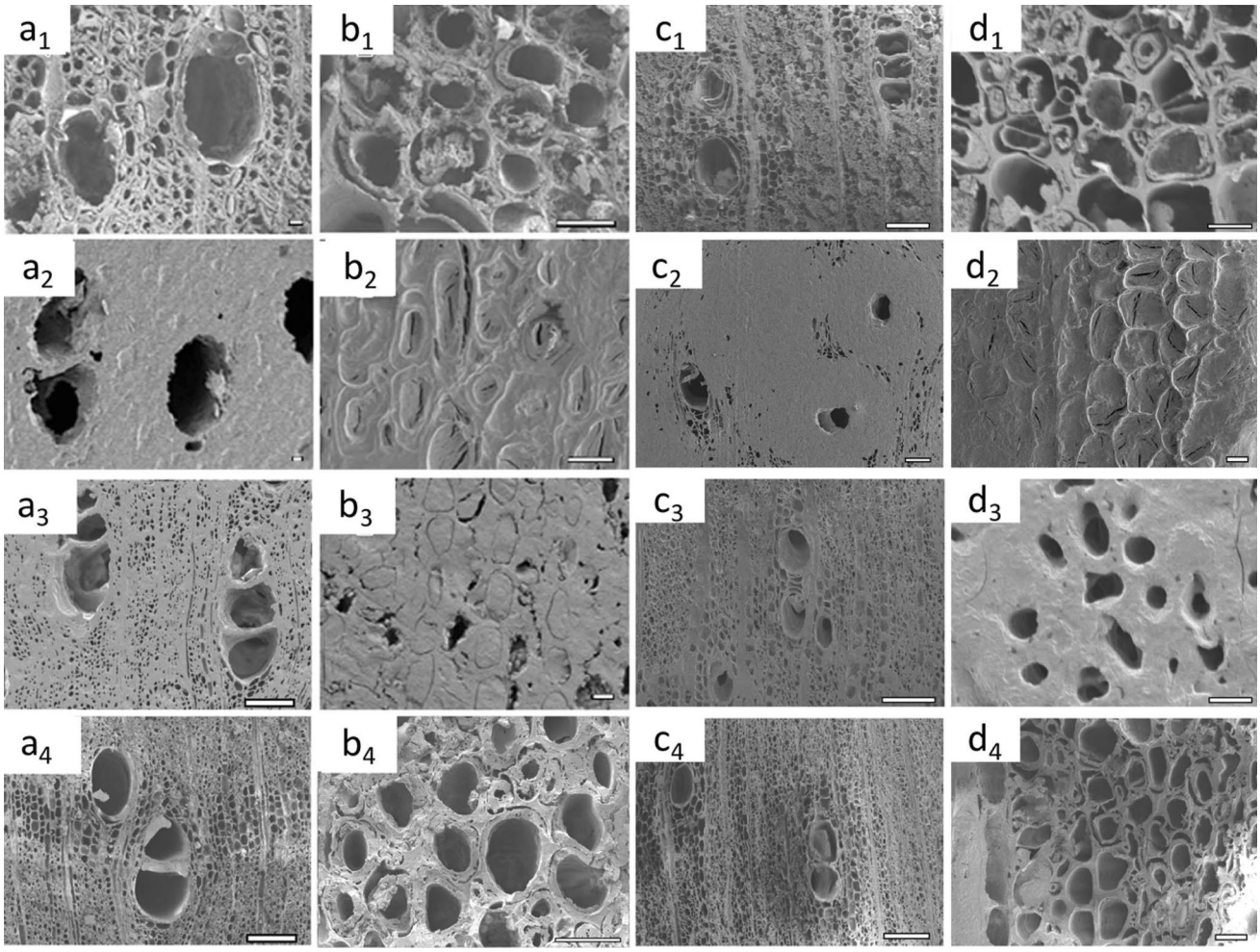

Fig. 6 Scanning electron microscopy images of waterlogged *Dysoxylum* sp. and *Albizia* sp. samples: $\mathbf{a_1}$, $\mathbf{b_1}$ untreated *Dysoxylum* sp. wood, $\mathbf{a_2}$, $\mathbf{b_2}$ *Dysoxylum* sp. treated with PEG, $\mathbf{a_3}$, $\mathbf{b_3}$ *Dysoxylum* sp. treated with trehalose, and $\mathbf{a_4}$, $\mathbf{b_4}$ *Dysoxylum* sp. treated with keratin, $\mathbf{c_1}$, $\mathbf{d_1}$ untreated *Albizia* sp. wood, $\mathbf{c_2}$, $\mathbf{d_2}$ *Albizia* sp. treated with PEG, $\mathbf{c_3}$, $\mathbf{d_3}$ *Albizia* sp. treated with trehalose, and $\mathbf{c_4}$, $\mathbf{d_4}$ *Albizia* sp. treated with keratin. For $\mathbf{a_1}$–$\mathbf{a_4}$ and $\mathbf{c_1}$–$\mathbf{c_4}$, the scale bars represent a distance of 100 μm; for $\mathbf{b_1}$–$\mathbf{b_4}$ and $\mathbf{d_1}$–$\mathbf{d_4}$, the scale bars represent 10 μm

esthetic appearance of woods is also a meaningful feature worth considering. The coloration of the as-treated samples is presented in Fig. 7. The dimensions of the WWs treated with PEG, trehalose, or keratin were well preserved, except for *Albizia* sp. preserved with keratin (Fig. 7b_4). The PEG-treated wood samples were unnaturally darker in color (Fig. 7a_2, b_2), while the trehalose-treated samples appeared slightly lighter (Fig. 7a_3, b_3). More interestingly, the color of WWs after keratin treatment was similar to that of modern wood (Fig. 7a_4, b_4). Such color appearance of treated woods seemed reflecting the degree of occupancy in cell lumina by treated chemical reagents.

In summary, all chemical treatment procedures provided satisfactory stabilizing effects. The ASE values reached above 82% except for the case of the heavily decomposed WW (U_{max} = 621%) treated with keratin, for which it was lower than 75%. Based on the improved dimensional stability of wood, shortened impregnation time, and natural color of treated samples, keratin can be considered as a promising impregnation agent for WW.

Conclusion

Treatment with PEG, trehalose, and keratin resulted in sufficient dimensional stabilities of WWs. The ability of keratin treatment to protect the dimensions of severely degraded WW was not as good as those of PEG or trehalose. The main purpose of WW conservation is to prevent dramatic dimensional changes of artifacts caused by the shrinkage and collapse of weakened cell walls upon drying. It is also important to reduce the impregnation time and protect the original appearance of historical wooden objects. Additionally, the retreatability of the treatment processes is also a

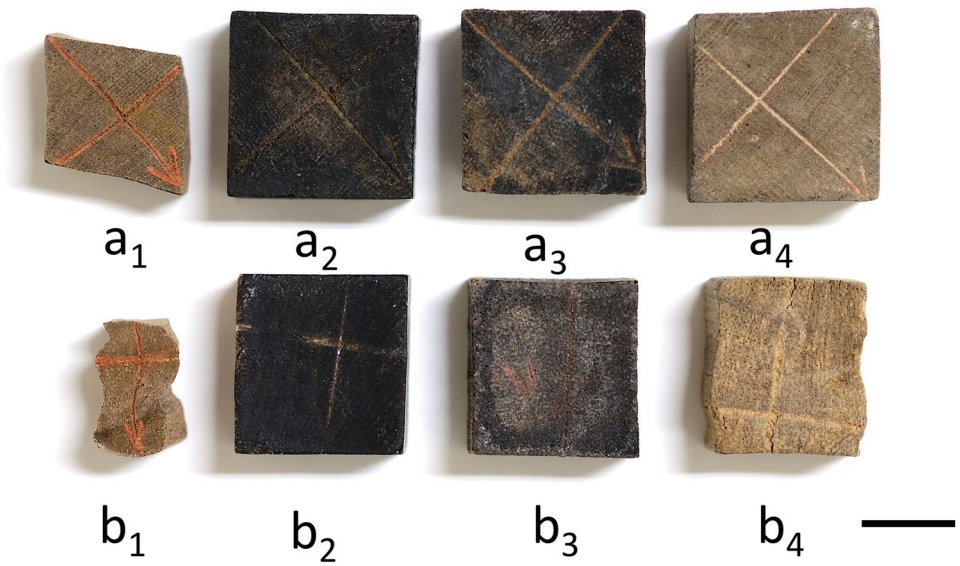

Fig. 7 Post-treatment appearance and coloration of the waterlogged *Dysoxylum* sp. and *Albizia* sp. samples after air drying: $\mathbf{a_1}$ untreated *Dysoxylum* sp., $\mathbf{a_2}$ *Dysoxylum* sp. treated with PEG, $\mathbf{a_3}$ *Dysoxylum* sp. treated with trehalose, and $\mathbf{a_4}$ *Dysoxylum* sp. treated with keratin; $\mathbf{b_1}$ untreated *Albizia* sp., $\mathbf{b_2}$ *Albizia* sp. treated with PEG, $\mathbf{b_3}$ *Albizia* sp. treated with trehalose, and $\mathbf{b_4}$ *Albizia* sp. treated with keratin. The solid bar below represents 1 cm

criterion used in the conservation and restoration of archeological artifacts. Keratin treatment shortened impregnation time and the good esthetic results obtained from this treatment. Furthermore, keratin was not filling the pores of the wood and the wood structure remains open, meaning it may be possible to retreat the sample if needed in the future. Due to the inherent limitations of WW materials, this study has investigated the impregnation of WWs with commonly used chemicals. Further examinations based on different experimental setups are also required. Of equal importance is the need to further investigate the effect of metal salt presented in keratin-treated wood to the future danger for wood degradation in long term. Although several further steps remain in the development of this method, the keratin treatment method presented in this study appears to have unique properties that can be applied practically for the conservation of WWs.

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