Investigation into the deterioration of Castanopsis hystrix buried wood by anatomical, chemical and thermal analysis

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Abstract
Comparative studies of the anatomy and composition had been investigated to learn about the degradation characteristics of Castanopsis hystrix wood. Its chemical degradation was analyzed with wet chemical analysis, Fourier-transform infrared analysis, x-ray diffraction analysis, and thermogravimetric analysis. Its morphology was observed with light microscopes and scanning electron techniques. It was found that the deformation and separation of cell walls have no effect on the identification of C. hystrix wood. The degradation of polysaccharides leads to the mechanical properties of C. hystrix buried wood decreased. The inclusions exist in cells of C. hystrix buried wood increased, and the dark color is related to the inclusions and ferric oxide. Furthermore, the hemicellulose was heavily degraded but there was no obvious degradation of crystalline cellulose. Large amounts of inorganic elements such as Fe and S were detected in the ash of C. hystrix buried wood.

1. Introduction
Buried woods are gradually formed by natural modification without any artificial modification [1]. They have been progressively degraded under unusual circumstances with poor oxygen, high pressure, and complicated microorganisms [2, 3]. The defined buried wood possessed exceptional fungi-resistant and good mechanical strength [4]. Due to the non-renewable and fascinating properties of buried wood, it is attractive to explore the formation process of buried wood. Nevertheless, not all species can persist in hostile environments over millions of years. As shown in figure 1, excavated buried wood possesses dark color in various degrees, implying physicochemical properties have gradually changed during the long time buried. This makes the wood display and long-term preservation more difficult. Understanding the degradation characteristics of wood is beneficial to wood conservation.

Chemical degradation played the most important role in the deterioration of archaeological wood [5–8]. It has been pointed out that the degradation is inseparable from microorganisms [7]. The deterioration was studied by the combination of chemical and anatomical results. The chemical degradation was analyzed with wet chemical analysis, Fourier-transform infrared analysis (FT-IR), x-ray diffraction analysis (XRD), and thermogravimetric analysis (TGA). The anatomy was observed with light microscopes (LM) and scanning electron (SEM) techniques.

At present, large works of Phoebe zhennan buried wood unearthed in Sichuan Province have been underway [8–13]. However, studies of other buried wood species has been quite limited. Physical and chemical properties vary greatly between species. Hence, diversified species of buried wood contributed to comprehensive mastering buried wood formation.

Castanopsis hystrix wood is one of the raw media for ships and furniture owing to its good fungi-resistant and perfect mechanical properties. In China, this species mainly grows in Guangdong, Hainan, mountainous areas of Guanxi, and South Yunnan [14]. In this paper, by comparing archaeological and modern C. hystrix wood, we try to explore the deterioration of buried wood and offer systematic and objective scientific of C. hystrix buried wood to buried wood research.
2. Experimental

2.1. Materials and reagents
The *C. hystrix* buried wood (BW) samples (around 2600 ± 200 years old) were obtained from Guangdong Province. The *C. hystrix* modern wood (MW) samples were growing in Guangdong Province. All the samples were air-dried before analysis. All chemical reagents used were of analytical grade.

2.2. Morphology
A combination of LM (LeicaDM1000LED) and SEM (ZEISS EVO 18 Special Edition) were used to determine the differences of BW from modern wood MW.

2.3. Organic chemical components analysis
The lignin, cellulose, and hemicellulose were determined according to NREL methods. Hot-water extractive, alcohol-toluene extractive, 1% sodium hydroxide solubility of the decayed woods was determined following GB/T 2677.1–1993 (in Chinese). All samples were analyzed in duplicate.

2.4. Inorganic chemical components analysis
The elemental composition of BW and MW were analyzed using an energy dispersive spectrometer (Oxford X-Max 20). The ash was obtained by the complete combustion of the wood samples. Then it was determined by EDS to explore the inorganic chemical components.

2.5. FT-IR analysis
The chemical structural groups’ spectrum of BW and MW were acquired by a Fourier-transform infrared spectrometer (Spectrum 100) using the KBr pellet technique.

2.6. XRD spectral analysis
An x-ray diffractometer (Ultima IV) was used to determine the crystallinity index (CrI) of the wood, which was calculated using the Segal [15] method according to the following formula:

\[
\text{CrI} = \frac{I_{002} - I_{\text{am}}}{I_{002}} \times 100\%
\]

where \(I_{002}\) is the intensity of the diffraction from the (002) plane, which is the maximum diffraction intensity, and it is representing both the crystalline and amorphous material of wood. \(I_{\text{am}}\) is the intensity of the diffraction from the plane at 2\(\theta\) = 18° representing only amorphous material.

2.7. TGA and DTG
The thermal properties were explored using sensitive thermobalance (PerkinElmer, Diamond, China). Air-dried wood powder (180–250 \(\mu\)m, 11% moisture content) was prepared. The samples of about 5 mg were placed on the thermobalance. Experiments were carried out at N\(_2\) atmosphere with a steady flow of 60 ml min\(^{-1}\) and a linear heating rate of 10 °C min\(^{-1}\). The temperature range from 35 °C to 800 °C.

3. Results and discussion
In this study, we selected the same species of modern (fresh standing) wood as the buried wood. The modern wood worked as the control sample, used to analyze the changes of *C. hystricis* buried wood from standing to being buried.
Table 1 compared the main chemical compounds content of BW and MW. The main components of the wood cell wall are cellulose, hemicellulose, and lignin. Wood properties depend on these compounds’ content. Cellulose is the skeleton of wood, hemicellulose acts like a bond, and lignin acts as hardening. For comparison, cellulose in BW was a bit higher than in MW. In contrast, hemicellulose in BW was significantly lower (by about 21.6%) than in MW.

3.1. Morphology
For the BW sample, the wood was hard and the new section appears dark brown (figure 2(a)), which is considered the surface was carbonized. The new section of BW appears red. Both BW and MW were hemi-ring-porous wood with obvious growth rings. For microstructural features of the BW (figures 3(a), (c), (e), the pores are round or oval, exclusively solitary (90% or more), in diagonal and/or radial patterns. Many tyloses can be seen in vessels. Perforation plates are simple. Intervessel pits are alternate. Axial parenchyma is in narrow bands or lines up. Fibers are thin- to thick-walled and septate. Wood rays are storied with numerous sedimentary, uniseriate rays are exclusive. There are crystals in enlarged cells.

Comparing the microstructure of BW samples with MW, we found that the wood microstructure of BW is comparable with MW (figures 3(b), (d), (f)). Generally, the wood is identified according to the main anatomical features including pore type, axial parenchyma type, and wood ray type. The suspected BW sample can be matched with MW sample through comparing the main features. However, there are some differences between BW and MW. In cell wall integrity, it can be observed that various cells in the BW samples are deformed, parts of different cell walls had significantly damaged and the number of cell contents increased. On the other hand, the contents (tyloses or gum) in vessels and parenchyma are notably increased. Furthermore, it is speculated that the dark color of BW is related to the enrichment of inclusions. It is speculated that water-induced migration and high pressure-induced compression make inclusions deposit. The darkening of the color during the burial is ascribed to the long-term oxidation of the organic matter [16].

In summary, the little differences between the BW and MW don’t exist in the main wood micro characteristics. Hence, there is no effect on the determination of wood species.

It is notable that there was no significant difference in the microstructures between water saturated and air dried modern wood cells. However, as for BW, in the process of air drying and water loss, the cell wall deformed simply under the action of capillary force (figure 4), which shows that the degradation of cell wall mechanical properties. We used the standard wood mechanics test method to determine the hardness of the buried wood, it is found that buried wood (7788.38 N) is harder than modern wood (6148.32 N) because the cell wall of buried wood shrunken significantly and densified under the air drying state, which makes the hardness of buried wood higher than modern wood macroscopically.

Figure 5 shows the ultrastructure of BW and MW. Figure 5(d) shows the intact cells in MW. However, as shown in figure 5(a), cells in BW were obviously compressed and deformed including all cell types, vessels, and wood fibers. Cells in BW had noticeably shrunk and deformed (figures 5(b), (e)), but the cell wall layer structure was clear and there was no indication of the destruction of the cell wall. Furthermore, inclusions in vessels of BW (figure 5(c)) were more than in that of MW (figure 5(f)). The pores of fibers appeared collapsed and deformed (figure 5(g)) while in MW appeared oval (figure 5(f)). Furthermore, the intercellular walls of BW fibers were separated and damaged (figures 5(h), (i)). Numerous inclusions were observed obviously in ray parenchyma cells (figures 5(i), (m), (j), (n)) of BW. However, we did not find any indication of microbial attack by electron microscopy.

3.2. Chemical compounds analysis
Table 1 compared the main chemical compounds content of BW and MW. The main components of the wood cell wall are cellulose, hemicellulose, and lignin. Wood properties depend on these compounds’ content. Cellulose is the skeleton of wood, hemicellulose acts like a bond, and lignin acts as hardening. For comparison, cellulose in BW was a bit higher than in MW. In contrast, hemicellulose in BW was significantly lower (by about 21.6%) than in MW.
MW. Lignin content in BW was about 13.07% lower than in MW. This is due to the hemicellulose being the most unstable compound. Hemicellulose is easy to be depolymerized by long-term burial in soil. Based on the results of SEM, there is no evidence that BW suffers from microbial attack. It could be concluded that the degradation of BW was mainly induced by chemical agents [13].

**Figure 3.** Microstructure of BW cross (a), radial (c) and tangential (e) section and microstructure of MW cross (b), radial (d) and tangential (f) section.

**Figure 4.** Water saturated BW (a) and air dried BW (b).
In BW, only the 1% NaOH solubility was higher than in MW. In 1% NaOH solubility, it mainly contained acid-soluble lignin, tannins, lipids, low molecular weight hemicellulose, and degraded cellulose [17].

Using an energy dispersive spectrometer, a qualitative and quantitative compositional analysis could be obtained and precise elemental composition of materials with the high spatial resolution was accomplished. Figure 6 showed the test area of EDS. Various inorganic compounds and metal elements exist in the ash [18]. Table 2 summarized the elemental analysis results. Fe (23.84%) and S (4.45%) can only be detected in BW, while no P and Cl exist in BW. On the other hand, the content of K and Mn was obviously decreased. Iron ions reacted with tannin could produce iron tannin. Iron tannin was a black complex, which explained why the BW was black.

After sufficient combustion, it is the most notable that the ash of BW was red apart from the gray ash of MW wood. This is mainly due to the enriched Fe being oxidized into ferric oxide appearing red.

![Ultrastructure of BW and MW](image)

**Figure 5.** Ultrastructure of BW and MW – (a), (d): The transverse section of BW and MW respectively. – (b), (e): The vessels and parenchyma cells in the transverse section of BW and MW respectively. – (c), (f): The vessels in the tangential section of BW and MW respectively. – (g): The deformed fibers cells in BW; - Intact oval fibers pores in MW; - (h), (i): Separated inner walls in BW and intact inner walls in MW respectively; - (j), (m): The tangential section of BW and MW respectively; - (j), (n): ray parenchyma cells of BW and MW respectively.

**Table 1.** The main chemical compounds of BW and MW.

| Name                  | MW/% | BW/% |
|-----------------------|------|------|
| hot-water extractive  | 7.15 | 3.25 |
| 1% sodium hydroxide solubility | 18.16 | 19.17 |
| alcohol-toluene extractive | 3.33 | 2.61 |
| cellulose             | 46.88 | 48.78 |
| hemicellulose         | 13.75 | 10.78 |
| acid-insoluble lignin | 33.64 | 38.70 |
| ash                   | 0.19  | 0.64  |
3.3. FT-IR analysis

Figure 7 depicts FT-IR spectra over a 4000–800 cm$^{-1}$ range to estimate the changes of the MW and BW chemical composition. To analyze the change of chemical composition between MW and BW, four characteristic absorption bands of holocellulose and two characteristic absorption ones of lignin are collected: 1740 cm$^{-1}$ for unconjugated C=O stretch of hemicellulose, 1372 cm$^{-1}$ for C–H deformation of cellulose or hemicellulose, 1160 cm$^{-1}$ for C–O–C vibration of cellulose or hemicellulose, 895 cm$^{-1}$ for C–H deformation of cellulose, 1510 cm$^{-1}$ for aromatic skeletal vibration of lignin and 1265 cm$^{-1}$ for C=O vibration of lignin [19–23].

The most significant difference between the spectra of the MW and BW samples is found around 1740 cm$^{-1}$, which in BW is nearly disappeared. This band is assigned to C=O stretching in unconjugated carbonyl, carboxyl, and ester groups. These groups mainly existed in hemicellulose. The absence of the peak at 1740 cm$^{-1}$ showed that BW possesses little hemicellulose [24]. It is further concluded that hemicellulose was largely degraded after burial.

| Element | MW percentage/% | BW percentage/% |
|---------|------------------|-----------------|
| C       | 9.83             | 9.44            |
| O       | 42.24            | 38.62           |
| Na      | 0.73             | 0.95            |
| Mg      | 5.43             | 3.54            |
| Al      | 0.30             | 1.18            |
| Si      | 0.47             | 1.56            |
| S       | /                | 4.45            |
| K       | 23.65            | 0.82            |
| Ca      | 11.37            | 14.18           |
| Mn      | 3.48             | 1.41            |
| Fe      | /                | 23.84           |
| P       | 1.10             | /               |
| Cl      | 1.39             | /               |
Whereas, the relative intensities of carbohydrate bands at 1372, 1160, and 897 cm$^{-1}$ are weaker in BW than in MW. However, the relative intensities of lignin bands at 1510 and 1265 cm$^{-1}$ undergo a remarkable increase after burial. This may be ascribed to the degradation of xylan in BW [25].

3.4. X-ray diffraction

Figure 8 depicted the XRD patterns of BW and MW. Obviously, there is no difference in the shape of the two 2θ diffraction intensity curves, whereas the diffraction intensity of the two samples is not the same. This indicated the CrI has changed [26]. CrI is used to indicate the relative rather than the absolute amount of the crystalline cellulose in the whole wood material. The CrI of BW was 54.94%, whereas the CrI of MW was 47.15%, indicating the crystallinity of BW was higher than MW. Due to the instability of amorphous cellulose and hemicellulose, it is more susceptible for them to be damaged during the buried condition [27]. On the other hand, hemicellulose possesses good water solubility and the highest susceptibility to biological degradation [24]. Therefore, we speculated that the higher BW crystallinity was mainly due to the degradation of amorphous
It can be inferred that the degradation of polysaccharides decreased the mechanical properties of wood cell wall.

3.5. TG and DTA measurements

The TG and DTG curves were used to analyze the thermal stability property of samples. The curves were obtained from the thermal decomposition of the wood samples under nitrogen (N₂) at heating rates of 5 °C·min⁻¹. The decomposition process is divided into three stages: before 200 °C (dehydration), from 200 to 430 °C (hemicellulose and cellulose), and over 430 °C (lignin) [28]. In figure 8 the TG and DTG curves of the BW and MW are portrayed. The test data are summarized in table, including the initial degradation temperature (Tᵢ), the temperature of maximum weight loss rate (T_max), and the char residue at 800 °C [29].

For all samples, the thermal degradation is just a one-step reaction. Before 200 °C, the dehydration process could be divided into losing the free water and the internal bound water, 100 °C is generally considered the point [16]. Water in BW is higher in the form of free water than in the MW. This conclusion is consistent with the higher CrI of BW.

After 200 °C with respect to MW, one major peak comprising a shoulder was present (figure 9(b)). The major peak could be attributed to the decomposition of crystalline cellulose, while the shoulder peak is ascribed to hemicellulose and an amorphous fraction of cellulose [30]. However, the shoulder is not only attributed to the hemicellulose but also the degradation of wood products, presumably both of cellulosic and phenolic nature. Hence the absent shoulder peak of BW may be explained by the lost hemicellulose and no present degradation products.

The MW began to decompose at 91.6 °C (Tᵢ) and exhibited the maximum weight loss rate at 353.9 °C (T_max), whereas for the BW, the values of Tᵢ and T_max are 79.6 °C and 343.6 °C, respectively. Besides, the BW showed a residual char of 31.0% at 800 °C compared to 13.0% for the MW, which increased the char residue rate by 138.5%. It is noteworthy that the Tᵢ and T_max of BW were reduced but the char rate was increased. It was presumed that the shifts of Tᵢ and T_max to lower temperatures and the growth of char residue rate were inseparable from the natural mineral inside the BW. This issue still needs further study.

4. Conclusions

After gradual natural aging, the main microstructure of Castanopsis hystrix buried wood didn’t change much. It is credible that identify the wood species by comparing the main anatomical features. The deformation and separation of cell walls do not affect the identification of wood species. The degradation of polysaccharides leads to the mechanical properties of wood decreased. Then the high pressure easily makes the cells deform and rupture. The rupture of the cell wall makes it easier for environmental materials to deposit in cell lumen. Thus deposits can be observed in most cells. The dark color is related to the inclusions and the organic oxidation. According to the results of FT-IR, XRD, and TGA, the dimensional stability and thermal stability of buried wood need further investigation.
Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

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