# Effects of extracts on color, dimensional stability, and decay resistance of thermally modified wood

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## Abstract

Thermal modification can enhance the color as well as improve the dimensional stability and decay resistance of wood without the use of chemical modifier, but the impact of extracts from thermally modified wood (TMW) on performance remains unclear. In order to utilize extracts to regulate the properties of the TMW or prepare the imitate TWM without mechanical loss, the effects of TMW acetone extracts on the color, dimensional stability, and decay resistance of TMW were investigated. The 180°C, 200°C and 220°C thermally modified spruce (*Picea asperata* Mast.) and poplar (*Populus tomentosa* Carr.)with or without acetone extract were used for CIELab system, anti-swelling efficiency(ASE), and decay resistance test. Fourier infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS) were used to characterize extracted and unextracted TMW. The results indicated that the extract showed positive effects on the color, dimensional stability (220°C), and decay resistance of TMW, and this effect weakened with the increase of temperature. The brightness difference ($\Delta L^*$) and the total color difference ($\Delta E^*$) of the TMW were reduced due to the removal of the extract, and the wood thermally modified at 180°C was the most significant. The removal of the extract will lead to the improvement of the dimensional stability of the TMW at 180°C and 200°C and the reduction of the TMW at 220°C. The decay resistance of wood thermally modified at 180°C, 200°C, and 220°C decreased after extraction, and was most significant at 180°C. The antifungal activity of the extract was related to the production of vanillin, isovanillic acid, syringic acid and syringaldehyde. Extract from TMW played an important role in the properties of TMW, and can be used to adjust its properties or as a natural wood modifier.


## 1. Introduction

The dimensional instability and biodegradability of wood limit its application (Spear et al. 2021). The dimensional stability of wood can be significantly improved by thermal modification, which is widely used in Europe and other regions (Dubey et al., 2011). In addition, the decay resistance (Chaouch et al. 2010; Tripathi et al. 2014) and surface color (Li et al. 2018; Nguyen et al. 2018) of wood can also be improved by heating with simple equipment, especially the sapwood of some species with poor decay resistance and color. Unfortunately, the mechanical strength of wood significantly decreased due to thermal modification and gradually reduces with increasing temperature (Korkut et al. 2008). The modulus of rupture (MOR) and modulus of elasticity (MOE) of wood treated at 200°C for 24 hours decreased by 44%-50% and 4-9% respectively (Bekhta and Niemz, 2003). Some study indicated that extracts from thermally modified wood (TMW) have a positive effect on its decay resistance and color (Esteves et al. 2008; Sandoval-Torres et al. 2010). The extract of TMW provide the possibility to regulate the properties of TMW and improve wood decay resistance and enhance wood color, and will eco-friendly and not reduce its mechanical strength (Bi et al. 2022).

The degradation of cellulose and hemicellulose due to thermal modification, which were generally considered as a prime key to improve decay resistance and reduce hygroscopicity (Candelier et al. 2016). The degradation of cellulose, hemicellulose, and lignin which are the nutritional sources of wood decay fungi, leads to the reduction of nutritional and hydrophilic hydroxyl groups (Costa et al. 2019; Hill et al. 2021). The reduction of hygroscopicity is not conducive to the expansion caused by the increase of moisture and to the growth of fungi and the transportation of small molecular substances that degrade the cell wall, thus improving the dimensional stability and corrosion resistance of wood (Sivrikaya et al. 2015). However, some researchers brought up that there was no significant correlation between wood hygroscopicity and decay resistance (Hakkou et al. 2006), and the decay resistance of TMW was also affected by pseudo-lignin and small molecular anti-fungal active compounds (Jiang et al. 2022). The new extracts with antifungal activity produced by thermally modification played an important role in improving the decay resistance (Kamdem et al. 2020; Chu et al. 2019). Isovanillic acid, vanillin, and other compounds in these extracts will inhibit the growth of wood decay fungi (Candelier et al. 2020; Bi et al. 2022). Vanillin having been proved to inhibit enzyme activity, damage cell membrane, ATP, and other physiological and biochemical indicators of wood decay fungi (Bi et al. 2021a). This indicated the utilization of the extract from thermally modified wood (TMW) can provide the possibility for the imitate TWM without mechanical loss.

Color is one of the main physical properties of wood. The chromophores and (or) auxochromes of hemicellulose, lignin and extract changed after heating, resulting in the color change of TMW (Qu et al. 2021). Extracts are often rich in compounds containing unsaturated conjugated structures such as tannin, phenol, stilbene and quinone, which are prone to change when heated (Mayer et al. 2006; Zhu et al. 2020). The new extracts produced by the degradation of the original wood extract and hemicellulose and lignin during the thermally modification process will lead to the change of chromogenic groups and chromogenic groups, thus changing the color of the wood (Chen et al. 2012; Gao et al. 2015).

However, the effect of extracts on color, dimensional stability, and decay resistance of TMW has not received sufficient attention and more research was needed. The objective of this study was to explore the influence of thermally modified wood extracts (air atmosphere) on its color, dimensional, and decay resistance. This study can provide a theoretical basis for extracts to modulate the color, dimensional stability,

and decay resistance of TMW. In addition, the processing residues of TMW can be reused and provide new materials for plant-derived wood modifiers.

## 2. Materials and methods

2.1. Materials

Wood preparation: Poplar (*Populus tomentosa* Carr.) and spruce (*Picea asperata* Mast.) trees were selected from plantations at Northwest A&F University in the Yangling District, Shaanxi Province, China. Clean, defect-free blocks of 20 mm × 20 mm × 10 mm (R × T × L) and 20 mm × 20 mm × 20 mm (R × T × L) blocks were cut from 45 cm diameter sapwood (stronger permeability and poorer durability) of poplar and spruce wood. The lumber was air-dried and then small shavings were cut from the lumber and ground to pass a 40-60 mesh screen. The wood blocks and ground wood were oven-dried at 60°C to 8 % moisture content and weighed (nearest 0.01 g).

The white-rot fungus, *Trametes versicolor* (L. ex Fr.) Quél. (Isolate # CFCC5336) and brown-rot fungus, *Gloeophyllum trabeum* (Pers.: Fr.) Murr. (Isolate # CFCC86617) were grown on 3.7% potato dextrose agar (PDA) at 28 ± 2°C and 80±5% relative humidity (RH) for 5-7 days before use.

2.2. Preparation of thermally modified wood and extraction

Acetone extract with strongest antifungal activity had the significant effect on the color of thermally modified poplar wood in our previous study, and it was selected as the extraction solvent (Bi et al., 2022 and 2021b). Some studies also indicated that wood extracts extracted with solvents with electrical constants between 4 and 6 have strong antifungal activity and relatively rich chromogenic and auxochrome (Mahlo et al. 2010; Candelier et al. 2020). The poplar wood blocks, spruce wood blocks, or ground wood with constant weight were respectively put into the oven with the side and top vents open, and thermally treated at 180°C, 200°C, or 220°C for 6 hours. The samples were cooled to 60°C then the TMW was stored in a desiccator. Twelve pieces of thermally modified poplar (TMP) and spruce (TMS) wood were placed in the extraction tube of soxhlet extractor, and 1000 mL of acetone was poured into round bottom flask of soxhlet extractor. The assembled soxhlet extractor was placed in a water bath and heated to produce four reflux changes per hour, and the extracted TMW samples were obtained after 72 hours of maintenance.

2.3. Color measurement

The color of wood will be enhanced after being heated, which was an eco-friendly and effective way to change the color of fast-growing wood species with poor color. The thermal degradation of the cellulose, hemicellulose, lignin, and extracts of original wood will produce new extracts, and the impact on the color of TMW was explored in this study.

The color coordinates ($L^*$, $a^*$, $b^*$) of the 20 mm × 20 mm × 20 mm (R × T × 50) TMP and TMS with or without acetone extraction were measured by the CIELab system with a CHNspec CS-802 Spectrophotometer (Hangzhou CHNspec Technology Co., Ltd., Hangzhou, Zhejiang, China) equipped with D65 light source (Bi et al. 2021b; Vuthiganond et al. 2020). The samples were measured at 10 degree observed angle with a 15 mm aperture at the point of measurement. Each treatment was repeated three times. The color coordinates were measured at three locations on each sample, and the mean value was calculated. The total color difference ($\Delta E^*$) was calculated according to the equation 1.

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad (1)$$

Where $\Delta L^*$, $\Delta a_*$ and $\Delta b^*$ are the differences of $L^*$, $a^*$ and $b^*$ between non-extracted and extracted samples, respectively. The positive number of $\Delta L^*$, $\Delta a^*$ and $\Delta b^*$ indicates that the color of the sample after extracting was tended to brighter, redder, or yellower than that before treatment. The negative number indicates darker, greener, or bluer.

2.4. Dimensional stability test of extracted and unextracted TMW

2.4.1 Anti-swelling efficiency of extracted and unextracted TMW

12 pieces of 20 mm × 20 mm × 20 mm extracted or unextracted TMP and TMS were placed in a temperature-humidity chamber at 22°C, and balanced for 4 days at 65%, 75%, 85%, 75%, and 65% relative humidity. Sample size and mass were measured after each humidity balance. The hygroscopicity of wood samples under various humidity conditions was calculated according to the eq. 2, and the

hygroscopicity analysis curve was drawn. The moisture absorption (MA) of the moisture absorption-desorption cycle and anti-swelling efficiency (ASE) of absorption was calculated according to eq. 3-4, and the results were averaged.

$$MA\ (\%) = \frac{M_a - M_b}{M_a} \times 100 \quad (2)$$

$$\Delta V\ (\%) = \frac{V_a - V_b}{V_a} \times 100 \quad (3)$$

$$\mathrm{ASE}\ (\%) = \frac{\Delta V_0 - \Delta V_1}{\Delta V_1} \times 100 \quad (4)$$

Where MA is moisture absorption of wood, %; $M_a$ is the mass of sample before each moisture balance, g; $M_b$ is the mass of sample after each moisture balance, g. $\Delta V$ is the change rate of wood volume,%; $V_a$ is the volume of sample before each moisture balance, $mm^3$; $V_b$ is the volume of of sample after each moisture balance, $mm^3$; ASE is anti-swelling efficiency of samples, %; $\Delta V_0$ is the change rate of original wood volume,%; $\Delta V_1$ is the change rate of treated wood volume, %.

2.4.2 Water contact angle of extracted and unextracted TMW

The SDC-100 contact angle meter was used to measure the water contact angle (°) of extracted or unextracted TMW. The samples were placed at 22°C and 65% relative humidity for one week to maintain constant weight before testing. A 5 μL drop of distilled water was deposited to the cross-section of the wood sample using the seat drop method and the water contact angle was recorded at 60 s.

2.5. Decay resistance test of extracted and unextracted TMW

Standardization XP CEN/TS 15083-1 (2006) were referenced in the decay resistance test and slightly modified. Decay tests were performed in Petri dishes (Bi et al. 2022). The potato dextrose agar (PDA) medium sterilized in a 121°C autoclave for 30 min was poured into a 90 mm glass petri dishes and cooled to solid state. The 4.4 mm disk was cut from active edge of the activated white-rot fungi (*T. versicolor*) and brown-rot fungi (*G. trabeum*) colonies and placed at the center of PDA medium. Those petri dishes incubated in darkness at 28°C and 80% relative humidity for 7 days. The 20 mm × 20 mm × 10 mm (R × T × L) extracted or unextracted TMW was wrapped in sterile gauze and sterilized in an autoclave at 105°C for 30 min. Sterilized samples were placed on a medium full of mycelium and incubated in the dark at 80% RH and 28°C for 15 weeks. The mycelium was scraped off the blocks and were oven dried (60°C) and weighed. The mass loss rate (ML) was calculated according to Eq. (5).

$$ML\ (\%) = \frac{m_1 - m_2}{m_1} \times 100\% \quad (5)$$

Where $m_1$ is the mass of the wood block before fungal exposure, $m_2$ is the mass of the wood block after fungal exposure.

2.6. Antifungal activity test of TMW extract

The inhibitory effect of acetone extracts from wood thermally modified at 180°C, 200°C and 220°C on the growth of wood decay fungi was used to investigate the effect of TMW extracts on the decay resistance of TMW. The acetone extracts of TMP and TMS were prepared by the ultrasound-assisted solvent extraction according to the previous method (Bi et al. 2022). TMP or TMS extracts were dissolved in anhydrous ethanol to prepare a 300 mg/mL stock solution. 107 μL, 213 μL or 427 μL of the stock solution was mixed with 7920 μL, 7814 μL or 7624 μL of sterilised PDA medium (80°C) to prepare extract-containing media at concentrations of 4 mg/mL, 8 mg/mL and 16 mg/mL respectively. The 4.4 mm disks were placed at the center of PDA medium and incubated in darkness at 28°C and 80% RH for 14 days. Each group was assessed on three plates per fungus. Colony diameter (mm) was measured and photographed at $2^{th}$, $4^{th}$, $6^{th}$, $8^{th}$, $14^{th}$ and $21^{th}$ days.

2.7. Chemical characterization

2.7.1 FTIR analysis

The extracted or unextracted thermally modified wood meals to be tested were placed in an oven at 105°C for 6 h to remove moisture. Those ground wood samples were subjected to FT-IR analysis using the KBr plate method on a Bruker Vertex 70 FTIR Spectrometer (Bruker Optics Ltd, Coventry, UK) The KBr plate were scanned 32 times at 4 $cm^{-1}$ resolution in the spectral region 4,000-400 $cm^{-1}$.

2.7.2 XPS analysis

10 mm x 1 mm x 10 mm (R x T x L) slices were cut from the radial faces of extracted or unextracted TMW. XPS analyses were carried out with a Thermo Escalab 250XI spectrometer (Thermo Fisher Scientific, Massachusetts, USA). 284.6 eV was used as an internal reference to correct the binding energies (Yan et al. 2021). The carbon spectrum (C1s) was de-convoluted into C1, C2, C3, and C4 peaks corresponded to C-C/C-C/C-H, C-O/C-O-C, -C-O/O-C-O, and O-C-O/C(=O)OH with binding energies (BE) of 284.6 eV, 286.4 eV, 287.8 eV, and 289.2 eV, respectively (Acheampong et al. 2018; Inari et al. 2011).

2.8 GC-MS analysis of extracts

The acetone extract of 180°C, 200°C and 220°C TMW (TMP and TMS) were dissolved in a methanol (GC) to prepare sample solutions of 1 mg/mL and the sample solutions were filtered through a 0.22 μm organic nylon filter at least 3 times to remove any particulate. The resulting filtrate was analyzed on a GCMS-QP2010 GC-MS spectrometer (Shimadzu Corporation, Kyoto, Japan) with a TG-5MS fused silica column (30 m × 0.25 mm id., film thickness 0.25 μm). The technical parameters were referenced to previous reports and their mass spectrum were identified using the National Institute of Standards and Technology library database (NIST08s. LIB). The top ten compounds in content were listed, and the results were only for reference.

2.9. Statistical analysis

The results were analyzed using a one-way ANOVA ($P = 0.05$) using the SPSS Statistics 22.0 software program. Results were expressed as mean ± SD, significant difference $P < 0.05$ (*) and highly significant difference $P < 0.01$ (**).

# 3. Results and discussion

3.1. Effects of extract on the color of thermally modified wood

The color of poplar and spruce wood were significantly changed after thermal modification and the total color difference ($\Delta E^*$) increased with the increase of treatment temperature, up to 53.7 and 49.6 at 220°C (Fig. 1 and Fig. 2). The color of poplar and spruce wood after thermal modification at 180°C tended to be darker, redder, and yellower, thermal modification at 200°C and 220°C tended to be darker, redder, and bluer, and spruce after thermal modification at 220°C was different from poplar and tended to be greener.

The effect of TMP or TMS acetone extracts on the color of these two thermally modified woods was similar, and their effect on spruce wood (Fig. 2) was stronger than that of poplar wood (Fig. 1). The effect of extracts on $\Delta a^*$ and $\Delta b^*$ of TMW were not significant and had no consistent pattern. The $\Delta L^*$ and $\Delta E^*$ of TMP and TMS wood were reduced after the removal of extract, and this effect was significant for wood thermally modified at 180°C. In addition, the effect decreased as the treatment temperature increased. This indicated that the extract had a positive effect on deepening the color of TMW, which was significant at 180°C temperatures. Only hemicellulose of wood underwent slight degradation among the three major components of wood at 180°C, with slight impact on wood color (Mehrotra et al. 2010; Ling et al. 2016). At this time, the change of the extract has a significant impact on the color of the TMW. Severe degradation of hemicellulose at temperatures above 200°C leaded to an increase in the content of lignin containing many chromogenic and auxochrome groups, which was the main reason for wood discoloration (Huang et al. 2010). At this point, the impact of the extract was not significant.

3.2. Effects of extract on the dimensional stability of thermally modified wood

3.2.1 Analysis of water contact angle and moisture absorption of extracted or unextracted TMW

The water contact angle (°) of the cross-section of wood significantly increased after thermal modification at 180°C, 200°C, and 220°C (Fig. 3). The water contact angles of TMP and TMS increased from 73.9° and 50.2° to over 100° at 60 s. The extracts had no significant effect on the hydrophobic angle of TMP, and the slight differences at different temperatures might be due to experimental errors.

The moisture absorption of poplar and spruce wood can be reduced by thermal modification under 180°C, 200°C and 220°C, especially at 200°C and 220°C (Fig. 4). The average moisture absorption of TMP and TMS samples at 220°C during the moisture absorption analysis cycle decreased from 10.8% and 10.9% (original wood) to 6.9% and 7.4%, respectively. The moisture absorption of TMP and TMS gradually decreased with the increase of thermal modification temperature, indicating that thermal modification will reduce the moisture absorption of wood regardless of whether the extract was removed. This is consistent with previous reports (Metsa-Kortelainen et al. 2006).

The hygroscopicity of poplar or spruce wood thermally modified at 180°C and 200°C was decreased induced by the removal of extract, while the hygroscopicity of 220°C TMP or TMS was increased by the removal of extractives. The effect of extracts on the moisture

absorption of TMS ($P$<0.05) was more significant than that of TMP ($P$ 0.05), and the hygroscopicity of the 180°C and 200°C TMS after removal of the extract decreased from 10.1% to 8.9% and 8.6% to 7.4%, respectively (Fig. 4D and E). This might be due to the different reactions in the thermal modification process caused by the different hemicellulose, lignin and extracts of spruce and poplar wood. For example, the mass loss after thermal modification and acetone extract yield of 180°C TMS (1.8% and 3.1%) were higher than those of TMP (1.2% and 2.8%), while acetone is a highly polar solvent and its extract is hydrophilic (Smeds et al. 2021; Korenman et al. 2010). The lower moisture absorption of TWS might be due to the removal of more acetone extracts with hydrophilic.

The results indicated that the removal of 180°C or 200°C TMS extracts would reduce the hygroscopicity of the TMS. The spruce thermally modified at lower temperatures (180°C and 200°C) can achieve the same hygroscopicity as the spruce thermally modified at higher temperatures by removing the extract.

3.2.2 Analysis of anti-swelling efficiency of extracted or unextracted TMW

The ASE indicates the ability of wood to resist deformation in high humidity environments, and a larger value indicates better dimensional stability. The average ASE of poplar wood can be improved by thermal modification at 180°C, 200°C, and 220°C (Fig. 5A), while the ASE of spruce wood can be improved by thermal modification at 200°C and 220°C (Fig. 5B). The ASE of wood thermally modified at 180°C and 200°C was improved after removing its extract, while the ASE of wood thermally modified at 220°C was reduced after removing its extract ($P$<0.05). This result was consistent with the conclusion of hygroscopicity and might be related to the different reactions of the main components of wood at different temperatures. The relative content of free hydroxyl groups in TMW and its acetone extracts at different temperatures was investigated to elucidate this result.

3.3. FTIR and XPS analysis of the extracted or unextracted TMW

3.3.1 FTIR analysis of the extracted or unextracted TMW

FTIR and XPS were used to analyze the free hydroxyl relative content of TMW with or without extraction at different temperatures. The characteristic peak intensities of 180°C and 220°C TMW decreased after extraction, especially for 220°C (Fig.6). The absorption peak at 3670-3230 $cm^{-1}$ was assigned to stretching vibration of hydroxyl groups, and the narrowing of the peak or shift towards a lower wavenumber indicated an increase in free hydroxyl groups (Gadhave et al. 2019). The hydroxyl stretching vibration peaks of 180°C TMP (3401 $cm^{-1}$) and TMS (3435 $cm^{-1}$) after removing the extract shifted towards low wavenumbers (3381 $cm^{-1}$ and 3389 $cm^{-1}$). This indicated that the removal of the extract at this temperature reduced the free hydroxyl content of the TMW, leading to a decrease of its hygroscopicity and an increase of its ASE. It was speculated that the acetone extract might have stronger hydrophilicity, and the free hydroxyl content of TMW decreased with the removal of the extract.

The hydroxyl stretching vibration peaks of 220°C TMP (3367 $cm^{-1}$) and TMS (3348 $cm^{-1}$) shifted towards high wavenumbers (3397 $cm^{-1}$ and 3356 $cm^{-1}$) with the removal of the extract, indicating that the free hydroxyl content of TMW increased due to the removal of the extract at this temperature. This was one of the reasons for the increase of moisture absorption and decrease of ASE of 220°C TMP after removing the extract. The characteristic peak at 1111 $cm^{-1}$ was assigned to the stretching vibration of the associating hydroxyl or glucose ring in cellulose (Lin et al. 2018). The weakening of the peak at 1111 $cm^{-1}$ of 220°C TMW after removing the extract indicated that removing the extract at this temperature will increase the free hydroxyl groups in the wood, leading to an increase in hygroscopicity. This might be due to the intense degradation of hemicellulose and the slight degradation of lignin at 220°C, and more free hydroxyl groups were exposed after the removal of extract.

3.3.2 XPS analysis of the extracted or unextracted TMS

The removal of extracts had a significant impact on the 180°C and 220°C TMS, and XPS was used to analyze the chemical composition of its surface (Fig.S1 and Table 1). The carbon spectrum (C1s) was de-convoluted into $C_1$, $C_2$, $C_3$, and $C_4$ peaks with $\sum X^2$ less than 40 (Fig. S1). The $C_1$, $C_2$, $C_3$, and $C_4$ peaks corresponded to C-C/C=C/C-H, C-O/C-O-C, -C=O/O-C-O, and O-C=O/C(=O)-OH with binding energies (BE) of 284.6 eV, 286.4 eV, 287.8 eV, and 289.2 eV, respectively (Acheampong et al., 2018; Huang et al., 2019). $C_1$ is mainly attributed to lignin and extracts of wood, such as fatty acids, fats, and waxes. $C_2$ and $C_3$ are mainly attributed to the hydroxyl of hemicellulose and cellulose and the carbonyl of hemicellulose and lignin, respectively. $C_4$ is mainly attributed to the ester and carboxyl groups of hemicellulose and extract (Lyu et al. 2019; Sernek et al. 2004; Ozen et al. 2014). The relative percentages of $C_1$ in 180°C TMS decreased compared to original spruce, which was attributed to the loss of extracts of spruce such as fatty acids, resin acids, and glycerol esters (Inari et al. 2011; Popescu et al. 2009). The relative percentages of C1 in 220°C TMS was higher than that of 180°C TMS, which was attributed to the degradation of hemicellulose led to a decrease in the relative percentages of $C_2$. The relative percentages of $C_4$ in spruce treated at 180°C and 220°C

increased, indicating the production of extracts containing ester and carboxyl groups after thermal modification. These extracts might have antifungal activity, making a partial contribution to improving the decay resistance of TMW.

The relative percentages of $C_2$ in 180°C TMS was decreased after removing the extract, indicating that there might be free hydroxyl groups in the extract of wood thermally modified at this temperature. The hydroxyl percentages of the TMW at 180°C decreased with the removal of the extract, resulting in a decrease in hygroscopicity. The relative percentages of $C_2$ in 220°C TMS was lower than that of the original spruce wood, while it decreased after removing the extract. This indicated that the hydroxyl content of the extract was lower at high temperatures, but it will expose more hydroxyl groups of TMW after removal of the extract, leading to an increase in hygroscopicity. This was consistent with the conclusion of the section 3.3.1. In addition, the relative percentages of $C_4$ in 180°C and 220°C TMS after removing the extract significantly decreased, indicating that the newly production extract that may have antifungal activity was extracted, which might lead to a decrease in wood decay resistance.

**Table 1** The relative percentages (%) with corresponding theoretical binding energies and bond types ($C_1$, $C_2$, $C_3$, $C_4$) for XPS scans of the C1s region of the original spruce, TMS and extracted TMS

| Groups | $C_1$ | $C_2$ | $C_3$ | $C_4$ |
|---|---|---|---|---|
| | C-C/C=C/C-H | C-O/C-O-C | O-C-O/C=O | O-C=O/C(=O)-OH |
| Original spruce | 55.5 | 26.4 | 11.1 | 7.0 |
| 180°C TMS | 39.1 | 35.6 | 10.5 | 14.8 |
| Extracted 180°C TMS | 52.7 | 34.9 | 7.1 | 5.4 |
| 220°C TMS | 50.4 | 25.9 | 12.4 | 11.4 |
| Extracted 220°C TMS | 45.9 | 30.1 | 14.5 | 9.5 |

3.4. Effects of extract on the decay resistance of thermally modified wood

The decay resistance of poplar and spruce wood to brown-rot fungi (*G. trabeum*) or white-rot fungi (*T. versicolor*) can be significantly improved by thermally modified at 200°C or 200°C and 220°C, respectively (Fig. 7). The decay resistance of the 180°C, 200°C and 220°C TMW were decreased after extracted, and the degree of decrease weakened with the temperature increasing.

The mass loss of 220°C TMP and TMS exposure to *T. versicolor* after 15 weeks of incubation was 14.0% and 3.5% (Fig. 7 A and C), significantly lower than the mass loss of original wood (39.0% and 24.0%). In addition, their mass loss exposure to *G. trabeum* after 15 weeks of incubation was 0.9% and 0.4% (Fig. 7 B and D), significantly lower than the mass loss of original wood (34.8% and 12.5%). The extract had inconspicuous effect on the decay resistance of TMW at this temperature. Some studies have indicated that the improvement in decay resistance of wood thermally modified at 220°C might be due to the combined effect of the degradation of three major nutrients leading to loss of nutrient sources, decreased hygroscopicity, formation of pseudo-lignin and antifungal active compounds (Jiang et al. 2022; Mohammed et al. 2006). For example, study has indicated that the moisture content and holocellulose of 230°C TMW (*Pinus spp.*, *Fraxinus mandshurica*, and *Quercus mongolica*) was significant decrease compared to 180°C TMW, which is only half of that of untreated wood (Li et al. 2009).

The decay resistance of poplar and spruce wood to *G. trabeum* fungi can be significantly improved by thermally modification at 200°C, but their decay resistance to *T. versicolor* cannot be improved. The positive effect of pseudo-lignin on the decay resistance of TMW to brown-rot fungi and little effect to white-rot fungi might be related to it (Jiang et al. 2022). In addition, hemicellulose and lignin were partially degraded at 200°C, and brown-rot fungi might lose more nutrients than white-rot fungi. The removal of extract had no significant effect on the decay resistance of 200°C TMW ($P$ 0.05), except for the decay resistance of TMP to *G. trabeum*. The mass loss of 200°C TMP after extraction exposed to *T. versicolor* and *G. trabeum* increased from 41.4% and 2.3% to 44.8% and 10.2%, respectively. The extract with antifungal activity was only one of the reasons contributing to the improvement of decay resistance of 200°C and 220°C TMW, and the role of extract needs to be further study.

Thermal modification of poplar and spruce wood at 180°C cannot effectively improve their decay resistance. The removal of extract had a significant impact on the decay resistance of 180°C TMP against *T. versicolor* and TMS against *G. trabeum* ($P$<0.05). The mass loss of TMP after extraction exposed to *T. versicolor* and *G. trabeum* increased from 32.5% and 16.9% to 47.4% and 22.2%, respectively. In addition, the mass loss of TMS after extraction exposed to *G. trabeum* increased from 14.8% to 27.1%, respectively. Jiang et al. (2022) indicated that

the pseudo-lignin produced by hydrothermal treatment of wood at 180°C was minimal and insufficient to affect the decay resistance of TMW. The degradation of the three major nutrients was weaker at this temperature and there was no significant improvement in hygroscopicity (Fig. 4). The results indicated that the extracts of 180°C TMP and TMS can significantly improve the decay resistance of 180°C TMW, and the extract played an important role in the decay resistance of TMW at this temperature.

3.5. Effect of thermally modified wood extracts on fungi growth

In order to further analyze the effect of TMW extracts on the decay resistance of TMW, the antifungal activity of 180°C, 200°C and 220°C TMW extracts was analyzed. The brown-rot fungi (*G. trabeum*) or white-rot fungi (*T. versicolor*) grew well on the non-amended control PDA, completely covering the agar over the 8-day incubation period. The average colony diameter (mm) of both fungi grown on PDA amended with 16 ,12 and 8 mg/mL extracts were shown in Fig. 8, Table S1 and Table 2. Both fungi grown on PDA amended with ethanol completely covered the agar over the 21-day incubation period. The average colony diameter of both fungi grown on PDA amended with 16 mg/mL extracts of 180°C, 200°C and 220°C TMW (TMP and TMS) were not increase (Fig. 8), indicating that all extracts at this concentration can completely inhibit the growth of wood decay fungi.

8 mg/mL extract of 220°C TMS cannot completely inhibit the growth of wood decay fungi, but their growth was slower than the control (Table S1). The growth of both tested fungi can be completely inhibited by other TMP and TMS extracts at different temperatures. Both tested fungi cannot be inhibited by 4 mg/mL extract of 180°C, 200°C and 220°C TMP and TMS (Table 2). However, their growth was slower that control and solvent control. The results indicated that the acetone extracts of TMW treated at 180°C, 200°C and 220°C showed satisfactory antifungal activity against wood decay fungi, and their growth was completely inhibited at 16 mg/mL. There was no significant difference in the antifungal activity of extracts from TMW treated at different temperatures. Ethanol extract of *Forsythia suspensa*, the essential oils of *Betula pendula* and *Syzygium aromaticum*, and water extract of teak (*Tectona grandis* L. f.) were reported to inhibit the growth of wood decay fungi at a concentration of 30 mg/mL, 10%, and 20 mg/mL, respectively (Zhao et al., 2021; Pánek et al. 2014; Brocco et al., 2017;). This result indicated that TMW extracts might have more potential as wood preservatives.

**Table 2** Average colony diameters (mm) of wood decay fungi grown on media containing 4 mg/mL of thermally modified poplar and spruce wood extracts at different temperatures (mm)

| Fungus | Extract | Temperature (°C) | Colony diameters (mm)[a] | | | |
|---|---|---|---|---|---|---|
| | | | 6 days | 8 days | 14 days | 21 days |
| White-rot fungi | Control | | 60.0 | 60.0 | 60.0 | 60.0 |
| *T. versicolor* | Ethanol | | 60.0 | 60.0 | 60.0 | 60.0 |
| | TMP extract | 180 | 21.7±1.9 | 31.1±1.4 | 60.0 | 60.0 |
| | | 200 | 19.4±0.7 | 31.9±1.1 | 60.0 | 60 |
| | | 220 | 25.3±3.8 | 34.9±6.6 | 60.0 | 60 |
| | TMS extract | 180 | 20.2±4.7 | 32.1±5.5 | 60.0 | 60 |
| | | 200 | 36.6±2.4 | 60.0 | 60.0 | 60.0 |
| | | 220 | 29.5±1.0 | 50.8±1.2 | 60.0 | 60.0 |
| Brown-rot fungi | Control | | 38.3±2.0 | 60.0 | 60.0 | 60.0 |
| *G. trabeum* | Ethanol | | 36.5±0.8 | 60.0 | 60.0 | 60.0 |
| | TMP extract | 180 | 16.9±0.6 | 16.6±1.4 | 30.1±4.4 | 45.6±3.7 |
| | | 200 | 24.7±3.4 | 25.7±4.7 | 50.3±2.5 | 60.0 |
| | | 220 | 24.1±1.4 | 25.5±0.5 | 60.0 | 60.0 |
| | TMS extract | 180 | 13.5±0.3 | 22.9±1.5 | 47.7±11.8 | 60.0 |
| | | 200 | 13.4±0.9 | 31.1±2.8 | 60.0 | 60.0 |
| | | 220 | 16.1±0.9 | 31.9±1.6 | 51.9±7.7 | 60.0 |

[a] The initial diameter of the fungal inoculum was 4.4 mm; The diameter of the petri dish was 60 mm; Values represent means of 3 replicates/fraction/ fungus

3.6. Chemical composition of extracts from thermally modified poplar and spruce wood

The primary components (top ten compounds) of original wood (Table S2) and 180°C, 200°C and 220°C TMW in the resulting GC-MS chromatograms were listed based on peak area (Table 3 and Table 4). The compound with the highest relative content (reference only) of acetone extract from poplar sapwood was 4H-1-Benzopyran-4-one, 2,3-dihydro-5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-, (S)-, followed by Naringenin, and the other compounds were various, including esters, organic acids, and alcohols. The extract of spruce sapwood contained 25% of 4'-methoxy-neneneea-2-hydroxystilbene, 8.9% of 2-(4-methylphenyl) benzoic acid, and other compounds were esters and phenols. The antifungal activity of these compounds against wood decay fungi has not been reported.

Aromatic aldehyde and aromatic acid compounds such as vanillin, syringaldehyde (benzaldehyde, 4-hydroxy-3,5-dimethoxy-), syringic acid (benzoic acid, 4-hydroxy-3,5-dimethoxy-), 4-ethoxybenzoic acid (benzoic acid, 4-hydroxy-), and isovanillic acid (3-hydroxy-4-methoxybenzoic acid) appeared in the acetone extract of poplar wood after thermal modification (Table 3). The vanillin, syringaldehyde, syringic acid, and isovanillic acid has been proved to show antifungal activity against wood decay fungi (Bi et al. 2021a; Esteves et al. 2008; Bi et al. 2022). These compounds provided antifungal activity for TMW extracts. The relative content of aromatic aldehydes and aromatic acids increased and then decreased with the increase of thermal modification temperature, and the content of aromatic acids was gradually higher than that of aromatic aldehydes.

**Table 3** Compounds identified in acetone extracts of TMP and their relative content (%)

| 180 | | | 200 | | | 220 | | |
|---|---|---|---|---|---|---|---|---|
| Rt/min | Compounds | Area% | Rt/min | Compounds | Area% | Rt/min | Compounds | Area% |
| 4.28 | Phenol | 51.6 | 4.29 | Phenol | 22.8 | 4.28 | Phenol | 8.3 |
| 9.19 | Vanillin | 3.3 | 14.56 | Benzoic acid, 4-hydroxy-3,5-dimethoxy- | 15.0 | 14.38 | Benzoic acid, 4-hydroxy-3,5-dimethoxy- | 7.2 |
| 12.51 | Benzaldehyde, 4-hydroxy-3,5-dimethoxy- | 2.8 | 12.54 | Benzaldehyde, 4-hydroxy-3,5-dimethoxy- | 9.1 | 15.24 | Ethyl phosphoric acid, di-TMS derivative | 5.9 |
| 10.47 | Methyl (3,4-dimethoxyphenyl) (hydroxy)acet | 2.4 | 11.24 | 3-Hydroxy-4-methoxybenzoic acid | 7.9 | 22.99 | Cyclononasiloxane, octadecamethyl- | 4.5 |
| 10.20 | Benzoic acid, 4-ethoxy- | 2.2 | 10.39 | Benzoic acid, 4-hydroxy- | 7.5 | 12.51 | Benzaldehyde, 4-hydroxy-3,5-dimethoxy- | 4.3 |
| 13.66 | 5-Methoxy-2-nitrobenzoic acid | 1.8 | 22.02 | 1,2-Benzenedicarboxylic acid, diisooctyl este | 3.7 | 16.08 | n-Hexadecanoic acid | 4.2 |
| 13.52 | 2-Propenal, 3-(4-hydroxy-3-methoxyphenyl) | 1.5 | 16.53 | 3,5-Dimethoxy-4-hydroxycinnamaldehyde | 3.5 | 24.74 | Epoxy-linalooloxide | 3.4 |
| 12.31 | Coniferyl aldehyde, methyl ether | 1.5 | 13.5 | Ethanone, 1-(4-hydroxy-3,5-dimethoxypheny | 3.3 | 13.94 | Benzoic acid, 4-hydroxy-3,5-dimethoxy-, hy | 3.0 |
| 16.51 | 3,5-Dimethoxy-4-hydroxycinnamaldehyde | 1.4 | 9.19 | Vanillin | 3.3 | 18.10 | Isotridecyl alcohol, trimethylsilyl derivative | 3.0 |
| 16.07 | n-Hexadecanoic acid | 1.4 | 10.21 | beta.-D-Glucopyranose, 1,6-anhydro- | 2.7 | 22.01 | Di-n-octyl phthalate | 2.5 |

The compounds with antifungal activity such as isovanillic acid, vanillin, syringic acid and syringaldehyde appeared in the acetone extract of spruce wood after thermal modification, which was the same as that of poplar (Table 4). The main compounds of TMS acetone extract were isovanillic acid and vanillin, while the TMP extract was mainly syringic acid. This was related to the lignin types of softwood (mainly guaiacyl propane) and hardwood (mainly guaiacyl and syringa oblata propane). The relative content of aromatic aldehyde and aromatic acid compounds increased and then decreased with the increase of thermal modification temperature. The results indicated that the antifungal activity compounds from lignin degraded such as isovanillic acid, vanillin, syringic acid, and syringaldehyde might be the source of antifungal activity of TMW extracts.

**Table 4** Compounds identified in acetone extracts of TMS and their relative content (%)

| 180 | | | 200 | | | 220 | | |
|---|---|---|---|---|---|---|---|---|
| Rt/min | Compounds | Area% | Rt/min | Compounds | Area% | Rt/min | Compounds | Area% |
| 4.29 | Phenol | 25.0 | 4.29 | Phenol | 25.0 | 11.45 | 3-Hydroxy-4-methoxybenzoic acid | 22.3 |
| 11.26 | 3-Hydroxy-4-methoxybenzoic acid | 11.2 | 11.26 | 3-Hydroxy-4-methoxybenzoic acid | 17.4 | 10.60 | D-Allose | 15.3 |
| 9.21 | Vanillin | 11.0 | 9.2 | Vanillin | 8.8 | 4.29 | Phenol | 9.4 |
| 19.88 | 1,1'-Biphenyl, 2,2'-dimethyl-6,6'-dinitro- | 6.6 | 10.21 | Ethanone, 1-(4-hydroxy-3-methoxyphenyl)- | 5.1 | 9.22 | Vanillin | 5.3 |
| 13.54 | 2-Propenal, 3-(4-hydroxy-3-methoxyphenyl) | 4.3 | 9.67 | beta.-D-Glucopyranose, 1,6-anhydro- | 3.8 | 9.94 | beta.-D-Glucopyranose, 1,6-anhydro- | 4.9 |
| 11.55 | Vanilic acid hydrazide | 2.3 | 14.45 | 3-Hydroxy-4,5-dimethoxybenzoic acid | 2.8 | 14.57 | Benzoic acid, 4-hydroxy-3,5-dimethoxy- | 4.2 |
| 10.22 | Ethanone, 1-(4-hydroxy-3-methoxyphenyl)- | 2.0 | 25.15 | 17-Methylandrosta-5,7,9(11)-trien-17-ol acet | 2.1 | 13.56 | 2-Propenal, 3-(4-hydroxy-3-methoxyphenyl) | 3.9 |
| 14.45 | Benzoic acid, 4-hydroxy-3,5-dimethoxy- | 1.7 | 10.31 | Benzoic acid, 4-hydroxy- | 2.0 | 9.87 | Phenol, 4-ethyl-2-methoxy- | 3.7 |
| 10.48 | Methyl (3,4-dimethoxyphenyl) (hydroxy)acet | 1.7 | 13.53 | 2-Propenal, 3-(4-hydroxy-3-methoxyphenyl) | 1.8 | 11.74 | 1,6-Anhydro-.alpha.-d-galactofuranose | 2.4 |
| 23.41 | 1,1'-Biphenyl-3,4,4'-trimethoxy-6'-formyl- | 1.6 | 12.52 | Benzaldehyde, 4-hydroxy-3,5-dimethoxy- | 1.5 | 10.23 | Ethanone, 1-(4-hydroxy-3-methoxyphenyl)- | 2.1 |

# 4. Conclusion

The effect of acetone extracts of thermally modified poplar and spruce wood on the color, dimensional stability, and decay resistance of thermally modified wood was studied. The results suggest the extract has a positive effect on the decay resistance, color, and dimensional stability (220°C) of thermally modified wood. The brightness difference $\Delta L^*$ and total color difference $\Delta E^*$ of TMW decreased with the extract removed, and this effect weakened with the increase of treatment temperature, significantly at 180°C. The dimensional stability of 180°C and 200°C TMW was improved due to the removal of the extract. The moisture absorption and anti-swelling efficiency of extracted 180°C TMP and TMS were increased from 20.6% and -6.9% to 34.2% and 14.1%, respectively. The removal of extracts will reduce the decay resistance of TMW. The mass loss of 180°C TMP exposed to white-rot fungi and brown-rot fungi increased from 32.5% and 16.9% to 47.4% and 22.2% after removing the extract, respectively. The extract showed satisfactory antifungal activity against wood decay fungi, and the compounds with antifungal activity such as vanillin, isovanillic acid, syringaldehyde and syringic acid in the extract played an important role.

# Declarations


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### Compliance with ethical standards

**Confict of interest** No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication.

### CRediT authorship contribution statement

**ZJ Bi:** Conceptualization, Methodology, Investigation, Writing - Original Draft, and Visualization. **XJ Zhou:** Writing – review & editing and Methodology. **J Chen:** Investigation (section 2.3 and 2.7) and Data Curation. **YF Lei:** Supervision and Validation. **L Yan:** Resources,

Supervision, Funding acquisition, Project administration. All authors have read and agreed to the published version of the manuscript.

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## Figures

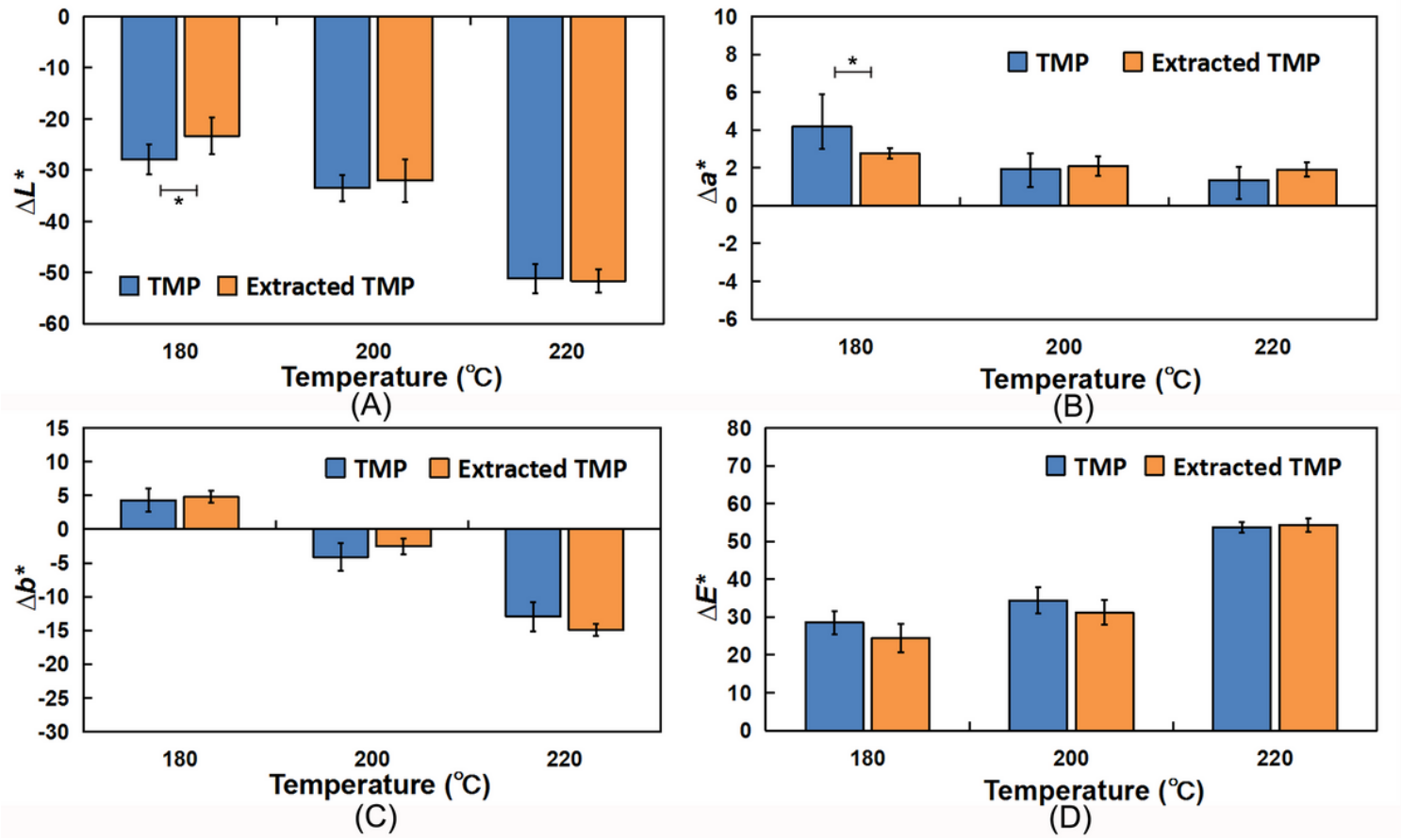


**Figure 1**

The lightness difference *ΔL** (A), red-green axis chromaticity index difference *Δa** (B), yellow-blue axis chromaticity index difference *Δb** (C), and the total color difference *ΔE** (D) of extracted/unextracted poplar wood thermally modified at 180°C, 200°C and 220°C

Note: ns means no significant difference, * means significant difference ($P$<0.05), and ** means extremely significant difference ($P$<0.01)

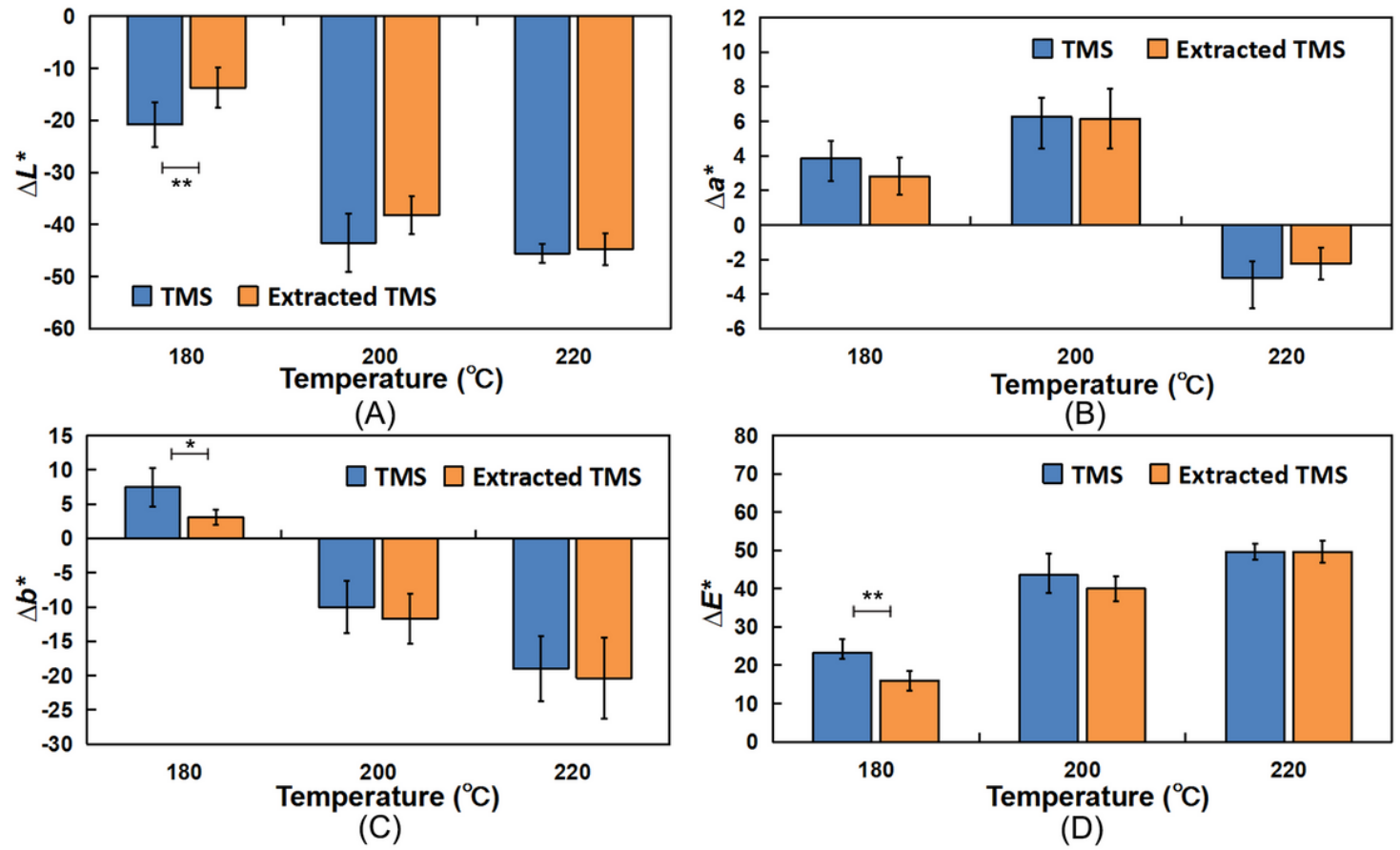


Figure 2

The *ΔL** (A), *Δa** (B), *Δb** (C), and *ΔE** (D) of extracted/unextracted spruce wood heat-treated at 180°C, 200°C and 220°C

Note: ns means no significant difference, * means significant difference ($P<0.05$), and ** means extremely significant difference ($P<0.01$)

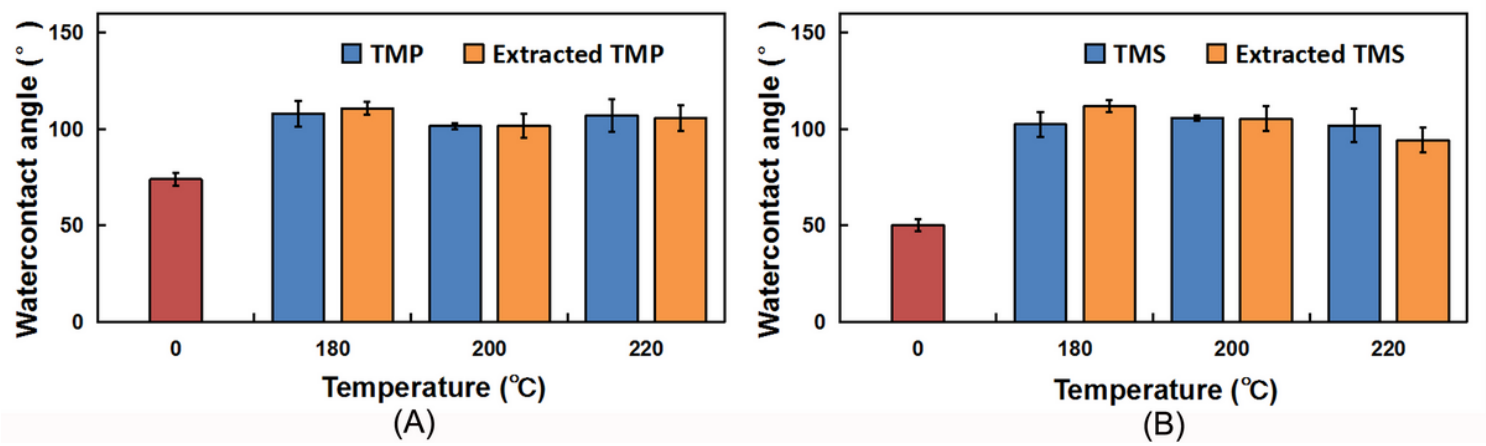


Figure 3

Water contact angle (°) at 60 s of cross-section of extracted/unextracted poplar (A) and spruce (B) thermally modified at 180°C, 200°C and 220°C

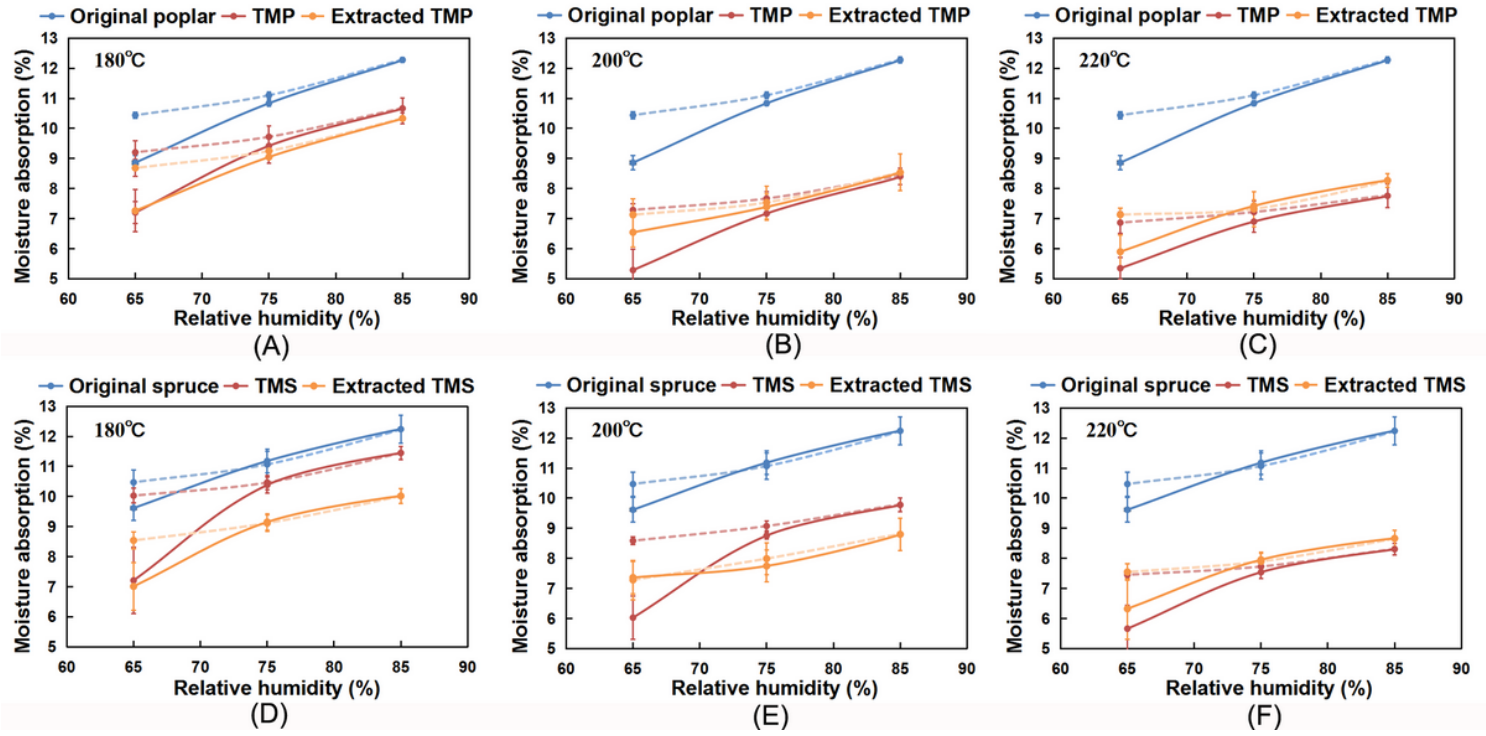


Figure 4

Moisture absorption (%) of extracted/unextracted poplar (A, B, C) and spruce (D, E, F) thermally modified at 180°C (A, D), 200°C (B, D) and 220°C (C, F) during hygroscopic desorption cycle

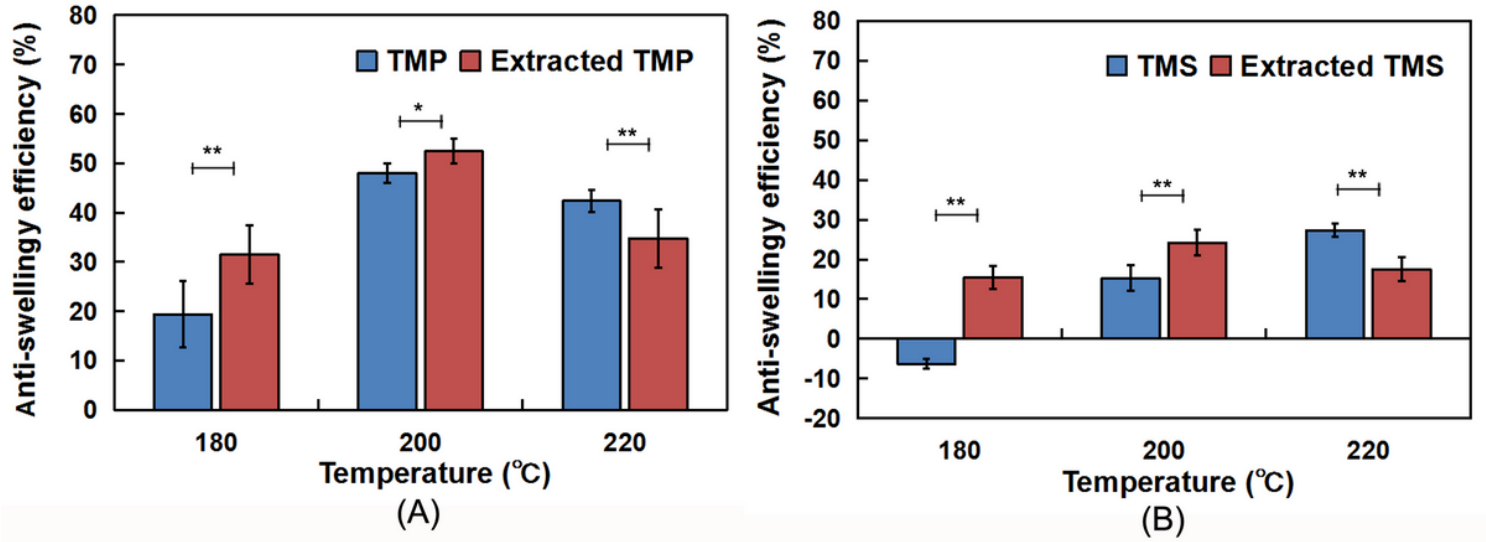


Figure 5

The average ASE of extracted or unextracted poplar (A) and spruce (B) wood thermally modified at 180°C, 200°C, and 220°C during hygroscopic process

Note: ns means no significant difference, * means significant difference ($P$<0.05), and ** means extremely significant difference ($P$<0.01)

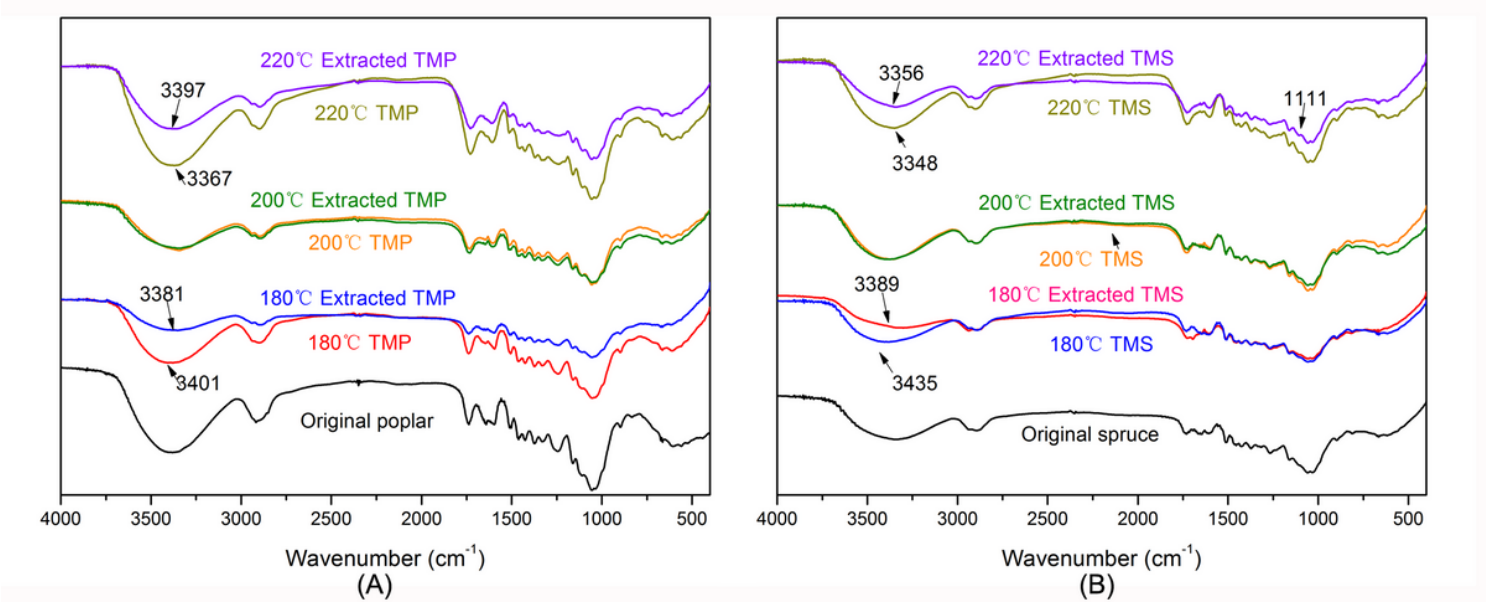


Figure 6

The FTIR spectrum of extracted/unextracted poplar (A) and spruce (B) wood thermally modified at 180°C, 200°C, and 220°C

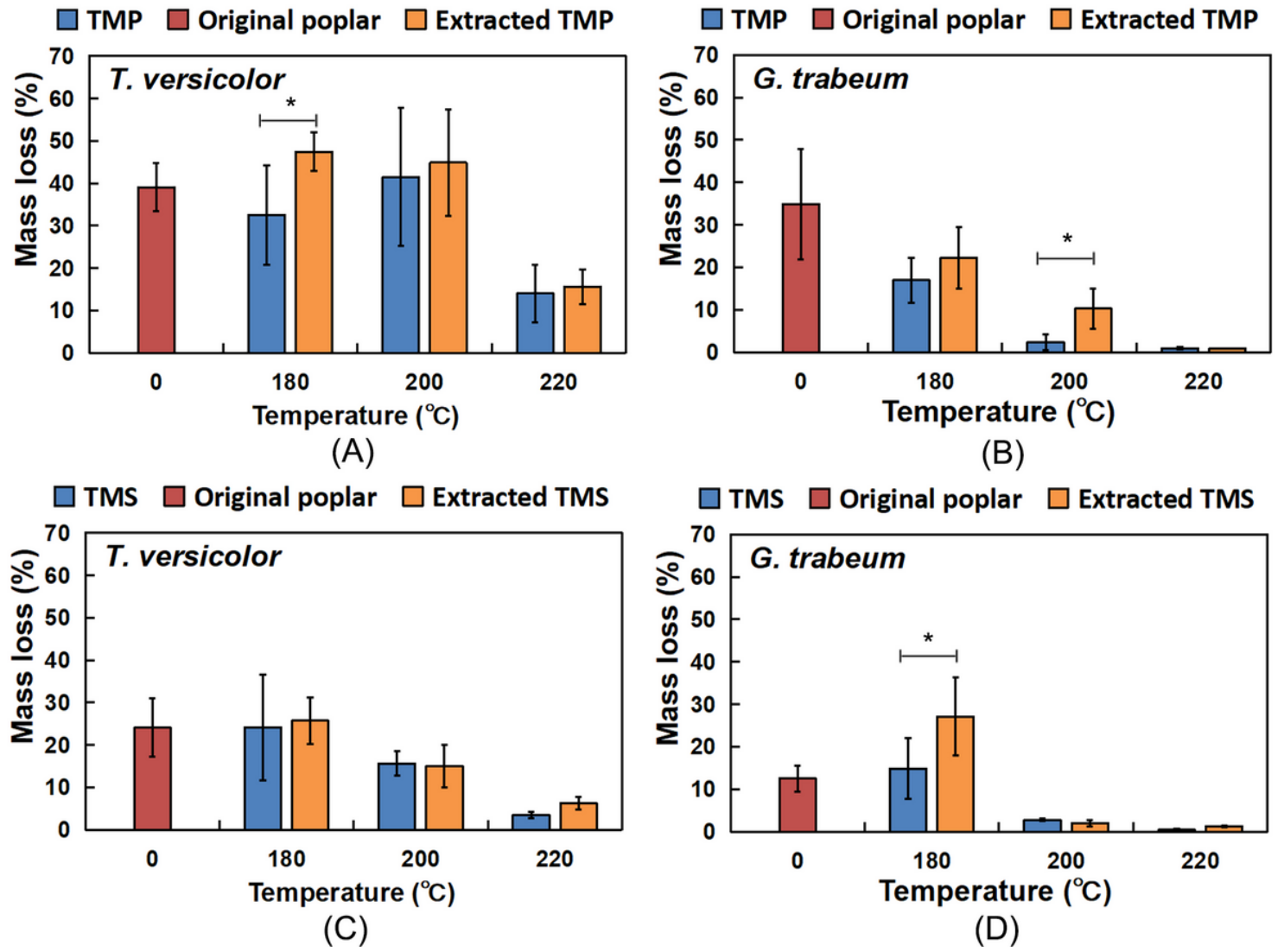


Figure 7

The mass loss of extracted/unextracted poplar (A, B) and spruce (C, D) wood thermally modified at 180°C, 200°C, and 220°C exposed to *T. versicolor* and *G. trabeum*

Note: ns means no significant difference, * means significant difference ($P$<0.05), and ** means extremely significant difference ($P$<0.01)

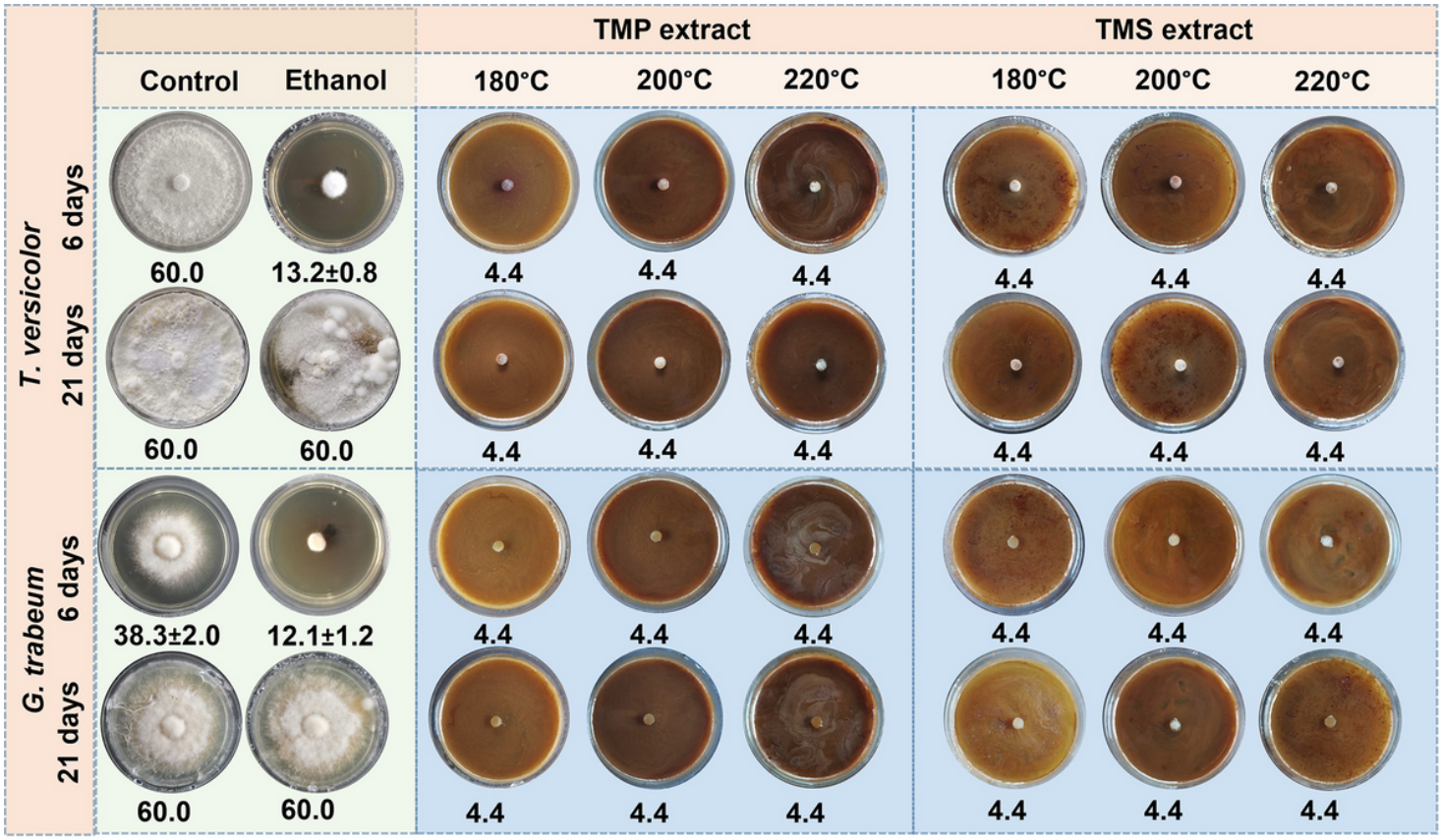


Figure 8

Photos and average colony diameters (mm) of wood decay fungi grown on media amended with 16 mg/mL of thermally modified poplar and spruce wood extracts at different temperatures

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.docx
- TableS2.docx
- Supplementarymaterial.docx