

# Effect of Hydrothermal Treatment with Different Aqueous Solutions on the Mold Resistance of Moso Bamboo with Chemical and FTIR Analysis

Dali Cheng, Shenxue Jiang, and Qisheng Zhang \*

Bamboo has received increased attention as a biomass material because it is fast growing and has good mechanical properties. But bamboo is very vulnerable to mold fungi, which greatly limits its applications. In this paper, bamboo was firstly hydrothermally treated at 140 °C by three different treatments: with water only, NaOH, and NaAc aqueous solution, then heat treated at relatively mild conditions (180 °C). Subsequently, the mold resistance of bamboo before and after the two-step heat treatment was investigated. The mechanism of mold resistance was analyzed by a bamboo chemical component analysis, FTIR spectroscopy. The results showed that strong degradation of hemicelluloses by heat treatment could inhibit mold growth to some extent. Moreover, the modification of lignin and the creation of phenolic compounds in the bamboo could prevent or slow down fungal growth.

*Keywords:* Moso bamboo; Mold resistance; Two-step heat treatment; Chemical analysis; FTIR

*Contact information:* Bamboo Engineering Research Center, College of Wood Science and Technology, Nanjing Forestry University, Nanjing, 210037, P.R. China; \*Corresponding author: zhangqs@njfu.com.cn

## INTRODUCTION

The world is facing a rapid decrease of forest resources, and the environment is suffering serious deterioration. Therefore, the development and exploitation of bamboo is of considerable importance. Bamboo is an important forest resource that grows abundantly in many tropical and subtropical regions of the world, especially in Asia. As a fast-growing material, bamboo has been widely used as a traditional material to make basic tools and furniture as well as a building material, due to its strength, surface hardness, and easy machinability.

In the 1980s, bamboo materials, such as bamboo curtain plywood, bamboo laminated board, reconstituted bamboo lumber, *etc.*, were successfully developed in China. However, like other biological materials, the main problem facing bamboo and bamboo products is the susceptibility of the material to attack by decay fungi and molds. The main bamboo cell wall components are similar to those of wood. Bamboo is primarily composed of the polymeric components cellulose, hemicellulose (mainly pentosan), and lignin, but the hemicellulose and lignin are quite different from those derived from wood. The hemicellulose structures of bamboo have been described as an intermediate between those of softwoods and hardwoods, and lignin from bamboo is characterized as typical gramineae lignin, composed of three phenylpropane units, p-coumaryl, coniferyl, and sinapyl alcohols (Fengel and Shao 1985; Liese 1987). Bamboo, in general, is chemically different from wood; in particular, it has a high content of sugar, starch, and protein (Sun *et al.* 2012), thereby possibly presenting degradation behavior that is distinct from that of wood. Mildew is apt to attack bamboo under high humidity

conditions. Mold growth on building materials not only causes aesthetic problems, but may also have negative health impacts. When mold spores are present in large quantities in the air, they may cause allergic reactions, asthma episodes, infections, and other respiratory problems (Dales *et al.* 1991; Yang *et al.* 2007a).

Traditionally, there are two types of approaches to bamboo mold prevention: physical methods, including high-temperature sterilization and immersion, and chemical modification with a mold-resistant agent. Physical methods limit insect and fungus attacks, but cannot solve general mold problems. Preservatives and antiseptics used for bamboo are toxic substances that check insect attack and the growth of fungus. However, the chemicals that kill the spores may be poisonous to human beings (Chen *et al.* 2012). In recent years, environmental issues have become more and more sensitive to society. Therefore, the ideal preservation for bamboo should have the following characteristics: environmentally friendly, stable, easily available, and low in toxicity.

High temperature heat treatment has been known for a long time as an effective method to modify the properties of wood (Seborg *et al.* 1953; Stamm 1956; Hillis 1984). The two-step heat treatment process involves a hydrothermolysis step with a successive high temperature step, and was found to be more efficient than a one-step heat treatment, with respect to improving the resistance to fungal attack. It has been shown that the wood's chemical composition plays an important role in mold growth during heat treatment (Tjeerdsma *et al.* 1998). FTIR spectroscopy is effective at analyzing the chemical structure of wood as a whole (Faix 1988) and has been widely used in thermal modification treatments (Pandey 1999; Hakkou *et al.* 2005; Boonstra and Tjeerdsma 2006).

Two-step heat treatment combines the above-mentioned physical and chemical methods of creating mold resistance and more easily provokes transformations of the chemical structure (autocatalytic reactions) in the aqueous solution, and consequently influences the properties of bamboo. Under hydrothermal treatment in aqueous conditions, hemicellulose (the most reactive component) is hydrolyzed into oligomeric structures and monomeric compounds (Carrasco and Roy 1992; Tjeerdsma and Militz 2005). During hydrothermal treatment conditions, acetic acid is formed as a result of cleavage of the acetyl groups of hemicelluloses and the pH of the solution is decreased to 3.5-4.5 (Fengel and Wegener 1984). Subsequently, partial hydrolysis of polysaccharides (especially hemicelluloses) and functional groups of lignin to hydrolysates leads to easier dissolution under the acidic conditions. It is thus evident that pH values of the treated solution have some influence on the properties of materials.

Research has determined the mechanical properties of materials by solution treatment. Bending tests and X-ray diffraction studies have been conducted on wood samples treated with various concentrations of inorganic and organic aqueous solutions to investigate the influence of solution treatment on the mechanical properties and the cellulose structure (Nakano *et al.* 2000; Ishikura *et al.* 2010; Ishikura 2011). However, reports on the influence of solution treatment on mold are very rare in the literature (Sun *et al.* 2006). Moreover, reports on chemical changes of bamboo in different aqueous solutions with high-temperature heat treatment and the relationship of the aqueous solution with mold are rare.

Two-step heat treatment was applied to bamboo modification in this study. The aim was to improve bamboo's resistance to mildew using a two-step heat treatment, to study the chemical transformations of the cell wall components during hydrothermal

treatment in different environments (water only, NaOH, and NaAc solution) and to theoretically demonstrate the correlation of these changes with bamboo mildew.

## EXPERIMENTAL

### Sample Preparation

Moso bamboo was obtained from a bamboo plantation in Anji, Zhejiang, China. The bamboo material was split lengthwise into strips with a thickness of 5.0 mm and a width of 20 mm. The length of the strip was approximately 220 mm. The moisture content of the specimens before treatment was 12 to 15%. Specimens were sorted into three treatment groups. Each group consisted of 12 test and 2 control samples.

### Two-step Heat Treatment

In the hydrothermal treatment, the bamboo strips were treated in an aqueous environment at super atmospheric pressure (0.2 to 0.3 MPa). This was done in a 5-liter laboratory stainless steel treated reactor (TFCF5-6.0). The treatment temperature was 140 °C. The three separate process media for hydrothermal treatment were water only, 1 wt% NaOH, and NaAc aqueous solution. A pH-meter was used to measure the pH value of the treated solution. Drying of the hydrothermolysed bamboo strips was performed in a conventional kiln at a relatively moderate temperature (80 °C). After drying, the bamboo samples were heat treated again in a high temperature kiln under atmospheric conditions, at a treatment temperature of 180 °C, for an effective treatment time of 2 hours. The untreated and treated samples were used for pH measurement. The pH of bamboo was determined by an extraction method (Humar *et al.* 2001).

### Mold Resistance Test

The bamboo strips were cut into 20 × 100 mm samples. Bamboo samples were exposed in an environment chamber to allow mold growth in a controlled temperature of 25 ± 1 °C and relative humidity of 95 to 98%. The mold resistance test for bamboo was carried out according to AWPA standard EN24-06. Samples were evaluated weekly for mold growth. Mold growth on each bamboo sample was visually rated using certain criteria (a complete description of visual rating scheme is given in the AWPA standard), shown in Table 1. The lower the infection value, the better the efficiency of treatment.

**Table 1.** Standard Method for Rating the Infection Value

Rating	Description
0	No visible growth
1	Mold covering up to 10% of the surface
2	Mold covering between 10% and 30% of the surface
3	Mold covering between 30% and 70% of the surface
4	Mold on greater than 70% of the surface
5	Mold on 100% of the surface

## Chemical Analysis of Bamboo Components

Before chemical analysis of the hydrothermally treated, two-step heat-treated, and untreated (control) bamboo samples, they were chopped to a length of 1 to 2 cm and ground in a plant mill to a homogeneous meal. The method for the quantitative determination of lignin was based on Klason's technique, involving hydrolysis with 72% sulfuric acid. The solution was then placed in an autoclave for one hour at 121 °C. The lignin content was determined as acid-insoluble Klason lignin and acid-soluble lignin by the NREL/TP-510-42618 method. Cellulose and hemicellulose content were calculated by the recovered sugar that was determined by HPLC analysis.

## FTIR Analysis

FTIR spectra of treated samples and untreated controls were measured by direct transmittance using the KBr pellet technique. Spectra were recorded using a Nicolet Impact 360 FTIR spectrometer. All the spectra were measured at a spectral resolution of 0.5 cm<sup>-1</sup>, and 40 scans were taken per sample.

## RESULTS AND DISCUSSION

### Chemical Analysis of Bamboo Components

Results of the chemical analysis of the bamboo components are given in Table 2.

**Table 2.** Chemical Analysis of Bamboo Components with Different Treatments

	Untreated	140 °C		140 °C (NaOH)		140 °C (NaAc)	
		Hydro-thermolysis	Heat treatment 180 °C	Hydro-thermolysis	Heat treatment 180 °C	Hydro-thermolysis	Heat treatment 180 °C
Glucan	42.35	43.22	44.25	43.63	45.63	43.85	44.00
Xylan	27.39	21.35	19.81	19.76	18.54	16.72	14.50
Arabinan	3.42	2.77	2.44	2.03	1.58	1.96	1.42
Klason lignin	23.95	25.53	27.67	27.33	29.84	26.8	28.46
Acid-soluble lignin	0.59	2.32	2.14	2.19	2.05	0.91	0.37

As can be seen from Table 2, the two-step heat treatment clearly affected the hemicelluloses of bamboo specimens. When it was hydrothermally treated at 140 °C in aqueous solution for 2 h, the xylan and arabinan content were lower in heat-treated bamboo than untreated bamboo. Hydrothermal treatment in water only, NaOH, and NaAc aqueous solution produced different decreases in xylan and arabinan contents. The lowest xylan and arabinan values were obtained from the NaAc aqueous solution treatment. The xylan and arabinan losses at 140 °C for 2 h were observed to be 16.72% and 1.96%, respectively. After heat treatment at 180 °C, the xylan and arabinan contents decreased to 14.5% and 1.42%, respectively. Hemicelluloses have lower thermal stability, are highly disordered, and are relatively easy to hydrolyze compared to celluloses (Yang *et al.* 2007b). In this case, it can be said that temperature might have a greater influence on hemicelluloses. Xylan (pentosan) is the most reactive bamboo hemicellulose and, in

general, pentosans are very sensitive to degradation and dehydration reactions (Manninen *et al.* 2002; Kotilainen 2000). The reduction of hemicellulose content was found to be higher than cellulose content.

The presence of water is important, since less degradation of hemicelluloses occur under dry and atmospheric conditions (Boonstra and Tjeerdsma 2006). Furthermore, hydronium ions generated by water auto-ionization and acetic acid cleavage are thought to act as catalysts in the reaction mechanism of the hydro-thermolysis stage. The pH value of hydrothermal treatment solution at 140 °C in process water was 4.75, as measured by a pH-meter. The solution's acidity is predominantly affected by the temperature, so the partial organic functional group of polysaccharides, particularly hemicelluloses and lignin, will be hydrolyzed in an acidic environment. The pH value of hydrothermal treatment solution changed to 6.01 and 5.23 after adding 1% NaOH and NaAc, which will neutralize the acidity of the solution. Therefore, the hydrolyzation of bamboo components (mainly hemicelluloses and lignins) would be weakened with the increase of pH value.

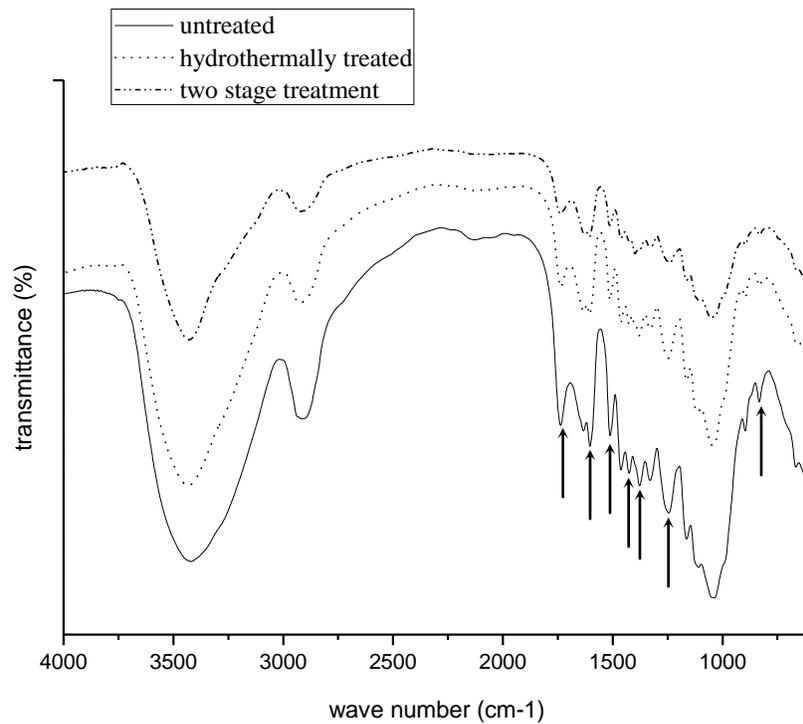
The glucan values were 43.22%, 43.63%, and 43.85%, corresponding to hydrothermal treatment with water only, NaOH, and NaAc aqueous solution. They increased slightly (around 3%) after hydrothermal treatment, and continued to increase to 44.25%, 45.63%, and 44% after high-temperature heat treatment. It can be concluded that the high-temperature heat treatment has an effect on degradation of cellulose. According to Torres *et al.* (1986), dissolution of cellulose is minimal at temperatures below 200 °C. Cellulose has a highly ordered crystalline structure that provides stability to the cellulose chains and protects them against acid attack during hydrolysis (Fengel and Wegener 1984; Efanov 2001).

The accessibility of the glucosidic bonds of cellulose is very limited when compared to those of hemicellulose. Moreover, it is difficult for the cellulose structure to change, such as the transformation of cellulose I into cellulose II (Revol and Goring 1981; Shiraishi *et al.* 1984; Murase *et al.* 1988; Kim 2005). This is thought to be due to matrix substances, such as lignin and hemicelluloses, in the cell walls that prevent cellulose from swelling in an alkali environment. According to researchers, the crystalline structure of cellulose does not change or even increase its crystallinity up to a certain temperature, depending on the conditions involved (Runkel and Wilke 1951).

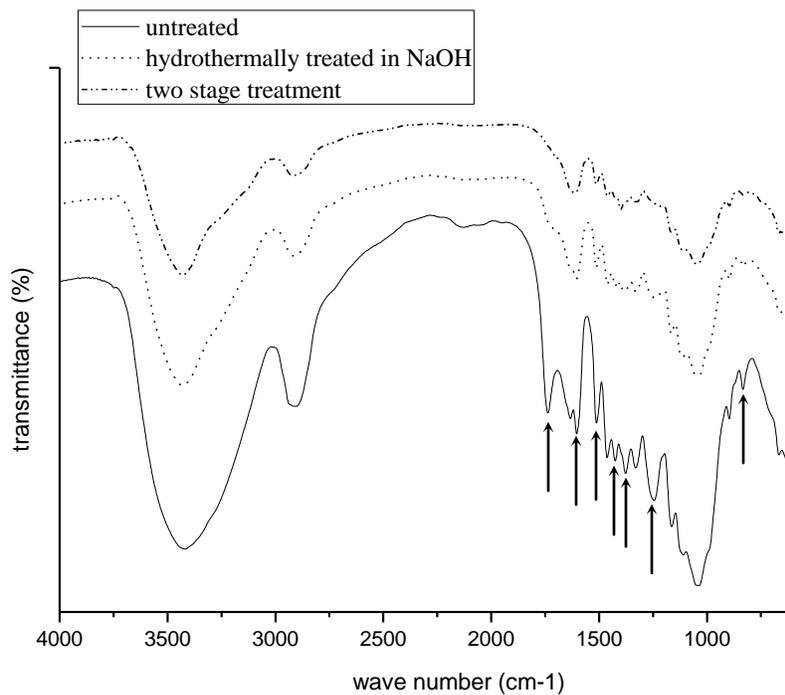
The Klason lignin values were 25.53, 27.33, and 26.8% after hydrothermal treatment with water only, NaOH, and NaAc aqueous solution. Additionally, they increased slightly to 27.67, 29.84, and 28.46%, respectively, after two-step heat treatment. Klason lignin values increased both after hydrothermal and two-step heat treatment, which has also been reported in the literature (Nuopponen *et al.* 2004). This is mainly caused by degradation of the hemicellulose during hydrothermal treatment and partially cellulose during heat treatment at 180 °C. Condensation reactions of lignin, as suggested by Tjeerdsma *et al.* (1998), probably also contributed to this higher lignin content. Though lignin has been seen to be the most thermally stable component of bamboo, various changes were observed, even at temperatures below 200 °C (Sibel *et al.* 2006). The lignin will degrade in alkaline environment because the C-C bond of lignin has less ability to resist alkali.

### FTIR Analysis

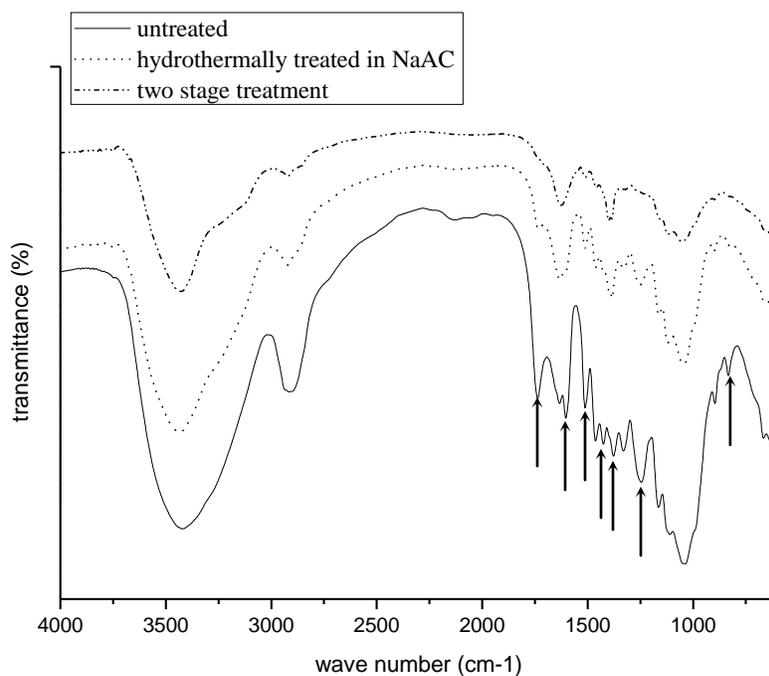
FTIR spectra of bamboo samples treated by water process, NaOH, NaAc aqueous solution, and two-step heat treatments are represented in Figs. 1 to 3.



**Fig 1.** FTIR spectra of untreated, hydrothermally treated in water, and two-step heat-treated bamboo



**Fig. 2.** FTIR spectra of untreated, hydrothermally treated in NaOH, and two-step heat-treated bamboo



**Fig. 3.** FTIR spectra of untreated, hydrothermally treated in NaAc and two-step heat-treated bamboo

Because of treatment, many characteristic bands were shifted at the peak maximum or the absorbance was changed. After hydrothermal treatment in NaAc, the bands at 1513, 1462, 1330, and 1164 shifted to 1509, 1458, 1338, and 1184 (Fig. 3), respectively. All of the bands were influenced by the transformation related to the change of intra- and intermolecular bonds. The characteristic bands located at 1513 and 1605  $\text{cm}^{-1}$  indicate the existence of aromatic rings and C-H bonds in the samples. The 1460  $\text{cm}^{-1}$  band corresponds to hemicellulose and methyl groups of lignin (Weiland and Guyonnet 2003). The appearance of bands in the range of 1330  $\text{cm}^{-1}$  shows the existence of syringyl groups (due to the S ring) in the lignin from bamboo. It has been reported that one of the characteristic IR spectral features of bamboo lignin (HGS type) was the presence of nearly equal absorption intensities at 1270  $\text{cm}^{-1}$  (due to the G ring in lignin) and 1230  $\text{cm}^{-1}$  (C-C plus C-O plus C=O stretch) (Faix 1991). Therefore, the broad 1245  $\text{cm}^{-1}$  band in this study may be the result of the 1270 and 1230  $\text{cm}^{-1}$  bands of approximately equal intensity overlapping.

The relative intensities of bands at 1513, 1462, 1330, and 1245  $\text{cm}^{-1}$  were all lower after treatment in the three aqueous solutions. Moreover, bands at 1740  $\text{cm}^{-1}$  for unconjugated C=O in xylans (hemicellulose) disappeared after hydrothermal treatment in NaOH and NaAc solutions. There was also a decrease of the small shoulder peak at 1164  $\text{cm}^{-1}$  in all the spectra of hemicellulose, corresponding to the glycosidic bond vibration of the arabinosyl side chain in the anomeric region (Xiao *et al.* 2001). The sharp band at 896  $\text{cm}^{-1}$  was due to the C-1 group frequency, which was characteristic of  $\beta$ -glycosidic linkages between the sugar units of hemicellulose (Kačuráková *et al.* 2000). The 896 and 833  $\text{cm}^{-1}$  bands, characteristic of lignin C-H vibration, both decreased after hydrothermal

treatment. The intensities of the aforementioned characteristic bands decreased further after two-stage heat treatment.

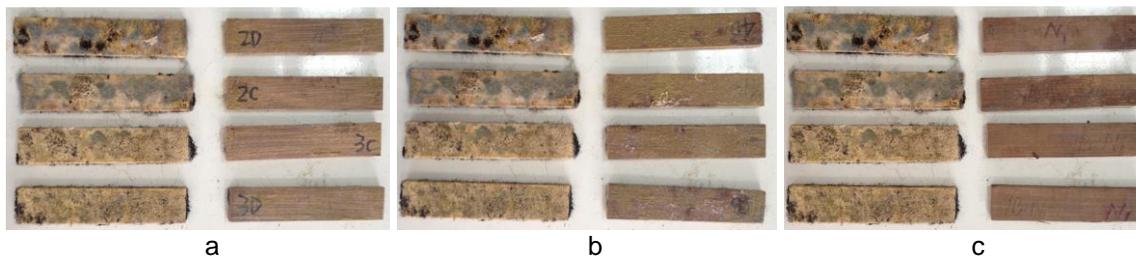
The  $1605\text{ cm}^{-1}$  band decreased in water and NaOH, while it disappeared after treatment in the NaAc solution and the  $1513\text{ cm}^{-1}$  decreased. These two bands correspond to C=C vibration of the aromatic skeleton of lignin. The intensity of the band at  $1425\text{ cm}^{-1}$ , assigned to  $\text{CH}_2$  bending vibration in crystallized cellulose I and amorphous cellulose mixture, decreased after hydrothermal treatment and disappeared after heat treatment in NaAc. When compared to the band at  $1430\text{ cm}^{-1}$ , the characteristic of crystallized cellulose, it is indicated that amorphous cellulose was more affected in salt solution. After treatment in NaOH, the O-H band of phenolic hydroxyl ( $1377\text{ cm}^{-1}$ ) and C-OH of aromatic phenyl ( $1245\text{ cm}^{-1}$ ) present in the lignin decreased. These phenomena implied that the sodium salt was formed by partial free phenolic hydroxyl reacting with NaOH. The modifications of lignin and the creation of several phenolic compounds after treatment could create a denaturation phenomenon and prevent or slow down fungal growth, since it can inhibit spore development.

### Mold-resistant Effect of Two-step Heat Treatment

The results of mold evaluation after two-step heat treatment (with different hydrothermal treatments) are summarized in Table 3. The pictures of mold on bamboo after 4 weeks are shown in Fig. 4.

**Table 3.** Mold-Resistance Rating for Bamboo Specimens after Two-step Heat Treatment

Specimen	Mold resistant rating (standard deviation)			
	Week 1	Week 2	Week 3	Week 4
Untreated bamboo	3.00(0.43)	5.00(0)	5.00(0)	5.00(0)
140 °C hydrothermal treatment	1.75(0.63)	3.00(0.43)	3.54(0.66)	3.92(0.28)
140 °C hydrothermal treatment (NaOH)	1.54(0.67)	2.16(0.39)	2.47(0.51)	2.86(0.58)
140 °C hydrothermal treatment (NaAc)	1.00(0)	1.12(0.29)	1.36(0.49)	2.04(0.29)



**Fig. 4.** Mold on bamboo of control sample and two-step heat treatment after 4 weeks (the first treatment is a: NaOH solution; b: water process; c: NaAc solution)

The results of mold evaluation of two-step heat treatment (with different hydrothermal treatment) are summarized in Table 3. The untreated bamboo samples showed little resistance to mold exposure and became rapidly covered with mold. After four weeks of exposure at conditions of  $25^{\circ}\text{C}$  and 95% relative humidity, hydrothermally treated bamboo samples showed some to high resistance to mold growth compared to the untreated samples. It has been shown that wood chemical composition plays an important

role in the mold growth on wood during heat treatment (Boonstra and Tjeerdsma 2006). The mold resistance rating of bamboo samples was higher after hydrothermal treatment in alkali and salt solutions. When 1% NaOH and 1% NaAc were separately added to the process water and heat treated at 140 °C, the mold resistance became much better. In this mild alkali condition, carbohydrate, in particular pentosans, underwent degradation and dissolution. Extractives were also, to a large extent, removed. The rating values of untreated, water only, 1% NaOH, and 1% NaAc aqueous solution-treated bamboo were 3, 1.75, 1.54, and 1, respectively, after one week of testing. The inherent mold resistance of heat-treated bamboo can be attributed to its chemical features. Bamboo exhibits high susceptibility to mold because of its high content of sugars and starches, which partially dissolve in solution.

In the moist treatment stage (hydro-thermolysis), hemicelluloses are depolymerized by hydrolysis reactions to oligomers and monomers. As can be seen from the chemical components analysis, the arabinose content decreased with heat treatment. The critical sugar in the hemicelluloses that may be the trigger for fungal attack is arabinose, as it is the only sugar in a less stable five-member ring, compared to sugars in a stable six-membered ring (Rowell *et al.* 2009). Acetic acid will be formed as a result of cleavage of the acetyl groups of hemicelluloses. The pH of solution decreased to the range 3.5 to 4.5. Subsequently, partial polysaccharides (especially hemicelluloses) and functional group of lignin hydrolyzed to hydrolysate dissolved easily in water at the influence of acid solution.

Xylan (pentosan) is the most reactive bamboo hemicelluloses and in general pentosans are very sensitive to degradation and dehydration reactions. Relatively extensive degradation of hemicelluloses, which constitute one of the main nutritive sources for fungi, and/or differences in the modification of the lignin network might explain the significant improvement of the resistance of heat-treated bamboo against mold.

The major factors in mold growth in bamboo are temperature, humidity, and the pH of the bamboo. The optimum temperature for bamboo mold fungi growth ranged from 25 to 30°C, humidity ranged from 80 to 95%, and pH ranged from 4 to 6. Hydrothermal treatment reduced the pH of bamboo to the range 2.5 to 4.5 due to the production of acetic acid and formic acid, whereas a pH of 5.0 to 5.5 was common for the untreated bamboo. Ran *et al.* (1997) studied the physiological characteristics of molds that infect bamboo; the optimum mold growth of five kinds of mold fungi, including *Aspergillus niger* V. Tiegh., *A. flavus* Link, *Rhizopus nigricans* Ehrenb., *Penicillium digitatum* Sacc., and *P. citrinum* Thom. was 4, 5, 4, 5 to 6, and 6, respectively. The pH following hydrothermal treatment in process water was 4.81; treatment with NaOH aqueous solution resulted in an almost neutral PH (6.03), and treatment with NaAc aqueous yielded a pH of 5.73, which was higher than the pH of untreated bamboo (5.25).

## CONCLUSIONS

1. Chemical analysis of the bamboo components showed that xylans in hemicelluloses were decreased greatly after treatment by a two-step heat treatment. The lignin content of treated bamboo increased, especially after the two-step heat treatment. This was mainly caused by degradation of hemicelluloses during hydrothermal treatment and partially cellulose during heat treatment at 180 °C. There was, however, a strong

indication that polycondensation reactions contributed to the increase of the lignin content, which also resulted in further cross-linking of the lignin network.

2. The two-step heat treatment improved the resistance to mold considerably.
3. FTIR results showed that heat treatment modified the chemical structure of bamboo. The concentration of acetyl groups ( $1740\text{ cm}^{-1}$ ) was found to be reduced after hydrothermal and two-step heat treatment. Significant changes occurred in the lignin component, as indicated by the considerable decrease in the intensities of the characteristic aromatic lignin peaks at  $1513$ ,  $1245\text{ cm}^{-1}$ , and other associated bands.
4. Strong degradation of hemicelluloses by the two-step heat treatment could inhibit mold growth to some extent. The modifications of lignin and the creation of phenolic compounds in the bamboo could prevent or slow down fungal growth.

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