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# ANTI-FUNGAL AND ANTI-TERMITE ACTIVITY OF EXTRACTIVES COMPOUNDS FROM THERMALLY MODIFIED ASH WOODS

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## ABSTRACT

Thermal modification of wood is a promising alternative to chemical and biocidal modification processes, increasing the biological durability and dimensional stability of wood. However, the wood-decay resistance properties of heat-treated wood are still not well known. The main objective of this study was to determine the biological resistance of heat-treated ash wood, and assess the antifungal and anti-termite activity of extractive compounds from heat-treated ash woods, depending on the intensity of the modification process (2 hours at 170, 200, 215, 228 (°C) - steam pressure). Untreated and heat-treated wood samples were extracted with water or acetone. The extracts were then used to determine inhibition effectiveness against white-rot (*Trametes versicolor*) and brown-rot (*Rhodonia placenta*) fungi. Whatman papers impregnated with extractives were used to evaluate the inhibition of termite feeding. Lastly, the extractives were analyzed by Gas Chromatography - Mass Spectrometry (GC-MS) and compared for their level of anti-termite and antifungal activity. The results showed that the degree of antifungal activity of these extracts depended on the solvent used during the extraction process and varied depending on heat treatment intensity. The extracts were more effective against brown-rot than white-rot fungi. However, the anti-termite activity of heat-treated ash wood extracts was not really significant. A GC-MS analysis showed that the main share of the extractives in untreated wood was removed. In addition, new chemical elements were generated by the thermal degradation of wood polymers (lignin and hemicelluloses), including aliphatic acids, monosaccharides and other products resulting from their dehydration reaction. The most abundant element was syringaldehyde, from lignin derived compounds, which might explain the antifungal activities of thermally treated ash wood extracts.

**Keywords:** Antifungal activity, extractive compounds, *Fraxinus* spp., heat treatment, termite resistance.

## 1. INTRODUCTION

Ash (*Fraxinus* spp.) is a significant hardwood resource largely used in the European wood industry. Given its very weak resistance to decay and insects, this timber is naturally in appropriate for class 3 use (EN 350 CEN 2016). Ash wood materials therefore need to be treated with biocide additives for outdoor use (Candelier *et al.* 2017; Gérard *et al.* 2017). Industrial wood protection methods usually focus on ways of impregnating active formulations containing biocides. However, ash wood is only a moderately treatable timber for both its sap and heartwood (EN350 CEN 2016; Gérard *et al.* 2017). In addition, due to their impact on human health, these chemicals are a subject of environmental pressures, which will restrict their use in the near future (Schultz *et al.* 2007). The Implementation of EU Environmental Legislation in recent years has resulted in the development of non-biocidal alternatives, such as thermal modification (Sandberg *et al.* 2016), chemical impregnation (Gérardin 2016; Mantanis 2017; Guo *et al.* 2018), or a combination of the two (Salman *et al.* 2016; Sandberg *et al.* 2017). Using antagonistic microorganisms, combined or not with biochemicals, is also considered as a promising ecofriendly way of protecting wood: (i) biocontrol microorganisms consume the available nutrients and produce biochemical compounds that are toxic and/or repellent to fungal decay and, (ii) the biochemical compounds include cell wall-degrading enzymes, siderophores, chelating iron and other antibiotics. This last wood preservation method is not yet industrialized and requires additional studies, especially regarding the environmental impact of these formulations (Susi *et al.* 2011).

On the other hand, thermal treatment of wood, using slow pyrolysis in an inert atmosphere, has been widely studied and has now been industrially developed around the world, mainly to increase wood durability against fungi in line with the process parameters (Calonego *et al.* 2010; Candelier *et al.* 2016). According to the literature (Kamdem *et al.* 2002; Weiland and

Guyonnet 2003), the improved resistance of heat-treated wood material to decay can be explained by the following four assumptions, all derived from chemical modifications of wood cell wall polymers due to their thermal degradation (Tjeerdsma and Militz 2005; Yildiz *et al.* 2006; Inari *et al.* 2007):

**(i)** The hydrophobic behavior of wood is increased, thus limiting water sorption within the material and reducing any fungal growth. This improved hydrophobicity reduces the capillary transfer of water into the thermally modified wood and thus limits fungal growth by removing the optimum conditions for its development (due to lack of water). In addition, heat-treated wood has a lower Fiber Saturation Point (FSP) than untreated wood, leading to better resistance to rot fungi (Weiland and Guyonnet 2003; Hakkou *et al.* 2006).

**(ii)** Wood polymers are modified and the enzymes involved in the fungal degradation of wood do not recognize it anymore as such. Firstly, the changes in lignin chemical composition prevent fungal enzymes from recognizing and attacking specific target molecules (Vallet *et al.* 2001; Lekounougou *et al.* 2009). Secondly, changes in the ligneous polymer network also seem to play a role in fungal inhibition. The improved resistance of modified wood to decay is mainly due to possible crosslinking between lignin and certain molecules derived from wood thermal degradation, such as furfural (Weiland and Guyonnet 2003). Such modifications lead to a substrate that is not recognized by the fungal enzyme system, resulting in an undecayed material (Hakkou *et al.* 2006). Moreover, cellulose may undergo an esterification reaction, due to the acetic acid generated during the thermal degradation of hemicelluloses (Tjeerdsma and Militz 2005; Gao *et al.* 2016).

**(iii)** Hemicelluloses are the main wood component degraded by thermal modification, inducing a significant loss in potential nutrients for fungal growth (Hakkou *et al.* 2006; Altgen *et al.* 2019). The chemical modification of polysaccharides is responsible for improving

resistance to wood decay, but cannot be the only factor, as lignin is also an important source of nutrients for white-rot fungi (Lekounougou *et al.* 2009).

**(iv)** New extractive, fungicidal substances are generated by wood thermal degradation and can serve to prevent decay. In fact, some extracts from heat-treated beech, poplar and maritime pine are already known to have antifungal activities (Kamdem *et al.* 2000; Peters *et al.* 2009; Lovaglio *et al.* 2017)). However, other studies showed that improvement in the resistance of beech wood to decay does not appear to be due to new extractible compounds formed during thermal treatment. Hakkou *et al.* (2006) reported that the new extractive compounds produced during heat treatment of beech wood, carried out between 200 °C and 280 °C, only had a slight effect on the heat-treated wood against *Trametes versicolor*. Kamdem *et al.* (2002) found similar results in a study focusing on heat-treated pine and spruce wood samples extracted with water and organic solvents.

The extractive content of heat-treated wood increased when treatment was carried out at low temperatures and decreased with treatment carried out at higher temperatures (> 220 °C). Most of the raw extractives disappeared and new compounds, such as anhydrosugars, mannosan, galactosan, levoglucosan and two C5 anhydrosugars, were generated. Syringaldehyde, sinapaldehyde and syringic acid appeared to be the products formed in the largest amounts, all of which came from lignin degradation (Esteves *et al*. 2008). This hypothesis regarding the formation of new extractives and their role in heat-treated wood durability therefore remains to be confirmed.

The resistance of thermally modified wood to termites has also been studied. Contrary to fungal resistance, the influence of heat treatment on wood resistance to termites appeared to be more variable depending on the processing conditions and wood species. In many cases, resistance to termites was random and, in some cases, even reduced (Doi *et al.* 1998; Momohara *et al.* 2003; Nunes *et al.* 2004; Sivrikaya *et al.* 2015; Salman *et al.* 2016). Some

studies revealed that thermally modified wood had lower termite resistance than untreated wood (Sivrikaya *et al*. 2015; Salman *et al.* 2016, 2017). Generally, termite survival levels reveal an effect of thermal treatment on the biology of these insects. Termite survival rates remained virtually constant for treatments carried out at a low temperature (180 °C to 210 °C) and were lower for higher treatment temperatures. Treatments over 200 °C induced crucial modifications to the wood material, possibly reducing suitability for termites (Candelier *et al.* 2017). As heat-treated wood is consumed when exposed to termites, the toxicity of ingested components may be the reason for the higher mortality rate observed (Surini *et al.* 2012) when treatments are carried out at high intensities (temperature – duration). In addition, when heat-treated and untreated control wood samples were exposed side-by-side (choice test), termites preferred to attack the untreated wood samples (Nunes *et al.* 2004).

In some cases, when the thermal modification process was carried out under optimum conditions, heat treatment enabled low natural durability timbers to be used by making them more resistant to decay, allowing them to be used in use classes 2 and 3 (use class 4 being excluded due to the occurrence of soft rots) (EN 335 CEN 2013), thus up-grading their economic value (Kamdem *et al*. 2002). These improved biological properties conferred to the wood by chemical modifications have a negative impact on the mechanical resistance of heat-treated wood (Dilik and Hiziroglu 2012; De Oliveira Araùjo *et al.* 2016). Surface hardness seems to be slightly enhanced, while other characteristics, such as bending and compression strengths, stiffness and shear strength, are considerably weakened depending on the kind of thermal processes and treatment intensities used (Boonstra *et al*. 2008; Hannouz *et al.* 2015).

The objective of this study was to investigate the antifungal and anti-termite activities of extracts from thermally modified ash wood depending on different treatment intensities. In order to ensure the reproducibility of the thermal treatment processes, as well as the quality of the treated wood products, the heat treatments were carried out under monitored conditions,

using different heating temperatures (170, 200, 200, 215 and 228 (°C)) and a fixed residence time (2 h).

## 2. MATERIALS AND METHODS

### 2.1. Wood samples

Untreated ash (*Fraxinus excelsior* L.) wood samples were obtained from a French wood company (Bois Durables de Bourgogne, 71120 Vendenesse-lès-Charolles, France). Twenty boards measuring 4000 x 110 x 25 ($mm^3$) [L x R x T] were selected. Special attention was paid to ensure these planks only had small variations in density (around 650 $kg/m^3$ ± 10 %) and a uniform width of annual rings. All the planks were sawn into two equal parts of 2 m in length. Half of each plank was used as reference material and the other half was thermally treated at different treatment intensities. The planks were then dried at 103°C in an industrial oven up to mass stabilization ($m_0$).

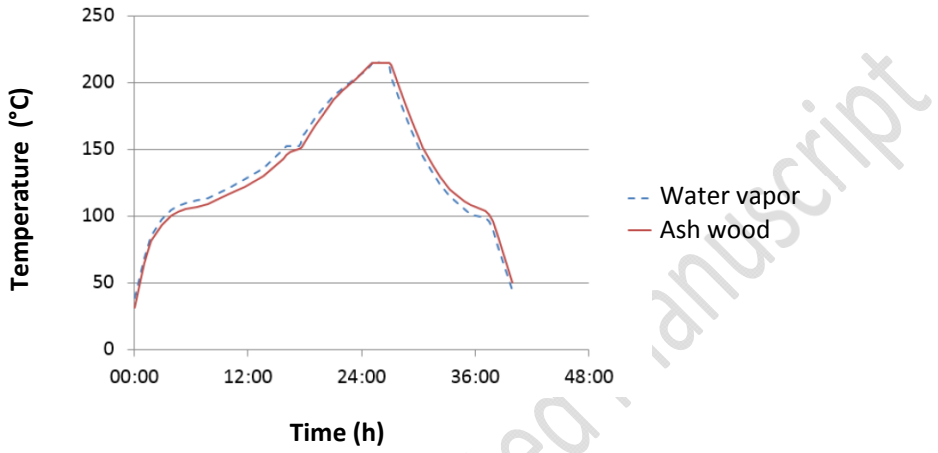
### 2.2. Heat treatment protocols

The thermal treatment processes were carried out at the same company. These operations were carried out in a 20 cubic meter industrial oven (Jartek®), in convection heat-transfer mode and under a steam pressure process [ThermoWood® method, Finnish Thermowood Association (2003)].

The temperatures of the wood core and that of the oven atmosphere were dynamically recorded and monitored throughout the treatment (Figure 1), in order to optimize the process and the final wood qualities.

The oven temperature was firstly increased rapidly (0,5 °C/min) to 100 °C, then slowly (0,2 °C/min) to 150 °C. This temperature level was maintained for 1 h. The temperature was finally increased (0,2 °C/min) to the desired level [from a low temperature (170 °C to 200 °C) to a high temperature (215 °C to 228 °C)] and kept at that constant level for 2 hours. Steam

169 was injected into the chamber to keep it oxygen-free and to influence chemical changes in the
170 wood. The heating system was then stopped and the wood samples were cooled to room
171 temperature in an oxygen-free atmosphere with a water spray.
172 Each heat treatment was carried out simultaneously on four ash wood planks.




173 **Figure 1:** Temperature kinetics of ash wood and water vapor during heat treatment carried
174 out at 215°C for 2 hours.
175

176 **2.3. Mass Loss (ML) due to wood thermal degradation**

177 Each raw ash wood plank measuring 2000 x 110 x 25 ($mm^3$) [L x R x T] was dried at 103 °C
178 up to mass stabilization and its anhydrous mass was measured ($m_0$). After a thermal
179 modification process each treated ash wood plank was again dried at 103 °C and its
180 anhydrous mass was re-measured ($m_1$). The Mass Loss (ML %) due to wood thermal
181 degradation depending on treatment conditions was determined by the following equation
182 (Eq.1).
183

184 $$ML(\%) = [(m_0 - m_1) \div m_0] \times 100 \qquad (1)$$

185

186 **2.4. Biological resistance tests on wood block samples**

187 ***2.4.1. Decay resistance***

188 Decay and termite resistance tests were carried out according to adaptations of XP CEN/TS
189 15083-1 (CEN 2006) and EN 117 (CEN 2012) standard criteria, respectively, in one of our
190 previous studies (Candelier *et al.* 2017). The protocols used for these durability tests are
191 detailed in that previous work.

192

193 **2.5. Extraction protocols**

194 All the experimental procedures used to determine extractive contents were adapted, with
195 minor modifications, from procedures found in the scientific literature (Rowell *et al.* 2005).
196 A mix of several pieces (One 20-cm long piece, per wood board) from untreated and heat-
197 treated planks was crushed and sieved to obtain particle sizes from to 0,2 and 0,5 mm, for
198 each temperature level. After a drying step at 103 °C up to mass stabilization ($m_2$), the
199 sawdusts (10 g) were separately extracted in a Soxhlet with acetone [Sigma Aldrich, 32201-
200 M] (6 h), or with hot water (2 x 6 h), and dried at 103 °C for 48 h to obtain the anhydrous
201 mass ($m_3$).
202 For untreated and heat-treated ash wood under varying temperature conditions, extractive
203 contents were determined by the following equation (Eq.2):

204 $$Ext.(\%) = [(m_2 - m_3) \div m_2] \times 100 \qquad (2)$$

205

206 **2.6. Antifungal activity test**

207 Antifungal activities against the growth of *Trametes versicolor* Quélet (TV) [Linnaeus, CTB
208 863 A] and *Rhodonia placenta* Coocke sensu J. Erikson (PP) [Fries, FPRL 280] were tested as
209 per Salem *et al.* (2014) with some variations.
210 Each Petri dish (9 cm diameter) was filled with 10 mL of malt-agar medium containing
211 1200 µL of the diluted extracts in water or acetone (Merck company, Darmstadt, Germany)

[C= 2,5% m/m] and left to solidify. These dilutions were chosen according to past studies (Esteves *et al.* 2011; Pal *et al.* 2011). A 1-cm square section of a seven-day-old culture of *Rhodonia placenta* or *Trametes versicolor* was placed in the center of the Petri dish and stored in a climatic chamber regulated at 22 °C ± 2 °C and 70 % ± 5 % relative humidity (RH) for seven days' incubation. Three replicates were carried out per diluted wood extract sample and per fungus. Three media free of extractives and without solvent were used as a control. In addition, three media free of extractives and with solvent (water or acetone alone) were used to check that the water and acetone solvents did not have any impact on the activity of each fungus. Following the fungal exposure period, the mycelium growth diameter was measured in millimeters, for the control medium ($M_c$) and the extract-supplemented medium ($M_t$). The percentage of mycelium growth inhibition was determined by equation 3 (Eq.3):

$$\% \, inhibition = \, [(M_c - M_t) \div M_c] \times 100 \qquad (3)$$

**2.7. Anti-termite activity test**

The anti-termite activities of each heat-treated ash wood extract were tested by screening tests. Anhydrous cellulose paper measuring 2,5 cm in diameter was weighed ($m_4$) and then impregnated with 70 µL of the diluted extracts in water or acetone [C= 2,5% m/m], air dried (20 °C ± 2 °C and 65 % ± 5 %) for 2 hours ($m_4$), then placed in the center of a Petri dish (5,5 cm diameter). Fifteen grams of wet sand (4 vol. sand/1 vol. water) was placed evenly around the paper and 20 termite workers (*Reticulitermes flavipes,* ex*. santonensis*) were added to each test set-up. Three replicates were carried out for each diluted wood extract. Three papers impregnated with water or acetone alone were tested to estimate the impact of the solvent on termite activity. Tests with water only were considered as a virulence control. For each test set-up, the paper samples were placed on a plastic grid. Lastly, three diet control set-ups containing only 15 g of wet sand and 20 termites were used to check termite survival without

any feeding possibilities/without any trophic sources. All test set-ups were kept at 27 °C and > 75 % RH (Figure 2).

Every two days, each test set-up was observed to check sand humidity, add water if needed and keep track of termite behavior and activity. When all the termites contained in the diet control set-ups had died, the test was stopped. The termite survival rate was then determined, the anhydrous mass of the cellulose papers was measured ($m_5$) and the Weight Losses ($WL_{term.}$ %) due to termite degradation were calculated by the following equation 4 (Eq. 4) :

$$WL_{term.}(\%) = [(m_4 - m_5) \div m_4] \times 100 \qquad (4)$$

**Figure 2:** Termite screening test set-ups at the start of termite exposure.

## 2.8. GC–MS analysis

The wood extracts (acetone and water) were analyzed on a Perkin Elmer Clarus 680 Gas Chromatograph (GC) with a fused silica DB-5MS [(diméthyl-/diphényl-polysiloxane, 95:5]

column (30 m, 0,25 mm, 0,25 μm), coupled with a Perkin Elmer Clarus SQ8 Mass Spectrometer (MS) and monitored by Turbo Mass v.6.1 software.

Before being injected into the GC-MS analyzer, the extractive substance samples were derivatized in order to improve the detection of all chemical compounds. To perform this silylation derivatization process, 2 mg of extract was solubilized in a glass tube with 50 μL of BSTFA + 1 % TMCS solution (Bistrimethylsilyltrifluoroacetamide + Trimethylchlorosilane) [Acros Organics]. The glass tube was sealed and dried in an oven at 70 °C for 120 minutes, then opened to evaporate the BSTFA. The derivatized wood extract was dissolved in 1 mL of $C_4H_8O_2$ solution (ethyl acetate [Acros Organics]). One μL of this solution was injected into the Gas Chromatograph at a 250 °C inlet temperature in splitless mode. Helium was used as the carrier gas. The temperature program was: 80 °C (2 min), 10 °C/min to 190 °C 15 °C/min$^{-1}$ to 280 °C - maintained for 10 minutes, 10 °C/min to 300 °C 15 °C/min$^{-1}$ - maintained for 14 minutes. A helium flow of 1 mL/min was used as the mobile phase. After this separation step, compounds were transferred to the Mass Spectrometer by a transfer line heated at 250 °C and ionization was achieved by the Electron Impact method (70 eV ionization energy).

The recognition of each component was achieved by comparing its mass spectrum with the NIST Library 2005 using NIST MS Search 2.0 (2011) software. The identification was deemed to be relevant when the comparison coefficient was higher than 900 (The comparison coefficient corresponds to the match factor between the mass spectrum obtained by a GC-MS analysis and the mass spectrum from the NIST MS Search 2.0 (2011). The match factors ranged from 0 to 1000, with 0 meaning no match and 1000 meaning a total match).

### 2.9. Determination of pH Value

pH measurements were conducted according to Wang *et al*. (2008). Untreated and heat-treated samples were ground and passed through 40-60 mesh screens. Three grams of oven-dried sawdust samples was soaked in 30 mL of distilled water and then stirred for 5 min, allowed to stand for 15 min, stirred for another 5 min, and then left to stand for another 20 min. After this procedure, the pH values of the liquid were determined using a pH meter (PH/mVmeter Knick 911 ATEX; Knick Elektronische™, Berlin, Germany).

### 2.10. Statistical Analysis

The impact of heat treatment intensity compared to the untreated ash wood on (i) the termite and fungal resistance of the wood samples, (ii) the content and (iii) the effects of water and acetone extractives on their antifungal and anti-termite efficiency were evaluated using an ANOVA (one-way analysis of variance) and Duncan's comparison test. These statistical analyses were carried out by the JMP 10.0.2 program (SAS 2012) by applying the Fisher test. The results were then ranked into several categories, from "a" to "e" for the water-extractives and from "A" to "E" for the acetone-extractives. The impact of a parameter on a system not connected by the same letter was considered as non-significant at the 5 % level.

## 3. RESULTS AND DISCUSSION

### 3.1. Biological resistance tests on wood block samples

Decay and termite resistance tests were previously undertaken in Candelier *et al.* (2017). In order to put into perspective, the biological durability of untreated and heat-treated solid wood with the following antifungal and anti-termite activities of the respective wood extractive fractions, this section focuses solely on the main results obtained by Candelier *et al.* (2017).

#### 3.1.1. Decay resistance

Similar results were found for both of the rots tested. However, *Rhodonia placenta* was the most aggressive rot on beech control samples (WL 49,9 %), whereas *Trametes versicolor* was

302 the most degrading rot on ash control samples (WL 48 %). Thermal modification increased
303 the durability of all wood materials, which was in agreement with previous studies (Kamdem
304 *et al.* 2002; Esteves and Pereira 2009). These results were expected as Rousset *et al*. (2004)
305 and Metsä-Kortelainen *et al.* (2005) also found that the thermal treatment of wood at high
306 temperatures increases the resistance of wood to decay.
307 According to Table 1, the thermal treatment carried out at temperatures over 200 °C conferred
308 the "very durable" durability class 1 to the heat-treated wood materials, according to the
309 classification method of XP CEN/TS 15083-1 (CEN 2006).
310
311 **Table 1:** Resistance of untreated and heat-treated ash wood block samples to *Trametes*
312 *versicolor* and *Rhodonia placenta* fungi and to *Reticulitermes flavipes* termite species [taken
313 from Candelier *et al.* (2017)].

| Wood species | Temperature of thermal modification process (°C) | Decay resistance | | | Termite resistance *(Reticulitermes flavipes)* | | |
|---|---|---|---|---|---|---|---|
| | | *Trametes versicolor* | *Rhodonia placenta* | Durability class | ML (%) | Survival rate (%) | Visual rating * |
| | | WL (%) | WL (%) | | | | |
| **Beech** | **Control** | 23,0 (0,13)a | 49,9 (0,11)a | 5 | 8,87 (1,98)abc | 69,50 (13,10)ab | 4 |
| **Pine** | **Control** | n.c | n.c | n.c | 11,12 (1,57)a | 64,50 (9,43)bc | 4 |
| **Ash** | **Control** | 48,0 (0,10)b | 39,7 (0,09)ab | 5 | 4,58 (0,57)d | 69,67 (8,33)ab | 4 |
| | **170** | 5,02 (0,05)c | 6,2 (0,12)c | 2 | 11,80 (2,60)a | 78,67 (4,16)a | 4 |
| | **200** | 2,8 (0,13)d | 2,2 (0,09)d | 1 | 11,60 (2,12)a | 71,33 ( 6,11)ab | 4 |
| | **215** | 1,5 (0,19)e | 0,9 (0,07)e | 1 | 10,86 (1,51)a | 56,67 (5,03)bcd | 4 |
| | **228** | 1,1 (0,10)f | 0,7 (0,05)e | 1 | 9,23 (1,97)ab | 51,33 (13,61)bcd | 4 (67%); 3 (33%) |

**WL% - Median values**
ML% - Average values
* "0" for no attack "1" for attempted attack, "2" for slight attack, "3" for average attack, "4" for a strong attack
** According to a one-way analysis of variance, systems not connected by the same letter are largely different at the 5 % level.

314
315 **3.1.2. Termite resistance**

316 Termite survival revealed an effect on termite biology. Although the survival rate was similar
317 for treatments at 170 ºC and 200 °C, it was lower above those temperatures (Table 1). Heat
318 treatments at temperatures over 200 °C caused critical changes to the wood and consequently
319 its durability could be improved. As the wood was nonetheless degraded, the toxicity of the

components consumed may explain the higher termite mortality rate (Surini *et al.* 2012) depending on the increase in heat treatment intensity. However, it was not particularly effective over a short time scale, as 51,33 % of the termites were still alive after the test, with no significant differences between treated and untreated wood. The current results are in agreement with those of Nunes *et al.* (2004), who studied the resistance to termites of the species *Reticulitermes grassei* with wood treated by the German method (OHT) and concluded that, in spite of the slightly higher mortality of termites in treated samples and a smaller weight loss, the differences were not significant.

### 3.2. Extractive content

For thermal treatment carried out at a low temperature (< 215 °C), the proportions of heat-treated wood extractives increased in comparison with those of untreated ash wood, whatever the solvent used during the extraction process (Figure 3). This result tallied with past studies, indicating an increase in extractive rate resulting from the generation of different degradation products (Poncsak *et al.* 2009; Esteves *et al.* 2008). A past study by Esteves *et al.* (2008), carried out on eucalypt wood, showed that thermal degradation of lignin and hemicelluloses led to an increase in extractive content. In fact, the formation of new extractive compounds arising from polysaccharide degradation at around 160 °C may be one of the reasons for that phenomenon. At higher temperatures (>215 °C), these new compounds are also generated but, under the heat effect, they are then converted into volatile products that leave the structure of the wood, thus causing a loss of matter, leading to a decrease in extractive content (Wang *et al.* 2016).

The relative percentage contents of extractives (Figure 3) increased at low treatment temperatures, reaching maximum values of 4,59 % and 2,19 % for thermal treatment carried out at 170°C and for water and acetone extractions, respectively. Beyond that temperature the

345 relative extractive percentage decreased.

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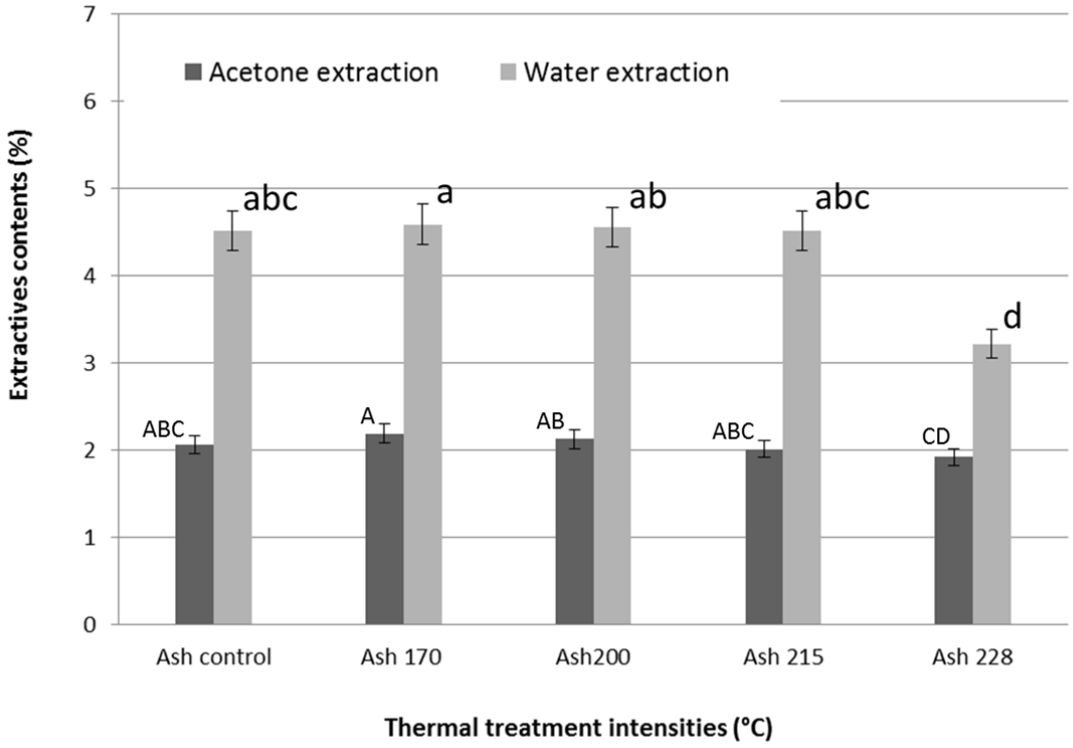


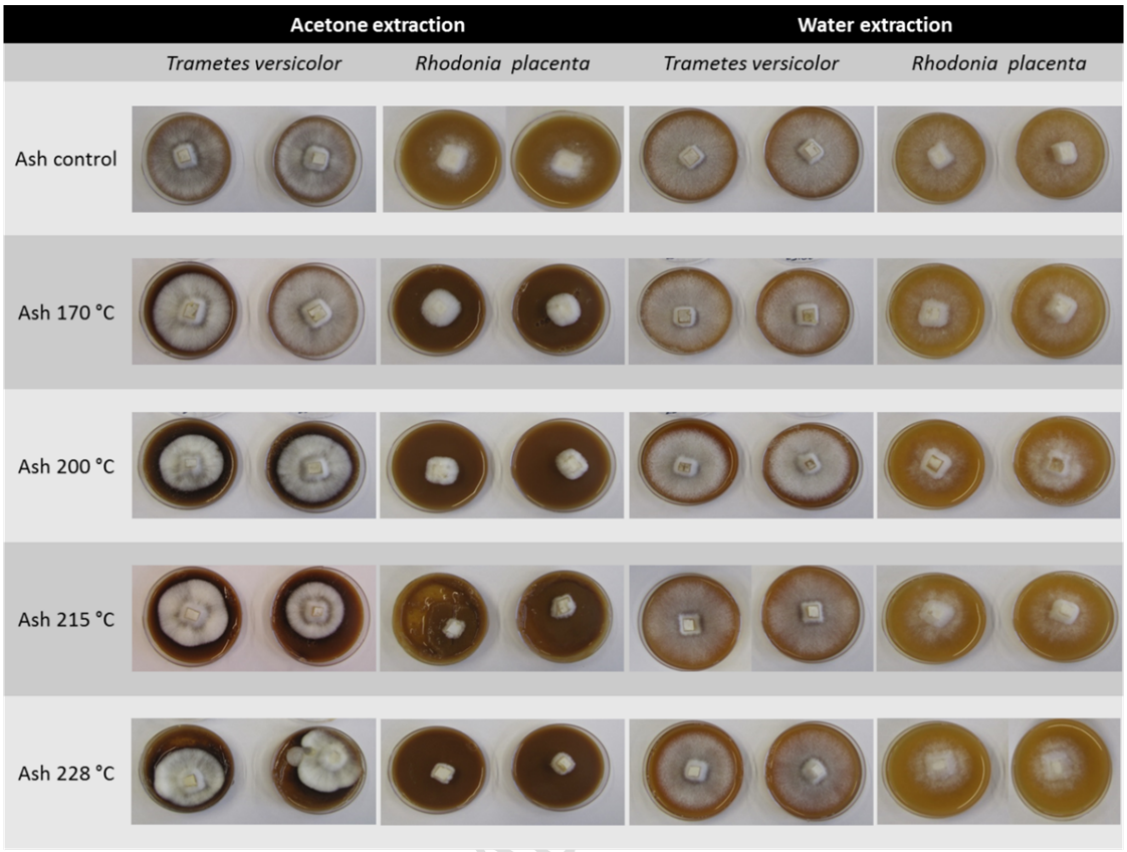

347 **Figure 3:** Extractive contents (%) of untreated and heat-treated ash woods at different
348 temperature levels (170 ºC to 228°C), depending on the extraction solvent used.

349

350 **3.3. Antifungal activity test**

351 Fungal activity was inhibited by the heat-treated and untreated ash wood extracts. Table 2 and
352 Figure 4 shows how fungal development was hindered depending on the extractive
353 compounds. However, for both of the fungus species tested, the effectiveness of the extracts
354 varied depending on the heat treatment temperature and the solvent used during the extraction
355 process. It clearly appeared that untreated and heat-treated ash wood extracts were more
356 efficient against brown-rot (*Rhodonia placenta)* than against white-rot (*Trametes versicolor)*
357 growth, whatever the extraction solvent used. In both cases, the efficiency of the extracts
358 seemed to be correlated to the heat treatment temperature.

359




360

361 **Figure 4:** *Trametes versicolor* and *Rhodonia placenta* growth depending on the inhibition of
362 untreated and heat-treated ash wood extracts (1200 µL), after seven days.

363

364 Table 2 indicates that heat-treated ash wood extracts were more efficient against *Trametes*
365 *versicolor* and *Rhodonia placenta* after 3,5 days than after 7 days, except for the modified ash
366 wood extracts extracted with acetone against *Rhodonia placenta.* It turned out that, 7 days
367 after fungal exposure, acetone-extracts from ash wood modified at a temperature of 228 °C
368 delayed the growth of *Trametes versicolor* and *Rhodonia placenta* most.

369

370

371

372

373 **Table 2:** Effectiveness of untreated and heat-treated ash wood extracts (1200 µL) against
374 *Trametes versicolor* and *Rhodonia placenta*.

| | | *Trametes versicolor* | | | | *Rhodonia placenta* | | | |
|---|---|---|---|---|---|---|---|---|---|
| Extraction method | Ash wood | 3,5 days | | 7 days | | 3,5 days | | 7 days | |
| | Thermal treatment temperature | Inhibition (%) | Standard deviation (%) | Inhibition (%) | Standard deviation (%) | Inhibition (%) | Standard deviation (%) | Inhibition (%) | Standard deviation (%) |
| | | | | | | | | | |
| **Acetone** | **0** | **24,01** $^{D}$ | *1,40* | **31,49** $^{C}$ | *1,02* | **42,05** $^{D}$ | *0,00* | **44,90** $^{E}$ | *0,00* |
| | **170** | **37,83** $^{BC}$ | *6,98* | **32,93** $^{BC}$ | *5,10* | **50,57** $^{C}$ | *2,41* | **61,22** $^{D}$ | *2,89* |
| | **200** | **44,74** $^{B}$ | *2,79* | **42,31** $^{B}$ | *2,04* | **42,05** $^{CD}$ | *9,64* | **68,37** $^{C}$ | *1,44* |
| | **215** | **48,68** $^{AB}$ | *8,37* | **46,63** $^{AB}$ | *8,37* | **60,80** $^{AB}$ | *2,41* | **76,53** $^{AB}$ | *1,44* |
| | **228** | **59,54** $^{A}$ | *6,98* | **51,68** $^{A}$ | *7,14* | **64,20** $^{A}$ | *2,41* | **79,63** $^{A}$ | *1,44* |
| | | | | | | | | | |
| **water** | **0** | **14,14** $^{e}$ | *1,40* | **31,49** $^{c}$ | *1,02* | **47,16** $^{a}$ | *2,41* | **14,29** $^{d}$ | *2,89* |
| | **170** | **20,07** $^{d}$ | *1,40* | **32,21** $^{abc}$ | *6,12* | **42,05** $^{b}$ | *0,00* | **24,49** $^{bc}$ | *2,89* |
| | **200** | **38,82** $^{bc}$ | *2,79* | **35,82** $^{b}$ | *1,02* | **45,45** $^{a}$ | *0,00* | **32,65** $^{a}$ | *5,77* |
| | **215** | **35,86** $^{ab}$ | *1,40* | **36,54** $^{ab}$ | *0,00* | **48,86** $^{a}$ | *0,00* | **35,71** $^{ab}$ | *1,44* |
| | **228** | **41,78** $^{a}$ | *1,40* | **40,14** $^{a}$ | *3,06* | **43,75** $^{ab}$ | *2,41* | **37,76** $^{a}$ | *1,44* |
| **Water cont.** | / | **0** | / | **0** | / | **0** | / | **0** | / |
| **Acetone cont.** | / | **0** | / | **0** | / | **0** | / | **0** | / |

375

376 The different degrees of fungal development for untreated and thermally modified wood
377 reflecting the contrasts in extractive chemical composition are displayed in Figures 6 and 7.
378 Although some effects of water and acetone extracts from heat-treated ash could be seen on
379 fungal growth depending on the treatment intensity, this phenomenon was not significant. It
380 appeared that the fungi adapted themselves to the new medium and regained normal growth
381 after a few days of incubation. In other words, heat-treated wood extracts seem to have
382 short-term fungistatic action.

383

384 **3.4. Anti-termite activity test**

385 Table 3 and Figure 5 show that the heat-treated ash extract yields and their anti-termite
386 activities were largely linked to the polar or nonpolar nature of the solvent used during the
387 extraction process. In fact, anti-termite compounds can have many varied chemical

388 characteristics and different polarity levels, which may or may not be soluble in a specific
389 solvent (Kadir *et al.* 2015).

390 The test was stopped after 21 days, when the termites in the diet control set-ups had died. The
391 results in Table 2 show that the anti-termite activity of the heat-treated ash wood extracts was
392 not significant.

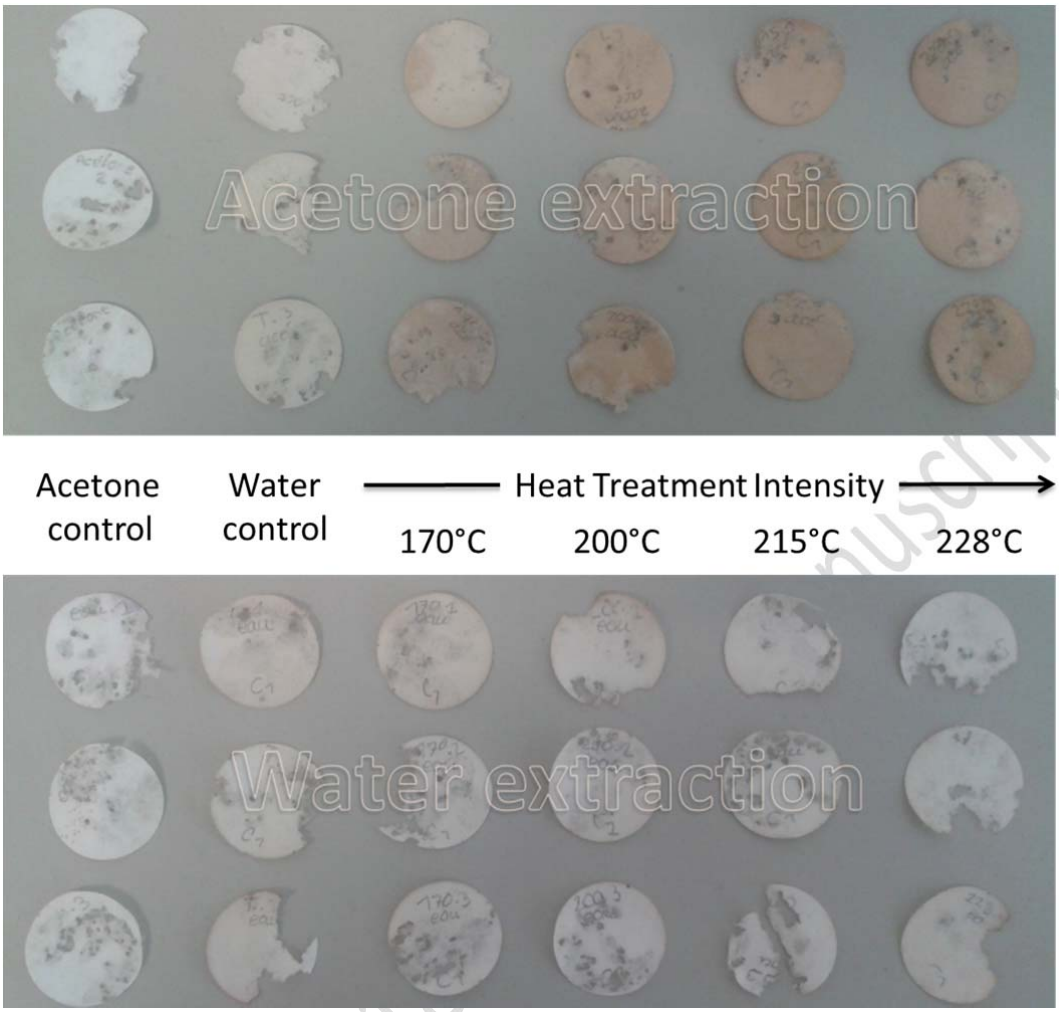
393 **Table 3:** Effectiveness of untreated and heat-treated ash wood extracts (1200 µL) against
394 *Reticulitermes flavipes*.

| Extraction solvent | Ash wood: Thermal treatment temperature | Weight loss (%) | *Standard deviation* (%) | Survival rate (%) | *Standard deviation* (%) |
|---|---|---|---|---|---|
| Acetone C=2,5% [m/m] | 0 | 45,25 $^{A}$ | *9,31* | 85,00 $^{A}$ | *5,00* |
| | 170 | 34,86 $^{AB}$ | *1,52* | 86,67 $^{A}$ | *7,43* |
| | 200 | 34,49 $^{AB}$ | *4,39* | 81,67 $^{AB}$ | *2,89* |
| | 215 | 32,17 $^{AB}$ | *4,02* | 76,67 $^{ABC}$ | *5,77* |
| | 228 | 31,26$^{B}$ | *1,84* | 86,67 $^{A}$ | *2,89* |
| Water C=2,5% [m/m] | 0 | 54,62 $^{abc}$ | *6,78* | 90,00 $^{a}$ | *5,00* |
| | 170 | 51,57 $^{c}$ | *3,48* | 73,33 $^{cd}$ | *2,89* |
| | 200 | 53,25 $^{c}$ | *3,36* | 80,00 $^{c}$ | *0,00* |
| | 215 | 59,55 $^{ab}$ | *2,52* | 76,67 $^{bc}$ | *5,77* |
| | 228 | 61,12 $^{a}$ | *3,05* | 90,00 $^{a}$ | *5,00* |
| Control | Acetone | 32,77 $^{AB/d}$ | *6,63* | 86,67 $^{A/ab}$ | 2,89 |
| | Water | 31,98 $^{ABC/de}$ | *6,29* | 88,33 $^{A/ab}$ | 5,77 |

395

396 For untreated and treated ash wood under different treatment intensities, the mass loss due to
397 termite degradation indicated that the relative extracts obtained with water were statistically
398 more attractive for termites and the termite survival rate was quite similar to that of the
399 control sample. This effect was related to the final temperature level of the thermal treatment.
400 On the other hand, the same extractives obtained with acetone seemed to have no significant
401 effect on termites compared to the water control samples (Figure 5).

402




403

404 **Figure 5:** Effectiveness of untreated and heat-treated ash wood extracts (1200 µL) against
405 *Reticulitermes flavipes*.

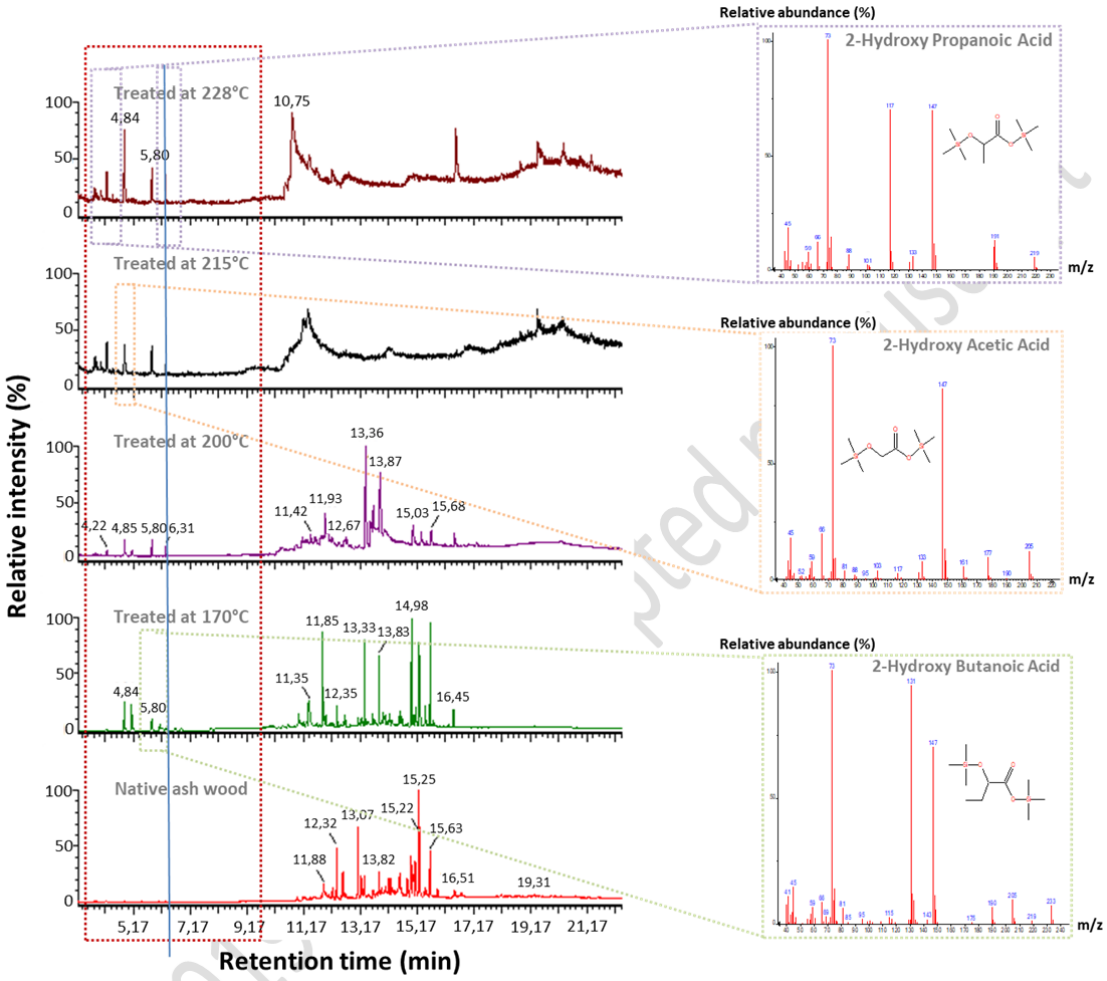
406 The termite survival rate revealed no significant effect on the biology of these insects. The
407 survival rate was not significantly dissimilar for the different treatments compared to the
408 control samples. The effects of untreated wood extracts on the termite survival rate were very
409 similar to those for heat-treated wood, confirming the innocuous property of these treated
410 wood extracts in terms of termite toxicity.

411

412 **3.5. GC-MS analysis**

413 **3.5.1. Water extractives**

414 The typical chromatograms from GC-MS analyses of the chemical composition of untreated
415 and thermally modified ash wood extracts by the water extraction process are presented in
416 Figure 6.




417

418 **Figure 6:** Untreated and heat-treated ash wood water-extractive composition identified by
419 GC-MS, depending on the thermal treatment temperature.

420

421 For untreated ash wood, the GC-MS analysis highlighted that almost all of the original water
422 extractives resulted from polysaccharides or their silylated fragments. Only the weak peak
423 observed at rt = 11,88 min corresponded to the antioxidant Tyrosol (4-
424 hydroxyphenylethanol).

For the ash wood treated at 170 °C, it was found that the respective water extracts mainly comprised low molecular weight organic 2-hydroxy-acids [propanoic acid (rt = 4,84 min and 6,09 min), acetic acid (rt = 5,10 min), butanoic acid (rt = 5,80 min)] (Table 5) and derivated phenylpropanoid compounds (rt = 11,00 min and 11,35 min). The presence of these acid compounds, decreasing the pH of the wood, may have played a role in fungal inhibition. Indeed, according to the results presented in Table 4, the pH values of ash wood decreased depending on the temperature used during the heat treatment process. The pH values varied from 5,16 for untreated wood to 3,92 for wood thermally modified at 228 °C. Similar trends were observed on softwood and hardwood species by Niemz *et al.* (2008), showing that the pH decreases with increasing heat treatment intensity.

**Table 4:** pH values of untreated and heat-treated ash wood.

| Wood species | Thermal treatment temperature (°C) | pH |
|---|---|---|
| **Ash wood** | 0 | 5,16 (0,15)[a] |
| | 170 | 4,51 (0,16)[b] |
| | 200 | 4,12 (0,17)[c] |
| | 215 | 4,03 (0,16)[cd] |
| | 228 | 3,92 (0,14)[cde] |

Indeed, Yalcin and Sahin (2015) reported that heat treatment conferred on narrow-leaved ash wood a pH that was less conducive to fungal growth, with the untreated wood pH of 5,7 falling to 3,9 in the modified wood, while the pH for optimum fungal growth is around 5-6 (Bozkurt *et al.* 1993). In addition, such a decrease in wood pH could disturb the enzymatic digestion of termites during their wood destruction (Lima *et al.* 2014). In fact, pH widely affects the activity of fungal enzymes. The pH dependence is usually due to the side groups of the amino acids. A diminution in pH changes the protonation pattern resulting in protein denaturation (in shape, charge and location within the substrate) (Purich 2010).

445 For ash woods treated at higher temperatures, from 200 °C to 228 °C, only traces of the
446 previously mentioned low molecular weight organic acids were observed. In addition, a large
447 peak located in the polysaccharide area, from rt = 10 min and rt = 13 min, was observed but
448 remained unidentified. These last peaks may have been due to the condensation of several
449 chemical compounds produced by wood thermal degradation and might also partially explain
450 the greater fungal resistance of wood thermally treated at high temperatures (Hakkou *et al.*
451 2006).

452

453 **3.5.2. Acetone extractives**

454 The typical chromatograms from GC-MS analyses for the chemical composition of untreated
455 and thermally modified ash wood extracts by the acetone extraction process are presented in
456 Figure 7.

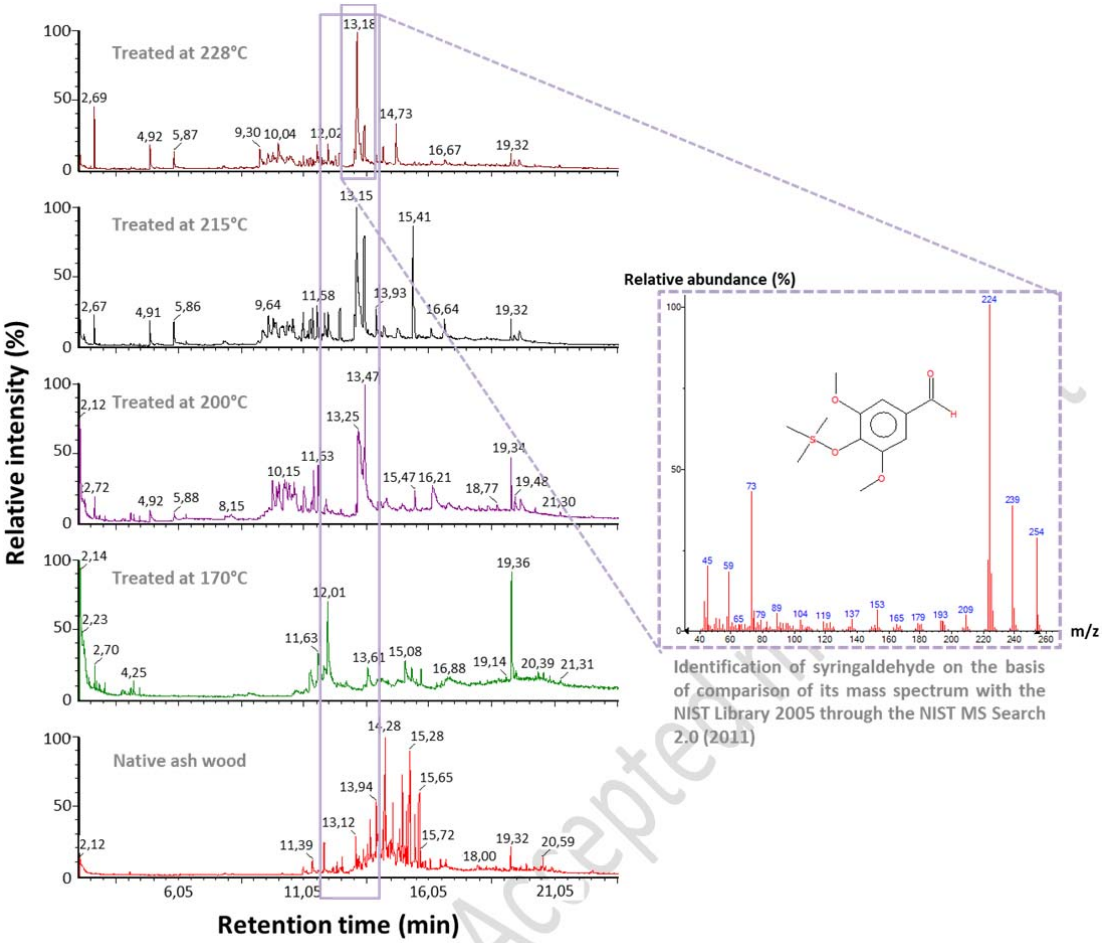



**Figure 7:** Untreated and heat-treated ash wood acetone-extractive composition identified by GC-MS, depending on the thermal treatment temperature.

The GC-MS analysis revealed that almost all of the untreated acetone-extractives disappeared after heat treatment, but on the other hand the thermal degradation of hemicelluloses and lignin generated new compounds. Monosaccharides and some products derived from their dehydration reactions, along with syringaldehyde (13,18 min, Figure 7), were the most abundant products generated by the heat treatment. Syringaldehyde appeared to be the product formed in the largest amounts, arising from lignin degradation, and was generally the major detected component according to the TIC chromatograms, Table 5.

471 **Table 5:** Retention time and Peak Intensities from GC-MS analyses of untreated and heat-

| Wood species | Thermal treatment temperature (°C) | Propanoic acid | | | | Acetic acid | | Butanoic acid | | Syringaldehyde | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Rt * (min) | Intensity** (%) | Rt (min) | Intensity (%)* | Rt (min) | Intensity (%) | Rt (min) | Intensity (%) | Rt (min) | Intensity (%) |
| Ash | **Control** | 4,21 | **1** | 6,31 | **1** | 4,84 | **5** | 5,8 | **2** | 13,12 | **28** |
| | **170** | 4,21 | **3** | 6,31 | **4** | 4,84 | **22** | 5,8 | **6** | 13,11 | **12** |
| | **200** | 4,22 | **9** | 6,31 | **12** | 4,85 | **18** | 5,8 | **19** | 13,25 | **67** |
| | **215** | 4,23 | **37** | 6,31 | **21** | 4,85 | **39** | 5,8 | **38** | 13,15 | **100** |
| | **228** | 4,23 | **39** | 6,32 | **37** | 4,84 | **78** | 5,8 | **41** | 13,18 | **100** |

* Rt = Retention time (in minutes) shows the time taken for the analytes to pass through the column and reach the mass spectrometer detector.
** Intensity (as a %) represents a reflection of the amount of a specific analyte that was present, compared to the predominant one (e.g. Intensity = 100%)

472 treated ash wood extracts.

473

474 Similar results for syringaldehyde extraction, by water and ethanol, from heat-treated wood
475 were found by Ibrahim *et al.* (2012). The syringaldehyde content increased depending on the
476 thermal treatment intensity, more particularly for wood treated at temperatures over 200 °C
477 (Figure 7), and might explain the fungal inhibition activities of the heat-treated wood
478 extractives. Indeed, previous studies highlighted the antifungal and anti-microbial activities of
479 the phenolic compound syringaldehyde. Murugesan *et al.* (2009) also showed that
480 syringaldehyde inhibited the growth of bacteria and of the fungus *Ganoderma lucidum* in
481 syringaldehyde-treated malachite green samples. De Souza *et al.* (2005) also reported that
482 syringaldehyde expounded anti-fungal activity against *Leucoagaricus gongylophorus*.
483 According to Ibrahim *et al.* (2012), syringaldehyde (also called 3,5-dimethoxy-4-
484 hydroxybenzaldehyde), has the same kind of structure as vanillin and is a unique natural
485 compound that has bioactive properties (antioxidant, antifungal, anti-microbial and anti-
486 tumorigenesis), which belongs to the phenolic aldehyde family. In a past study,
487 syringaldehyde displayed a successful role in inhibiting fungal growth rates (Kelly *et al.*
488 2008). Its fungicidal effects were mainly due to aldehyde moiety and the hydroxyl substituent

present in syringaldehyde (De Souza *et al.* 2005). Total hydroxyl groups from lignin also had a large impact on cellulase adsorption and enzymatic hydrolysis occurring during fungal degradation (Yu *et al.* 2014), and more particularly acetosyringone, vanillin and syringaldehyde (Qin *et al.* 2016). Ximenes *et al.* (2011) confirmed that syringaldehyde and vanillin inhibit cellulose hydrolysis. Although syringaldehyde displayed insecticidal properties against *Acanthoscelides obectus* beetles in a past study (Regnault-Roger *et al.* 2003), the concentrations tested in this work did not make it possible to confirm its insecticidal behavior on termite activity.

## 4. CONCLUSIONS

Heat treatment improved wood durability, clearly increasing resistance to brown and white rots, but it had only a slight effect in improving resistance to termites. The process carried out at 228 °C was found to give ash wood the best biological resistance properties. However, it can be expected that such treatment will greatly degrade the mechanical properties of the wood.

In addition, the results presented in this work confirmed that thermal treatment generates new extractive substances within the modified wood material, depending on the treatment temperature level, which could act as fungistatics and prevent fungus from growing, improving the resistance of the modified wood to decay. The antifungal activity of heat-treated ash wood extracts varied depending on heat treatment intensity and the solvent used during the extraction process, but these effects were not efficient over the long term and were not significant. It also clearly appeared that extracts from heat-treated ash wood were more efficient against brown-rot growth than against white-rot growth. The analysis of these extractive compounds showed that the most abundant was syringaldehyde. In addition, the syringaldehyde content increased in line with the thermal treatment intensity, whatever the extraction process used. Although syringaldehyde has shown insecticidal properties in past

studies, it could not be shown in our study that it has an insecticidal role against termites. The generation of low molecular weight organic acids might decrease the pH of wood, also impacting fungal inhibition and the enzymatic digestive system of termites. Lastly, the condensation of several chemical compounds from wood thermal degradation might also partially explain the better decay resistance of wood treated at high temperature. However, taken separately, the extractive compounds might act differently from the same extracts present within the wood. The increased durability of heat-treated ash wood is due to thermal degradation of wood cell wall polymers involving a combination of modifications: hydrophobic behavior, lignin modification, hemicellulose degradation and also extractive generation. To conclude, the results obtained in this study showed that the extractive compounds of heat-treated wood have a slight impact on the better durability of thermally modified wood, but are certainly not the only reason.

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