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3 **ANTI-FUNGAL AND ANTI-TERMITE ACTIVITY OF EXTRACTIVES**
4 **COMPOUNDS FROM THERMALLY MODIFIED ASH WOODS**
5

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19 **ABSTRACT**

20 Thermal modification of wood is a promising alternative to chemical and biocidal
21 modification processes, increasing the biological durability and dimensional stability of wood.
22 However, the wood-decay resistance properties of heat-treated wood are still not well known.
23 The main objective of this study was to determine the biological resistance of heat-treated ash
24 wood, and assess the antifungal and anti-termite activity of extractive compounds from heat-
25 treated ash woods, depending on the intensity of the modification process (2 hours at 170,
26 200, 215, 228 (°C) - steam pressure). Untreated and heat-treated wood samples were extracted
27 with water or acetone. The extracts were then used to determine inhibition effectiveness
28 against white-rot (*Trametes versicolor*) and brown-rot (*Rhodonina placenta*) fungi. Whatman
29 papers impregnated with extractives were used to evaluate the inhibition of termite feeding.
30 Lastly, the extractives were analyzed by Gas Chromatography - Mass Spectrometry (GC-MS)
31 and compared for their level of anti-termite and antifungal activity. The results showed that
32 the degree of antifungal activity of these extracts depended on the solvent used during the
33 extraction process and varied depending on heat treatment intensity. The extracts were more
34 effective against brown-rot than white-rot fungi. However, the anti-termite activity of heat-
35 treated ash wood extracts was not really significant. A GC-MS analysis showed that the main
36 share of the extractives in untreated wood was removed. In addition, new chemical elements
37 were generated by the thermal degradation of wood polymers (lignin and hemicelluloses),
38 including aliphatic acids, monosaccharides and other products resulting from their
39 dehydration reaction. The most abundant element was syringaldehyde, from lignin derived
40 compounds, which might explain the antifungal activities of thermally treated ash wood
41 extracts.

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

44 **1. INTRODUCTION**

45

46 Ash (*Fraxinus* spp.) is a significant hardwood resource largely used in the European wood
47 industry. Given its very weak resistance to decay and insects, this timber is naturally in
48 appropriate for class 3 use (EN 350 CEN 2016). Ash wood materials therefore need to be
49 treated with biocide additives for outdoor use (Candelier *et al.* 2017; Gérard *et al.* 2017).
50 Industrial wood protection methods usually focus on ways of impregnating active
51 formulations containing biocides. However, ash wood is only a moderately treatable timber
52 for both its sap and heartwood (EN350 CEN 2016; Gérard *et al.* 2017). In addition, due to
53 their impact on human health, these chemicals are a subject of environmental pressures, which
54 will restrict their use in the near future (Schultz *et al.* 2007). The Implementation of EU
55 Environmental Legislation in recent years has resulted in the development of non-biocidal
56 alternatives, such as thermal modification (Sandberg *et al.* 2016), chemical impregnation
57 (Gérardin 2016; Mantanis 2017; Guo *et al.* 2018), or a combination of the two (Salman *et al.*
58 2016; Sandberg *et al.* 2017). Using antagonistic microorganisms, combined or not with
59 biochemicals, is also considered as a promising ecofriendly way of protecting wood: (i)
60 biocontrol microorganisms consume the available nutrients and produce biochemical
61 compounds that are toxic and/or repellent to fungal decay and, (ii) the biochemical
62 compounds include cell wall-degrading enzymes, siderophores, chelating iron and other
63 antibiotics. This last wood preservation method is not yet industrialized and requires
64 additional studies, especially regarding the environmental impact of these formulations (Susi
65 *et al.* 2011).

66 On the other hand, thermal treatment of wood, using slow pyrolysis in an inert atmosphere,
67 has been widely studied and has now been industrially developed around the world, mainly to
68 increase wood durability against fungi in line with the process parameters (Calonego *et al.*
69 2010; Candelier *et al.* 2016). According to the literature (Kamdern *et al.* 2002; Weiland and

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

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

80 **(ii)** Wood polymers are modified and the enzymes involved in the fungal degradation of wood
81 do not recognize it anymore as such. Firstly, the changes in lignin chemical composition
82 prevent fungal enzymes from recognizing and attacking specific target molecules (Vallet *et al.*
83 2001; Lekounougou *et al.* 2009). Secondly, changes in the ligneous polymer network also
84 seem to play a role in fungal inhibition. The improved resistance of modified wood to decay is
85 mainly due to possible crosslinking between lignin and certain molecules derived from wood
86 thermal degradation, such as furfural (Weiland and Guyonnet 2003). Such modifications lead
87 to a substrate that is not recognized by the fungal enzyme system, resulting in an undecayed
88 material (Hakkou *et al.* 2006). Moreover, cellulose may undergo an esterification reaction,
89 due to the acetic acid generated during the thermal degradation of hemicelluloses (Tjeerdsma
90 and Militz 2005; Gao *et al.* 2016).

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

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

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

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

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

119 studies revealed that thermally modified wood had lower termite resistance than untreated
120 wood (Sivrikaya *et al.* 2015; Salman *et al.* 2016, 2017). Generally, termite survival levels
121 reveal an effect of thermal treatment on the biology of these insects. Termite survival rates
122 remained virtually constant for treatments carried out at a low temperature (180 °C to 210 °C)
123 and were lower for higher treatment temperatures. Treatments over 200 °C induced crucial
124 modifications to the wood material, possibly reducing suitability for termites (Candelier *et al.*
125 2017). As heat-treated wood is consumed when exposed to termites, the toxicity of ingested
126 components may be the reason for the higher mortality rate observed (Surini *et al.* 2012)
127 when treatments are carried out at high intensities (temperature – duration). In addition, when
128 heat-treated and untreated control wood samples were exposed side-by-side (choice test),
129 termites preferred to attack the untreated wood samples (Nunes *et al.* 2004).

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

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

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

146 **2. MATERIALS AND METHODS**

147 **2.1. Wood samples**

148 Untreated ash (*Fraxinus excelsior* L.) wood samples were obtained from a French wood
149 company (Bois Durables de Bourgogne, 71120 Vendennes-lès-Charolles, France). Twenty
150 boards measuring 4000 x 110 x 25 (mm³) [L x R x T] were selected. Special attention was
151 paid to ensure these planks only had small variations in density (around 650 kg/m³ ± 10 %) and a uniform width of annual rings. All the planks were sawn into two equal parts of 2 m in
152 length. Half of each plank was used as reference material and the other half was thermally
153 treated at different treatment intensities. The planks were then dried at 103°C in an industrial
154 oven up to mass stabilization (m_0).

156

157 **2.2. Heat treatment protocols**

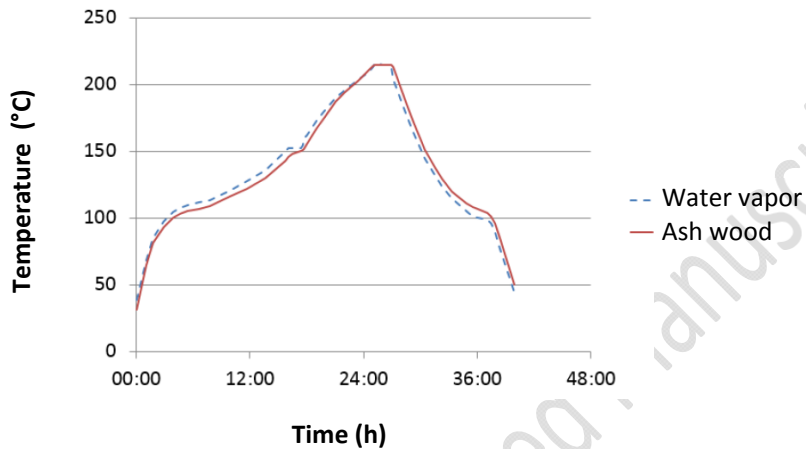
158 The thermal treatment processes were carried out at the same company. These operations
159 were carried out in a 20 cubic meter industrial oven (Jartek[®]), in convection heat-transfer
160 mode and under a steam pressure process [ThermoWood[®] method, Finnish Thermowood
161 Association (2003)].

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

165 The oven temperature was firstly increased rapidly (0,5 °C/min) to 100 °C, then slowly (0,2
166 °C/min) to 150 °C. This temperature level was maintained for 1 h. The temperature was
167 finally increased (0,2 °C/min) to the desired level [from a low temperature (170 °C to 200 °C)
168 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 (mm³) [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

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

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

205

206 **2.6. Antifungal activity test**

207 Antifungal activities against the growth of *Trametes versicolor* Quélet (TV) [Linnaeus, CTB
208 863 A] and *Rhodonina 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)

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

$$223 \quad \% \text{ inhibition} = [(M_c - M_t) \div M_c] \times 100 \quad (3)$$

224

225 **2.7. Anti-termite activity test**

226 The anti-termite activities of each heat-treated ash wood extract were tested by screening
227 tests. Anhydrous cellulose paper measuring 2,5 cm in diameter was weighed (m_4) and then
228 impregnated with 70 µL of the diluted extracts in water or acetone [C= 2,5% m/m], air dried
229 (20 °C ± 2 °C and 65 % ± 5 %) for 2 hours (m_4), then placed in the center of a Petri dish (5,5
230 cm diameter). Fifteen grams of wet sand (4 vol. sand/1 vol. water) was placed evenly around
231 the paper and 20 termite workers (*Reticulitermes flavipes*, ex. *santonensis*) were added to each
232 test set-up. Three replicates were carried out for each diluted wood extract. Three papers
233 impregnated with water or acetone alone were tested to estimate the impact of the solvent on
234 termite activity. Tests with water only were considered as a virulence control. For each test
235 set-up, the paper samples were placed on a plastic grid. Lastly, three diet control set-ups
236 containing only 15 g of wet sand and 20 termites were used to check termite survival without

237 any feeding possibilities/without any trophic sources. All test set-ups were kept at 27 °C and >
238 75 % RH (Figure 2).

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

244
$$WL_{term.}(\%) = [(m_4 - m_5) \div m_4] \times 100 \quad (4)$$

245



246

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

248

249 2.8. GC–MS analysis

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

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

254 Before being injected into the GC-MS analyzer, the extractive substance samples were
255 derivatized in order to improve the detection of all chemical compounds. To perform this
256 silylation derivatization process, 2 mg of extract was solubilized in a glass tube with 50 μL of
257 BSTFA + 1 % TMCS solution (Bis(trimethylsilyl)trifluoroacetamide + Trimethylchlorosilane)
258 [Acros Organics]. The glass tube was sealed and dried in an oven at 70 $^{\circ}\text{C}$ for 120 minutes,
259 then opened to evaporate the BSTFA. The derivatized wood extract was dissolved in 1 mL of
260 $\text{C}_4\text{H}_8\text{O}_2$ solution (ethyl acetate [Acros Organics]). One μL of this solution was injected into
261 the Gas Chromatograph at a 250 $^{\circ}\text{C}$ inlet temperature in splitless mode. Helium was used as
262 the carrier gas. The temperature program was: 80 $^{\circ}\text{C}$ (2 min), 10 $^{\circ}\text{C}/\text{min}$ to 190 $^{\circ}\text{C}$ 15
263 $^{\circ}\text{C}/\text{min}^{-1}$ to 280 $^{\circ}\text{C}$ - maintained for 10 minutes, 10 $^{\circ}\text{C}/\text{min}$ to 300 $^{\circ}\text{C}$ 15 $^{\circ}\text{C}/\text{min}^{-1}$ -
264 maintained for 14 minutes. A helium flow of 1 mL/min was used as the mobile phase. After
265 this separation step, compounds were transferred to the Mass Spectrometer by a transfer line
266 heated at 250 $^{\circ}\text{C}$ and ionization was achieved by the Electron Impact method (70 eV
267 ionization energy).

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

274

275 **2.9. Determination of pH Value**

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

282

283 **2.10. Statistical Analysis**

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

292

293 **3. RESULTS AND DISCUSSION**

294 **3.1. Biological resistance tests on wood block samples**

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

299 **3.1.1. Decay resistance**

300 Similar results were found for both of the rots tested. However, *Rhodonía placenta* was the
301 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 (<i>Reticulitermes flavipes</i>)		
		<i>Trametes versicolor</i>	<i>Rhodonia placenta</i>	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 °C 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

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

328

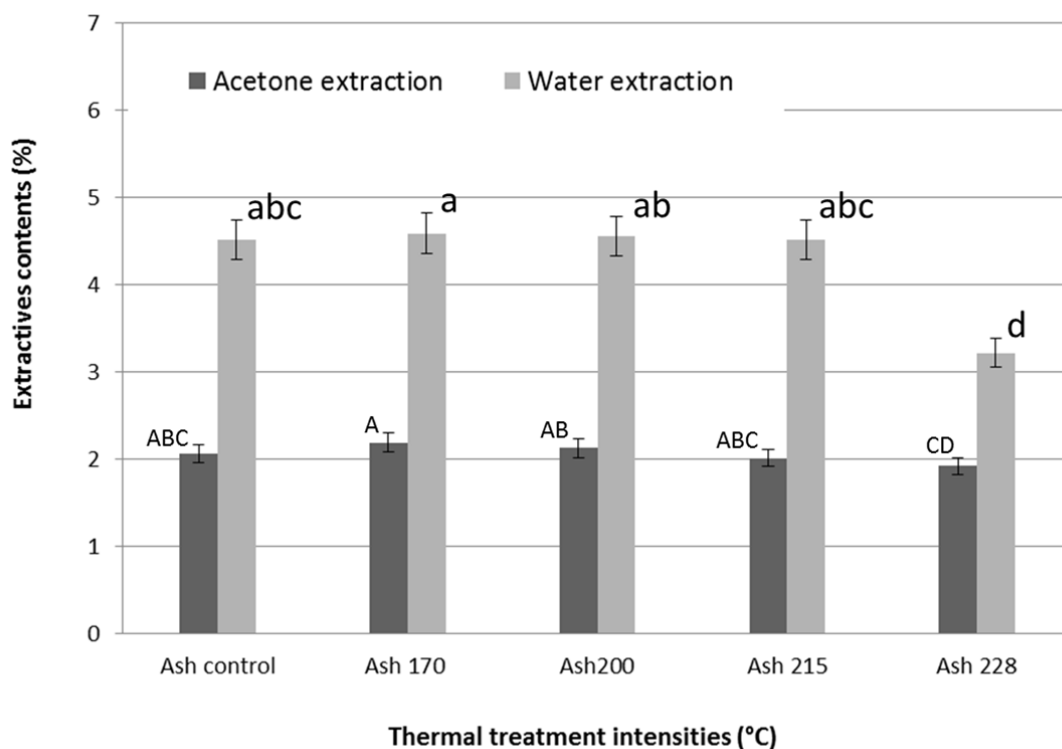
329 **3.2. Extractive content**

330 For thermal treatment carried out at a low temperature (< 215 °C), the proportions of
331 heat-treated wood extractives increased in comparison with those of untreated ash wood,
332 whatever the solvent used during the extraction process (Figure 3). This result tallied with
333 past studies, indicating an increase in extractive rate resulting from the generation of different
334 degradation products (Poncsak *et al.* 2009; Esteves *et al.* 2008). A past study by Esteves *et al.*
335 (2008), carried out on eucalypt wood, showed that thermal degradation of lignin and
336 hemicelluloses led to an increase in extractive content. In fact, the formation of new extractive
337 compounds arising from polysaccharide degradation at around 160 °C may be one of the
338 reasons for that phenomenon. At higher temperatures (>215 °C), these new compounds are
339 also generated but, under the heat effect, they are then converted into volatile products that
340 leave the structure of the wood, thus causing a loss of matter, leading to a decrease in
341 extractive content (Wang *et al.* 2016).

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

345 relative extractive percentage decreased.

346

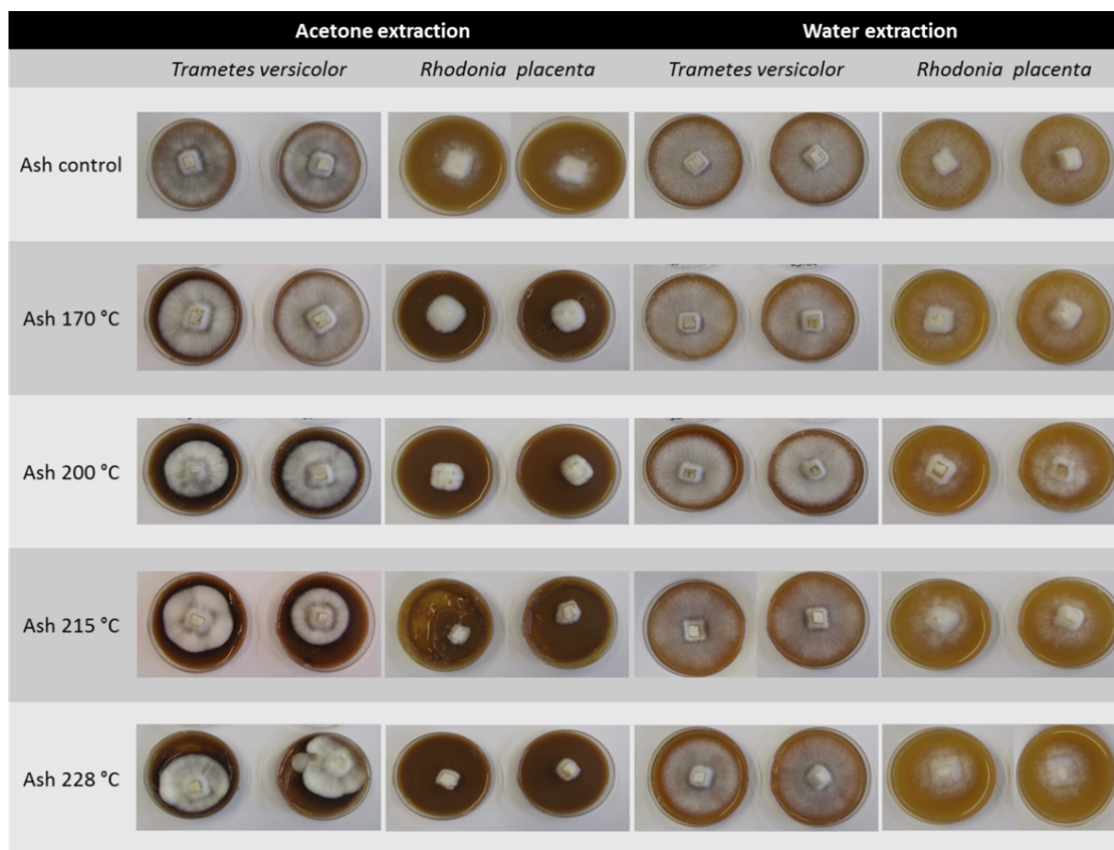


347 **Figure 3:** Extractive contents (%) of untreated and heat-treated ash woods at different
348 temperature levels (170 °C to 228°C), depending on the extraction solvent used.
349

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 μ L), 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 *Rhodonía placenta*.

Extraction method	Ash wood	<i>Trametes versicolor</i>				<i>Rhodonía placenta</i>			
		3,5 days		7 days		3,5 days		7 days	
		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 μ L) against
 394 *Reticulitermes flavipes*.

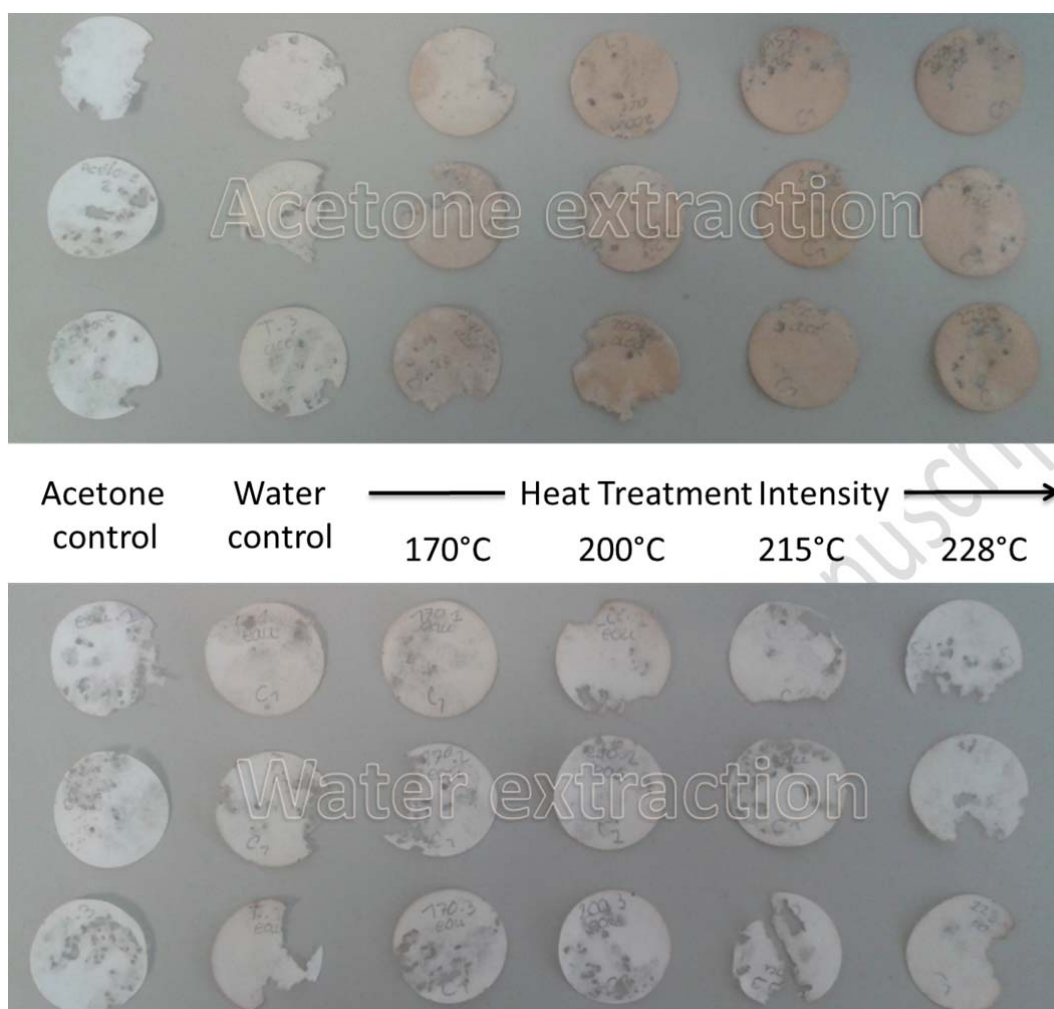
Extraction solvent	Ash wood	Weight loss (%)	Standard deviation (%)	Survival rate (%)	Standard deviation (%)
	Thermal treatment temperature				
Acetone C=2,5% [m/m]	0	45,25 ^A	9,31	85,00 ^A	5,00
	170	34,86 ^{AB}	1,52	86,67 ^A	7,43
	200	34,49 ^{AB}	4,39	81,67 ^{AB}	2,89
	215	32,17 ^{AB}	4,02	76,67 ^{ABC}	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*.

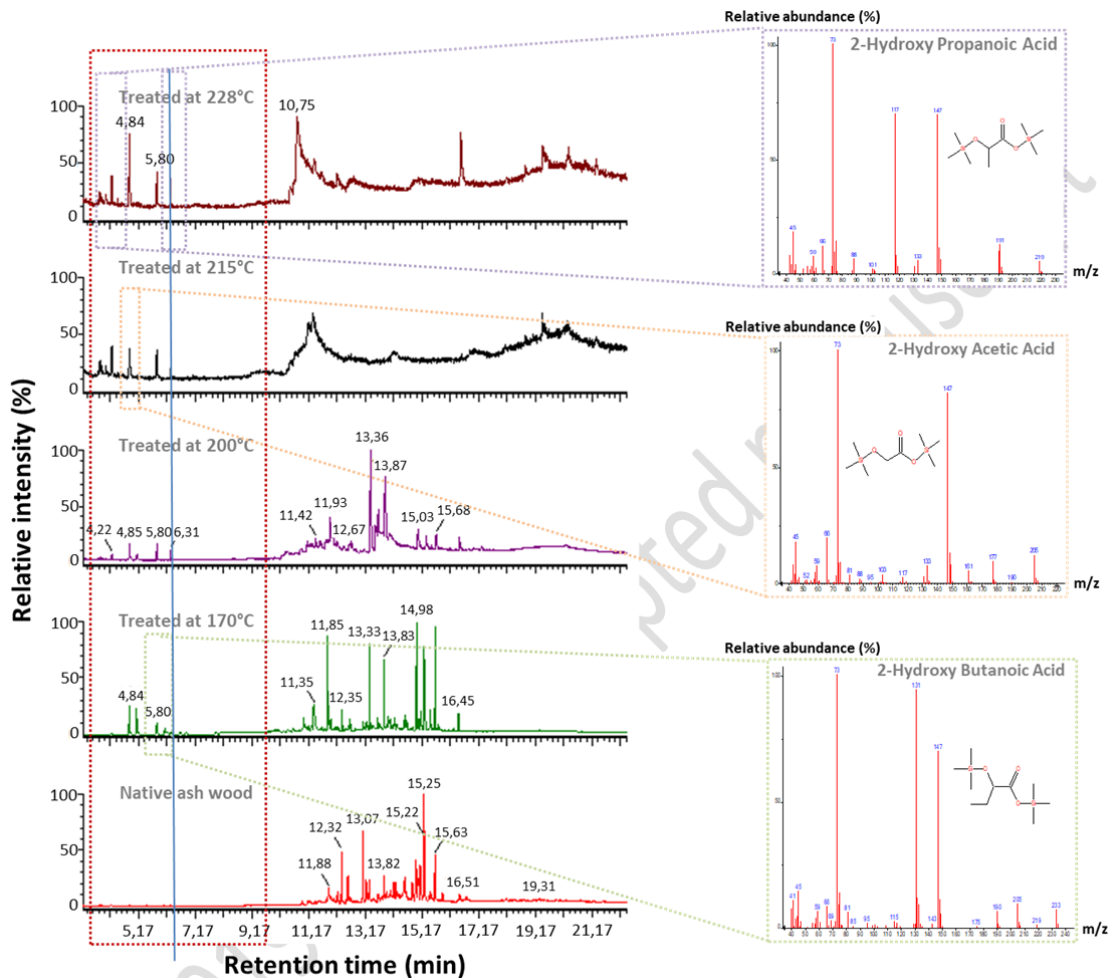
406 The termite survival rate revealed no significant effect on the biology of these insects. The
407 survival rate was not significantly dissimilar for the different treatments compared to the
408 control samples. The effects of untreated wood extracts on the termite survival rate were very
409 similar 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).

425 For the ash wood treated at 170 °C, it was found that the respective water extracts mainly
 426 comprised low molecular weight organic 2-hydroxy-acids [propanoic acid (rt = 4,84 min and
 427 6,09 min), acetic acid (rt = 5,10 min), butanoic acid (rt = 5,80 min)] (Table 5) and derivated
 428 phenylpropanoid compounds (rt = 11,00 min and 11,35 min). The presence of these acid
 429 compounds, decreasing the pH of the wood, may have played a role in fungal inhibition.
 430 Indeed, according to the results presented in Table 4, the pH values of ash wood decreased
 431 depending on the temperature used during the heat treatment process. The pH values varied
 432 from 5,16 for untreated wood to 3,92 for wood thermally modified at 228 °C. Similar trends
 433 were observed on softwood and hardwood species by Niemz *et al.* (2008), showing that the
 434 pH decreases with increasing heat treatment intensity.

435 **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}

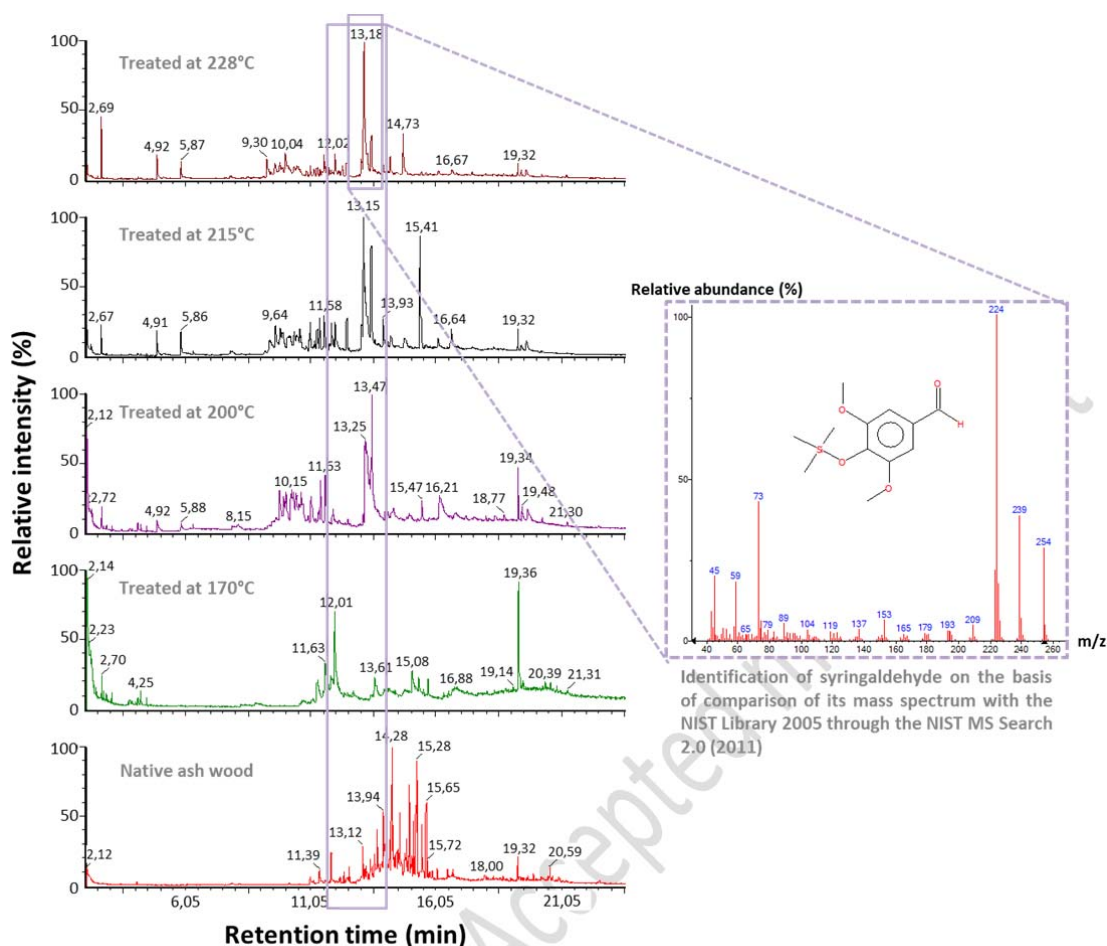
436
 437 Indeed, Yalcin and Sahin (2015) reported that heat treatment conferred on narrow-leaved ash
 438 wood a pH that was less conducive to fungal growth, with the untreated wood pH of 5,7
 439 falling to 3,9 in the modified wood, while the pH for optimum fungal growth is around 5-6
 440 (Bozkurt *et al.* 1993). In addition, such a decrease in wood pH could disturb the enzymatic
 441 digestion of termites during their wood destruction (Lima *et al.* 2014). In fact, pH widely
 442 affects the activity of fungal enzymes. The pH dependence is usually due to the side groups
 443 of the amino acids. A diminution in pH changes the protonation pattern resulting in protein
 444 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.



457

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

460

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

468

469

470

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

489 present in syringaldehyde (De Souza *et al.* 2005). Total hydroxyl groups from lignin also had
490 a large impact on cellulase adsorption and enzymatic hydrolysis occurring during fungal
491 degradation (Yu *et al.* 2014), and more particularly acetosyringone, vanillin and
492 syringaldehyde (Qin *et al.* 2016). Ximenes *et al.* (2011) confirmed that syringaldehyde and
493 vanillin inhibit cellulose hydrolysis. Although syringaldehyde displayed insecticidal
494 properties against *Acanthoscelides obectus* beetles in a past study (Regnault-Roger *et al.*
495 2003), the concentrations tested in this work did not make it possible to confirm its
496 insecticidal behavior on termite activity.

497

498 **4. CONCLUSIONS**

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

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

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

527
528
529

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