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Chemistry and ecotoxicity of heat treated pine wood extractives

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Abstract

Pine (*Pinus pinaster*) wood was heat treated in an autoclave for 2-12 hours at 190-210 ºC. Hemicelluloses were the first compounds affected by the treatment. In general, the sugar decrease was higher for arabinose and galactose followed by xylose and mannose. Lignin started to degrade for small mass losses but at a slower rate than hemicelluloses, and cellulose only degraded significantly for severe treatments. Almost all of the original extractives disappeared and new compounds arose like anhydrosugars and phenolic compounds. The compounds that might leach from heat treated wood were mainly those identified in the water and ethanol extracts, all of which were not harmful at the existing concentrations, thereby reinforcing the wood heat treatment as an environmental benign process.

Introduction

Heat treatment is by now a well known and commercial wood modification process that improves wood properties, changing low value species into higher value materials. The main known processes are Rectified Wood in France, Thermowood in Finland, Plato wood in Holland and OHT in Germany.
All these treatments decrease equilibrium moisture content and increase dimensional stability of wood as reported by many authors (Jämsä and Viitaniemi 2001; Yildiz 2002; Esteves et al. 2007a, b; 2008a). Durability is also enhanced mainly against rot (Kamdem et al. 2002; Hakkou et al. 2006) but termite resistance does not increase. The resistance against weathering shows a slight improvement (Jämsä et al. 2000) along with a decrease in wood wettability (Pétrissans et al. 2003; Hakkou et al. 2005). The major disadvantage of heat treatment is the reduction of wood strength mainly due to the decrease of bending strength (Kim et al. 1998; Bengtsson et al. 2002). A review on wood heat treatment with a description of the major publications in this field has been recently published by Esteves and Pereira (2009).
The heat treatment causes a chemical change in the wood and studies on the chemical composition of treated wood have been reported by several authors. The chemical modification starts with the degradation of hemicelluloses by deacetylation followed by depolymerization catalyzed by the released acetic acid, giving origin to some low mass extractable compounds (Tjeerdsma et al 1998; Sivonen et al. 2002; Nuopponen et al. 2004). At the same time carbohydrate dehydration decreases the overall content of hydroxyl groups (Weiland and

Guyonnet 2003) and leads to the formation of aldehydes like furfural and hydroxymethylfurfural, respectively from pentoses and hexoses (Tjeerdsma and Militz 2005).

Although cellulose is more resistant than hemicelluloses, there is degradation of the amorphous cellulose and therefore an increase of cellulose crystallinity (Bhuiyan and Hirai 2000). Lignin is affected through cleavage of β-O-4 linkages and in softwood lignin there is also a reduction of methoxyl content leading to a more condensed structure (Wikberg and Maunu 2004; Tjeerdsma and Militz 2005; Boonstra and Tjeerdsma 2006).

In relation to extractives, the most volatile compounds leave the wood while others are degraded. As a result of these reactions the overall content of hydrogen and oxygen is reduced in relation to carbon (Bourgois and Guyonnet 1988). The compounds that are volatilized during the treatment can be recovered and do not constitute an environmental hazard. Graf et al. (2005) analyzed the gaseous emissions from six woods (spruce, fir, larch, oak, ash and robinia) during thermal treatment and concluded that about 80% of the products were acetic acid, furfural, some furfuryl derivatives and also several mono-, sesqui- and diterpenes. There are still some emissions of volatile organic compounds from heat treated wood in service but in accordance with Manninen et al. (2002) for Scots pine wood they are about eight times less than those from air-dried untreated wood. One of the compounds released from treated wood, 2-furacarboxyaldehyde, is irritating for the respiratory system, but the same happens with the monoterpenes from untreated wood (Norbäck et al. 1995). Esteves et al (2008b) studied the extractive content and composition of heat treated *Eucalyptus globulus* wood and concluded that the extractive content increased in the beginning of the treatment and decreased afterwards. Almost all of the original extractives disappeared and new compounds were formed including monosaccharides and their dehydratation products, as well as syringaldehyde, syringic acid and sinapaldehyde as the most prominent compounds.

This work focus on the extractives that are present in *Pinus pinaster* heat treated wood and that might leach when wood is in service. This is an important aspect since heat treated wood is considered environmentally benign because no toxic chemicals are added to wood as in traditional preservation methods but the

chemical changes induced by the heat treatment may originate new compounds, which may be unsafe to humans or to the environment.

Experimental

Heat treatment and sample preparation

Cubic sapwood samples with approximately 40 mm edge of maritime pine (*Pinus pinaster* Aiton.) wood from the Portuguese region of Águeda were submitted to a heat treatment in an autoclave during 2 to 12 h at 190 ºC, 200ºC and 210ºC. Four replicates were made for each treatment condition. The autoclave with 0.5 m height and square area of 1 m^2 was heated by a mixture of superheated and saturated steam to the desired temperature. A sleeve with a flow of superheated steam was used to maintain temperature at a constant value. After the treatment the samples were put in a dry environment, cooled and weighted. Mass loss was determined in relation to dry wood.
Samples of untreated and heat treated wood were milled, screened and the 40-60 mesh fraction was used for chemical analysis, in accordance with Tappi T 264 MAC-88.

Extractive content

The extractive content was determined by successive Soxhlet extraction of about 3 g of each sample using dichloromethane, ethanol and water. Extractions were made in 250 ml soxhlets with 150 ml of solvent during 10 h for dichloromethane and 20 h for ethanol and water. The extracts were concentrated in a rotary evaporator, dried in an oven at 40 ºC overnight, and followed by 1 hour at 100 º C. The percentage of extractives in each solvent was determined gravimetrically in relation to initial dry mass, according to Tappi T 204 Mac-88. All samples were analysed in duplicate.

Extractive composition

The composition of dichloromethane, ethanol and water extractives of the heat treated pine wood samples that were analysed corresponded to treatments at 190ºC during 2, 6 and 12 h and at 210ºC during 12 h, with mass losses of 0.4%,

3.5%, 3.7% and 6.7%, respectively. For ethanol and water extracts only the samples with 3.5% and 6.7% mass loss were analysed. The samples were analysed in duplicate.

The volume of the dichloromethane, ethanol and water solutions necessary to contain about 3 mg of solid extract was evaporated in a rotary evaporator under vacuum until a volume of about 1 ml. The bath temperature was kept below 40 ºC, with a 65 mbar vacuum for water, 175 mbar for ethanol and 900 mbar for dichloromethane. The sample was moved to a vial and dried under a nitrogen flow. The vials were kept overnight in an oven at 40ºC with a Petri dish containing P_2O_5, cooled in a desiccator and weighed in an analytical Mettler H31AR balance, with a precision of ± 0.0001g.

Samples were derivatized with 10 µl of pyridine and 10 µl of BSTFA (N, O-bis-trimethilsylil-trifluoroacetamide) for each mg of dry extract. The vials were closed and kept for 20 min in an oven at 60ºC, cooled down and injected in a chromatograph HP6890A with a mass detector 5973 Agilent and an Agilent Db-5ms column. The injection of 1 µl was made in splitless mode. The injector was kept at 320 ºC and column and detector at 325 ºC. The program used for dichloromethane and ethanol extractives was similar, starting at 100 ºC during 5 min, followed by an increase of 5ºC/min until 320 ºC and remaining at this temperature for 15 min. For water extractives the initial temperature was 145ºC during 3 min, followed by an increase of 5 ºC/min up to 310 ºC and 2 min at this temperature.

Extractive compounds were identified by comparing their EI mass spectra with library published spectra, and with the spectra obtained from standard compounds. Extractive composition was determined by peak area integration with no further correction for eventual differences in their response factors

Klason lignin determination

The samples for lignin determination were kept in an oven at 60 ºC overnight, followed by 1 hour at 100 ºC. After that 350 mg were weighed into a small glass vessel and 3 ml of iced sulphuric acid at 72% were added. The vessels were kept in a thermostatic bath at 30ºC during one hour, mixing every 10 minutes. The samples were transferred to 100 ml Schott flasks and 84 ml of distilled water was added. After autoclaving during one hour at 120ºC, the flasks were cooled with

ice and the samples filtered with a nr. 4 crucible, dried and weighed. The filtered solution was kept for sugar determination. The determination of soluble lignin was done by removing 2 ml of the filtered solution, diluting to 20 ml and measuring the absorbance at 205 nm in a spectrophotometer in accordance with Tappi UM 250 “Acid-soluble lignin in wood and pulp”. The analyses were made in duplicate.

Determination of monosaccharides

After the hydrolysis, distilled water was added to the filtered solution until reaching 250 ml and 100 ml were removed to a 250 ml Erlenmeyer adding 2 ml of 1% inositol as internal standard. The solution was neutralized with barium hydroxide and centrifuged. Sodium borohydride was added to the clean solution and after 2 h glacial acetic acid was joined until gas release stopped. The resulting solution was evaporated in a rotator evaporator at 40ºC between 800 and 30 mbar vacuum until syrup. After that 10 ml of methanol were added and evaporated at 45ºC and 250 mbar. The last step was repeated with another 10 ml of methanol. After evaporation the samples were dried in an oven at 100 ºC during 15 min. The samples were derivatized by acetylation and injected in a gas chromatograph HP 5890A with a S2330 column and ionization flame detector. The injector and detector were kept at 250ºC while the temperature of the oven initiated at 225 ºC during 1 min, followed by an increase of 5 ºCmin^{-1} up to 250 ºC and remaining at this temperature for 3 min.
The content of monosaccharides was determined according to Tappi 249 cm-00. The response factors were 1.17 for arabinose, 1.03 for xylose, 0.99 for mannose, 1.00 for galactose and 0.91 for glucose. The analyses were made in duplicate.

Ecotoxicity

For the ecotoxicity tests 2 g of dried wood were extracted as mentioned before and the extracts were concentrated to 50 ml. The strain of *Bacillus stearothermophilus* and the conditions for its maintenance and growth have been described previously (Jurado et al. 1987; Monteiro et al. 2008). Liquid cultures were started with an early stationary inoculum and were grown in 300 ml Erlenmeyer flasks containing 50 ml of growth medium (diluted L-Broth), at 65 ºC

and shaken at 100 rpm in a GFL 1083 water bath shaker. Water and ethanol pine wood extracts were added to the growth medium in order to obtain the concentrations indicated in the figures. No ecotoxicity tests were made with dichloromethane extracts because the preliminary tests showed that dichloromethane itself was very toxic to the bacteria. A 2% (v/v) concentration represents 2 ml of extract for 100 ml of medium solution. In experiments with ethanol pine wood extracts, control cultures were grown in a medium without pine wood extractives, but with 2% (v/v) ethanol (e.g. the maximum amount of solvent used). The bacterial growth was measured by turbidimetry at 610 nm in a Jenway 6505 UV/vis spectrophotometer.

Results and discussion

Chemical composition

Chemical composition was determined for untreated and for heat treated wood and the results are presented on Table 1. Although 3% mass loss is considered as the least mass loss necessary to improve wood properties by heating, the treated wood samples were selected to represent different severities of the heat treatment, from mild (< 1% mass loss) to more severe conditions (> 6% mass loss). In general, heat treated wood had more extractives, apparent lignin and cellulose and less hemicelluloses. The wood chemical composition changed with the heat treatment, as a result of the different thermal resistance of the chemical compounds.

Hemicelluloses were the first compounds to degrade. The degradation of hemicelluloses starts by deacetylation followed by depolymerization catalyzed by the released acetic acid as reported earlier. Even for small mass loss (0.4%) there was a decrease of arabinose and galactose content resulting from the degradation of pine arabinogalactan and possibly arabinan even thought this last one represents less than 0.3%. By increasing treatment severity, xylose and mannose content decreased, corresponding to the degradation of arabinoglucuronoxylan followed by galactoglucomannan. For instance at 3.5% mass loss, xylose content decreased about 21% in relation to initial content while mannose decreased only

5%. The higher degradation of xylan in relation to mannan is in accordance with several authors (Alén et al 1995; Nuopponen et al 2004).

The thermal degradation of hemicelluloses usually yields small mass carbohydrates and other compounds, depending on the temperature. Some extractable compounds that were found in all of the extracts from heat treated samples, even for small mass loss (0.4%), seem to result from the degradation of hemicelluloses, e.g. galactosan and two C_5 anhydrosugars identified in the dichloromethane extract that probably come from galactose, arabinose and xylose degradation. Mannosan, probably from mannose degradation, was only found for higher mass losses (Tables 3-4). These sugars can be found in a compilation of the main compounds resulting from polysaccharide pyrolysis (Faix et al. 1991a, b) showing that despite the lower temperature used for these treatments, heat treatment might be considered a low temperature pyrolysis.

Cellulose was little affected by the mild treatments, as confirmed by the increase of glucose content. Moreover, levoglucosan which is often considered as the main product of cellulose thermal degradation (Gao et al. 2003) was found only in small amount in the heat treated samples with small mass losses. In the heat treated samples with mass losses higher than 6.7%, levoglucosan was noticeable, corresponding to 15.3% of the dichloromethane extract and 28.3% of the ethanol extract (Tables 3-5). This means that pine wood cellulose will only be significantly attacked for heat treatments that induce mass losses of this order of magnitude. Therefore cellulose content increased with the treatment not because of an increase on cellulose content but rather due to the relative higher degradation of hemicelluloses.

Acid insoluble lignin of treated pine wood increased with the severity of the heat treatment. This does not mean that there is an increase on the amount of lignin itself, but instead that the reduction of polysaccharides was higher. Also this increase may derive from humidification products of carbohydrates and condensation reactions that increase the amount of acid insoluble material in heat treated wood (Tjeerdsma et al. 1988). This explains also the increase of the sum of chemical composition between untreated and treated wood.

The increase in phenolic compounds in the dichloromethane extract for small mass losses (0.4%) suggests that there was already lignin degradation at this stage as reported by some authors (Windeisen et al. 2007, Esteves et al. 2008b). Several

extractable phenolic compounds appeared in this extract like catechol, vanillin, vanillic acid, 3-vanillyl propanol and coniferyl aldehyde. These compounds are generally referred as resulting from lignin pyrolysis (Faix et al. 1990a, b) and are not found in polysaccharide pyrolysis (Faix et al. 1991a, b), which means that there was already some lignin degradation for small mass losses. The majority of these compounds can also be identified in the smoke of forest fires and surrounding air (Graham et al. 2002).

Figure 1 presents the variation of pine wood extractives in function of mass loss with the heat treatment. In untreated pine wood, extractives were mainly polar compounds: on average untreated wood had 1.2%, 0.8% and 0.6% extractives soluble in water, ethanol and dichloromethane respectively. With heating, the total content of extractives increased, reaching maximal values of 7.8% in heat treated samples at about 3-4% mass loss. After that, the content of extractives decreased. Similar results were reported for heat treated eucalypt wood by Esteves et al. (2008b). This increase followed by a decrease suggests that there is an equilibrium between the degradation or volatilization of original extractives and the appearance of extractable compounds resulting from lignin and polysaccharide degradation. With the increase of treatment severity, the newly formed compounds will degrade to volatiles that leave the wood leading to a decrease on extractable compounds. This can be confirmed by the number of compounds identified in the samples with 6.7% mass loss which is much higher than those found in samples with smaller mass losses.

The content in dichloromethane extractives increased to a maximal value of 2% until about 3.5% mass loss, decreasing afterwards. The ethanol extractives increased to a maximum amount of 2.5%. The major increase in extractive content was due to water extractives, possibly due to the formation of polar compounds resulting from the degradation of cell wall components, as reported by Rosa and Pereira (1994) for the thermal degradation of cork.

Extractive composition

Table 2 presents the extractive contents in dichloromethane, ethanol and water of the analysed samples. The results obtained for the extractive composition are summarised in Tables 3-5 where the percentages shown at the head of the tables

represent the respective mass losses due to heat treatment. Since extractives were determined by GC-MS after derivatization, the non derivatizable compounds were not taken into account. Also, the extraction method did not allow full recovery of the extremely volatile compounds.

The extractive content and composition of pine wood samples were similar to the values reported for this species by several authors (Hemingway et al. 1973; Esteves et al. 2005). The dichloromethane extractives were mainly resin acids, fatty acids, small amounts of phenolic compounds, some glycerides and cyclic sugars. The major compounds identified in the dichloromethane extracts of untreated and heat treated wood samples are presented in Table 3.

Of the original compounds in the dichloromethane extract of untreated wood, fats were the first compounds to disappear with the heat treatment, followed by fatty acids, although some were still found for the most severe treatments. Resin acids content increased in treated samples in the beginning of the treatment as a result of the decrease of other compounds, decreasing afterwards. These results are in accordance with Nuopponen et al. (2003) who reported that for heat treated *Pinus sylvestris* wood at 200 ºC resin acids leave the heartwood to the sapwood and at higher temperatures disappear from the wood.

New compounds were already detected in the dichloromethane extract of heat treated wood samples with only 0.4% mass loss, mainly phenolic compounds such as catechol, vanillin, vanillic acid, 3-vanillyl propanol and coniferyl aldehyde, as well as anhydrosugars, such as levoglucosan (vestiges), two C_5 anhydrosugars which probably correspond to 1-5-anhydroarabinofuranose and 1-5-anhydro-β-D-xylofuranose and galactosan (1,6-anhydro-α-D-galactopyranose) . With the increase of treatment severity, both phenolic compounds and anhydrosugars contents increased.

Table 4 presents the composition of the ethanol extracts of untreated and heat treated wood.

The increase of ethanol extractives was mainly due to phenolic compounds and anhydrosugars. The increase of phenolic compounds until about 3.5% mass loss was due to the increase of vanillin, vanillic acid, 3-vanillyl propanol and over all to the appearance of coniferyl aldehyde. The content of anhydrosugars in the ethanol extract of heat treated wood increased due to the C_5 anhydrosugars, already identified in the dichloromethane extract. Some 3-deoxy acid sugars were

also found in this extract, such as 3-deoxy-D-erythro-pentoic- acid-γ- lactone, 3-deoxy-D-ribo-hexonic- acid-γ- lactone and 3-deoxy-D-arabino-hexonic acid. In accordance with Luijkx et al. (1995), hydrothermolysis of cellulose can lead to the formation of 3-deoxy-D-hexonic acid sugars since these compounds result from the heat degradation of glucose, mannose and fructose by adding a water molecule.

Table 5 presents the composition of water extracts for untreated and heat treated pine wood. The water extract corresponded to 1.2% of untreated wood and 2.3% and 3.8% of treated wood with 3.5% and 6.7% mass loss.

The water extract was only partially derivatizable and an insoluble residue remained at the bottom of the vial. The derivatizable compounds of untreated wood were mainly phenolic compounds and sugars and the increase in water extractives in the treated samples was due to mono and disaccharides constituents of hemicelluloses in open and close forms. The identified compounds represented small amounts but most of the nonderivatizable compounds were probably oligosaccharides resulting from the degradation of polysaccharides that could not be volatilized due to their high mass.

The properties of heat treated wood imparted by the treatment are a result of chemical modification. The lower amount of hemicelluloses, which are the most hygroscopic compounds in wood, and the dehydratation reactions occurring in the matrix are responsible for the steep decrease on equilibrium moisture content and consequent increase on dimensional stability in conjunction with the higher cellulose crystallinity. The extensive decrease on hemicellulose content is also the reason for the high reduction on mechanical properties mainly bending strength as reported before for decayed wood (Winandy and Lebow 2001). Other mechanical properties like MOE or compression strength are less affected since they depend more on cellulose and lignin content.

Ecotoxicity

Ecotoxicity tests are important to evaluate the effects of chemical compounds that leach from wood. The major leachable compounds are found in the water and ethanol extracts so the tests were made with both extracts for untreated and heat treated pine wood using a thermophilic eubacterium *Bacillus stearothermophilus*. This bacterium is frequently used to determine the toxic effects of lipophilic

compounds, e.g. citostatics (Luxo et al. 2000), antiarrythmics (Rosa et al. 2000) and pesticides (Monteiro et al. 2005) and is considered an indicator of the impact on the soil and aquatic ecosystems. The grown and the adaptive process of this bacterium were well characterized by Jurado et al. (1987). This bacterium presents several advantages from the commonly used *Vibrio fischeri*. This is a thermophilic bacterium that is not contaminated by common bacteria, it exists in the soil participating on the biodegradation of compounds and it is representative of both soil and aquatic ecosystems while *Vibrio fischeri* is more adequate for salted mediums. Another advantage is that the wood extracts are coloured and so less suitable for luminescence measurements.

The comparison between untreated and heat treated wood extracts was made with the same amount of extracted wood (2 g) concentrated in 50 ml, in order to compare the eventual increase in toxicity for the same wood mass.

Figure 2 represents the effects of the increasing concentrations of water (A, B) and ethanol (C, D) extracts of untreated (A, C) and heat treated pine wood (B, D) on the optical density (O.D.) of the liquid cultures of *B. stearothermophilus*, as a function of time. The optical density is proportional to the cell density in the liquid cultures and therefore represents the bacterial growth.

No significant effects on the bacterial growth were observed for both untreated (A) and heat treated pine wood (B) showing that the water soluble compounds of both extracts were not toxic for bacteria. This was to be expected since the water extractives presented on Table 5 are mainly sugars, which are not toxic to bacteria. Some of these compounds might even provide additional nutrients for bacteria growth.

In relation to the ethanol extracts of untreated and heat treated wood, the increasing concentrations affected the bacterial growth. The specific growth rate and the final cell density decreased with the increase of extract concentration for untreated pine wood. The addition of 2% (v/v) of ethanol extract of untreated pine wood led to an inhibition of the specific growth rate of 23.8 % (in relation to initial) and a decrease of the optical density in the stationary phase. Differently, for heat treated pine wood, the increased extract concentration led to a gradually increase of the lag phase length and a decrease of the specific growth rate. For instance, an addition of 2% (v/v) of ethanol extract for heat treated pine wood doubled the length of the lag phase and decreased the specific growth rate with a

19.7% inhibition without any effects on the final cell density (Table 6). The different behaviour of bacterial growth for untreated and heat treated wood reflects the differences in extract composition presented in Tables 3 to 5. Even though there were some effects on bacterial growth due to the increased heat treated ethanol extracts they were not significant, because after a while bacteria adapted themselves to the new media and regained a normal growth. The effects of untreated wood extracts on bacterial growth were even more pronounced than for treated wood, confirming the benign nature of heat treatment.

Conclusions

Pinewood chemical composition changed with the heat treatment by the degradation of both structural and extractable compounds. Hemicelluloses were the first to degrade simultaneously with the removal of fats and fatty acids followed by resin acids. Lignin degraded already for mild heat treatments with small mass losses, leading to formation of soluble phenolic compounds, while cellulose degradation required heat treatments with higher mass losses with the formation of levoglucosan. The increase in extractive content was due to water extractives, representing more than 50% of the total, mostly sugars. The overall toxicological results obtained with water and ethanol extracts indicate that the heat treatment of pine wood does not induce additional toxicity to bacteria. These results reinforce the environmental benign nature of heat treated wood and the fact that the potentially leachable compounds are not harmful.

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Fig. 1 Variation of extractive content in dichloromethane, ethanol, water and total for heat treated pine wood versus mass loss resulting from the treatment

Fig. 2 Effects of the water extractives of untreated (A) and heat treated (B) pine wood on the growth of cultures of *Bacillus stearothermophilus*. C and D show, respectively, the effects of the ethanol extractives of untreated and heat treated pine wood on bacterial growth

Table 1. Chemical composition of untreated and heat treated pine wood to different mass losses.

Treatment mass loss (%)	Chemical composition (%)								
	Extractives	Klason lignin	Soluble lignin	Total lignin	Glucose	Xylose	Mannose	Galactose	Arabinose
0.0	2.6	27.1	0.47	27.5	42.9	4.3	12.6	2.1	1.5
0.4	4.8	28.3	0.51	28.8	44.7	4.3	12.8	2.0	1.2
3.5	5.9	29.7	0.53	30.3	46.9	3.5	12.3	1.6	0.7
7.7	4.8	33.7	0.66	34.4	48.6	2.4	10.8	1.0	0.4

Table 2. Extractive contents in dichloromethane, ethanol and water of the analysed samples.

Treatment mass loss (%)	*Dichloromethane*	*Ethanol*	*Water*	*Total*
0.0	0.6	0.8	1.2	2.6
0.4	0.6	1.4	2.8	4.8
3.5	1.6	2.0	2.3	5.9
3.7	1.4	1.6	4.9	7.8
6.7	0.4	2.0	3.8	6.2

Table 3. Dichloromethane soluble extractives of untreated and oven and autoclave heat treated pine wood ordered by retention time (Rt) and analyzed as trimethylsilyl derivatives by GC chromatography. Vest=vestigial amounts, less than 0.1%.

Rt (min)	Compound	Extract (%)				
		Initial	0.4%	3.5%	3.7%	6.7%
16.32	Glycerol	1.5	1.6	Vest	0.6	-
17.80	Catechol	-	1.2	0.6	3.1	2.8
22.70	Anhydrosugar (C5)	-	1.5	0.4	3.7	7.4
23.22	Anhydrosugar (C5)	-	2.8	0.7	7.1	10.6
23.31	4-Hydroxy-pentanoic acid	-	0.6	0.1	1.5	0.6
23.83	Vanillin	-	1.4	0.6	4.7	17.4
24.97	Maleic acid	-	Vest	-	1.0	-
25.90	4-Hydroxybenzoic acid	-	-	-	Vest	0.3
26.45	Galactosan	-	0.5	0.2	1.6	6.3
27.00	Levoglucosan	-	Vest	0.1	0.4	15.3
27.10	3-Deoxy-D-erythro-pentoic-acid-γ-lactone	-	-	-	Vest	0.6
27.44	Mannosan	-	-	-	0.1	0.9
28.90	Vanillic acid	Vest	0.4	0.2	1.0	3.2
29.55	Azelaic acid	0.4	Vest	Vest	0.3	-
30.03	3-Vanillyl propanol	-	1.2	0.5	2.9	6.0
30.86	Coniferyl aldehyde	-	6.6	3.2	19.3	7.3

31.60	Syringic acid	Vest	-	-	0.1	0.3
32.67	Pentadecanoic acid	Vest	-	-	-	-
34.63	Palmitic acid	5.7	0.9	0.2	0.6	0.2
37.68	Linoleic acid	1.0	Vest	-	0.1	-
37.83	Oleic acid+11-trans-octadecenoic acid	28.8	5.1	0.9	2.3	0.3
38.20	Stearic acid	0.2	Vest	Vest	0.1	-
39.70	Pimaric acid	3.6	3.6	2.8	1.4	-
39.96	Sandaracopimaric acid	0.4	Vest	0.3	0.2	-
40.26	Isopimaric acid	3.0	2.8	2.1	1.4	0.2
40.52	Resin acid	0.5	10.5	17.9	5.4	1.5
41.00	Dehydroabietic acid	39.3	52.8	48.7	26.5	7.0
41.54	Abietic acid	0.5	-	0.1	0.7	-
	Non identified compounds (%)	15.1	6.5	20.4	14.0	11.9
	Identified compounds (%)	84.9	93.5	79.6	86.0	88.1

Table 4. Ethanol soluble extractives of untreated and oven and autoclave heat treated pine wood ordered by retention time (Rt) and analyzed as trimethylsilyl derivatives by GC chromatography. Vest=vestigial amounts, less than 0.1%.

		Extract (%)		
Rt (min)	Compound	Initial	3.5%	6.7%
10.2	2-Hydroxypropanoic	4.3	3.5	0.9
10.6	Acetic acid	-	4.1	1.2
16.3	Glycerol	19.5	2.1	Vest
17.6	Butanedioic acid	0.2	0.6	Vest
22.7	Anhydrosugar C5	-	9.5	7.4
23.2	Anhydrosugar C5	-	6.7	3.3
23.8	Vanillin	0.1	0.8	0.6
25.2	Arabinofuranose	1.3	0.8	0.9
25.5	Arabinofuranose	-	0.2	0.1
25.9	4-Hydroxybenzoic acid	0.1	-	-
26.5	Galactosan	0.1	6.4	9.7
27.0	Levoglucosan	0.1	2.2	28.3
27.1	3-Deoxy-D-erythro-pentoic acid- γ-lactone	-	0.2	0.5
27.4	3-Deoxy-D-erythro-pentoic-acid- γ-lactone	0.3	0.3	0.4
27.4	Mannosan	-	0.4	1.7
27.7	Cyclic sugar	18.2	-	-

28.9	Vanillic acid	0.2	0.8	0.6
29.0	3-Deoxy-D-ribo-hexonic-acid	-	0.9	3.4
29.1	Homovanillic acid	-	Vest	-
29.1	3-Deoxy-D-arabino-hexonic acid	-	1.8	5.3
29.4	3-Deoxy-D-arabino-hexonic acid	-	3.0	6.3
29.5	D- Fructose	1.2	0.4	-
30.0	3-Vanillyl propanol	0.2	2.0	1.4
30.2	Inositol	40.5	32.6	19.1
30.9	Coniferyl aldehyde	-	4.8	0.3
31.4	Glucopyranose	0.2	-	-
31.5	Galactonic acid	0.2	-	-
32.2	Glucose	1.4	0.4	-
35.3	Inositol	0.3	0.2	0.2
37.7	Linoleic acid	0.2	-	-
37.8	Oleic acid	1.6	-	-
40.9	Dehydroabietic acid	0.1	0.7	0.2
	Non identified compounds (%)	9.9	14.6	8.1
	Identified compounds (%)	90.1	85.4	91.9

Table 5. Water soluble extractives of untreated and oven and autoclave heat treated pine wood ordered by retention time (Rt) and analyzed as trimethylsilyl derivatives by GC chromatography. * Base peak. Vest= vestigial amounts less than 0.1%.

Values correspond to percentage

		Extract (%)		
Rt (min)	Compound	Initial	3.5%	6.7%
12.94	2-Hydroxypentanodioic acid	-	-	1.0
13.64	Arabinofuranose	21.8	44.4	9.2
14.35	β-L-Arabinopyranose	3.1	8.1	3.4
14.72	Arabinofuranose	-	-	3.1
14.83	α-D-Arabinopyranose	5.3	10.9	2.0
15.31	Mannosan	-	-	2.2
15.70	Anhydrosugar C6	-	-	Vest
15.84	D-Xylopyranose	-	-	0.6
16.06	Non identified compound (97)*	1.2	6.3	3.0
16.82	Glucuronic acid	-	-	1.0
17.07	Vanillic acid	Vest	2.8	2.0
17.23	Homovanillic acid	-	-	0.5
17.65	D-Galactose	-	-	3.0
17.84	β-D-Galactofuranose	-	-	0.8
18.20	3-Vanillyl propanol	1.9	4.0	1.2
18.52	3-Deoxy-arabinohexonic acid	-	-	0.7

18.63	3-Deoxy-arabinohexonic acid	-	-	1.0
18.73	3-Deoxy-arabinohexonic acid	-	-	3.0
19.44	Glucose	-	-	0.5
19.61	Arabinose	-	1.9	1.5
19.70	Glucose	-	-	1.7
20.98	D-Galactose	-	-	0.5
22.69	Hexadecanoic acid	-	3.6	2.2
22.73	Myo-inositol	4.1	-	-
26.18	Octadecanoic	-	7.6	5.5
30.18	4-Hydroxymandelic acid	2.4	-	-
30.39	α-4-Benzeneacetic acid	1.6	-	-
31.16	Vanillyl ethanediol	17.7	5.4	0.4
31.41	Vanillyl ethanediol	20.0	5.0	0.6
-	Disaccharides	6.7	0.0	20.6
	Non identified compounds (%)	15.5	6.3	31.6
	Identified compounds (%)	84.5	93.7	68.4

Table 6. Specific growth rate inhibition by water and ethanol extracts

	Specific growth rate inhibition (% of control)			
Extract % (v/v)	Water		Ethanol	
	Untreated	Heat treated	Untreated	Heat treated
0.0	0	0	0	0
0.2	-6.1	-4.3	-1.6	2.4
0.6	0.4	-3.1	-7.7	-6.6
1.0	0.8	-3.9	-3.9	-11
1.5	-0.8	1.1	-11.6	-16.7
2.0	-4.9	1.9	-23.8	-18.7