Wood Sci Technol 37 (2003) 39–46
DOI 10.1007/s00226-002-0160-6

Original

Analysis of acetylated wood by electron microscopy

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Abstract The properties of acetylated solid wood were investigated earlier, in particular the anti-shrink efficiency and the resistance against decay. This study focuses on the possible changes and damage to the wood structure due to an acetylation process leading to weight per cent gains of up to 20%. Electron microscopy (SEM and TEM) was used to investigate the fine structure of acetylated beech, pine and spruce. Cell wall swelling was observed, but no evidence of damage could be seen as a result of the acetylation procedure. The fine structure of the wood tissue such as the pits and the thin parenchyma walls appeared untouched.

Introduction

Chemical modification of wood aims to enhance the properties of wood related to hygroscopicity and durability. This applies to wood and wood products—avoiding the introduction of toxic compounds (Rowell 1975,1983; Goethals and Stevens 1994; Hon 1996; Kumar 1994). Wood acetylation is

Received: 18 May 2000 / Accepted: 5 December 2001
Published online: 9 April 2003

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The authors would like to thank Jan van der Heijden (Wageningen) for his help with the SEM analysis and Anja Geitmann [Laboratory of Experimental Plant Morphology and Cell Biology (EPC), Wageningen University and Research Centre] for the preparation of samples for TEM analysis.

probably the most investigated approach to wood modification. In this modification process, hydroxyl groups in lignin and holocellulose react with acetic anhydride and are replaced by acetyl groups (Rowell et al. 1993, 1994). During the reaction of cell wall components with acetic anhydride, acetic acid is formed as a by-product. Many experiments on the acetylation of wood have been performed on small wood parts, e.g. fibres, chips, flakes, veneers. But recently, successful acetylation trials on solid wood have also been undertaken (Goldstein et al. 1961; Larsson and Simonson 1994; Militz 1991; Beckers and Militz 1994). Acetylation of wood leads to a permanent swelling of the cell wall accompanied by a weight per cent gain (WPG) due to the higher molecular weight of the acetyl group, in contrast to the smaller hydroxyl group. As a result, the anti-shrink efficiency (ASE) and the resistance against decay are considerably improved (Beckers et al. 1994, 1995; Chow et al. 1994; Imamura and Nishimoto 1987; Larsson and Simonson 1994; Rowell and Plackett 1988).

The use of elevated temperatures and the presence of acetic acid during an acetylation process combined with the resulting cell wall swelling might induce changes or even damage tissue at the cell wall level. In this case, changes in the mechanical properties are likely to occur. However, many studies on the mechanical properties of acetylated solid wood have indicated that the effect of acetylation on the strength properties is negligible (Akitsu et al. 1993; Larsson and Tillman 1989; Larsson and Simonson 1994; Liu et al. 1994; Rowell 1991). Only a few studies have indicated slight effects. Ramsden et al. (1997) reported a slight reduction in the tensile modulus of acetylated Scots pine. Larsson and Simonson (1994) observed a slight reduction in the modulus of elasticity (MOE) and modulus of rupture (MOR) in acetylated pine, and some decrease in the MOR of hardwood was reported by Dreher et al. (1964).

This study was performed to determine whether any changes occur at an anatomical/ultrastructural level during acetylation, which might effect the mechanical properties. Wooden samples were prepared and investigated for three different species acetylated to different degrees. During the investigations, electron microscopy was used to study the fine structure of acetylated wood. The aim was to gain knowledge of the possible changes to the structure and to help explain the changes in wood properties due to acetylation.

Material and methods

Wood species

Stakes from acetylated and non-treated Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*) and beech (*Fagus sylvatica*) were investigated. These species were chosen for analysis for two reasons:

1. Considerable knowledge on the acetylation of these species has been gained in recent trials (Beckers and Militz 1994).
2. The effect of acetylation can be studied more effectively because of the low natural dimensional stability and low durability against attack by micro-organisms for these three species. Samples 25 mm×50 mm×800 mm in dimension were acetylated for each of the species.

Acetylation

For the acetylation of the wood, acetic anhydride was used in a non-catalysed acetylation process. A two-step treatment was used:

- *Step 1*: Impregnation of wood with acetic anhydride and reaction at 120°C with a residence time of 16 h followed by oven drying
- *Step 2*: Impregnation and reaction with acetic anhydride at 120°C for 3 h.

All treatments were performed at SHR Timber Research in Wageningen, The Netherlands. The acetylation procedure and the removal of residual acetic acid and unreacted anhydride were similar to those described by Beckers and Militz (1994). After acetylation, the most highly acetylated part of the samples—the outer 5 mm—was used for further analysis. The acetyl content was determined [by high-performance liquid chromatography (HPLC)] to be 20.0% for beech, 17.8% for pine and 15.6% for spruce.

Electron microscopy

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used to analyse the fine structure of the wood. The samples for the SEM analysis were boiled in water for 15 min (microwave), then shock frozen using liquid nitrogen. The shock freezing was performed to obtain a temporary embedding of the samples using the frozen water in the cell lumina as an embedding media. The samples surfaces were then cut with a razor blade, they were then oven-dried and sputter coated with gold–palladium. Scanning analysis was performed using a JEOL-instrument (JSM 5200), with magnification up to 10,000×. The images were digitally recorded and stored on a personal computer.

The samples for TEM analysis were dehydrated in an ethanol series and embedded in Spurr resin (Spurr 1969). Samples 80 nm thick were cut using a rotary microtome and then stained with potassium permanganate ($KMnO_4$). This gives a high contrast to lignin-rich structures such as middle lamellas and cell corners (Donaldson 1992). The TEM analysis was performed with a JEOL-electron microscope (JEM 1200EX II) at 80 kV and magnification up to 20,000×. Due to the time-consuming preparation of samples, only one acetylated sample and one non-treated wooden sample were investigated for both spruce and beech.

Results

The electron microscopy study focused on the cross and radial section of the wood, since most features of wood's fine structure such as pits and ray parenchyma can be sufficiently investigated within these sections. The chosen acetylation treatments did not lead to visible changes or damage to the wood structure. Only slight alterations in colour occurred, as also reported in earlier studies (Rowell 1983). The SEM analysis did not reveal any defects in the wood structure, even at high WPGs. However, in the latewood, swelling of the cell wall into the lumina was observed in some samples (Fig. 1). Thin-walled ray parenchyma and epithelia cells appeared unaffected. Resin canals of spruce and pine were often lacking resin, which can be a result of being dissolved in the acetic anhydride during treatment (Fig. 2).

Analyses of the pits and pit membranes in spruce did not reveal any differences from those of natural, non-treated xylem tissue. All the features investigated appeared to be unaffected by the process conditions used (Fig. 3, 4, 5). The tori of bordered pits in earlywood of spruce and pine did not reveal any degradation (Fig. 3). Bordered pits in the earlywood from both spruce and pine were aspirated. Even the large window pit membranes of Scots pine were not damaged (Fig. 4). The fine structure of the tertiary wall of these pine samples appeared

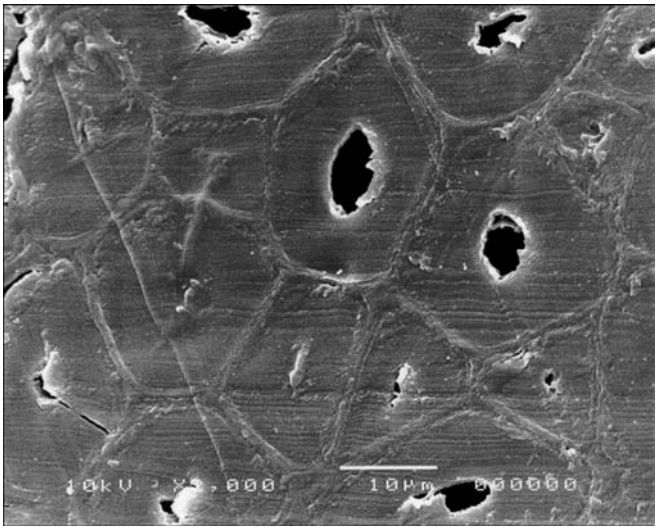

Fig. 1. Acetylated beech (WPG between 18% and 20%). Cross-sectional view of swollen fibre tissue. Some lumina are completely closed

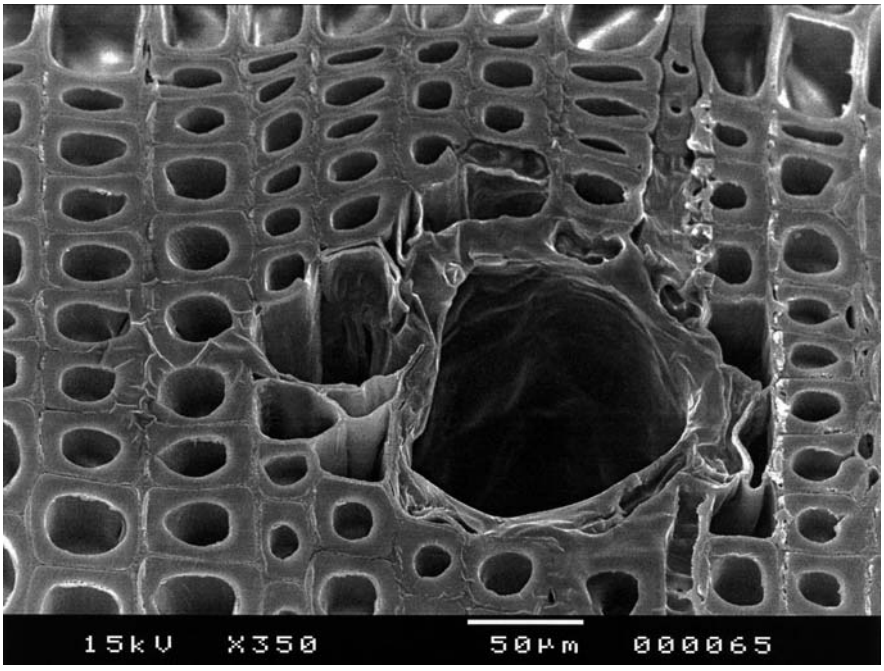

Fig. 2. Acetylated Scots pine (WPG between 16% and 18%). Cross-sectional view of latewood with a resin canal. Epithelia cells are untouched and partly filled with resin

untouched. Vestures in beech vessels (Fig. 5) and in the bordered pit chambers of pine remained untouched.

Any changes within the cell wall due to the impact of the chemicals and high temperatures used during acetylation should be revealed by analysis using TEM.

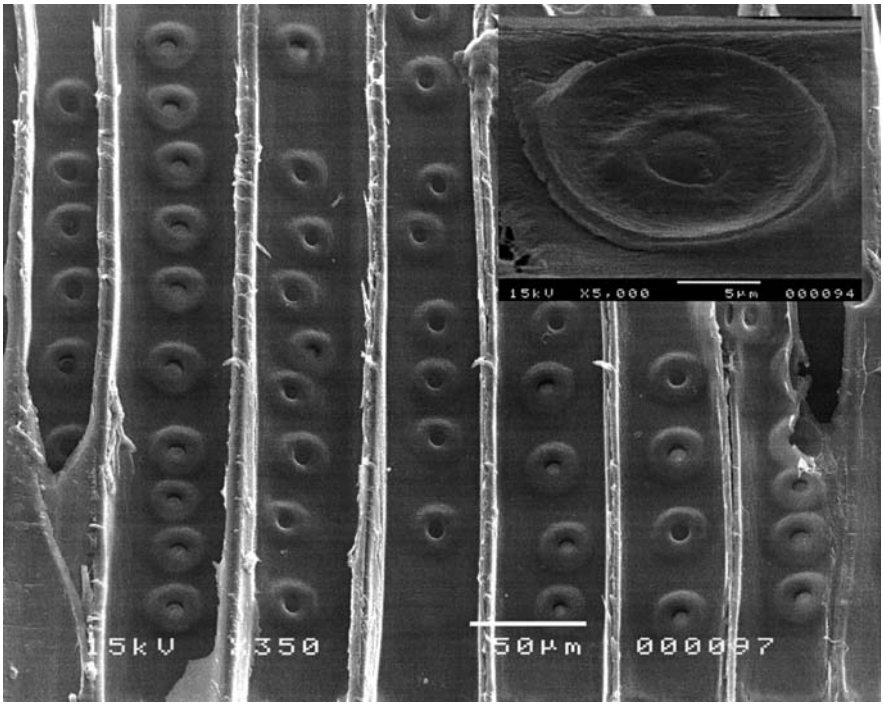

Fig. 3. Radial section of acetylated Norway spruce (WPG 16%). Bordered pits are undamaged. *Window*: opened bordered pit chamber with intact torus and margo

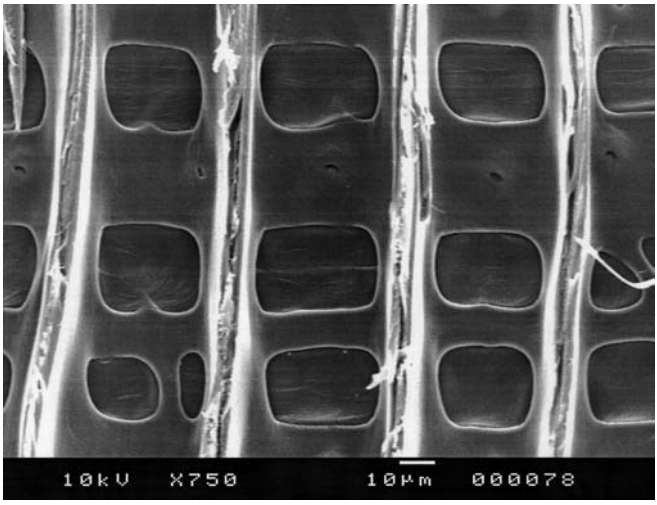

Fig. 4. Radial section of acetylated pine wood (WPG between 16% and 18%). Window pits with undamaged membranes

Changes such as detachment of different cell wall layers were not observed. Staining with $KMnO_4$ did not indicate any difference in contrast, which might be due to changes in lignin (Fig. 6).

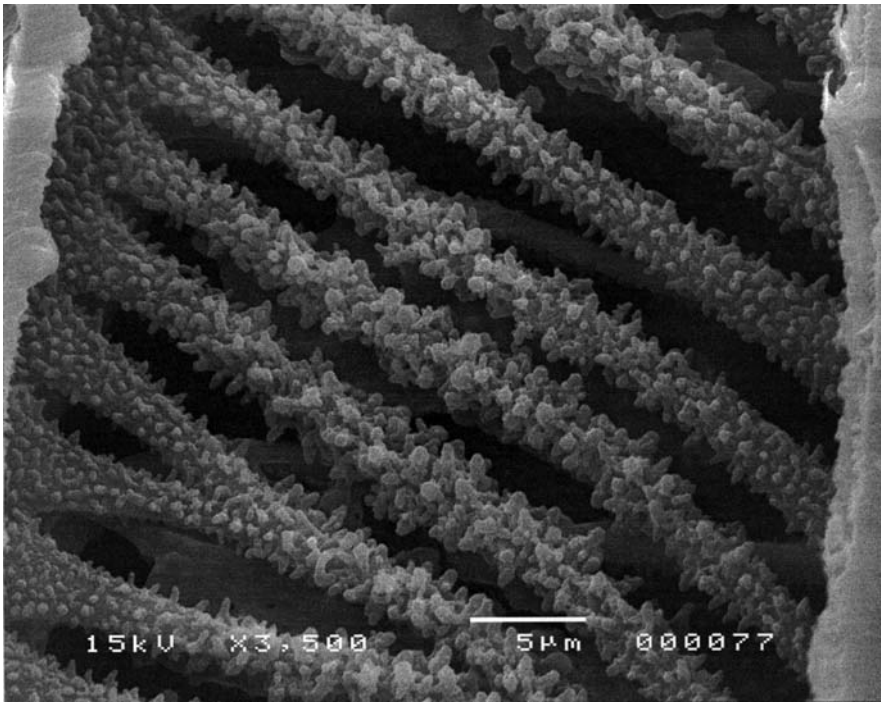

Fig. 5. Longitudinal section of acetylated beech wood (WPG between 18% and 20%). Scalariform vessel perforation with vestures, unaffected by the treatment

Discussion

From the theoretical point of view, chemical modification might cause changes in the fine structure of wood due to:

1. The mechanisms related to swelling of the cell wall; and
2. The impact of acid or basic agents on the matrix polymers in the cell wall alone or in combination with high temperature.

Swelling of the wooden cell wall is quite a common phenomenon, mostly related to the adsorption of water. Uptake of water leads to bulking of the cell wall until fibre saturation occurs. Frequent swelling and shrinkage might cause plastic deformations to the structure (Matejak 1982). In contrast to reversible swelling by sorption of water, chemical wood modification results in a permanent bulking effect (Rowell 1983). Some chemical modification processes introduce molecules into the cell wall, which are capable of exceeding fibre saturation and swelling the tissue beyond the "green" volume of wood, which can cause severe damage. This was reported by Rowell et al. (1976) for Southern pine treated with epoxides and by Rowell and Ellis (1981) for wood modification with isocyanates. It is possible for checks to occur within the latewood cell walls after bulking exceeds fibre saturation, but to date such effects have not been reported for acetylation.

In this study, swelling of tissue was revealed by SEM, but the addition of acetyl groups to the cell wall matrix did not swell the cell wall beyond fibre saturation and thus did not become critical. This is probably because acetylation is a single-site reaction, where no cross-linking or polymerisation of agents occurs, which

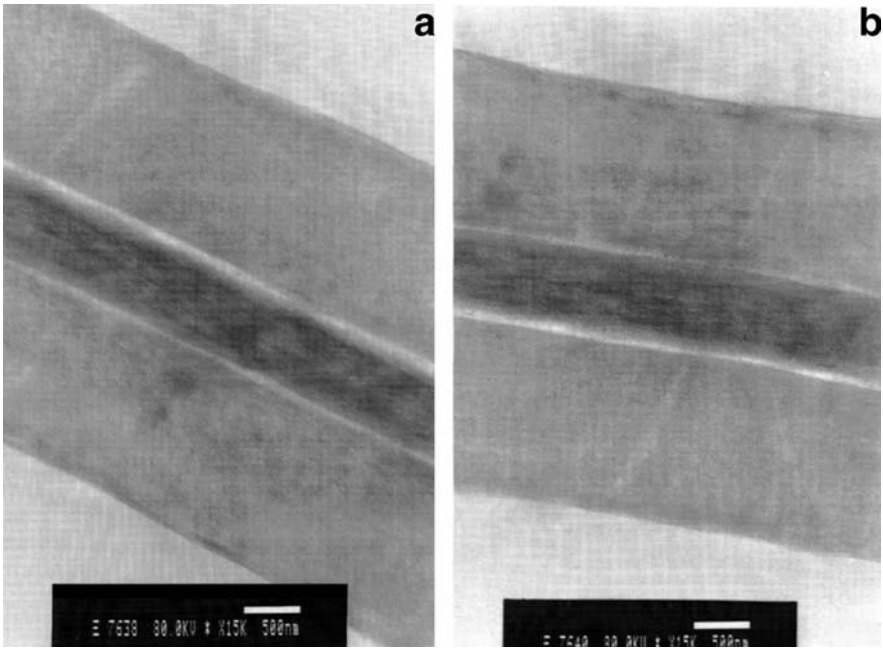

Fig. 6a, b. TEM-micrographs of a non-treated (**a**) and an acetylated (**b**) tracheid cell wall of the same tree ring. The WPG of the acetylated sample was 17%. The stronger lignified areas appear darker due to permanganate contrast. *ML*, middle lamella; *S2*, secondary wall 2

may cause internal stress within the cell walls. Furthermore, the swelling of the wood—due to acetylation—is equal to the swelling that would have been caused by water which is no longer taken up by the wood. Therefore, acetylated wood with an ASE of 100% (not achievable in practice) will be swollen equally to untreated wood at fibre saturation point, and swelling beyond fibre saturation as a result of the acetylation itself is not possible.

Acetic reaction conditions often limit the process of chemical wood modification (Rowell 1983). Stevens and Paramswaran (1981) reported hydrolytic activity of formaldehyde gas on the matrix substances of wood. In this study, acetic acid did not have any visible effect on the wood's ultrastructure even after a process that included a long period at elevated temperatures.

It can be concluded that acetylation using uncatalysed acetic anhydride using the right process conditions is very unlikely to result in any damage or alteration to the ultrastructure of the wood.

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