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Physico-mechanical and decay resistance properties of bio-resin modified wood

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PHYSICO-MECHANICAL AND DECAY RESISTANCE PROPERTIES OF BIO-RESIN MODIFIED WOOD

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Submitted by

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Abstract

This thesis reports investigations into the use of bio-resin to modify wood for the improvement of physical, mechanical and decay resistance properties. A bio-based resin system was developed by ozonolysis process from the cashew nut shell liquid (CNSL) a natural, plant derived, renewable material composed of different phenolic constituents. The fungitoxicity of the raw CNSL in different forms, solvent extracted (*i*CNSL) and technical (*t*CNSL) were examined by DPPH free radical scavenging assay, agar plate assay and decay resistance in the EN 113 agar block test. The CNSLs showed insufficient fungitoxicity for complete inhibition of fungal growth and decay resistance of Scots pine sapwood against brown rot (*Coniophora puteana*, *Postia placenta*) and white rot (*Trametes versicolor*, *Pleurotus ostreatus*). Though *t*CNSL (cardanol rich) showed lesser antioxidant and antifungal properties than *i*CNSL, the *t*CNSL was used for subsequent work due to its hydrophobicity and availability.

The CNSL resin was prepared and impregnated as an alcoholic solution into the sapwood of Scots pine (*Pinus sylvestris*), Obeche (*Triplochiton scleroxylon*) and Gmelina (*Gmelina arborea*) using vacuum at -0.7 bar for 30 min and cure at 120 °C for 24 hours. This specification of impregnation was followed throughout the study. The resin modification moderately improved the physical and moisture related properties i.e. specific gravity, void volume, dimensional stability and water repellency. The CNSL resin was not leachable from the modified wood and was stable to hydrolysis as tested according to EN 84 water leaching test and also a 5 cycle water soak/oven dry test. The viscoelastic properties of the CNSL resin modified wood were examined by the dynamic mechanical thermal analyser (DMTA) which revealed the glass transition (T_g) of the modified wood between 60-70 °C due to the relaxation of the CNSL resin. The storage modulus (E') which indicate the modulus of elasticity of resin modified woods measured at room temperature (20-25 °C) did not significantly improve but among the modified wood species, Scots pine had the best modulus of elasticity.

Oxidative degradation experiments of CNSL resin modified Scots pine sapwood micro-veneers (100 µm thick) were conducted to investigate the effect of the hydroxyl radicals generated by the Fenton's reaction on the mass and zero-span tensile strength. A large impact on the minimum weight losses and improved tensile strength with increased WPG was found in modified micro-veneers though after a longer duration of exposure a substantial strength loss (30-40%) occurred. An EN 113 decay resistance test was conducted with the same test fungi as used in the fungitoxicity test of CNSL. The CNSL resin modification with the limited WPG did not achieve full decay protection in any of the wood species in valid decay tests. Full protection threshold WPGs were established by extrapolation of data using regression analysis. Thresholds varied between the wood and fungal species. The decay resistance was successfully increased by investigating an alternative approach of treating wood with the bio-based CNSL resin combined with different antioxidants (AOs). The combined effect of the resin and antioxidants has improved the reduction of mass losses in comparison with the treatment with AOs and resin alone. AOs with high free radical scavenging property (propyl gallate, ferulic acid) showed better combined performance with CNSL resin.

The distribution of the CNSL resin into the modified woods were investigated using light microscopy and FTIR analysis. The microscopic evidence revealed that the resin largely located in the lumen either by surface coating or filling and good distribution was found in Scots pine. The FTIR spectra of resin modified woods showed some changes in different bands which are mainly attributed to the functional groups of incorporated CNSL resin in wood.

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List of Abbreviations

µg	Microgram
µl	Microliter
µm	Micrometre
µM	Micromolar
AAI	Antioxidant activity index
ANOVA	Analysis of variance
AO	Antioxidant
ASE	Anti-swelling efficiency
ATR	Attenuated total reflection
BC	Bulking coefficient
BHT	Butylated hydroxyl toluene
CCA	Copper chromium arsenic
CNSL	Cashew nut shell liquid
DDT	Dichloro diphenyl trichloroethane
DMDHEU	Dimethylol dihydroxy ethylene urea
DMTA	Dynamic mechanical thermal analyser
DP	Degree of polymerization
DPPH	2, 2-diphenyl-1-picrylhydrazyl
E'	Storage modulus
E"	Loss modulus
EMC	Equilibrium moisture content
EN	Euro norm
ENV	European pre-standard
FTIR	Fourier transformer infrared radiation
GPa	Giga Pascal
h	Hour
IC	Inhibitory concentration
*i*CNSL	Solvent extracted CNSL
IMS	Industrial methylated spirit
Kg m^{-3}	Kilogram per meter cube
kN	Kilo newton
MA	Malt agar
MC	Moisture content
MF	Melamine formaldehyde

mg	Milligram
ml	Millilitre
ML	Mass loss
mM	Millimolar
mm	Millimetre
MMF	Methylate melamine formaldehyde
MUF	Melamine urea formaldehyde
OD	Oven dry
OH	Hydroxyl radical
Pa	Pascal
PEGMA	Polyoxyethylene glycol methacrylate
PF	Phenol formaldehyde
PVAc	Polyvinyl acetate
R^2	Regression coefficient
RH	Relative humidity
ROS	Reactive oxygen species
SD	Standard deviation
SG_b	Basic specific gravity
SG_{OD}	Oven dry specific gravity
SSL	Spent sulphite liquor
*t*CNSL	Technical CNSL
T_g	Glass transition temperature
UF	Urea formaldehyde
UV	Ultra violet
VC	Volume change
VC_{max}	Maximum volume change
V_{rel}	Relative volume
WA	Water absorption
WL	Weight loss
WPG	Weight percent gain
WRE	Water repellent efficiency
WS	Water soak

The following publications have been presented in conferences based on the work of this thesis by the author.

1. Ashaduzzaman, M., Hale, M.D., Tverezovskiy, V. and Ormondroyd, G.A. (2013). *Effect of bio-resin from cashew nut shell liquid (CNSL) on decay resistance properties of wood*. Proceedings of the 44th Annual Conference of International Research Group on Wood Protection, IRG/WP 13-40649, Stockholm, Sweden, June 16-20, 2013.
2. Ashaduzzaman, M. and Hale, M.D. (2013). *Does cashew nut shell liquid (CNSL) have any potential as wood preservative*? 2nd International Conference on Biodeterioration of Wood and Wood Products, Tartu, Estonia, April 23-27, 2013.

Chapter 1

Introduction

1.1. Background and need for research

In recent years, there has been increasing concern for the preservation of the environment and sustainability of resources. Utilization of forest resources is receiving renewed interest as an alternative to non-renewable resources in material technology. In this sense, wood is considered as a future oriented material even though it is one of the oldest and most common structural and building materials. At present, particularly in tropical parts of the world, forests continue to decline, whilst this is not offset by increase in forest plantations of rapidly growing species and managed forest in developed countries. The demand for wood products cannot be met even with the fast growing trees. Fast grown wood, particularly juvenile core wood, is characterized by low density, inferior mechanical properties and low decay resistance. Lower than desired durability has been addressed through the use of preservatives, traditionally a mixture of chemicals toxic to the environment and mankind. Thus the search for environmentally less aggressive wood treating alternatives has been a central research subject over the last few years in Europe (Van Acker and Hill, 2003).

Conventional wood preservative use is widespread because of the declining availability of naturally durable wood and use of plantation timber with high sapwood content. In the past 10 years a major wood preservative, chromated copper arsenate has been removed from service amid concerns about environmental and health and safety aspects and another preservative, creosote, is severely restricted in its availability and use. Numerous organic biocides (dieldrin, lindane, pentachlorophenol, tri-butyl tin oxide) have been withdrawn to make way for safer alternatives but there have also been concerns regarding the efficacy and costs of their replacements. Then there are issues regarding end of life cycle disposal, and treated wood may be classified as a toxic waste as they were initially treated with environmentally impacting persistent formulations. It is most unlikely that new biocides will be developed specifically for wood preservation because the market is too small. These significant changes in the timber preservation sector have resulted in a significant increase in interest in wood modification (Hill, 2006).

Among the different wood modification technologies, chemical, thermal and impregnation methods have already been established commercially, although these methods have advantages and disadvantages when their properties are considered. The first to be developed commercially were thermally modified woods (ThermoWood, PlatoWood), which add dimensional stability but do not achieve high decay resistance and may cause undesirable changes in mechanical properties. Acetylated wood, marketed as Accoya, has shown excellent dimensional stability and resistance to decay. In addition a number of modified woods based on the impregnation technology have also been introduced into the market. The impregnation of wood through the furfurylation process produced 'Kebony' and 'Keywood' as commercial products. Using oligosaccharides in combination with melamine resins and other additives produce the products e.g. 'Indurite', 'Kurawood' and 'Lignia'. A new product originated by impregnation of DMDHEU named 'Belmadur' has recently been commercialized (Hill, 2009; 2011).

Generally, the impregnation modification of wood is one of the passive modification methods and defined as "any method that results in the filling of the wood substance with an inert material (impregnant) in order to bring about a desired performance" (Hill, 2006). This category of modification does not necessarily require changes in the chemical structure of the wood cell wall. Resin treatment is considered a most useful method among several other processes of impregnation modification and has been widely studied using a variety of resins of synthetic origin, including epoxy resins, phenolic resins, melamine resins, urea resins, polyurethane pre-polymers and unsaturated polyesters (Pittman, 1994). Phenol-formaldehyde resin-forming systems with low molecular weights are the most successful thermosetting agents used in resin modification of wood.

Limited work using natural or bio-resin systems has been done to identify these as potential wood protection agents. Van Acker *et al.* (1999) evaluated six natural resins using both a pure culture Basidiomycete test according to EN113 and a natural microflora ENV807 soil bed test. The use of natural resins is important for a number of reasons including those positives relating to the use of natural resources / sustainable renewable resources, co-products from other processes, to negative concerns about the use of synthetic resin systems involving health, safety and environmental issue, end of life concerns and recycling and chemical legislation (BRE, 2007).

This study looks at a resin system based on a natural source, renewable and available from the commercial point of view. Cashew nut shell liquid (CNSL) is a natural renewable product extracted from the shells of the cashew nut, a delicate and valuable food item and is available as a by-product from the cashew nut processing industries. It is a caustic, viscous, dark liquid, and is a natural source of saturated and unsaturated long chain phenols (Andrade *et al*., 2011). CNSL is a mixture of different phenolic compounds, anacardic acid, cardol, and cardanol and 2 methyl cardol (Tyman and Kiong, 1977). The CNSL may be extracted in two different ways: extracted by solvent extraction where the product is called immature or natural (*i*CNSL) and extracted by roasting the nut shell at high temperature, which is a by-product of the cashew industry called technical (*t*CNSL). The phenolic nature and unsaturation in the side chain of CNSL, offers reaction sites both on the aromatic ring and on the side chain, which allows the synthesis of different resin types (Kumar *et al.*, 2002).

This renewable material has wide applications in the form of brake linings, surface coatings, paints, and varnishes but the main applications of CNSL are in the polymer industry (Menon *et al.*, 1995). Compared with conventional phenolic resins, CNSL polymer has improved flexibility (due to the internal plasticization effect of the long chain) and better processability. It is also reported that the side chain imparts a hydrophobic nature to the polymer, making it water repellent and resistant to weathering (Kumar *et al*., 2002).

From the moisture sorption and decay resistance points of view, impregnation modification with resins is generally inferior to chemical modification and reaction within the cell wall because the inside of the cell wall is only coated, while chemical modification can achieve reaction throughout the cell wall and thus achieve low moisture sorption at modest chemical add-on (e.g. acetylation with acetic anhydride shows low sorption at 8 weight per cent gain) while higher amounts of 20 WPG are required for high decay resistance under severe ground contact exposure (Larsson *et al*., 2000). Although acetylation has little adverse effect on mechanical properties, it shows no improvement whereas resin modification can improve a number of strength properties. Thus impregnation modification with bio-resins may prove advantageous, if it can be made to give acceptable performance in terms of decay resistance and reduced cell wall sorption (either in terms of rate or overall end-point).

Fungal decay of wood results from the wetting of the wood to a critical value whereby there is sufficient water both in the wood lumens and in the cell wall to allow decay to progress. Other aspects are of importance too and too much water, or other factors which impede

oxygen availability and gaseous exchange may impede fungal respiration, growth and limit decay processes. Fungal hyphae in the cell lumens release enzymes and oxidative free radicals which break down the wood cell wall polymers to soluble compounds which the fungi can assimilate. Conventionally, fungal decay can be prevented by the inclusion of toxic compounds which are released when the fungi colonise the wood cell lumens and these typically contain copper and co-biocides to deal with copper tolerance and insects, but modern approaches attempt to reduce reliance on toxicity. The approach which is investigated at in this study combined a bio-resin modification approach with the inclusion of antioxidants e.g. gallic acid, propyl gallate, quercetin etc. as these have shown some efficacy and may form a component of natural decay resistance in heartwoods which show high decay resistance.

1.2. Structure of this thesis

Chapter 1 (this chapter) covers the general introduction, structure of the thesis and the objectives of this study.

Chapter 2 presents a literature review of the subject matter of this study covering general information about wood and its structure, problems associated with wood utilization, natural durability and wood preservation, impregnation modification and its properties and the importance of bio-resin and cashew nut shell liquid.

Chapter 3 characterizes the antioxidant and antifungal properties of CNSL. Antioxidant activity was evaluated by the DPPH free radical scavenging assay, while agar plate assay and agar block tests were used to examine antifungal properties. This chapter provides the preliminary information regarding the fungitoxic properties of the CNSL against the wood decay fungi.

Chapter 4 reports the physical properties i.e. weight percent gain (WPG), oven dry specific gravity (SG_{OD}), volume change (VC%) and leachability of resin; moisture related properties i.e. dimensional stability, stability to hydrolysis and water repellent efficiency of CNSL resin modified wood. This chapter also describes the formulation of the CNSL resin system. A characterization of these properties of modified wood is addressed.

Chapter 5 describes the viscoelastic properties of the CNSL resin modified wood which is evaluated by dynamic mechanical thermal analyser (DMTA). This provides the thermo-mechanical behaviour of modified wood.

Chapter 6 deals with the oxidative degradation of wood modified with CNSL resin. The oxidative degradation of the modified wood is assessed by the Fenton's reaction. The effect of hydroxyl radicals generated by Fenton's reagent on the mass and tensile strength is assessed to mimic the initial decay mechanism.

Chapter 7 describes the resistance of CNSL resin modified wood against wood decay fungi assessed by the EN113 protocol.

Chapter 8 reports on the additive effect of CNSL resin combined with different antioxidant compounds (free radical scavengers) on the decay resistance properties of wood. This chapter provides an alternative approach of wood protection.

Chapter 9 examines the distribution of CNSL resin in the impregnated wood by light microscopy and also provides information about the change of wood cell wall chemical structure due to resin modification evaluated by FTIR spectroscopy.

Chapter 10 draws together the general discussion of the results from the earlier chapters, final conclusion and scope of further study.

1.3. Aim and objectives

The overall aim of this study is to investigate the potential of bio-resin developed from CNSL for modifying the wood properties i.e. physical, thermo-mechanical and decay resistance and thus produce a high performance material. The specific objectives expected to be achieved at the end of the study, are:

a) Establish an understanding of processing techniques needed for obtaining bio-resin (CNSL resin) modified wood.
b) Screening the antioxidant and antifungal properties of CNSL to find out its effectiveness as a wood protecting agent.
c) Evaluate the physical, moisture related, thermo-mechanical and decay resistance properties for determining the qualities of CNSL resin modified wood.
d) Assess the oxidative degradation of CNSL resin modified wood by Fenton's reagents.
e) Investigate the additive effects of CNSL resin in combination of antioxidants to provide additional preservative properties to the modified wood.

Chapter 2

Review of literature

2.1. Wood as a versatile building material

Wood has been a very important material for the development of mankind since early times when man discovered fire and started to use tools. Wood can have excellent mechanical properties; it is recyclable, renewable, it is an energy store, a fibre source and it is biodegradable. It sequesters carbon and requires little energy to process into a value added commodity. A number of these attributes make it an economic material of choice for many purposes and worldwide many people are employed in the forest and related industries. Further downstream wood and its major structural components: cellulose, hemicellulose and lignin, can be utilized to produce many beneficial chemicals for industrial purposes, such as ethanol for biofuel and platform chemicals for synthesis into other materials such as bio plastics.

When wood is used as a constructional material one of its drawbacks is its response to water: below fibre saturation point cell wall moisture changes can lead to dimensional changes and above fibre saturation point decay by fungi. Insects too may be limited by very low moisture but may be active in relatively dry wood. The heartwood from a number of tree species shows some resistance to decay, although that from fast growing plantation species is less so, particularly because it may have a large proportion a lot of susceptible sapwood and less durable juvenile wood. The common solution to the fungal decay problem is to treat with preservatives where a hazard assessment dictates necessity to increase decay resistance, and with appropriate construction techniques and assessment of the extent of wetting likely to be achieved in service, and with surface protection using paints or stains.

Why is wood such a versatile material? Its success depends partly on its remarkable strength to weight ratio and workability. As a structural material, wood has high strength per unit weight and can be easily shaped and fastened. The colour patterns and textures of wood are often pleasing leading to uses for many decorative purposes. It has good insulating properties and is warm to the touch. Wood is available in a wide range of colours, textures, densities and chemical compositions supporting a wide range of significant uses (Hoadley, 1980).

Chemically, wood is composed of mostly carbon, hydrogen, and oxygen which are then arranged to cellulose, hemicellulose, and lignin; the former two comprise about 70% of the dry wood weight and are both made up of sugar units, principally glucose with lower amounts of mannose, xylose arabinose and galactose (Haynes, 2003). So, the wood polymer sugar molecules potentially present an appetizing food source if they can be released from the polymer as monomers and dimers. In order to do this they need to circumvent the protective effect of the lignin complex. In the presence of sufficient oxygen from the air and sufficient water, wood can be biodegraded. While this may ultimately desirable at the end of life, especially within a forest ecosystem, the breakdown of wood products in service, or biodeterioration, is a cause of safety issues and economic losses. Its service life can be severely limited at sites and situations where the moisture content is constantly suitable for decay and the wood has little natural decay resistance or preservative induced resistance. Because there is a worldwide need for large quantities of wood fibre, fast growing, multi-purpose plantations are necessary; these often produce resources which require improvement.

From the perspective of carbon storage and sequestration forests and forest products are of immense importance. Growing trees can soak up a great quantity of the greenhouse gas, carbon dioxide (CO_2), and lignin, its breakdown products and similar metabolites form long term carbon storage compounds in forest soils. Where wood is used from the forest, longer term carbon storage can result, particularly where the material is prevented from decaying by appropriate usage and preservation (ECCM, 2006). Indeed approximately half of the dry weight (49%) of wood is carbon and thus a cubic metre of wood of typical density, 500 kg m^{-3} comprises approximately 250 kg of carbon. Thus, a single tree of 4 m^3 can easily be a store of a tonne of carbon above ground. When accompanying soil carbon and root biomass are accounted for it is considerably more.

2.2. Structure and formation of wood

Wood (i.e. secondary xylem) has a complex biological structure composed of different cell types and chemistries, acting together to serve tree life functions of support, transport of solutes and storage. It is produced by the seed-bearing plants and belonging to the Spermatophytae. Wood properties are governed by the wood structure, particularly by its anatomical organization and cell wall ultrastructure. Generally, four orders of structural variation can be recognised, macroscopic, microscopic, ultrastructure and molecular (Dinwoodie, 2000). The following section provides a brief summary of the wood structure and

its formation. In this respect there are a good number of excellent texts which deal with this subject, which give a detailed description of wood structure (e.g. Stamm, 1964; Hoadley, 1980; Sjostrom, 1983; Fengel and Wegener, 1989; Zabel and Morrell, 1992; Eaton and Hale, 1993; Walker, 1994; Desch and Dinwoodie, 1996; Dinwoodie, 2000; Hon and Shiraishi, 2001; Bowyer *et al.*, 2007).

Wood is classified into soft- and hardwoods; the former produced by gymnosperms (i.e. conifers) and the latter by angiosperms (i.e. deciduous or broad-leaf trees). Woody trees comprise many species and altogether *ca*.30 000 angiosperm and *ca.* 500 conifer tree species are currently known, with the majority of the angiosperms growing in tropical regions. Gymnosperms tree genera include the pines (*Pinus*), spruces (*Picea*), and firs (*Abies*) while typical temperate / boreal angiosperm genera include birches (*Betula*), beeches (*Fagus*), oaks (*Quercus*) and poplars (*Populus*). Hardwood trees lose their leaves during the autumn while the conifers are normally evergreen except for certain genera like larch (*Larix*) that lose their needles during autumn and grow new needles in the spring.

Structurally each tree can be divided into several main parts commonly referred as the crown, trunk (i.e. stem) and root system; each part in turn composed of different tissues that are ultimately comprised of individual wood cells. The trunk (Figure 2.1) may be divided into: a) the bark, which is dead and provides protection from physical, mechanical and biological damage; b) the phloem which is living and allows for the transport of nutrients and storage products; c) the vascular cambium, a thin layer of cells which by repeated division produces phloem cells to the outside and xylem (secondary) to the inside, and d) the secondary xylem which constitutes the bulk of the woody material. The xylem is normally divided into the sapwood and heartwood; the former composed of living and dead cells and the latter comprised of normally entirely dead cells. Finally, pith is normally present at the centre of the trunk and represents tissues developed during the initial year of tree growth, formed by the apical meristem.

The xylem is normally organized into concentrically orientated rings which are distinct in softwoods and ring porous hardwoods. In temperate climates or in climates where there is a significant seasonal weather, e.g. drought, these are referred to as annual growth rings; each ring representing one year's growth. Wood is composed of a variety of different cell types (softwoods: tracheids and parenchyma; hardwoods: vessels, fibres, tracheids and

parenchyma) having different roles in the living tree (i.e. support, transport, storage) and which can behave differently in different wood products.

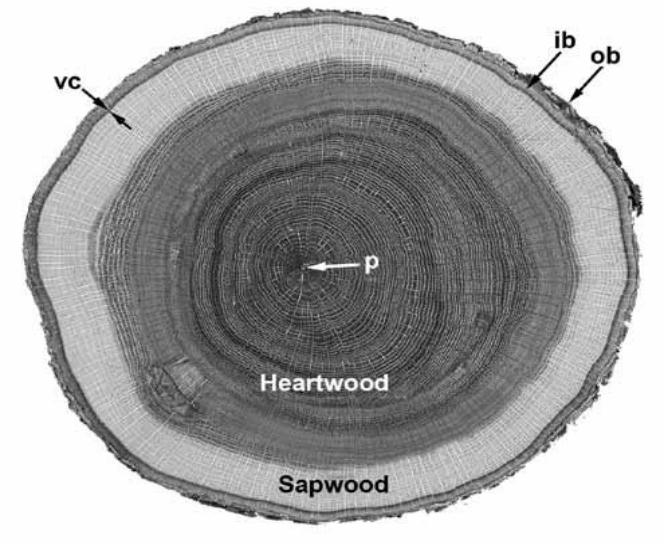


Figure 2.1: Macroscopic view of the transverse section of *Quercus alba* trunk presenting outer bark (ob), inner bark (ib) and vascular cambium (vc), inside the vascular cambium shows the sapwood, which can be easily differentiated from the darker heartwood. The centre of the trunk is pith (p), which is hardly visible in the centre of the heartwood (Forest Products Laboratory, 2010).

Wood is composed of highly ordered axial and radial cell systems (Figures 2.2, 2.3). The axial system predominates and it is composed of primarily elongated cells orientated in the longitudinal direction of the trunk. Wood cells are produced in the vascular cambium from two special mother meristematic cell types known as the fusiform and ray initials; the former giving rise to all cell types of the axial and the latter the radial cell systems in both hard- and softwoods.

These cells vary in both size and shape (i.e. cell wall thickness and cell length, see below), features which are related to their function, with thick-walled, narrow lumen cells providing mechanical support and strength to the tree and thin-walled wide lumen cells primarily providing liquid transport and the storage of nutrients. The radial system is orientated perpendicularly to the tree and is comprised of rays that form horizontal files of cells extending from the bark to the pith (primary rays) or to specific annual rings (secondary rays). The major function of the rays is to store and redistribute storage materials (e.g. starch). Rays may contribute 5–11 % of the total softwood volume and up to 30 % in hardwoods (e.g. *Quercus* spp.).

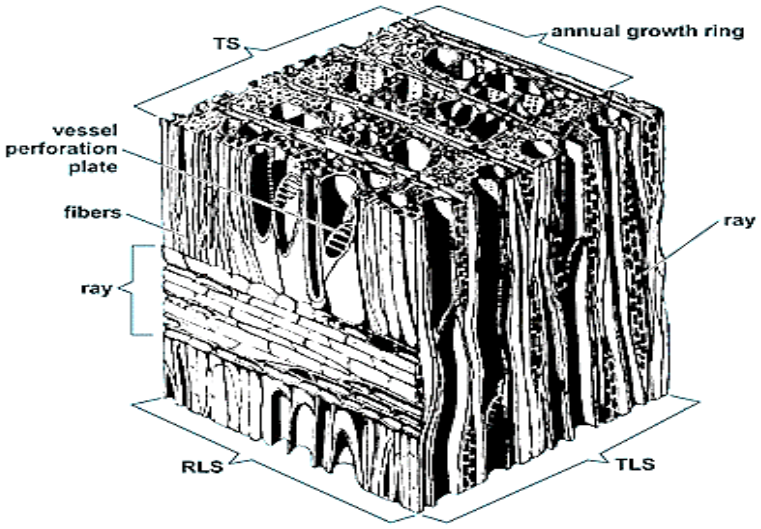


Figure 2.2: Structure of a typical hardwood, RLS = radial longitudinal section; TLS = tangential longitudinal section; TS = transverse section (Miller, 2002).

Softwoods are considered to have a much simpler structure than hardwoods and are composed of minimum number and uniform cell types (normally 3 axial and 2 radial). In contrast hardwoods are normally comprised of a greater number of axial (5–6 axial and 2 radial) cell types with much greater cell morphology; nevertheless all cell types are derived from the two meristematic cell types named above.

Softwood xylem is composed of limited number of cells known as tracheids, parenchyma and epithelial cells with tracheids normally forming between 90–95 % of the total cell volume and ray parenchyma between 5–10 %. Because of their uniformity (homogeneity) and simplicity, the structure in softwoods tends to appear similar in appearance.

Hardwoods are more advanced and complex in their general anatomical organization than softwoods. In addition they have a larger number of different cell types than softwoods including vessels, fibres (libriform fibres and fibre tracheids), and parenchyma (longitudinal and ray cells). Vessels represent the main conducting element in hardwoods and the cells have entirely open or perforated ends, with species with open ends (e.g. *Quercus* spp.) considered to be more evolutionarily advanced. Both the size and cellular morphology of the vessels varies between hardwood species. Vessels appear in transverse sections of wood as holes, and for this reason hardwoods are known as “porous woods” in contrast to the “non-porous” softwoods.

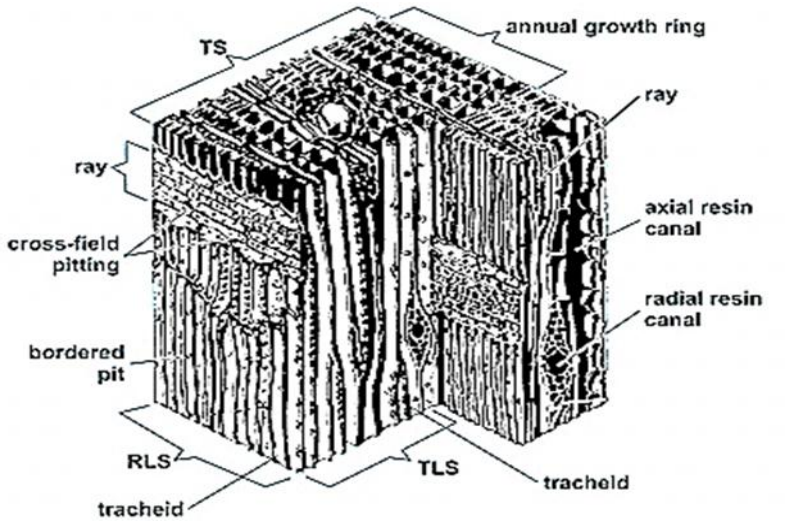


Figure 2.3: Structure of a typical softwood. RLS = radial longitudinal section; TLS = tangential longitudinal section; TS = transverse section (Miller, 2002).

Pits are one of the most characteristic microstructures that occur in cell walls of both soft- and hardwoods. They represent canals that allow the flow of liquids both laterally and vertically through the cell walls and essentially consist of a pore and a membrane. Normally in living cells and frequently even after death the membrane, of variable permeability, divides the cells. Pits have a variety of shapes and sizes that together with their location on wood cells can be used as a diagnostic feature for wood classification. Pits of adjacent cells are normally always paired thereby forming pit pairs – simple, bordered and half-bordered pit pairs. All pits have essentially two main components: the pit cavity and pit membrane (Figure 2.4). The pit membrane involves of primary wall and middle lamella and since the pits occur in pairs the membrane is composed of two primary walls and middle lamella.

2.3. Wood cell wall chemistry

The cell walls of wood consist of 3 main structural chemical components e.g. cellulose, hemicelluloses and lignin. The lumen is a void space but the cell wall is a highly regular structure, which varies between cell types, species, and even in softwoods and hardwoods. However, generally common features underline the structure. In simplified terms the cellulose develops a skeletal matrix which is surrounded and covered by the hemicelluloses and lignin. Generally, cellulose is comprised of glucose units which are ordered into chains with the smallest building element represented by the elementary fibril bundles of some 36 parallel aligned cellulose molecules (Ding and Himmel, 2006). Cellulose microfibrils (aggregated fibrils 5-10 nm in diameter, Brown *et al*., 1996; Jarvis, 2003) are visible using electron

microscopy and more recently atomic force microscopy, and may be aggregated further into macrofibrils and lamellae, the latter organized into a concentric arrangement around the wood cell wall layers. The hemicelluloses are amorphous and are associated and orientated around the cellulose while lignin is amorphous and isotropic and encrusts both the hemicelluloses and cellulose. The chemical composition of wood cell wall layers varies between different cell types and between soft- and hardwoods.

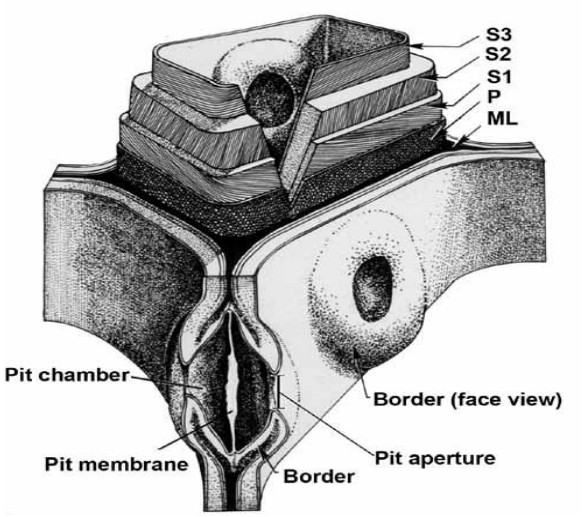


Figure 2.4: The wood cell wall with the structure of a bordered pit showing different layers of cell wall e.g. middle lamella (ML), primary wall (P) and the three layers of the secondary walls: S1, S2, and S3 (Forest Products Laboratory, 2010).

The compound middle lamella region is rich in lignin and contains the highest lignin content (g/g) in wood cell layers but also contains pectin and cellulose. The primary cell wall contains high levels of pectin as well as the glycoprotein extensin – thought to hold the cellulose microfibrils in their criss-cross network- and also the hemicellulose xyloglucan. The layers of the secondary cell wall (S1, S2, and S3) also vary in their chemical composition with the concentration of lignin being greater in the S1 layers than S2 and S3, and the total amount of cellulose and hemicelluloses being greater in the S2 than either S1 or S3 layers. This can only be recognized as a general trend and exact chemical analyses of the individual wall layers may vary between species and growth conditions.

The heartwood serves primarily as a support tissue and is present in all trees although the age at which formation begins varies according to wood species and prevailing environmental conditions. During heartwood formation secondary metabolites known as extractives are deposited in the cell lumens and cell walls making the wood less permeable and more durable (i.e. to wood decay by fungi, bacteria and animals). In certain softwoods and hardwoods like pines and oaks the heartwood is more easily recognized by its darker colour while in other

woods like spruces and birches the difference is not readily apparent. The main difference between the sapwood and heartwood is the death of the parenchyma cells and cessation of water conduction. In softwoods bordered pits become closed (i.e. aspirated) and encrusted with extractives and in some hardwoods the vessels become blocked by tyloses. The mechanisms behind the increase in natural durability of heartwood against wood decay is a feature of considerable interest as an understanding of the process represents a novel way for developing environmentally acceptable methods of producing wood of high durability without the need for wood preservative application.

2.4. Problems associated with the utilization of wood

A variety of problems are commonly associated with the utilisation of wood in service and of interest in this study are dimensional stability, weathering and decay resistance. Under certain situations fire resistance is also an issue.

2.4.1. Dimensional stability of wood

Wood in an unstressed state may undergo dimensional changes following variations in its moisture content and/or temperature between the dry state and fibre saturation point (FSP) (Dinwoodie, 2000). Wood is a hygroscopic material because the cell wall polymers, especially hemicellulose, have a number of free available hydroxyl groups (OH). The OH groups of cell wall polymers attract the atmospheric water of the surrounding air and form hydrogen bonds. In a given set of environmental condition of temperature and humidity the dry wood can absorb moisture until it reaches the equilibrium moisture content (EMC), with the surrounding atmosphere. Such woods, when placed in an atmosphere with constant lower relative humidity, will lose moisture until the equilibrium is reached. Thus, in any combination of relative humidity and temperature there is a corresponding EMC where there will be no inner or outer diffusion of water (Dinwoodie, 2000).

When water is lost from the green wood, there is no change of wood volume until it reaches the fibre saturation point (FSP). The FSP is the moisture content of the wood cell wall when there is no free water in the voids but the cell walls are saturated with bound water. The FSP ranges from 20 to 50% moisture content of the dry weight basis depending on the wood species and the method of measurement of fibre saturation point (Feist and Tarkow, 1967; Hill, 2006). Moisture is exists in wood as free water (liquid water or water vapour in cell lumen and cavities) and as bound water (held by intermolecular attraction within cell walls). The

moisture losses below FSP where it loses bound water results in shrinkage. Conversely, as water enters the cell wall structure, the wood will swell. Shrinkage and swelling is a reversible process in small pieces of stress-free wood (Bowyer *et al.*, 2007).

As wood is anisotropic the dimensional changes in shrinkage and swelling are different in the three principal planes of wood, i.e. longitudinal, tangential and radial. Apart from the movement in service the dimensional changes can cause distortion when logs are converted into planks and dried, dependant on the location within the log (Figure 2.5). With respect to dimensional stability, the tangential shrinkage is about double of the radial shrinkage, and the longitudinal shrinkage is negligible for most woods (e.g. Stamm, 1964; Hoadley, 1980; Desch and Dinwoodie, 1996; Dinwoodie, 2000; Bowyer *et al.*, 2007). Despite this, because in construction long spans of timber are used movement may be of significance in some applications.

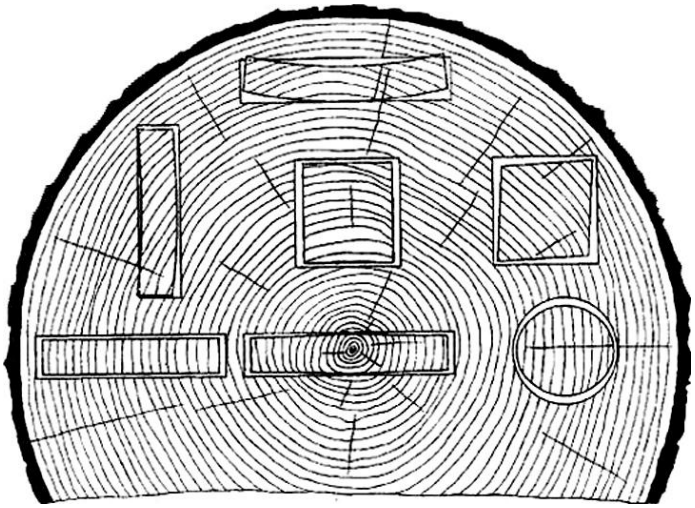


Figure 2.5: The characteristic of shrinkage and distortion of flat, square, and round pieces of wood as affected by direction of growth rings (Forest Products Laboratory, 2010).

Wood is much more permeable to both water vapour and liquid water in the longitudinal direction than the tangential or radial directions. Due to this anisotropy, the longitudinal flow paths are very important for the wetting of wood exposed to the atmosphere (Miller and Boxall, 1984). When water is added to the wood cell wall, its volume increases almost proportionally to the water volume added (Stamm, 1964). This swelling of wood continues until the cells reach the FSP, and the water beyond this FSP is free water in the void structure. As this process is reversible, the wood shrinks due to loss of moisture below the FSP. The dimensional instability limits wood from various applications where the movement of material due to changes in moisture content cannot be accepted (Stamm, 1964).

When water enters into the wood cell wall it causes swelling and it is the hemicellulose rich areas which are attractive to water and water is initially absorbed by hydrogen bonding onto the hemicellulose hydroxyl groups. Subsequent further uptake by the wall may result in the formation of polymolecular water. These events cause the cell wall microfibrils to move apart. Although the cellulose also has considerable hydroxyl functionality the crystalline portions are so closely hydrogen bonded together that it is inaccessible to water. Lignin has few free hydroxyl groups so contributes little to swelling. As stated above the cell wall of wood swells more in the radial and tangential directions than the longitudinal direction. This is because of the low winding angle of the microfibrils in the S2 layer.

The dimensional stability of wood is the measure of extent of swelling resulting from the ultimate uptake of moisture. A variety of terms have been used for describing the degree of dimensional stability of wood by various treatments. The most common term for denoting this feature is termed as antishrink efficiency (ASE), can be misleading as it seems to apply on shrinking and not on swelling although the abbreviation is acceptable because ASE might also refer to anti-swelling efficiency (Rowell and Banks, 1985).

2.4.2. Weathering of Wood

When wood is subjected to exterior exposure it will be subjected to weathering, the combined physico-chemical effects, mechanical effects and surface biology. There is an evident need for enhancing the resistance of wood to weathering (Hon, 1995; 2001). This has moved from a largely empirical body of accumulated practical knowledge to an increasingly sophisticated science, employing the most advanced techniques of physics and chemistry.

Generally, weathering is used to define the slow degradation of the surface of wood exposed to the weather, i.e. the combined environmental effects of light (particularly UV light), temperature changes, water fluctuations (rain, relative humidity, mechanical erosion (wind, particles, surfactants, mechanical wear by walking and other human activities) and surface active biological agents (Feist, 1990). Additionally atmospheric pollution may have a direct effect or by modifying another agent, such as acid rain. Extractives and applied preservation systems may also be subject to UV photodegradation and leaching losses. The degradation of wood exposed to exterior conditions occurs due to the UV component of the solar spectrum. This degradation is principally limited to the lignin component of wood, and results in a steady release of polysaccharide rich wood cells, which are then consequently removed from the wood surface by rain and wind erosion (Hill, 2006) and also by foraging insects, e.g. wasps.

Photo-oxidation of the lignin reduces its molecular weight and it becomes soluble so that the middle lamella is destroyed.

The degradation starts instantly after the wood is exposed to sunlight. Initially, the colour changes and the surface fibres loosen and erode, but the process is quite slow. The weathering can take more than 100 years to decrease the thickness of a board by 5–6 mm. In addition, other processes also occur with the slow erosion process. By this time the wood may develop checks and raised grain. Blue stain fungi can also colonize the surface and discolour the wood. The boards may produce cup and warp, particularly for decking applications. The other weathering factors such as growth of fungal stain, splitting, checking, and warping, are frequently more important than the photo-oxidation alone, but these processes often perform together to degrade the surface (Feist and Hon, 1984; Feist, 1990; Hon, 2001).

2.4.3. Wood spoilage and decay

There are numerous organisms from different environments that live on or within wood and use wood either as a home or as a primary food source. A useful definition was that of Hueck (1965, 1968) where he defined biodeterioration (as distinct to biodegradation) as "*any undesirable change in the properties of a material caused by the vital activities of organisms*". In the context of wood this encompasses the decay-like activities of fungi, bacteria, insects and marine borers and spoilage by stain, mould fungi and pit degrading bacteria. Biodeteriorgens (organisms causing biodeterioration) are very dependent on temperature, moisture conditions and aeration conditions to grow in wood. Wood decay by fungi is the major type of damage to wood in use in temperate terrestrial environments whereas termites may be important in tropical situations.

Fungal decay

Decay essentially is the result of wood digestion by fungi (Zabel and Morrell, 1992). The slow, progressive digestion of the wood causes a continuum of changes in its appearance, mechanical, physical and chemical properties. Only limited groups of fungi possess the ability to digest wood. Various groups of fungi attack the wood cell wall constituents in different ways and sequences that result in several types of decay. Decay fungi are single-celled or multicellular filamentous organisms that use wood as food. The fungal spores are spread by soil, wind, water, insects, or animals. They germinate on moist, susceptible surfaces, and the hyphae spread throughout the wood. These hyphae secrete enzymes involved either directly

(hydrolases) or indirectly (oxidative enzyme systems) in the attack of the cell wall components and cause wood to deteriorate. After serious decay, new spore producing structures or fruiting bodies may form.

Three main types of fungal decay are recognised. White and brown rots are principally caused by fungi from the Basidiomycota and soft rot are principally caused by fungi from the Ascomycota and their asexual counterparts from the Deuteromycota, the fungi imperfecti. The brown-, white-, and soft-rot fungi have enzymatic systems which include cellulases and hemicellulases. Some fungi from each of the group can use single electron oxidation systems for modifying lignin (Eaton and Hale, 1993). The decay fungi require food material (hemicellulose, cellulose), oxygen (air), the right temperature (10-40 ˚C but optimally 20-30 ˚C), and moisture (above the FSP) to grow. The free water must be present (from rain, condensation and wet ground contact) for the FSP to be reached for decay. Dry wood will usually have no more than 20% moisture content, so decay cannot occur (Eriksson *et al.*, 1990; Zabel and Morrell, 1992; Eaton and Hale, 1993).

2.4.3.1. White rot fungi

White rot is so-called due to the bleaching of the wood which may become apparent with more advanced white rot decay by some white rot fungi, notably *Trametes versicolor.* The bleaching many be from lignin removal and black zone lines may develop where different individuals or species of fungi interact and compete (Eaton and Hale, 1993). White rot decay generally occurs on hardwoods but can also be found on softwoods. The degraded woods do not crack across the grain as seen with brown rot but the wood generally keeps its outward dimensions but feels very spongy when pressed. As the decay progresses, the strength properties decrease gradually, although toughness of wood may be lost at an early stage.

The white rot fungi may decompose all of the structural components (cellulose, hemicellulose, and lignin) of wood, but they are noted in their abilities to degrade and deplete lignin and aromatics (Ibach, 2005) at rates faster than other organisms. A distinction can be made into white rot fungi which simultaneously degrade all of these components at a similar rate, simultaneous white rot, for example as caused by *Trametes versicolor*, and preferential lignin degradation where delignification is concomitant with a reduction in hemicellulose content, a so-called selective decay, for example, as caused by *Ceriporiopsis subvermispora*.

Microscopic examination may reveal cell wall penetration with widening bore holes and erosion around the bore holes, erosion of the cell wall from hyphae on the wood cell lumens

resulting in cell wall thinning and in some instances cell separation caused by middle lamella delignification during a more lignin selective form of decay.

In simplistic terms the micromorpholgy of simultaneous decay is a cell wall erosion initiating from troughs formed around the hyphae (Bravery, 1968) and subsequent cell wall thinning while selective, preferential decay is seen as cell wall separation due to middle lamella delignification which is also accompanied by the loss of lignin in the secondary cell wall layers. Some fungi may perform a mixed selective and simultaneous decay within different regions of the wood.

White rot fungi often may produce considerable amounts of extracellular polysaccharides which may retain the high molecular weight cell wall degrading enzymes (e.g. cellulases, hemicellulases, and peroxidases), metabolically expensive enzymes, close to the hyphal surfaces, while lower molecular weight, mediator driven, and delignification systems may be more diffusible and may be responsible for wood cell wall delignification.

2.4.3.2. Brown rot fungi

Brown rot decay is so-called because of the darkening which occurs during decay, which is a result of the removal of the wood polysaccharides and the presence of degraded oxidised lignin. Subsequent drying and the loss of wood substance, especially the cellulose causes the wood to shrink and as it has little residual strength cross-grain cracking and longitudinal cracking occurs. It is weak and easily crumbles between the fingers resulting in a fine powder. When subjected to mechanical testing it is can be shown to have lost much of its toughness at very low weight losses (less than 5%) (Armstrong and Savory, 1959; Henningsson, 1967; Wilcox, 1978; Green *et al*., 1991; Winandy and Morrell, 1993; Curling *et al*., 2001) and it is thus considered a serious form of decay from a structural standpoint.

If examined by light microscopy brown rotted wood may show widened bore hole formation through the cell walls and loss of bordered pit membranes in softwoods (Wilcox, 1978; Schwarze, 2007) and cell wall shrinkage, but the use of polarised light may reveal loss of birefringence due to the removal of the cellulose. When the S2 layer is examined by scanning electron microscopy a porous structure is revealed, indicating considerable internal damage.

The brown rot fungi decompose the polysaccharides (cellulose and hemicelluloses) from wood, which then leaves the modified lignin remaining and making the wood browner in colour (Ibach, 2005). Brown rot fungi largely colonize softwoods, but they can also be found

on hardwoods, even durable hardwoods. For example the dry rot fungus, *Serpula lacrymans* may be found on oak in historic buildings in the UK. The strength properties of decayed wood decreases quickly in the early stages of decay (Ibach, 2005) where hemicellulose degradation predominates (Winandy and Morrell, 1993; Curling *et al.*, 2001). The hemicellulose constituents are degraded initially and this is followed by cellulose degradation. Since the cellulose microfibrils are enveloped in hemicellulose, this may be a serious stage during fungal degradation (Green and Highley, 1997). The brown rot fungi first use a small, diffusible low molecular weight system linked to a Fenton hydroxyl radical system (Chelator mediated Fenton system) to penetrate into the fine pore structure of the cell wall and depolymerize the cellulose in the cell wall and then apply endo-cellulases for further decomposition. Some, but not all, brown rot fungi are also known to produce cellobiohydrolases, typically those from the Coniophoraceae. Brown rot decay is not localised around hyphae and the brown rot fungi are not noted to produce large amounts of extracellular polysaccharides to retain their degradative enzymes. The currently held view of the brown rot decay mechanism in respect of lignin attack (which has been around since the 1970s), suggests that brown rot fungi do degrade lignin but do not metabolise it, instead they polymerise it into a recalcitrant lignin-like structure (Arantes and Goodell, 2014).

2.4.3.3. Soft rot fungi

Soft rot fungi are normally regarded as fungi of the Ascomycota and related asexual fungi of the Deuteromycota which occur usually in constantly wet wood, but they may also appear on the surfaces that encounter wet-dry cycling and may dominate at the surfaces of preservative treated wood. The typical soft rot decay is a shallow softening at the wood surface when wet, but the undecayed wood underneath is still firm and progressively deeper shows no microscopic features of decay. If in running water the soft rot inhabited the surfaces are weak and may be eroded resulting is losses in wood dimensions, and allowing decay to continue. When dried, decayed surfaces may show fine longitudinal and cross cracking. The wood decayed by soft rots becomes darker (dull-brown to blue-grey).

When examined by microscopy wood decayed by soft rot fungi may show various decay features including blue-stain like cell wall penetration, cavity formation in the S2 and S1 layers, cell wall erosion of various morphologies and general loss of birefringence when examined by polarised light microscopy which has been likened to a form of brown rot attack (Eaton and Hale, 1993). Although soft rot is essentially caused by the types of fungi cited above, cavity

formation in the S2 layer has also been reported in both brown and white rot fungi, and may be related to the damper, lower oxygen tension conditions which may occur. Soft rot fungi typically produce a range of cellulases, hemicellulases (Eaton and Hale 1993) and some soft rot fungi are reported to be able to degrade lignin, albeit slowly. The soft-rot fungi have a system to release lignin in wood to then allow the cellulases access to substrate (Ibach, 2005).

2.4.4. Decay mechanisms

Wood cell walls can only be regarded as a solid material down to a certain level, beyond which they consist of the chemical constituents and a variety of spaces, which give rise to an internal micropore structure. The early view of breakdown of cell wall polymers by hydrolytic enzymes alone is inadequate because the pores are regarded as being too small for the enzyme systems to rapidly move within the wall, so either alternative low molecular weight systems are involved or a series of surface modifications need to occur in concert to remove the cell wall components, in a stepwise manner. Cowling and Brown (1969) pointed this out and this has been a central theme of the understanding of both white and brown rot decay types, ever since. In the event highly diffusible low molecular weight systems have been shown to operate with both white and brown rot fungi which, dependant on the decay system operating change the pore structure and increase subsequent enzyme accessibility.

The review will only address the generality of decay mechanisms because recent genomic studies (Riley *et al*., 2014) suggest that there is considerable overlap in the Basidiomycota decay types, i.e. the white and brown rots. In terms of wood decay the fungi of the Basidiomycota, the brown and white rots, are the most significant and these have been used within this work, so that detailed review of soft rot mechanisms is not included here.

2.4.4.1. Decay mechanisms by white rot fungi

White rot fungi usually have a complete cellulase complex and a series of hemicellulases, but also have various enzymes which are involved in lignin degradation. The hemicellulose appears to serve as a carbohydrate source (energy and carbon source) for the fungus as lignin degradation occurs and is depleted (Eriksson *et al*., 1990). With white rot the mechanisms may be different with some of the different activities expressed during decay. Simultaneous decay may occur by cell wall erosion, involving all cell wall polymers, while delignification of the cell wall and middle lamella may be a more selective form of decay involving hemicellulose and

lignin. This attack within sound cell walls, per se, requires low molecular weight degradative agents rather than enzymes to initiate decay and this is achieved during ligninolytic attack.

Where enzymes have access to the cellulose substrate the white rot fungi express a range of extracellular enzymes involved in cell wall polysaccharide breakdown including glycoside hydrolases (GH), carbohydrate esterases (CE) and polysaccharide lyases and cellobiose deoxygenase (CDH) and lytic polysaccharide monooxygenase (LPMO). Greater diversity is present in wide genome studies (Hori *et al.*, 2013). In addition carbohydrate binding systems are also present (CBM) which appear to be present with white- not brown rot fungi.

The most important and best understood group of the cell wall degrading enzymes are the glycoside hydrolases, which work by a synergistic action to break down cellulose i.e. endo-1,4-β-glucananses, exo-1,4-β-glucananses and 1,4-β-glucosidases. The endo-1,4-β-glucananses hydrolyse parts of the chain of cellulose along the length of crystallite, releasing shorter chain oligosaccharides, while the exo-1,4-β-glucananses generally bind onto non-reducing ends of the chain and move towards the end and cleaving off cellobiose units. The 1,4-β-glucosidases can hydrolyse large oligomers and cellobiose to monomers (i.e. glucose), which is taken up by the fungal hyphae. Oxidative enzymes e.g. cellobiose oxidase, glucose oxidase and cellobiose dehydrogenase may also be involved, but these are involved in shunt reactions to avoid the build-up of breakdown products (Eriksson *et al*., 1990; Eaton and Hale, 1993). The enzymatic decay mechanism of hemicelluloses is similar to cellulose except that the synergistic action of a series of enzymes is not necessary because hemicelluloses are non-crystalline. The enzymes also operate endo- and exo- to the hemicelluloses which are used by fungi (Eriksson *et al*., 1990).

Lignin represents a difficult structure to degrade and powerful oxidants have been implicated (Hammel and Cullen, 2008). Several high oxidation potential class II peroxidases are expressed and commonly found in the genomes of white rot fungi (Riley *et al.* 2014), and comprise Lignin peroxidase (LiP), Manganese dependant peroxidase (MnP) and in some white rot fungi, versatile peroxidase (VP), for example in *Pleurotus* spp. (Martinez, 2002; Boada *et al*., 2005; Tsukihara *et al*., 2008). Laccases (lac) are common but are also present in some brown rot fungi (Riley *et al*., 2014) and are not found in all white rot fungi, e.g. *Phanerochaete chrysosporium* (Martinez *et al.*, 2004). Laccase in wood decay fungi has been known for a long time which was mentioned by Bavendamm (1928) and its presence was widely used as an indicator of white rot. Its role in lignin degradation was thought to have been largely restricted

to monomer degradation, due to its considerably lower oxidative potential than the peroxidase lignin degrading enzymes, although more recent work has promoted the role of laccases in the presence of redox mediators and iron reducing systems.

Although LiP was the first major lignin degrading enzyme to be isolated (simultaneously published by Glenn *et al.*, 1983; Tien and Kirk, 1983) it turned out that this was not universally present in white rot fungi (e.g. *Pleurotus ostreatus* and related species) and a second enzyme, MnP, discovered slightly later (Glenn and Gold, 1985) and is thought to be more widespread and possibly of greater importance. However the peroxidases are thought to be responsible for large scale lignin degradation due to their higher redox potential.

The peroxidases can oxidise phenolic and non-phenolic (Cα–Cβ cleavage) compounds within the lignin structure. MnP, oxidises manganese from Mn II to Mn III and this later oxidises lignin phenolics to phenoxy radicals. The phenoxy radicals ultimately initiate depolymerisation reaction within lignin. This is a low molecular weight diffusible system which can attack the wood cell wall from within. On the other hand, laccase can also produce phenoxy radicals similar to MnP (Eriksson *et al*., 1990; Reid, 1995). Versatile peroxidase is thought to have lower activity and its role requires further clarification (Hammel and Cullen, 2008).

LiP is oxidised by hydrogen peroxide which then itself oxidises two substrate molecules, forming cationic substrate free radicals intermediates which spontaneously fragment. Most of the scissions occur between the Cα–Cβ to give benzaldehydes which may be further oxidised by other reactions to benzoic acid structures (Hammel *et al*., 1986).

2.4.4.2. Decay mechanisms by brown rot fungi

The polysaccharides are rapidly depolymerised and the degree of depolymerisation (DP) of cellulose is also reduced while at the same time the weight losses are typically low (Cowling, 1961; Eriksson *et al*., 1990). The decay starts mainly in the S2 layer of the wood cell wall leaving the S3 layer of the lumen surface un-decayed (Highley *et al*., 1985; Eriksson *et al*., 1990; Kim *et al*., 1991; Arantes *et al*., 2011). The hyphae of brown rot fungi mostly grow in the wood cell lumens (Kuo *et al*., 1988) and the degradation is not restricted to the immediate surface of the hyphae, but the degradative system diffuses away from the hyphal surfaces, thus indicating that degradative agents have considerable ability for diffusion, and where possible into the wood cell wall.

Many studies have not detected cellobiohydrolase activity in brown rot fungi although they may be present in the Coniophoraceae (Nilsson, 1974; Highley, 1988; Eaton and Hale, 1993; Schmidhalter and Canevascini, 1993) and other fungi including *Fomitopsis palustris* (Yoon and Kim, 2005) and *Gloeophyllum trabeum* (Cohen *et al.*, 2005). Thus many lack the synergistic endo- and exo- glucanase for the enzymatic degradation of the crystalline cellulose that white rot fungi have (Enoki *et al*., 1990; Backa *et al*., 1992; Goodell *et al*., 1997; Hyde and Wood, 1997) and that the enzyme complexes characterised in the brown rot fungi are essentially too large to penetrate into the wood cell wall to reach and degrade cellulose in sound wood cell walls (Cowling and Brown, 1969; Goodell *et al*., 1988; Flournoy *et al*., 1991) alternative theories involving low molecular weight systems have been examined. Cowling and Brown (1969) suggested that the Fenton chemistry is the possible mechanism of the brown rot decay and this has been followed since.

The brown rot fungi are known to produce extracellular hydrogen peroxide using glyoxal oxidases and copper radical oxidases and the hydrogen peroxide is believed to participate in the brown rot decay. Koenigs (1972, 1974a, b) reported that the brown rot fungi produce extracellular hydrogen peroxide and wood contain iron ions and suggested that the Fenton's reaction may involve in the brown rot decay ($H_2O_2 + Fe^{+2} \rightarrow Fe^{+3} + OH^- + {}^{\bullet}OH$). Hydroxyl radicals are the most powerful oxidising systems of living cells. The hydroxyl radicals produced from the Fenton's reaction were thus proposed to cleave long chain molecules of cellulose into small fragments. It was later demonstrated that molecular oxygen may take part in the production of superoxide radicals which may be the precursor of hydrogen peroxide in brown rot decay processes (Enoki *et al*., 1990; Lu *et al*., 1994). The hydroxyl radicals were then thought to be able to diffuse into the wood cell wall and initiate decay.

Questions have been raised about the stability of the hydroxyl radicals themselves, in that they would need to be stable for long enough for them to diffuse into the wood cell walls as they have a half-life in the order of around 10^{-9} s (Goodell *et al*., 1997). Thus more stable radicals would have to be generated which could diffuse into the cell wall and then regenerate hydroxyl radicals within the lignocellulose/hemicellulose S2 wood cell wall matrix. A simple system of this has been proposed by Arantes and Goodell (2014) which is summarized in Figure 2.6 and this involves the production of hydrogen peroxide in the cell lumens and the generation of hydroxyl radicals inside the wood cell wall.

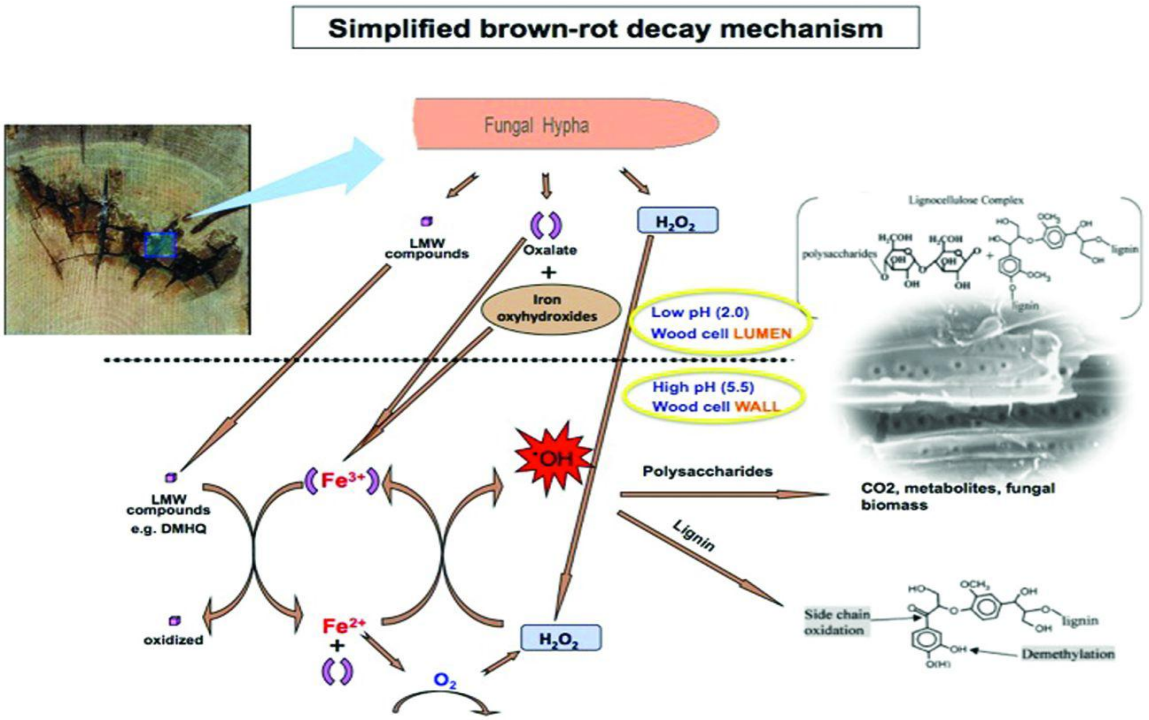


Figure 2.6. The simplified mechanism for the role and transport of hydroxyl radicals in the Fenton mediated brown rot decay system as supported by Arantes and Goodell (2014).

This pulls together a series of ideas involving modified hydroxyl radical theories which have been developed by Goodell, co-workers and others over a number of years. Initial work looked at the role of siderophores in the sequestration of iron (Jellison *et al.*, 1991) but this has assumed a more active role where some compounds are released by the fungus which have potentially more active roles as mediators, including 2,5-dimethoxyhydroquinone (DMHQ), isolated from *G. trabeum* by Paszczynski *et al*. (1999) and subsequently shown to be present in other brown rot fungi. The brown rot fungi generate a low pH in the lumen by means of oxalic acid which combines with iron, low molecular weight iron reducing compounds (LMW) and hydrogen peroxide. All of these diffuse into the cell wall where the DMHQ sequesters Fe^{3+} from the oxalate Fe complexes and reduces this to Fe^{2+}. The Fe^{2+} participates in the Fenton reaction inside the wall and generates the hydroxyl radicals causing the resultant effects on the lignocellulosic complex of glycosidic bond cleavage, lignin demethylation and depolymerisation. Lignin itself is now thought to be more degraded than was previously realised but may repolymerise following Fenton hydroxyl radical attack (Goodell *et al.*, 1997).

Thus it is concluded that these decay types involve oxidative free radical generating systems and enzymes which directly attack the wood cell wall substrates so that an inclusion of an oxidative system into the theme of this thesis is important.

2.5. Natural durability of wood

The durability of a wood species to attack by degrading organisms such as termites, powder-post beetles, marine borers and fungi will determine its natural durability. The ability of a wood species to resist biodeterioration for a specific period of time is known as "natural durability" or "natural decay resistance". This definition only refers to heartwood and excludes species with no distinction between heartwood and sapwood (Wong *et al.*, 2005). In service, wood needs to have durability fit for purpose and the risk of wetting determines the level of durability required (EN 350-1). When the risk of wetting is high and a long service life is required, high durability wood is required. Inadequate durability could mean early failure and a short service life. Therefore, natural durability against decay is an important property in wood. The heartwood of some species e.g. teak (*Tectona grandis*) and greenheart (*Ocotea rodiaei*) can last for decades in regions of high decay hazard, while other species requires quick conversion and drying after felling if the risks of spoilage and or decay need to be avoided, e.g. birch (*Betula* spp.) and pine sapwood (*Pinus* spp.) (Eaton and Hale, 1993).

Some wood species produce extractive compounds which can protect the wood; these are considered the principal source of decay resistance for many species. The extractives are produced when the living ray cells of the inner sapwood zone die and form the non-living heartwood. As the sapwood dies in wood species, a series of reactions take place in the parenchyma cells of wood rays and converts the stored starch and sugars into a wide range of compounds, some of which are toxic and become the constituents of new heartwood. Generally, the sapwood of all species has low natural durability (Toole, 1970; Eslyn and Highley, 1976). The heartwood of many species has a distinctive dark colour, while in some other species it is difficult to differentiate the colour from sapwood. The decay resistance of wood varies among the species, individual trees, and within the individual trees (Scheffer and Cowling, 1966).

The climate conditions in combination with particular types of wood degrading organisms endemic to a specific geographical area interact to influence the natural durability of wood species. Wood used in tropical conditions generally degrades much quicker than in temperate regions (Wong *et al*., 2004). The tropical wood species vary extremely in their resistance to decay, but only a few of them are resistant against the termites. Generally, the natural durability of wood species is evaluated by different standard methods of field and laboratory tests as appropriate to different exposure situations and environments, and these procedures

rate wood species into durability classes. In ground contact field test results, the durability of wooden stakes differs between temperate and tropical regions due to temperature and water availability, producing diverse rates of biodegradation. An example for in-ground durability classification of wood species against Basidiomycete (white and brown rot) or soft rot decay (temperate problem) and termite decay combinations (humid tropical problem) is shown in Table 2.1.

Table 2.1: The service life criteria for evaluating in-ground durability of wood species (test stakes) in temperate and tropical regions compared with the mass loss criteria of laboratory durability test (wood blocks).

Natural durability or decay resistance class[4]	**Temperate climate (England) service life[1]**	**Tropical climate (Malaysia) service life[2]**	**Laboratory test mass loss (%)[3]**	**Laboratory test mass loss* (EN 350-1)[4]**	**Laboratory test mass loss (%) (ASTM)[5]**
1. Very durable	>25 yr	>10 yr	0, or negligible	x ≤ 0.15	0 – 10%
2. Durable	15 – 25 yr	5 – 10 yr	<5%	0.15 < x ≤ 0.30	11 – 24%
3. Moderately durable	10 – 15 yr	2 – 5 yr	6 – 10%	0.30 < x ≤ 0.60	25 – 44%
4. Slightly durable	5 – 10 yr	<2 yr	11 –30%	0.60 < x ≤ 0.90	>44%
5. Not durable	<5 yr	-	>30%	x > 0.90	-

* Average corrected mass loss of test specimens/ average mass loss of reference specimens.
[1]Eaton and Hale, 1993; [2]Wong *et al.*, 2005; [3]Findlay, 1985; [4]EN350-1,1994; [5]ASTM, 1981

The natural durability of wood is mostly dependent on the presence of extractive compounds in heartwood. The conventional view, held during the last century was that the extractives are toxic but other mechanisms are now being added to this, including antioxidant activity (Schultz and Nicholas, 2000) and the polymerisation of molecules with the wood structure. Furthermore heartwood formation results in the drying of the wood and in some hardwoods the formation of tyloses in vessels (e.g. white oaks), whilst in softwoods pit aspiration and encrustations on these occur. Thus heartwood durability is complex and may involve a variety of different factors i.e. biocidal, antioxidant, and metal chelating mechanisms, moisture exclusion and cell wall moisture exclusion. Many studies have focused on the isolation of the wood extractives and its effect on the durability of wood, i.e. flavonoids, quinones, stilbenes etc. (Schultz *et al.*, 1995; Chang *et al*., 2000; Ohmura *et al*., 2000; Wang *et al*., 2004; Dungani *et al*., 2012).

Schultz and Nicholas (2000) reported that the flavonoids can protect heartwood from the fungal degradation by a dual mechanism – fungicidal and free radical scavenger (antioxidant).

Gupta and Prakash (2009) reported that flavonoids have natural antioxidant properties and ability to scavenge of free radicals. Specially, it is well known that the extractives have excellent free radical scavenging and metal chelating capabilities. As discussed for brown rot decay, free radical systems may be very important for brown rot decay and antioxidants are thus important.

Schultz and Nicholas (2000, 2002) proposed an alternative approach to develop new wood protection methods by studying the natural durability mechanism of heartwoods of some wood species. The authors suggested that the extractives present in heartwood protect it from the fungal colonization and degradation due to having fungicidal and possible other non-biocidal properties (antioxidants/free radical scavenging) which work synergistically.

It is also found that the fungal degradation of wood involves different metals (Eriksson *et al*., 1990). The phenolic extractives are able to form complexes with metals (metal chelator), thus metal chelation could be another means of protecting wood by extractives. Schultz and Nicholas (2002) further studied the synergistic effect of various organic fungicides, with different antioxidants in combination to metal chelators using different wood species against the wood decay fungi. The study gave enhanced decay resistance when compared with the results of organic fungicides alone and suggested the possible trio-mechanism of heartwood protection by fungicidal, free radical scavenging and metal chelating activities. Several other studies have also concentrated on this issue of using synergistic effects to develop new wood protection strategies (Mabicka *et al*., 2005; Gao *et al*., 2007, Stirling *et al*., 2007).

2.6. Wood preservation and environmental legislation

The sapwood of all wood species is susceptible to the attack of wood destroying organisms but the heartwood, as discussed above, differs in its resistance to decay depending upon the wood species. Many heartwoods are durable enough for use in adverse situations without preservative treatment, but others are no more durable than sapwood. In many cases when a tree trunk is converted to the sawn product, both sapwood and heartwood are present in the same piece of timber. So, even if the heartwood is durable, the presence of a substantial proportion of sapwood may dictate that to meet end-use requirements, the wood will need preservation if the wood is exposed to a decay risk environment. The European Committee for Standardisation, (Technical Committee CEN/TC38) has adopted the following definition for a wood preservative.

"*Wood preservatives are active ingredient(s) or preparations containing active ingredient(s), in the form in which they are placed on the market, and which are, on the basis of the properties of their active ingredient(s), intended either to prevent wood-destroying or wood-disfiguring organisms (fungi, insects and marine borers) from attacking the wood and wood-based products or to combat an attack of these organisms*."

Wood preservation is a constantly developing technology. This can be demonstrated by the changes in the active ingredients used. The changes may be introduced by shifting the availability, efficiency, economics, safety and/or environmental considerations. In many cases, particularly for the environmental considerations, such changes are demanded through different legislation. While some compounds such as mercuric chloride, dieldrin, lindane, DDT, pentachlorophenol and tri-n-butyl tin oxide have long since ceased to be used, mainly because safer and less environmentally damaging systems were developed, more recently chromated-copper-arsenate has been withdrawn from use by legislation.

Rising concerns for the environmental acceptability and safety of many biocides was already important and discussed earlier (Hilditch, 1983). There has for a long time been an increasing general need to improve wood protection methods that meet a number of criteria including environment and health, appropriate degrees of protection for specified end-uses and economic viability. Biocide issues which have vexed the industry also include leaching losses and uncontrolled emissions and spillage in use (i.e. into watercourses, soil water and river ecosystems) and end-of life cycle disposal issues. Such concerns have led to the banning of CCA or have strictly limited its use to specific products or market sectors. There have been more concerns raised on the use of chromium and arsenic as wood preservatives in places where there is a high probability of human exposure to treated wood (McNeill, 1990).

The acknowledgement of preservative treated wood as hazardous waste generally leads to two options for the wood protection technology: reduce the waste generation chemicals or substitute alternative and safer chemicals for the traditional preservatives of wood. The latter option leads to the wood modification, which offers future prospects for the wood preservation industry. This can be in parallel with other methods of imparting the performance of wood, including its durability. This alternative method can use non-toxic materials e.g. bulking agents and/or water repellents that modify the wood chemically and thus the wood becomes unattractive to wood destroying organisms (Kumar and Morrell, 1993).

2.7. Wood modification

Conventional wood modification includes three principal strategies i.e. chemical, thermal and impregnation modification. The first two share a common aim to modify the molecular structure of the wood cell wall constituents, while impregnation modification may not necessarily achieve cell wall modification and may only achieve some lumen filling. The properties of wood can be improved considerably by changing the cell wall hydrophilic hydroxyl- (OH) groups into more hydrophobic groups (Homan and Jorissen, 2004). Although the wood modification has been a subject of great deal of study at the academic level for more than 50 years, it is only recently that there has been major commercial development (Hill, 2006) and in 2011 Accoya, acetylated radiata pine, became available worldwide.

Different definitions of wood modification are reported in old and recent literature. In some cases, they include a change in the chemical structure of wood cell wall components. Recent insights have revealed that in many cases it is difficult to obtain these criteria (Hill *et al.,* 2004b). A wider definition of the wood modification includes a treatment of wood to improve its properties, but that does not contain a product that has toxic residues. This excludes the use of biocidal treatments and thus separates the wood preservation from the wood modification (Van Acker and Hill, 2003).

Hill (2006) defined wood modification as a means of altering the material to ameliorate or overcome one or more disadvantages of wood. In a more specific term "*wood modification involves the action of a chemical, biological or physical agent upon the material, resulting in a desired property enhancement during the service life of the modified wood. The modified wood should itself be non-toxic under service conditions and, furthermore, there should be no release of any toxic substances during service, or at end of life following disposal or recycling of the modified wood. If the modification is intended for improved resistance to biological attack, then the mode of action should be non-biocidal*".

The different wood modification technologies have been acknowledged for a long time, but no economic or environmental urgency was developed in these technologies in the past. Recently, there has been growing legislative and environmental pressure for biocide and preservative use based on traditional biocides which have created new opportunities for wood modification. At present some processes for modifying wood have been commercialised at the industrial scale (e.g. Accoya: acetylation, furfurylation, and various heat treatments) and

continuous advances in the process development will make these materials more common in future (Hill, 2009).

The modification of wood has been classified early on by Norimoto and Gril (1993) mainly in two ways, active and passive modification, by referring to changes occurring at the cell wall level (Table 2.2). Wood modification involves active modifications which result in a change of chemical nature of the material, but in the passive modification, the change in properties is effected without alteration of the chemistry of the material.

Table 2.2: Classification of wood modification methods (Norimoto and Gril, 1993).

Division	Type	Class
Active	Chemical modification	Cell wall
		Surface
	Thermal modification	Cell wall
	Enzymatic modification	Surface
Passive	Impregnation modification	Cell wall fill
		Lumen fill

Wood modification can improve many important properties of the wood including dimensional stability, permeability, hardness, UV stability and biological durability. To control the moisture uptake into and loss from wood is a very effective way for protecting wood. Different wood modification processes have been confirmed at the laboratory and at semi-industrial scales and continuous advances are going on in the process of development. Chemical modification of wood has been the subject of many literature reviews, including acetylation, furfurylation and N-methylol (DMDHEU) (Rowell, 1977, 1983; Banks, 1990; Takahashi, 1993; Banks and Lawther, 1994; Kumar, 1994; Sasaki and Kawai, 1994; Hon, 1995; Militz *et al.*, 1997; Suttie, 1997; Birkinshaw 1998). Thermal modification of wood is another interest in wood modification sector and has been practised for a long time. This is the most developed modification technology in the commercial sense, with production starting in Finland in the late 1990s (Hill, 2009). Besides these two well established modification methods there are some other methods that have been developed including enzymatic modification and surface modification (Hill, 2006).

2.7.1. Impregnation modification

The impregnation of the wood cell wall with different types of chemicals is a very broad area that continues to attract interest. Impregnation involves the impregnation of the wood cell lumens with a monomer solution. This may polymerise only in the lumen or it may also diffuse

into the cell wall and polymerise there as well. It is however, important to note that an impregnation of the cell wall with the modifying agent is required in order to bring about desirable property changes and that furthermore this impregnant should ideally be non-leachable (Hill, 2009). All such impregnation modification technologies use low molecular weight monomeric compounds. Examples of these modification methods developed in the past are the Impreg and Compreg technologies developed at the FPRL Madison WI USA which use phenol formaldehyde resin impregnation followed by heat or heat combined with pressure curing to make a modified wood product (Stamm and Seborg, 1941).

This category of modification does not necessarily involve changes to the chemical composition of the wood cell wall. The principle behind impregnation modification is explained by Hill (2006) as "to impregnate the cell wall of wood with a chemical, or a combination of chemicals, that then react so as to form a material that is locked into the cell wall. For this to occur, it is necessary that during the impregnation phase that the cell wall is in a swollen state, so as to ensure accessibility to the impregnant". He also mentioned that the presence of non-leachable material in the cell wall of wood can affect the physical and biological properties of wood through different mechanisms (see Hill, 2006).

2.7.2. Properties of resin modified wood

The resin treatment is the most useful method among several other process of impregnation modification. As with many early research areas in the field of wood modification, Stamm and co-workers performed much of the work in resin impregnation at the Forest Products Laboratory in Madison (Stamm and Seborg, 1941). Beside this, several other processes like dimethylol dihydroxy ethylene urea (DMDHEU), furfuryl alcohol, maleic acid with glycerol, maleic anhydride with polyglycerol, N-methyl acrylamide, and impregnations using silicon-containing compounds have been developed as impregnation modification (Hill, 2006).

The preliminary work on resin treated solid wood was directed towards increasing dimensional stability, which was done by filling the voids of the wood with resin and therefore stopping the ingress of moisture. Stamm and Seborg, (1936) suggested that the permanency of resin bonded to the -OH groups of cellulose would give a far more permanent effect that with other materials such as waxes. Resin can impart decay resistance in two ways; either by simply blocking the micropores in the wood, making the wood cell wall impervious to water, or by acting as a toxic chemical barrier that kills the fungi (Hill, 2006).

The treatment of wood with different resins have been widely studied, including the use of phenolic resins, urea resins, melamine resins, epoxy resins, polyurethane pre-polymers and unsaturated polyesters (Pittman, 1994). The principles for enhancing the durability of wood using in particular waterborne resins impregnated into the wood were described by Rapp and Peek (1995). Treatment with polymethylmethacrylate achieved the WPGs of *ca.* 160 and produced a highly water repellent composite due to the filling of cell lumen, but the dimensional stability was found to be lower than other modified woods because the polymer could not penetrate into the cell walls. The modified material (acrylic wood) exhibits good mechanical properties and can be used in parquet flooring, musical instruments and sports equipment (Rowell and Konkol, 1987).

Polyethylene glycol (PEG) is a water soluble polymer which is impregnated into wood by diffusion and can be accelerated by elevating the temperature and PEG solution concentration (Stamm, 1964). With a WPG of 25-30 achieved, the PEG remains water soluble and it was leached out after drying. However wood impregnation with PEG can impart very good dimensional stability, as ASE values increased, and the treatment reduced the occurrence of checks during drying (Norimoto and Gril, 1993). Wood impregnated with glycols is usually finished with a surface coating to seal them. This has been used in archaeology for treating water-logged wood materials, as in the Mary Rose project (Stevenson, 1995). It is also used for treating the cross-section plaques, table tops, rifle stocks, and the green wood sections of bowls and other turnings (Rowell and Konkol, 1987).

Wood has been treated with thermosetting and fibre penetrating resin system followed by curing without compression (Stamm and Seborg, 1962). The phenol formaldehyde (PF) resin forming systems with low molecular weights are by far the most successful thermosetting systems for modifying wood properties. The PF resin can enter into the wood cell walls, which results in an improvement of the heat resistance, electrical insulation properties and decay resistance. The dimensional stabilization of particleboards and solid wood surfaces treated with PF results in good mechanical performance and was thought to be worthy for commercial application (Norimoto and Gril, 1993). The PF resin with a low molecular weight in aqueous solution has been used to treat veneers for plywood and particleboards which achieved improved dimensional stability and biological resistance to fungi and termites (Kajita and Imamura, 1993).

The melamine resins also were advanced as resin treatments for the improvement of wood durability and hardness in the application of parquet flooring (Rapp and Peek 1996). Wood treated with melamine formaldehyde (MF) and melamine urea formaldehyde (MUF) resin systems showed an impressively improved dimensional stability, and resistance to weathering and fire (Pittman, 1994). The MF resins had good weather and moisture resistance, were stable to ultraviolet light and also had excellent fire retardant properties. Conversely, some of the mechanical properties of wood were diminished due to the rigid and brittle nature of the cured resin in the wood. This negative effect could be overcome by using blends of resins (MUF and MF). Generally, the differences in the WPG are evaluated by the resin type which is impregnated into the wood and indicated the degree of ASE that will be advised to the final product. These resins have the capability not only to exclude the moisture from wood but also to interfere with some of the fungal degradation mechanisms.

Many authors have reported on microbial resistance of resin modified wood (Stamm and Baechler, 1960; Van Acker and Stevens, 1989; Takahashi and Imamura, 1990; Ryu *et al.*, 1991; Militz, 1993; Rapp and Peek, 1995, 1996; Sailer *et al.*, 1998; Ritschkoff *et al.*, 1999; Van Acker *et al*., 1999; Deka and Saikia, 2000; Westin *et al.*, 2004). Stamm and Baechler (1960) reported the decay resistance of Sitka spruce wood modified by 5 different techniques including PF resin impregnation and found that weight loss due to decay by *Lenzites trabea* (=*Gloeophyllum trabeum*) became negligible when the ASE reached to 70% or higher, summarized that the decay resistance of PF impregnated wood increased as a result of the physical blocking of wood cell wall -OH groups. The effect of the molecular weight of resin on the decay resistance of PF treated wood has been a subject of many investigations. Furuno *et al.* (2004), Ryu *et al.* (1991) and Takahashi and Immamura (1990) reported that decay resistance is higher with low molecular weight resin systems than higher molecular weight resins.

Rapp and Peek (1996) investigated the decay resistance by fungi of Scots pine sapwood treated with methylol-substituted melamine-based resins. Some samples were impregnated with aqueous solution of resin and after curing, were exposed to *Coniophora puteana* and *Trametes versicolor*. Good fungal protection was achieved with the resin treatment, even after leaching in accordance to EN84 protocol. Lukowsky and Peek (1998) reported that the decay resistance of MMF resin-treated Scots pine sapwood was improved when the resin immersion periods were extended up to 24 hours and over.

Van Acker *et al.* (1999) studied the decay resistance of the MMF treated Scots pine and beech in accordance to EN113 and ENV807 protocols. The study revealed that resin retentions in excess of 50 kg m^{-3} were found to be required in order to provide satisfactory protection to the wood. Westin *et al.* (2004) reported that Scots pine was resistant after MMF resin treatment when assessed in laboratory pure basidiomycetes test. The wood specimens were impregnated with 7.5, 15 and 25% MMF solution by the full cell process with a vacuum of 30 minutes and 4 hour pressure at 8 bars. Maximum weight losses of 4.2% were found when exposed to *Trametes versicolor* and a lower 1.6% was found when exposed to *Postia placenta*.

Work done at a similar time as a European project "Natural resins as a potential wood protecting agent" evaluated a whole range of resin based on natural compounds and their usability as wood protection agents by means of a Basidiomycete test (EN113) and a soil bed test (ENV807). Six resin treatments were tested at three concentrations giving various treatment levels (Van Acker *et al.*, 1999). The results revealed that none of the resin treatments had potential to be used under use class 4 conditions, some showed potential as wood protecting agent for use class 3, including modified tall oil rosin, DMDHEU and MMF. Resin treatment has been found to improve to a significant degree the resistance to attack of termites. Deka and Saikia (2000) reported that the thermosetting resin treated wood was resistant to attack by termites.

2.8. Bio-resin

The idea of wood adhesives from natural and renewable raw materials has been a topic of significant interest for several decades. The level of interest and development has depended on demand and economic factors. The use and application of adhesives based on the natural renewable resources by many industries and the general public is often considered as a new approach which requires novel technologies and methods for implementation (Dunky *et al.* 2002). The major thrust to date in bio-adhesives or bio-resins was started by the oil crisis of the early 1970s, though this interest waned as oil prices stabilized.

However there is a new demand for bio-derived products, including resins, driven by consumer demand as well as environmental considerations. When considering bio-derived adhesives, it is necessary to establish the broadness of the definition. If considering the major component of the adhesive, the bio-derived material may be referred to as non-petroleum based. The term 'bio-derived adhesive' has come to be used in a very well-specified and narrow sense to only include those compounds of natural, non-mineral or non-petroleum

based origin, that can be used either in their original (natural) condition or after small modifications and capable of reproducing the behaviour and performance of the synthetic resins (BRE, 2007). Such a definition limits the number of materials that can currently be included to tannins, lignins, carbohydrates, unsaturated oils, liquefied wood and wood welding by self-adhesion. Other materials have been identified and widely used in the past, and work continues into adhesives manufactured using proteins, blood and collagen.

2.8.1 Classification of bio-resin

The term 'natural adhesive' or 'bio-resin' includes material of any polymeric and pre-polymeric monomer or oligomer derived from the natural sources that will cure to form a durable bond. Though this class of adhesives also includes animal by-products e.g. blood, collagen and fish glues, this section will only discuss adhesives derived from plants.

Plant derived adhesives generally separate roughly into one of the four categories outlined below (Table 2.3). At present only small amounts of tannin and lignin are utilised in the wood based industries. Tannins are utilized in the southern hemisphere mainly confined to panel products industries within South Africa, Australia and South America. Lignin (both organosolv and spent sulphite liquor, SSL, from the pulp and paper mills) has been used as a co-reactant with the commercial PF to achieve specific properties (Sellers *et al*., 1994; Wang *et al*., 2009). However few examples of true commercial lignin based resins can be found, but use of tannin and lignin as natural phenolic replacements is increasing. Again very few instances exist of practical wood glues from triglycerides and polysaccharides. Cashew Nut Shell Liquid (CNSL) has attracted commercial interest to replace synthetic phenol resin systems (Dunky *et al*., 2002; BRE, 2007) and is one of several examples of compounds that have potential as new resin systems (Table 2.3).

Table 2.3: Potential plant derived compounds for using bio-resin (Dunky *et al*., 2002).

Categories of bio-resin	**Types**
Tannins	Hydrolysable Condensed
Lignins	Spent sulphite liquor (SSL) Organosolv
Carbohydrates	Starch Cellulose Hemicellulose
Unsaturated oils	Oil seed triglycerides Cashew nut shell liquid (CNSL)

2.8.2. Cashew Nut Shell Liquid (CNSL)

Cashew nut shell liquid (CNSL) is a natural and renewable product obtained from the shells of cashew nut. With its natural form, the crude CNSL is a mixture of different phenolic compounds. However, the main constituents are anacardic acid, cardol, cardanol and 2-methyl cardol (Figure 2.8). Due to its phenolic nature and unsaturation of the side chain, the CNSL has reaction sites both on the aromatic ring and the side chain. This makes CNSL a suitable raw material for a wide range of reactions. CNSL can react with active methylenes e.g. formaldehyde or hexamethylene tetramine through the hydroxyl group (OH) and then undergo additional polymerization via the unsaturation present in the side chain (Mahanwar and Kale, 1996). Therefore, different types of resins can be synthesized from CNSL, its isolated constituents (cardanol), or from chemically modified CNSL. The synthesis and properties of resins developed from CNSL and different aldehydes, styrenated CNSL-formaldehyde resins, polyurethanes, acetal resins, acrylic resins, and a variety of resins have been reported (Mahanwar and Kale, 1996).

CNSL is available naturally in many parts of the world e.g. Brazil, India, Bangladesh, Tanzania, Kenya, Mozambique, and in the tropical regions of Africa, south-east and far-east Asia. The cashew (*Anacardium occidentale*) is a tropical evergreen tree (Figure 2.7a) of Brazilian origin and the most abundant member of the Anacardiancae family. It is now grown in many other tropical countries due to Portuguese traders (Tyman and Kiong, 1977). Other parts of the cashew plant including the cashew apple, cashew nuts and extracted CNSL are also shown in Figure 2.7 (b-d).

The major constituents of the CNSL have been characterized by Tyman (1975) and Murthy *et al.* (1965) using ultraviolet and infrared spectroscopy, mass spectroscopy, H-NMR, and coupled with appropriate chromatography. The CNSL contains four major components, i.e. anacardic acid, cardanol, cardol, and 2-methyl cardol (Figure 2.8). Based on the mode of extraction from the cashew nut shell, CNSL is classified into 2 types e.g. solvent-extracted CNSL (*i*CNSL) and technical CNSL (*t*CNSL) (Table 2.4). Among the CNSL constituents, anacardic acid possesses antimicrobial, anti-acne, and many other medicinal properties (Kubo *et al.* 1993). Cardol is a dihydric component that accounts for CNSL's vesicant activity and toxicity (Wasserman and Dawson, 1948). With its polymerizable phenolic group and its side chain, cardanol has been widely studied for its polymeric properties (in the form of phenol-formaldehyde resins) (Kumar *et al.*, 2002).

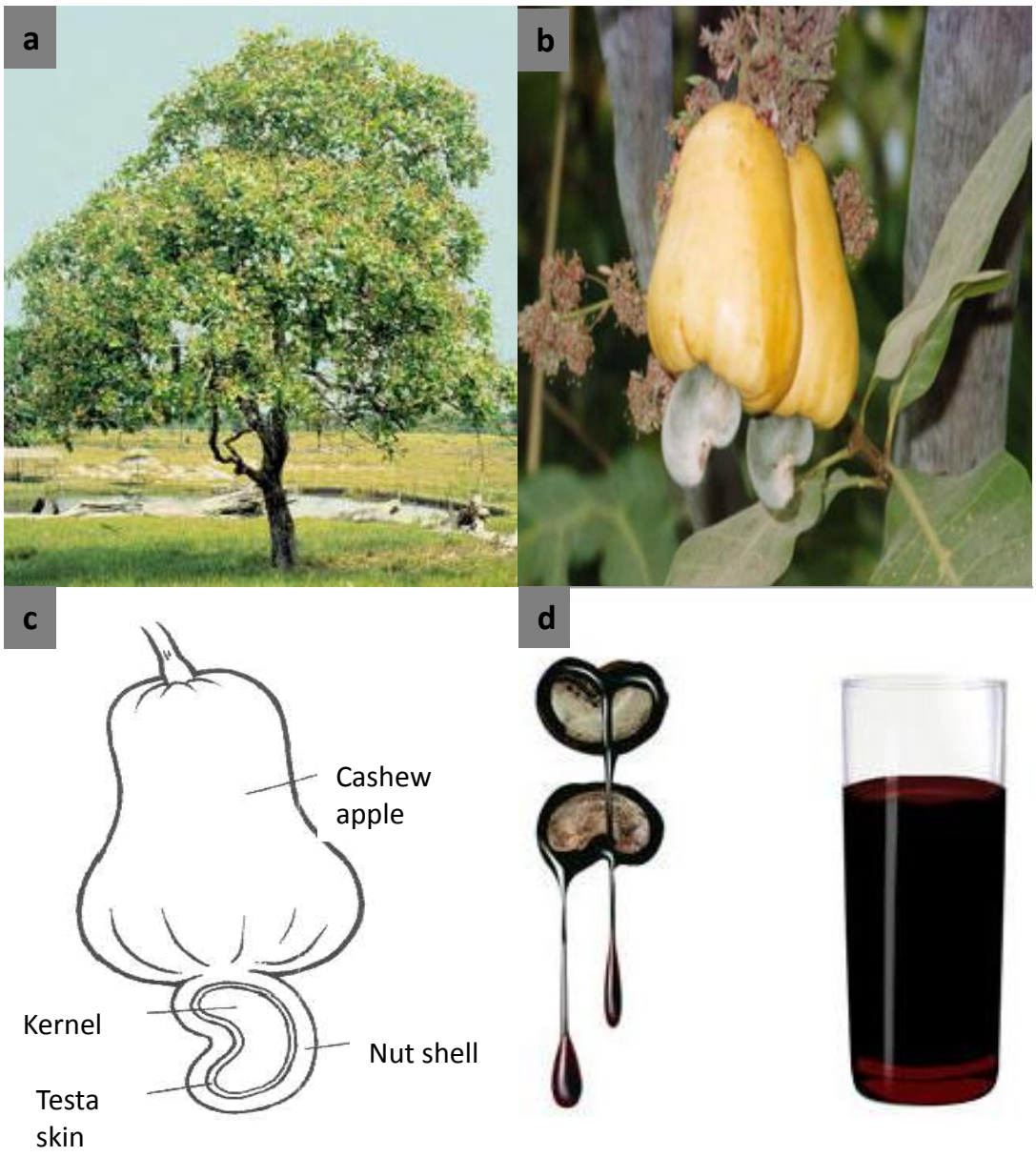


Figure 2.7: General pictorial information of CNSL, (a) The cashew tree, (b) cashew apple with nut from end of fruit, (c) cross section of the cashew nut and (d) appearance of the dark brown coloured cashew nut shell liquid (CNSL). (Sources: Ramanan *et al*., 2008; Bala Chem, 2011; Fruitpedia, 2011).

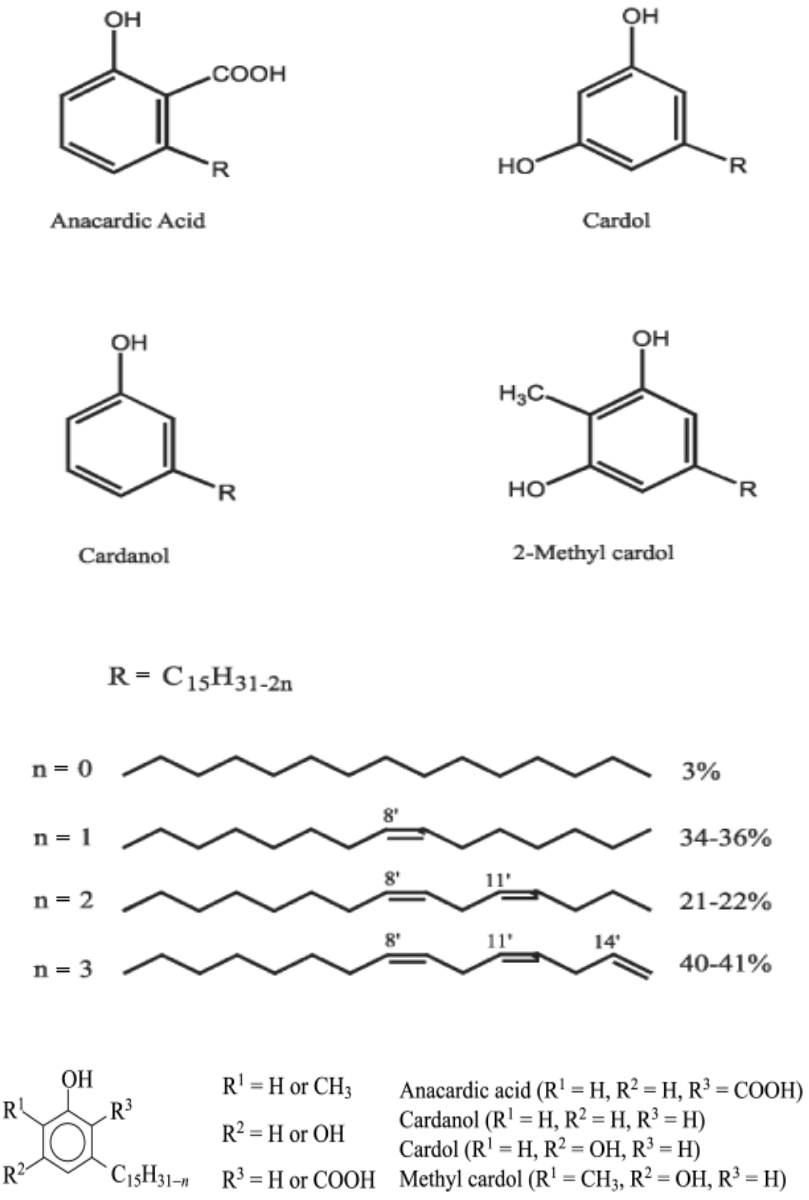


Figure 2.8: Structure of CNSL (Cashew nut shell liquid) constituents (Kumar *et al*., 2002).

Table 2.4: Chemical composition of CNSL obtained by different extraction process (Kumar *et al.* 2002; Das *et al*., 2004).

Factors	**Solvent extracted (*i*CNSL)**	**Technical (*t*CNSL)**
Method of extraction	Extraction in Organic solvent	Shell roasting at 180-185 °C
Anacardic acid	60-65 %	-
Cardanol	10%	60-65%
Cardol	15-20%	15-20%
2-methyl cardol	-	trace
Polymeric material	trace	10%

Resins made from this renewable material have wide applications including surface coatings, paints, varnishes, and brake linings, but the main applications of CNSL are in the polymer industry (Menon *et al.*, 1995). Compared with conventional phenolic resins, CNSL polymer has improved flexibility (because of the internal plasticization effect of the long side chain) and this improves processability. The side chain of CNSL imparts a hydrophobic nature to the polymer and this makes it water repellent and resistant to weathering (Pillai *et al.*, 1980). The low "fade" character on friction is a significant property of the CNSL polymer, makes it an essential additive for most organic brake lining formulations (Wilson, 1975). CNSL based resins have excellent resistance to the softening action of the mineral oils and high resistance to acids and alkalis (Knop and Scheib, 1979). The CNSL polymers also have useful features such as electrical and heat resistance (Anon, 1978) and, as discussed below, biological activity.

2.8.3. Bio-protection of wood by CNSL

As mentioned earlier that among the CNSL constituents, anacardic acid possesses antimicrobial, anti-acne and many other medicinal properties, while Cardol accounts for CNSL vesicant (blistering) activity and toxicity, although in rats 5 g /kg is tolerated (Suresh and Kaleysa, 1990). The CNSL has potential for use as a biocide to protect wood form bio-deterioration by termites (Lepage and De Lelis, 1980) and fungi. Adetogun *et al.* (2009) reported the evaluation of CNSL as an anti-fungal wood preservative, where test blocks of *Triplochiton scleroxylon* (Obeche) were vacuum treated with different concentrations of CNSL in ethanol. Supposedly substantial penetration and absorption of *i*CNSL was reported and weight loss was prevented in a laboratory test with the white rot fungi *Coriolopsis polyzona, Pycnoporus sanguineus, Ganoderma lucidum* and *Lenzites palisoti* (=*Trametes*) at solution concentrations of 16%, where 12% was inadequate to control *C. polyzona*. Here it is difficult to interpret their results as they state that the wood was treatable and was well treated by a vacuum system but only achieved a retention of 7 kg m^{-3} at a solution strength of 32%. No WPG data were reported but if a 32% ethanolic solution gave a retention 7 kg m^{-3} and the reported density of Obeche is 380 kg m^{-3} this would equate to a WPG of 1.8, and at half of that (i.e. 16% pro rata), 0.9 would seem impressive.

Venmalar and Nagaveni (2005) studied copperised CNSL (extracted in hot kerosene) as a wood preservative to protect rubber wood after dip or vacuum impregnation. Better decay resistance was found with CNSL combined with copper than with the pure CNSL alone although again the interpretation of retentions is difficult. Mwalongo *et al.* (1999) reported

that the wood of *Pinus ponderosa* and *Populus tremuloides* (aspen) were least termite damaged after 108 days exposure in Tanzania when treated with 40% CNSL with 2% $CuCl_2$ (w/w).

Early studies in Brazil by Lepage and De Lelis (1980) suggested that CNSL painted onto wooden structures prevented termite attack and subsequently a number of studies have focused on the potential of the CNSL as an insecticide to protect wood from termite attack in other locations (Remadevi *et al*., 2005; Remadevi and Muthukrishnan, 2007; Asogwa *et al.*, 2007). Unfortunately the above treatment solutions strengths do not concur with the normal industry standard methods of expressing treatments; different woods have different densities and permeabilities. Thus treating with certain solution strengths is more or less meaningless because different amounts of a substance or formulation will end up in the material and little attention has even been given to visual assessment of depth of penetration. Industrially the amount of preservative applied is expressed as an uptake, loading or retention, and penetration depths are also considered (Table 2.5). In lab decay resistance tests small blocks are treated and full penetration is often assumed with proscribed test species, e.g. Scots pine sapwood.

Table 2.5: Properties use to evaluate the efficacy of resin (preservative) treatment.

Properties of treatment	**Remarks**
Uptake	Assesses the amount of preservative taken up in a given weight (conditioned or oven dry) or volume of wood
Loading (or retention)	Uses the uptake x the solution strength to calculate the amount of preservative present and can be expressed as weight/ volume industrially. Researchers often express this as weight/ weight because they may be working with woods of different density
Weight per cent gain (WPG)	Assesses the dry weight differences between before and after treatments, expressed as a percentage of increased dry weight.

Chapter 3

Screening of antioxidant and antifungal properties of cashew nut shell liquid (CNSL)

3.1. Introduction

Natural crude cashew nut shell liquid (CNSL) is a mixture of different phenolic compounds i.e. anacardic acid, cardanol, cardol and 2-methyl cardol (Tyman and Kiong, 1977). The phenolic components of CNSL have an unsaturated long side chain of 15 carbon atoms which offers reaction sites on the aromatic ring and also on the side chain. Resins can be thus be synthesized from CNSL and its isolated constituents (cardanol).

CNSL is produced as a by-product of the cashew nut industry. According to the extraction method utilized, CNSL can be classified into two types, solvent extracted and natural or immature (*i*CNSL) and technical (*t*CNSL) CNSL. The *i*CNSL is obtained by extraction in a solvent and the *t*CNSL is obtained by a heating process, and is the most widely available CNSL. The chemical constituents of these two types of CNSL are variable (see Table 2.4 in chapter 2) due to the decarboxylation of anacardic acid to cardanol by the thermal treatment (Tyman *et al*., 1989; Philip *et al*., 2008; Gandhi *et al*., 2012).

Among the CNSL constituents, anacardic acid possesses antifungal (Himejima and Kubo, 1991; Prithiviraj *et al*., 1997) and antioxidant (Kubo *et al*., 2006; Trevisan *et al*., 2006) properties. Cardol, the other major component, is reported to have other general properties including antimicrobial and antitumor, larvicidal and molluscicidal (Lomonaco *et al*., 2009; Pimentel *et al*., 2009; Tocco *et al*., 2009). Some studies also cite the antioxidant, antifungal, antibacterial, larvicidal and molluscicidal properties of CNSL regardless of its isolated constituents (Casadei *et al*., 1984; Evans and Raj, 1988; Souza *et al*., 1992; Kubo *et al*., 1993, Weerasena *et al*., 1993; Kubo *et al*., 2006; Khanna *et al*., , 2009; Andrade *et al*., 2011; Oliveira *et al*., 2011). In addition, in some cases the CNSL was also found effective against fungal wood decay (Venmalar and Nagaveni, 2005; Adetogun *et al*., 2009; Adetogun, 2011) and termite attack (Mwalongo *et al*., 1999; Asogwa *et al*., 2007) as a natural preservative.

The major antifungal studies done earlier (Himejima and Kubo, 1991; Prithiviraj *et al*., 1997) indicated toxicity against human and plant pathogenic fungi but specific information regarding wood decay protection are difficult to interpret because the reports only state the solution

strengths used in specific wood species and no retention data is stated. Furthermore the type of CNSL is not stated and it is possible that the antifungal and antioxidant properties of the CNSL may be variable as because the proportion of the chemical constituents are different between the *t*CNSL and *i*CNSL.

It has been stated in the literature review that the natural durability of heartwood may be a result for not only fungitoxicity (e.g. thujaplicin) but of antioxidant activity (e.g. taxifolin) (Scheffer and Cowling, 1966) this chapter sets out to explore these activities of both types of CNSL. Thus, this chapter reports an investigation of antifungal and antioxidant properties of different types of CNSL (*t*CNSL and *i*CNSL). The fungi-toxicity of CNSL was tested by agar plate assay against brown rot (*Coniophora puteana* and *Postia placenta*) and white rot (*Trametes versicolor* and *Pleurotus ostreatus*) fungi. The decay resistance of CNSL treated Scots pine (*Pinus sylvestris*) wood was also examined by EN113 protocol. Investigation of the antioxidant properties of different types CNSL was also done followed by the DPPH free radical scavenging assay.

3.2. Materials and methods

3.2.1. Chemicals

Cashew nut shell liquid (CNSL) was used in two different types, technical (*t*CNSL) and solvent extract or natural (*i*CNSL) as a condensed liquid. All other chemicals used for the experiment were of analytical purity grade and purchased from Sigma-Aldrich, UK. Fungal growth media were supplied by Oxoid, UK and deionised water was used for all experiments. The *t*CNSL was supplied by The Biocomposites Centre, UK as "cardanol rich CNSL, Grade 1, batch 423" from Rishabh Resins and Chemicals, Hyderabad, India in a 200 kg volume and was supplied through Canopus Trade links Ltd., Chennai. It was shipped by Bibby distribution services to Allport Ltd, Tilbury. The *i*CNSL was supplied from Biocomposites stock by Dr. Viacheslav Tverezovskiy.

3.2.2. Fungi

Wood decay fungi were obtained from the Mycology lab of School of Environment, Natural Resources and Geography of Bangor University, UK. The fungal strains used in this experiment include two brown rot fungi*, Coniophora puteana* (Schum.) Karst., strain FPRL 11E and *Postia placenta* (M.J. Larsen & Lombard, current name =*Rhodonia placenta*), strain FPRL 280; and two white rot fungi, *Trametes versicolor*, (L.) Lloyd., strain CTB 863A and *Pleurotus ostreatus*

(Jacq.) P. Kumm., strain FPRL 40C. The cultures of all fungi were maintained on 2% malt extract agar (2% malt extract, 2% mycological agar) at 22°C and sub-culturing was done at 2 week intervals.

3.2.3. Antioxidant assay

The antioxidant assay of the both CNSLs was determined by the way of free radical scavenging activity using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) discoloration method as described by Scherer and Godoy (2009). Gallic acid and Quercetin were used as standards and/or control for the CNSL antioxidant assay. Solutions of different concentrations were prepared by dissolving the test components i.e. CNSLs and standards in methanol (100%). Aliquots of about 0.1 ml of methanolic solutions of the tested CNSLs and standards were each added to 3.9 ml of the DPPH methanolic solution. The DPPH solution of 0.124 mM was prepared by dissolving 24 mg in 500 ml of methanol. A blank control of 0.1 ml pure methanol added to 3.9 ml of DPPH solution was used. After 30 min of incubation at ambient temperature, the absorbance was measured at 517 nm using a Bio-Tek micro-plate spectrophotometer (model PowerWave XS). The tests were carried out in triplicate. The radical scavenging activity was calculated by the following formula:

Percentage Inhibition, I (%) = $[(A_0-A_1)/A_0] \times 100$ …….. (3.1)

Where, A_0 was the absorbance of the control or blank and A_1 was the absorbance of the tested compound at different concentrations.

The IC_{50} (Inhibitory concentration of the tested compounds, or standards, necessary to reduce the absorbance of DPPH by 50%) was calculated graphically using a calibration curve by plotting the extract concentration versus the corresponding radical scavenging activity (Chen *et al*., 2013). The antioxidant activity was expressed as the antioxidant activity index (AAI) (Scherer and Godoy, 2009). The AAI was calculated considering the mass of DPPH and mass of the tested compound in the reaction which results a constant for each compound. This is independent of the concentration of the DPPH and sample used. The AAI was calculated by the following formula.

Antioxidant Activity Index, AAI = Final concentration of DPPH (µg/ml)/IC_{50} (µg/ml) …. (3.2)

3.2.4 Agar plate assay

The agar plate test was conducted using a mycelial diameter growth inhibition technique to evaluate antifungal activity of different grades of CNSL against the brown- and white rot wood decay fungi. For the growth study fungi were grown on 2% malt extract agar (MEA) medium containing *t*CNSL (technical), *i*CNSL (solvent extract or natural) and a mixture of both grades as *it*CNSL (50: 50). These three types of CNSL were initially dissolved in Industrial Methylated Spirit (IMS) (100%) to make a geometric series of alcoholic solutions. The alcoholic solution of CNSL compounds were diluted with autoclaved growth media, MEA to make a final concentration of 0, 2.5, 5, 10, 20, 40 and 80 µl/ml (CNSL volume to growth medium volume). The medium was poured into Petri dishes (90 mm diameter) providing a minimum depth of 3-4 mm and left overnight at room temperature for cooling and evaporation of the solvent. Petri-dishes containing MEA and IMS without CNSL were used for controls. Each fungus was then centrally inoculated in triplicate into Petri dishes with single 5 mm^2 plugs taken from the margins of actively growing cultures. Inoculated plates were incubated in the 22 °C and 65 ± 5 % relative humidity. The diameters (mm) of the fungal mycelia were measured every day at right angles across the colonies and were averaged. When fungal mycelia reached the edges of the control dishes, the antifungal indices were calculated. The antifungal index was calculated by the following formula:

Antifungal Index, AI (%) = (1-Da/Db) x 100 …… (3.3)

Where, Da is the diameter growth of fungal mycelia in the experimental dishes (in mm) and Db is the diameter growth of fungal mycelia in the control dishes (in mm).

3.2.5. Agar block test

Agar block test was carried out in accordance to EN113 protocol (1996) with some modification in wood block size and the duration of fungal. As this is wood modification study so the oven dry weight (treated) were known before the exposure to the decay fungi. Thus direct calculation of mass loss was possible in this study which also differs from the EN113 protocol. Decay resistance imparted by both CNSL (*i*CNSL and *t*CNSL) was determined by exposing the CNSL treated Scots pine (*Pinus sylvestris* L.) sapwood blocks against two brown rot (*C. puteana* and *P. placenta*) and two white rot (*T. versicolor* and *P. ostreatus*) fungi. Samples of clean, straight grained, defect free sapwood of Scots pine were prepared to dimensions of 20 x 20 x 5 mm (radial x tangential x longitudinal). This block size was chosen to

ensure even penetration of the CNSL solution into the blocks. Prepared wood samples were kept in a climate chamber at 20 °C temperature and 65% relative humidity.

Wood blocks were dried in an oven at 105 °C for 24 hours. Following removal from the oven, the wood blocks were allowed to equilibrate to ambient temperature by placing them in a desiccator over silica gel. After cooling, the blocks were weighed in grams to 4 DP to an accuracy of 0.1 mg. The test specimens were vacuum impregnated with alcoholic solution of CNSL of different solute content in industrial methylated spirit (IMS) to achieve different level of weight percent gain (WPG). The wood samples treated with IMS were used as control. The impregnation was carried out in a vacuum desiccator at a -0.7 bar vacuum for 30 minutes. Air was admitted into the desiccators and the samples left in the impregnating solution for 2 hours at ambient temperature to allow for uptake of CNSL solution. Following the impregnation, the excess solution was blotted off and the samples were then left in a fume cupboard for an hour to allow the IMS to evaporate. The samples were then oven dried at 105 °C for 24 hours and then transferred to desiccators and placed over silica gel to cool. The samples were then reweighed as detailed above. Weight percent gain (WPG) of CNSL impregnated wood samples was determined by using the following formula:

$$WPG\ (\%) = [(W_1 - W_0)/W_0] \times 100 \quad \ldots\ldots (3.4)$$

Where, W_1 is the oven-dry weight of the CNSL impregnated wood and W_0 is the corresponding oven-dry weight of un-impregnated wood.

Prior to exposure, the samples were reconditioned in climate chamber at 20 °C temp and 65% RH for 2 weeks until they reached equilibrium moisture content. The treated samples and their control counterparts were individually bagged and then sterilised by gamma irradiation (2.5 Mrad) at Synergy Health, Reading (formerly Isotron Ltd.). The fungi were grown on 4% malt agar in 500 ml squat jars, 60 ml per jar. Jars were autoclaved at 121 °C for 20 minutes for sterilization, prior to inoculation. After cooling the jars, pellets of fungus were aseptically transferred from the petri dish cultures. The jars were then transferred to the climate chamber and left to grow for 2 weeks. Once the fungi had grown to cover the agar, an autoclave sterilised geotextile polypropylene mesh was placed over the fungus surface and the blocks were placed on top of this. When resealed the assembled jars were incubated for a further 10 weeks. For each fungus, 6 replicates (n=6) of each treatment were exposed. Additional sets of sterile controls were also placed in exposure jars, but without fungal inoculation to assess the operational control losses.

After the exposure period the samples were removed from the jars and blocks were wiped to remove fungal mat. The blocks were then weighed to 4 DP before drying in oven at 105 °C for 24 hours. The samples were then cooled and reweighed to find out the mass loss and post decay moisture content. The decay resistance of CNSL treated wood against different decay fungi was determined by the mass loss when compared to the control wood blocks. The effect of moisture on decay activities of the CNSL treated wood blocks was determined by evaluating the post decay moisture content of the wood blocks. The percentage of mass loss (ML) due to decay by fungus was determined by using the following formula:

$$ML\ (\%) = [(M_1 - M_2)/M_1] \times 100 \quad \ldots\ldots (3.5)$$

Where, M_1 is the oven-dry mass of wood sample prior to decay exposure and M_2 is the oven-dry mass of the corresponding sample after exposure.

The percent of moisture content (MC) of the wood samples after exposure to decay fungi was calculated by using the following formula:

$$MC\ (\%) = [(W_2 - W_3)/W_3] \times 100 \quad \ldots\ldots (3.6)$$

Where, W_2 is the wet weight of the wood samples after exposure to decay fungi and W_3 is the oven-dry weight of the corresponding samples.

3.2.6. Statistical analysis

The data obtained were analysed by the multifactor analysis of variance (ANOVA) test using IBM® SPSS® Statistics 20 to determine the statistical difference followed by Tukey's multiple comparison. The results with $P<0.05$ were considered to be significantly different.

3.3. Results

3.3.1. Antioxidant properties

The free radical scavenging effect of both *t*CNSL and *i*CNSL were assessed by DPPH assay using gallic acid and quercetin as standard antioxidants. In all cases antioxidant activity was shown which increased curvilinearly with increasing concentration (Figure 3.1). The most effective antioxidants were gallic acid (93.1% at 80 µg/ml) followed by quercetin (72.3% at 80 µg/ml), although, both *t*CNSL and *i*CNSL showed activity of 17.3% and 18.5% respectively but statistically this was not significantly different ($P>0.05$). Furthermore, no significant differences ($P>0.05$) were found when this was examined for interactions between concentrations and compounds of CNSL.

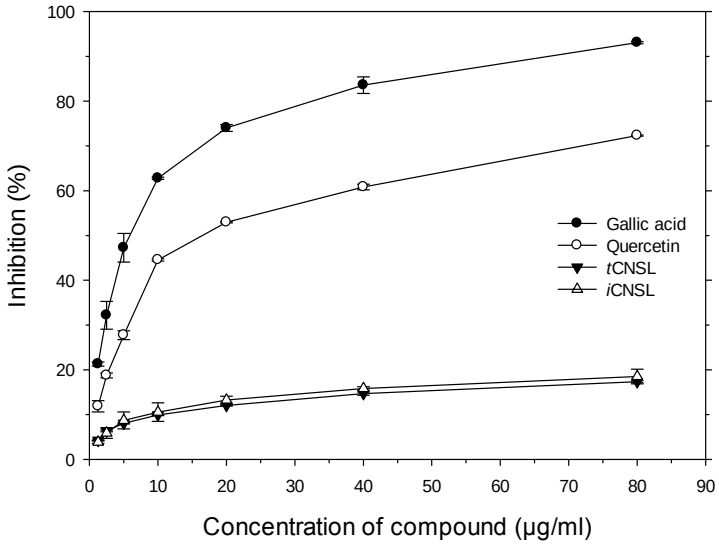


Figure 3.1: Inhibition percentage of DPPH (2, 2-diphenyl-1-picrylhydrazyl) by *t*CNSL, *i*CNSL, gallic acid, and quercetin (n=3, error bar=±SD).

Table 3.1: Antioxidant properties gallic acid and quercetin from the DPPH free radical scavenging assay.

Compounds	**IC_{50} (µg/ml)**	**AAI[a]**	**R^2 value[b]**
Gallic acid	5.39	8.90	0.9917
Quercetin	9.63	5.08	0.9902

[a]AAI value was calculated by the formula 3.2, [b] R^2 value is the regression coefficient.

The concentrations providing 50% inhibition (IC_{50}) in the DPPH assay were calculated for gallic acid and quercetin (Table 3.1) which again showed gallic acid was strongest antioxidant. This was also true for the antioxidant activity index (AAI). All the compounds showed good regression coefficient value providing the IC_{50} value calculation confidence (Table 3.1). It was not appropriate to calculate IC_{50} for *t*CNSL and *i*CNSL as their radical scavenging activity was too low (Figure 3.1).

The DPPH inhibition index (I %) showed the capacity of the compound to reduce the DPPH radicals (or not) in a fixed concentration level. In this case, increasing compound concentration increased the inhibition percentage. The IC_{50} shows the compound concentration necessary to reduce or decrease the initial DPPH concentration by 50%. However, there is a deficiency in comparing the antioxidant potential of the compounds due to researchers using different DPPH concentrations as the result will be different. Thus, the AAI relates the DPPH concentration used in the assay with IC_{50} of the compound, resulting in a constant data for each compound (Scherer and Godoy, 2009).

3.3.2 Agar plate test

The growth rates were assessed by measuring the diameter of the fungal colonies (mm) and expressing the result as mm per day. The results of growth rate of the brown rot fungi (*C. puteana* and *P. placenta*) and white rot fungi (*T. versicolor* and *P. ostreatus*) with the different types of CNSLs are presented in Tables 3.2 and 3.3. The fungal growth rates decreased with increasing concentration of all three types of the CNSLs with all of the fungi. Statistically significant differences ($P<0.001$) have been found between the CNSL types and the concentration levels on the growth rate for both brown rot and white rot fungi. Among the CNSLs, *i*CNSL provided significantly higher effect on the growth reduction of brown rots (*C. puteana* and *P. placenta*) and white rots (*T. versicolor* and *P. ostreatus*) than *t*CNSL and *it*CNSL.

For better understanding of the efficacy of the CNSLs, antifungal activity was examined by determining the antifungal index (AI) according to the formula 3.3. The results of the antifungal indices of the *t*CNSL, *i*CNSL and *it*CNSL against the brown rot (*C. puteana* and *P. placenta*) and white rot fungi (*T. versicolor* and *P. ostreatus*) are presented in the Figure 3.2. It is evident that the *i*CNSL had a greater effect on the reduction in fungal growth than *t*CNSL or *it*CNSL and this effect increased with increasing concentration, but declined in magnitude. This was true for both the brown- and white rot fungi. Pictorial view of growth inhibition of brown and white rot fungi is presented in Figure 3.3 and 3.4

Table 3.2: Mean fungal growth rate of brown rot fungi on CNSL added malt agar media.

Concentration of compound (µl/ml)	**Growth rate (mm/day) of brown rot fungi**					
	C. puteana			***P. placenta***		
	***t*CNSL**	***i*CNSL**	***it*CNSL**	***t*CNSL**	***i*CNSL**	***it*CNSL**
Control	10.8 (±0.0)^{a}	10.8 (±0.0)^{a}	10.8 (±0.0)^{a}	10.8 (±0.0)^{a}	10.8 (±0.0)^{a}	10.8 (±0.0)^{a}
2.5	9.2 (±0.2)^{b}	8.9 (±0.2)^{b}	9.4 (±0.1)^{bc}	9.8 (±0.2)^{b}	9.7 (±0.1)^{b}	9.7 (±0.1)^{b}
5	9.0 (±0.3)^{bc}	8.7 (±0.1)^{bc}	9.0 (±0.3)^{bc}	9.6 (±0.1)^{bc}	9.3 (±0.4)^{bc}	9.5 (±0.1)^{b}
10	8.8 (±0.1)^{bc}	8.3 (±0.2)^{cd}	8.8 (±0.1)^{cd}	9.5 (±0.3)^{bc}	9.0 (±0.4)^{cd}	9.3 (±0.1)^{bc}
20	8.5 (±0.3)^{cd}	8.0 (±0.2)^{d}	8.4 (±0.3)^{de}	9.2 (±0.2)^{cd}	8.6 (±0.1)^{de}	8.9 (±0.3)^{cd}
40	8.3 (±0.2)^{d}	7.8 (±0.3)^{de}	8.3 (±0.2)^{e}	8.8 (±0.1)^{de}	8.4 (±0.2)^{de}	8.7 (±0.2)^{de}
80	8.0 (±0.1)^{d}	7.3 (±0.2)^{e}	7.7 (±0.2)^{f}	8.4 (±0.3)^{e}	8.0 (±0.4)^{e}	8.3 (±0.3)^{e}

Values in parenthesis refer to the SD and n=3. The growth rate among the CNSLs are significantly different ($P<0.001$). Number followed by different letters are significantly different at the level of $P<0.05$ according to Tukey's test.

Table 3.3: Mean fungal growth rate of white rot fungi on CNSL added malt agar media.

Concentration of compound (µl/ml)	Growth rate (mm/day) of white rot fungi					
	T. versicolor			*P. ostreatus*		
	***t*CNSL**	***i*CNSL**	***it*CNSL**	***t*CNSL**	***i*CNSL**	***it*CNSL**
Control	10.8 (±0.0)^{a}	10.7 (±0.0)^{a}	10.8 (±0.0)^{a}	9.6 (±0.0)^{a}	9.6 (±0.0)^{a}	9.6 (±0.0)^{a}
2.5	10.3 (±0.1)^{b}	9.7 (±0.2)^{b}	10.0 (±0.1)^{b}	9.1 (±0.1)^{b}	9.0 (±0.1)^{b}	9.0 (±0.2)^{a}
5	10.0 (±0.1)^{c}	9.2 (±0.2)^{bc}	9.7 (±0.2)^{bc}	8.6 (±0.3)^{bc}	8.4 (±0.2)^{c}	8.4 (±0.3)^{b}
10	9.8 (±0.1)^{cd}	8.9 (±0.1)^{bc}	9.4 (±0.1)^{c}	8.2 (±0.2)^{cd}	7.8 (±0.2)^{d}	8.1 (±0.2)^{bc}
20	9.6 (±0.1)^{d}	8.6 (±0.5)^{cd}	8.9 (±0.1)^{d}	7.8 (±0.3)^{de}	7.3 (±0.1)^{e}	7.7 (±0.2)^{cd}
40	9.1 (±0.1)^{e}	8.5 (±0.2)^{cd}	8.7 (±0.1)^{d}	7.5 (±0.2)^{e}	7.0 (±0.3)^{e}	7.1 (±0.2)^{d}
80	8.8 (±0.3)^{e}	8.1 (±0.6)^{d}	8.1 (±0.1)^{e}	6.8 (±0.2)^{f}	6.3 (±0.1)^{f}	6.7 (±0.1)^{e}

Values in parenthesis refer to SD and n=3. The growth rate among the CNSLs are significantly different (P<0.001). Number followed by different letters are significantly different at the level of P<0.05 according to Tukey's test.

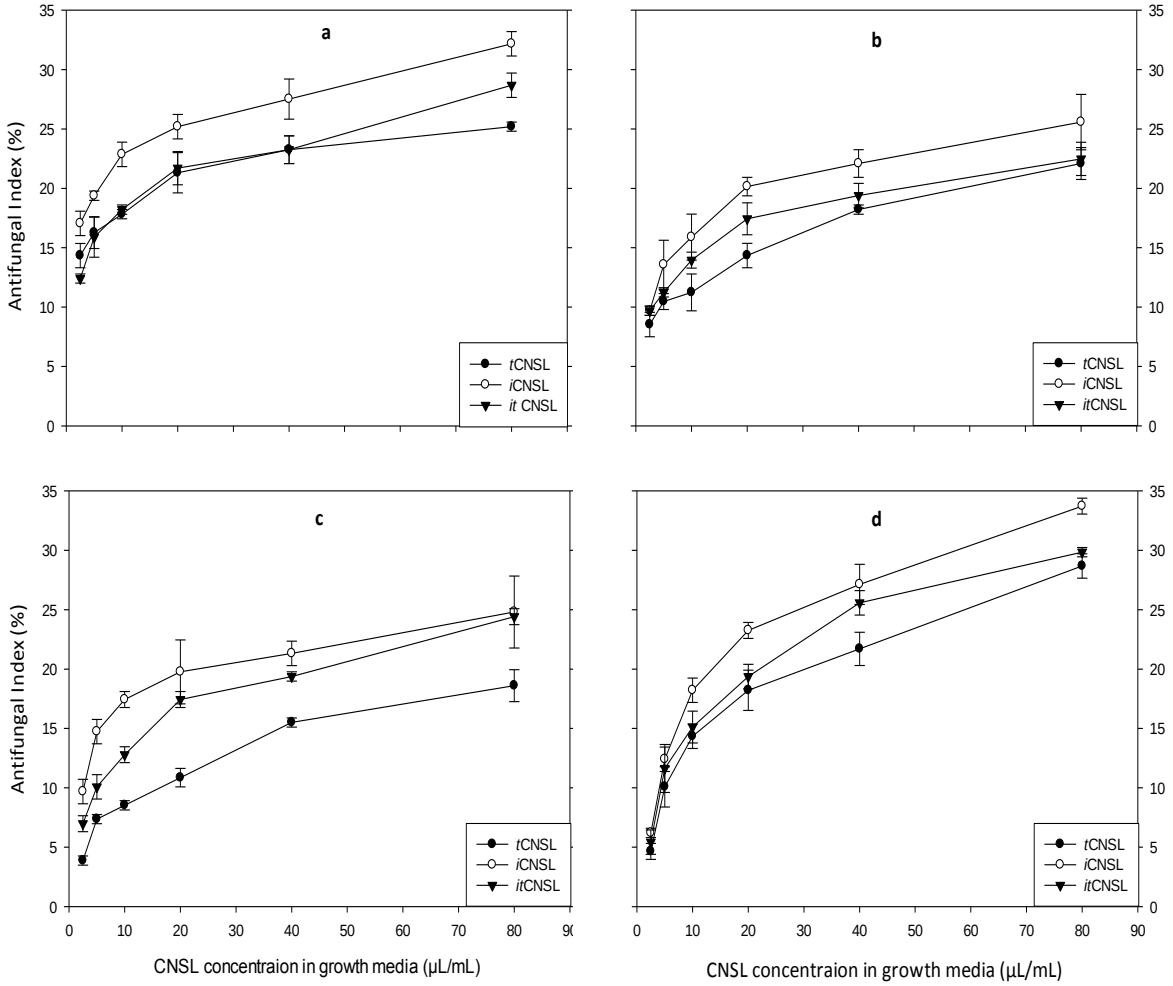


Figure 3.2: Antifungal activity of three CNSL types, *t*CNSL, *i*CNSL and a mixture of both (50: 50) *it*CNSL against brown rot fungi (a) *C. puteana*, (b) *P. placenta* and white rot fungi (c) *T. versicolor,* (d) *P. ostreatus* (n=3, error bar=±SD).

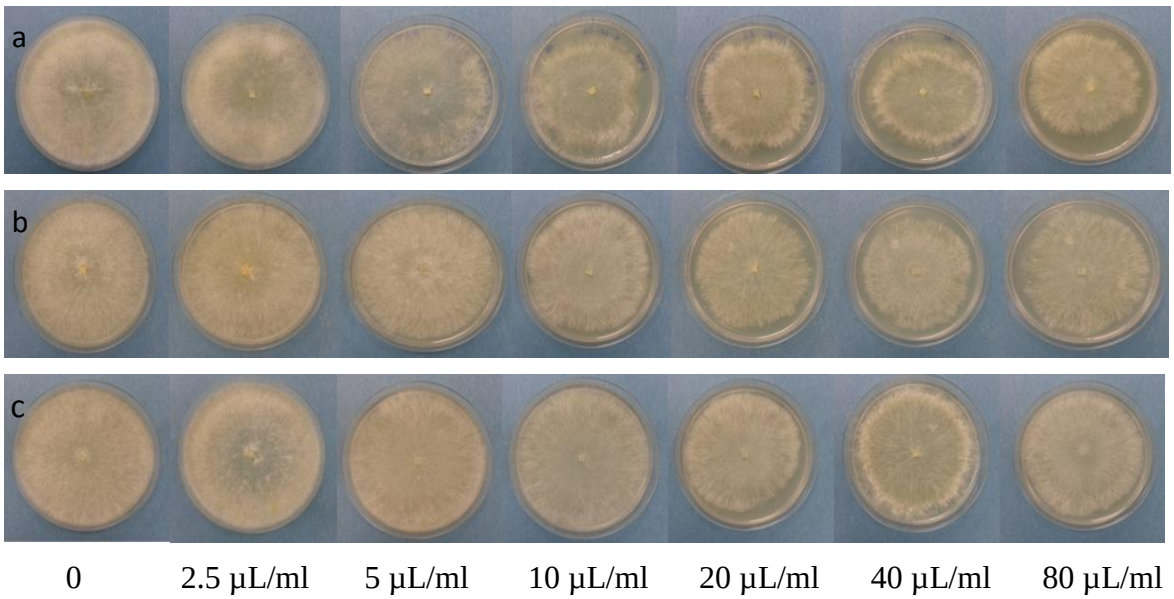


Figure 3.3: Inhibitory concentrations, from 0 (control) to 80 µL/mL (progressing left to right) (a) *t*CNSL, (b) *i*CNSL and (c) *it*CNSL against the brown rot fungi *Coniophora puteana* growing on 2% MEA media.

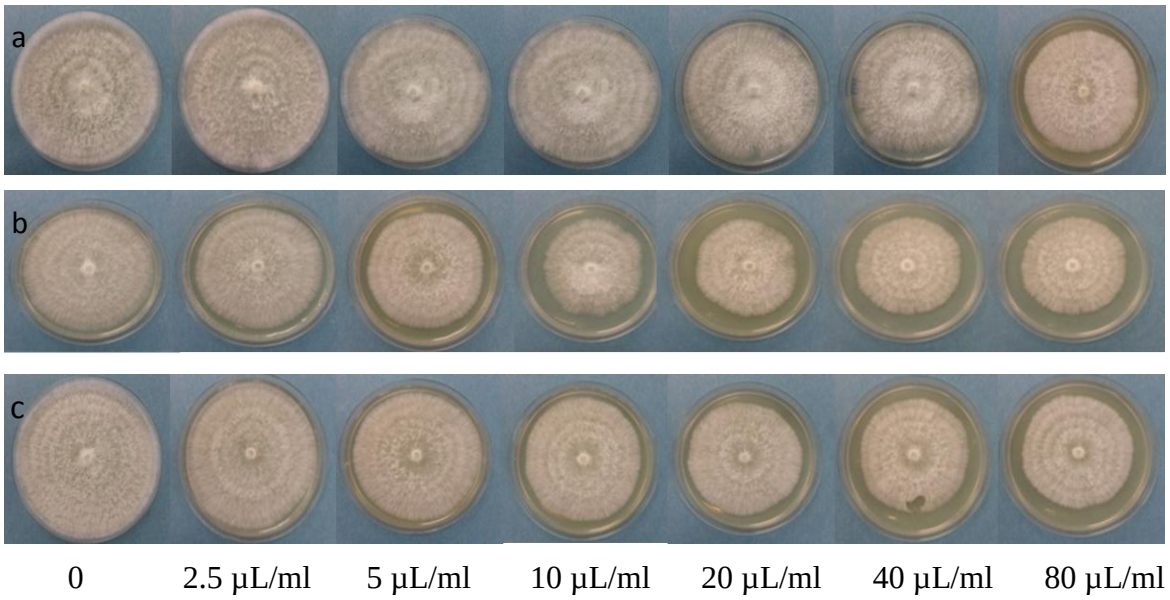


Figure 3.4: Inhibitory concentrations, from 0 (control) to 80 µL/mL (progressing left to right) (a) *t*CNSL, (b) *i*CNSL and (c) *it*CNSL against the white rot fungi *Pleurotus ostreatus* growing on 2% MEA media.

Growth inhibition was statistically significantly different ($P<0.001$) between the CNSL types and the concentration levels for both brown rot and white rot fungi, but the interaction of concentrations and CNSL types was insignificant ($P>0.05$) for the same, which means that the effect of concentrations on the fungal growth inhibition has no significant interaction between the CNSL types. The relative effect of the different CNSLs was greatest on *P. ostreatus* followed by *C. puteana*. The least effect was on *T. versicolor* and *P. placenta*.

3.3.3. Agar block test

3.3.3.1. Treatment retentions: WPG

The results obtained by agar plate assay (agar and fungitoxic system) are not necessarily indicative of the performance of a compound or formulation when tested on wood, so that more realistic results have been obtained by impregnating wood and agar block test was performed. The results of the two CNSL treatments of Scots pine are presented in Table 3.4 as mean weight percent gain (WPG). Treatment of both of the CNSLs with 5, 10, 20 and 40 concentrations resulted in linear increase in WPG in the Scots pine wood blocks. Higher WPGs were achieved in treatment with *i*CNSL than *t*CNSL. Control wood blocks treated with 0% CNSL concentration (treated in IMS) showed no WPG (when expressed to 2 decimal points).

Table 3.4: Weight percent gain (WPG) of oven dried Scots pine (*Pinus sylvestris*) wood after treated with different types of CNSL (n=30).

CNSL Concentration (%)	Mean weight percent gain (WPG)	
	***i*CNSL**	***t*CNSL**
Control (IMS)	0.0 (±0.0)	0.0 (±0.0)
5	4.4 (±0.6)	10.0 (±0.6)
10	12.9 (±1.3)	18.6 (±1.0)
20	24.6 (±1.5)	24.2 (±1.9)
40	49.1 (±2.7)	42.4 (±3.0)

Values in parenthesis represent the standard deviation of weight percent gain

3.3.3.2. Weight loss in decay test

Data points represent the mean mass loss (ML) against the respective mean weight percent gain (WPG) of the CNSLs treatment (Figure 3.5). The decay tests gave high weight losses (>20%) in the control blocks (Figure 3.5) for three of the fungi but *P. ostreatus* only achieved 6.6%. The treatment with either of the CNSL types reduced the mass loss with the increase of WPG of Scots pine wood when exposed to the brown rot fungi (*C. puteana* and *P. placenta*) and the white rot fungi (*T. versicolor* and *P. ostreatus*). The decay reduction curves for the white rot fungi did not show the same linearity. With *T. versicolor* a 2% weight loss, close to zero value, was achieved with both CNSL types at the highest WPG values. Because the weight losses for the untreated controls of *P. ostreatus* were too low conclusions regarding thresholds are unreliable but very low weight losses were achieved at the highest WPG values. Statistically significantly difference ($P<0.001$) has been found between the CNSL types and the WPG level on the mass losses for all the fungi tested.

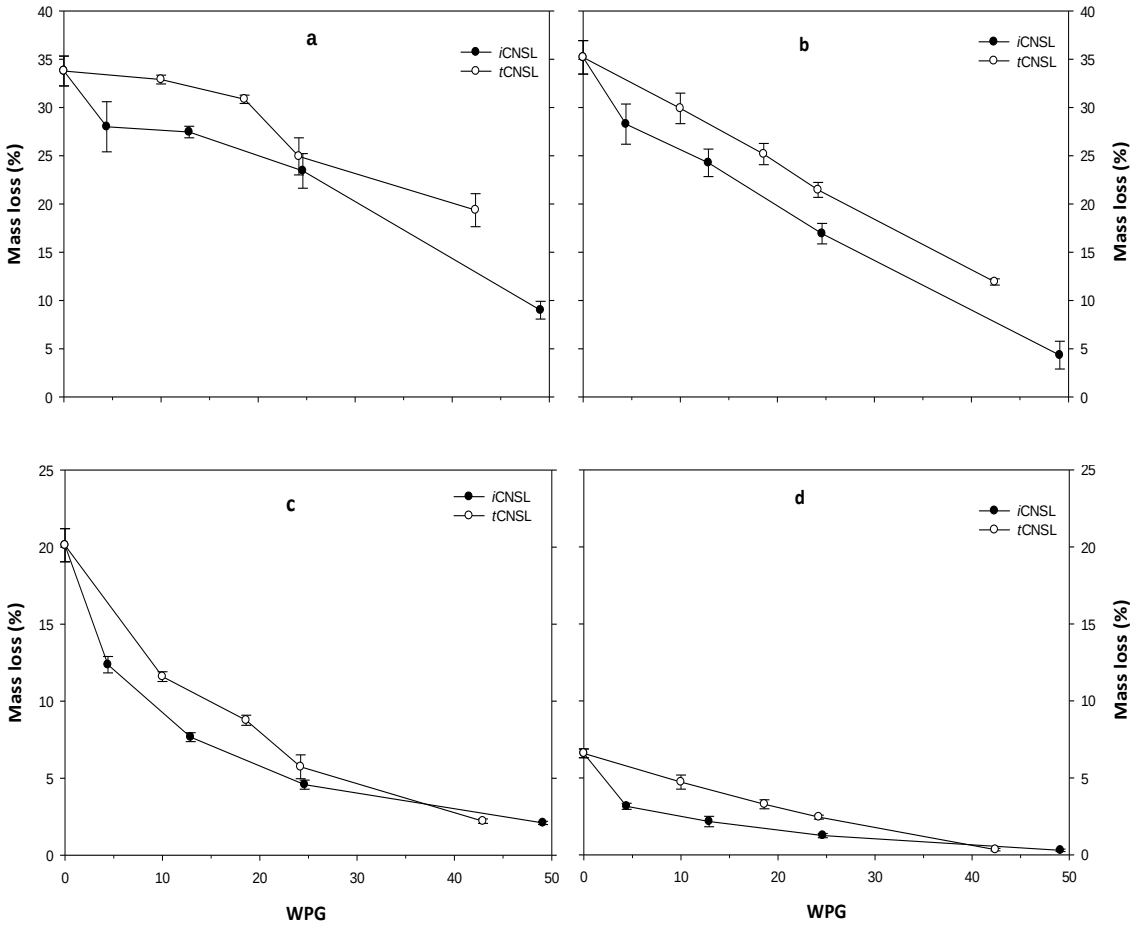


Figure 3.5: Mass loss (ML) of Scots pine (*Pinus sylvestris*) wood blocks treated with *t*CNSL and *i*CNSL after 10 weeks exposure to brown rot fungi (a) *C. puteana* , (b) *P. placenta* and white rot fungi (c) *T. versicolor*, (d) *P. ostreatus*. Control (0% WPG) values are IMS treated wood blocks (n=6, error bars=±SD).

3.3.3.3. Moisture content in decay test

The post decay moisture content (MC) was determined at the end of decay test to find out the effect of moisture content on the mass loss of treated wood blocks. The sterile control sets of CNSLs (*t*CNSL and *i*CNSL) treated wood blocks run without fungal inoculation showed that the moisture content at the end of the exposure of 10 weeks decreased with the increase of WPG (Figure 3.6). The untreated blocks showed adequate moisture content for decay test. The *t*CNSL at the higher WPGs reduced the moisture content more than *i*CNSL.

The mean moisture contents of the fungus exposed wood blocks (Figure 3.7, a-d) showed similar behaviour but the moisture contents were higher, particularly for the IMS treated control blocks, with the difference declining with increasing WPG. The mean final moisture contents of the control wood treated with IMS (0 WPG) obtained 67.7%, 92.6%, 58.5% and 51.0% for *C. puteana*, *P. placenta*, *T. versicolor* and *P. ostreatus* respectively.

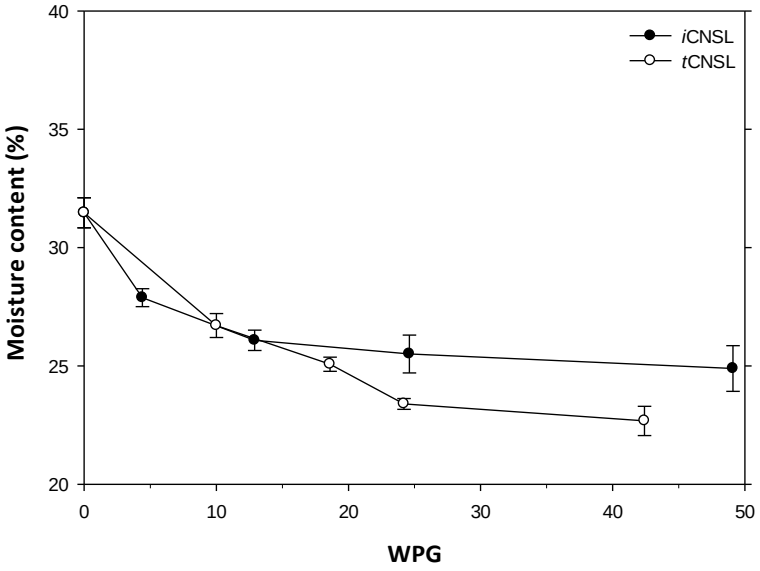


Figure 3.6: Reduction of the moisture content of the sterile control *t*CNSL and *i*CNSL treated Scots pine (*Pinus sylvestris*) wood blocks (n=6, error bars=±SD).

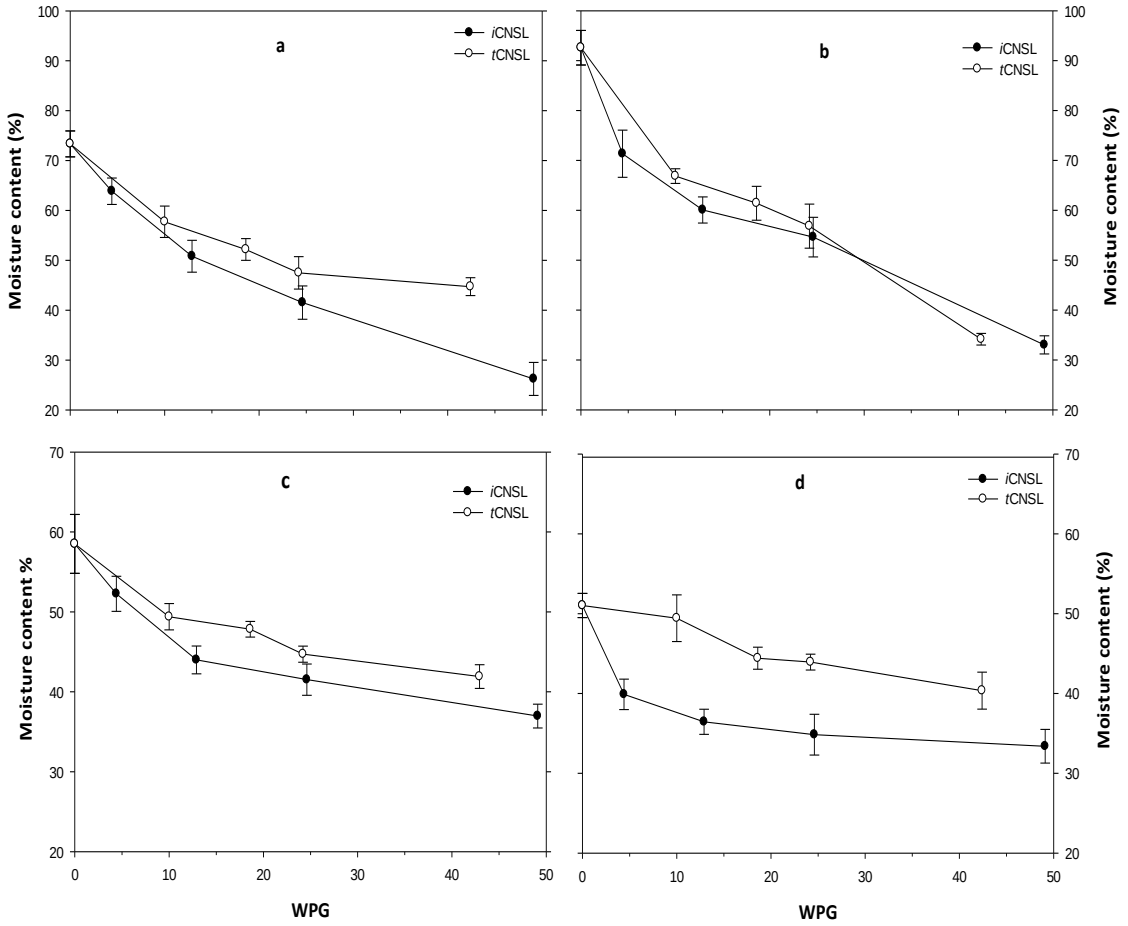


Figure 3.7: Reduction of the moisture content of Scots pine (*Pinus sylvestris*) wood treated with *t*CNSL and *i*CNSL at the end of the decay test exposure to brown rot fungi (a) *C. puteana*, (b) *P. placenta* and white rot fungi (c) *T. versicolor*, (d) *P. ostreatus*. Control (0 WPG) values are IMS treated wood blocks (n=6, error bars=±SD).

With *C. puteana*, *i*CNSL had a greater effect on the final moisture content than *t*CNSL, different to the sterile controls, but this wasn't seen with *P. placenta.* Statistically significant difference ($P<0.001$) on MC has been found between the CNSL types and WPG levels for both the brown rot fungi. The interaction between CNSL types and WPG level had also significant effect ($P<0.05$) on the final moisture content of the treated wood blocks at the end of the decay test. With the white rot fungi *T. versicolor*, statistically significant difference ($P<0.001$) was only found between the WPG levels but not between CNSL types.

3.4. Discussion

3.4.1. Free radical scavenging activities of CNSL

The *t*CNSL and *i*CNSL showed antioxidant properties, though less effective than the standards gallic acid and quercetin. The antioxidant properties of CNSLs may be due to its phenolic constituents. Generally, the antioxidant properties of phenols are due to their oxy-reduction properties which allow them to act as reducing agents, hydrogen donors, single and triple oxygen eliminators or decomposing peroxides (Osawa 1994; Guerra, 2001). Evans *et al*. (1996) also reported that phenolic hydroxyls may have redox properties and act as antioxidants. Some studies have shown that unsaturation of the long side chain of 15 carbon atoms of CNSL are associated with the antioxidant activity (Kubo *et al*., 2006; Rodrigues *et al*., 2006). The alkyl and peroxy radicals may be trapped by allyl substituents and thus may provide an antioxidant function. Some studies have reported that anacardic acid, the major constituent of *i*CNSL has a higher antioxidant capacity as compared to cardols and cardanol (Kubo *et al*., 2006; Trevisan *et al*., 2006; Stasiuk and Kozubek, 2010). In the present study, there was no significant difference between the two types of CNSL. However, Oliveira *et al*. (2011) reported that free radical scavenging activity of cardanol was higher than the cardol and anacardic acid, when they evaluated the antioxidant properties of CNSL obtained by solvent extraction (*i*CNSL). Again, Andrade *et al*. (2011) found antioxidant properties with *t*CNSL but this was much less intense than their standard compounds rutin and ascorbic acid.

In most cases, where *i*CNSL and anacardic acid showed higher antioxidant properties they were evaluated by the enzyme xanthine oxidase assay (Kubo *et al*., 2006; Trevisan *et al*., 2006; Stasiuk and Kozubek, 2010) rather than the DPPH assay. It is possible that antioxidant mechanisms for the inhibition of DPPH free radicals may differ from the enzymatic methods where inhibition was measured on xanthine oxidase rather than scavenging of hydroxyl

radicals. Kubo *et al*. (2006) reported that anacardic acid prevents the formation of superoxide radicals but does not trap reactive oxygen, and other previous studies revealed that the phenolic lipids present in CNSL are related to the antioxidant properties. This is also revealed from the present study as similar results were found for the antioxidant properties of anacardic rich solvent extract *i*CNSL and cardanol rich technical *t*CNSL.

3.4.2 Antifungal activities of CNSL

The results of the agar plate assay, as evidenced by the antifungal index (AI), showed that both types of CNSL reduced the rate of hyphal extension of both the brown and white rot fungi and are thus assumed to have a fungitoxic effect. The most effective was *i*CNSL, which consistently showed significantly greater inhibition than the other CNSLs.

The mixed *it*CNSL showed better activity than the *t*CNSL. Thus it is obvious that the *i*CNSL is the most effective as an antifungal compound against the wood decay fungi, and this was expected because the *i*CNSL has a higher proportion of anacardic acid than the *t*CNSL. The antifungal properties of solvent extract CNSL, i.e. *i*CNSL, was reported by Khanna *et al*. (2009). The study concluded that a distinct range of antifungal properties was found for *i*CNSL on the growth various moulds (*Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Curvularia* sp. and *Fusarium* sp.). The fungitoxic effect was due to triterpenoids, phenolics and volatile oil constituents isolated from the ethanolic extraction of CNSL rather than from cardanol and anacardic acid. The antifungal activity of anacardic acid against some fungi was also reported by Himejima and Kubo (1991), Prithiviraj *et al*. (1997). Prithiviraj *et al*. (1997) reported that anacardic acid was highly inhibitory to spore germination in *Colletotrichum capsici,* followed by some other plant pathogenic fungi including *Fusarium udam, Fusarium oxysporum, Alternaria alternata, Alternaria brassicae* and *Curvularia lunata*.

The present study also showed that *t*CNSL had a growth reduction effect. So, apart from the anacardic acid the two other major constituents of CNSL, cardanol and cardol have an effect. Literature reports that cardol is in equal proportions in both *i*CNSL and *t*CNSL (Kumar *et al*., 2002 and Das *et al*., 2004). Cardol also demonstrated good larvicidal activity against *Artemia salina*, followed by anacardic acid and cardanol, when all of these phenolics isolated from solvent extracted CNSL (Oliveria *et al*., 2011).

It is probable that the antifungal property of the CNSLs is due to bioactive components either individually or in combination. Phenolics are well-known antifungal compounds present in plants and the constituents of CNSL (anacardic acid, cardol and cardanol) are rich in phenolics. The antifungal index data did not show clear difference in the effect of fungitoxicity of CNSLs on brown rot and white rot fungi. The highest growth inhibition was against *P. ostreatus* while the lowest inhibition was with *T. versicolor*. Growth inhibition of both the brown rot fungi by all the CNSLs provided similar efficacy. The toxic effect of CNSLs on the brown and white rot fungi was variable with fungal species used.

3.4.3. Decay resistance of CNSL treated wood

It should be pointed out that the agar block test done in accordance to a modified EN113 protocol (smaller blocks, 10 weeks exposure time) and revealed the CNSLs had a significant effect on the decay resistance of Scots pine (*P. sylvestris*) sapwood, but that despite high WPGs complete protection (where high decay in the untreated controls was not achieved). The *i*CNSL was statistically significantly more effective than *t*CNSL in reducing the mass loss of wood against brown rot fungi (*C. puteana* and *P. placenta*) and it was more effective against *P. placenta* than *C. puteana*. With the white rot fungi both the CNSL types gave equal performance and there was no significant difference between the two types.

The decay resistance data obtained from the decay test against the *P. ostreatus* is not usable because it gave low mass losses in the solvent (IMS) treated controls (Figure 3.5d). Various reasons may account for this, including the use of a softwood substrate, where this fungus is naturally found on hardwoods, and it is often the case that hardwoods are more rapidly decayed than softwoods by white rot fungi (Zabel and Morrell 1992; Eaton and Hale, 1993). This fungus is used in testing composite board materials; typically those bonded with PF resins and were thus chosen for this work. In the board test standard (BS 1982, 1990) wetted vermiculite is added to test jars over agar to wet the blocks up, so this test may have remained too dry for the fungus; however nearly 60% moisture content was shown here (Figure 3.7) which should have been adequate for decay. The geo-textile support has been widely and successfully used in this laboratory over several years but not necessarily with this fungus.

The higher decay resistance properties of *i*CNSL treated wood may be due to the presence of anacardic acid in *i*CNSL is likely to have antifungal properties similar to those previously reported (Himejima and Kubo, 1991; Prithiviraj *et al*., 1997; Khanna *et al*., 2009).

From the mass loss data (Figure 3.5) it is evident that at some WPG, protection threshold could be established for each fungus, corresponding to the point where no mass loss occurred. Eaton and Hale (1993) reported that a mass loss of less than 3% is employed in the EN113 protocol. In the case of brown rot fungi, *i*CNSL treatment of 49.1% WPG was very close to the threshold for *P. placenta* (4.3% ML). But the treatment with *t*CNSL failed to meet the threshold level in exposure to both brown rot fungi. On the other hand, the threshold levels were found by the treatment with *t*CNSL and *i*CNSL against both the white rot fungi. The threshold for *t*CNSL and *i*CNSL treatment against *T. versicolor* was met at 42.4% and 49.1% respectively (2.2% and 2.1% ML). If there is any confidence in the data for *P. ostreatus* the decay protection threshold was lower at 18.6% and 12.9% WPG for *t*CNSL and *i*CNSL respectively.

Phenolic compounds present in plant materials are associated with decay resistance (Scheffer and Cowling 1966; Hart and Hillis 1974). The fungitoxic mechanism of phenolic compounds is due to the inactivation of fungal enzymes that contain –SH group on their active sites (Cowan, 1999). Juven *et al*. (1994) also reported that the phenolic compounds permeabilised the membrane and caused leakage of intracellular constituents. The effect of CNSLs against the wood decay fungi is thus probably due to the phenolic components, principally anacardic acid, cardanol and cardol. In this case the interaction with the fungi may be at the level of interference with the extracellular wood decay enzymes systems. However we have also provided evidence for some toxicity, i.e. reduction in growth rate, and also some interference with the free radical decay system of these fungi.

3.4.4. Effect of moisture content on the wood decay resistance

The moisture content of the decayed wood blocks was found to be lowered by increasing WPG, in line with the reduction in mass loss in the decay test, although it is difficult to separate cause and effect. The interaction of the water and wood is of major interest for the fungal decay. Generally, no wood can be colonized and decayed unless it moisture content is 20% or above or around fibre saturation point (FSP) (Eaton and Hale, 1993). Some studies also showed that wood decaying fungi of the Basidiomycota are particularly sensitive to low moisture potential of the wood substrate (Griffin, 1977; Boddy, 1983).

In this study, there was an adequately high moisture content value observed in the sterile exposed untreated control blocks of around 34% (Figure 3.6) which is a confirmation of favourable growing condition for the fungi. As the WPG increased the final moisture contents

of these decrease to values approaching in the worst case close to 23% but this is expressed in wood with a WPG above 40%, so the actual wood moisture is somewhat higher, i.e. adequate for decay initiation. At the end of the decay test, the moisture contents of the Scots pine wood treated with both *t*CNSL and *i*CNSL also showed declining values with increasing WPG. The low uptake of moisture in both *t*CNSL and *i*CNSL treated sterile control wood blocks (Figure 3.6) may be a result of the hydrophobic nature of CNSL. It is assumed that the CNSLs may have a water repellent activity which lowers the moisture of wood and slows the activity of decay fungi. Literature evidence reports that CNSL is hydrophobic and this is imparted due to the presence of the unsaturated side chain. This makes it water repellent and resistant to weathering (Tyman, 1975; Pillai *et al*., 1980; Kumar *et al*., 2002).

3.5. Conclusions

1. Though the CNSLs (*t*CNSL and *i*CNSL) have potential antioxidant properties, they were much less intense than the standard antioxidant compounds gallic acid and quercetin. From the DPPH free radical scavenging assay it was evident that the DPPH inhibition of *i*CNSL is no higher than the *t*CNSL when considered statistically, although the IC_{50} and AAI values of the *i*CNSL were higher than the *t*CNSL.

2. It is seems that the better antioxidant activity of *i*CNSL is due to the assumed presence of anacardic acid which is almost absent in *t*CNSL. Earlier studies claimed the antioxidant properties of anacardic acid and solvent extracted natural CNSL in which anacardic acid is profound. This study also revealed that *t*CNSL (cardanol rich) also has DPPH inhibition potential. It is also claimed in other studies that cardanol has better antioxidant properties than anacardic acid and cardol. Irrespective of the chemical composition of CNSL another factor may responsible for free radical scavenging capability and the unsaturated long side chain of 15 carbon atoms of CNSL constituents may be the principal reason of imparting antioxidant activity.

3. The fungal mycelia growth rate and antifungal activity were also assessed by CNSL inoculated malt agar media assay. This investigation revealed that *i*CNSL had a significant effect on the growth reduction of both brown rot (*C. puteana* and *P. placenta*) and white rot fungi (*T. versicolor* and *P. ostreatus*). Further, its antifungal effect was significantly higher than *t*CNSL against all of the fungi tested. It can be noted that the fungal growth reduction was significantly improved when both the CNSLs were mixed at 50: 50 ratio (*it*CNSL) and this

phenomenon was more significant on the growth of the *T. versicolor*. The fungal growth rate was significantly more reduced on white rot fungi than the brown rot fungi, but the inhibition of fungal growth by the CNSLs was found to vary between the wood decay fungi tested.

4. From the aforesaid results it can be postulated that there might be a correlation between the free radical scavenging activities and the antifungal properties. The radical scavenging properties of the tested CNSLs might have direct contribution to their antifungal properties. It is also thought that the antifungal properties of the CNSLs are due to the bioactive constituents (e.g. anacardic acid, cardol) present in CNSL.

5. Some improved decay resistance of Scots pine was imparted by the impregnation treatment with both the CNSLs. The efficacy of the CNSLs was more effective on white rot fungi than the brown rot fungi on Scots pine. The effect of *i*CNSL on the reduction of mass loss was better than *t*CNSL in exposure to brown rot fungi. The result of this investigation also reflected that the difference of decay resistance is due the chemical composition of CNSL and the overall decay resistance capacity of the CNSL is its phenolic constituents.

6. Moisture content at the end of the decay revealed the hydrophobic nature of CNSL might associated with decay resistance but that it is difficult to separate cause and effect. It may contribute as an addition to the toxic nature of CNSL. The final moisture contents of the CNSL treated wood blocks were reduced with increasing WPG against all the decay fungi. This hydrophobicity of CNSL is explained due the unsaturated long side chain of the CNSL constituents. This is of great interest for synthesising CNSL based resin and modify wood properties by impregnation modification and discussed in later chapters.

Chapter 4

Physical and moisture related properties of CNSL resin modified wood

4.1 Introduction

Modification of wood properties by impregnation is a method to enhance the physical, mechanical and decay resistance properties of wood. Unlike the chemical modification, this type of modification does not necessarily involve chemical changes within wood cell wall. Hill (2006) described the principle of impregnation modification as chemical or combination of chemical impregnated into the wood cell wall and reaction so as to form a material that is locked in the cell wall. Resin treatment has proven to be one of the most useful methods among several other process of impregnation modification.

Wood treatment with resins has been widely studied going back a long time, using epoxy resins, phenolic resins, melamine resins, urea resins, polyurethane pre-polymers and unsaturated polyesters. Most of the resin modification work performed has focused on the dimensional stabilization and water repellency and/or reduction of the hygroscopicity of wood (Stamm and Seborg, 1939; Inoue *et al*., 1993; Pittman, 1994; Rapp and Peek, 1995; Sailer and Rapp, 1997; Lukowsky *et al*., 1998; Deka and Saikia, 2000; Lukowsky, 2002; Ohmae *et al*., 2004; Ormondroyd, 2007). Much of this has used formaldehyde based phenol, melamine, urea, melamine-urea, methylolated melamine resin systems. Rapp and Peek (1995) evaluated 30 resin systems including some formaldehyde based system but these gave poor dimensional stability results. To date phenol formaldehyde resin modified wood has provided the best dimensional stability to wood when treated with low molecular weight type resin systems (Furuno *et al*., 2004). Gabrielli and Kamke (2010) reported that the phenol formaldehyde enhances dimensional stability and mechanical properties to densified wood by the viscoelastic thermal compression process. Jinshu *et al*. (2007) showed the improvement of wood properties when modified with urea formaldehyde with nano-SiO_2.

The use and application of resins based on natural and renewable resources is often thought of as a new approach that requires novel technologies and methods to implement it (Dunky *et al.* 2002). Cashew nut shell liquid (CNSL) is categorised as one of the plant derived sources for natural or bio based resins alongside tannins, lignins, carbohydrates, unsaturated oils and

liquefied wood (BRE, 2007). As CNSL major components possess a phenolic nature and an unsaturated side chain, it offers reaction sites on the aromatic ring and also on the side chain, which makes it a suitable raw material for variety of reactions (Mahanwar and Kale 1996). Therefore, different types of resins and polymers have been synthesized from CNSL, its isolated constituents, principally from cardanol, or from chemically modified CNSL (Swain *et al*., 1994; Bhunia *et al*., 1998; Mohapatra *et al*., 1998; Ikeda *et al*., 2002; Mythili *et al*., 2004; Suresh and Kishanprasad, 2005). The cardanol rich technical CNSL (*t*CNSL) is preferable for resin formulation due to its availability and extraction as it is produced as a by-product from the cashew nut processing industries.

Ozonolysis of unsaturated vegetable oils by reacting ozone with alkene compounds in the presence of participating co-reactants forms reaction products which are particularly suitable for use in the formation of resin (Graham and Tyman, 2002; Fitchett *et al*., 2003). Ozonolysis of CNSL forms ozonolysis reaction products (ozonides) which can be then be treated under reducing conditions to form CNSL aldehydes, which are used to form adhesives (Khan *et al*., 2002; Fitchett *et al*., 2003). The reaction scheme for this is shown (Figure 4.1). The aim of this study was to prepare CNSL resin by the ozonolysis process and impregnate this into wood to improve the dimensional stability and water repellency and also reduce the hygroscopicity of wood. The impregnation modification with CNSL resin is expected to improve other physical properties of wood and thus this is an opportunity to characterize these physical properties after resin modification.

4.2 Materials and Methods

4.2.1 Wood samples

Three wood species, one softwood and two hardwoods were selected for this study. Samples from sapwood of Scots pine (*Pinus sylvestris* L.), Obeche (*Triplochiton scleroxylon* K. Schum.) and Gmelina (or Gamar) (*Gmelina arborea* Roxb.) were prepared to dimensions of 20 x 20 x 5 mm (radial x tangential x longitudinal). All samples used in the experiment were selected with the grain direction parallel to the tangential surface and free from any defects (e.g. knots, resin pockets). After sanding to remove loose fibres, the wood samples were stored in a climate chamber at 20 °C and 65% relative humidity (RH) until they reached equilibrium moisture content.

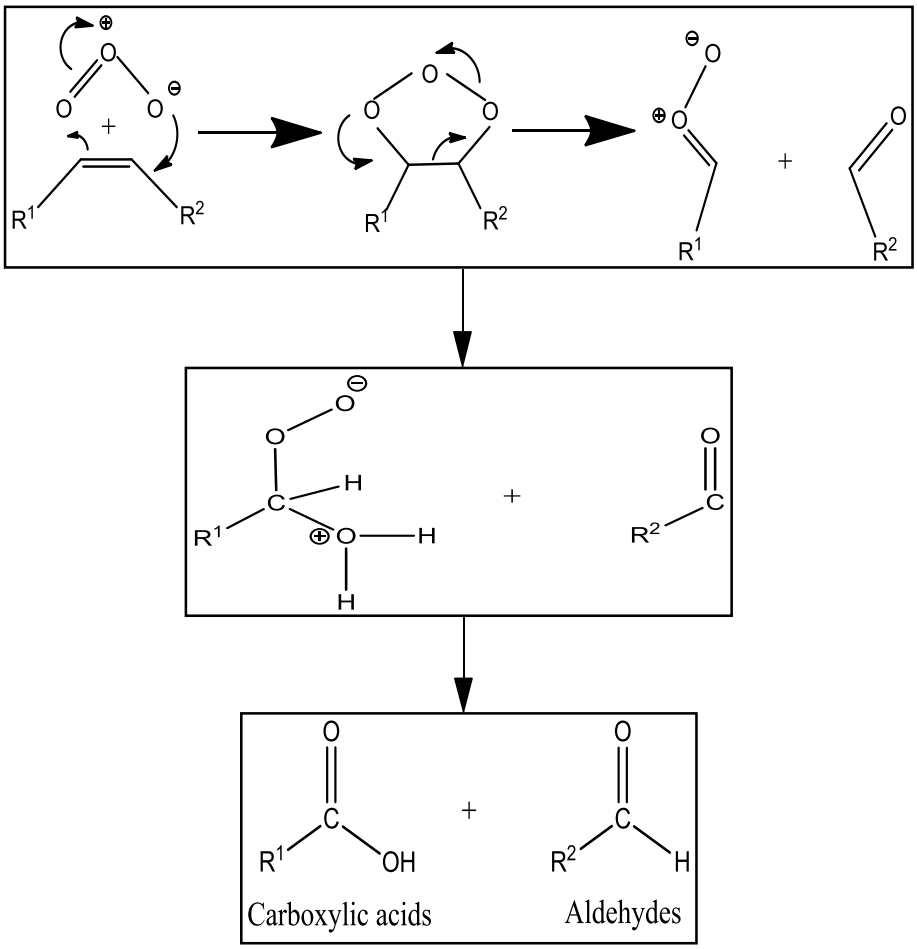


Figure 4.1: Chemical reaction of *t*CNSL to develop CNSL resin system by ozonolysis (Tverezovskiy, 2012).

4.2.2 Preparation and properties of CNSL resin

A resin system was synthesized from technical grade CNSL (*t*CNSL) by the ozonolysis process, where resin forming aldehydes and other compounds were derived after decomposition of ozonolysis products (or ozonides). Ozonides were formed by reacting ozone with unsaturated oil-like compounds in CNSL. Ozonolysis of CNSL comprised reacting together ozone, CNSL and water as a participating co-reactant in sufficiently low quantities (ratio water to CNSL) to allow CNSL to act as a solvent. This minimised the formation of unwanted by-products. Water is used as a participating co-reactant and vehicle for ozonolysis which leads to the formation of hydro-peroxides. The process used in this study for the formulation of resin from CNSL is adapted from the processes of Khan *et al*. (2000a, b), Fitchett *et al*. (2003). The ozonolysis was carried out with laboratory facilities and supervision of the BioComposites Centre.

250 g of *t*CNSL was mixed with 50 g deionised water. The mixture was placed in a 700 ml reactor flask fitted with 4-necked lid equipped with an overhead mechanical stirrer, thermometer and gas inlet-outlet tubes. Before introducing ozone, the mixture at 50-60 °C

was stirred at a speed 200 rpm and this was gradually increased up to 250 rpm. Ozone was bubbled through the mixture at 0.43 g/min (Oxygen flow rate 5 L/min) for 120 minutes from an ozone generator (Azcozone, Azco Ltd.). The temperature of the reaction mixture was maintained in a cold water bath at a temperature high enough to reduce the viscosity of the reaction mixture but low enough to avoid decomposition of ozonides. At the end of the reaction time the mixture was stirred for further 10 minutes to strip residual ozone. The principal components of this reaction mixture were peroxides including peroxy hemi-acetals.

Stabilization of the ozonolysis reaction products (ozonides) was performed by heat treatment. After finishing the ozonolysis reaction, the water bath was removed and the reactor flask was heated by an electric heater at 100 °C for 30 minutes. The ozonides were stirred vigorously during heating. This treatment brings thermal decomposition of the principal reaction products, such as 1-(1-hydroxyalk-1-yl peroxy)-alc-1-ols, and also free hydroxyalkyl-peroxides and secondary ozonides. Heat treatment also liberated gases like O_2 and CO_2 generated from decomposition reactions. The heat treated product contains a mixture of various aldehydes and carboxylic acids. The weight of the final products was measured to be 270 g, giving a yield of 90%. The characteristics of the resin produced are reported in Table 4.1.

Table 4.1: Characteristic of resin obtained from technical cashew nut shell liquid (CNSL) by ozonolysis reaction process (Tverezovskiy, 2012).

Property	**CNSL resin**
Viscosity	2000 (centipoises)
Molecular weight	100 to 400
Solid content	95%
pH	5.0 -6.0 (acidic)
Density	1.047 (gm cm^{-3})
Solubility	Organic solvent

4.2.3. Resin impregnation

The weight and dimensions of the test samples were measured before and after oven drying. All wood blocks of the three species were dried in an oven at 105 °C for 24 hours. Following removal from the oven, the wood blocks were allowed to equilibrate to ambient temperature by placing them in a desiccator over silica gel. After cooling, the blocks were weighed to 0.1 mg. The dimensions of the oven dried wood samples were measured by using digital calliper to 0.1 mm. The test specimens were vacuum impregnated with alcoholic solutions of CNSL resin of 0%, 5%, 10%, 20% or 30% solute content in industrial methylated spirit (IMS) to achieve different amounts of weight percent gain (WPG). The impregnation was carried out in a vacuum desiccator at a -0.7 bar vacuum for 30 minutes. Air was admitted into the desiccators

and the samples were left submerged in the impregnating solution for 2 hours at ambient temperature to allow uptake of the CNSL resin solution. Wood blocks of different species were impregnated separately. Following the impregnation, the excess solution was blotted off and the samples were then left in a fume cupboard for an hour to allow the IMS to evaporate. The samples were then cured at 120 °C for 24 hours in an oven. After curing the samples were then cooled, weighed and measured as above.

4.2.4. Evaluation of the physical properties of modified wood

The change of wood physical properties as a result of resin modification were assessed by evaluating the weight percent gain (WPG), oven dry specific gravity (SG_{OD}) of wood before and after resin modification, percentage of void volume of wood before and after resin modification and percentage volume change (VC). Weight and volume of 10 replicates of each resin modification from each species were measured to find the above mentioned properties.

Weight percent gain (WPG) of resin modified wood samples was determined by using the following formula:

$$WPG\ (\%) = [(W_2 - W_1)/W_1] \times 100 \quad \ldots\ldots (4.1)$$

Where, W_2 is the oven dry weight of the resin modified and W_1 is the corresponding oven dry weight of unmodified wood sample.

Volume change (VC) due to the resin modification was determined by using the following formula:

$$VC\ (\%) = [(V_{mod} - V_{unmod})/V_{unmod}] \times 100 \quad \ldots\ldots (4.2)$$

Where, V_{mod} is the volume of oven dry modified and V_{unmod} is the corresponding oven dry unmodified wood samples.

The ratio of theoretical and measured volume change (V_{rel}) due to the CNSL resin modification was determined from the following formula:

$$V_{rel} = V_{theor}/V_{meas} \quad \ldots\ldots (4.3)$$

Where, V_{theor} is the theoretical volume increase (weight of CNSL resin in wood/density of the CNSL resin) and V_{meas} is the measured volume increase due to resin modification.

SG_{OD} before and after resin modified wood samples were determined by using the following formula:

$$SG_{OD} = [(W_2/V_2)/1\ g\ cm^{-3}] \quad \ldots\ldots (4.4)$$

Where, W_2 is the oven dry weight of (resin modified or unmodified) and V_2 is the corresponding oven dry volume of (resin modified or unmodified) wood sample.

The percentage of SG_{OD} change due to the resin modification was determined by using the following formula:

SG_{OD} change (%) = [(SG_{ODmod} - $SG_{ODunmod}$) $SG_{ODunmod}$] x 100 …… (4.5)

Where, SG_{ODmod} is oven dry specific gravity of resin modified and $SG_{ODunmod}$ is the corresponding oven dry specific gravity of unmodified wood sample.

Void volume before and after resin modified wood samples was determined by using the following formula:

Void volume (%) = [1-(SG_{OD} /1.50)] x 100 …….. (4.6)

Where, SG_{OD} is the oven dry specific gravity of (resin modified or unmodified) wood and 1.50 is the constant for the specific gravity of the wood cell wall material (Dinwoodie, 2000).

Percentage of void volume change due to the resin modification was determined by using the following formula:

Void volume change (%) = [($Vvol_{mod}$ – $Vvol_{unmod}$)/$Vvol_{unmod}$] x 100 …… (4.7)

Where, $Vvol_{mod}$ is the void volume of modified and $Vvol_{unmod}$ is the corresponding void volume of the unmodified wood sample

4.2.5 Leachability of the resin

The leachability of the CNSL resin from the modified wood specimens of all wood species was tested according to BS EN 84. 10 cured, oven dried and cooled replicates from each resin modified wood were weighed to 0.1 mg. Samples from the different variable sets (species x treatment WPG) were impregnated under vacuum with deionised water separately for 20 minutes and submerged under water for 2 hours. The containers were refilled with water to a ratio of 5 volumes of water to one volume of wood and the samples were kept immersed for 14 days in climate chamber at 20 °C and 65% relative humidity (RH). Nine cycles of water changes were made with cycles 1 and 2 at the end of first and second days and the other 7 cycles in the remaining 12 days at not less than 1 day and not more than 3 days intervals. The samples were then oven dried. Examination of the data revealed that there were losses from the control samples so that the data had to be corrected to account for losses from the wood.

The leachability of the impregnated CNSL resin was determined from the following formula:

Leachability (%) = [{(W_2 –W_3)-CL}/ (W_2-W_1)] x 100…… (4.8)

Where, W_1 is the oven dry weight of unmodified wood, W_2 is the oven dry weight of modified wood before the leaching test, W_3 is the oven dry weight of modified wood after the leaching test and CL is the control losses for the individual species based on the average of the control (IMS treated) leached block.

The WPG of wood before and after leaching was determined from the formula 4.1.

4.2.6. Dimensional stabilization

Dimensional stabilization was determined by the cyclic water soaking and drying method (Hill *et al*., 2004a; Hill and Mallon, 1998; Hill and Jones, 1996; Rowell and Ellis, 1978). Following oven drying, cooling and determination of the weight and dimensions of 5 replicates of each resin modification, the samples were vacuum impregnated for 30 minutes with deionised water for the water soak test. All the wood samples were soaked for 5 days at room temperature. The water soaked weight and volume was determined. Following measurement the samples were oven dried for 2 days at 105 °C to a constant weight. The samples were then re-weighed and their dimensions were measured again. This procedure was repeated for a total of 5 cycles of oven-dry (OD) and water soak (WS).

The volumetric swelling co-efficient of the resin modified wood was determined from the following formula:

S_{mod} (%) = [(Vws_{mod} – Vod_{mod})/Vod_{mod}] x 100 …… (4.9)

Where, Vws_{mod} is the water saturated volume of the resin modified wood and Vod_{mod} is the oven dry volume of the resin modified wood.

The volumetric swelling co-efficient of the unmodified wood was determined from the following formula:

S_{unmod} (%) = [(Vws_{unmod} – Vod_{unmod})/Vod_{unmod}] x 100 …… (4.10)

Where, Vws_{unmod} is the water saturated volume of the unmodified wood and Vod_{unmod} is the oven dry volume of the unmodified wood.

The anti-swelling efficiency of the resin modified wood was determined from the following formula:

ASE (%) = [(S_{unmod} – S_{mod})/S_{unmod}] x 100 …….. (4.11)

Where, S_{unmod} is the volumetric swelling co-efficient of the unmodified wood and S_{mod} is the volumetric swelling co-efficient of the resin modified wood.

The stability to hydrolysis was evaluated by determining the weight loss (WL) after each cycle of the water soaking and drying. The weight loss was calculated by the following formula.

$WL\ (\%) = [(Wod_{bef} - Wod_{aft})/Wod_{bef}] \times 100$ …….. (4.12)

Where, Wod_{bef} is the oven dry weight before water soaking and Wod_{aft} is the oven dry weight after water soaking of the resin modified wood.

4.2.7. Water absorption and water repellent efficiency

The water absorption of the CNSL resin modified wood samples and its water repellent efficiency were determined by the cyclic water soaking and drying method as mentioned earlier (section 4.2.6).

The water absorption (WA) of the resin modified and unmodified wood samples were determined by the following formula.

$WA\ (\%) = [(W_{ws} - W_{od})/W_{od}] \times 100$ …….. (4.13)

Where, W_{ws} is the water saturated weight of the (modified or unmodified) wood samples and W_{od} is the oven dry weight of (modified or unmodified) wood samples.

The water repellent efficiency (WRE) of the resin modified wood samples was determined by the following formula:

$WRE\ (\%) = [(WA_{unmod} - WA_{mod})/WA_{unmod}] \times 100$ …….. (4.14)

Where, is WA_{unmod} the water absorption of the unmodified (control) wood samples and WA_{mod} is the water absorption of the resin modified wood samples.

4.2.8. Statistical analysis

The data obtained were analysed by the one way analysis of variance (ANOVA) test using IBM® SPSS® Statistics 20 to determine the statistical difference followed by Tukey's multiple comparison. The results with $P<0.05$ were considered to be significantly different.

4.3. Results

4.3.1. Characterization of physical properties of CNSL resin modified wood

4.3.1.1. Weight percent gain (WPG)

The results of the mean weight percent gain (WPG) due to CNSL resin modification of Scots pine, Obeche and Gmelina wood are shown in the Table 4.2. The WPG of the resin modified wood increased with the increasing concentration of CNSL resin in the treatment solution. Impregnation modification of all the species with 5, 10, 20 and 30% CNSL resin resulted in

linear increases in WPG in the wood samples. Control wood blocks of all species treated with 0% resin concentration (i.e. impregnated in IMS) showed no WPG. At all the resin treatment levels, the WPGs of the Scots pine were at least twice that of Gmelina, and Obeche was intermediate. The WPGs achieved in different treatment levels showed significant differences ($P<0.05$) for all the three wood species. Significantly higher WPGs were achieved in Scots pine than the other two wood species (Scots pine [highest value =27.9]>Obeche [highest value =15.5]>Gmelina [highest value =9.4]). With the low resin solution concentrations Scots pine gave 5.5, Obeche 3.9 and Gmelina 2.7 WPG.

Table 4.2: Mean weight percent gain (WPG) of the oven dried samples of the three wood species due to the CNSL resin impregnation modification.

Wood species	Resin Concentration (%)	WPG
Pinus sylvestris	0 (Control)	0.0 (0.0)
	5	5.5 (0.6)a
	10	10.3 (1.1)b
	20	16.6 (0.7)c
	30	27.9 (1.7)d
Triplochiton sclerexylon	0 (Control)	0.0 (0.0)
	5	3.9 (0.4)a
	10	6.8 (0.7)b
	20	9.3 (0.6)c
	30	15.5 (2.3)d
Gmelina arborea	0 (Control)	0.0 (0.0)
	5	2.7 (0.3)a
	10	4.4 (0.3)b
	20	6.9 (0.6)c
	30	9.4 (0.9)d

Values in parenthesis refer to ±SD and n=10. WPG achieved were statistically significantly different ($P<0.05$) for all of the wood species. Number followed by different letters for each wood species are significantly different at the level of $P<0.05$ according to Tukey's test.

4.3.1.2. Volume change

The percent of volume change (VC) in wood samples of Scots pine, Obeche and Gmelina due to the CNSL resin modification as determined using the measurement of the external dimensions of the wood blocks is shown in Figure 4.2. Data points represent the individual wood samples and best fit line has been drawn through the data points for all of the wood species (Figure 4.2 a, b and c). There is a clear, positive linear relationship between the VC% and WPG although the linearity is less good at the higher WPG values for Scots pine and Obeche.

The mean percent of volume change due to the resin modification is shown in Table 4.3. The mean VC% increases with the increased WPG. The highest VC% found, at highest treatment

levels in Scots pine, Obeche and Gmelina, were 7.1%, 5.5% and 5.5% respectively. The change of volume due to resin modification was no greater than ca. 7.1% even at the highest WPG level of 28. It was observed from the VC% data that there is little difference in the behaviour of the samples between the wood species when the VC% values are compared at equivalent WPG values.

Statistically significant differences ($P<0.05$) have been found for the effect of WPG on the VC% for the three wood species (Table 4.3). Higher VC% values were found for Scots pine than the other two wood species, but this was at higher WPG values. With the other two species the VC% changes are similar at each resin WPG, and when similar WPG values are compared to the Scots pine there is little difference in VC%, for example, volume increase of ca. 5% at a WPG of *ca.* 10 for all of the tested wood species.

The ratio of theoretical to measured volume increase (V_{rel}) due to the CNSL resin loading or WPG is also presented in the Table 4.3. The theoretical volume increase is obtained by dividing the density of CNSL resin (Table 4.1) by the weight gain of wood due to modification (Formula 4.3). All V_{rel} values increased with increasing WPG. Scots pine showed V_{rel} values greater than 1 throughout, indicating that less resin has gone into the cell wall than expected. The values for Obeche and Gmelina are lower than 1 at the lower WPG values but approach 1 at the higher WPG values. This will be more fully discussed later.

Table 4.3: Effect of CNSL resin modification on the percent volume change (VC) and ratio of theoretical to measured volume (V_{rel}) of three wood species.

Wood species	**WPG**	**VC (%)**	**V_{rel}**
Pinus sylvestris	5.5	2.2 (0.5)a	1.02
	10.3	4.1 (0.6)b	1.03
	16.6	5.7 (0.8)c	1.19
	27.9	7.1 (0.5)d	1.50
Triplochiton scleroxylon	3.9	1.8 (0.7)a	0.66
	6.8	3.5 (0.4)b	0.74
	9.3	5.2 (1.3)c	0.78
	15.5	5.5 0.5)c	0.90
Gmelina arborea	2.7	1.7 (0.4)a	0.70
	4.4	3.3 (0.3)b	0.74
	6.9	4.4 (0.3)c	0.96
	9.4	5.5 (0.4)d	1.03

Values in parenthesis refer to ±SD and n=10. The VC% are significantly different ($P<0.05$) between the WPGs for all of the wood species. Number followed by different letters for each wood species are significantly different at the level of $P<0.05$ according to Tukey's test.

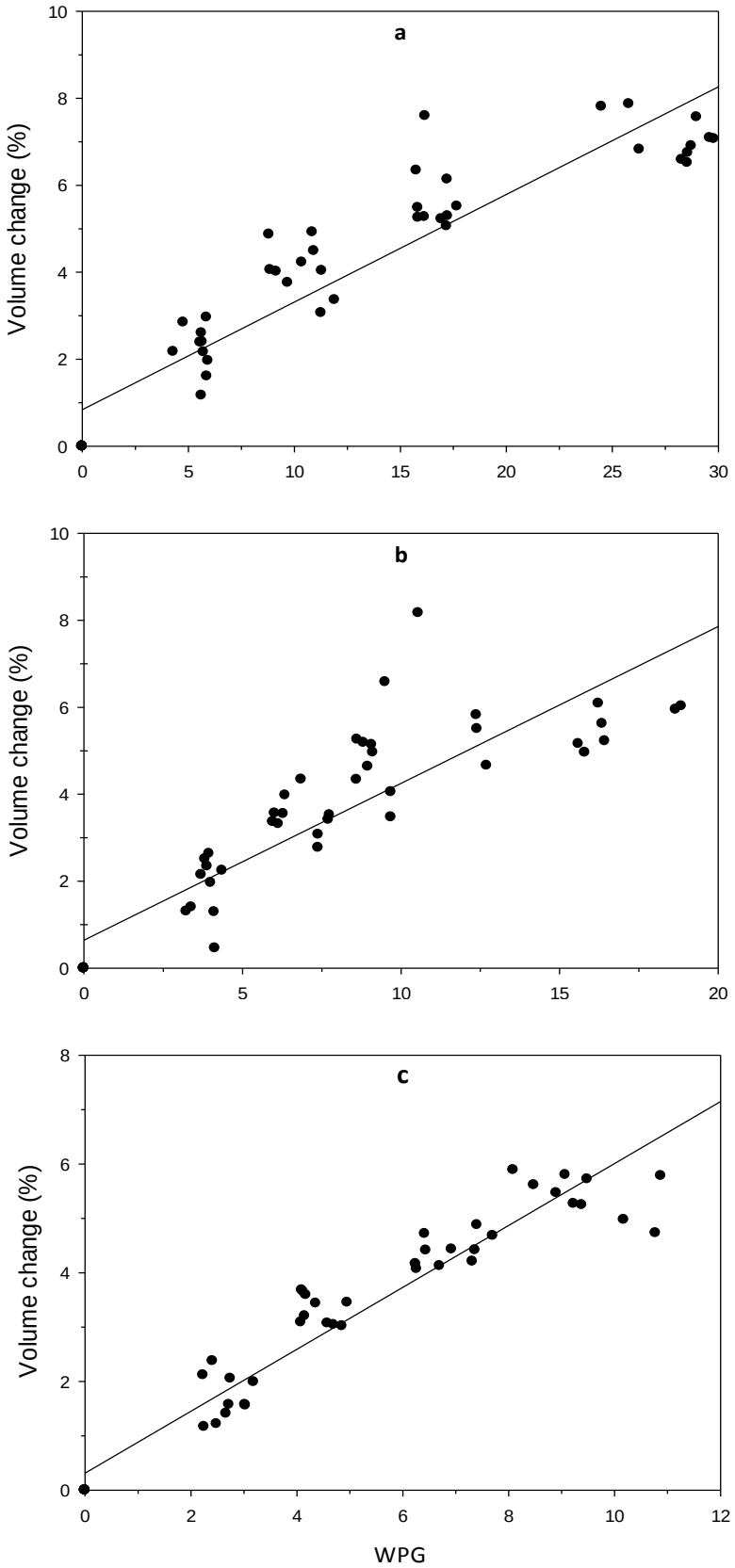


Figure 4.2: Relationship between percentage volume change and weight percent gain (WPG) of (a) *Pinus sylvestris*, (b) *Triplochiton scleroxylon* and (c) *Gmelina arborea* wood blocks due to the impregnation modification with CNSL resin.

4.3.1.3. Specific gravity and void volume

The basic and oven dry specific gravities of the sapwood of these species were measured before modification (Table 4.4) and they are higher in unmodified Scots pine sapwood than Gmelina but the lowest was in Obeche.

Table 4.4: Basic and oven dry specific gravity of the sapwood of three species used in the CNSL resin impregnation experiment.

Wood species	Specific gravity	
	Basic	Oven dry
Pinus sylvestris	0.39	0.41
Triplochiton sclerexylon	0.33	0.34
Gmelina arborea	0.36	0.37

The basic and oven dry specific gravity was measured by the equation 4.4 where, oven dry weight for both cases and green volume and oven dry volume was used for the calculation of basic and oven dry specific gravity respectively.

The effect of CNSL resin modification on the oven dry specific gravity (SG_{OD}) of Scots pine, Obeche and Gmelina (Table 4.5) is as expected, the SG_{OD} increases with increasing WPG. Among the three wood species, Scots pine showed significantly higher $SG_{OD.}$ The CNSL resin loading (WPG) had a significant ($P<0.05$) effect on the SG_{OD} of Scots pine wood, but not on the SG_{OD} of Obeche and Gmelina wood. The percentage increment of SG_{OD} of the individual sample block was also calculated from the SG_{OD} of CNSL resin modified wood blocks in comparison to the SG_{OD} of the corresponding unmodified wood blocks. The increment of the SG_{OD} was achieved *ca.* 20% due to the CNSL resin impregnation modification. The highest SG_{OD} increment (ca. 20%) was found in Scots pine. Both Obeche and Gmelina showed similar ca. 10% increment of SG_{OD}. The effect of CNSL resin loading on the increment of SG_{OD} was significant as it showed a significant difference ($P<0.05$) for all of the wood species.

The results of the void volume of the CNSL resin modified wood are also shown in Table 4.5. The void volume of the resin modified wood decreased with the increasing WPG in all three wood species. The void volume was decreased 72.9% to 68.1% in Scots pine, 78.0% to 75.4% in Obeche and 75.4% to 73.0% in Gmelina modified wood. The effect of WPG on the percentage void volume was statistically significant ($P<0.05$) in Scots pine wood, but not in Obeche and Gmelina. The percentage reduction of void volume due to the CNSL resin impregnation modification was also measured from the difference of the void volume of modified wood blocks and the void volume of the corresponding unmodified wood blocks (Table 4.5). As expected the reduction of the void volume was greater in Scots pine than the other two species, however, Gmelina showed greater void volume reduction than Obeche

with less resin loading. The effect of CNSL resin modification (WPG) on the reduction of the void volume was also significant (P<0.05) for all three wood species.

Table 4.5: Effect of CNSL resin impregnation modification on the specific gravity and void volume of the three tested wood species.

Wood species	WPG	Specific gravity (oven dry)	Increment of specific gravity (%)	Void volume (%)	Reduction of void volume (%)
Pinus sylvestris	0.0	0.41 (0.4)a	0.0 (0.0)a	72.9 (2.7)a	0.0 (0.1)a
	5.5	0.42 (0.4)a	3.2 (0.9)b	71.8 (2.5)a	1.2 (0.2)b
	10.3	0.44 (0.1)ab	6.0 (1.4)c	71.0 (2.6)ab	2.3 (0.3)c
	16.6	0.45 (0.2)ab	11.9 (1.3)d	70.4 (0.9)b	4.2 (0.5)d
	27.9	0.48 (0.4)c	19.4 (1.9)e	68.2 (1.5)b	7.0 (0.7)e
Triplochiton sclerexylon	0.0	0.34 (0.5)a	0.0 (0.0)a	78.0 (2.9)a	0.0 (0.2)a
	3.9	0.35 (0.3)a	3.1 (1.2)b	76.8 (2.1)a	0.9 (0.4)b
	6.8	0.36 (.03)a	3.2 (0.9)b	75.8 (2.0)a	1.0 (0.2)c
	9.3	0.36 (0.3)a	5.8 (3.8)c	75.8 (1.8)a	1.7 (0.9)d
	15.5	0.37 (.03)a	9.2 (1.6)d	75.4 (1.9)a	2.7 (0.5)e
Gmelina arborea	0.0	0.37 (0.5)a	0.0 (0.0)a	75.4 (2.9)a	0.0 (0.5)a
	2.7	0.39(0.3)a	1.5 (0.5)a	74.1 (1.6)a	0.5 (0.2)b
	4.4	0.39 (0.4)a	3.4 (1.0)b	74.1(2.3)a	1.1 (0.3)c
	6.9	0.40 (0.4)a	7.4 (2.1)c	73.6 (2.7)a	2.4 (0.5)d
	9.4	0.40 (0.3)a	10.0 (1.4)d	73.0 (1.7)a	3.2 (0.4)e

Values in parenthesis refer to ±SD and n=10. The effect of WPG on specific gravity and void volume were significantly different (P<0.05). Number followed by different letters for each wood species are significantly different at the level of P<0.05 according to Tukey's test.

4.3.2. Leaching

4.3.2.1. Leachability of resin

The results of the leachability of the CNSL resin after the impregnation modification in Scots pine, Obeche and Gmelina wood is shown in Figure 4.3. The leaching of control wood blocks of the each species showed losses may be due to the loss of soluble hemicelluloses and wood extractives. So, the losses of the control wood blocks was accounted as a correction value to the amount of resin leached out from the treated wood blocks. Though, the "apparent" leaching of CNSL resin gradually increased with the increase of resin WPG, but close examination of the raw data shows that the actual losses were similar in some cases to the losses of the control blocks. Thus it may be concluded that there is minimum losses of resin and this phenomenon was found for all the three wood species tested.

Between the wood species, higher amounts of CNSL resin was leached out from the Gmelina modified wood than Obeche and Scots pine (Figure 4.3). At the lowest WPG, leachability of CNSL resin was 1.9%, 2.5% and 3.5% which only increased to 3.1, 4.2% and 5.3% when WPG reached to the maximum for Scots pine, Obeche and Gmelina modified wood. The effect of

WPG on the leachability of CNSL resin from the modified wood blocks were not statistically significantly different (P>0.05) for the three wood species.

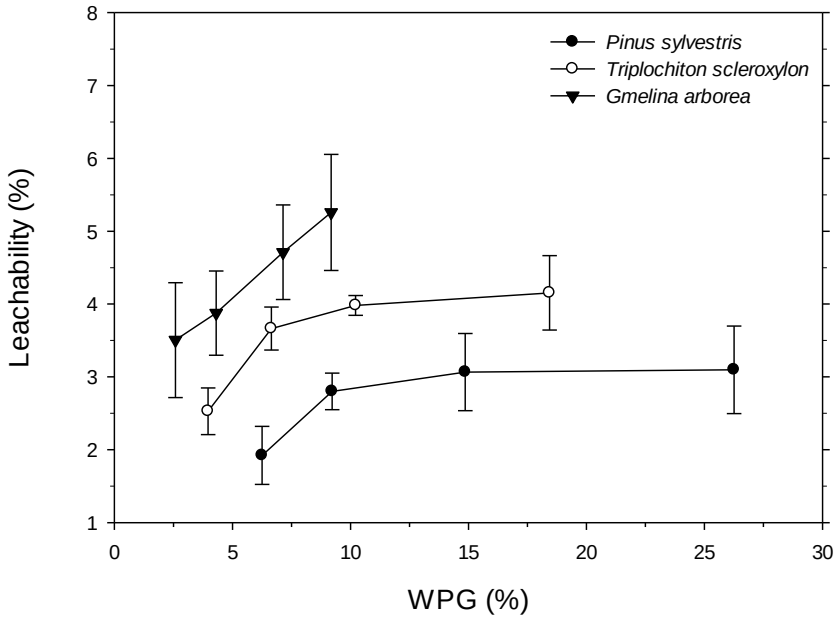


Figure 4.3: Leachability of the CNSL resin from the impregnation modified *Pinus sylvestris*, *Triplochiton scleroxylon* and *Gmelina arborea* wood (error bars = ±SD, n=10).

4.3.2.2. Reduction of weight percent gain (WPG)

The effect of the water leaching test on the WPG of CNSL resin modified wood of Scots pine; Obeche and Gmelina are presented in Figure 4.4. It is apparent that the leaching caused a small WPG reduction to the CNSL resin modified wood. It is also evident that the reduction of the WPG also occurred in the control (IMS treated) wood blocks for all of the wood species tested. The negative WPG value of the control wood blocks indicated the possible reduction of the substances from wood due to the water leaching test. After leaching the modified wood finally exhibited WPGs of 5.2, 8.1, 13.4 and 24.6 for Scots pine; 3.2, 5.5, 8.8 and 16.9 for Obeche; 2.0, 3.1, 5.6 and 7.7 for Gmelina wood. There is little difference between the amounts of loss, irrespective of WPG or wood species.

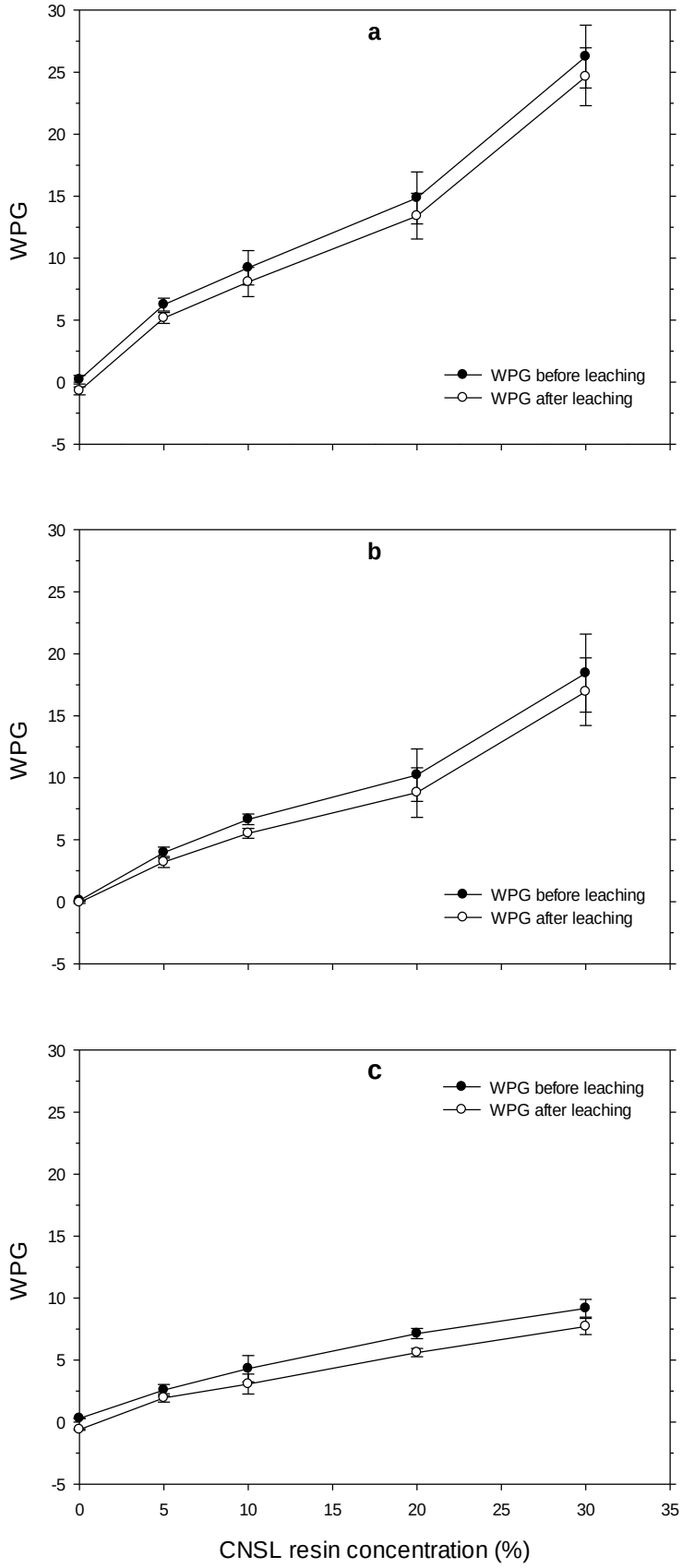


Figure 4.4: Mean weight percent gain (WPG) of (a) *Pinus sylvestris*, (b) *Triplochiton scleroxylon* and (c) *Gmelina arborea* wood treated with various concentrations of CNSL resin before and after leaching test according to EN 84 protocol (error bars = ±SD, n=10).

4.3.3. Dimensional stability

4.3.3.1. Volume changes due to water soak and oven dry cycle

The effect of successive water soak (WS) and oven dry (OD) cycles on to the volume of CNSL resin modified wood for Scots pine, Obeche and Gmelina is shown in Figure 4.5. The changes of volume between the WS and OD cycle were calculated from the water saturated and oven dry volume of samples of the modified wood at different resin loading (WPG) from the water soak and dry dimensional stability test. In most instances, unmodified wood blocks did not survive well as checking and splitting often occurred, but the CNSL resin modified wood blocks were undegraded. From the Figure 4.5 it is obvious that the OD volumes prior to water soaking are larger than the subsequent OD volume for all modified woods. Similarly, the initial WS volumes of the resin modified wood after the 1st oven dry cycle are greater than the following WS volumes. It is also observed that there are only minor variations in both the WS and OD volumes between the subsequent cycles.

Much of the data shows a general decrease in water saturated and oven-dry volumes over the cycles. Another feature worthy of mention is that there were no variations in both the OD volumes and WS volumes between the cycles.

4.3.3.2. Volumetric swelling co-efficient and anti-swelling efficiency

The results of the volumetric swelling coefficient (S) and anti-swelling efficiency (ASE) of the CNSL resin modified wood samples of Scots pine, Obeche and Gmelina at WS/OD cycles are shown in Tables 4.6 and 4.7. The average volumetric swelling co-efficient or anti-swelling efficiency represents the mean value of 5 WS/OD cycles at each resin loading (WPG) level. The values at the 0% WPG represent the S% of the unmodified or control samples of the tested wood species.

Generally, the S% reduced with the increasing WPG in each successive WS/OD cycle (Table 4.6). This tendency of reducing S% was found for all of the wood species. However, there is no specific trend of S% either increasing or decreasing with each progressive WS/OD cycle for each WPG level. This phenomenon is also observed for all of the wood species tested. A statistically significant difference ($P<0.05$) has been found for the effect of WPG on the S% in each cycle, as well as on the average S% of the 5 consecutive cycles in all wood species.

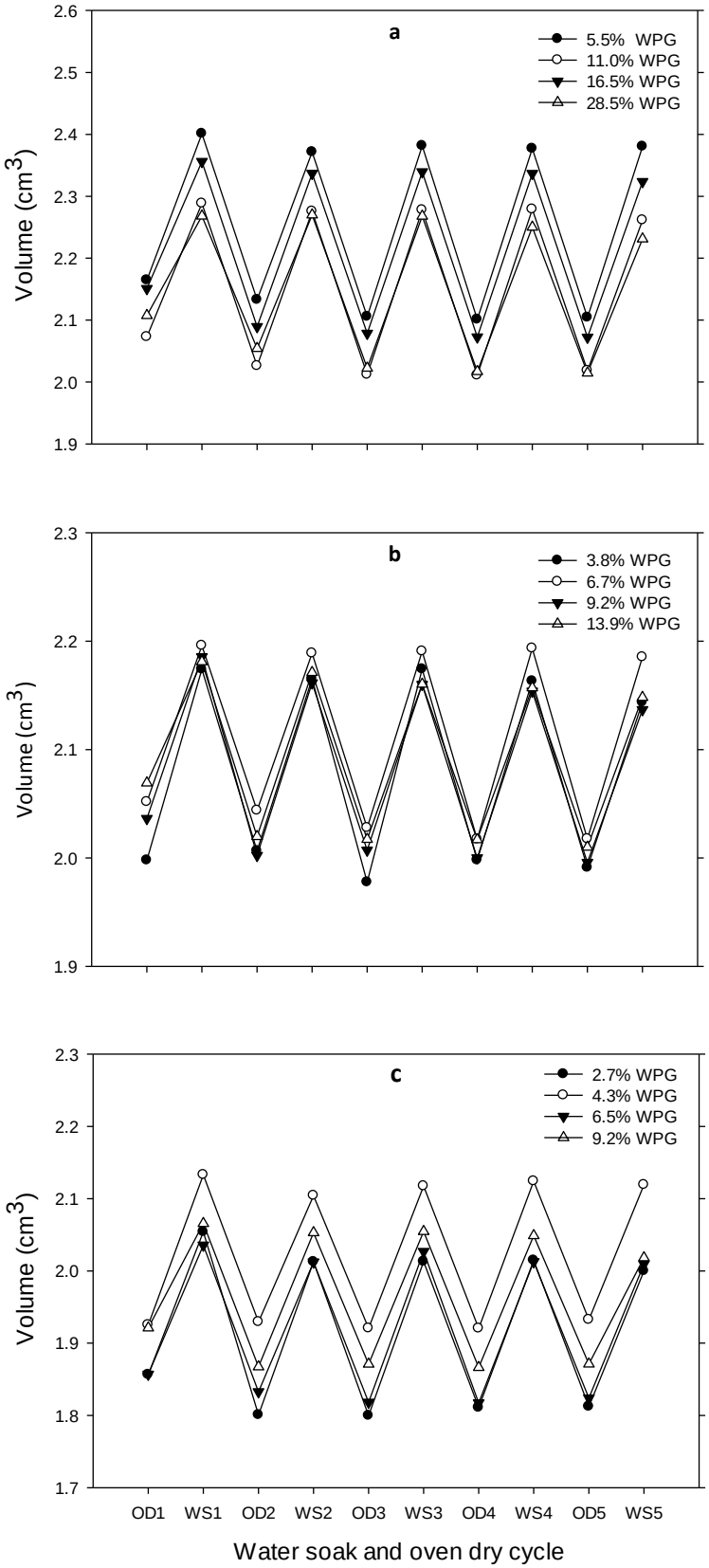


Figure 4.5: Changes in water saturated and oven dry volumes for CNSL resin modified (a) *Pinus sylvestris*, (b) *Triplochiton scleroxylon* and (c) *Gmelina arborea* wood samples at different water soak and oven dry cycle (n=5).

Table 4.6: Volumetric swelling co-efficient, S (%) as determined from the successive WS and OD cycle of the dimensional stabilization test of CNSL resin modified wood.

Wood Species	WPG	Volumetric swelling co-efficient (%)					
		Cycle					Average
		1	2	3	4	5	
Pinus sylvestris	0.0	14.5 (0.9)a	14.8 (1.4)a	15.9 (2.3)a	15.2 (2.3)a	15.4 (1.4)a	15.2 (0.8)a
	5.5	11.0 (2.9)ab	11.2 (1.0)a	13.2 (3.4)a	13.2 (2.8) ab	13.2 (1.4)b	12.3 (2.0)b
	11.1	10.4 (0.3)b	12.3 (2.2)b	13.2 (2.0)a	13.3 (0.5) ab	12.1 (0.9)bc	12.3 (0.9)b
	16.5	9.5 (0.7)b	11.9 (1.2)b	12.6 (1.1)a	12.8 (0.7)ab	12.1 (0.9)bc	11.8 (0.7)b
	28.5	7.6 (3.1)b	10.5 (2.9)b	12.1 (2.4)a	11.6 (0.9)b	10.7 (0.9)c	10.5 (1.2)c
Triplochiton sclerexylon	0.0	9.9 (1.0)a	10.0 (0.8) a	10.1 (0.4)a	11.5 (0.9)a	9.0 (1.2)a	10.1 (0.3)a
	3.8	8.8 (0.7) ab	7.9 (0.8)b	8.9 (1.6)b	8.3 (0.6)b	7.7 (0.9)a	8.3 (0.3)b
	6.7	7.0 (0.6) ab	7.1 (0.8)b	8.0 (0.5)b	8.7 (0.4)bc	7.7 (0.9)ab	7.7 (0.5)b
	9.2	6.2 (2.9)ab	8.0 (1.1)b	7.6 (0.3) b	7.7 (0.7)bc	7.1 (0.6)b	7.3 (0.7)c
	13.9	5.4 (0.7)b	7.5 (1.1)b	7.1 (0.4)b	7.0 (0.8)c	6.9 (0.3)b	6.8 (0.3)c
Gmelina arborea	0.0	12.0 (0.9)a	13.3 (2.2)a	12.7 (0.9)a	12.5 (3.4)a	12.1 (0.8)a	12.5 (1.0)a
	2.7	10.7 (2.2)ab	11.8 (1.8)ab	10.9 (1.2)a	10.3 (1.6)a	10.4 (1.0)a	10.8 (1.4)ab
	4.3	10.2 (2.1)ab	10.7 (0.3)b	10.3 (0.4)ab	10.6 (0.8)a	9.6 (1.6)a	10.3 (0.7)b
	6.5	9.7 (2.4)ab	9.8 (1.6)b	10.5 (0.6)bc	10.3 (1.1)a	9.6 (1.0)a	10.0 (0.7)bc
	9.2	7.5 (2.4)b	10.0 (2.0)b	9.8 (0.9) c	9.8 (2.5)a	7.9 (1.6)b	9.0 (0.9)c

Values in parenthesis refer to ±SD and n=5. The average S% is of cycle 1 to 5. The effect of WPG on S% is significantly different ($P<0.05$) for all of the wood species. Number followed by different letters for each wood species are significantly different at the level of $P<0.05$ according to Tukey's test.

Table 4.7: Anti-swelling efficiency, ASE (%) as determined from the successive WS and OD cycle of the dimensional stabilization test of CNSL resin modified wood.

Wood Species	WPG	ASE (%)					
		Cycle					Average
		1	2	3	4	5	
Pinus sylvestris	5.5	24.5 (3.2)a	24.5 (3.2)a	17.0 (3.1)a	13.3 (1.3)a	14.5 (2.1)a	18.8 (2.5)a
	11.1	28.2 (2.1)a	16.8 (2.7)a	16.7 (3.6)a	12.5 (2.1)b	21.6 (2.6)a	19.2 (1.9)a
	16.5	34.2 (4.5)a	20.1 (3.6)a	20.9 (2.8)a	16.3 (2.6)b	21.1 (2.7)ab	22.5 (3.6)ab
	28.5	47.9 (1.6)b	28.9 (2.9)a	23.5 (2.1)a	24.1 (2.3)b	30.1 (2.8)b	30.9 (2.0)b
Triplochiton sclerexylon	3.8	10.5 (3.2)a	20.6 (3.9)a	11.1 (2.4)a	28.2 (2.3)a	14.7 (4.4)a	17.0 (0.8)a
	6.7	28.6 (2.6)b	28.7 (3.8)a	20.1 (2.4)b	24.3 (1.6)a	14.4 (1.0)a	23.2 (1.6)b
	9.2	37.3 (1.8)bc	20.3 (4.9)a	24.3 (1.3)c	33.4 (2.9)ab	21.4 (3.2)a	27.3 (2.0)b
	13.9	45.1 (3.2)c	24.9 (2.3)a	29.4 (1.7)c	39.7 (3.3)b	23.5 (1.5)a	32.5 (1.3)c
Gmelina arborea	2.7	11.1 (4.2)a	10.9 (3.1)a	14.7 (2.1)a	17.4 (2.7)a	14.3 (3.8)a	13.7 (3.5)a
	4.3	14.8 (3.8)b	19.5 (1.0)a	19.5 (1.2)a	14.8 (2.8)a	20.2 (2.6)ab	17.8 (2.0)ab
	6.5	19.3 (2.3)b	26.1 (5.4)a	17.7 (1.0)a	17.6 (2.2)a	20.9 (0.5)ab	20.3 (2.2)ab
	9.2	37.3 (4.2)b	25.0 (2.8)a	22.9 (3.2)a	21.1 (2.4)a	35.1 (2.7)b	28.3 (3.2)b

Values in parenthesis refer to ±SD and n=5. The average ASE is of cycles 1 to 5. ASE are significantly different ($P<0.05$) between the resin loadings in particular cases for all of the wood species. Number followed by different letters for each wood species are significantly different at the level of $P<0.05$ according to Tukey's test.

The ASE of the CNSL resin modified wood is also generally increased with the increasing WPG (Table 4.7) when each successive cycle is examined in isolation but at each WPG the ASE (%) varied highly between each cycle. In Scots pine the ASE generally declined after the first cycle while in the other two species the ASE values are variable throughout. The effect of WPG on the ASE was significantly different ($P<0.05$) in cycle 1, 4 and 5 for Scots pine; cycle 1, 3 and 4 for Obeche; cycle 1 and 5 for Gmelina. The average ASE of 5 consecutive cycles did show a significant difference ($P<0.05$) between the WPGs for the three wood species.

The unmodified or control wood showed higher S% value for Scots pine (15.2%), Obeche (10.1%) and Gmelina (12.5%) than the CNSL resin modified wood of the same. Increasing resin WPG clearly has an important effect in imparting dimensional stability (Figure 4.6 a, S% and b, ASE %; Table 4.6 and 4.7). The lowest S (6.8%) was found in Obeche wood at 13.9 WPG, while Gmelina provided 9% volumetric swelling coefficient with at 9.2 WPG. It was much less effective in Scots pine, only provided 10.5 % swelling coefficient at 28.5 WPG, a resin loading more than double in Obeche and Gmelina.

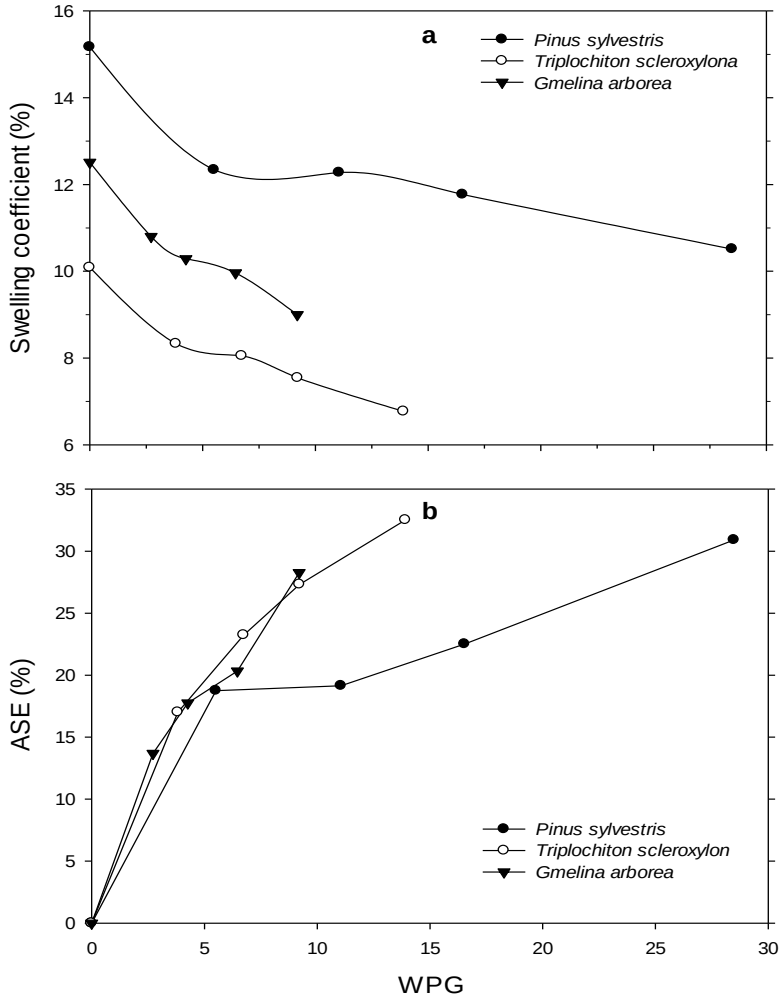


Figure 4.6: Relationship between the WPG and (a) volumetric swelling coefficient (S%) and (b) anti-swelling efficiency (ASE) for *Pinus sylvestris, Triplochiton scleroxylon* and *Gmelina arborea* modified wood with CNSL resin.

In terms of ASE (%) the highest WPG values achieved were around 30%, although in Scots pine this was at much higher WPG. At lower WPG values the effect was more or less linear for the hardwoods but tailed off at higher WPG for the Obeche. The improvement of dimensional stability in Obeche and Gmelina modified wood were found with low resin loadings (WPG).

The relationships between the volume change (VC%) and the S% and ASE% however show a different picture (Figure 4.7 a, b). Though, the S% decreased and the ASE% increased and showed a close relationship with volume change for all the wood species.

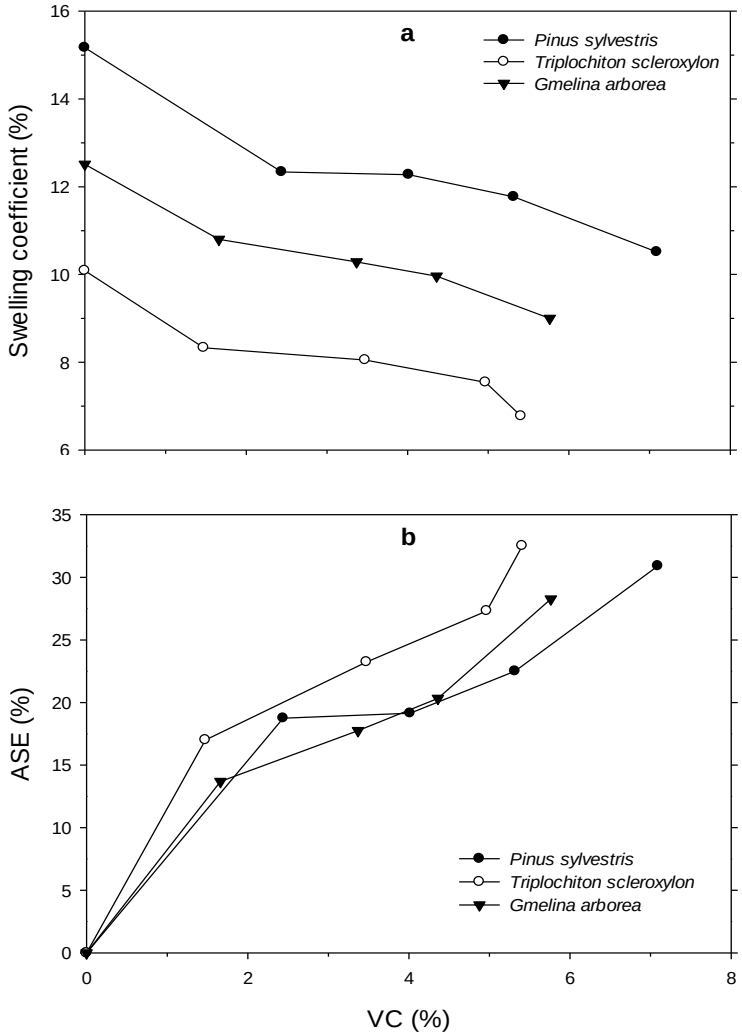


Figure 4.7: Relationship between the VC (%) and (a) volumetric swelling coefficient (S%) and (b) anti-swelling efficiency (ASE%) for *Pinus sylvestris, Triplochiton scleroxylon* and *Gmelina arborea* modified wood with CNSL resin.

4.3.4. Stability to hydrolysis

The stability to hydrolysis of CNSL resin modified wood was evaluated by calculating the percentage of weight losses (WL %) recorded during the cyclic water soaking and oven dry dimensional stability test. The results of the weight losses at different resin loading (WPG) of resin modified Scots pine, Obeche and Gmelina wood with the successive WS/OD cycle are shown in Table 4.8.

Table 4.8: Weight losses (WL) of CNSL resin modified wood as determined from the successive WS and OD cycle of the dimensional stabilization test.

Wood Species	WPG	Weight losses (%)					
		Cycle					Total
		1	2	3	4	5	
Pinus sylvestris	0.0	1.5 (0.2)a	1.0 (0.2)ab	0.4 (0.1)a	0.1 (0.1)a	0.3 (0.2)a	3.2 (0.3)a
	5.5	1.2 (0.3)a	1.0 (0.1)a	0.2 (0.1)ab	0.4 (0.1)ab	0.4 (0.1)a	3.1 (0.2)a
	11.1	1.0 (0.4)a	1.1 (0.1)a	0.4 (0.1)b	0.4 (0.1)b	0.2 (0.1)a	3.1 (0.5)a
	16.5	1.2 (0.4)a	1.0 (0.1)ab	0.5 (0.1)b	0.3 (0.1)b	0.3 (0.1)a	3.1 (0.3)a
	28.5	1.4 (0.2)a	0.7 (0.2)b	0.4 (0.1)b	0.4 (0.1)b	0.3 (0.1)a	3.2 (0.3)a
Triplochiton sclerexylon	0.0	1.0 (0.5)a	0.9 (0.4)a	0.3 (0.1)a	0.1 (0.1)ab	0.1 (0.1)a	2.5 (0.1)a
	3.8	1.1 (0.5)a	0.8 (0.1)a	0.2 (0.1)ab	0.1 (0.1)ab	0.3 (0.1)b	2.5(0.1)a
	6.7	2.1 (0.4)a	0.8 (0.1)ab	0.3 (0.1)ab	0.2 0.1)bc	0.2 (0.1)a	3.5(0.1)a
	9.2	2.9 (0.8)a	0.6 (0.1)ab	0.3 (0.1)ab	0.2 (0.1)c	0.1 (0.1)a	4.1 (0.9)a
	13.9	1.0 (0.1)a	0.3 (0.1)b	0.4 (0.1)b	0.3 (0.1)c	0.1 (0.1)a	2.1(0.1)a
Gmelina arborea	0.0	2.2 (0.5)a	0.8 (0.1)a	0.3 (0.1)a	0.1 (0.1)a	0.0 (0.1)a	3.4 (0.5)ab
	2.7	1.0 (0.5)b	1.1 (0.1)b	0.2 (0.1)ab	0.1 (0.1)ab	0.1 (0.1)a	2.5 (0.8)ab
	4.3	1.0 (0.5)b	0.9 (0.1)ab	0.3 (0.1)ab	0.1 (0.1)ab	0.1 (0.1)a	2.4 (0.4)ab
	6.5	2.7 (0.9)b	0.8 (0.1)a	0.3 (0.1)ab	0.2 (0.1)bc	0.2 (0.1)a	4.2 (1.0)b
	9.2	2.3 (1.4)b	0.6 (0.1)c	0.5 (0.1)b	0.2 (0.1)c	0.1 (0.1)a	3.7 (1.4)ab

Values in parenthesis refer to ±SD and n=5. The total WL is sum of cycles 1 to 5. WLs are significantly different ($P<0.05$) between the WPGs in particular cases for the wood species. Number followed by different letters for each wood species are significantly different at the level of $P<0.05$ according to Tukey's test.

In general, the wood blocks of all species modified with CNSL resin showed greatest WL after the first water soak and oven dry cycle of the test. In most instances, WL of all wood samples declined with each successive cycle. With Scots pine the total WL losses were similar for each WPG but in the Obeche the losses increased and then declined while in Gmelina the total varied. Statistically significant difference ($P<0.05$) was found in WL between the WPG levels in some of the cycles. But the total WL was not significantly different ($P>0.05$) in any of the wood species.

4.3.5. Water absorption and water repellent efficiency

The water absorption and water repellent efficiency of the CNSL resin modified wood blocks are also evaluated from the water soak and oven dry cycle of dimensional stability test. The results of the water absorption (WA) and water repellent efficiency (WRE) of Scots pine, Obeche and Gmelina wood modified with CNSL resin with successive cycles are shown in Table 4.9 and 4.10 respectively.

Table 4.9: Water absorption of the CNSL resin modified wood as determined from the successive WS and OD cycle of the dimensional stabilization test.

Wood Species	WPG	Water absorption (%)					
		Cycle					Average
		1	2	3	4	5	
Pinus sylvestris	0.0	195.7 (24.0)a	200.8 (25.8)a	203.6 (26.3)a	209.8 (27.4)a	206.5 (26.5)a	203.3 (25.9)a
	5.5	147.0 (6.3)b	172.1 (7.2)b	179.7 (6.7)ab	183.8 (8.3)ab	184.6 (7.4)ab	173.5 (6.9)b
	11.1	126.2 (10.9)bc	154.7 (10.3)b	164.7 (13.4)b	166.2 (12.1)b	170.6 (13.0)b	156.5 (11.8)b
	16.5	126.4 (10.6)bc	152.6 (7.5)b	159.3 (5.8)b	165.9 (6.5)b	168.8 (3.7)b	154.6 (6.1)b
	28.5	108.3 (6.0)c	120.9 (10.4)c	124.2 (9.3)c	131.9 (6.2)c	141.7 (6.7)c	125.4 (6.9)c
Triplochiton sclerexylon	0.0	196.0 (36.9)a	218.1 (36.1)a	217.8 (40.7)a	223.5 (38.4)a	224.1 (34.3)a	215.9 (37.2)a
	3.8	161.1 (15.0)a	167.0 (16.1)b	176.5 (17.1)ab	183.4 (14.7)ab	183.6 (16.6)ab	174.3 (15.6)b
	6.7	159.9 (16.4)a	162.8 (15.1)b	174.5 (19.6)ab	183.2 (9.6)ab	188.3 (19.1)b	173.7 (15.9)b
	9.2	155.5 (20.7)a	155.6 (22.8)b	171.6 (23.2)b	181.2 (22.0)b	182.9 (17.6)b	169.4 (19.6)b
	13.9	104.9 (12.1)b	105.6 (5.9)c	107.0 (3.4)c	116.8 (2.6)c	122.2 (3.9)c	111.3 (4.5)c
Gmelina arborea	0.0	179.9 (16.2)a	170.7 (28.5)a	157.6 (26.2)a	149.3 (21.0)a	166.4 (30.6)a	164.8 (23.7)a
	2.7	175.7 (9.4)a	154.8 (3.8)b	141.9 (2.2)b	145.9 (10.5)a	155.9 (3.7)a	154.8 (5.0)a
	4.3	172.3 (5.1)a	142.1 (5.2)b	140.4 (10.3)b	143.5 (6.7)a	154.5 (4.0)a	150.5 (5.4)a
	6.5	169.0 (8.0)a	141.9 (18.0)b	137.6 (25.5)b	141.3 (34.7)b	151.1 (14.8)a	148.2 (13.2)b
	9.2	165.3 (10.4)a	126.9 (9.0)c	118.0 (13.9)c	134.9 (15.7)b	138.8 (9.7)b	136.8 (12.9)b

Values in parenthesis refer to ±SD and n=5. The average WA is of cycles 1 to 5. WA is significantly different ($P<0.05$) between the WPGs in particular cases for all of the wood species. Number followed by different letters for each wood species are significantly different at the level of $P<0.05$ according to Tukey's test.

Table 4.9 shows that the WA decreases with increasing WPG but increases with each WS/OD cycle. The lowest WA was found in Obeche modified wood followed by Scots pine and Gmelina. The effect of WPG on WA was found significantly different ($P<0.05$) in most of the cycles and in average for all of the modified wood except cycle 1 for Gmelina modified wood.

The WRE also increases with increasing WPG. This was found for each successive WS/OD cycle for all wood species. In Scots pine modified wood the WRE decreased with each cycle at each WPG, but in Obeche and Gmelina wood there no such trend was observed. Among the species, Obeche modified wood obtained the highest average water repellent efficiency (48.4 %) at 13.9 WPG. While a 38% WRE was found at 28.5 WPG in Scots pine modified wood (Table 4.10). The lowest WRE was observed with Gmelina modified wood, 17% at low 9.2 WPG.

Table 4.10: Water repellent efficiency (WRE) of the CNSL resin modified wood as determined from the successive WS and OD cycle of the dimensional stabilization test.

Wood species	**WPG**	**WRE (%)**					
		Cycle					**Average**
		1	**2**	**3**	**4**	**5**	
Pinus sylvestris	5.5	24.9 (3.2)a	14.3 (3.6)a	11.7 (3.3)a	12.4 (3.9)a	10.6 (3.5)a	14.8(3.4)a
	11.1	35.5 (5.6)b	22.9 (2.2)b	19.1 (6.5)ab	20.8 (5.8)b	17.4 (6.3)ab	23.2(5.7)b
	16.5	35.4 (5.4)b	24.0 (1.6)b	21.8 (2.8)b	20.9 (3.0)b	18.2 (1.7)b	24.1(3.0)b
	28.5	44.7 (3.0)c	39.8 (2.3)c	39.0 (4.5c	37.2 (2.9)c	31.4 (3.2)c	38.4(3.4)c
Triplochiton sclerexylon	3.8	17.8 (7.6)a	23.4 (7.6)a	18.9 (7.8)a	17.9 (6.6)a	18.1 (7.4)a	19.2(7.2)a
	6.7	18.4 (8.3)b	25.4 (6.9)a	19.9 (9.0)a	18.0 (4.3)a	16.0 (8.5)b	19.5(7.4)b
	9.2	20.7 (10.5)b	28.7 (10.4)a	21.2 (10.6)a	18.9 (9.8)a	18.4 (7.8)b	21.6(9.0)b
	13.9	46.5 (6.2)c	51.6 (2.7)b	50.9 (1.5)b	47.7 (1.2)b	45.5 (1.7)c	48.4(2.1)c
Gmelina arborea	2.7	2.3 (5.2)a	9.3 (2.2)a	10.0 (1.4)a	2.3 (7.0)a	6.3 (4.4)a	6.1(3.0)a
	4.3	4.2 (2.8)a	16.8 (3.1)b	11.0 (6.5)b	3.9 (4.5)b	7.2 (5.4)a	8.6(3.3)a
	6.5	6.1 (4.4)a	16.9 (4.7)b	12.7 (7.2)b	5.4 (10.4)b	9.2 (8.9)a	10.0(6.4)b
	9.2	8.1 (5.7)a	25.7 (5.6)b	25.2 (8.8)b	9.7 (4.7)b	16.6 (5.8)b	17.0(4.7)b

Values in parenthesis refer to ±SD and n=5. The average WRE is of cycles 1 to 5. WRE is significantly different ($P<0.05$) between the resin loadings in particular cases for all of the wood species. Number followed by different letters for each wood species are significantly different at the level of $P<0.05$ according to Tukey's test.

The effect of WPG on the WRE was found to be significantly different ($P<0.05$) in most of the individual cycles and on average for all of the wood species. The relationship between the WPG and WA, and WRE of the CNSL resin modified Scots pine, Obeche and Gmelina wood is shown in Figure 4.8. The increase of WPG causes the decrease of WA and increase of WRE for all of the wood species. The lower WA and higher WRE were obtained from the Obeche modified wood followed by Scots pine and Gmelina.

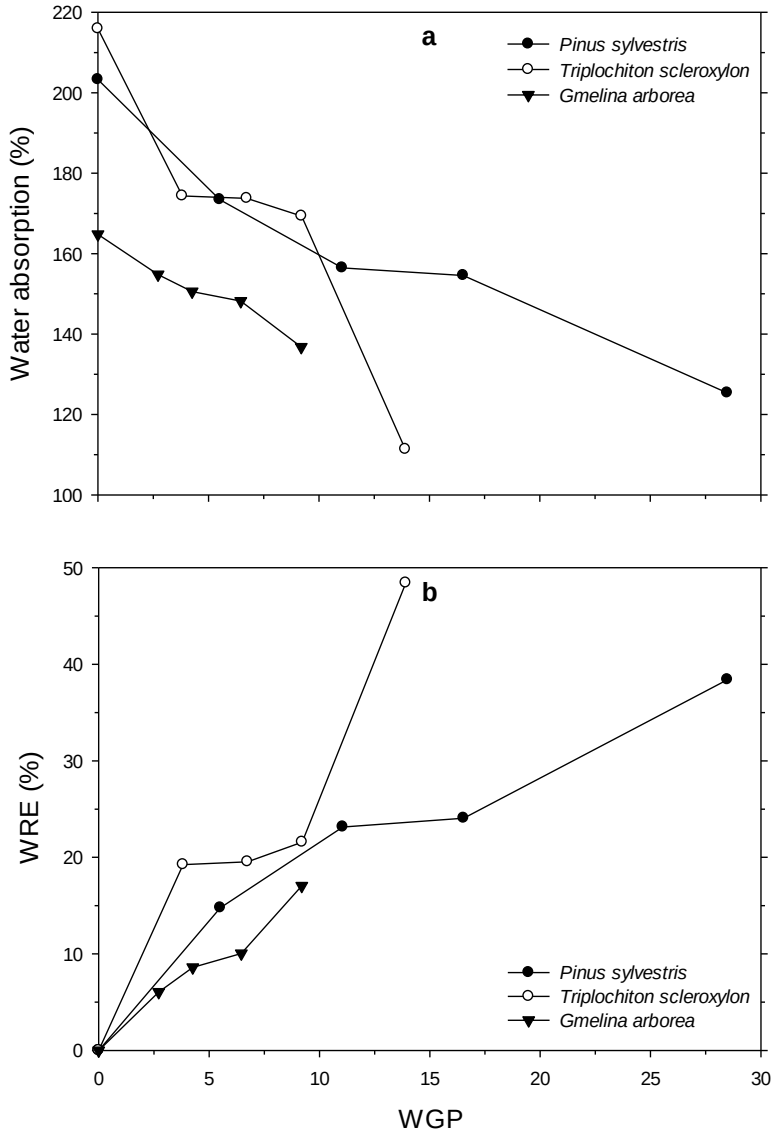


Figure 4.8: Relationship between the WPG and (a) water absorption (WA), and (b) water repellent efficiency (WRE) for *Pinus sylvestris, Triplochiton scleroxylon* and *Gmelina arborea* modified wood with CNSL resin.

4.4. Discussion

4.4.1. Physical properties improvement due to CNSL resin modification

4.4.1.1. Weight percent gain

Weight percent gain (WPG) is commonly used to define the loading of modification of wood with impregnates and is equivalent to a mass/mass loading (g kg $^{-3}$), whereas commercially a mass volume loading (kg m^{-3}) is used for conventional wood preservatives. The SG differences between untreated and treated woods are effectively mass volume loadings. Different levels of WPG were successfully achieved in all species by varying the solution concentration and linear relationships were established for concentration and WPG, however the species behaved quite differently: in that the most permeable species, Scots pine, achieved significantly higher WPG (ca. 28) in comparison to Obeche (ca. 15) and Gmelina (ca. 10). Generally, the differences of WPG were anticipated as the wood permeabilities are different. Although Islam *et al*. (2012) found a correlation between the WPG and density of wood and Yap *et al*. (1990) showed similar, in the current work the high permeability of the softwood, Scots pine is an overriding factor. Obeche however, of lower density (0.33) did achieve higher WPG (15.5) than the higher density Gmelina (SG_b 0.36, 9.4 WPG). There is however little indication of the location of the resin inside the wood cells and in the hardwoods there may be a greater loading within vessels rather than within other cell types (fibres, parenchyma).

4.4.1.2. Volume change

Hill (2006) stated that volume change (VC) or bulking coefficient (BC) is an important indicator that chemical reaction has occurred inside the cell walls rather than just within the cell lumens. Thus, wood not only gained weight but also swelled due to modification indicating the impregnant has penetrated into the cell wall. In this study, CNSL resin impregnation showed linear relationships between the VC and WPG for the different wood species (Figure 4.2). This indicates that higher amounts of CNSL resin are located in the wood cell wall with the increased WPG.

Despite lower permeabilities and lower VC_{max} values for Obeche and Gmelina (5.5%, both) than Scots pine (7.1%), at equivalent VC values (e.g. 5.7, 5.5 and 5.5, Table 4.3) the WPG for Gmelina is considerably lower. This may indicate that much of the resin did not enter the cell wall, particularly at the higher WPG values in Scots pine. Conversely, this may indicate that there is better cell wall penetration in Gmelina, but this will be discussed later (section 9.3.5) in the context of overall distribution where Gmelina showed poor depth of treatment.

The results of the V_{rel} (theoretical/measured volume) also confirm that a lower proportion of resin is located in the cell wall as the WPG increased. If the entire resin was located within the cell wall at every level of modification, a value of one would be found for each V_{rel} and that did not change with resin loading. Hill and Mallon (1998) mentioned that this method focuses on the contribution of the volume of the impregnants to the volume increase due to impregnation modification.

The V_{rel} values are close to unity at up to 10.3 WPG for Scots pine but increase at higher values. In Obeche and Gmelina the values increase to close to (Obeche 0.9) or just above (Gmelina 1.03) unity with increasing WPG. The higher than unity values for V_{rel} in Scots pine and possibly Gmelina show the measured increase of volume is less than the theoretical value of volume increased and indicates that some of the resin was not located within the wood cell wall but is also located in the lumen. Furthermore, the lower than unity values of V_{rel} in Obeche and Gmelina at lower WPGs indicate that the volume change is not due solely to the presence of resin in the cell wall. If all the resin was located in the cell wall at each level of treatments then a value of unity would be found for Vrel and that would not change with the WPG (Hill and Jones, 1996; Hill *et al*., 2004a).

The ability of CNSL resin molecules to penetrate into the wood cell wall is an important question itself. Hill (2006) mentioned that molecules of diameter 2-4 nm or more are unable to penetrate the cell wall micropores as this is the maximum diameter of a typical cell wall micropore. In a study of the penetration of organic liquids, Mantanis *et al*. (1994) reported that liquids with molar volume of larger than 100 cm^3 mol^{-1} (estimated 0.68 nm molecular diameter) have limited capability to penetrate into the cell wall of oven dried wood. But it was not possible to calculate the accurate molecular volume of the CNSL resin as it contains different functional groups which were roughly estimated to molecular weight 100 – 400 (Table 4.1). So, it can be assumed that some portion of the cell wall micropores were inaccessible to the resin due to their size.

4.4.1.2. Change of specific gravity and void volume

The impregnation modification with CNSL resin has significantly affected the oven dry specific gravity (SG_{OD}) of wood of Scots pine, Obeche and Gmelina. It is found that the improvement of SG of ca. 20% was found in Scots pine which is almost double of the improvement in Obeche and Gmelina (both show 10%). It should be noted that the SG_{OD} was increased with the increase of resin loading or WPG in to the wood. This means that a greater amount of CNSL

resin was located in the Scots pine wood than the other two wood species. The increase of SG_{OD} of modified wood is also attributed due to the specific gravity of the CNSL resin itself. This fact is accounted as the lower SG_{OD} of Obeche unmodified wood was increased to almost similar amount of increase of SG_{OD} of Gmelina (10%). Hence, the SG_{OD} of unmodified Gmelina wood is higher than the unmodified Obeche wood.

Increasing amounts of CNSL resin after treatment leaves a lower residual void volume. The greatest void volume reduction was found in Scots pine wood (7%) with lower amounts in Obeche and Gmelina at ca. 3% reduction. The reduction of the void volume is thus related to the increase of SG_{OD} of wood of the tested wood species. The effect of WPG on the reduction of void volume due to the modification in Scots pine, Obeche and Gmelina wood is also significant. The reduction of void volume is an indication of the amount of resin in cell lumen rather than the micro voids of the cell wall. Cell wall micropores may also be considered as void space but these will change with swelling and the entry of solvent and resin into the cell wall.

4.4.2. Leaching of CNSL resin

Hill (2006) mentioned that the effectiveness of impregnation modification relies on stability to leaching in service. In this study, the EN 84 water leaching test revealed that a small amount of CNSL resin was leached out from the modified wood blocks (Figure 4.3). The effect of WPG on the amount of leachable resin was not significantly different indicating that the bulk of impregnated resin was substantially fixed in the wood. Modified Gmelina showed significantly higher leaching, followed by Obeche and Scots pine. This may indicate more unfixed CNSL resin in cell lumen or cell wall micropores of Gmelina modified wood blocks.

It is also noted that the difference of WPG of modified wood before and after the leaching test was not significant (Figure 4.4). In almost each case the difference of WPG was found equal at each resin loading level, at ca. 2%. It was also evident that the WPG was decreased in the control wood blocks due the leaching which indicate that the leaching of resin was accompanied with the water soluble fragments of wood. Small variation of WPG was observed for the three wood species, although the difference in WPG is higher in Gmelina modified wood. This small weight loss during the water leaching may be the loss of water soluble wood extractives and wood degradation products, possibly also hemicelluloses rather than resin components.

4.4.3. Dimensional stabilization

4.4.3.1. Volume changes due to water soak and oven dry cycle

In this study, cyclic volume changes were observed with exposure of the resin modified woods to wetting and drying cycles (soaking and oven drying), and there was a general decrease in amplitude with each cycle. A possible reason for this might be that the OD and WS volume decrease to losses of CNSL resin caused by hydrolysis of the resin, but Hill (2006) claimed that if the OD and WS volumes decrease at an equal rate due to loss of the most thermo-labile component of wood i.e. hemicellulose, but not to loss of the modifying components. It is also important to note that if the impregnated agent is being lost due to the cyclic test, the OD volume would typically decrease and WS volume increase. Earlier work of Hill and Jones (1996) and Hill and Mallon (1998) showed this using anhydride modification, although the reverse was also found in these studies when succinic anhydride and heptanoic anhydride was used to modify wood.

Many earlier studies have reported that the first cycle of such tests usually produces unrepresentative data (Hill and Jones, 1996; Hill and Mallon, 1998; Hill *et al*., 2004a) and thus ignored. Rafidah *et al*. (2006) pointed that when wood samples are measured after solvent extraction and oven drying, they exhibit volumes higher than the volume measured after drying from a cell wall water saturated state. As a result a higher WS volume and lower OD volume are observed in the first cycle, but in this study the volume of modified wood samples in OD and WS volume after the first cycle has been found consistent with the consecutive cycles. Despite this it is possible that there might be non-bonded but cell wall bulking agents remaining after the treatment and these leached out in the first cycle causing a lower OD volume and higher WS volume (Rowell and Ellis, 1978).

4.4.3.2. Volumetric swelling co-efficient and anti-swelling efficiency

The CNSL resin brought a modest improvement in dimensional stability to the three tested wood species. As the WPG increases the S% reduces and ASE% increases in all wood species (Figure 4.6). Among the species a significantly higher dimensional stability was found in Obeche wood, combined with a lower S% of the control wood blocks. Both the Obeche and Gmelina wood showed greater dimensional stability with low WPGs than the Scots pine wood. This is because the control blocks of these woods also showed higher dimensional stability than the Scots pine controls. Even at the highest WPG (28.5) the Scots pine modified wood did not provide a better ASE value than the modified hardwoods. This may be because much of

the resin was polymerised and located in the wood cell lumens rather than in the cell walls, so did not contribute to the dimensional stability.

The CNSL resin imparted increased dimensional stability of wood due to incorporation of the resin in the cell wall so far and permanent cell wall swelling (bulking) under the dry condition. The permanent swelling or bulking was increased with the increase of resin loading or WPG (Figure 4.2). Many workers have reported that the ASE is strictly correlated to the bulking of the wood cell wall by chemical add on (Hill and Jones, 1996; Hill and Mallon, 1998; Hill *et al*., 2004a; Xiao *et al*., 2010). Owing to the effect of cell wall bulking the ASE increased with the increase of WPG. To find out why the CNSL resin modification imparts dimensional stability to the CNSL modified wood the ASE value was plotted against the VC% (bulking) and this showed a close relationship (Figure 4.7).

When the average of the five successive cycles is examined a modest dimensional stability of ca. 30% is found for all wood species, but a higher ASE value was observed in the first cycle (Table 4.7). Moreover, no steady loss of ASE was found in the cyclic test (Table 4.7). This can be explained as the loss of ASE was not due to the loss of modifying agents rather a loss of wood extractives and / or the wood hemicellulose. This is further observed when the results of the weight loss due to hydrolysis are evaluated in the next section.

It is very difficult to compare the dimensional stabilization data between the different resin systems reported in the literature as the results do not follow a standard set of procedures and differences in wood species, dimensions, treatment procedures, loadings and test methods, often involving only one a cycle dimensional stability test.

A verity of resin system was also applied to improve the dimensional stability of wood. Rapp and Peek (1995) reported that a maximum of only 20% ASE was achieved when wood was impregnated with 30 different water based resin system at up to ca 250 kg m^{-3} retention in Scots pine and beech wood. Most of their resin system showed poor penetration into the wood blocks and thus poorly imparted dimensional stabilization. In another study Sailer and Rapp (1997) found methanol etherified melamine formaldehyde resin provided the best ASE at 25% of four impregnated wood-resin systems tested. However, good dimensional stabilization was reported when wood was modified with different formaldehyde based resins including phenol formaldehyde (PF), melamine formaldehyde (MF) and urea formaldehyde (UF) (Deka and Saikia, 2000). They reported that ca. 70% ASE was achieved with PF and MF,

and 50% ASE with UF, when the wood of *Anthocephalus cadamba* was treated by these resin at 34 WPG. This resin loading gave a volume increase of 9 to 14%.

PF resin was a promising dimensional stable agent in an earlier study (Stamm and Seborg, 1939). Gabrielli and Kanke (2010) and Furuno *et al*. (2004) also found 70% ASE by modifying with PF resin, but most of these works were done with a low molecular weight resin system. Ohmae *et al*. (2002) also found a greater ASE (74%) with a low molecular weight PF resin at a 30% WPG. The low molecular weight resin system can penetrate the wood cell wall and thus contributed to the dimensional stability as bulking is related to the ASE. The molecular weight of CNSL resin is quite large and varies from 100 to 400. This may also reflect on the resin loading and volume change results, as CNSL resin modified wood in this study achieved low WPG and VC.

4.4.4. Stability to hydrolysis

Stability to hydrolysis is particularly important when a realistic multi-cycle WS and OD method is used (Hill, 2006) because leaching of modifying chemical will be evident as the test proceeds. In this study, the greatest weight loss was found in first WS/OD cycle of the test and thereafter it was lower for subsequent cycles and in total over all of the cycles loss values of 2.1% to 4.2% occurred (Table 4.8). This weight loss is not accompanied by the loss of ASE over the successive cycles which suggests that there was minimal loss of resin from the cell wall.

In some cases of the cyclic test the WPG has a significant effect on the weight loss but when the total weight loss is examined over the five WS/OD cycles the effect of WPG was insignificant on weight loss except for Gmelina wood. At 6.5 WPG the weight loss of modified wood was significantly higher than the control wood blocks in Gmelina wood but weight loss of modified wood was almost equal to the control wood blocks of Obeche and Scots pine. This weight loss data is in agreement that found in the EN84 leaching test (section 4.3.2, Figure 4.3), on the first cycle, although it increased when the sum of the five cycles is considered. Without a full compositional analysis of leachate is not possible to be certain but it is assumed that the weight losses of the cycles is associated with the wood losses, rather than resin losses. Once again this could be water soluble fragments of hemicellulose and other wood extractives. A small portion of sapwood extractives can be leached out as the test specimens were not solvent extracted before the resin impregnation.

4.4.5. Water repellency

The water repellency of CNSL resin modified wood was evaluated by measuring the water absorption (WA, Table 4.9) and water repellent efficiency (WRE, Table 4.10). The water repellency of wood is improved due to the modification with CNSL resin. The WA was reduced and WRE was increased with the increase of resin loading or WPG. Resin modified wood blocks of the three tested species showed significantly lower WA than their matching control wood blocks. It is apparent that WPG has large influence on the water repellency of modified wood. In the cyclic test no consistent trend of increasing or decreasing of water repellency was observed in the subsequent cycles suggesting that the reduction of water repellency of the resin modified wood was not due to loss of impregnated resin rather than loss of soluble component of wood materials. Significantly greater water repellency was found in Obeche modified wood where ca. 50% WRE was achieved with a comparatively lower WPG (13.9) than Scots pine which only achieved 38.4% at ca. double the WPG (28.5). Among the species Gmelina showed lowest water repellency since it obtained lowest WPG, but at equivalent values of WPG it showed lower water repellency (cf. Obeche 6.7 and 9.2 WPG with Gmelina 6.5 and 9.2 WPG).

Yang *et al*. (2013) suggested that improved water repellency in modified wood is a complex phenomenon which involves bulking of the cell wall or lumen or cross-linking of the wood polymers and or hemicellulose degradation. Baysal *et al* (2004) found chemical bonding between the cross-linking agents and wood cell wall when modified wood with furfuryl alcohol catalysed by borates. A similar observation was reported by Jinshu *et al*. (2007) that hydrophilic groups of wood cell wall were replaced by functional groups of modifiers namely urea formaldehyde resin and nano-SiO_2.

The decrease of WA and increase of WRE of CNSL resin modified wood can be attributed to the hydrophobic nature of CNSL resin which impedes water absorption and improves repellency. The interaction by reaction or blocking of the OH groups available to water in wood cell wall polymers with the functional groups of the CNSL resin may have been considered to effectively improve water repellency (Rowell and Banks 1985). From the volume change data it can be assumed that the lumens are occupied by the hydrophobic side chain of the CNSL resin and thus, there is little possibility of cross linking of the resin with the water assessable sites of wood cell wall. In addition the capillaries of the lumens in wood might be blocked by the CNSL resin and thus water is lost from its main channel.

4.5. Conclusions

Impregnation modification with CNSL resin has improved the physical and moisture related properties of Scots pine, Obeche and Gmelina wood, although not in equal amounts relative to the different WPG achieved.

1. The WPG and VC linearly increased with increased of resin concentration. Significantly greater WPG occurred in Scots pine wood than Obeche and Gmelina mainly due to permeability differences. VC also increased to ca. 7% in Scots pine and ca. 5% in Obeche and Gmelina wood which indicates that some amount of resin penetrated into the wood cell wall which is a prerequisite of modification to enable dimensional stability of wood. However the V_{rel} results reveal that large portion of resin can be located in the cell lumens. There is the possibility that the greater molecular volume of the CNSL resin components are unable to penetrate the microvoid networks of the wood cell wall because of their size. The CNSL resin is composed of long unsaturated side chain, which creates the bulk of the macro void volume of the wood.

2. The SG_{OD} and void volume are largely influenced by the CNSL resin impregnation. SG_{OD} is increased in Scots pine (20 %) double that of the Obeche and Gmelina and thus gave greater densification. As the specific gravity increased the void volume of the modified wood decreased due to the resin loading. The reduction of the void volume is in effect the gross macro void volume of wood that is occupied by the CNSL resin and consequently a greater void volume reduction was found in Scots pine than Obeche and Gmelina.

3. CNSL resin leachability according to EN84 was minimal. This was further substantiated by the cyclic soaking and oven drying test where little loss occurred. Performance of the impregnation modification of wood is largely depends on the non leachability of the impregnants. The small leaching losses which occurred could be explained as losses of wood extractives and soluble storage polysaccharide and hemicellulose losses as the control blocks showed similar losses. Some minor losses could also be explained by unpolymerised resin components but this was not thought to have occurred.

4. The CNSL resin imparted moderate dimensional stability to the three wood species. The ASE increased with the increased WPG. Surprisingly and significantly higher dimensional stability was found in Obeche wood than Scots pine although it had a lower WPG and provided lower bulking than the Scots pine wood. In addition to the effect of the resin there was a contribution from the wood itself, where the control blocks of Obeche showed a lower

volumetric coefficient of swelling. The reduction of the ASE over the successive WS and OD cyclic test did not provide any specific trend suggested that this reduction is not due to the loss of resin rather than the loss of wood extractives and or wood hemicellulose. This is not the loss of dimensional stability of the modified wood as the OD and WS volumes decrease at an equal rate over the five successive cycles.

5. This is further supported from the results of the hydrolysis test, as found that the weight loss due to the cyclic test was neither controlled by the cycles (hydrolysis) nor the resin loading. The CNSL resin provided moderate dimensional stability to wood as the mean ASE value of five successive cycles was used in this study though the ASE value of the first cycle was significantly higher for all of the wood species. This is considered in respect of the long term stability of wood in service; many previous studies have only considered one cycle, which has subsequently shown to be unrepresentative of the dimensional stability of wood.

6. The water repellency (WRE) of wood is also upgraded due to the CNSL resin modification. Obeche provided better water repellency than the other two wood species. The WRE was greatly influenced by the WPG but not by the cycles of water soaking. It can be assumed that the hydrophobic nature of CNSL resin is chiefly responsible to impart the water repellency in modified wood.

Chapter 5

Dynamic mechanical thermal properties of CNSL resin modified wood

5.1. Introduction

Wood is considered a unique polymeric composite consisting of several polymeric materials i.e. cellulose, hemicelluloses and lignin. The response to external stress applied to wood is a consequence of interactions of these wood cell wall polymers (Kelley *et al*., 1987). Like other polymeric materials, wood also demonstrates viscoelastic behaviour meaning that it has elastic and viscous properties. This is why if any external stress is applied to wood it will result in strain but with the progress of time this strain will extend, which is termed creep. Moreover, viscoelastic materials also experience relaxation phenomena associated with molecular mechanisms which change considerably with temperature. The amorphous components of wood (hemicelluloses and lignin) go through several thermal transitions from the glassy to the rubbery state which is termed glass transition (T_g) (Kelley *et al*., 1987). These transitions mark a change in the mechanical and other properties of wood (Backman and Lindberg, 2002).

The dynamic mechanical thermal analysis (DMTA) is an excellent technique to determine the thermal and mechanical properties of wood which is another way to investigate the viscoelastic properties of wood. This method is also reported to provide valuable information regarding the wood polymer relationship, structure and morphology (Manchado and Arroyo, 2000). DMTA has been used by many workers to investigate the viscoelastic properties of wood, mainly to understand the structure of wood polymers and the effect of moisture on its properties (Salmen, 1984; Birkinshaw *et al*., 1986; Kelley *et al*., 1987; Backman and Lindberg, 2001), but the most popular use of dynamic mechanical analysis is in the viscoelastic study of adhesives and other polymers, particularly to study the compatibility in polymer blends (Manson and Sperling, 1976; Wetton, 1993; Nielsen and Landel, 1994; Chen and Gardner, 2008). This method can also be used to investigate the viscoelastic properties of other ligno-cellulosic materials like bamboo and cane (Obataya and Norimoto, 1999; Liu *et al*., 2012).

DMTA is also used to understand the adhesion between wood and polymers at the cell wall level. Significant work has been carried out to obtain information needed in the application of glues and paints to wood. In most cases changes in T_g are observed which indicate the degree

of interaction between wood and polymers. Early work by Handa *et al.* (1980) showed interactions between wood and polyoxyethylene glycol methacrylate (PEGMA) gave lowered peaks from 230 to 200 °C and attributed this to the cellulose T_g and interactions in the cell wall. Backman and Lindberg (2002, 2004) studied the interaction of wood with polyvinyl acetate glue, polyurethane-alkyd lacquer and polymethylmethacrylate polymer by DMTA and found that decreases in T_g were accompanied by good adhesion at the cell wall level. DMTA has also been usefully used to study the dynamic mechanical thermal properties of newly developed extruded nylon-wood composites (Chen and Gardner, 2008), and viscoelastic properties of tropical wood polymer composites (WPC) made of oxidized sodium metaperiodate and phenyl hydrazine (Hamdan *et al*., 2010).

Resin modification can improve the mechanical properties of wood but other forms of modification i.e. chemical and thermal may have adverse effects on its mechanical properties. The study of thermo-mechanical properties or the viscoelastic properties of resin modified wood has not been reported to date. It will be an opportunity to study the thermo-mechanical properties of CNSL resin modified wood by using DMTA. This chapter will also investigate the compatibility of CNSL resin with wood polymers as it will provide an insight of interaction or possible cross-linking of resin with the wood cell wall.

5.2. Concept of DMTA

The DMTA is a technique in which a small sinusoidal stress is applied to the test specimen and the resulting strain is measured. The load is applied at a constant frequency with the temperature is increased at a constant rate. The measurement of stress is defined by mainly two moduli to describe the viscoelastic properties of wood, the storage modulus (*E'*) and loss modulus (*E"*). An important characteristic of DMTA of viscoelastic material is that it can determine the loss factor (tan δ), the ratio between the loss modulus and storage modulus. Generally, the viscoelastic materials have a combination of elastic and viscous properties, with stress and strain out of phase by an angle (δ) less than 90° which is termed as loss factor or tan δ.

The storage modulus is reported over the range of temperatures studied and provides the material stiffness at different temperatures. The loss modulus is explained as an energy loss which is transformed to heat on 'work' in the material. As mentioned earlier, the viscoelastic material experiences relaxation phenomena as a function of temperature e.g. glass transition

temperature (T_g). T_g and other transitions can be determined from the DMTA based on the change in mechanical properties. The criteria for identifying T_g is usually either a) the inflexion point corresponding to a sharp drop in storage modulus or b) peak of the corresponding loss modulus or c) peak of the loss tangent (tan δ) (Nielsen, 1974; Chen and Gardner, 2008), but Herzog *et al*, (2005) reported that the T_g value found from storage modulus and from tan δ have significant difference so that in the present study the T_g value found from tan δ is used.

The tan δ varies considerably with the change of temperature. Kelley *et al*. (1987) reported that the temperature at which the T_g occurred is largely subjected to the individual components of wood, i.e. cellulose, hemicellulose and lignin, the frequency of the oscillation and also the heating rate also affects the results of DMTA (Chen and Gardner, 2008). The presence of moisture in wood also affects the glass transition temperature which is widely studied by several workers (Handa *et al*., 1980; Back and Salmen, 1982; Kelley *et al*., 1987; Liu *et al*., 2012). In totally dry wood, cellulose, hemicellulose and lignin have T_g temperatures of 200-250 °C, 150-220 °C and 130-205 °C respectively. As the wood becomes wetter the T_g and other transition peaks are lowered.

5.3. Materials and methods

5.3.1. Wood samples and preparation

Four wood species, one softwood and three hardwoods were selected for this study. Sapwood samples of Scots pine (*Pinus sylvestris* L.), Obeche (*Triplochiton scleroxylon* K. Schum.), Gmelina (*Gmelina arborea* Roxb.) and Alstonia (*Alstonia scholaris* L. R. Br.) were prepared to dimensions of 4.5 x 2.5 x 46 mm (radial x tangential x longitudinal). The hardwood Alstonia was introduced in this experiments as it was evident that the other two hardwood species, Obeche and Gmelina were not sufficiently permeable and did not impregnate well with CNSL resin (chapter 4). Thus, it was expected that more permeable Alstonia will be treated well and produce a good WPG values. All samples used in the experiment were selected with straight grain free from any defects (e.g. knots). After sanding to remove loose fibres, the wood samples were stored at 20 °C and 65% relative humidity (RH) until they reached equilibrium moisture content.

5.3.2. Resin impregnation

The wood specimens were vacuum impregnated as described in chapter 4, section 4.2.3 with alcoholic solutions of CNSL resin of 0%, 10%, 20% or 30% solute content in industrial methylated spirit (IMS) to achieve different weight percent gain values (WPG). After the resin curing the modified wood specimens were stored at 20 °C and 65% RH for a further two weeks.

5.3.3. Dynamic mechanical thermal analysis of CNSL resin modified wood

The mechanical- thermal properties of CNSL resin modified woods were measured using a dynamic mechanical thermal analyser, TTDMA from Triton Technology, Grantham, UK. The storage modulus, loss modulus and loss factor (tan δ) were determined under the sinusoidal load in a three point bending mode with span length of 30 mm. Three frequencies, 0.1, 1 and 10 Hz were used to provide an oscillating force with a heating rate 5 °C/min. Measurements were conducted at temperatures between -150° to 150° C and data were collected at a thermal scan of 2 °C/min. The specimens were clamped into the test equipment immediately after transfer from conditioned storage chamber to avoid moisture changes. The specimens were cooled rapidly in liquid nitrogen. The oven dry basis moisture content of unmodified and modified wood specimens were ca. 8-9% and ca. 5-6% respectively of the four wood species. A minimum of 3 specimens per treatment per species were tested, and the mean value of storage modulus at room temperature was calculated as well as glass transition temperature of the modified wood and the glass transition of the untreated wood.

5.3.4. Statistical analysis

The data obtained were analysed using IBM® SPSS® Statistics 20 by the one way analysis of variance (ANOVA) test to determine the statistical difference followed by Tukey's multiple comparison test. The results with $P<0.05$ were considered to be statistically significantly different.

5.4. Results

The impregnation modification gave a range of WPG values for the different wood species tested, in line with values achieved previously, i.e. 11.4, 16.9 and 21.6 WPG for Scots pine; 7.8, 12.6 and 17.6 WPG for Obeche; 4.6, 6.9 and 10.8 WPG for Gmelina; and 17.4, 31.4 and 42.5 WPG for Alstonia wood respectively. WPG values for Alstonia wood were higher than the previous maximum of Scots pine. The wood samples of the above mentioned species were also treated with only alcoholic solution (0%) is used as unmodified or control for the DMTA measurement.

5.4.1. DMTA measurement on wood modified with CNSL resin

Graphical representation of the dynamic mechanical thermal properties of unmodified and CNSL resin modified Scots pine, Obeche, Gmelina and Alstonia wood are presented in the Figure 5.1 (a, b, c, d), 5.2 (a, b, c, d), 5.3 (a, b, c, d) and 5.4 (a, b, c, d) respectively.

The results of DMTA analysis shows the temperature dependence of storage modulus (*E'*) and loss modulus (*E"*) of the unmodified and modified wood samples when flexed along the tangential direction at a moisture content of ca. 9% and ca. 6% respectively after conditioning.

Although the results of the DMTA analysis presented in Figures 5.1 to 5.4 only show one sample run in the DMTA test the other values were in accordance. The mean values per treatment per wood species have been used to determine the glass transition (T_g) of unmodified and CNSL resin modified wood and in the storage modulus (*E'*) of the unmodified and CNSL resin modified wood at room temperature.

In Scots pine, a substantial decrease of storage modulus (*E'*) was observed over the entire temperature range in both the unmodified and CNSL resin modified wood. The *E'* decreased with temperature increase for each of the 3 frequencies applied. In each case of resin modification, higher *E'* values were recorded at 10 Hz, followed by 1 and 0.1 Hz. Irrespective of frequency, the *E'* of unmodified wood decreased from 1.2 GPa at -150 °C to 0.6 GPa at 150 °C. A better *E'* was found when Scots pine wood was modified with CNSL resin at different WPGs (Figure 5.1 b, c, d). At the highest WPG (21.6) the *E'* dropped from 1.4 GPa to 0.6 GPa over the same temperature range irrespective of frequencies applied, but a sharp loss of *E'* was found from 50 to 90 °C temperature range in modified wood.

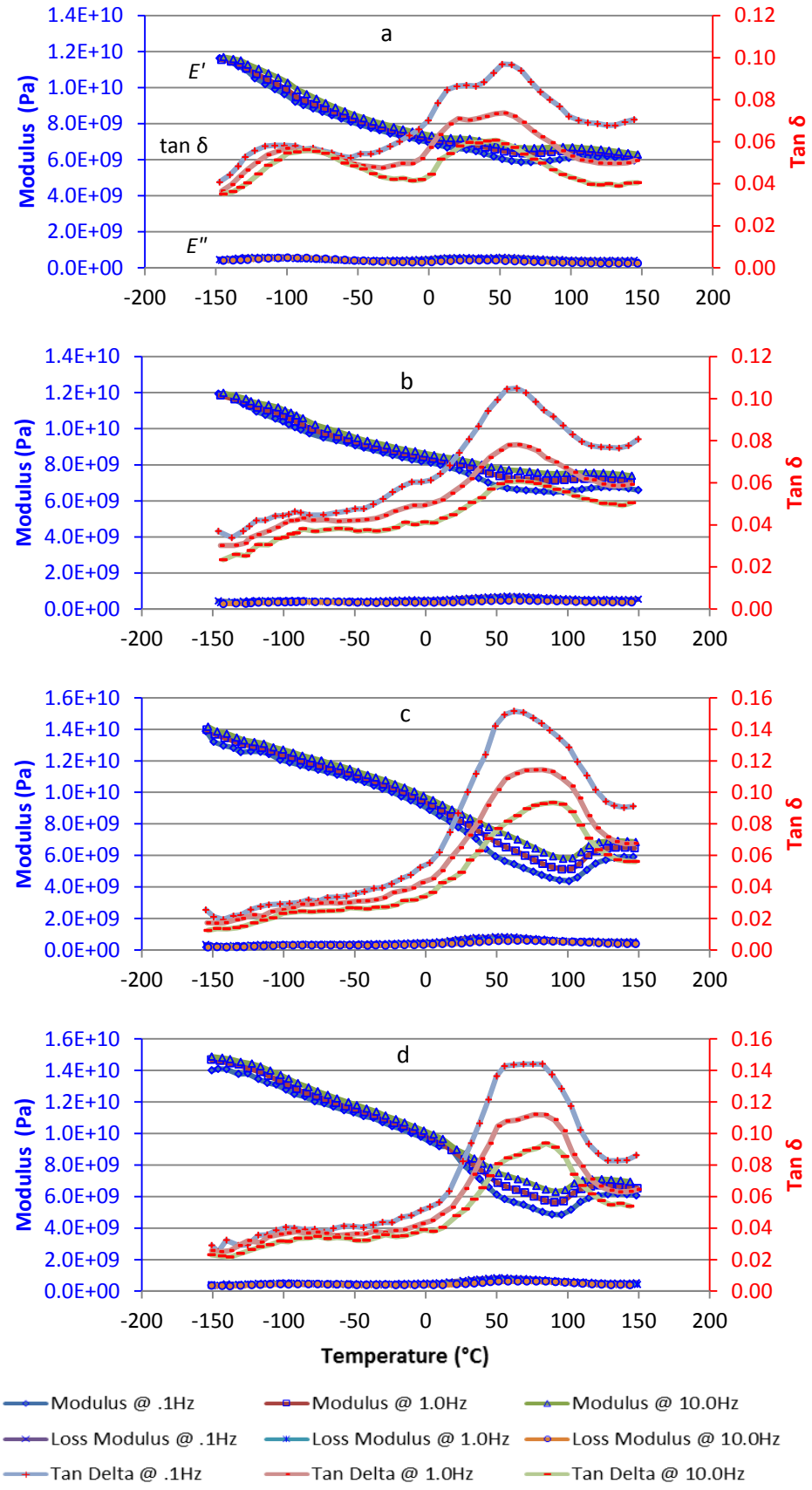


Figure 5.1: Dynamic thermo-mechanical properties of Scots pine (*Pinus sylvestris*) wood (a) unmodified, (b) 11.4 WPG, (c) 16.9 WPG and (d) 21.6 WPG. The curves represent storage modulus (E'), loss modulus (E") and loss factor (tan δ) at different temperatures and frequencies.

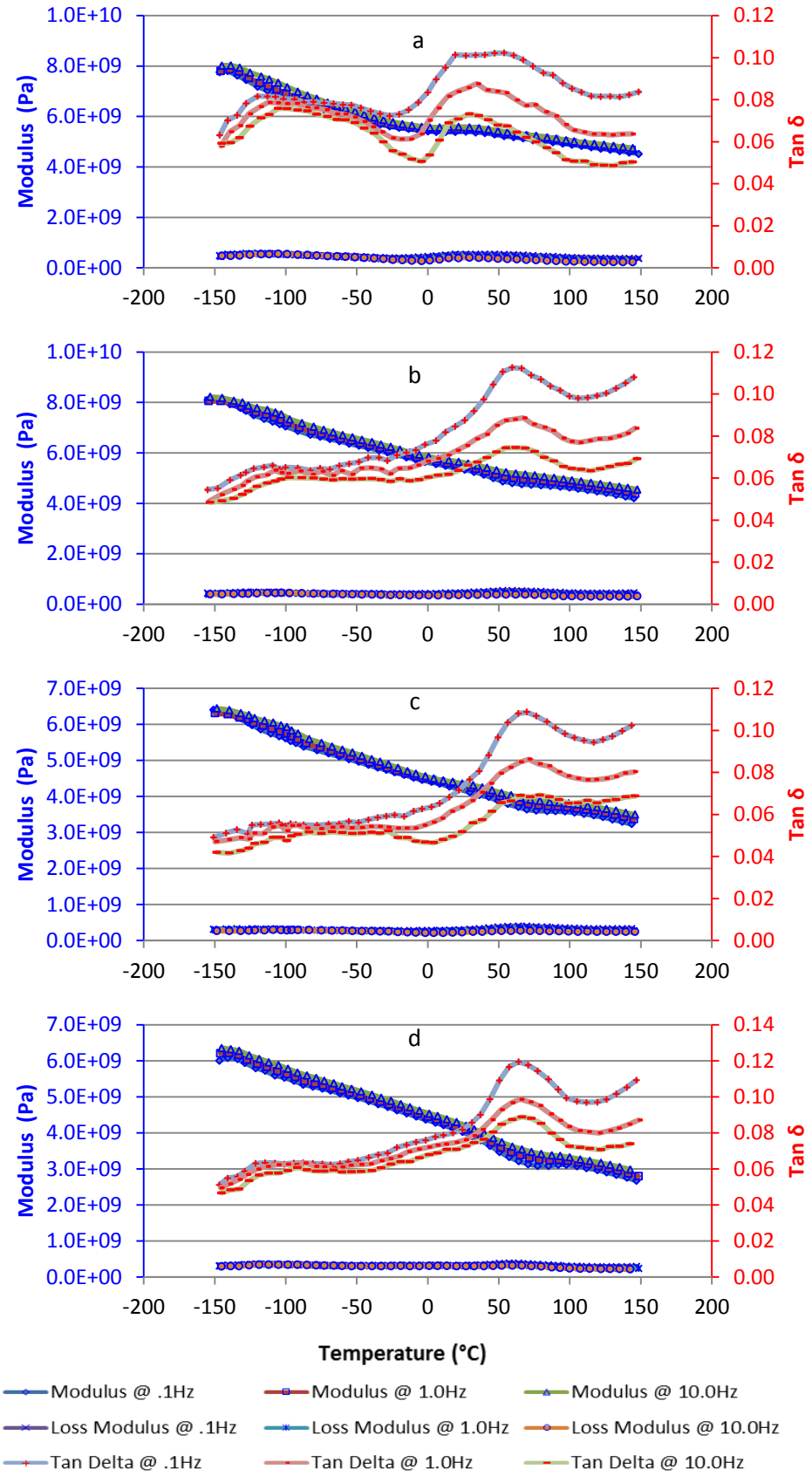


Figure 5.2: Dynamic thermo-mechanical properties of Obeche (*Triplochiton scleroxylon*) wood (a) unmodified, (b) 7.8 WPG, (c) 12.6 WPG and (d) 17.6 WPG. The curves represent storage modulus (E'), loss modulus (E") and loss factor (tan δ) at different temperatures and frequencies.

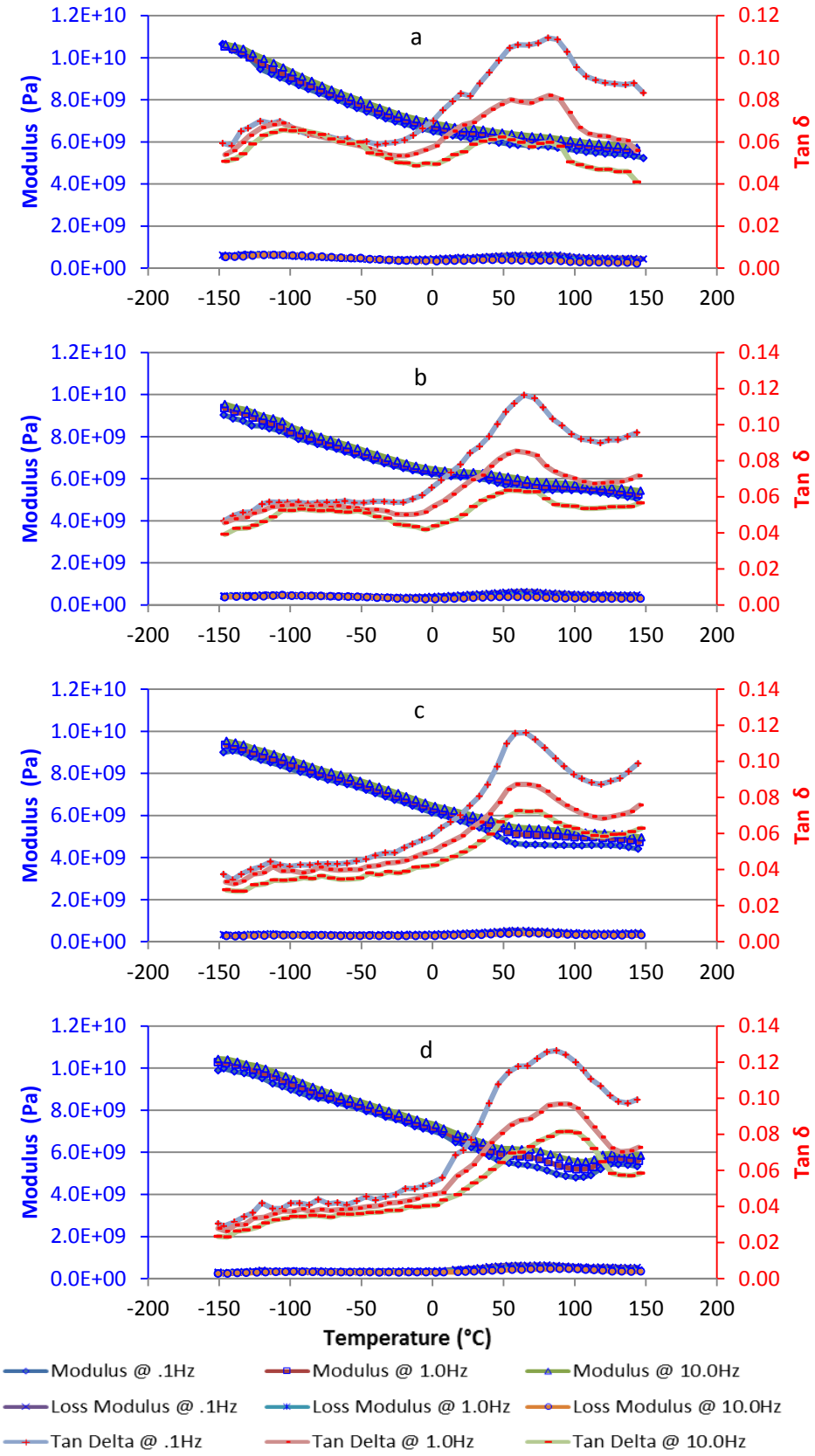


Figure 5.3: Dynamic thermo-mechanical properties of Gmelina (*Gmelina arborea*) wood (a) unmodified, (b) 4.6 WPG, (c) 6.9 WPG and (d) 10.8 WPG. The curves represent storage modulus (E'), loss modulus (E") and loss factor (tan δ) at different temperatures and frequencies.

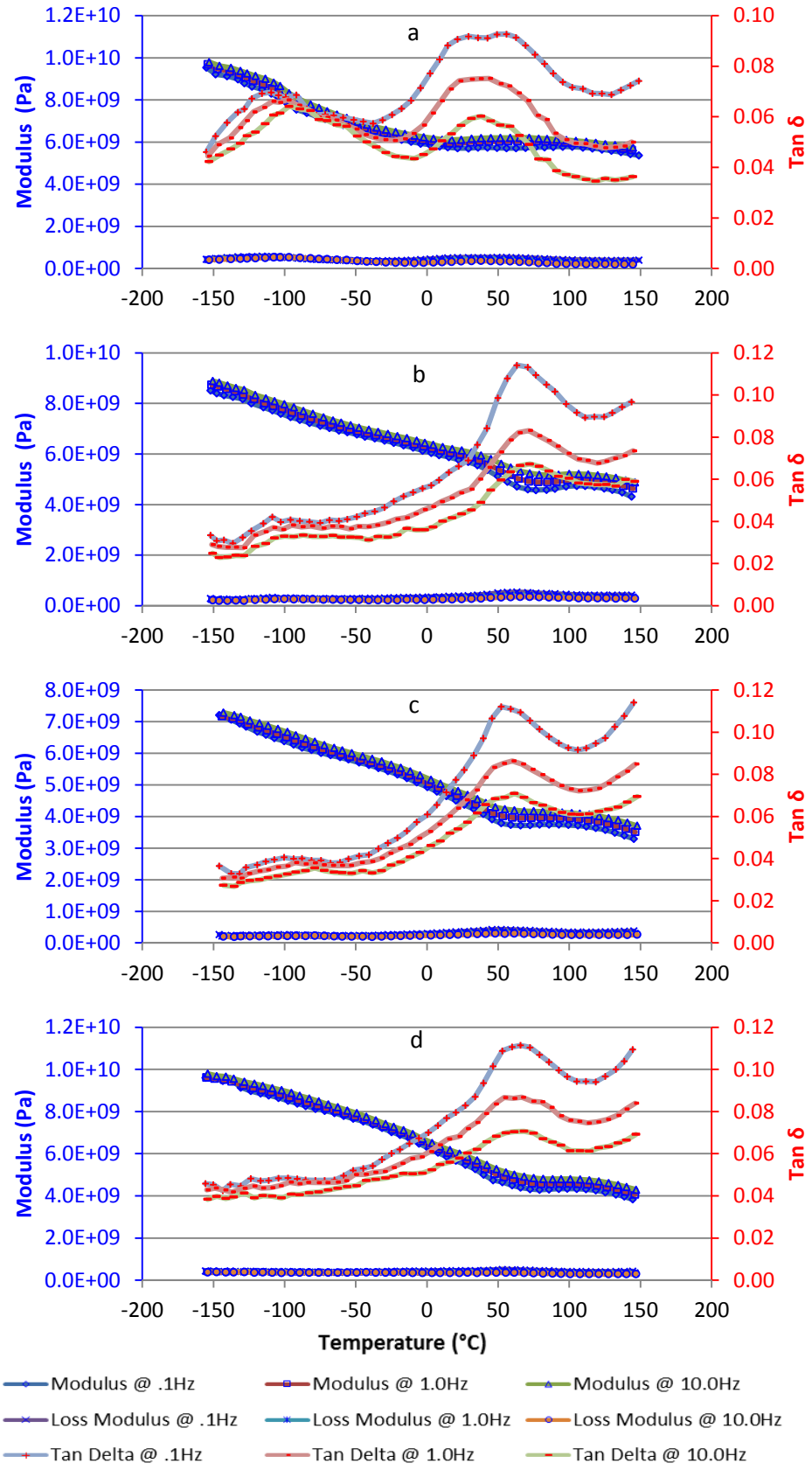


Figure 5.4: Dynamic thermo-mechanical properties of Alstonia (*Alstonia scholaris*) wood (a) unmodified, (b) 17.4 WPG, (c) 31.4 WPG and (d) 42.5 WPG. The curves represent storage modulus (E'), loss modulus (E") and loss factor (tan δ) at different temperatures and frequencies.

The tan δ (loss factor) of unmodified Scots pine wood showed three dominant peaks due to the wood polymer relaxation at each frequency, with the location of these tan δ peaks shifting sometimes due to the frequencies. The height of the tan δ peaks also varied due to the effect of frequency. Instead of showing three peaks the modified wood samples showed one strong tan δ peak at a different temperature and these increased in height with reducing frequencies. At the highest WPG the tan δ was also wide, possibly consisting of two superimposed peaks in the 50-90 °C temperature range. The tan δ peaks temperature corresponded well with the *E'* deflection temperature range of the resin modified Scots pine wood. The loss modulus (*E"*) curve also showed inflections in those temperature ranges corresponding to the tan δ peak and *E'* deflection.

The *E'* of Obeche unmodified and modified wood also decreased with temperature increase but much reduction was observed in modified wood in the temperature range 50 to 90 °C. In both unmodified and modified wood the *E'* decreased from 0.8 GPa to 0.6 GPa over the temperature scan (Figure 5.2 a, b, c, d). In unmodified wood, two broad tan δ peaks were observed at low and high temperatures which arise due to polymer relaxation. The tan δ peak shifted in modified wood from the unmodified wood and the tan δ temperature was also changed between unmodified and modified wood. The height of the tan δ peak also increased with the increase of WPG in the modified Obeche wood. In this case, the effect of frequencies on the storage modulus, loss modulus and loss factor was also evident.

In Gmelina wood, the decrease of *E'* was also observed for both unmodified and modified wood. Unmodified wood storage modulus decrease from 1 GPa to 0.5 GPa and modified wood storage modulus decrease from 1 GPa to 0.6 GPa over the temperature scan from -150 to 150 °C. The *E'* in the modified wood showed a sharp deflection from 50 to 100 °C temperatures and then increased. Unlike the Obeche wood, Gmelina unmodified wood showed three tan δ peaks because of the relaxation of the wood polymers (Figure 5.3a) but as like Scots pine a wide tan δ peak was also found in modified wood at different temperatures. The height of the tan δ peak in the modified wood increased with increased WPG (Figure 5.3 b, c, d) at all frequencies.

In Alstonia wood, the decrease of *E'* is also evident for unmodified and modified wood over the temperature scan. In both cases, the reduction of *E'* was found from 0.9 GPa to 0.5 GPa over the temperature range studied (Figure 5.4 a, b, c, d). The deflection in *E'* curve was found at the temperature range 50 to 90 °C in modified wood samples. Two tan δ peaks were found

at high temperature at 0.1 Hz but only one peak was observed at the 1 and 10 Hz frequencies. Tan δ peak at lower temperature level was found in all frequency levels. The tan δ peaks shifted to a higher temperature in modified wood with a wide peak from unmodified wood to the CNSL resin modified wood. As the WPG increased the height of the tan δ at each frequency also increased.

5.4.2. Glass transition of unmodified wood

It is important to understand the dynamic mechanical thermal behaviour of unmodified wood to differentiate the behaviour of the wood polymers (cellulose, hemicelluloses and lignin) from the applied polymers or resin, which may also show relaxation phenomena. As mentioned earlier each amorphous component of the wood polymers exhibit thermal transitions and relaxation, i.e. the transition from glassy to rubbery state, the glass transition (T_g). Generally, the T_g of the unmodified wood was measured by the tan δ (loss factor) peaks observed during the temperature scan. Conventionally, the tan δ peaks are labelled to α, β and γ in order to decreasing temperature. In wood these may refer to hemicellulose, cellulose or lignin. In wood where all three components are present some peaks may be superimposed. For clarity in some cases α, β and γ notation has been generalised to demonstrate trends between wood species and α or β may be omitted. Table 5.1 shows the mean value of the tan δ peaks of the unmodified wood samples of Scots pine, Obeche, Gmelina and Alstonia at the three different frequencies.

Table 5.1: Loss factor (tan δ) peak temperature (°C) for control (untreated) wood at different frequencies (Hz).

Species	Loss factor (Tan δ) peak	Tan δ peak temperature (°C)		
		0.1 Hz	1 Hz	10 Hz
Pinus sylvestris	α	55.6	54.7	53.5
	β	20.0	19.1	-
	γ	-102.8	-100.8	-91.2
Triplochiton scleroxylon	α	-	-	-
	β	44.9	32.3	29.2
	γ	-114.9	-111.6	-100.0
Gmelina arborea	α	78.5	75.2	71.2
	β	56.3	55.3	49.4
	γ	-104.7	-103.4	-97.7
Alstonia scholaris	α	56.2	-	-
	β	23.6	29.7	32.9
	γ	-108.6	-102.0	-92.2

In Scots pine two tan δ peaks occur at temperatures between 20 to 80 °C and one tan δ peak occurs at a lower temperature between -115 to -90 °C (Table 5.1). The α peak was observed at 55.6, 54.7 and 53.5 °C at frequencies of 0.1, 1 and 10 Hz respectively. No β peak was found when temperature scanned at 10 Hz but 0.1 and 1 Hz β peaks occurred at 20° and 19.1 °C respectively. At the lower temperature range, the γ peak was observed at -102.8, -100.8 and -91.2 °C in frequencies of 0.1, 1 and 10 Hz respectively. The maximum peaks (α and β) shifted from higher to lower temperatures when the frequencies were increased from 0.1 to 10 Hz. The reverse trend was found in case of the γ peak.

In Obeche wood, no particular α peak was observed at higher temperatures at any frequency but the plot showed a shoulder (Figure 5.2 a, b, c, d) and consequently no peak maximum was recorded (Table 5.1). However, a very strong β peak was found at 44.9, 32.3 and 29.2 °C at frequencies of 0.1, 1 and 10 Hz respectively. The γ peak was observed at -114.9, -111.6 and -100.0 °C in frequencies of 0.1, 1 and 10 Hz respectively. The increase of frequency also shifted the β peak from to lower temperature but the γ peak shifted to a higher temperature.

In Gmelina wood, the α peak is observed at 78.5, 75.2 and 71.2 °C in frequencies of 0.1, 1 and 10 Hz respectively. A second peak was observed close to the α peak but the temperature range shifted to a higher temperature than the β peak temperature range found in Scots pine wood. The β peak found in Gmelina wood ranged from 49.4 to 56.3 °C temperature irrespective of frequency. The γ peak was found at -104.7, -103.4 and -97.7 °C at frequencies of 0.1, 1 and 10 Hz respectively.

In Alstonia wood, the α peak was only observed at 0.1 Hz, but for the other two frequencies it was missing. The β peak is present at 23.6, 29.7 and 32.9 °C in frequencies of 0.1, 1 and 10 Hz respectively and this β peak is very strong at 1 and 10 Hz. The low temperature γ peak is found at -108.6, -102.0 and -92.2 °C which were shifted due to increase of frequencies from 0.1 to 10 Hz.

5.4.3. Glass transition of CNSL resin modified wood

The glass transition (T_g) of CNSL resin modified wood was measured from the loss factor (tan δ) peak temperature as it provide practical information for identifying the relaxation temperature (T_g) of the resin applied in wood. The mean T_g of the CNSL resin modified Scots pine, Obeche, Gmelina and Alstonia are presented in the Table 5.2.

Table 5.2: Glass transition (T_g) temperature of CNSL resin modified wood at different frequencies.

Wood species	WPG	T_g (^{0}C) at different frequency		
		0.1 Hz	1 Hz	10 Hz
Pinus sylvestris	11.4	59.8	63.5	67.0
	16.9	62.6	68.3	71.2
	21.6	62.7	70.6	76.4
Triplochiton scleroxylon	7.8	62.4	65.9	67.1
	12.6	65.5	66.6	68.0
	17.6	66.5	69.3	70.6
Gmelina arborea	4.6	64.7	65.8	66.8
	4.8	67.6	68.9	70.1
	10.8	69.2	70.6	71.6
Alstonia scholaris	17.4	62.8	64.0	65.2
	31.4	62.3	63.4	64.8
	42.5	68.1	69.3	70.3

The T_g of CNSL resin modified wood varied with WPG, wood species and frequency (Table 5.2) and of these the greatest and significant differences in WPG levels were found between the wood species. The T_g was shifted to high temperature as the WPG of the modified wood increased. This phenomenon is observed for most of the wood species except in Alstonia at the 31.4 WPG (Table 5.2. The T_g of the all CNSL resin modified wood species also increased when the frequency increased.

The T_g temperature varied from 59.8 to 76.4 °C in Scots pine, 62.4 to70.6 °C in Obeche, 64.7 to 71.6 °C in Gmelina and 62.3 to 70.3 °C in Alstonia modified wood irrespective of resin loading and frequency. The T_g temperature shifts with increasing WPG in most wood species at a given frequency but no specific order of shifting T_g was found with the increase in WPG between the wood species, i.e. wood with lower WPG (Obeche and Gmelina) and showed T_g at high temperatures than the wood with higher WPG (Scots pine and Alstonia).

It is also important to note that the height of the tan δ peak increased with the increasing WPG all wood species and was found at all frequencies (see figures 5.1-5.4). It is evident that at the T_g temperature of CNSL resin modified wood, the glass transition, causes the storage modulus (E') to drop significantly to a greater extent than the T_g events seen for unmodified wood (Figure 5.1 to 5.4). The magnitude of this E' decrease in the increased resin loading for all of the wood species tested.

5.4.4. Storage modulus (*E'*) at room temperature

Generally, higher storage modulus (*E'*) value indicates higher modulus of elasticity of the material. The DMTA data of storage modulus can give insight into the mechanical properties at normal working condition (room temperature). The *E'* of the unmodified and CNSL resin modified wood was measured from the *E'* curve over the temperature scan at room temperature (20-25 °C). The mean *E'* value at room temperature for Scots pine, Obeche, Gmelina and Alstonia unmodified and modified wood at different frequencies are presented in Table 5.3. The WPG of 0 indicated the unmodified or control wood samples of the tested species.

Table 5.3: Mean storage modulus (*E'*) of CNSL resin treated wood at room temperature (20-25 °C) in different frequencies.

Wood Species	WPG	*E'*(GPa) at different frequency		
		0.1 Hz	1 Hz	10 Hz
Pinus sylvestris	0	0.63	0.66	0.70
	11.4	0.70	0.72	0.75
	16.9	0.74	0.73	0.76
	21.6	0.75	0.74	0.77
Triplochiton scleroxylon	0	0.40	0.42	0.42
	7.8	0.41	0.43	0.43
	12.6	0.45	0.46	0.47
	17.6	0.46	0.47	0.48
Gmelina arborea	0	0.52	0.54	0.55
	4.6	0.54	0.55	0.56
	4.8	0.57	0.58	0.60
	10.8	0.66	0.59	0.69
Alstonia scholaris	0	0.46	0.48	0.50
	17.4	0.50	0.52	0.54
	31.4	0.55	0.57	0.59
	42.5	0.56	0.58	0.60

The results of the *E'* at room temperature show that a low gradual increase of *E'* was found in CNSL resin modified wood as the WPG of the modified wood increased. This phenomenon is observed in all the modified wood species. The *E'* of the modified wood for all the species is slightly higher than the unmodified wood samples of the same species but statistically this is not significant ($P>0.05$) because the contribution to stiffness from wood species is dominant. This insignificant difference was found for wood species in all the frequencies.

The storage modulus varies with test frequency, as the frequency increased the *E'* increased in all the wood species at most WPGs. The increased *E'* value with the frequency increase is also evident in unmodified wood samples. The highest storage modulus was found in Scots

pine modified wood with a WPG among the wood species. Meanwhile, with a low resin loading Gmelina wood showed better *E'* value than Alstonia modified wood, and at high loadings the Alstonia was still only similar to Gmelina although Alstonia took up significantly more CNSL resin. The lowest storage modulus is found in Obeche modified wood.

5.5. Discussion

5.5.1. Glass transition of unmodified wood

Three tan δ peaks (α, β and γ) were found in the unmodified woods due to the glass transition of hemicellulose, lignin and molecular relaxation. The temperature at which these transitions occurred was found to depend on the frequency of the oscillation (Table 5.1). Kelley *et al*. (1987) reported that the glass transition temperature as well as its intensity is dependent on the nature of wood constituents and the concentration of water or other plasticiser molecules such as ethylene glycol. Generally, these transitions in wood are found in high temperature region. These formed due to the relaxation of the amorphous component i.e. hemicelluloses and lignin of the wood cell wall components.

In this study, all the wood species tested showed transition at low temperature range, termed as γ peak, of the tan δ from -90 to -115 °C, irrespective of wood species and the applied frequencies. The tan δ peak in this temperature range has reported earlier by many workers for different wood species. Backman and Lindberg (2002) found a tan δ peak at -80 °C of Scots pine wood in both the radial and tangential direction when tested at a frequency of 1 Hz, which is associated due to the movement of the methylol groups coupled with water molecules. Kelley *et al*. (1987) found a tan δ peak in the low temperatures from -90 to -110 °C for Spruce and Maple wood at 10% moisture content. This behaviour of transition at low temperatures is due to the secondary dispersion involving only small scale molecular motion.

Maeda and Fukada (1987) claimed that the peak at -105 °C for dry wood is due to the movement of the methylol side chain. In general, the methylol group is present in all the three main polymers found in wood. The γ peak is also dependent on the moisture content of the wood samples as it is associated with the methylol group bonded to water molecules (Handa *et al*., 1980). It is also stated that the peak moves from -40 to 80 °C when moisture content increased to 8% due to the increased motion of chain segment and the complex of wood polymers and water (Backman and Lindberg, 2001). The moisture content of the unmodified

wood in this study was 9% and revealed that the γ peak occurred between -90 to -115 °C for all of the wood species and frequencies studied. It is associated with the methylol groups of the wood polymers and wood polymers and water complexes formed between these functional groups and water.

In this study, two tan δ peaks (α and β) at high temperatures were observed for most of the tested unmodified wood species. The α peak is found in Scots pine wood at ca. 55 °C and in Gmelina wood ca. 75 °C irrespective of the frequencies applied. For the other two wood species Obeche and Alstonia, no clear α peak was seen in the temperature scan but they did produce a very strong β peak. The β peak moved from 45 to 30 °C as the frequencies increased in Obeche wood. The Alstonia wood only produced α peak at 56 °C at 0.1 Hz. The β peak is limited to 30 °C in Alstonia wood.

The two tan δ peaks evident at high temperatures are due to transition from the amorphous hemicelluloses and lignin. Backman and Lindberg (2001) found a β peak at 3 to 8 °C in the tangential direction of Scots pine wood, and observed the drop of *E'* at this transition temperature; they proposed that is due to the glass transition of hemicellulose. They also claimed a α transition peak at 40 to 60 °C when the sample was conditioned at higher relative humidity, and this transition peak was shifted to the 80 °C or over in the samples conditioned at lower relative humidity. Kelley *et al*. (1987) also reported the glass transition temperature of hemicelluloses at 20 °C and lignin at 80 °C in Spruce wood at a moisture content of 10% and 1 Hz frequency. The strong β peaks in Obeche and Alstonia are found 10 °C higher than in Scots pine and 20 °C lower than in Gmelina. The β peak of Gmelina wood is higher in comparison to other three species at ca. 50 °C but close to the α peak. In a study Birkinshaw *et al*. (1986) also found the glass transition of hemicellulose at about 50 °C.

The α transition varied from 55 to 75 °C between Scots pine and Gmelina wood and is due to the glass transition of lignin. This is supported by the findings of Olsson and Salmen (1997) who found the T_g of lignin in moist wood (i.e. not absolutely dry) lies between 60 to 90 °C. Lignin has glass transition in the temperatures 60 to 200 °C depending upon the moisture content and measuring methods (Salmen, 1990). Salmen (1984) also reported the T_g of lignin at 100 °C in Norway spruce measured longitudinally at a frequency of 10 Hz.

Several differences of α and β transitions among the wood species tested in this study are evident which can be related to change of wood density and the frequency of crosslinking in lignin (Kelley *et al*., 1987). The greater heterogeneity between softwood and hardwood lignin

is attributed to the formation of distinct lignin domains. In general, the softwoods and hardwoods contain 25-35% and 18-25% lignin of the wood dry weight respectively (Kollmann and Cote, 1968). The softwoods lignins are consist predominantly of guaiacyl moieties which are a polymerization product of coniferyl alcohol whereas hardwoods lignins consist of syringyl-guaiacyl moieties, a co-polymer of sinapyl and coniferyl alcohols (Rowell *et al*., 2005). This probably affected the differences in transition temperatures between the tested wood species, softwood Scots pine and hardwoods Obeche, Gmelina and Alstonia. The difference between hardwoods may also relate to the ratio of syringyl-guaiacyl moieties as this co-polymer type varies from 4: 1 to 1: 2 for different lignins (Sarkanen and Ludwig, 1971).

5.5.2. Glass transition of CNSL resin modified wood

All the CNSL resin modified wood samples provided a single strong tan δ peak in the high temperature area which is limited to 60 to 70 °C irrespective of wood species, though the T_g was shifted to higher temperature as the WPG of the resin modified wood increased (Table 5.2). It is assumed that the tan δ peaks temperature could be changed in modified wood from the unmodified wood due to the CNSL resin itself and the effect of modification on the wood. Generally, the tan δ peak which originated from the wood polymer relaxation particularly from the hemicelluloses is found in same temperature range (50 °C) (Back and Salmen, 1982; Birkinshaw *et al*., 1999). In this study, the unmodified wood β peak found due to the hemicelluloses relaxation at an temperature range 20 to 30 °C, though the α peak is found at higher temperature range 60 to 75 °C particularly in Scots pine and Gmelina wood. In the resin modified wood of all species showed a single dominant T_g temperature between 60 to 70 °C. So, it is assumed that the peak of resin modified wood is due to the function of CNSL resin relaxations. Backman and Lindberg (2004) found the tan δ peak change from 56 to 40 °C when Scots pine wood was treated with PVAc and tested tangential direction.

The main tan δ peak (T_g) which is found in CNSL resin modified wood is due to the relaxation of CNSL resin impregnated into wood. There may be double tan δ peaks due to the superimposed relaxation from the pure polymer and that from the interaction of wood polymers (Backman and Lindberg, 2002). The T_g peak temperature decrease was reported when untreated wood was treated with PEGMA due to the interaction with the wood cell wall and disturbance to the arrangement of lignin, cellulose and hemicelluloses by extending the intermolecular distance (Backman and Lindberg, 2004). The interaction of resins or polymers with wood is usually observed by changing the loss factor peak (tan δ), though the

distinguishing the movement of hemicellulose peaks is difficult due to interaction of PEGMA polymers with hemicellulose (Backman and Lindberg, 2002).

If a polymer is compatible with wood, it tends to show an increase or decrease in T_g temperature due to its interaction with wood polymers. Backman and Lindberg (2002) found the T_g decreased when measured in polyurethane alkyd lacquer treated wood compared to pure lacquer. The T_g decrease of adhesives or polymers treated wood from the T_g of pure polymer or adhesive is due to increase of free volume to the impregnated polymer. The molecular mobility is dependent on the availability of free volume at some temperature (Nielsen and Landel, 1994). The concept of free volume of the polymers indicates the segment size voids in polymer chain. The increase of free volume of the polymer is caused either by the development tensile force during the curing of polymer or the presence of plasticizers (Nielsen and Landel, 1994; Oksman and Lindberg, 1995). The low molecular weight compounds of the wood can affect polymers and introduce a plasticization effect.

Chen and Gardner (2008) also reported that the T_g decreased from the pure polymers of nylon to the nylon wood composites due to the additives of composites, moisture in the sample and wood filler constituents. Generally, the decrease of T_g is correlated with good adhesion to wood on the cell wall level, which implies some kind of bond formation (Backman and Lindberg, 2004). On the other hand, disturbing the arrangement of wood polymers can also lower the T_g of polymer treated wood (Handa *et al*., 1980).

It was not possible to measure the T_g of pure CNSL, though it is assumed that the T_g of CNSL resin modified wood decreased from the T_g of pure CNSL resin, which is evident by the fact that the T_g temperature of CNSL resin modified wood was decreasing as the resin loading or WPG of the modified wood decreased (Table 5.2). The change in tan δ peak temperature is an indication of change in molecular mobility in the resin. It is also evident that with the decreasing of resin loading the tan δ peak height decreased (Figure 5.1 to 5.4). This lowering of peak height reflects the lower amount of resin in wood (Backman and Lindberg, 2002). Obataya *et al*. (2003) also reported that the tan δ peak of the glucose penta acetate treated acetylated wood was shifted smoothly with the increasing WPG. The impregnants act as a plasticizer due to the interaction with wood cell wall polymers.

5.5.3. Storage modulus of wood modified with CNSL resin

The considerable decrease of storage modulus (*E'*) of both modified and unmodified wood over the entire temperature range (Figures 5.1 to 5.4) suggests that wood endured a transition (T_g) from glassy to rubber state. The onset temperature for this drop in storage modulus is important for analysing the modified wood behaviour which is related to the mechanical behaviour as well. Backman and Lindberg (2001) found the elastic properties (*E'*) of Scots pine wood at radial and tangential direction was a few percent different from the Young's modulus and thus argued that the DMTA is very reproducible in term of mechanical behaviour of wood.

The decreases of *E'* over the thermal scan are not due to thermal degradation of wood constituents but are attributed to the transition of wood constituents. In the unmodified woods no substantial loss in *E'* was evident over the temperature at γ transitions but the dramatic decrease in *E'* was associated with α and β transitions (amorphous material relaxation). Moreover, the *E'* of unmodified woods was relatively stable on heat scan in these transition temperatures but the CNSL resin modified wood showed a marked decrease in *E'* in the transition temperature 60 to 70 °C for all the wood species. The resin modified wood also showed a steep rise in tan δ peak in this transition temperature.

To understand the elastic properties of CNSL resin modified wood, the *E'* value at room temperature (20 to 25 °C) was examined from the *E'* curves over thermal scan (Table 5.3) and revealed no significant variation of *E'* at room temperature between the resin modified and unmodified wood. This indicated that the CNSL resin has no particular effect on the elastic properties of wood at room temperature. This is possible if the amorphous wood constituents are in the glassy state, and not plasticized by polymers or resin at room temperature (Obataya *et al.*, 2003). The CNSL resin modified wood showed a sharp *E'* at high temperature which implies that the resin can be acted as a plasticizer at these high temperature where the amorphous molecules in the wood are sufficiently mobile.

5.6. Conclusions

1. The dynamic mechanical thermal analysis provided valuable information regarding the viscoelastic properties of CNSL resin modified wood. The glass transition temperature (T_g) and storage modulus (E') of modified and unmodified wood provided variable values due to the increase of oscillation frequencies for all the wood species tested i.e., Scots pine, Obeche, Gmelina and Alstonia.

2. All the unmodified wood species showed a tan δ peak at low temperatures (γ peak) due to the movement of the methylol groups of wood polymers which also coupled with water molecules. At the high temperatures two tan δ peaks were found for most of the wood species due to the wood polymer relaxation, these were T_g, the α peak for lignin and β peak for hemicellulose relaxation. These relaxation temperatures were shifted among the wood species owing to the difference of wood density and frequency of the crosslinking within lignin or composition of hemicelluloses. The heterogeneity of formation of amorphous polymer domains between softwood and hardwood also affect the shifting of T_g temperature of the wood species.

3. Due to the modification of wood with CNSL resin at different WPG the T_g of the modified wood showed only one relaxation at high temperature level suggested that the transition is for the resin relaxation. The T_g temperature increased with increasing resin loading in the wood and established a correlation with the resin loading and T_g temperature. It is assumed that the T_g of the resin modified wood was decreased from the T_g of the pure CNSL resin. The DMTA measurement of T_g is a method to study the molecular interactions at the molecular level of polymer chain segmental stability. The decreased T_g of resin in modified wood could relate to the increase of free volume of the CNSL resin caused by tensile stress created during the curing of the resin and/or effect of the resin as a plasticizer.

4. The E' of both modified and unmodified woods decreased with the increasing temperature due to the transition of the wood polymers. Though the unmodified wood E' drop at T_g temperature is stable, a dramatic drop of E' was found in modified wood at T_g temperature. Even so, the E' at room temperature provided no significant variation of elastic properties between modified and unmodified woods of the same species. Among the wood species Scots pine modified wood showed better elastic properties than the other three woods.

Chapter 6

Oxidative degradation of wood modified with CNSL resin

6.1 Introduction

Wood decay fungi secrete hydrolytic and oxidative enzymes which participate in biodegradation of ligno-cellulosic material (Hammel *et al*., 2002). The extracellular cell wall degrading enzymes produced by fungi are too large to penetrate deep into the wood cell wall because the smaller pore size of the unmodified wood cell wall do not permit the penetration by enzymes to reach the cell wall polymers (Cowling, 1961; Cowling and Brown, 1968; Highley *et al*,. 1983; Goodell *et al*., 1988; Flournoy *et al*., 1991) and this is true for the early stages of decay, where little decay has occurred to open up the internal pore structure (Daniel *et al*., 1990; Kim *et al*., 1993; Blanchette, 1997). Thus the initial degradation of wood cell wall polysaccharides does not occur by direct enzyme attack alone. With brown rot fungal decay the degradation is not solely restricted to regions around the hyphal surface, and studies by light and electron microscopy (Fuzakawa, *et al*., 1976; Kim *et al*. 1991; Srebotnik and Messner, 1991) confirm this indicating that the degrading agents are capable to diffuse into wood cell wall. Therefore, the degradation of polysaccharides by brown rot fungi must employ non-enzymatic process in the initial stage of degradation.

Reactive oxygen species (ROS) are possible candidates produced by fungi, which initiate decay within the secondary wood cell wall (Hammel *et al*., 2002). Among the ROS produced from the fungi of the Basidiomycota, the hydroxyl radicals ($^{\bullet}OH$) are mostly focused though the other ROS, peroxyl radicals ($ROO^{\bullet}$) and hydroperoxyl radicals ($^{\bullet}OOH$) produced by fungi might also attack wood polysaccharides. Many studies also reported the production of hydroxyl radicals by white rot fungi (Backa *et al*., 1993; Tanaka *et al*., 1999) and brown rot fungi (Backa *et al*., 1992; Goodell *et al*., 1997; Hyde and Wood, 1997; Kerem *et al*., 1999; Jensen *et al*., 2001) in relation to wood degradation.

A pathway for hydroxyl radical production was established by Fenton's reaction (H_2O_2 + $Fe^{+2} \rightarrow Fe^{+3}$ + OH^- + $^{\bullet}OH$) which is involved in the initial wood decay by brown rot fungi. The Fenton reaction generates $^{\bullet}OH$ radicals from H_2O_2 which can produce secondary oxygen species when oxidizing organic molecules (Hammel *et al*., 2002). Koenigs (1972a) reported that brown rot fungi involved the Fenton's reaction as they produce extracellular hydrogen

peroxide and the wood contains iron involved in the Fenton reaction with cellulose degradation. Iron is present in wood which is found predominantly in the form of insoluble ferric (hydro) oxides. Oxalic acid, a prime metabolite of brown rot fungi is considered as reagent for reducing Fe^{+3} to Fe^{+2} for use in Fenton's reaction (Schmidt *et al*., 1981). Subsequently it has been demonstrated that the biochelators, a 2,5-dimethoxy-1,4-benzoquinone and 4,5-dimethoxycatechol, low molecular weight are produced by the brown rot fungus *Gloeophyllum trabeum* and functions to mediate the reaction with iron in the initial stage of polysaccharides degradation and also reduce ferric iron to ferrous iron (Goodell, *et al*., 1994; 1997). Wood degraded by reactive products of Fenton's reaction exhibited unique features similar to that of brown rot fungi both physically and chemically (Highley, 1980; Schmidt *et al*., 1981).

Thus Fenton's reagent is a key component as a model of brown rot decay of wood and the rapid strength loss attributed to the degradation of wood polymers. The reaction between H_2O_2 and ferrous ions generates highly oxidative hydroxyl radicals, which can cleave glycosidic bonds of polysaccharides, carbonyl group and carboxyl groups (Kirk *et al*., 1991). Wood reacted with Fenton's reagent showed severely degraded hemicelluloses then cellulose (Koenigs 1974a).

Many researchers have used the micro-veneers (thin veneer strip) to examine the wood degradation by decay fungi and thus study the effectiveness of wood protection system through the measurement of tensile strength loss (Bravery and Grant, 1971). Banks and Evans (1984, 1988) initially monitored the loss of tensile strength of wood micro-veneer weathered by water. The loss in tensile strength of the micro-veneer was also monitored to evaluate the wood degradation of modified and unmodified wood during weathering (Evans *et al*., 2000, 2002; Derbyshire *et al*., 1995; Turkulin *et al*., 2004; Xie *et al*., 2005, 2007, 2010). Most of these studies used zero-span tensile strength to evaluate the degradation parameters. In general, tensile strength of micro-veneers determined in a zero-span mode evaluates the strength of single fibres in comparison to bending strength which is also influenced by the adhesion between the fibres (Winandy and Rowell, 2005). Moreover, good correlation between the strength loss of micro-veneers and the degree of polymerization of polysaccharides was established (Derbyshire and Miller, 1981). It was also found that the tensile strength loss of wood is related to the degradation of cellulose in the cell wall after exposure to reduced oxygen species (Xie *et al.*, 2010). The tensile strength loss of the micro-veneers was also used

to study the degradation potential of hydroxyl radicals driven by Fenton chemistry (Takahashi *et al.,* 1989; Mai *et al*., 2010; Xie *et al*., 2009, 2010).

This chapter reports the effect of the hydroxyl radicals generated by the Fenton reaction on the mass and zero span tensile strength of CNSL resin modified wood micro-veneers. This was chosen because it has the potential to provide rapid results.

6.2. Materials and Methods

6.2.1. Wood species and micro-veneer preparation

Scots pine (*Pinus sylvestris*) sapwood, straight grained and free of knots and other defects, was used in this study. The wood blocks were cut, 100 mm x 40 mm x 10 mm (longitudinal x tangential x radial), with their growth rings inclined at about 5° to the tangential direction. The wood blocks were immersed in deionized water in a vacuum for 30 minutes and then kept under deionized water for 14 days under ambient pressure and room temperature with daily water changes. Micro-veneers of 100 µm in thickness were microtomed from the radial surface of the wood blocks as described by Evans and Banks (1984, 1988). The micro-veneers were cut by disposable microtome blades (Cambridge Instruments No.818) and a blade holder (Reichert-Jung). The thickness of the micro-veneers was checked by a dial gauge micrometre and those with greater than 5 µm deviation were rejected. The micro-veneers were air dried, placed flat and straight in glass sheet in a dark climate chamber (22 °C and 65% RH) for 14 days. After drying the micro-veneers were stored in sealed paper envelopes and shielded from light.

6.2.2. Chemicals

The CNSL resin was prepared from *t*CNSL (technical cashew nut shell liquid) by ozonolysis process at the BioComposites, Bangor, UK Laboratory (see chapter 4, section 4.2.2). Hydrogen peroxide (H_2O_2) (30%), ferrous sulphate heptahydrate ($Fe_2SO_4.7H_2O$), acetic acid (glacial), sodium acetate were obtained from Sigma-Aldrich Co. UK. Chemicals were used in this study as received.

6.2.3. Micro-veneer impregnation

The micro-veneers were vacuum impregnated with alcoholic solutions of CNSL resin of 5%, 10%, 20% or 30% solute content in industrial methylated spirit (IMS) to achieve different amounts of weight percent gain (WPG). Micro-veneers treated with 0% CNSL is used as

untreated control. The resin impregnation was carried out according to procedure described in chapter 4, section 4.2.3. After the curing of the modified wood specimens were stored in the climate chamber for two weeks.

6.2.4. Treatment with Fenton's reagent

The oxidative degradation of wood micro-veneers was performed with Fenton's reagent of consisting of 0.44 mM $Fe_2SO_4.7H_20$ and 1% H_2O_2 in 0.1 M acetate buffer at a pH 4.2 as proposed by Koenigs (1974b). The micro-veneers were separated into two groups consisting of those treated with Fenton reagent and a pH 4.2 buffer control. The micro-veneers were treated in 250 ml Erlenmeyer flasks containing 100 ml solution and 3 micro-veneers. A total of 6 micro-veneers were treated for each treatment. The micro-veneers were immersed in the acetate buffer and Fe^{+2} salt containing flasks and then Fenton's reaction was initiated by adding H_2O_2 into the flasks. The flasks were gently shaken in a dark water bath at 30 °C for 6, 12, 24 and 48 h. The pH 4.2 buffer control micro-veneers were treated likewise. After treatment the micro-veneers were washed with deionized water.

6.2.5. Determination of weight loss

The micro-veneers were dried in oven at 105 °C for 6 h and cooled in a desiccator before and after the treatment with Fenton's reagent. The micro-veneers were weighed and reweighed to 0.1 mg after oven drying. The percentage weight loss of micro-veneers due to oxidative degradation was determined according to equation 4.12 (chapter 4).

6.2.6. Determination of zero-span tensile strength

The tensile strength at zero-span of micro-veneers was determined with a Pulmac paper tester (Pulmac International Inc. USA). The rate of loading was 80 kPa per second and initial clamping pressure was 0.55 MPa. The tensile strength retention of the Fenton's reagent treated micro-strips was related to the tensile strength of micro-veneers which were incubated in acetate buffer served as control. The tensile strength of the control micro-veneers was set to 100%.

6.2.7. Statistical analysis

The data obtained were analysed by the multifactor analysis of variance (ANOVA) test to determine the statistical difference followed by Tukey's multiple comparison using IBM® SPSS® Statistics 20. The results with $P<0.05$ were considered to be statistically significant difference.

6.3. Results

6.3.1. Weight loss due to oxidative degradation

The CNSL resin impregnation of the Scots pine micro-veneers achieved a range of weight percent gains (WPGs) values (Table 6.1). The weight losses due to the Fenton's reagent oxidative degradation at different duration of reaction and the acetate buffer controls are also presented in Table 6.1. In acetate buffer reagent the weight losses of micro-veneers are inconsistent in that they are not proportional to reaction time or WPG. Statistically, significant difference ($P<0.001$) was found among the resin loading (WPG) and among the reaction durations on the weight loss of the micro-veneers. The effect of interaction of resin loading and the reaction durations was also significant ($P<0.001$) on the weight losses of micro-veneers. In most of the cases, the weight losses of the resin modified micro-veneers do not vary from the unmodified veneers except the weight losses at a WPG level of 24.9. Substantial weight losses ca. 4.5% in contrast to the unmodified veneers was observed in this WPG irrespective of the reaction duration. Similar values were reported for longer reaction times and the highest WPG.

Table 6.1: Weight losses of CNSL resin modified micro-veneers treated in acetate buffer with and without Fenton's reagent at different treating durations.

Reagents	WPG	Weight losses (%) at different reaction time (h)			
		6	12	24	48
Buffer reagent	0 (unmodified)	1.47 (0.4)a	1.96 (0.2)a	3.17 (0.3)bc	2.23 (0.5)c
	9.8	0.99 (0.4)a	1.39 (0.3)a	0.89 (0.3)a	0.84 (0.1)a
	24.9	4.48 (0.1)b	4.63 (0.4)b	4.30 (0.4)c	4.31 (0.3)c
	32.1	2.03 (0.4)a	2.31 (0.1)a	2.28 (0.7)bc	1.85 (0.5)b
	37.2	1.76 (1.0)a	1.62 (1.2)a	4.26 (0.9)c	4.15 (0.1)c
Fenton's reagent	0 (unmodified)	3.00 (0.4)b	14.93 (0.7)d	53.34 (5.9)c*	58.84 (6.5)c*
	9.8	2.11 (0.3)a	7.32 (0.6)c	10.73 (1.3)b	15.97 (1.8)b
	24.9	4.05 (0.2)c	6.59 (0.2)bc	8.01 (1.7)b	12.75 (0.8)b
	32.1	4.48 (0.1)c	5.58 (0.7)b	8.83 (1.4)b	12.20 (0.8)b
	37.2	1.42 (0.4)a	2.73 (0.3)a	4.93 (0.5)a	6.70 (0.4)a

Values in parenthesis refer to ±SD and n=6. Number followed by different letters for each reaction time are significantly different at the level of $P<0.05$ according to Tukey's test. * Samples exposed to Fenton's reagent for 24 and 48 h were severely degraded and only fractions of wood material were measured.

Weight losses with the Fenton's reagent were dramatic, particularly in unmodified micro-veneers. In the untreated controls most of the degradation occurred within 24 h (53%) and

doubling the reaction time only resulted in a further 5.5% weight loss to 58.4%, thus for comparisons of weight loss 24 h has been selected. In the modified wood the weight losses also increased with the increasing reaction duration but decreased with the increasing WPG and a substantial reduction of the weight loss was seen in CNSL resin modified micro-veneers even at low WPG. The effect of resin loading and reaction duration were statistically significant ($P<0.001$) on the weight losses of the micro-veneers. Significant difference ($P<0.05$) was also found in the interaction of the resin loading and Fenton's reaction duration on the weight losses of the micro-veneers.

6.3.2. Zero-span tensile strength of CNSL resin modified micro-veneers

The tensile strength at zero-span mode of CNSL resin modified micro-veneers is presented in Figure 6.1. A gradual increase of tensile strength was found with the increase of resin loading (WPG) in the micro-veneers. The unmodified veneers provided the tensile strength of 8.3 kN/m and which is increased up to 11.3 kN/m at the highest WPG level of 37.2. This is suggested that there is a good correlation between the resin modification and the tensile strength at zero-span. Statistically significantly difference ($P<0.05$) was found between the WPG levels of resin modification on the tensile strength of the micro-veneers, but the effect of resin loading is much higher over 24.9 WPG and above.

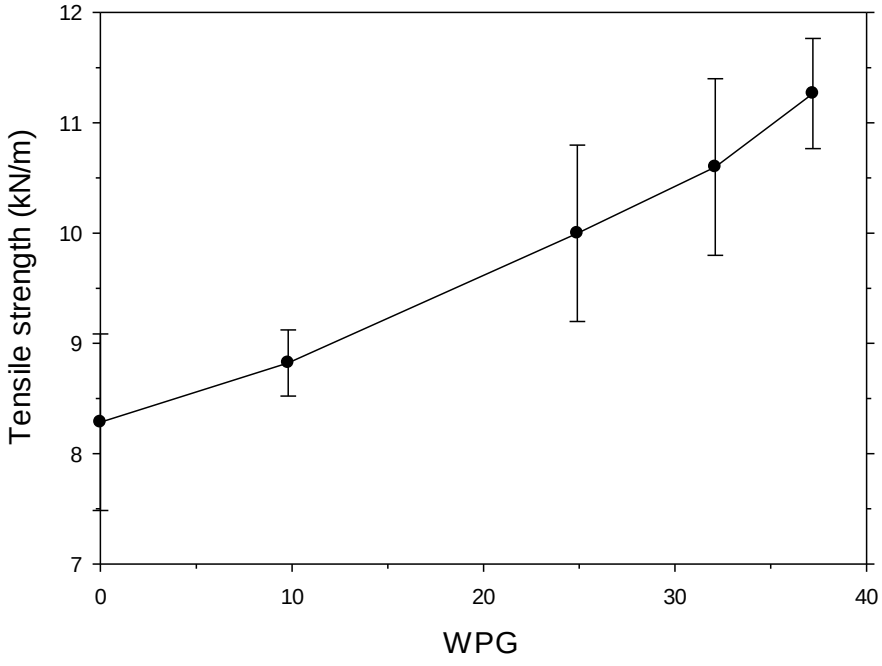


Figure 6.1: Tensile strength as determined at zero-span mode of the CNSL resin modified micro-veneers (error bars = ±SD, n=6).

6.3.3. Loss of tensile strength due to Fenton's reaction

The loss of the tensile strength caused by Fenton's reagent in acetate buffer solution and the acetate buffer controls is shown in Table 6.2. Only minor losses in the tensile strength of the unmodified micro-veneers occurred due to the effect of pH 4.2 buffers only and these losses increased with increasing exposure time. No "zero time" control was included, i.e. dipped in the buffer, rinsed and tested after conditioning, but the untreated control in section 6.3.2 gave a lower value (8.3 kN/m) than the 6 h values (8.93 kN/m).

Table 6.2: Loss of tensile strength of the CNSL resin modified and unmodified micro-veneers due to the treatment in acetate buffer with and without Fenton's reagent at different treating durations.

Reagents	WPG	Tensile strength (kN/m) at different reaction time (h)			
		6	12	24	48
Buffer reagent	0 (unmodified)	8.93 (0.5)a	8.12 (0.5)a	8.10 (0.6)a	8.04 (0.7)a
	9.8	9.41 (1.8)a	8.97 (0.3)a	8.81 (1.2)a	8.23 (1.2)a
	24.9	9.59 (0.5)a	9.47 (1.0)a	9.25 (0.2)a	8.84 (1.1)a
	32.1	10.61 (1.6)a	10.05 (0.6)a	9.59 (1.2)a	9.31 (0.7)a
	37.2	11.03 (1.1)a	10.41 (1.1)a	10.30 (0.3)a	10.08 (0.4)a
Fenton's reagent	0 (unmodified)	7.04 (1.1)a	4.88 (1.3)a	-*	-*
	9.8	8.26 (0.4)ab	7.53 (0.3)ab	6.86 (0.5)a	5.03 (1.1)a
	24.9	8.69 (0.2)ab	7.66 (0.5)b	6.96 (0.6)a	5.99 (1.2)a
	32.1	10.14 (0.9)bc	9.05 (1.7)b	7.52 (1.4)a	6.08 (1.5)a
	37.2	10.78 (1.1)c	9.76 (0.4)b	8.99 (0.7)b	7.04 (1.7)b

Values in parenthesis refer to ±SD and n=6. Number followed by different letters for each reaction time are significantly different at the level of $P<0.05$ according to Tukey's test. * Samples exposed to Fenton's reagent for 24 and 48 h were severely degraded and unable to measure the tensile strength at zero span.

CNSL resin modified micro-veneers also incurred minor tensile losses due to the effect of pH 4.2 buffer only and these also increased with increasing exposure time. The observation that there was an increase at the shortest buffer exposure time as compared to the unexposed modified strips was only valid for the low WPG (9.8) and at higher WPG there were little differences. From the statistical study, neither the resin loadings (WPGs) nor the reaction durations have a significant effect ($P>0.05$) on the tensile strength of the micro-veneers due to treatment with acetate buffer solution. Also, the interaction of the WPGs and reaction durations did not show significant difference ($P>0.05$) on the tensile strength of the micro-veneers after the buffer treatment.

As with the weight loss, the effect of the Fenton's reagent on the tensile strength of the untreated micro-veneers was dramatic and increased with time so that at 24 h the tensile strength of test strips could not be assessed. For the modified strips the tensile strength also decreased with increasing reaction time but overall higher values were seen for higher WPG. Statistically both the resin loading and the reaction duration have significant effect ($P<0.001$) on the tensile strength of the micro-veneers. Moreover, the interaction of the resin loading and the reaction duration showed significant difference ($P<0.001$) on the tensile strength of the micro-veneers due to the treatment with Fenton's chemistry.

6.3.4. Strength retention

The tensile strength retention of the CNSL resin modified micro-veneers were evaluated from the tensile strength at zero-span of the veneers treated with Fenton's reagent in comparison to the tensile strength of the veneers treated only in acetate buffer solution. The results of the strength retention of the modified micro-veneers at different treating duration are presented in Figure 6.2. Increasing WPG was effective at maintaining more of the modified strength of the micro-tensile strips at all of exposure times to the Fenton's reagent.

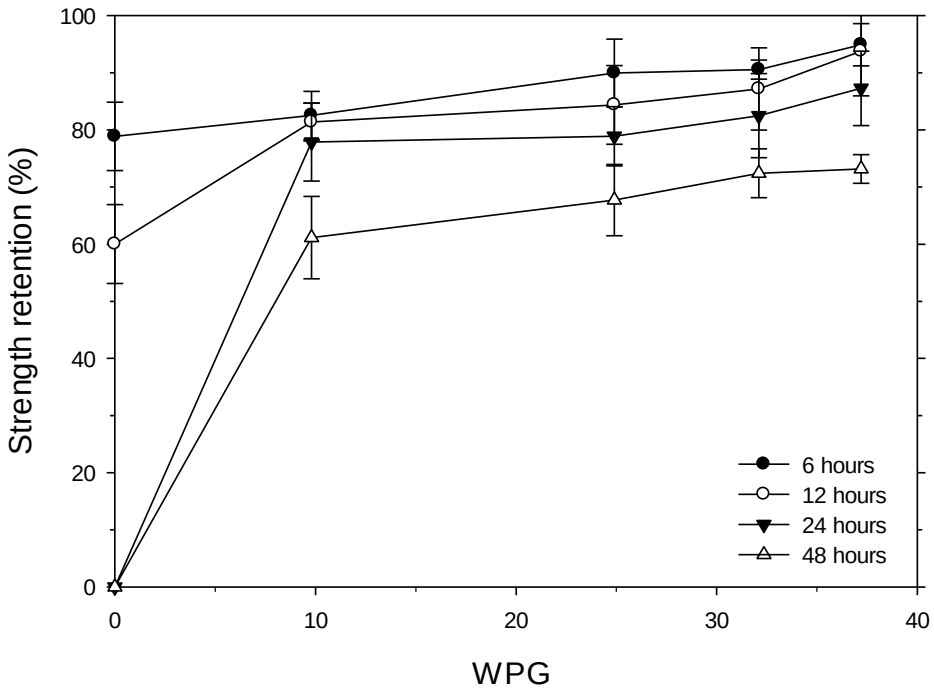


Figure 6.2: Tensile strength retention of CNSL resin modified micro-veneers exposed in acetate buffer to Fenton's reagent for 6-48 h (error bars = ±SD, n=6).

6.4. Discussion

The method used was successful at inducing both weight loss and tensile strength losses in untreated veneers and follows previous studies cited in the introduction. The number of veneers in each flask was chosen so as to leave an excess of reagent because work by Xie *et al.* (2010) had indicated that the concentration of H_2O_2 reduced to half of the original when the wood micro-veneers in the reaction mixture were increased from 1 to 6. In this study, 3 micro-veneers were treated in the reaction mixture during the test and thus assuming that they influenced the loss of tensile strength.

6.4.1. Weight loss

Minor weight losses occurred with the acetate buffer exposed samples, but these did not follow a distinctive pattern of exposure time and WPG, and the losses are thought to be leaching losses of wood extractives and unreacted resin, as discussed in chapter 4, for a more neutral pH system. The highest weight losses were observed at 24.9 WPG which is difficult to explain. Possibly at this WPG there is more resin located on the surface of the micro-veneers which was leached out in the buffer solution. Thus, this is assumed that the weight losses found in resin modified micro-veneers is not solely caused by oxidation but also the leaching of unreacted resin and hydrolysis of the wood components at the pH value used.

During the first 6 h of the Fenton's reagent exposure of the unmodified control and resin modified micro-veneers the weight losses of the micro-veneers were not significant, but by 12 h substantial weight losses were observed with the controls (Table 6.1) and with increasing WPG the weight loss values decreased. By 24 h very high weight losses (53.34%) had occurred in the controls but even at the lowest WPG the weight losses were small (10.73%) at about a fifth of the control value. At the highest WPG; the losses were minimal (4.93%) and similar to the buffer alone (4.26%) losses. It is clear that resin modification has a large impact on weight loss even at low WPG and has an impact on the oxidative degradation of Scots pine sapwood. Previous studies have used this degradation system to achieve similar results. Xie *et al*. (2012) studied the weight losses (and tensile strength) of Scots pine micro-veneers due to Fenton's reagent in acetate buffer. They showed that the chelator 2, 3 DHBA reduced the weight losses caused by the oxidative degradation of the Fenton's reaction.

6.4.2. Tensile strength

Strength losses are induced by hydrolysis of cell wall polysaccharides or the deposition of chemical agents within the wood cell wall (Rowell, 1998). In conventional wood preservation systems based on aqueous solutions of metal compounds small reductions in strength are widely recognised. The loss of tensile strength by chemical modification is also evident as Xie *et al*. (2007) found strength losses, measured by zero-span, of up to 50% with the micro-veneers of Scots pine modified with DMDHEU. This strength loss was attributed to the hydrolysis of wood cell wall polysaccharides. In addition, Militz (2002) has commented that the strength properties can decrease with increasing treatment temperature, particularly for thermal modification. When heat treatment is applied the MOR is particularly prone to loss (Gonzalez-Pena and Hale, 2007).

In contrast to strength losses with some types of chemical and with thermal modification of wood, in this study this resin modification provided statistically significantly improved tensile strength to micro-veneers (Figure 6.1) and this increases in a linear fashion with increasing WPG, so that in proportional terms there is a 36% increase in tensile strength between untreated and the highest WPG (37.2). The improvement may purely be that there is a densification effect and that there is a greater amount of material under test in the cross section of the zero-span. This effect would be expected irrespective of location, i.e. veneer surface, wood cell lumens or within the wall.

6.4.3. Loss of tensile strength

There was little effect of the control (acetate buffer alone) on the tensile strength of the micro-veneers, irrespective of WPG and exposure period to the acetate buffer (Table 6.2). In contrast, the Fenton's reagent (in combination with the buffer) caused a massive loss in tensile strength of the untreated micro veneers and the loss of strength was severe even after 12 h exposure, and a complete loss occurred by 24 h (Table 6.2). The modified micro-veneers however retained 60 to 70% of their modified tensile strength values with 24 h exposure, irrespective of the WPG (Figure 6.2).

The hydroxyl radicals produced through Fenton reaction are the strongest oxidants in the biological system (Koppenol and Liebmen, 1984). These radicals can mineralize organic matter to CO_2 and water (Pignatello *et al*., 2006) and thus are attributed to the cause of tensile strength loss. Oxidative degradation was evident during the experiment and was visible as a brown discoloration of the unmodified veneers and the development of bubbles (CO_2) on the

veneer samples. Colour change observations were difficult in the darker brown modified wood but the CO_2 evolution was less in the modified wood.

The high strength loss can be attributed to greater ability of the hydroxyl radicals to penetrate the wood cell wall matrix, depolymerise cell wall polymers, particularly solubilising the hemicelluloses and reducing the degree of polymerisation of the cellulose (Mai, 2010). Depolymerisation of wood polysaccharides is directly related to the loss of tensile strength of wood micro-veneers (Derbyshire and Miller, 1981; Derbyshire *et al*., 1996, 1997; Turkulin *et al*., 2004). Early work by Koenigs (1974b) reported that the degree of depolymerisation of cellulose in wood treated with Fenton's reagent increased rapidly at low weight loss and thereafter gradually reduced. The Fenton system also solubilized hemicelluloses of sweet gum and Loblolly pine wood more readily than cellulose (Koenigs, 1974a).

That there is a significant decrease in the oxidative decay induced tensile strength loss of treated strips leaves the question of mechanism of protection. The hydroxyl radicals are very small in nature and should be able to adequately penetrate the wood cell wall matrix structure unless physically or by some other means blocked. The protective effect of the low WPG might be considered greater than could be expected by a purely physical blocking effect, unless the blocking is purely at the surface alone, i.e. effectively denying access to the bulk of the veneer. Instead another option, that combining some cell wall and lumen blocking effect and another action, that of an antioxidant, might be considered and these will be discussed more fully in chapter 10 where the results of the antioxidant work in chapter 3 and the microscopic distribution of the resin (chapter 9) are considered together. The tensile strength retention of the modified micro-veneers is attributed to the protection of the depolymerisation of the wood polysaccharides by the CNSL resin system.

This protection could be as a result of physical blocking by the resin of the cell wall sites and/ or it could be as a result of metal binding by the phenolic component, ultimately reducing the availability of Fe for participation in the Fenton reaction. Work by Xie *et al* (2010) showed that the tensile strength loss of micro-veneers decreased with increasing concentrations of a phenolic, 2,3-dihydroxybenzoic acid, a metal chelator, which was thought to reduce the availability of iron for the reaction. Reducing the availability of Fe in the system by chelation however may not be the entire action of 2,3-dihydroxybenzoic acid, as it has been reported to have antioxidant activities too (Sroka and Cisowski, 2003). The tensile strength loss of the micro-veneers was found decreased with the increasing concentration of 2,3-

dihydroxybenzoic acid (Xie *et al*., 2012). They concluded that this reduced the cellulose degradation and the degree of wood degradation in the Fenton system.

6.5. Conclusions

1. The oxidative degradation of CNSL resin modified micro-veneers of Scots pine wood have been assessed via Fenton's reaction to mimic the mechanism of degradation process of many biological systems, particularly the decay mechanism of the brown rot fungi. The Fenton reagent generates highly oxidative hydroxyl radicals (•OH) which can cleave the glycosidic bonds of wood polysaccharides and initiate decay by fungi of the Basidiomycota, prior to action by cell wall degrading hydrolases. Later attack by hydrolases is made possible because cell wall pore sizes increase to sizes large enough to allow enzyme access. Thus, the treatment with Fenton's reagent of the CNSL modified wood is a rapid, dependable, bio-system free method which provides useful information regarding the initial decay mechanism of CNSL resin modified micro-veneers by measuring mass changes and the tensile strength changes at zero-span mode.

2. The oxidative degradation provided low weight losses to the resin modified micro-veneers exposed to Fenton's reagent for up to 12 h but longer periods of exposure showed severe weight losses of the unmodified controls and low weight losses, thus good protection of the resin modified veneers. This protection was dose related but low levels of modification gave a disproportionately high response. The weight losses of the resin modified veneers is attributed to not only to the depolymerisation and solubilisation of cellulose but also contributed by the loss of water soluble wood extractives and wood degradation products probably hemicelluloses.

3. The tensile strength at the zero-span mode of the resin modified and unmodified micro-veneers were decreased with the increasing of treatment duration. It is also evident that the resin modified veneers retained much strength with the increasing resin loading in comparison to the unmodified veneers. Unlike the strength losses caused by some forms of chemical modified wood, the CNSL resin modified veneers showed improved tensile strength properties.

4. Treatment of unmodified micro-veneers with Fenton's reagent for longer periods led to a complete loss of tensile strength but a substantial tensile strength was retained in resin modified veneers for these exposure periods to the Fenton's reagent. This is much evident

when the strength retention was evaluated in comparison to the tensile strength of the veneers from the buffer solution. Much of the strength losses were found in the treatment period from 24 h but below this limit the strength loss was not prominent. The strength retention was increased rapidly at a modification of 24.9% WPG and then increased gradually in all the reaction period. Though, 30 to 40% strength loss was found in modified veneers at longer Fenton's reaction which is attributed to the depolymerisation of the cellulose as it is well established that the loss of tensile strength is correlated to the polymerization of wood cell wall polysaccharides.

Chapter 7

Decay resistance of CNSL resin modified wood

7.1. Introduction

Conventional preservative treated timber has widely been considered as hazardous waste which has led to the substitution with alternative chemical systems for wood preservation. Traditionally wood preservatives have been selected on their biocidal activity but do not alone change the water related behaviour of wood i.e. dimensional stabilization, moisture sorption. Wood modification offers a viable alternative not only improving its dimensional stability but also improving its decay resistance or durability. Modification can use low toxicity materials, such as bulking agents and water repellents, or modify the wood cell wall chemically so as make less able to sustain wood-destroying organisms (Kumar and Morrell, 1993). The impregnation of the wood cell with chemical which enter the wood cell wall is a very broad area, which has attracted wide interest (Hill, 2006). Resin treatment is a useful method of impregnation modification to impart the decay resistance properties of wood.

Hill (2006) states that resin can impart decay resistance in two ways; either by simply blocking the micropores in the wood, making the wood cell wall impervious to water or by acting as a toxic chemical barrier that kills the fungi. The ingress of fungal metabolites which degrade the cell wall polymeric components of wood can be reduced or prevented by the impregnating monomers which are ultimately polymerized in the wood cell wall. The impregnated polymers can block the cell wall micropores network through which the degradative agents diffuse (Eaton and Hale, 1993). The other mechanism for improved decay resistance of impregnate modified wood including the resin treatment has been suggested due to the reduction of moisture content that impedes fungal colonization (Sailer and Van Etten 2004; Hill *et al*., 2005; Ormondroyd, 2007; Verma *et al*., 2009).

Many authors have reported on microbial resistance of resin modified wood (Stamm and Baechler, 1960; Takahashi and Imamura, 1990; Ryu *et al.,* 1991; Militz, 1993; Rapp and Peek, 1995, 1996; Sailer *et al.,* 1998; Westin *et al.,* 2004), these studies only concentrate on synthetic resin systems derived from petrochemical resources. Some natural resin systems have also been evaluated for their potential as wood protecting agents including tall oil rosin, modified tall oil rosin, modified linseed oil, hemp oil and rapeseed oil (Ritschkoff *et al.,* 1999; Van Acker

et al., 1999; Spear *et al.,* 2006). Van Acker *et al*. (1999) studied six natural resin systems using biocidal activity criteria to evaluate their potential as wood protecting agents. Most of the resins were not sufficiently effective to give establish the threshold toxic limits needed for total decay protection although, treatment with DMDHEU at higher WPG reduced the mass loss to close to the threshold limit. Silicon compounds and silanes have been shown to successfully impart water repellency, hydrophobation and decay resistance as non-biocidal systems (Hill *et al*., 2004a; Ghosh *et al*., 2008; Weigenand *et al*., 2008).

CNSL resin is a bio-based resin synthesised by ozonolysis from cashew nut shell liquid, a naturally occurring mixture of phenolics. The resin was found hydrophobic (chapter 4) and imparts dimensional stabilization and water repellency to modified wood. In this chapter *t*CNSL (technical) was selected because it was available in quantity and can be made into a resin. The aim of this chapter is to assess the decay resistance properties of CNSL resin modified wood against standard test decay fungi of the Basidiomycota.

7.2. Materials and Methods

7.2.1. Decay fungi

The fungi used for decay test (Table 7.1) were maintained on 2% malt 2% agar at 22 °C.

Table 7.1: Fungi used in the decay test performed in accordance to the EN113 protocol.

Fungus	Strain	Decay type
Coniophora puteana	FPRL 11E	Brown rot
Postia placenta	FPRL 280	Brown rot
Trametes versicolor	CTB 863A	Simultaneous white rot
Pleurotus ostreatus	FPRL 40C	Simultaneous white rot

7.2.2. Wood samples

Samples from sapwood of Scots pine (*Pinus sylvestris*), Obeche (*Triplochiton scleroxylon*) and Gmelina (*Gmelina arborea*), straight grained and free from knots, were prepared to dimensions of 20 x 20 x 5 mm (radial x tangential x longitudinal). After sanding to remove loose fibres, the wood samples were stored in a climate chamber at 20 °C and 65% relative humidity (RH) until they reached equilibrium moisture content (EMC) and their weights were recorded in g to 4 decimal places.

7.2.3. CNSL resin preparation

A bio based resin system was synthesized from *t*CNSL (technical grade) by ozonolysis process at the BioComposites Centre, UK as described in section 4.2.2 (chapter 4).

7.2.4. Resin impregnation

The wood specimens were vacuum impregnated with alcoholic solutions of CNSL resin of 0%, 5%, 10%, 20% or 30% solute content in industrial methylated spirit (IMS) to achieve different amounts of weight percent gain (WPG). The resin impregnation was carried out according to procedure described in chapter 4, section 4.2.3. Weight percent gain (WPG) of resin impregnated samples was determined by using the equation 4.1 (chapter 4).

7.2.5. Wood decay test

Wood samples were exposed to the fungi (Table 7.1) based on the EN 113 protocol (1996) with some modifications (see section 3.2.5, chapter 3). Prior to exposure, the samples were conditioned in climate chamber (20 °C and 65% RH) for 2 weeks, although their oven dry weights were previously recorded and used for subsequent mass loss (ML) calculations. The treated samples and their untreated controls were individually bagged and then gamma irradiated (2.5 Mrad) at Isotron Ltd. The fungi were grown on approximately 60 ml of 4% malt agar in 500 ml squat jars for 2 weeks at 22 °C, 65% RH. Once the fungi had grown to cover the agar the blocks were placed on a sterilised (autoclaved) polypropylene mesh (a geo-textile weed suppressing felt) inside the jar over the fungal mycelia mat. For each fungus, 6 replicates (n=6) of each treatment were exposed to decay for 12 weeks. Additional sets of sterile controls were run without fungal inoculation to assess the operational control losses.

The test block sizes were of 20 x 20 x 5 mm (radial x tangential x longitudinal) rather than the EN113 standard sample size of 50 x 30 x 15 mm chosen to ensure that even penetration of CNSL resin throughout the wood samples. After the exposure period the samples were removed from the jars and blocks were wiped to remove surface mycelia. The blocks were then reweighed before drying in oven at 105 °C for 24 hours. After drying and cooling in a desiccator the samples were then reweighed and mass losses and decay moisture contents were determined. These were expressed as percentage ML and dry mass moisture content using standard formula described in equation 3.5 and 3.6 (chapter 3).

7.2.6. Statistical analysis

The data obtained were analysed using IBM® SPSS® Statistics 20 by the one way analysis of variance (ANOVA) test to determine the statistical difference followed by Tukey's multiple comparison. The results with $P<0.05$ were considered to be statistically significantly different.

7.3. Results

7.3.1. CNSL resin modification

Results of resin impregnation of three different wood species Scots pine, Obeche and Gmelina are present in Table 7.2. Treatment of all the species with 5, 10, 20 and 30% CNSL resin resulted in linear increases in WPG in the wood blocks. Higher WPG was achieved in Scots pine than the other two wood species (*P. sylvestris* >*T. scleroxylon* >*G. arborea*). Control wood blocks of all species treated with 0% resin concentration (i.e. impregnated in IMS) showed no WPG.

Among the species only Scots pine achieved weight gain over 20% and in all cases the WPG of Scots pine was double than Gmelina, whereas the WPG of Obeche was also significantly higher than Gmelina and lower than Scots pine. Overall the lowest weight gain was found in Gmelina wood blocks (2.1%). These differences in WPG were anticipated as the wood density and permeability are generally somewhat different between the wood species and are similar but lower than the test with pure CNSL (chapter 3).

Table 7.2: Mean weight percent gain (WPG) of oven-dried wood samples impregnated with CNSL resin for the decay resistance test.

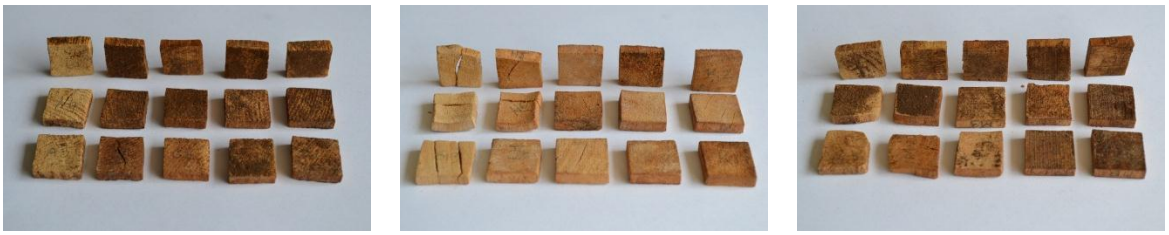
CNSL Resin Concentration (%)	**Mean weight percent gain (WPG)**		
	P. sylvestris	***T. scleroxylon***	***G. arborea***
Control (IMS)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
5	5.2 (0.4)	3.0 (0.6)	2.1 (0.4)
10	10.8 (0.8)	7.7 (1.0)	4.6 (0.6)
20	16.6 (1.8)	10.5 (1.2)	6.6 (0.7)
30	24.0 (1.6)	15.6 (1.0)	9.7 (0.7)

Values in parenthesis represent the standard deviation of weight percent gain

7.3.2. Visual observations of the decayed blocks

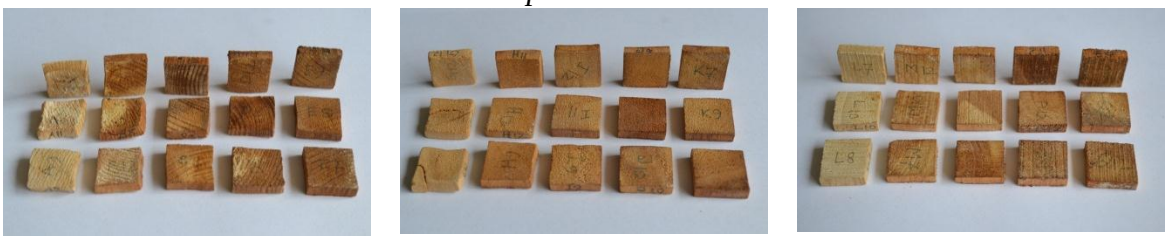
All blocks were colonised by the decay fungi and prior to removal extensive mycelia were present with the white rot fungi. After oven drying at end of the decay test (Figure 7.1) the *C. puteana* caused a darkening of the wood (Figure 7.1. a-c), particularly for Scots pine. With both brown rot fungi shrinkage was apparent for the solvent controls and lower WPG values. At the higher WPG blocks, where the treatment caused appreciable darkening of the wood blocks, the fungi caused some bleaching and this was particularly noticeable for *P. ostreatus*.

C. puteana



(a) Scots pine (b) Obeche (c) Gmelina

P. placenta

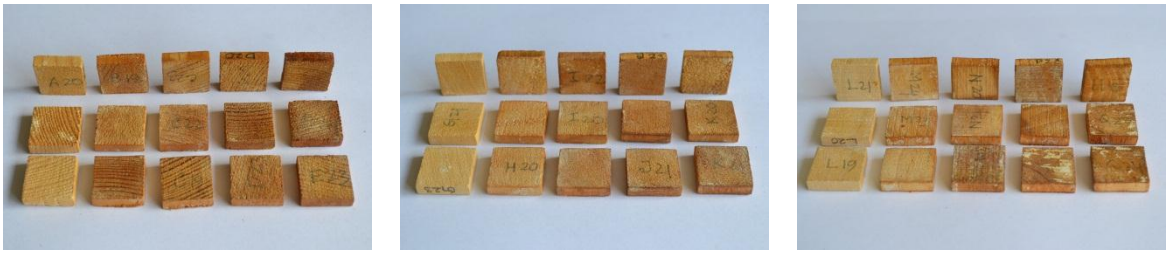


(d) Scots pine (e) Obeche (f) Gmelina

T. versicolor

(g) Scots pine (h) Obeche (i) Gmelina

P. ostreatus



(j) Scots pine (k) Obeche (l) Gmelina

Sterile controls

(m) Scots pine (n) Obeche (o) Gmelina

Figure 7.1: Appearance CNSL resin modified wood samples after 12 weeks exposure to brown rot and white rot fungi. Samples arranged left to right in order of treatment solution strength (i.e. WPG).

7.3.3. Mass losses of the CNSL resin modified wood

The CNSL resin modification reduced the mass loss with the increasing WPG in all wood species and all decay fungi (Figure 7.2) but no threshold protection values were achieved and extrapolated data will be introduced in the next section. Data points represent mean percent of mass loss (ML) against respective mean weight percent gain (WPG) of resin treatment. Three of the decay fungi (*C. puteana, P. placenta* and *T. versicolor*) performed well in the tests, achieving ML of over 25% on all no resin controls wood species (Figure 7.1 a, b, c) but *P. ostreatus* only gave results of below 10% on untreated controls and the highest ML was on Gmelina (9%). With the other decay fungi Gmelina gave MLs of over 40%.

In Scots pine modified wood the mass losses decreased with the increasing WPG for all of the fungi tested (Figure 7.2a). The mass losses caused by brown rot decay only decreased slightly with increasing resin content, even at the highest WPG of 24%. Both brown rot fungi showed similar response (25 to 30% ML at the highest WPG) with slightly higher decay overall caused by *C. puteana.* It is very difficult to assume that full protection can be achieved against either brown rot decay fungi with this resin system alone. Better decay resistance in Scots pine was achieved when exposed to white rot fungi. Treatment at WPG of 24 against *T. versicolor* provided a low ML of 7.8% though the full protection was not achieved at this level. The best protection (2% ML) of the treatment at WPG 24% was achieved when exposed to *P. ostreatus*, but, as stated above, this fungus only gave low ML overall. Statistically significant differences ($P<0.001$) of ML among the WPG levels were found for all four fungi. The *Post hoc* multiple comparisons of the ML also revealed that the decay resistance at 24 WPG was significantly lower ($P<0.05$) against all of the fungi tested.

In Obeche resin treatment resulted in a more marked reduction in ML with the increase of WPG against all fungi studied (Figure 7.2b), although the effect on the brown rot fungi was more marked, especially against *P. placenta*; a WPG of 15.6 reduced the ML to only 7.1%. With *C. puteana* the ML was reduced to 16.1% at this WPG. At this highest WPG *T. versicolor* gave the highest ML (19.1%). The other white rot fungus, *P. ostreatus,* failed to produce sufficient decay in untreated control Obeche blocks, even less occurred than in Scots pine. The effect of resin treatment (WPG) on the ML is effective as significant differences ($P<0.001$) were found between the WPGs for both the brown rot and white rot fungi. According to the *Post hoc* multiple comparisons (Tukey's), the highest WPG of 15.6 provided significant ($P<0.05$) higher decay resistance for all fungi.

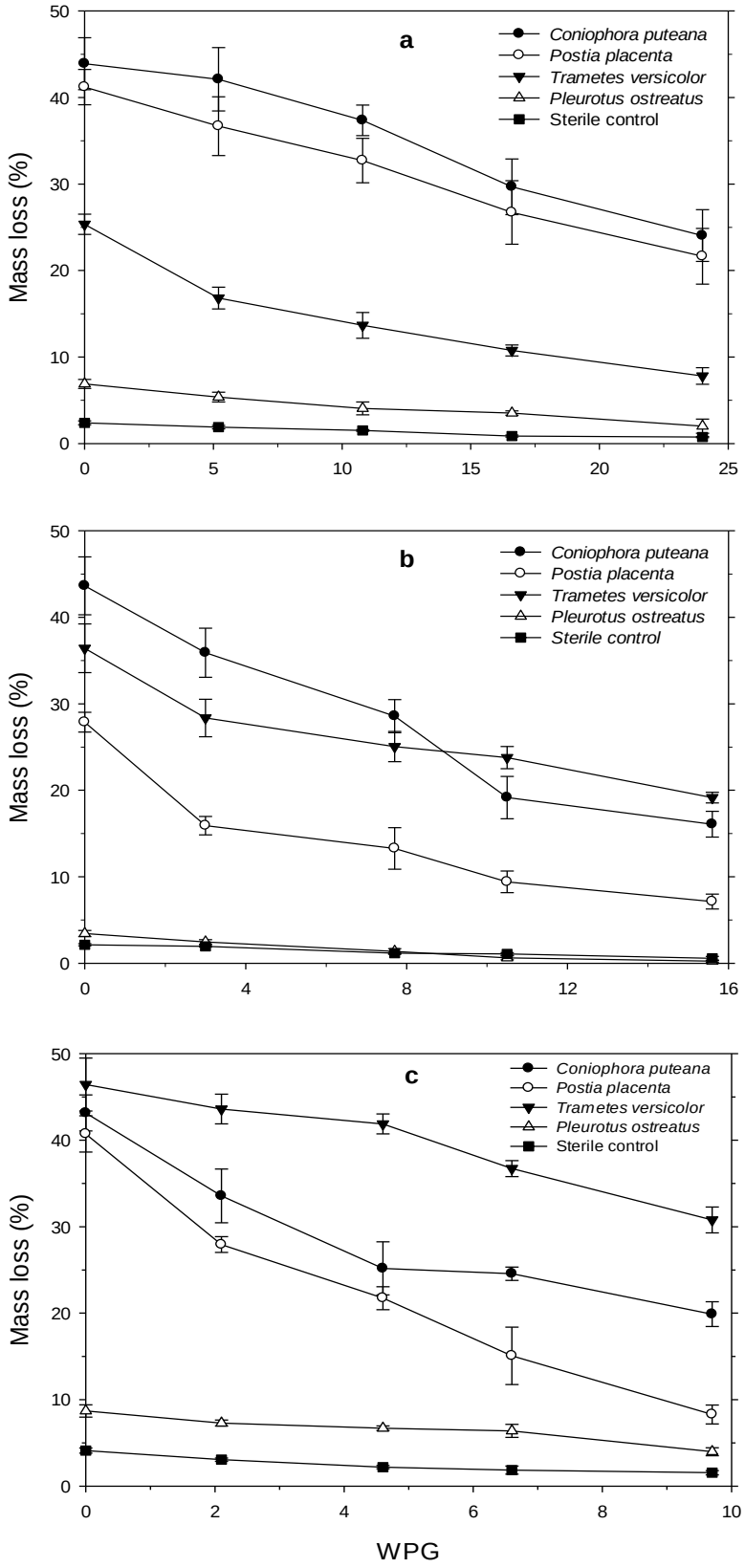


Figure 7.2: Mass loss of (a) Scots pine (b) Obeche and (c) Gmelina wood blocks modified with CNSL resin after 12 weeks exposure to brown rot and white rot decay fungi and sterile control (without decay fungi). Controls are IMS treated samples (error bars = ±SD, n=6).

Gmelina showed the lowest decay resistance in the tests and also the lowest uptakes of the resin (Figure 7.2c, Table 7.2) and despite this, the effect of increasing WPG on the brown rot fungi was more pronounced. At the highest WPG of 9.7 (less than half of the Scots pine WPG 24.0), ML values of 19.9% and 8.3% were seen for *C. puteana* and *P. placenta* respectively. With *T. versicolor* the ML of Gmelina only slightly reduced with the increase of WPG and a high value of 30.8% ML occurred at the highest WPG level. It is unlikely that even a considerably higher WPG alone would achieve adequate protection against *T. versicolor*. A very low ML (4%) was found with the highest WPG (9.7%) when exposed to *P. ostreatus*, but as mentioned above low overall ML occurred with this fungus. Significant differences ($P<0.001$) in the ML were found due to the effect of WPGs for all the fungi and as like the other two wood species and at highest WPG of 9.8 showed a significantly higher ($P<0.05$) effect on the reduction of the ML for all the fungi tested.

An additional set of samples as sterile control were exposed as the same manner of the decay test but without fungal inoculation for all the three wood species provided some useful information regarding the decay resistance of the CNSL resin modified wood. Results showed very little ML occurred in these samples due to the operational loss of the test, but this did decrease with the increase of WPGs (Figure 7.2 a, b, c). The effect of resin loadings is significant ($P<0.001$) on the ML of the sterile control blocks for all three wood species. Again, in this case, the resin loading at the highest level for specific wood species also provides significantly higher effect ($P<0.05$) on the ML of the sterile control wood blocks.

7.3.4. Threshold for total decay protection

It is evident that resin treatment at all the WPG values tested failed to meet the requirements for establishing a threshold against the selected brown rot and white rot decay fungi. This would correspond to the point at which no ML occurred or to a ML of less than 3% (as employed in the EN113 protocol) (Eaton and Hale, 1993). However some of the highest WPGs were close to the threshold limit, for example, Scots pine wood in exposure to *T. versicolor*, or Obeche and Gmelina wood in exposure to *P. placenta*. To improve the interpretation an examination of the effect of resin loading (WPGs) on mass loss was made using linear regression analysis from the whole ML data, presented as means in Figure 7.2. The data indicates a positive relationship between extent of resin modification and decay resistance (Table 7.3). Threshold values (at extrapolated 0% mass loss) and linear regression functions of particular fungi for all the three wood species to the specific WPG were noted (Table 7.3).

Results show a good indication of linear relationship in decay resistance (ML) of Scots pine, Obeche and Gmelina in exposure of decay fungi to the increased extent of resin modifications (WPGs). However examination of the linear plots show that in many instances the untreated control blocks gave higher decay than a linear extrapolation to the y-axis would suggest (e.g. Obeche, *P. placenta*: actual ML 28%, extrapolated ML 24%) so that the extrapolations to the x-axis give lower threshold values. The threshold values showed high variability between the brown rot (*C. puteana and P. placenta*) and white rot fungi (*T. versicolor and P. ostreatus*) than among the different modified wood species.

Resin modified Scots pine wood blocks exposed to brown rot fungi showed higher threshold WPG values of 52.4 and 50 for *C. puteana and P. placenta* respectively, while, less modification would theoretically be required to achieve the total protection against the white rot fungi. Conversely, much lower threshold WPG values were found for the brown rot fungi in Obeche modified wood, ca. 20% WPG in comparison to the white rot fungus, *T. versicolor* at 35 WPG. In Gmelina wood the theoretical thresholds for the brown rot fungi were even lower at WPGs of 17.4 and 12 for *C. puteana* and *P. placenta* respectively. The threshold WPGs for white rots *T. versicolor* and *P. ostreatus* were similar, at 27.2% and 28.2% respectively.

Table 7.3: Parameters of the simple linear regression function between wood decay resistance (ML) and extent of resin modification (WPG) for the wood decay fungi used against the three wood species. Threshold (**WPG at which 0% ML**) values were calculated by the extrapolation of respective data by using the regression equation.

Wood species	**Fungi**	**Linear regression function**		**Threshold**
		Regression equation	**R^2**	**(WPG)**
Pinus sylvestris	*C. puteana*	y = -0.8660x + 45.349	0.84	52.4
	P. placenta	y = -0.8268x + 41.305	0.88	50.0
	T. versicolor	y = -0.6841x + 22.638	0.87	33.1
	P. ostreatus	y = -0.1926x + 6.5637	0.86	34.1
Triplochiton scleroxylon	*C. puteana*	y = -1.8802x + 42.665	0.89	22.7
	P. placenta	y = -1.2445x + 23.968	0.82	19.3
	T. versicolor	y = -0.9658x + 33.783	0.81	35.0
	P. ostreatus	y = -0.2084x + 3.1807	0.90	15.3
Gmelina arborea	*C. puteana*	y = -2.2893x + 39.849	0.81	17.4
	P. placenta	y = -3.0778x + 37.079	0.88	12.0
	T. versicolor	y = -1.7635x + 48.035	0.91	27.2
	P. ostreatus	y = -0.4624x + 8.8050	0.85	28.2

7.3.5. Durability classification

A durability classification has been derived following the method used in EN-350-1 (1994) by using the X factor of the mass losses of the CNSL resin modified woods, as a relative comparison (Table 7.4). The X factor of the mass losses is derived by comparing the ML of unmodified or control wood samples to the resin modified samples at each WPGs from the same species. From the X factor, durability ratings have been derived (Table 7.5) as regarded the durability classes of European Standard EN 350–1 (1994).

Table 7.4 Ranking the decay resistance of CNSL resin modified woods using X factor based on the mass loss.

Wood species	WPG	Brown rot		White rot	
		C. puteana	*P. placenta*	*T. versicolor*	*P. ostreatus*
Pinus sylvestris	5.2	0.96	0.89	0.66	0.78
	10.8	0.85	0.79	0.54	0.59
	16.6	0.68	0.65	0.42	0.51
	24.0	0.55	0.53	0.31	0.29
Triplochiton scleroxylon	3.0	0.82	0.57	0.78	0.72
	7.7	0.66	0.48	0.69	0.41
	10.5	0.44	0.34	0.65	0.19
	15.6	0.37	0.26	0.53	0.07
Gmelina arborea	2.1	0.78	0.69	0.94	0.84
	4.6	0.58	0.53	0.90	0.77
	6.6	0.57	0.37	0.79	0.73
	9.7	0.46	0.20	0.66	0.46

Table 7.5: Durability Classification of the CNSL resin modified wood exposed to decay fungi based on the X factor in accordance to EN 350-1 (1994).

Wood Species	WPG	Brown rot		White rot	
		C. puteana	*P. placenta*	*T. versicolor*	*P. ostreatus*
Pinus sylvestris	5.2	5	4	4	4
	10.8	4	4	4	4
	16.6	4	4	4	4
	24.0	3	3	3	2
Triplochiton scleroxylon	3.0	4	3	4	4
	7.7	4	3	4	3
	10.5	3	3	4	2
	15.6	3	2	3	1
Gmelina arborea	2.1	4	4	5	4
	4.6	3	4	5	3
	6.6	3	3	4	3
	9.7	3	2	4	3

1= very durable, 2= durable, 3= moderately durable, 4= slightly durable, 5= non-durable

In this study, Scots pine modified wood provided class 3 (moderately durable) against both the brown rots and white rots, with a comparable high resin loading (24 WPG). The durability class increased for Obeche modified wood against the brown rot fungi and showed class 2 (durable) at higher WPG values e.g. for exposure to *P. placenta*. Durability against *T. versicolor* showed similar to that of Scots pine modified wood but with the lower resin modification (15.6) of Obeche wood. Surprisingly, the Gmelina wood with a low WPG (9.8) showed a better durability rating against brown rots (class 2 and 3 against *P. placenta* and *C. puteana* respectively) than Obeche and Scots pine. However, the durability ranked 4 (slightly durable) against *T. versicolor* with this low level of resin modification.

7.3.6. Moisture content of CNSL resin modified wood after the decay test

The mean moisture content in resin modified and untreated controls wood samples of Scots pine, Obeche and Gmelina after 12 weeks exposure to test fungi and sterile control are shown in Figure 7.3. Adequate moisture contents were achieved in the untreated sterile control test blocks (33.6%, Scots pine, 37.8% Obeche and 64% Gmelina) and as the WPG increased due to the modification with resin, the final moisture content (MC) at the end of test decreased. At the maximum WPGs for the three different wood species (24, 15.6, and 9.7), apparently low MCs, 15.9, 19.7 and 21.7 % for Scots pine, Obeche and Gmelina respectively were achieved, although these effectively refer to both wood and resin moisture contents.

In the presence of fungi, moisture contents were elevated as compared to the sterile control blocks and these also decreased with increasing WPG. In Scots pine wood, the MC reduced from over 100% in untreated blocks to ca. 30% at the higher level of modification against both brown rot fungi (*C. puteana and P. placenta*) while, the MC reduced from 51 and 42 % in untreated blocks to similar values to the sterile controls (23-24%) with the white rot fungi (Figure 7.3a). In Obeche, the MC reduction against the brown rot fungi is more pronounced than with the white rot fungi. MC reduced to ca. 23% in both brown rots but to 32% with the white rot fungus *T. versicolor* at the highest modification level of Obeche wood (Figure 7.3b).

Although the reductions in MC with increasing WPG were evident for Gmelina, the data showed more variability. Where high ML occurred, high MC also occurred but the behaviour switched between the two brown rot fungi. At the highest WPG, MC values of ca. 20, 30 and 45 % were found for *P. placenta*, *C. puteana* and *T. versicolor* respectively. The MC of *P. ostreatus* on Gmelina showed similar values to the sterile controls and overall the values on the other wood species were only marginally higher than the sterile controls.

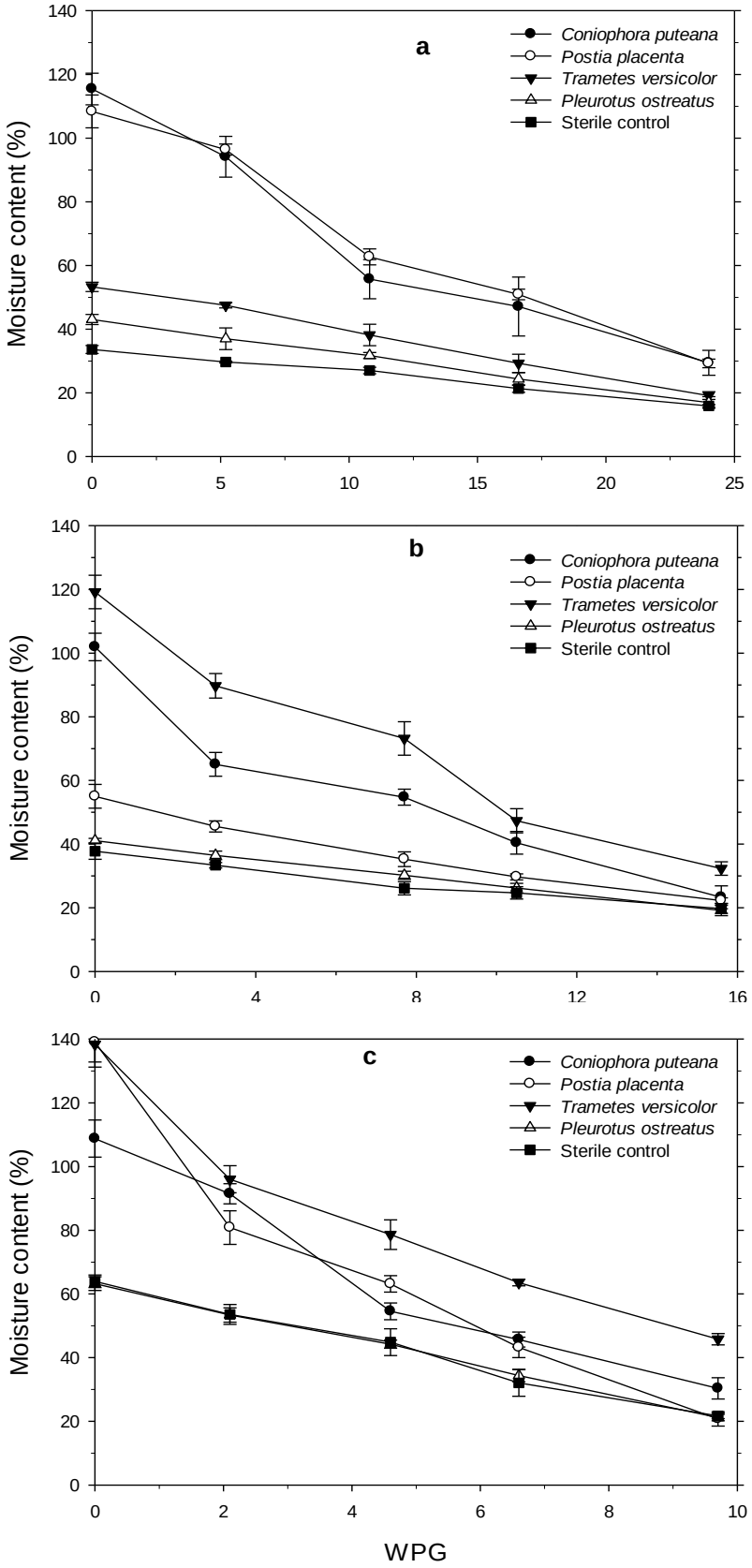


Figure 7.3: Reduction of moisture content (MC) with increasing WPG of CNSL modified wood at the end of the decay test (a) Scots pine, (b) Obeche and (c) Gmelina (error bars = ±SD, n=6).

Statistically significant differences (P<0.001) between the resin loadings (WPGs) on the final MC of decayed wood samples were found against all of the fungi tested, and the sterile controls for all the three wood species. In all cases, resin loading at the highest point (24%, 15.6% and 9.8%) showed a significantly higher effect (P<0.05) on the reduction of the MC for Scots pine, Obeche and Gmelina wood respectively according to the *Post hoc* multiple comparison (Tukey's).

7.4. Discussion

7.4.1. Effect of CNSL resin on wood decay resistance

Although the CNSL resin system reduced the mass losses of decayed wood, total decay protection was not achieved in any of the wood species against any successful decay fungi tested. The data from *P. ostreatus* should be ignored for this purpose because it is clear that *P. ostreatus* failed to give adequate mass losses to the untreated control blocks. Similar performance of *P. ostreatus* had previously been observed when the fungus was inoculated in Scots pine wood treated with *i*CNSL and *t*CNSL (chapter 3).

Conventionally, a threshold is established where the data exceeds the threshold thereby a threshold can be reliably shown. Alternatively, estimation of efficacy using regression techniques has also been reported (Van Acker and Stevens 1989; Gezer *et al*. 1999; Williams and Hale, 1999; Van Acker *et al*. 1999; Hill *et al*. 2004a; Ormondroyd 2007). In this work by using the regression analysis it can be assumed that a full protection threshold could be established by extrapolation of the data points. The extrapolation suggested that full protection in Scots pine and Obeche may occur at WPGs in excess of 30 against *T. versicolor*. The extrapolation also suggested that WPGs of ca. 20 for both brown rot fungi may impart full protection to Obeche wood. In Gmelina even lower thresholds were found for *P. placenta* (12 WPG), *C. puteana* (ca. 17 WPG) and *T. versicolor* (ca. 27 WPG).

Hill (2006) stated that much higher resin loadings (WPGs) are required for the protection of hardwoods than softwoods. In the current work, despite showing low decay resistance when untreated, the hardwoods, Obeche and Gmelina, showed better decay resistance with low resin loadings, particularly against the brown rot fungus *P. placenta,* and mass losses were reduced to ca. 7%, but at the same WPGs the mass losses were considerably higher against the white rot fungus, *T. versicolor* (19%). On the other hand, in Scots pine the decay resistance

showed better improvement against white rot than the brown rots. This can be explained basic decay type preferences: the hardwoods are preferentially decayed by white rot fungi and softwoods by brown rot fungi (Zabel and Morrell 1992; Eaton and Hale, 1993). Although this is a simplistic view often lignin types and quantities are used to explain these differences (Sarkanen and Ludwig, 1971; Rowell *et al*., 2005).

Hill (2006) mentioned that where there is sufficient space for access, low molecular weight resins are polymerized within the cell wall micropores and locked in a place due to entanglement of the resin system with the cell wall polymeric materials, or that chemical bonds form between the resin and the wood cell wall polymers. In the present work it is not clear whether the CNSL resin formed any chemical bonds with the wood cell wall polymers, but resin may cause cell wall micropore blocking by its passive presence in microvoids. With chemical modification by the formation of chemical bonds with cell wall polymers, decay resistance is imparted due to the cell wall bulking, which reduces the size and number of the wood cell wall micropores (Papadopoulos and Hill, 2002; Hill *et al*., 2005). The volume change (bulking) of CNSL resin modified wood was studied in chapter 4 (section 4.3.12) and it was revealed that the CNSL resin did not bulk the wood cell wall but located in the cell lumen. This would thus block some of the accessible cell wall micropores. However it is assumed that most of the CNSL resin molecules are larger than cell wall micropores, which hinder their entry into the cell wall micropores (see chapter 4). Presumably, most of the resin fills part of the macro-void volume of wood, cell lumens.

Hill *et al*. (2004) reported that complete decay resistance was achieved against the brown rot and white rot fungi by impregnation of alkoxysilane coupling agents at the WPG where the impregnants were largely located in lumen and the inner cell wall surface rather than deeply throughout the cell walls and thus concluded that full protection can be achieved by a combination of cell wall blocking to micropores and a barrier effect of the lumen surface by the silanes coating.

The resin may also form a physical barrier to the hyphal growth, and thus reduce the colonization of the fungi, because the fungus must establish in the wood cell lumens before it can release degradative agents and start cell wall degradation (Eriksson *et al*., 1990). For example DMDHEU impregnated into wood increased decay resistance due to alteration of the cell wall and reduced fungal colonization occurred (Verma *et al*., 2009). The lower colonization in the DMDHEU treated wood reduced the mass loss. It is difficult to assess the effect of CNSL

resin on the fungal colonization as no detailed study was made in this work involving the microscopy of colonisation at different point of time during a decay test.

Cell wall blocking is of lesser relevance where decay is driven by hydrolytic enzymes as the enzymes are too large to penetrate into the cell wall, but it is of greater importance where low molecular weight agents are concerned, especially in the early stages of decay. So, the initial degradation potential of wood is due to the low molecular degradation agents produced by fungi which was proposed and established in particular to brown rot fungi through the Fenton's reaction mechanism. This is examined in earlier chapter 6 and it was observed that the CNSL resin cannot completely protect the wood cell wall to penetrate by the OH radicals, which degrade wood cell wall polysaccharides through depolymerisation. Thus, it is assumed that the CNSL resin only penetrates into the cell wall at the lumen surface and may also forming a thin layer within the lumen. Thus the coating forms a protective layer but only a small region of the cell wall micropores, i.e. those that are immediately accessible from the lumen are filled. Again, it is possible that the CNSL resin also provides some extent of direct physical barrier to penetration by fungal hyphae into the cell wall from the cell lumens. This would in effect reduce colonisation.

7.4.2. Effect of moisture content of wood on decay resistance

The lowered uptake of moisture in resin modified sterile control samples after the decay test may be explained by the hydrophobic nature of CNSL resin system and macro-pore blocking, i.e. blocking the movement of water into the blocks. These are also assumed to be partially responsible for the improvement in decay resistance. Some of the reduction may simply be a feature of increased weight of a hydrophobic material within the block so giving a distorted view of moisture content, however this only accounts for a minor part of the lowered moisture content and a worst case scenario calculation on the highest treatment level sterile control (Scots pine) suggests that the average difference is only about 2.8% and would give a moisture content of 18.7% at the highest WPG in the Scots pine sterile controls (i.e. based on the control wet weight minus the resin weight and expressed as a percentage of the original block weight). In general, wood is not adequately colonized and decayed unless its moisture content is 20% or more and for the wood cells to be around fibre saturation point (FSP) (Eaton and Hale 1993). The post decay moisture contents at the highest WPGs of many of the tests showed a decrease to around 20% where the amount of decay is lowest. Of course this is only the end of decay moisture content and may not truly reflect earlier moisture contents as moisture losses may

have occurred during the decay test. However where appreciable decay occurs higher moisture contents are seen. This may be because the moisture contents are elevated by the decay fungi themselves and by the reduced wood mass.

Rapp and Peek (1996) reported the effect of moisture regime change on the decay resistance of melamine resin treated Scots pine sapwood against *C. puteana* and argued that the increased decay resistance of treated wood was due to the change of moisture regime (low moisture content) and not by the poisoning the fungi. Ormondroyd (2007) studied wood decay resistance of Scots pine and Beech treated with urea formaldehyde, melamine formaldehyde and melamine-urea formaldehyde and concluded that the decay resistance was due to the physical blocking of OH groups in the cell wall and the inhibition of moisture ingress into cell wall and thus making wood substrate unsuitable for decay.

So it is likely that the CNSL resin is too large to substantially penetrate the cell wall and the bulk of the resin is located in the cell lumen. This may provide a physical barrier to the OH groups of the wood cell wall and ultimately reduce wood cell wall swelling and the moisture ingress and thus reduce the mass loss of decayed wood. The final moisture contents are directly, linearly correlated with the mass loss of the CNSL resin modified decayed wood (Figure 7.4). The CNSL resin modified wood samples also showed water repellency when tested in water soak method (chapter 4) at WPGs similar to the WPGs of modified wood exposed to the decay fungi in this experiment. So, it is assumed that the CNSL resin is hydrophobic in nature, which is attributed to the side chain of CNSL (Pillai *et al.* 1980). Stamm and Baechler (1960) studied the decay resistance of phenol-formaldehyde wood and found the relationship between the swelling reductions of the treated wood to the low mass losses due to decay. This finding is later addressed by Hill (2002) and suggested that due to the swelling reduction there is insufficient moisture contained in the cell wall to support decay. Dimensional stability of the CNSL resin modified wood was also studied in chapter 4 and revealed that the resin increased stability to wood by reducing the swelling in water soak test.

7.5. Conclusions

1. Threshold wood protection levels were not reached in valid fungal tests at the WPGs provided by the treatments used. Where they were the tests were deemed invalid due to low decay in the untreated controls (e.g. not *P. ostreatus*). The limited WPGs of the modified wood are probably due to the comparatively large molecular size of the CNSL resin system which give the treatment solution higher viscosity and thus lowers penetration.

2. In this work the apparent thresholds for the softwood were much higher than for the hardwoods, although the brown rot fungi proved problematic to control. The extrapolation of mass loss data revealed that ca. 30% WPG will reduce the mass losses against *T. versicolor* for all the three wood species i.e. Scots pine, Obeche and Gmelina. In the case of brown rot decay, a very high threshold required for Scots pine (ca. 50%) but less than 20% WPG threshold can provide complete protection to decay in hardwoods Obeche and Gmelina.

3. It is assumed that CNSL resin cannot block the wood cell wall micropores and thus cannot prevent penetration of the low molecular diffusible agents for initial decay followed by the enzymatic degradation and fungal colonization in the wood. In some cases the limited WPGs of resin modified wood reduced the mass losses particularly the brown rots in Obeche and Gmelina which indicated that the CNSL resin provides a barrier to the fungal hyphae penetration into the wood cell wall in the later stage decay.

4. In the present study, it is clear that the ultimate uptake of moisture by the resin modified is reduced, provided that the resin is polymerized in the wood (probably in lumen) and which is not hydrophilic. The resin may form a physical barrier to the cell wall OH groups and resist moisture ingress thus make the wood unsuitable for decay. In many cases the mass losses were reduced to the lowest limit when final moisture content of the decayed samples showed to the lowest value. The reduction of the mass losses of the CNSL resin modified wood showed a good relation to the low moisture content of the decayed wood.

Chapter 8

Additive effect of CNSL resin combined with antioxidants to improve the decay resistance of wood

8.1. Introduction

The development of environmental friendly wood protection systems have become important to reduce the impact of artificially durable wood throughout its life cycle and opposed to conventional wood preservatives. Recently, wood protection systems have been directed towards the combining more than one organic biocide in water borne formulations in a cocktail involving environmentally benign and inexpensive agro-chemicals with non-biocidal additives to increase efficacy and/or other properties (Schultz *et al.*, 2004).

Schultz and Nicholas (2000, 2002) proposed an alternative approach to protect wood from biodegradation by some of the mechanisms involved in heartwood durability. It has been long known that wood extractives are fungitoxic (Tsoumis, 1991; Schultz *et al.*, 1995; Yen *et al.*, 2007) and insecticidal (Ohmura *et al.*, 2000; Morimoto *et al.*, 2006) and more recently their role as antioxidants (AOs) has been proposed (Larson, 1988; Gao *et al.*, 2007; Huang *et al.*, 2009). Antioxidants can synergistically work to protect the heartwood against fungal colonization (toxicants) and degradation (AOs). As the initial stages of fungal degradation of wood take place through oxidative reactions (Goodell *et al.*, 1997; Hyde and Wood, 1997; Kerem *et al.*, 1999; Jensen *et al.*, 2001; Hammel *et al.*, 2002), some AOs have been screened as promising additives for development of novel and environmentally benign protection systems for wood.

Combining different AOs including butylated hydroxyl toluene (BHT), propyl gallate, octyl gallate, irganox 1076 with various organic biocides have enhanced the antifungal effects against the decay fungi (Schultz and Nicholas, 2000, 2002; Mabicka *et al.*, 2005; Hsu *et al.*, 2007). Moreover, the combined effect of the natural antifungal compound cinnamaldehyde (Wang *et al.*, 2005; Cheng, 2006) with the AOs catechin, quercetin and eugenol were examined to find the synergistic effect on the wood decay fungi (Hirasawa and Takada, 2004; Yen *et al.*, 2008). It was reported that cinnamaldehyde with eugenol showed a strong synergy which was attributed to the radical scavenging effect along with the interference of fungal cell wall synthesis and fungal cell wall destruction. Schultz *et al.* (2004) reported that combination of

organic biocides with commercial AOs generally improved biocidal efficacy 2 to 3 times higher against the wood decay fungi when assessed in a short term laboratory test. The combined effect also enhanced the decay resistance in the field test after 2 to 4 years.

From the decay resistance point of view impregnation modification with resins is generally inferior to chemical modification due to lack of reaction within the cell wall as the inside of the cell wall is only coated. The decay resistance of impregnation modified wood with CNSL resin, a bio-based resin system was investigated in chapter 7 revealed that the CNSL resin can improve the decay resistance but complete protection was not achieved. Furthermore, the oxidative degradation of CNSL resin modified wood was investigated in chapter 6 where it was revealed that the resin cannot totally impede extracellular OH radical ingress into the wood cell wall.

Conventionally fungal decay can be prevented by the inclusion of toxic compounds, typically copper and co-biocides, this approach attempts to reduce reliance on toxicity and alternative approach is investigated in this chapter which combines the bio-based CNSL resin modification and different AOs. The aim of this study is to investigate the additive effect of CNSL resin combined with AOs to improve the decay resistance of wood.

8.2. Materials and methods

8.2.1. Chemicals

The CNSL resin was prepared from *t*CNSL (technical cashew nut shell liquid) by ozonolysis process at the BioComposites Centre, Bangor, UK Laboratory (chapter 4, section 4.2.2). Gallic acid ($C_7H_6O_5$), Ferulic acid ($C_{10}H_{10}O_4$), Caffeic acid ($C_9H_8O_4$), Quercetin ($C_{15}H_{10}O_7$) and Propyl gallate ($C_{10}H_{12}O_5$) were obtained from Sigma-Aldrich Co. UK. Chemicals were used in this study as received. Spent sulphite liquor (SSL) phenolics were solvent extracted from the crude SSL, industrial black liquor and by product obtained from the cooking processes in pulp and paper industries. The SSL phenolic extracts were collected from the SSL of *Eucalyptus globulus* supplied from Greece (Tverezovskiy, 2013).

8.2.2. Wood samples

Samples from sapwood of Scots pine (*Pinus sylvestris*), Obeche (*Triplochiton scleroxylon*) and Gmelina (*Gmelina arborea*), straight grained and free from knots, were prepared to dimensions of 20 x 20 x 5 mm (radial x tangential x longitudinal). After sanding to remove

loose fibres, the wood samples were stored in a climate chamber at 20 °C and 65% relative humidity (RH) until they reached equilibrium moisture content.

8.2.3. Decay fungi

Wood decay fungi and their strains used in this experiment include brown rot fungus, *Coniophora puteana* strain FPRL 11E and white rot fungus, *Trametes versicolor*, strain CTB 863A. The cultures of all fungi were maintained on 2% malt extract, 2% agar medium at 22 °C.

8.2.4. Antioxidant assay

The AO properties of gallic acid, ferulic acid, caffeic acid, quercetin, propyl gallate and SSL phenolic extracts were determined by the way of free radical scavenging activity using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) discoloration method as described in the chapter 3, section 3.2.3. The DPPH inhibition (I %), IC_{50} (Inhibitory concentration reduce 50%) and AO activity index (AAI) were calculated according to equations 3.1 and 3.2 (chapter 3).

8.2.5. Impregnation with CNSL resin and antioxidants

The wood specimens were vacuum impregnated with alcoholic solutions of CNSL resin of 10% solute content in industrial methylated spirit (IMS) containing different AOs. 100 mM/L of each commercial AO was added to the resin solution prior to the impregnation, shaken until the complete dilution of the AO components with the resin solution. The SSL phenolic compound was added to the resin solution at 10% (v/v). A separate set of wood blocks were also impregnated with only AO compounds of different type (without resin). Each of the AO compounds was added to the IMS in same amount that was used in resin solution and diluted in the similar way mentioned earlier. The wood samples treated with IMS were use as control (untreated) and 10% resin solution as control (resin treated). The resin impregnation was carried out according to procedure described in chapter 4, section 4.2.3. It is assumed that the 10% solution strengths gave a similar WPG to those achieved earlier (Table 4.2).

8.2.6. Wood decay test

Wood samples were exposed to brown rot (*C. puteana*) and white rot (*T. versicolor*) based on the EN 113 protocol (1996). The decay test was carried out according to process described in chapter 7, section 7.2.5. The mass losses of the decayed wood samples were determined and expressed as percentage mass loss using standard formula described in equation 3.5 (chapter 3).

8.2.7. Statistical analysis

The data obtained were analysed using IBM® SPSS® Statistics 20 by the one way analysis of variance (ANOVA) test to determine the statistical difference followed by Tukey's multiple comparison. The results with $P<0.05$ were considered to be statistically significant difference.

8.3. Results

In the results of this chapter the effect of AOs on the decay resistance is looked at in various ways. Initially the potency of a variety of AOs is examined using a DPPH assay (section 8.3.1) and this is followed by a wood decay test and looks at ML data for AO: resin combinations. Initially these data are examined as percentage ML (ML as AOs + wood: section 8.3.2; ML as resin + AOs + wood: section 8.3.3) but then they are examined as indices to determine their effects relative to the controls and expressed as percentages. The first of these shows the reduction in mass loss for each of the AO: wood combinations referenced against their controls (8.3.4, Table 8.2) using the data from section 8.3.2 and then the same transformation is done for the AO plus resin data (8.3.4, Table 8.3) using the data from section 8.3.3. The data from these (Tables 8.2 and 8.3) is then compared to show the difference between AO alone and AO plus resin (Table 8.4). Finally the additive effect of the combination is presented (Table 8.5) where ML of each AO with the resin is expressed as the percentage of the respective control (i.e. untreated).

8.3.1. Antioxidant assay

The free radical scavenging effect of the AO compounds assessed by DPPH assay (Figure 8.1) as expected show that as AO concentration increases the percentage of DPPH inhibition (I %) increases. At the highest concentration most of the AOs showed the highest radical scavenging effect. A ca. 90% DPPH inhibition for all compounds tested except for Ferulic acid (60%).

Table 8.1 represents the AO properties i.e. the inhibitory concentration of DPPH providing 50% (IC_{50}) and AO activity index (AAI) of the tested compounds. On this basis the most effective AO properties were gallic acid, ferulic acid and propyl gallate with IC_{50} values of 8.24, 8.67 and 8.75 µg/ml and AAI values of 5.94, 5.65 and 5.59 respectively. A moderate AO property was found in quercetin and with IC_{50} value of 10.84µg/ml and AAI values of 4.67. Comparatively lower AO properties were found in caffeic acid and SSL phenolic extracts; they had with higher IC_{50} and lower AAI values than the other compounds tested.

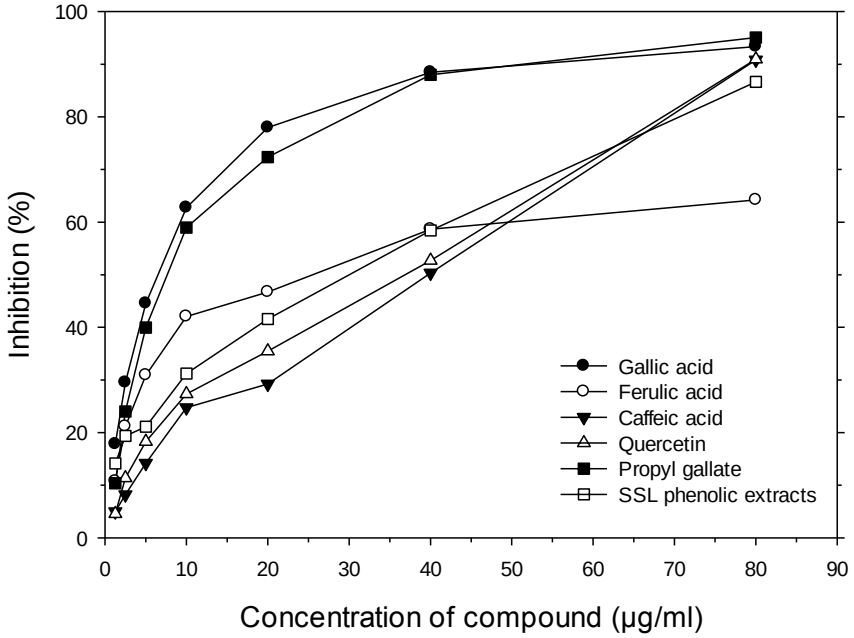


Figure 8.1: Inhibition percentage of DPPH (2, 2-diphenyl-1-picrylhydrazyl) by different antioxidant compounds.

The DPPH inhibition index (I %) showed the capacity of the compound to reduce the DPPH radicals at a fixed concentration. The IC_{50} shows the compound concentration necessary for reduce the initial DPPH concentration by 50%. Thus, the AAI relates the DPPH concentration used in the assay with IC_{50} of the compound, resulting in constant data for each compound (Scherer and Godoy, 2009).

Table 8.1: Antioxidant properties of compounds from the DPPH free radical scavenging assay.

Compounds	IC_{50} (µg/ml)	AAI
Gallic acid	8.24	5.94
Ferulic acid	8.67	5.65
Caffeic acid	23.80	2.05
Quercetin	10.84	4.67
Propyl gallate	8.75	5.59
SSL phenolic extracts	20.28	2.41

8.3.2. Mass losses of the wood treated with antioxidants

The mass losses of wood blocks of Scots pine, Obeche and Gmelina treated with the different AOs alone (without CNSL resin) exposed to *C. puteana* and *T. versicolor* (Figures 8.2a and 8.2b respectively) performed well giving high untreated control mass losses (ML) and the test is accepted as valid, i.e. over 20% ML. The mass loss of each set of treated wood blocks run in decay test without fungi as sterile controls (Figure 8.2c) show very low operational mass losses of the wood blocks, less than ca. 2%, so for the purposes of further data analysis have not

been subtracted and have been are ignored for this and the subsequent data for AO plus resin (Figure 8.3.c).

With *C. puteana* ML of ca. 45% were recorded for Scots pine, Obeche and Gmelina control wood and with *T. versicolor* ca. 45% on Gmelina. Values of ca. 35% were recorded for Scots pine and Obeche control wood samples. Given these differences some proportional data processing, as detailed earlier, will be presented below.

The raw data shows that the only AO consistently effective at reducing ML caused by *C. puteana* in all three wood species was propyl gallate and the other AOs were not very effective (Figure 8.2a). Propyl gallate treatment reduced the ML to 8%, 1.9% and 6.3% for Scots pine, Obeche and Gmelina respectively. There is little variation within each set so the test has more or less behaved uniformly although ferulic acid showed some ML reduction against *C. puteana*. Here it was most effective in Gmelina (ML ca 16%), and much less so in Scots pine (ML ca 32%). Obeche wood blocks treated with SSL phenolic extracts provide a low ML (ca 14%) against *C. puteana*. Statistically, significant difference ($P<0.001$) in ML between the AO treatment has been found for all the three wood species. Propyl gallate treated wood blocks for the three species showed significantly lower ($P<0.05$) ML against *C. puteana*.

The AOs were more effective in reducing ML of the treated wood blocks against *T. versicolor* (Figure 8.2b). Greater variation of the ML were found between the wood species with exposure to *T. versicolor* and the MLs are greater in Gmelina untreated and treated wood than the other two wood species. Propyl gallate was again the most effective in reducing the MLs of the treated wood blocks for all the three wood species, although this was less effective than for *C. puteana* at ca. 13% and 17% MLs in Scots pine and Obeche (respectively) and 27% in Gmelina. The ML differences between the AO treatments and the three wood species are statistically significant ($P<0.001$) and propyl gallate treated wood blocks showed significantly lower ($P<0.05$) MLs for all the three wood species against *T. versicolor*.

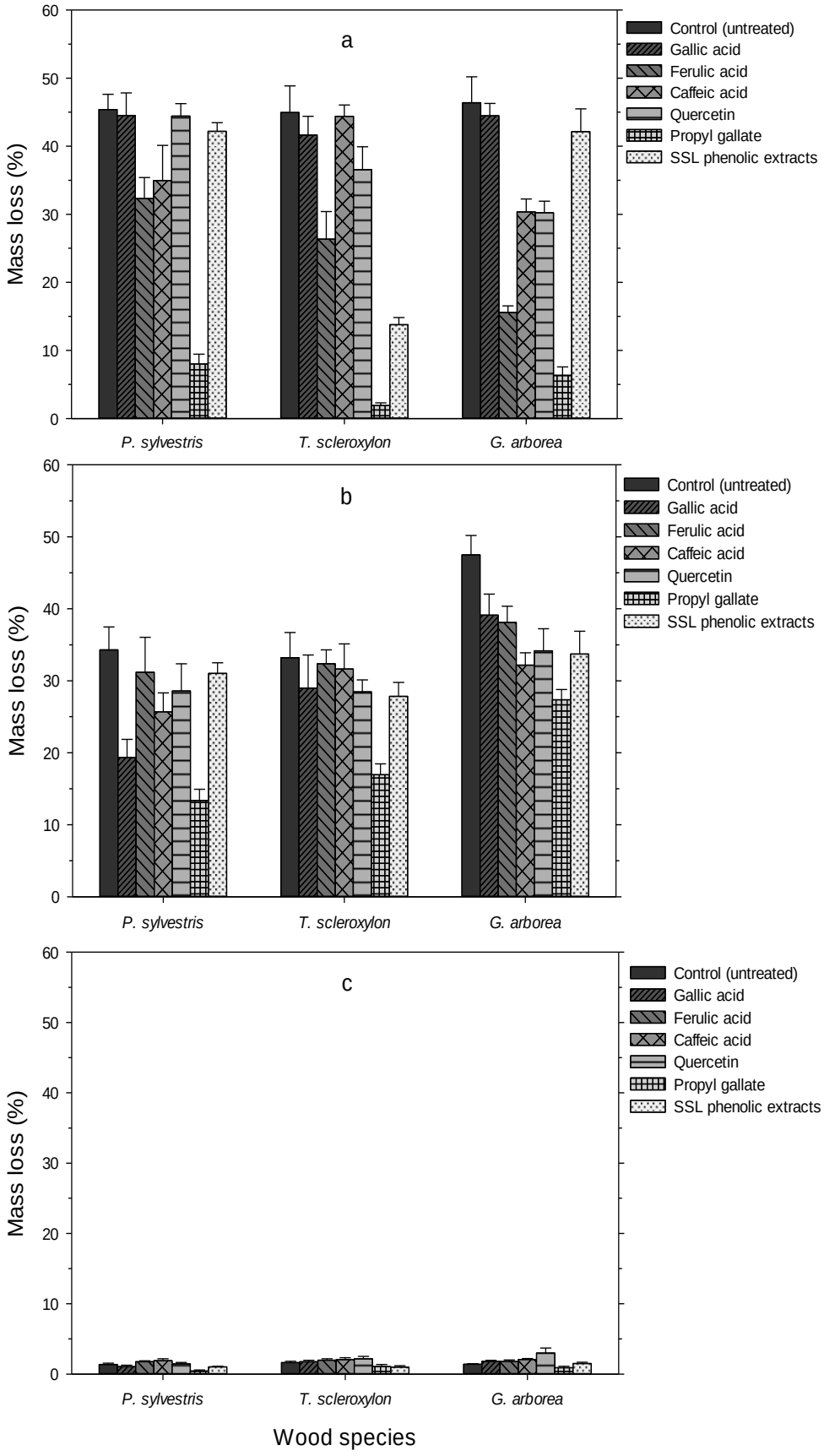


Figure 8.2: Mean mass losses of wood treated with different antioxidant compounds (without CNSL resin) after 12 weeks exposure to decay fungi a) *C. puteana*, b) *T. versicolor* and c) Sterile control (without fungal inoculation) (n= 6, error bar= ±SD).

8.3.3. Mass losses of wood treated with CNSL resin combined antioxidants

The combined effect of CNSL resin and AOs on the MLs of the treated wood blocks of Scots pine, Obeche and Gmelina against *C. puteana* and *T. versicolor* are presented in Figure 8.3. Wood blocks treated with only CNSL resin were used as the control for this test. The wood blocks showed lower ML when AOs were added with CNSL resin for all the three wood species against both the fungi, *C. puteana* and *T. versicolor*, although in some instances they were only marginally lower than the controls. The amount of decay caused by *T. versicolor* on the AO plus resin treated was substantially lower in Scots pine than in the AO alone controls.

In general, the combined of the resin and the AO was more effective in Gmelina than Scots pine or Obeche. Among the AOs used, propyl gallate showed more effect with CNSL for all three wood species against *C. puteana*. Furthermore propyl gallate was more effective in Scots pine and Obeche (<1% ML) than in Gmelina (3% ML) although SSL phenolic extracts gave an effect in Obeche, and ferulic acid gave an enhanced effect in Gmelina against *C. puteana*. Statistically significantly differences ($P<0.001$) in ML were found among the treatments and significant lower ML ($P<0.05$) was found in wood blocks treated with propyl gallate and CNSL resin.

The MLs of the combination (wood: resin: AO) were lower against *T. versicolor* than *C. puteana* for all the wood species although the ML of wood blocks treated with only CNSL resin is much lower with *T. versicolor*. Scots pine and Obeche wood blocks showed lower MLs than Gmelina but the effect of AOs was generally less in Scots pine and Obeche as compared to their controls (i.e. resin alone) and a wider range of differences were seen Gmelina. Propyl gallate showed the lowest ML when combined with resin in all the three wood species, although in Gmelina still gave appreciable ML. Ferulic acid showed lower ML in Gmelina wood and SSL extract showed a good reduction in Obeche. Statistically significantly difference ($P<0.001$) in mass losses between the treatment has been found for all the three wood species and propyl gallate and resin treated wood blocks showed significant lower ($P<0.05$) mass losses for all the three wood species against *T. versicolor*.

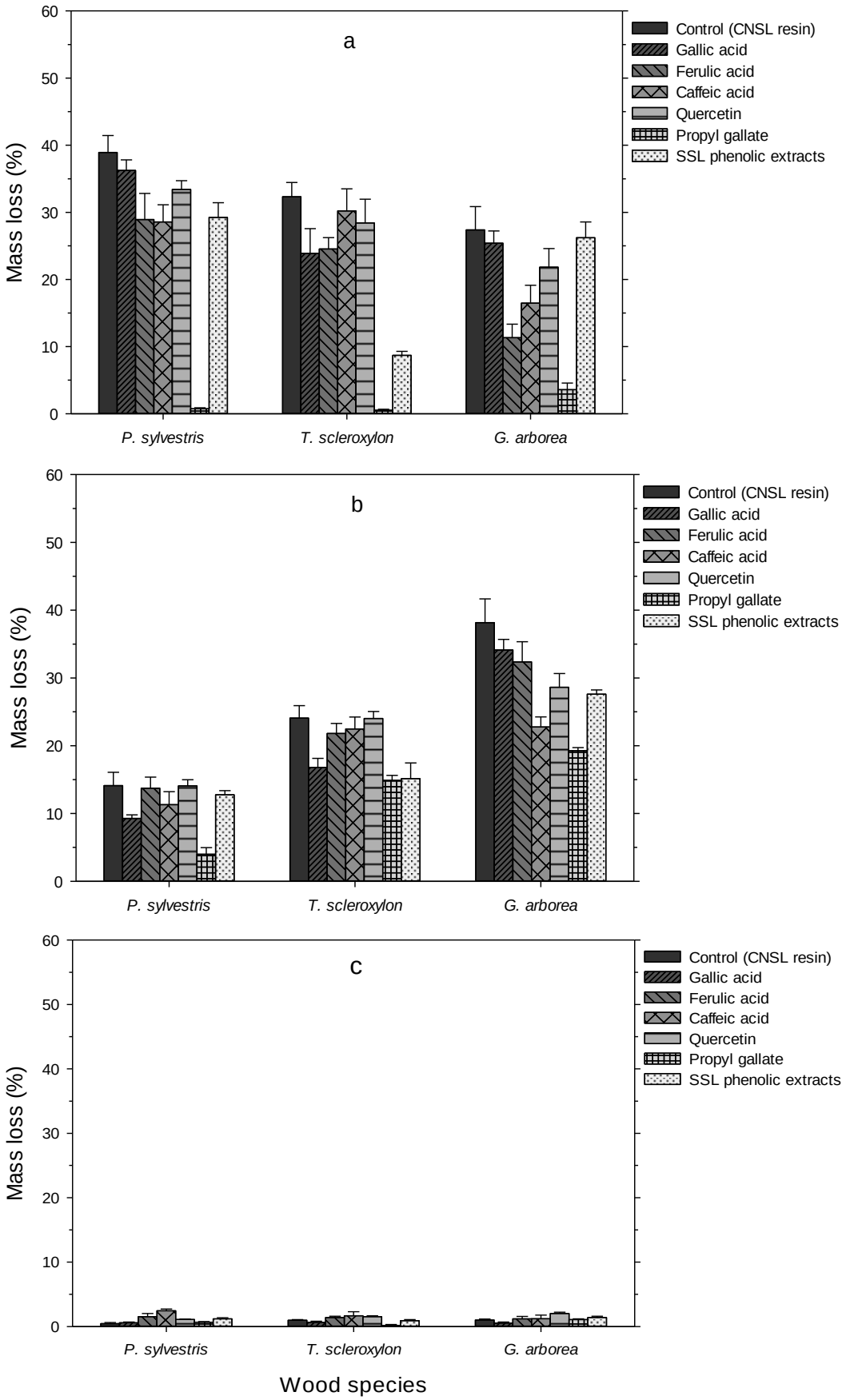


Figure 8.3: Mean mass losses of wood treated with different antioxidant compounds combined with CNSL resin after 12 weeks exposure to decay fungi a) *C. puteana*, b) *T. versicolor* and c) Sterile control (without fungal inoculation) (n= 6, error bar= ±SD).

8.3.4. Effect of antioxidants, CNSL resin and their combination on mass loss

To account for different levels of decay in the control blocks the results can be prepared as an index; by expressing the percentage of ML reduction of the treated wood blocks against the control wood blocks. Two tables of proportional data are presented, one examining the effect of AOs without resin (Table 8.2) and with resin (Table 8.3). In each case the controls are 100%, so that lower numbers equate to greater reduction in mass loss.

Table 8.2: Reduction of mass loss (%) of the wood of different species due to the effect of antioxidant without CNSL resin on the decay fungi. Results in **bold** indicate the best values, underlines, second best.

Fungi	Antioxidants	*P. sylvestris*	*T. scleroxylon*	*G. arborea*
C. puteana	Gallic acid	98.1	92.6	95.9
	Ferulic acid	71.2	58.6	33.6
	Caffeic acid	77.0	98.8	65.4
	Quercetin	97.8	81.3	65.2
	Propyl gallate	**17.6**	**4.2**	**13.6**
	SSL phenolic extracts	92.9	30.6	90.8
T. versicolor	Gallic acid	56.4	87.3	82.4
	Ferulic acid	90.9	97.5	80.2
	Caffeic acid	74.9	95.3	67.8
	Quercetin	83.4	85.8	71.9
	Propyl gallate	**39.0**	**51.0**	**57.6**
	SSL phenolic extracts	90.5	83.9	71.0

Table 8.3: Reduction of mass loss (%) of the wood of different species due to the effect of antioxidant combined with CNSL resin on the decay fungi. Results in **bold** indicate the best values, underlines, second best.

Fungi	Antioxidants	*P. sylvestris*	*T. scleroxylon*	*G. arborea*
C. puteana	Gallic acid	93.1	73.9	92.7
	Ferulic acid	74.3	75.9	41.3
	Caffeic acid	73.4	93.5	60.1
	Quercetin	85.8	87.9	79.6
	Propyl gallate	**1.9**	**1.6**	**13.1**
	SSL phenolics extracts	75.2	26.9	95.6
T. versicolor	Gallic acid	65.7	69.8	89.5
	Ferulic acid	97.2	90.6	84.8
	Caffeic acid	80.0	93.3	59.6
	Quercetin	99.6	99.7	75.0
	Propyl gallate	**28.3**	**61.9**	**50.5**
	SSL phenolic extracts	90.3	62.9	72.4

From Tables 8.2 and 8.3 it is clear that only propyl gallate is effective at reducing the ML in both treatments, with AOs alone (Table 8.2) and AOs combined with CNSL resin (Table 8.3). This phenomenon is found with all test combinations, i.e. *C. puteana* and *T. versicolor* for all the three wood species but in general, the propyl gallate is more effective against *C. puteana*

than *T. versicolor*. Other AOs are not consistently as good as pointed out above. Ferulic acid showed some effectiveness for Gmelina wood when exposed to *C. puteana*. The SSL phenolic extracts only showed effectiveness on Obeche wood with exposure to *C. puteana*. Generally, the ML reduction due to treatment with AOs in both cases (with or without resin) is more effective against *C. puteana* than with *T. versicolor*.

A further index can be made to show the degree of effect between the resin and AOs. This can be done by comparing the ML of AOs alone and AOs with resin expressed as a percentage ([ML AO/ ML AO+RESIN]*100, Table 8.4).The effect of CNSL resin on reduction of ML is high when combined with propyl gallate particularly in Scots pine wood against both fungi and also showed activity against *C. puteana* on Obeche but activity was not substantial otherwise. In general, the resin gave better ML reduction in Scots pine wood against *T. versicolor*.

Table 8.4: Reduction of mass loss (%) of the wood of different species due to the effect of CNSL resin compared between treatment with or without antioxidants on the decay fungi. Results in **bold** indicate the best values.

Fungi	Antioxidants	*P. sylvestris*	*T. scleroxylon*	*G. arborea*
C. puteana	Control	85.7	71.9	59.1
	Gallic acid	81.4	57.4	57.1
	Ferulic acid	89.5	93.1	72.7
	Caffeic acid	81.7	68.1	**54.3**
	Quercetin	75.2	77.7	72.2
	Propyl gallate	**9.3**	**26.8**	56.7
	SSL phenolic extracts	69.4	63.2	62.2
T. versicolor	Control	41.2	72.5	80.4
	Gallic acid	48.0	57.9	87.3
	Ferulic acid	44.0	67.4	84.9
	Caffeic acid	44.0	71.0	70.7
	Quercetin	49.2	84.3	83.8
	Propyl gallate	**29.8**	88.0	**70.4**
	SSL phenolic extracts	41.1	**54.4**	81.9

The additive effect of CNSL resin with AOs on the percentage reduction of the ML is assessed by comparing the mass loss of control (untreated) to the ML of wood treated with CNSL resin combined AOs ([ML AO+RESIN/ ML No AO or RESIN]*100) (Table 8.5). A greater mass loss reduction is found with resin combined with propyl gallate for the three wood species but this is more effective against *C. puteana* than *T. versicolor*. Overall, the additive effect of resin and AOs are greatest with Scots pine in exposure to *T. versicolor* and Gmelina in exposure to *C. puteana*. In one instance SSL phenolic extracts showed some additive effect with CNSL resin.

Table 8.5: Reduction of mass loss (%) of the wood of different species due to the additive effect of CNSL resin combined with antioxidants on the decay fungi. Results in **bold** indicate the best values.

Fungi	Antioxidants	*P. sylvestris*	*T. scleroxylon*	*G. arborea*
C. puteana	Gallic acid	79.8	53.1	54.7
	Ferulic acid	63.7	54.6	24.4
	Caffeic acid	63.0	67.2	35.5
	Quercetin	73.6	63.2	47.0
	Propyl gallate	**1.6**	**1.1**	**7.7**
	SSL phenolic extracts	64.5	19.4	56.5
T. versicolor	Gallic acid	27.1	50.6	71.9
	Ferulic acid	40.0	65.7	68.2
	Caffeic acid	32.9	67.7	47.9
	Quercetin	41.0	72.3	60.3
	Propyl gallate	**11.6**	**44.9**	**40.6**
	SSL phenolic extracts	37.2	45.6	58.2

8.4. Discussion

8.4.1. Effect of antioxidants on wood decay resistance

The effect of AOs on wood decay resistance was higher in combination treatment with CNSL resin rather than without resin (Figures 8.2 and 8.3) although, some of the AOs effectively reduced the MLs of the treated wood blocks when AOs were used alone (e.g. propyl gallate). In general, the AOs tested are more effective against brown rot fungus, *C. puteana* than the white rot fungus, *T. versicolor* and this is found both with AOs with or without CNSL resin. When the percentage of mass loss reduction is calculated from the respective control treatment, there was no overall difference between the wood species (Tables 8.2 and 8.3).

Among the AOs, propyl gallate was found very effective in all three wood species: applied with or without CNSL resin (Tables 8.2 and 8.3). While, propyl gallate showed good AO properties (propyl gallate 8.75) examined in DPPH assay (Table 8.1) some of the other AOs with similar IC_{50} values (Table 8.1: gallic acid 8.24, ferulic acid 8.67) were ineffective in reducing the ML in the decay experiment. Ferulic acid did have some effect on ML reduction for hardwood species Obeche and Gmelina against to *C. puteana* but only gallic acid showed some effect with Scots pine against *T. versicolor*. Unfortunately, quercetin with a moderate AO property (AAI, 4.67) did not provide reduction of the ML in either cases of treatment, with or without resin. Interestingly, the SSL phenolic extract with lower AO properties (AAI, 2.41) was only effective against *C. puteana* in Obeche wood.

Hsu *et al.* (2007) found no antifungal effect with AOs when applied alone against the wood decay fungi in an agar plate test but when the AOs were combined with organic biocides the antifungal properties were enhanced. They found very low antifungal properties of propyl gallate (100 µm/ml concentration) against the white rot fungi (*Lenzites betulina* and *T. versicolor*) and brown rot fungi (*G. trabeum* and *Laetiporus sulphureus*). In a subsequent study, Hsu *et al.* (2009) reported that propyl gallate reduced the ML of wood to less than 3% against *G. trabeum* and *T. versicolor* when impregnated into *Cryptomeria japonica* and *Liquidambar fomosana* wood blocks but it showed poor fungicidal activity when examined in agar plate test and thus they concluded that the effective ML reduction is due to the excellent AO properties of propyl gallate which provided the preservative effect. Schultz and Nicholas (2002) also reported that an enhanced effectiveness against the decay fungi when propyl gallate was used in combination with different biocides assessed by the strength loss reduction test of the decayed wood.

Quercetin did not perform well in this study but it was thought that it would have provided some decay resistance to the wood. Quercetin is found in heartwood extractives of a variety of species (Hillis, 1987) and it has good AO properties. Heartwood extractives are known to retard wood decay (Scheffer and Cowling, 1966, Hillis 1987). Although quercetin has been reported to possess antifungal properties (Hiraswa and Takada, 2004), Yen *et al.* (2008) found no antifungal properties of quercetin against *L. betulina* and *L. sulphureus* in an agar plate test. The mechanism of free radical scavenging is quite different to that of biocidal mechanisms in reducing the fungal decay. So, it is difficult to compare the antifungal properties of quercetin in this study. However quercetin did not show any decay resistance enhancement in either of the fungi tested for the three wood species although it has good AO properties (4.67 AAI).

The SSL phenolic extracts were used in this study because of its composition of different compounds including *m*-coumaric acid, *p*-coumaric acid, caffeic acid, ferulic acid, gallic acid and protocatechuic acid and having good AO properties of 3.29 AAI, (Tverezovskiy, 2013). Many of these compounds are widely used as AOs for different purposes (e.g. gallic acid, ferulic acid). Faustino *et al.* (2010) extracted phenolics from SSL which was produced from *Eucalyptus globulus* pulp and found AO properties of AAI ranged from 2.2 to 3.4. Scherer and Godoy (2009) stated that compounds having AAI value greater than 2 showed strong AO activity. Though, in this study SSL phenolic extracts showed AAI value of 2.41 but in comparison to the other AO compounds it is not strong. It is probably that the lower free

radical scavenging activity is the reason why the SSL phenolic extracts did not show overall effectiveness on reduction of the ML by the decay fungi.

The percentage of ML reduction due to the treatment with AOs with or without CNSL resin has revealed that AOs with higher free radical scavenging properties showed better ML reduction of the treated wood blocks e.g. (propyl gallate, ferulic acid). This may be attributed to quenching of the free OH radicals produced by the decay fungi and thus this protects the wood cell wall from the depolymerisation of the cell wall components which takes place at the early stage of wood decay. Moreover, the AOs were found more effective against the brown rot fungus, *C. puteana* than the white rot fungus, *T. versicolor* which is a good indication that the protection mechanism involves an AO effect. It has been established the brown rot fungi produce extracellular OH radicals in the initial stage of brown rot decay (Goodell, *et al.*, 1994; 1997).

8.4.2. Effect of CNSL resin on wood decay resistance

The effect of CNSL resin on wood decay resistance was discussed in chapter 7. In this study treatment with a 10% resin solution which is used as control in this chapter (Figure 8.3) showed similar ML values to that study, i.e. (ch 8 vs ch 7): 38% vs 37%, 32% vs 29 and 27% vs 25% against *C. puteana*, and 14% vs 14%, 24% vs 25 and 38% vs 42% against *T. versicolor* for Scots pine, Obeche and Gmelina wood respectively. The effect of CNSL resin on reduction of the ML is assessed in the treatment with AOs to understand the effect of resin only excluding the effect of AOs for improving the decay resistance. Overall the resin was found effective more against *T. versicolor* than *C. puteana*. Among the wood species resin treated Scots pine showed more resistance to decay This is expected due to the difference of wood permeability which is higher in Scots pine and thus higher WPGs were retained in Scots pine wood. It was already discussed in earlier chapters that the WPGs of the Scots pine wood is higher than the Obeche and Gmelina. It was also found that decay resistance is increased with the increasing WPG of CNSL resin treated wood (chapter 7). On the other hand, this is not true for the woods exposed to *C. puteana*, where Obeche and Gmelina showed higher mass loss reduction due to the effect of CNSL resin. This is also observed in the earlier studies (chapter 7) and attributed to the choice of wood species against the decay fungi as brown rots do not prefer hard wood species.

8.4.3. Additive effect of CNSL resin combined antioxidants on wood decay resistance

The ML reduction is much improved when the wood blocks were treated with CNSL resin combined with some of the AOs (Figure 8.3, Table 8.5). Table 8.2 and 8.3 revealed the effect of AOs treatment with or without CNSL resin on the ML reduction of the wood blocks and found that propyl gallate and ferulic acid were better at reducing the ML losses but gallic acid with greater AO properties was not effective alone. However there was an enhanced effect of gallic acid and resin on ML reduction. Some improvement was also observed for other AOs with lower free radical scavenging capacity of quercetin, caffeic acid and SSL phenolic extracts.

Most of the AOs were more effective against *C. puteana* than *T. versicolor* when the ML reduction was assessed due to the treatment with AOs with or without resin (Tables 8.2 and 8.3). However when the mass loss reduction due to the effect of CNSL resin was examined the decay resistance was better against *T. versicolor* than *C. puteana* (Table 8.4). Moreover, no specific difference in ML reduction was noticed between the wood species due to the effect of AOs but a variable effect of CNSL resin was found to the ML reduction which is attributed to the resin loading and variations of softwood and hardwood. The ML reduction with the woods treated with resin and AOs showed that most of the AOs work well with resin against *T. versicolor* for Scots pine wood, but with exposure to *C. puteana* they work well in Gmelina wood. This is attributed to the effect of CNSL resin and AOs.

In many cases, AOs with high free radical scavenging properties also gave higher decay resistance (Table 8.5) and indicated that the AO mechanism works against the decay fungi that produced free OH radicals in process of wood decay. On the other hand the mechanism of decay resistance CNSL resin modified wood may be due to the hydrophobic nature of the resin system and provides a barrier to ingress of moisture and thus making unsuitable for fungal growth. As it was previously found that the CNSL resin cannot block the wood cell wall micropores (chapter 4) and thus allow the extracellular OH radicals produced by the decay fungi to penetrate the wood cell wall which started the oxidative degradation in wood (chapter 6) in the earlier stage of decay. Thus, it is assumed that the reduction of the ML of the wood treated with the CNSL resins and AO compounds are due to the combined effects of free radical scavenging provided by the AOs and water repellent properties of the hydrophobic CNSL resin system.

8.5. Conclusions

1. Among the AOs tested propyl gallate was found most effective in reducing the mass losses either treated with CNSL resin or alone.

2. Generally, the AOs are more effective against *C. puteana* than *T. versicolor* when treated without CNSL resin for all of the wood blocks.

3. No specific difference between the wood species in exposure to either fungus was found attributed to the mass loss reduction due to the effect of treatment with AO with or without CNSL resin.

4. The effect of CNSL resin is also observed excluding the effect of AOs on the mass loss reduction of the wood against both the fungi. But the mass loss reduction was higher in Scots pine wood against *T. versicolor* and in Gmelina against *C. puteana*. Similar results were also found in previous study done with CNSL resin alone and provided that the resin impregnation also contributed the improvement of decay resistance.

5. Improved decay resistance was found due to the additive effect of CNSL resin combined with different AOs. Best decay resistance was achieved with the treatment with CNSL resin and propyl gallate which showed good free radical scavenging activities. Other AOs having higher radical scavenging properties also improved decay resistance due to the enhancement of CNSL resin e.g. gallic acid and ferulic acid and quercetin. However, some of these AOs did not show effectiveness (ML reduction) when applied without CNSL resin.

6. Due to the combined effects of CNSL resin and AOs, better performance occurs in Scots pine wood with exposure to *T. versicolor* while in Gmelina better ML performance occurs with exposure to *C. puteana*. This phenomenon is also observed with the effect of CNSL resin alone but not with AO alone. This is further indication of the performance of CNSL resin and AOs on the mass loss reduction of wood.

7. In general, the AOs with better free radical scavenging properties showed good performance with CNSL resin to improve the decay resistance of wood. This is suggested that the AOs compound is active in the initial oxidative degradation process of fungal decay of wood and scavenge the extracellular OH radicals produced by decay fungi, particularly brown rot.

Chapter 9

Examining the evidence for distribution of CNSL resin into wood by FTIR spectroscopy and light microscopy

9.1. Introduction

Fourier transform infrared (FTIR) spectroscopy is a useful method to produce information regarding the structure of chemical constituents of wood. The FTIR is used to determine the intensity of specific bonds and functional groups within the polymeric structure. It has been found effective for analysing the chemical structure of wood as a whole (Faix, 1992). The spectra are measured directly from the surface of the solid wood by using ATR while the structure of the wood is maintained. FTIR technique is also useful to identify the chemical changes which have occurred in wood due to a treatment or modification. Analysing the entire polymeric complex is advantageous to understand the behaviour of the modified wood.

FTIR has previously been used to characterize the chemistry of wood (Faix, 1992; Pandey, 1999; Stevanic and Salmen, 2009). This technique has been also used for wood surface characterization for estimating lignin and carbohydrate compounds in wood and other lignocellulosic materials (Backa and Brolin, 1991; Pandey and Theagarajan, 1997). It is also used to analyse the chemical changes which take place during weathering, decay and other chemical treatments (Pandey and Pitman, 2003; Pandey, 2005; Tjeerdsma and Militz, 2005 Verma *et al*., 2009; Fackler *et al*., 2010; Salla *et al*., 2012; Wu *et al*., 2012; Pries *et al*., 2013).

Light microscopy of wood can be useful to a certain extent to examine the distribution of a chemical applied, both at a gross level and within the anatomy. In addition X-ray emissions from wood treated with exotic, heavier elements (than C, H and O, the main constituents of wood) have been used within electron microscopy to examine the micro-distribution of preservatives, particular those containing copper. Generally, the light microscopy permits rapid view of many cells with a moderate specimen preparation (Schwarze, 2007).

In this study FTIR analysis was carried out to the CNSL resin modified wood blocks and micro-veneers to find out the chemical changes or transformation of the cell wall compounds of wood and thus obtaining evidence of CNSL resin distribution into the wood. The resin modified wood samples were also examined without significant magnification and as sections by light

microscopy at different magnification to examine the distribution of the CNSL resin within the wood structure.

9.2. Materials and methods

9.2.1. Sample preparation for FTIR spectroscopy and light microscopy

The CNSL resin modified wood blocks with different concentration of resin solution and unmodified blocks (IMS) (as described in chapter 4) of Scots pine (*Pinus sylvestris*), Obeche (*Triplochiton scleroxylon*) and Gmelina (*Gmelina arborea*) were used for FTIR spectroscopy. Scots pine micro-veneers modified with CNSL resin and unmodified veneers (as described in chapter 6) were also examined.

9.2.2. Pure CNSL resin sample preparation for FTIR spectroscopy

Pure CNSL resin samples were prepared by curing 5 g of pure CNSL resin in an oven at 120 °C temperature for 24 hours. The liquid CNSL resin was placed over glass plates and then transferred to the oven for drying. After removal from the oven the resin was a flat, circular small disk and was subsequently kept in the desiccator for cooling.

9.2.3. Wood sample preparation for macro-examination

Blocks were split in either the radial or tangential plane across the centre and oriented, exposed surface uppermost. They were then photographed with a Canon digital SLR equipped with a 100 mm macro lens at closest distance, with a ruler marked in mm as a size reference.

9.2.4. Wood sample preparation for light microscopy

The CNSL resin modified wood blocks at the highest modification level (WPG) and the unmodified blocks (IMS) (as described in chapter 4) of Scots pine, Obeche and Gmelina were used for examining in light microscopy. The wood blocks of both modified and unmodified were softened by soaking in deionized water for 24 hours and then cut into small pieces approximately 7 mm^2 with a razor blade. Thin transverse sections (<20 µm) of wood were microtomed by disposable microtome blades (Cambridge Instruments No.818) and a blade holder (Reichert-Jung). The randomly located sections were then placed on glass slide with a drop of deionized water, covered with cover glass and transferred for microscopic observation. An additional set were prepared from the blocks used for macro-examination without soaking, and thin sections were cut by hand from the centre of the exposed 20 mm surface, specifically to examine the nature of resin penetration into the blocks.

9.2.5. FTIR spectroscopy

The FTIR spectra of CNSL resin modified woods (blocks and veneers) and pure CNSL resin were measured by direct transmittance from the wood and resin surface by using attenuated total reflection (ATR) technique. The spectra were recorded using a Nicolet 8700 FTIR Spectrometer (Thermo Scientific, Madison, USA) equipped with Gladi ATR (Pike Technology, Madison, USA). All spectra were measured at a spectral resolution of 4 cm^{-1} at the rate of 32 scan per measurement in the mid-IR region from 4000 to 400 cm^{-1}. The background spectra were collected with the empty ATR unit.

9.2.6. Light microscopy

Light microscopy of the CNSL resin modified wood and unmodified wood was carried out by a Leica DMLB microscope (Leica Microsystem GmbH, Germany) equipped with GXCAM-9 digital camera (GX Optical, UK). The prepared slides of wood sections were then viewed under the microscope at different magnification of X10, X40, X63 and X100.

9.3. Results and Discussion

9.3.1. FTIR of unmodified Scots pine micro-veneer

A FTIR spectrum of unmodified Scots pine micro-veneer is presented in Figure 9.1. The spectrum of Scots pine wood shows a strong hydrogen bonded O-H stretching absorption at 3332 cm^{-1} and a prominent C-H stretching absorption at 2888 (Pandey and Pitman, 2003; Pandey, 1999; Wu *et al*., 2012). Pandey (1999) mentioned that many sharp and discrete absorption bands in the fingerprint region of 1800-900 cm^{-1} are due to the various functional groups present in the wood constituents. Stevanic and Salmen (2009) also reported the spectral signals related to absorption from cellulose and hemicelluloses (glucomannan and xylan) and lignin in the wave number rages from 1800 to 720 cm^{-1}.

Absorption bands assigned to lignin appear at 1647 cm^{-1} for C-O stretching vibration (conjugated) (Pandey, 1999, Pandey and Pitman, 2003), 1590 cm^{-1} and 1509 cm^{-1} for aromatic skeletal vibration in lignin (Faix, 1992, Pandey and Srinivas, 2013), 1452 cm^{-1} for C-H deformation in lignin (methyl and methylene) (Faix, 1992, Pandey, 1999, Pandey and Pitman, 2003, Wu *et al*., 2012), and 1262 cm^{-1} for C-O of guaiacyl ring and C-O stretching of lignin (Wu *et al*., 2012; Pandey and Srinivas, 2013). An absorption band appears at 1311 for C-O of syringyl ring derivatives (Pandey and Pitman, 2003).

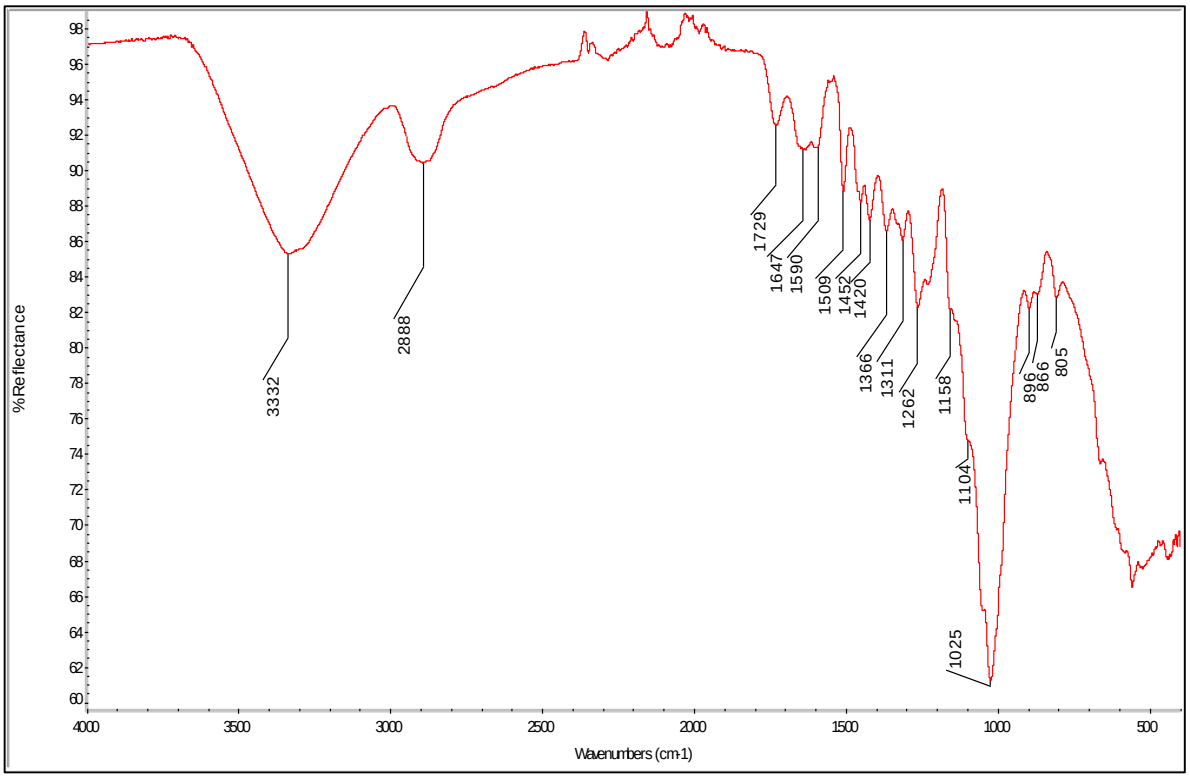


Figure 9.1: FTIR spectra of unmodified Scots pine (*Pinus sylvestris*) micro veneer.

The other absorption bands assigned to polysaccharides, mainly hemicellulose, appear at 1729 cm^{-1} for C=O stretching vibration (unconjugated) in xylan (Pandey and Pitman, 2003; Stevanic and Salmen, 2009; Wu *et al*., 2012), 1420 cm^{-1} for C-H deformation in carbohydrate (Pandey and Pitman, 2003), 1366 cm^{-1} for C-H deformation vibration in cellulose and hemicellulose (Pandey, 1999), 896 cm^{-1} for C-H deformation in cellulose (Pandey and Pitman, 2003) and 866 cm^{-1} and 805 cm^{-1} for glucomannan (Stevanic and Salmen, 2009; Fackler *et al*., 2010). The C-O-C vibration at 1158 cm^{-1}, O-H association and C-O stretching at 1048 cm^{-1} are due to the wood polysaccharides (cellulose and hemicellulose) (Stevanic and Salmen, 2009; Srinivas and Pandey, 2012). In general, the major part of absorption in wood is assigned to C-O stretching in primary alcohol at 1025 cm^{-1} (Pandey, 1999).

9.3.2. FTIR of CNSL resin

The FTIR spectra of pure CNSL resin is presented in Figure 9.2. In general, the area between 4000 and 1400 cm^{-1} is an area particularly useful for identification of functional groups. This area indicated absorptions which are due to the extension modes. On the other hand, the area between 1400 and 400 cm^{-1} are very complicated because of many stretching and bending absorptions for different organic functional groups which occurs in this area (Coates, 2000).

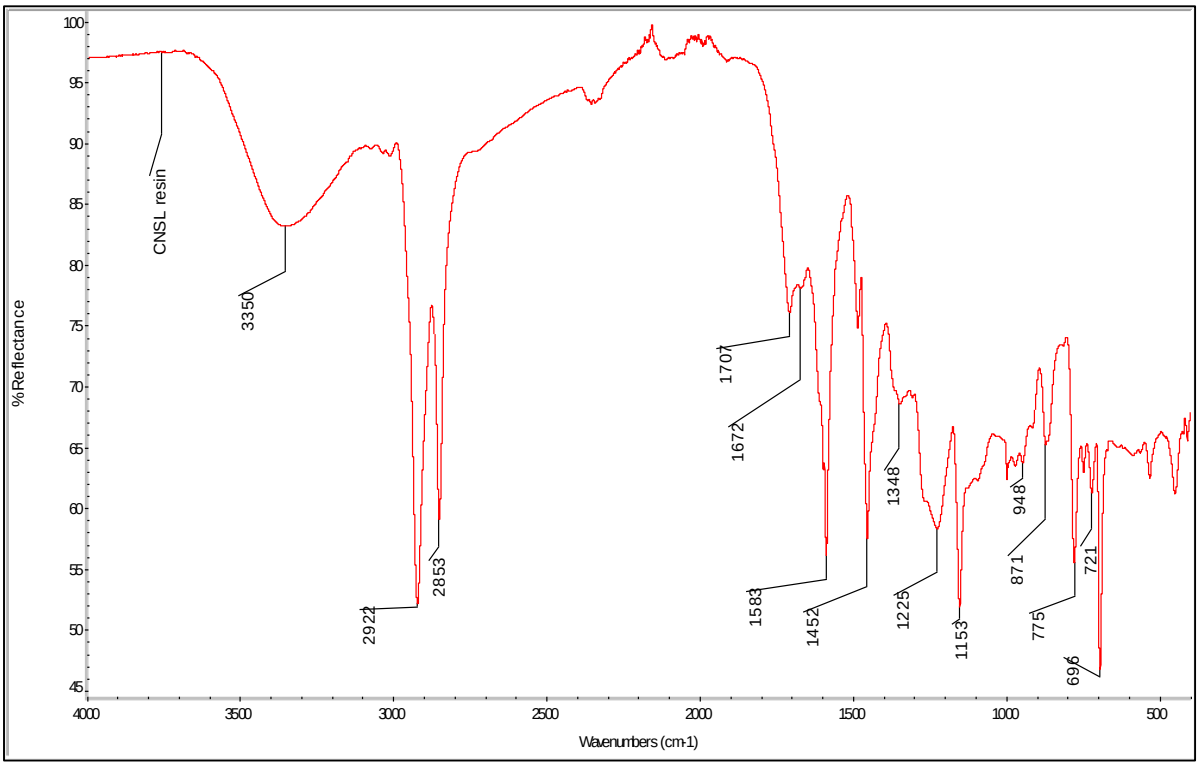


Figure 9.2: FTIR spectra of pure CNSL resin.

The broad absorbance at 3350 cm^{-1} for O-H stretching vibration is due to the polymeric O-H which indicates the phenolic compounds (Mwaikambo and Ansell, 2001; Das *et al*., 2004; Risfaheri *et al*., 2009). The strong absorbance band at 2922 cm^{-1} and 2853 cm^{-1} for C-H vibration due to the presence of alkanes (Das *et al*., 2004; Silverstein *et al*., 2005). Additional bands at slightly higher wave number are known to indicate the presence of C-H stretching in phenolic compounds however these were weak in the spectrum. Absorbance band at 1707 cm^{-1} appears for C=O stretching vibration due to the presence of aldehydes, 1672 cm^{-1} for O-H and C=O stretching vibration due to the presence of carboxylic acid and derivative esters (Das *et al*., 2004; Silverstein *et al*., 2005).

The strong absorbance bands at 1583 cm^{-1} and 1452 cm^{-1} are assigned for aromatic skeletal vibration of C=C bonds and its branching (Mwaikambo and Ansell, 2001; Risfaheri *et al*., 2009; Silverstein *et al*., 2005). Absorbance bands assigned to carboxylic acid appear at 1348 cm^{-1} for C-O-H bending and at 1225 cm^{-1} for C-O stretching. In general, bands between 1300 cm^{-1} and 900 cm^{-1} appear for C-H stretching and O-H deformation due to presence of primary, secondary and tertiary alcohols and bands between 900 cm^{-1} and 650 cm^{-1} for C=O, C-O stretching due to presence of aromatic esters (Das *et al*., 2004). According to Mwaikambo and Ansell (2001) bands found at 948 cm^{-1}, 775 cm^{-1}, 721 cm^{-1} and 696 cm^{-1} refer to vinyl vibration due to C=C double bonds within the side chain.

9.3.3. FTIR of CNSL resin modified micro-veneer of Scots pine

The FTIR spectra of CNSL resin modified micro-veneer of Scots pine wood are presented in Figure 9.3. The wide absorbance band appears at 3332 cm^{-1} for O-H stretching in the modified and unmodified micro-veneers of Scots pine wood. The phenolic compound of the CNSL resin was assigned in similar wave numbers but the band is shifted from 3350 cm^{-1} in pure CNSL resin (Figure 9.2) to 3332 cm^{-1} when impregnated into wood. The intensity of this band is not variable between the unmodified and modified wood veneers which indicate that the limited changes occur to the OH groups of the wood cell wall.

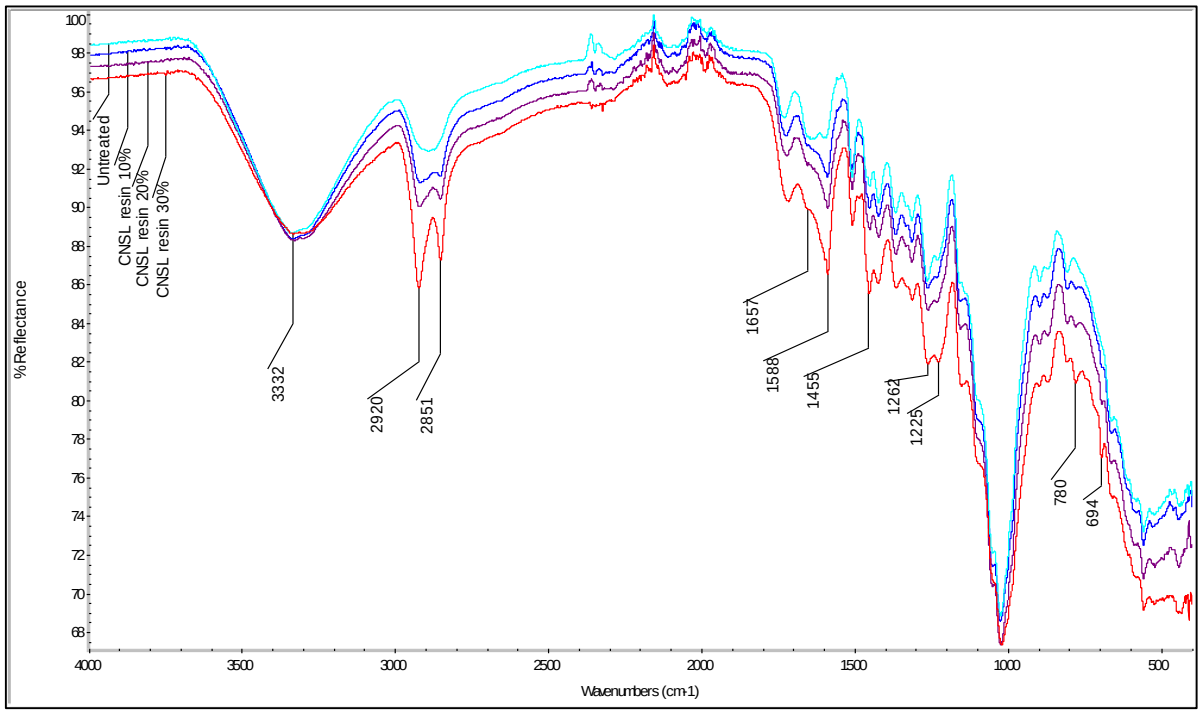


Figure 9.3: FTIR spectra of Scots pine (*Pinus sylvestris*) CNSL resin modified micro-veneers.

Shift or change has been observed in band at 2888 cm^{-1} of unmodified veneer to the two sharp bands at 2920 cm^{-1} and 2851 cm^{-1} of the modified veneers. These absorbance bands of the pure CNSL resin were found in exactly the same location assigned for C-H stretching due to the presence of alkane branch chain of phenolic compounds (Figure 9.2), while a single wide band is found in unmodified wood. The intensity of these two bands in modified wood veneer is increased with the increasing CNSL resin into wood.

A significant change has been observed at 1657 cm^{-1} and 1586 cm^{-1} of the CNSL resin modified wood. These bands were assigned due to lignin in unmodified wood appear for C-O stretching vibration (conjugated) and aromatic skeletal of lignin respectively. In modified wood spectra the 1657 cm^{-1} band decreased in intensity and completely merged with the band 1586 cm^{-1}.

The intensity of the band 1586 cm^{-1} is increased with the increasing CNSL resin into wood. In the pure CNSL resin, a strong absorbance band was also found at 1583 cm^{-1} due to the aromatic skeletal vibration of C=C bonds.

A minor change has been observed at 1265 cm^{-1} and 1225 cm^{-1} of the modified wood, the bands are little shifted and the intensity was changed from the unmodified bands. In unmodified wood these bands are appear due to the C-O guaiacyl ring and C-O stretching of lignin. In pure CNSL resin absorbance band was appear at 1225 cm^{-1} due to the C-O stretching of carboxylic acid. Change of band intensity is also found at 780 cm^{-1} and 694 cm^{-1} of the CNSL resin modified wood. No such absorbance was particularly found in unmodified wood samples but in pure CNSL resin strong absorbance was found at 775 cm^{-1} and 696 cm^{-1} due to the vinyl vibration of the side chain of C=C double bonds (Mwaikambo and Ansell, 2001) and/or C-H out of plane due to aromatic compounds (Silverstein *et al*., 2005).

9.3.4. FTIR of CNSL resin modified wood blocks

The FTIR spectra of CNSL resin modified wood blocks of Scots pine, Obeche and Gmelina are presented in Figure 9.4, 9.5 and 9.6 respectively. The changes in Scots pine modified wood is evident at absorbance bands of 2918 cm^{-1}, 2846 cm^{-1}, 1585 cm^{-1}, 1452 cm^{-1}, 1228 cm^{-1} and 776 cm^{-1}. Almost identical absorbance band was appear in the spectra of the CNSL resin modified micro-veneer of Scots pine and discussed earlier (Figure 9.3). A little variation of the band intensity at 1452 cm^{-1} is observed in modified blocks than the unmodified blocks. This band is assigned to lignin due to C-H deformation in the unmodified wood whereas a very strong absorbance band at 1452 cm^{-1} is found in pure CNSL resin due to aromatic skeletal vibration of C=C bonds.

The chemical changes are evident in Obeche modified wood blocks with CNSL resin (Figure 9.5) at 2917 cm^{-1}, 2848 cm^{-1}, 1726 cm^{-1}, 1586 cm^{-1}, 1452 cm^{-1}, 780 cm^{-1} and 694 cm^{-1}. The changes of absorbance at 2917 cm^{-1} and 2848 cm^{-1} are also found in Scots pine wood and arise due to the branch chain of phenolic compounds of the CNSL resin (Figure 9.2) and discussed earlier in section 9.3.3. The other changes of the above mentioned wave numbers are also found in Scots pine wood blocks and micro-veneers and discussed (9.3.3). A significant change is observed at 1726 cm^{-1}, the intensity of the band absorption is decreased from the unmodified Obeche wood. This absorbance in native wood is assigned to xylan (hemicellulose) appeared due to C=O stretching vibration (unconjugated). In pure CNSL resin absorbance is found at 1707 cm^{-1} for C=O stretching vibration due to the presence of aldehydes.

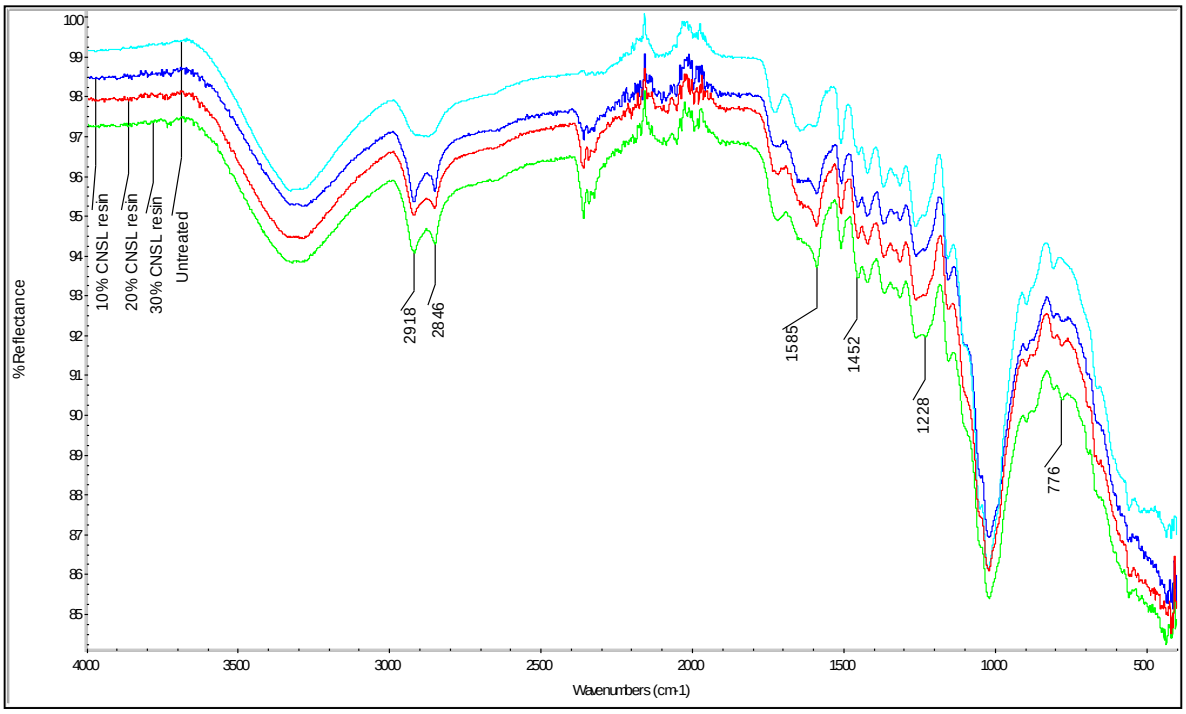


Figure 9.4: FTIR spectra of CNSL resin modified Scots pine (*Pinus sylvestris*) wood blocks.

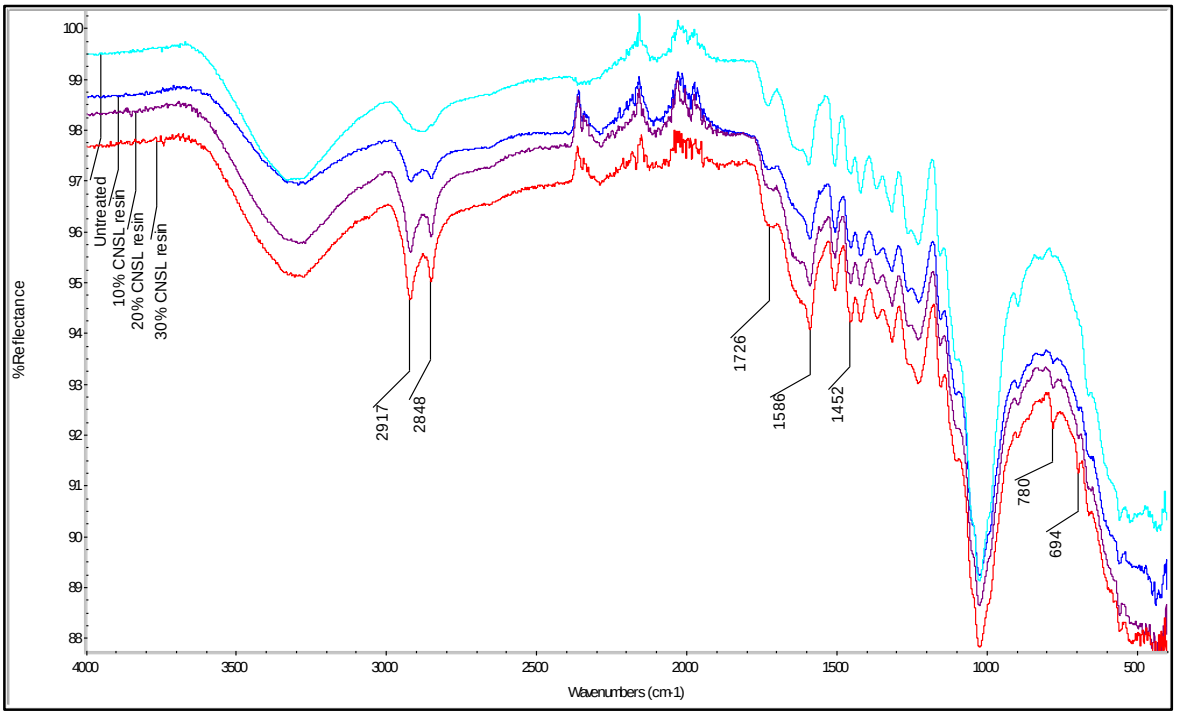


Figure 9.5: FTIR spectra of CNSL resin modified Obeche (*Triplochiton scleroxylon*) wood blocks.

Similar changes in chemical composition of the Gmelina modified wood blocks with CNSL resin are found at 2917 cm^{-1}, 2848 cm^{-1}, 1586 cm^{-1}, 1455 cm^{-1}, 778 cm^{-1} and 694 cm^{-1} (Figure 9.6) as found in Scots pine and Obeche wood. Two more changes are also found in Gmelina modified wood at 1501 cm^{-1} and 1101 cm^{-1}. The intensity of absorption is decreased at 1501 cm^{-1} from the unmodified to modified Gmelina wood. This absorption band appears in unmodified wood

due to aromatic skeletal vibration of lignin. Little absorption change is observed at 1101 cm^{-1} which does not appear in Scots pine wood but low absorption is found in case of Obeche. This absorption band is appearing due to the O-H association in cellulose and hemicellulose.

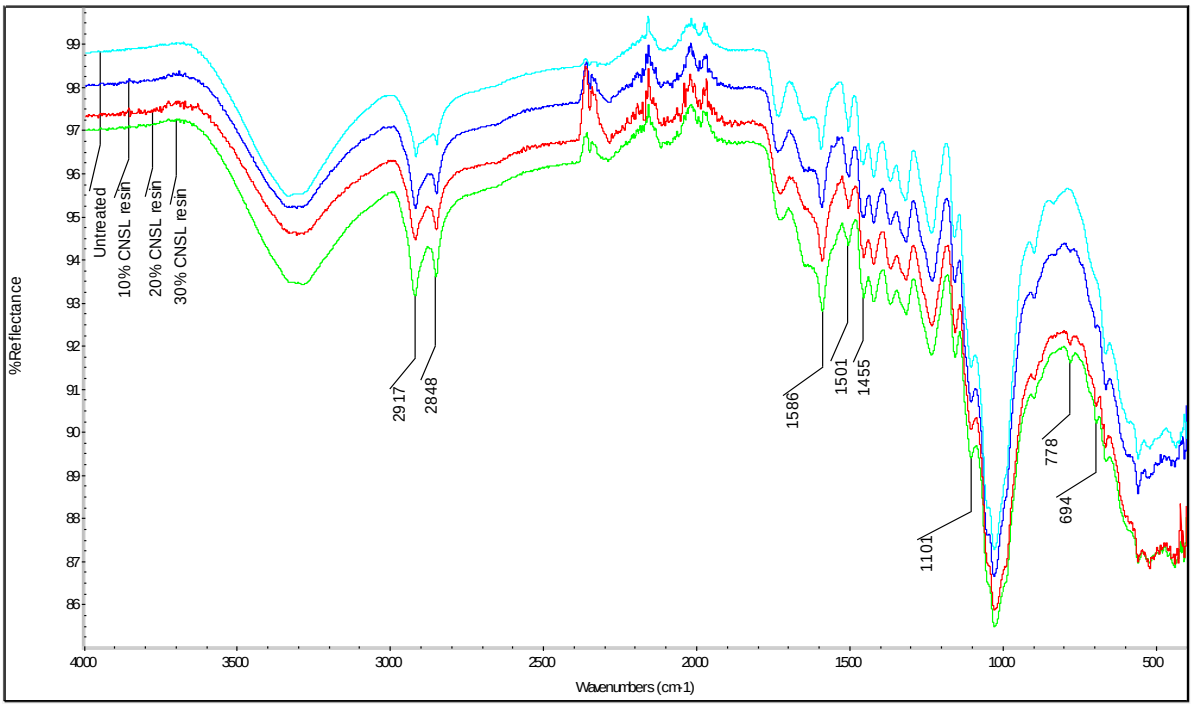


Figure 9.6: FTIR spectra of CNSL resin modified Gmelina (*Gmelina arborea*) wood blocks.

9.3.5. Macro appearance of the CNSL resin modified wood

The internal appearance of the three wood species (Figure 9.7) showed marked difference between the softwood and the two hardwoods. Resin modified Scots pine showed darkening at the wood surface (Figure 9.7a) and a more or less brown coloration throughout the wood, with slightly darker appearance in the rays. Although the two hardwoods showed darkening at the surface (Figure 9.7, b, c), the zone beneath the surface showed little evidence of penetration and only the vessels were markedly coloured, in some instances, obviously filled with resin. Between the two hardwoods a greater number of vessels were stained in Obeche and in some instances the colouration ran entirely through the block. The penetration through the longitudinal surface, i.e. the darkening, in the Obeche was a thin darker line while in Gmelina it appeared to have penetrated deeper and was more diffuse. The longitudinal surfaces of all of the blocks showed a slightly rough surface with the fibres oriented away from their normal orientation, indicative of saw damage.

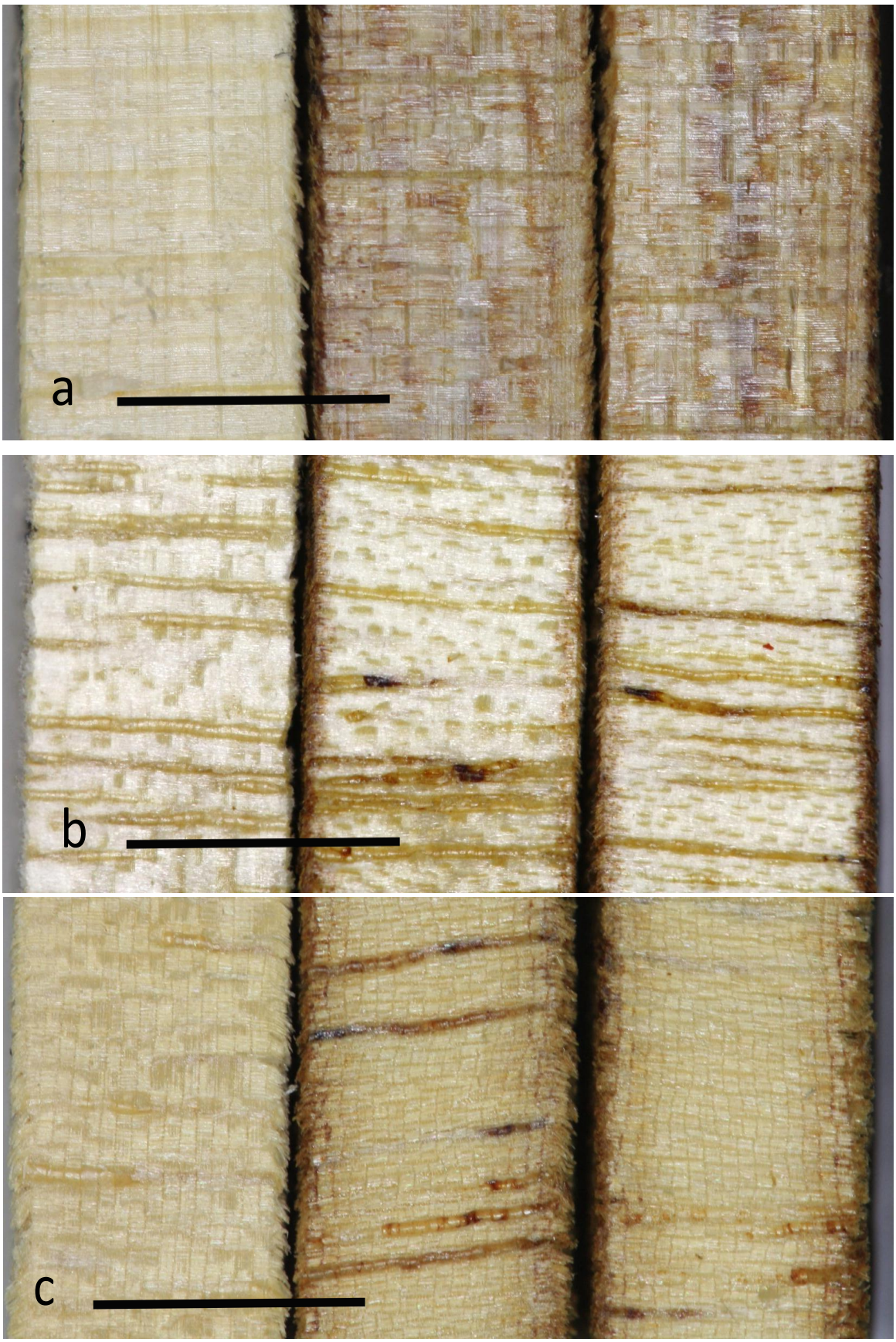


Figure 9.7: Internal appearance of untreated (left) and treated blocks (middle, right) after splitting showing the macro distribution of resin, (a) Scots pine, (b) Gmelina, (c) Obeche (scale bar = 5 mm). The block longitudinal orientation is horizontal.

9.3.6. Microscopy of the CNSL resin modified wood

The purpose of the microscopy was to examine the distribution of the resin within the wood at the tissue distribution level and, if possible to determine whether there was any evidence of cell wall uptake. The highest resin loadings were sectioned.

Given the initial observations of wide scale distribution in Scots pine (Figure 9.7a) it was not surprising to see evidence of resin in most of the tracheids examined (Figure 9.8). There is clearly staining visible in many of the cells which was not visible in the controls (cf. Figure 9.8 a, b untreated vs c, d treated). Many of both the early- and latewood tracheids were completely filled with resin at the point examined while others showed coating on the inside of the lumens. In cases where lumens were filled they were often seen to follow a line across the growth ring, presumable following a ray within the vicinity. Closer examination of the cells showing a lumen coating (Figure 9.8 d, e, f), suggested that the coating was only on the lumen surface, rather than showing substantial evidence of cell wall penetration. In some instances the coating appeared to have finished residing at the cell corners (Figure 9.8.e). Some cells examined left some doubt as to whether the cell walls had been penetrated but careful examination of adjacent cells (Figure 9.8 f) showed that this most likely a lumen coating or very limited near lumen surface penetration.

Examination of the longitudinal sections showed the damage alluded to in the macrophotography and that at the block ends there was a high penetration of resin (Figure 9.8 g), and also at sporadic areas within the block. The longitudinal sections were not prepared by long term pre-soaking and did not reveal high quality micrographs but the when mounted in water recalcitrant air pockets, indicative of hydrophobic surfaces were noted in the tracheids exposed on the transverse (Figure 9.8 h).

From the macroscopic appearance Obeche was expected to show a very poor resin distribution but instead showed better penetration. The untreated wood showed a few large vessels (Figure 9.9 a, b), multiseriate rays and numerous thin walled fibres. Examination of treated wood (Figures 9.9 c-h) showed that, as expected the vessels were lined with resin.

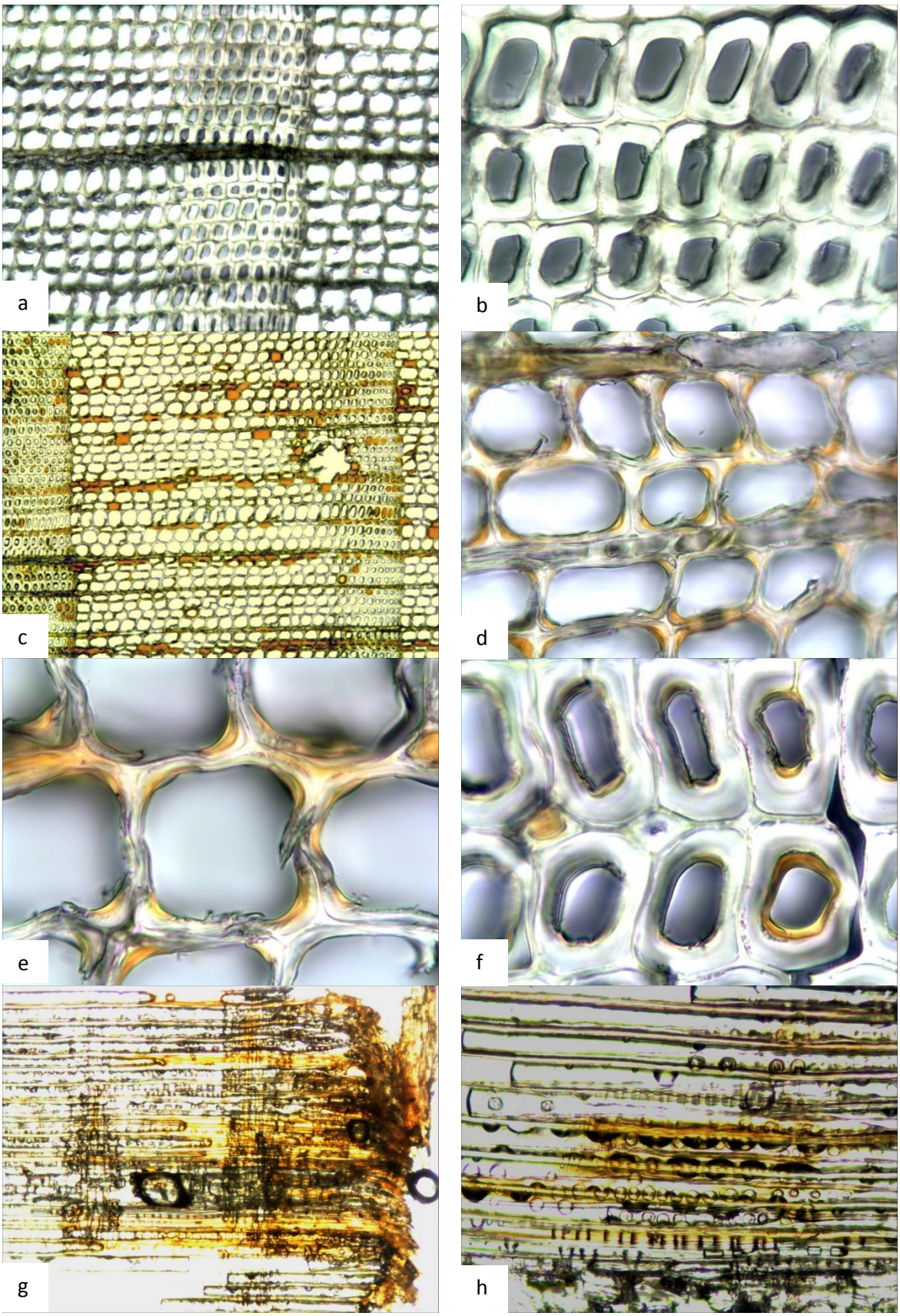


Figure 9.8: Light microscopy of untreated (a, b) and treated Scots pine (c-h) showing resin distribution.

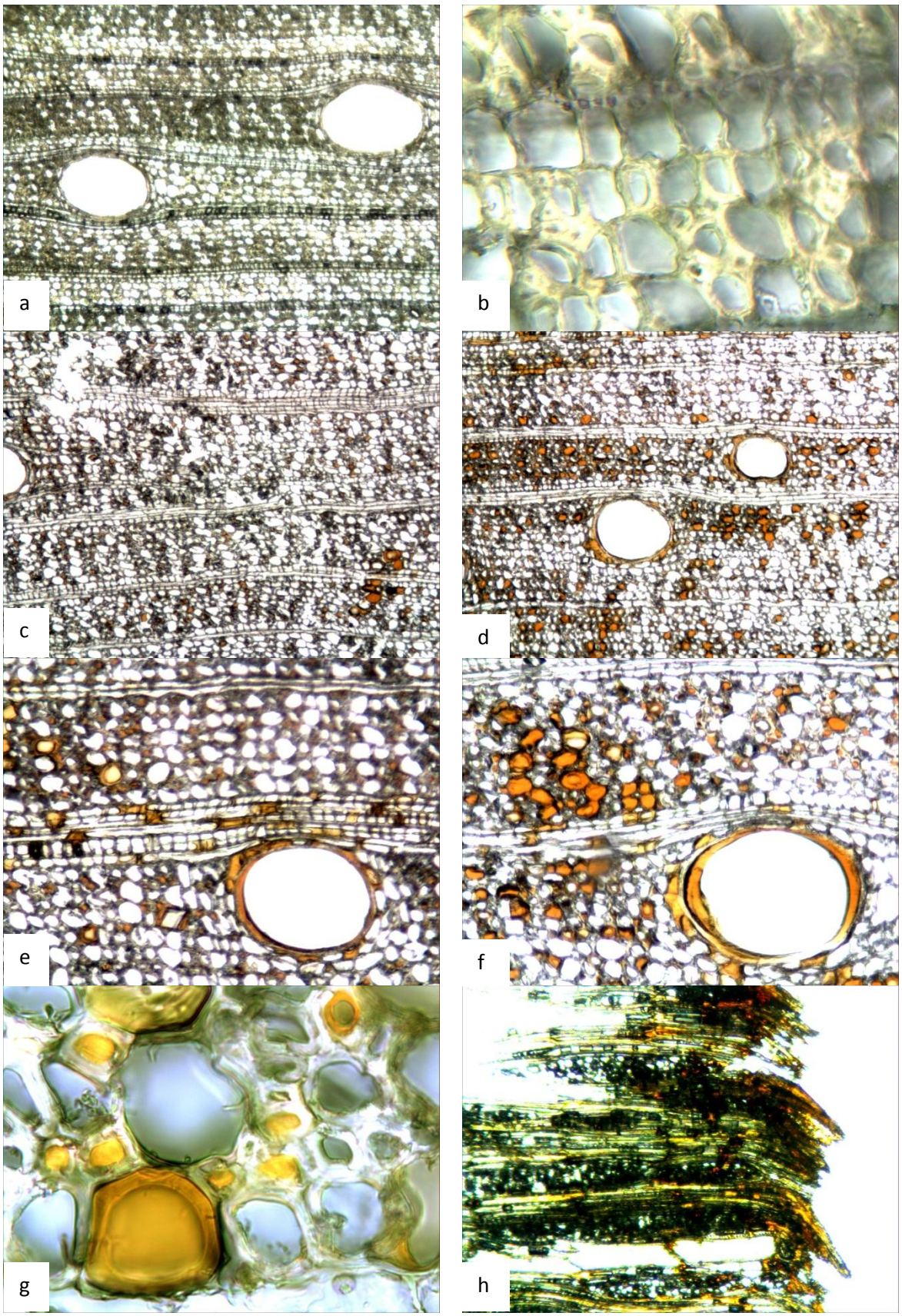


Figure 9.9: Light microscopy of untreated (a, b) and treated Obeche (c-h) showing resin distribution.

Many of the fibres were well treated (Figure 9.9 c, d) either by total lumen filling or by lumen surface coating (Figure 9.9 e-g) and potentially more convincing evidence for cell wall penetration was seen (Figure 9.9 g) although this was uncommon. Ray cells were poorly penetrated although some cells showed lumen filling (Figures 9.9 e, f). Longitudinal sections showed damage to the cells at the block ends and high levels of resin penetration at the block ends (Figure 9.9 h).

As with the macroscopic examination of Obeche, Gmelina was expected to show poor resin penetration when examined by light microscopy and this was indeed the case. Untreated Gmelina showed a higher proportion of vessels than Obeche (Figure 9.10, a) and multiseriate rays. The fibres were thicker walled than Obeche (Figure 9.10 b). Examination of the treated Gmelina showed sporadic and a few scattered cells had been visibly treated (Figure 9.10 c-h). Vessels would be expected to show good penetration but his was not universally the case (Figure 9.10 c, d) and while some vessels were filled or had some obvious lumen lining, some were completely empty of resin. Amongst the fibre rich tissue areas some cells had been impregnated with resin and well filled these cells appeared to be thinner walled and may be parenchyma cells. As with the other blocks examined damage at the block ends was evident, but higher resin uptake could be seen in this region.

Given what is known about the permeability of Scots pine sapwood the good distribution of resin throughout the blocks explains why in earlier chapters high WPG have been achieved. The Hardwoods show patchier penetration beyond the surface layers. Obeche appears to show a better resin distribution than Gmelina, which again is consistent with WPG data. With Gmelina it is however surprising how poorly the resin has been distributed in the wood and even some vessels are poorly penetrated.

In this microscopic study no convincing evidence of wood cell wall penetration by resin has been shown, although it may have occurred to a limited extent at the lumen surfaces where resin penetrated the lumens. Other microscopic approaches may reveal whether resin has entered the wood cell wall and to what extent. It is suggested that Raman confocal microscopy may resolve this issue, and these are suggested as options for further study (Matsumura *et al*., 1999; Gierlinger *et al*., 2005; Cyr *et al*., 2008; Sing *et al*., 2009).

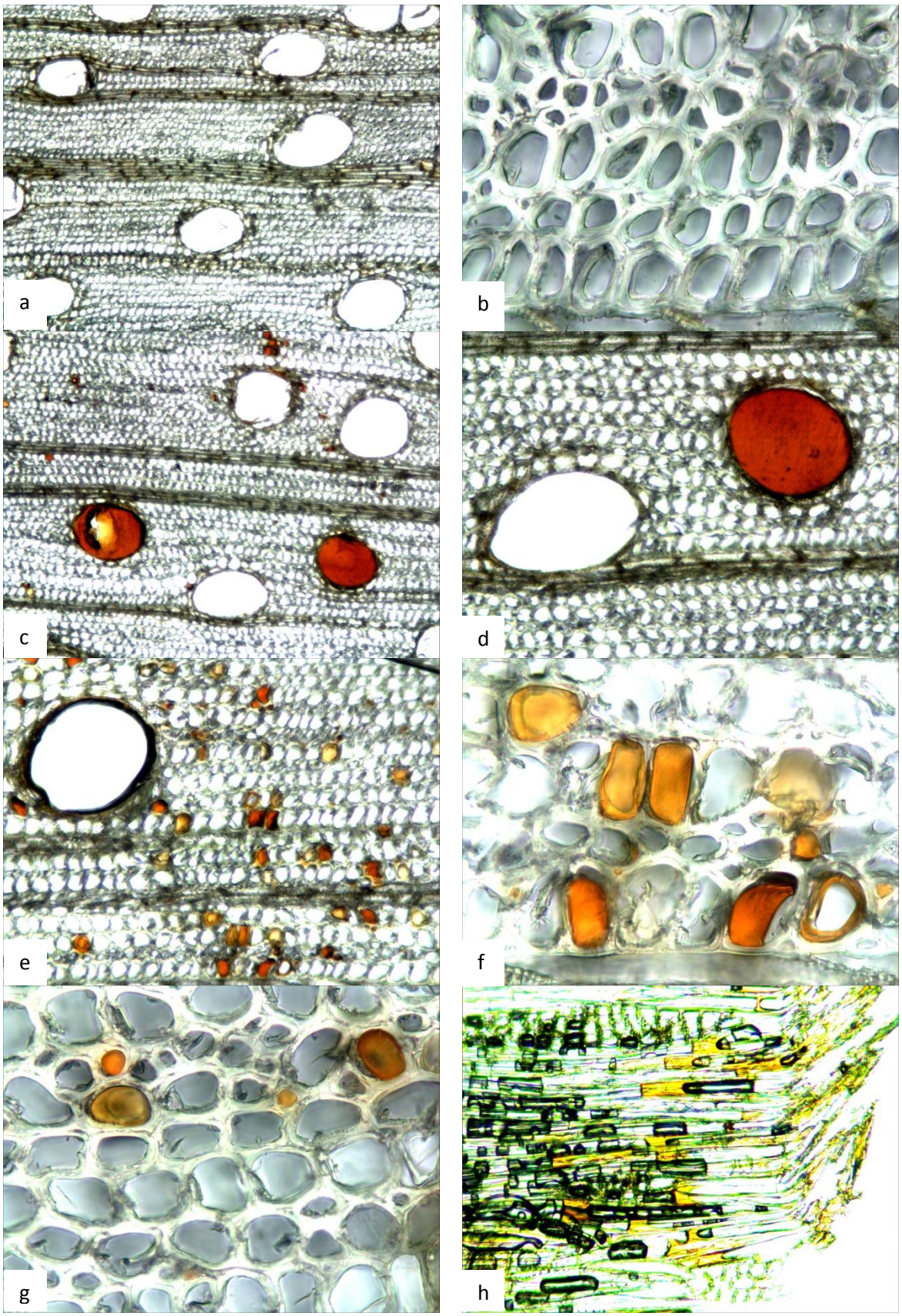


Figure 9.10: Light microscopy of untreated (a, b) and treated Gmelina (c-h) showing resin distribution.

9.4. Conclusions

1. The FTIR spectra of CNSL resin modified wood showed changes in bands in comparison to the spectra of unmodified wood and the pure CNSL resin. There is no substantial change was evident in the accessible OH groups of the cell wall as the absorption band (3332 cm^{-1}) is not different between the modified and unmodified wood. This caused complication as the pure CNSL resin also showed absorption band at the same area of spectra (3350 cm^{-1}) due to the presence of phenolic compounds. Significant changes of bands and intensity were found at 2918 cm^{-1} and 2846 cm^{-1} in Scots pine and 2917 cm^{-1} and 2848 cm^{-1} in Obeche and Gmelina due to the incorporation of the CNSL resin in wood attributed to the branch chain of phenolic compound of the CNSL resin.

2. The other major changes occur in the bands 1657 and 1585-87 is difficult to explain the reason as both the unmodified and pure CNSL resin showed band in these region due to the aromatic skeletal vibration (lignin in wood). Obeche modified wood showed some chemical changes in 1726 cm^{-1} (due to xylan of hemicellulose of wood), a band is also found here in pure CNSL resin due to aldehydes. Two significant absorbance bands appear in most modified wood at 776-780 cm^{-1} and 694 cm^{-1} due to the presence of aromatic compound of the CNSL resin.

3. The Scots pine wood treated with CNSL resin showed better distribution of resin throughout the wood in comparison to the hardwoods, Obeche and Gmelina. In hardwoods the resin is penetrated mostly in vessels and a diffuse penetration was observed. In Scots pine treated wood resins were distributed mainly in the early- and latewood tracheids and in some cases resins were found in cell corners. In Obeche treated wood many fibres were also treated along vessels with resin either by lumen filling or coating. In Gmelina treated wood penetration of CNSL resin was sporadic and the vessels are filled with resin but not universal. Substantial evidence of cell wall penetration by resin is not clear even in the Scots pine wood.

Chapter 10

General discussion, conclusions and future study

10.1. General discussion

A schematic diagram which summarises the overall approach of this study is presented in Figure 10.1.

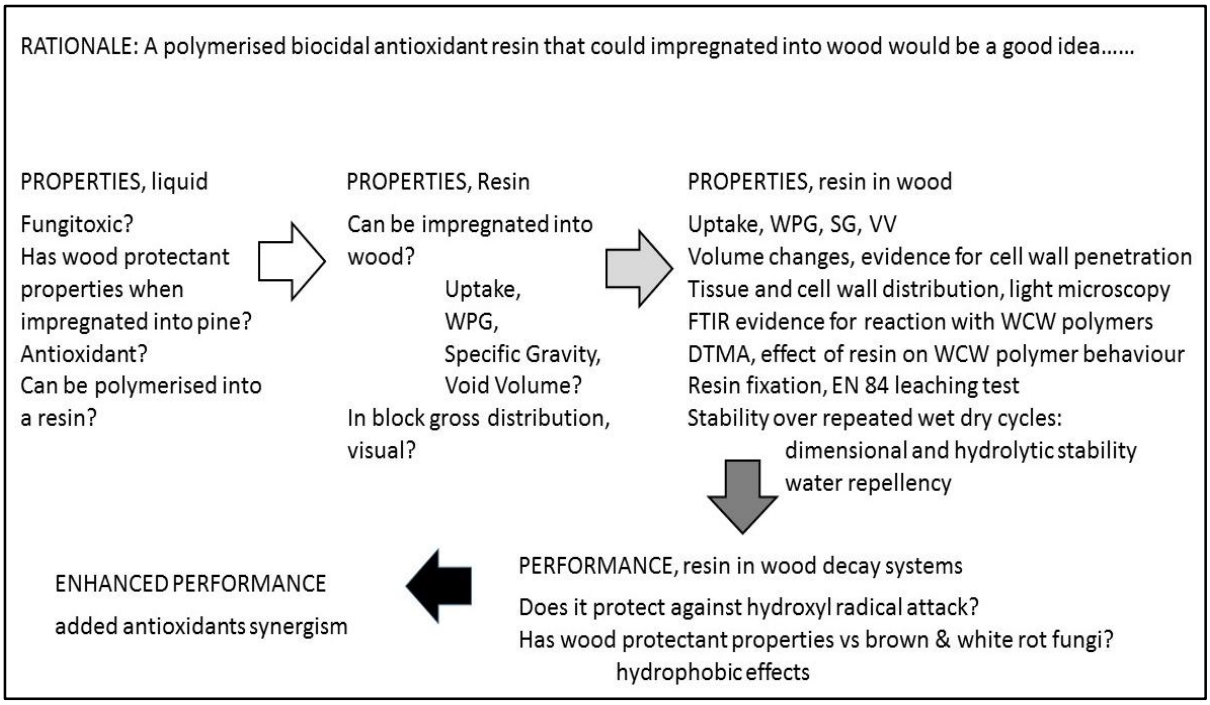


Figure 10.1: Schematic diagram of the approach used in this discussion, detailing the grouping of ideas.

Rationale

Earlier work by Roszaini (2011) on the decay resistance of south east Asian hardwoods indicated that the most durable species, Chengal (*Neobalanocarpus heimeii*), had a high quantity of extractives with high antioxidant activity and analysis of the cold methanol extractives indicated that some of the active antioxidants were polymerised within the wood, and that the extractives were not proven to be fungitoxic, thus it was interesting to explore polymerised antioxidants, with the advantages of low leachability and low mammalian toxicity. Earlier work (Venmalar and Nagaveni, 2005; Adetogun, 2011) had shown some potential of CNSL as a wood preservative, either alone or in the presence of copper. So in this thesis the benefits of including an antioxidant polymer into the wood was examined, in the form of CNSL resin. A body of evidence has been amassed in this thesis which characterises

the performance of a bio-resin modified wood in terms of decay resistance, antioxidant properties, resin distribution, physical and mechanical properties. Fungitoxicity and decay studies were completed on the CNSL alone, resin and resin plus more potent antioxidants against various standard brown and white rot decay fungi.

Fungitoxicity of CNSL types in agar plate assay against brown and white rot fungi

It was decided to use CNSL as it was readily available in Bangor (as *t*CNSL) and was an appropriate material as a by-product, from various developing countries. It was reported to be fungicidally effective at reportedly low retentions/WPG and its constituent molecules looked as if they had some potential as antioxidants. However when tested in this study for fungitoxicity the native, unresinified liquid was less effective than expected so that comparative work was done between the *t*CNSL and the *i*CNSL. In chapter 3 both the CNSL types (*i*CNSL and *t*CNSL) and their combination showed antifungal activity in agar plate assay evident by reducing the rate of fungal growth to some extent but total inhibition of the fungal growth was not achieved in either of the CNSL types (Figure 3.2). Among the CNSLs, *i*CNSL provided better antifungal properties due to the greater quantities of anacardic acid (Prithiviraj *et al*., 1997; Khanna *et al*., 2009) but *t*CNSL also showed some antifungal properties. So, it is likely that the limited fungitoxicity of CNSLs is due to bioactive phenolic components either individually or in combination (i.e. anacardic acid, cardanol and cardol). The antifungal activity did not show a clear difference in the effect of fungitoxicity of CNSLs on brown rot (*C. puteana* and *P. placenta*) and white rot fungi (*T. versicolor* and *P. ostreatus*).

Efficacy of CNSL alone as a wood preservative system in Scots pine against brown and white rot fungi

Chapter 3 also contains some baseline data on whole wood was also gained by looking at the ability of the *t*CNSL and *i*CNSL to protect wood when impregnated into blocks. Due to high viscosity these were formulated in IMS, the blocks were thin in the axial direction (as have been widely used for initial studies on chemical modification) and retentions were controlled using solution strength. IMS is a swelling solvent opening up the possibility of some cell wall penetration. In the event Scots pine was highly permeable and was treated throughout, at least in the cell lumens. Higher WPGs were achieved with the *i*CNSL. An agar block weight loss test following EN113 showed reduced mass losses with the increasing WPG but complete decay protection was not achieved even with a very high WPG (49.1 for *i*CNSL and 42.2 for *t*CNSL) for all the brown rot and white rot fungi. As expected *i*CNSL provided more effective

reduction of mass loss than *t*CNSL but this was only for the brown rot fungi where the *i*CNSL were close to 3% ML but the *t*CNSL failed. With the white rot fungi, both CNSLs gave similar performance (Figure 3.5) and protection was achieved, but only *T. versicolor* gave valid results. Untreated blocks exposed to *P. ostreatus* were not adequately decay for the test to be considered valid. The effect of CNSLs against the wood decay fungi is attributed to the phenolic moiety of the molecule, as phenolic compounds are frequently regarded as fungicidal for example in heartwood (Hart and Hillis, 1974; Scheffer and Cowling, 1966).

Also included in chapter 3 are some preliminary results of antioxidant studies which measured the DPPH free radical scavenging ability of *t*CNSL and *i*CNSL and the raw CNSLs proved to have some AO activity but this was low in comparison to the reference compounds, gallic acid and quercetin. Generally, the antioxidant properties of CNSL are due to presence of phenolic constituents of anacardic acid, cardanol and cardol (Osawa, 1994; Evans *et al*., 1996; Guerrra, 2001). Earlier studies supported that *i*CNSL showed higher antioxidant activity than *t*CNSL (Kubo *et al*., 2006; Trevisan *et al*., 2006) as the former contains higher proportion of anacardic acid while the *t*CNSL is composed mainly of cardanol. However in this study, *t*CNSL also showed some antioxidant activities and statistically no significant difference was found between the antioxidant activities of *i*CNSL and *t*CNSL. So, irrespective of the chemical composition of CNSL another factor may have contributed to the free radical scavenging activity. The presence of unsaturated long side chain of the CNSL's chemical constituents has been claimed in earlier studies (Kubo *et al*., 2006; Rodrigues *et al*., 2006).

Impregnation of Scots pine, Obeche and Gmelina with CNSL resin, WPG and within block distribution

Subsequently, chapter 4, resin was formulated from *t*CNSL due to availability. This was impregnated in IMS solutions into one softwood and two hardwoods of tropical origin, Obeche and Gmelina. The resin was not novel and had been characterised previously (Khan *et al*., 2000 a, b; Fitchett *et al*., 2003). The impregnation was highly successful in a permeable standard test species, Scots pine, but due to tissue distribution issues and aspects of hardwood anatomy was less successful in the hardwoods Obeche and Gmelina. At the highest solution strength (30% v/v) Scots pine achieved a significantly higher WPG (ca. 28) than Obeche (ca. 15) and Gmelina (ca. 10). In later studies (chapter 5) successful impregnation was achieved in a different tropical hardwood, Alstonia (*Alstonia scholaris*).

Specific gravity and void volume

The SG_{OD} and void volume are largely influenced by the CNSL resin impregnation. SG_{OD} is increased in Scots pine (20 %) double that of the Obeche and Gmelina (10%) and thus gave greater densification. As the specific gravity increased the void volume of the modified wood decreased due to the resin loading (Table 4.5). The reduction of the void volume is in effect the gross macro void volume of wood that is occupied by the CNSL resin and consequently a greater void volume reduction was found in Scots pine than Obeche and Gmelina.

Cell wall penetration: evidence from volume changes

Evidence was sought for cell wall penetration by looking at the volume changes (VC%) of the wood following treatment. The resin impregnation increased the volume of wood blocks (external dimension measurements) and a linear relationship was found between the VC% and WPG for the three wood species (Figure 4.2). Lower permeability gave lower VC_{max} values, Obeche and Gmelina (ca. 5.5%) while in the more permeable Scots pine higher VC_{max} values (7.1%). The results of the V_{rel} (theoretical/measured volume) indicated that a lower proportion of resin is located in the wood cell wall than might be expected and much of the resin is located in the cell lumen, particularly at the higher WPG values (Table 4.3).

Resin distribution in the wood blocks, light microscopy

Distribution within the blocks was further examined by microscopy (chapter 9). This revealed high variation, even in Scots pine, some early- and latewood tracheids (Scots pine) were partially and completely filled with resin but careful examination showed that the evidence for distribution other than in the lumen is unconvincing, and probably lumen coating/filling and minor penetration occurred (Figures 9.8-9.10). In other cases the fibres (Obeche) and vessels (Obeche and Gmelina) are also treated with resin either by total lumen filling or by lumen surface coating and the treatment distribution in Gmelina was poor and sporadic. For the hardwoods, where resin had penetrated it was concluded that cell wall penetration was not evident. More appropriate microscopic techniques, possibly the conventional EDAX techniques with an exotic “heavy” label like S or Cl could be used or other techniques looking at refractive index differences or Raman confocal microscopy (Gierlinger and Schwanninger, 2006). The ability of CNSL resin molecules to penetrate into the wood cell wall is an important question which will be addressed later as it is likely that the shape and molecular size of the resin molecule exclude them from significant cell wall penetration into the cell wall micropores.

FTIR evidence for reaction with cell wall polymers

In previous studies, where wood has been modified by a variety of processes, FTIR, in various ways, has been used to collect evidence relating to the cell wall changes, e.g. chemical modification (Williams and Hale, 1999), thermal modification (Gonzalez-Pena and Hale, 2010) and various decay types (Faix *et al*., 1991, Pandey and Pitman, 2003), so that its use here is of relevance to determine whether cell wall penetration and possibly reaction had occurred.

The study relating to the FTIR spectra of the CNSL resin modified wood blocks of the three tested species (Scots pine, Obeche and Gmelina) and modified Scots pine micro-veneers revealed some changes in bands that are evident in resin modified wood as compared to the unmodified wood and pure CNSL resin spectra (chapter 9). Although no substantial changes were evident in the accessible OH groups of the wood cell wall in both cases (wood blocks and micro-veneers) but a significant changes was found in bands at 2846/2848 to 2917/2918 cm^{-1} of the spectra for all the wood species and this changes is attributed due to the branch chain of phenolics of the CNSL resin, which is almost absent in unmodified wood but also found in the pure CNSL resin spectra (Figure 9.3-9.6). The other changes are found in some bands which are difficult to explain the causes of, as these are in the same location of spectral bands found for both wood and resin i.e. 1585, 1452, 1225/1228 cm^{-1}. Some other bands are also found in modified woods solely due to the incorporation of CNSL resin in wood. Overall, the absorption bands which appeared in the resin modified wood spectra are mainly due to the presence of the different functional groups of the CNSL resin but in some cases bands are appeared due to the interference of CNSL resin functional groups with the wood cell wall polymers. Thus the evidence only shows inclusion of resin into the wood rather than substantial reaction with wood cell wall polymers.

Dynamic mechanical thermal analysis, effect on wood cell wall polymers

Dynamic mechanical thermal analysis (DMTA) of the wood was also done to examine whether the resin affected the cell wall polymers. In the event, evidence suggested that it masked it rather than changing it in a way which would suggest cell wall reaction.

The DMTA of unmodified wood revealed three tan δ peaks (α, β and γ) due to the glass transition of lignin, hemicellulose and molecular relaxation. The γ peak occurred at lower temperature scan (-90 to -115 °C) irrespective of wood species and frequencies. The α peak of tan δ found from ca. 55 to 75 °C between Scots pine and Gmelina wood due to the lignin

relaxation. The other two species, Obeche and Alstonia, did not show α transition but produced wide β transitions confined to temperatures of 30 to 45 °C (Table 5.1).

The α tan δ peak of the CNSL resin modified woods shifted to a particular temperature range 60 to 70 °C for all the wood species. This change can be attributed to the glass transition (T_g) of the CNSL resin itself and the effect of modification on wood. Earlier studies has suggested that if the polymer is compatible with wood, it tends to show an increase for decrease of T_g temperature from the pure polymer to the modified wood (Backman and Lindberg, 2002; Chen and Gardner, 2008). Here the tan δ (T_g) temperature and peak height decreased as the WPG decreased in all the wood species but overall no clear relationship between WPG and T_g was found. The decreases of storage modulus (*E'*) of modified and unmodified wood over the thermal scan are attributed to the transition of the wood constituents (amorphous material relaxation). The elastic properties (*E'*) of CNSL resin modified wood were examined at room temperature (20-25 °C) revealed no significant difference between the unmodified and modified woods.

Efficacy of fixation of resin within the blocks, EN 84 leaching

Any wood preservation system needs to be stable within the wood and not lost by dissolving into the surrounding water but may need to be able to release active ingredients when challenged by decay organisms or their decay machinery, of free radicals, acids and enzymes. To test the behaviour in water, leaching tests, a prerequisite for some EN 113 decay tests was done following an EN 84 protocol.

The impregnated CNSL resin was found non-leachable from the wood when examined according to the EN 84 protocol. The effect of WPG on the amount of the leachable resin was not significantly different (Figure 4.3) which means that the bulk of the impregnated resin is substantially located and fixed in wood. There is little variation in WPG before and after the leaching test (Figure 4.4) which was also influenced by the loss of water soluble wood sugars and extractives. However, among the wood species, Gmelina showed higher leaching.

Stability over repeated wet-dry cycles: dimensional stability, hydrolytic stability and water repellency

Other water stability tests were made to examine water repellency, the stability of the resin and the dimensional stability of the wood, where repeated wetting and drying cycles challenge stability to hydrolysis and dimensional stability. The stability to hydrolysis of the CNSL resin

inside the wood was evident when weight loss was calculated in the subsequent WS and OD cycles (Table 4.8). The greatest weight loss was found in first cycle and thereafter lowers for the subsequent cycles. In most of the cases, the WPG has no significant effect on the weight loss and in many cases the weight loss of modified wood was equal to the control wood blocks. Thus, it is assumed that the difference of WPG found in leaching test and the weight loss found in cyclic test is mainly the non-bonded resin leached out in cycle 1 accompanied with some water soluble fragments of hemicellulose. A small portion of other sapwood extractives can also be leached out as the test specimen were not solvent extracted before the resin impregnation.

The CNSL resin brought a modest dimensional stability of ca. 30% for all wood species. Higher dimensional stability was found in Obeche wood with a lower WPG combined with lower swelling coefficient (S%) in comparison to Scots pine with high WPG. As the resins are mostly polymerized in the cell lumen they made little direct contribution to improve the dimensional stability. Again, no steady loss of ASE was found in the cycle test (Table 4.7) suggested that this is attributed to the loss of wood extractives and hemicellulose. Hill (2006) mentioned that this is actually not the loss of dimensional stability of the modified wood as the OD and WS volumes decrease at an equal rate over the five successive cycles (Figure 4.5).

The water repellency of wood is also improved due to the modification with CNSL resin as the WA was reduced and WRE was increased with the increasing WPG. As like the dimensional stability, higher water repellency was found in Obeche and lowest in Gmelina modified wood (Table 4.9 and 4.10). The reduction of the water repellency in the subsequent cycles was not attributed to the loss of resin rather than the loss of soluble components of wood material, as mentioned earlier.

Ability to protect against Fenton's reagent attack: brown rot mimic, weight and tensile strength loss

As this study aimed to look at bio-resins and antioxidant effects a system was used to mimic part of the brown rot decay mechanism and provide useful quantitative results, the Fenton's reagent was used to show the ability to protect the wood. The treatment with Fenton's reagent of the CNSL modified wood is a rapid, dependable, bio-system free method which provides useful information regarding oxidative degradation of the initial decay mechanism of CNSL resin modified micro-veneers by measuring mass changes and the tensile strength changes at zero-span mode. The CNSL resin modified wood micro-veneers provided a large

impact on the weight losses even at low WPG in reaction with Fenton's reagent while severe weight losses of unmodified control veneers were found by 24 h of reaction (Table 6.1). The tensile strength of the modified veneers was decreased with the increasing reaction duration but decrease with the increase of WPG. Treatment of unmodified micro-veneers with Fenton's reagent for longer periods completely lost any tensile strength but a substantial tensile strength was found in resin modified veneers at the same reaction duration (Table 6.2). Though, 30 to 40% strength loss was found in modified veneers at longer Fenton's reaction which is attributed to the depolymerisation of the cellulose as it is well established that the loss of tensile strength is correlated to the depolymerisation of wood cell wall polysaccharides (Figure 6.2). In contrast to strength losses with some types of chemical and with thermal modification of wood, in this study this resin modification provided improved tensile strength to micro-veneers (Figure 6.1). It is unfortunate that local testing facilities for further zero span testing were not available as a variety of resin, WPG and antioxidant variable could have been examined further.

The reality of fungal decay on resin treated wood however gave different results, whereby degradation by the Fenton reagent was controlled at lower WPG, at least initially, than were necessary to prevent fungal decay in agar block tests. The micro-veneer approach has one major disadvantage in this respect in that the sections are up to 4 cells thick and are probably well encapsulated by the resin, and the reactive surface area is high.

Decay of resin treated wood: resistance to brown and white rot decay

Where decay in resin modified wood is concerned the CNSL resin system reduced the mass losses of decayed wood but the total decay protection was not achieved in any of the wood species against any successful decay fungi tested (Figure 7.2). Full protection threshold was established by extrapolation of data using regression analysis (Gezer *et al*. 1999; Williams and Hale, 1999; Van Acker *et al*. 1999; Hill *et al*., 2004; Ormondroyd 2007). A very high threshold was required for Scots pine (ca. 50 WPG) but less than 20 WPG provided complete protection to decay in the hardwoods Obeche and Gmelina against the brown rot fungi. In case of white rot fungus, *T. versicolor,* ca. 30 WPG provided complete protection for all the three wood species. In this study, the hardwoods, Obeche and Gmelina showed better decay resistance with low resin loadings, particularly against the brown rot fungus *P. placenta*. On the other hand, Scots pine modified wood showed better resistance against white rot fungus, *T. versicolor.*

Cell wall bulking or lumen filling and effects on decay resistance

Other studies with chemically modified wood have shown that decay resistance is imparted by cell wall bulking, which reduces the size and number of the wood cell wall micropores (Papadopoulos and Hill, 2002; Hill *et al*., 2005). It is evident from the volume change study (chapter 4) and microscopic study (chapter 9) that the CNSL resin has very limited penetration into the cell wall micropore matrix but is polymerized in the lumen giving at least a coating (in case of hardwoods vessels coating, see Figure 9.9 and 9.10). Moreover, the oxidative degradation by Fenton's reagent study of the modified micro-veneers (chapter 6) revealed that the low molecular weight agents like OH radicals can penetrate into the resin modified wood with prolonged exposure and cause degradation of the cell wall. Thus, the initial degradation process employed by most fungi through low molecular weight degradation agents cannot be prevented by the CNSL resin as the resin largely provides only a lumen coating, which is discontinuous, especially in Gmelina which did not treat well. It is expected that aside for treatment issues of resin distribution at the anatomical level, the larger molecular weight of the CNSL resin may hinder the entry of the resin into the wood cell wall and as a result complete decay resistance was not achieved in this study.

Hydrophobicity of the resin: effects on decay resistance

The decay resistance achieved may also be due to the hydrophobicity of the resin. There is some doubt about the interpretation of end of decay test moisture contents because when they are low it does not necessarily mean that they have been low throughout the test, especially after a prolonged exposure period, however in this test they have been interpreted as real and the resin treated wood blocks showed low moisture contents at the end of the decay test. This is also evident in resin modified sterile control wood blocks (Figure 7.3). Thus lowered moisture content to below fibre saturation point suggests that the resin forms more than just a simple physical barrier, but one that is capable of protecting the hemicellulose hydroxyl groups and reducing cell wall swelling. Consequently this would interfere with the decay systems and would provide reduced mass loss during decay exposure. A more severe wetting test regime, such as the vermiculite "white rot additive" system used in the UK board test material standard (BS1982: 1990, Part 1) and used by Forster (1998) for acetylated wood may provide useful data here.

Resin and antioxidants: effects on decay reduction

In this study an alternative approach was investigated (chapter 8), the additive effects of bio-based CNSL resin with different antioxidants (AOs) to improve the decay resistance of wood. Control data with no AO (Figure 8.1) gave similar results to generate in the earlier test (Figure 7.2), but fewer fungi were used for test. A *key* point is that the combined effect of resin and AOs on wood decay resistance was higher than alone (Figures 8.2 and 8.3). Some of the AOs alone effectively gave large reduction in the MLs of the treated wood blocks, particularly propyl gallate (Figure 8.1, Table 8.1). The percentage of ML reduction due to the treatment with AOs with or without CNSL resin has revealed that AOs with higher free radical scavenging properties showed better ML reduction e.g. (propyl gallate, ferulic acid). The AOs were found more effective against the brown rot fungus, *C. puteana* than the white rot fungus, *T. versicolor* and this may be because brown rot fungi produce extracellular OH radicals in the initial stage of brown rot decay (Arantes and Goodell, 2014) so this result is important and that it works, still needs considerable further work in a variety of ways (further work section).

Apart from the good activity of the propyl gallate resin combination some of the other AOs showed lesser effect with resin (Figure 8.3, Table 8.5) and attention is drawn to ferulic acid with *C. puteana* with Gmelina, to SSL phenolic extract with Obeche for both fungi and gallic acid with *T. versicolor*. Some improvement was also observed for other AOs with lower free radical scavenging capacity of quercetin and caffeic acid. The ML reduction with the woods treated with resin and AOs showed that most of the AOs work well with resin against *T. versicolor* for Scots pine wood, but with exposure to *C. puteana* they work well in Gmelina wood. This is attributed to the combined effect of CNSL resin and AOs. Thus, it is assumed that the reduction of the ML of the wood treated with the CNSL resins and AO compounds are due to the combined effect of free radical scavenging provided by the AOs, the barrier effects offered by the presence of resin inside the wood cells and water repellent properties of the hydrophobic CNSL resin system.

Thus it is concluded that although this in not a perfect system, in that the chosen resin system is not effective alone, it is bio-based and other bio-based systems could be used to lock and release antioxidant systems to reduce the impact of decay fungi. At the onset it had been expected that CNSL oil would have given better decay resistance properties, better AO properties and that selection of a resin building block with better AO properties could be beneficial.

10.2. Conclusions

The aim of the work in this thesis was to investigate the physico-mechanical and decay resistance properties of bio-resin modified wood and the following conclusions can be drawn:

1. The bio-based CNSL resin prepared by the ozonolysis process gave a viscous liquid of high molecular weight, hydrophobic and thermosetting resin system and needed to be dissolved in IMS to lower the viscosity for treatment.

2. The raw CNSL of both type (*i*CNSL and *t*CNSL) do not have enough fungitoxicity to achieve complete decay resistance against the wood decay fungi of the Basidiomycota.

3. The CNSL resin is located largely in the wood cell lumen either by coating the lumen surface or filling it entirely. Limited evidence has been found regarding entry into the wood cell wall and micropore blocking of the wood cell wall.

4. The CNSL resin modification moderately improved the physical properties e.g. WPG, VC, SG_{OD}, void volume, and moisture related properties e.g. dimensional stability, water repellency.

5. The leachability of the CNSL resin was minimal according to EN 84 test. The resin also found stable to hydrolysis when tested in five consecutive WS/OD cycles.

6. The glass transition (T_g) of the CNSL resin modified woods were found at 60-70 °C temperature. The storage modulus, *E'* (elastic properties) of CNSL resin modified wood was not particularly increased at room temperature. Among the species, Scots pine modified wood showed better elastic properties.

7. Threshold levels of complete decay protection were not reached at the WPGs provided by the resin modified wood against any decay fungi in any of the species. The CNSL resin only provided a barrier to fungal hyphae in the cell lumen either by surface coating or filling, and by modifying the moisture behaviour of the wood so that it was more hydrophobic.

8. CNSL resin modification improved the tensile strength of the wood when examined micro-veneers at zero-span mode. Minimum weight loss and better strength retention was found in modified micro-veneers in exposure to oxidative reaction with Fenton's reagent but with prolonged exposure of the wood greater strength losses occurred, due to the depolymerisation of wood cell wall polysaccharides.

9. Decay resistance was improved due to the effect of CNSL resin combined with antioxidants (AOs). The AOs with high free radical scavenging ability showed better performance with CNSL resin even with low WPG. Propyl gallate was found most effective in reducing mass losses among the five AO tested.

10. Among the wood species, Scots pine modified wood was improved in WPG, VC, SG_{OD}, void volume and elastic properties. On the other hand, Obeche modified wood was improved in dimensional stability, water repellency and decay resistance. But, Gmelina modified wood found most inferior and only showed improvement in decay resistance.

10.3. Future study

1. In this study, a clear indication of hydrophobic nature of the CNSL resin has been found but unfortunately although some sorption isotherm work was initiated it was not completed so use of dynamic vapour sorption (DVS) would be useful to explain the wood-water relationships as this has direct relationship to the dimensional stability and decay resistance of wood. It may also be possible to analyse data using the prescribed model for isotherm fitting and determination of monolayer and polylayer moisture content of the modified wood.

2. This study has found that the CNSL resin impregnation was not sufficient to some low permeable hardwood species, particularly Obeche and Gmelina provided low WPG throughout the study. Although Alstonia was introduced at a later stage a future work should involve more permeable hardwoods i.e. beech, rubber wood, although there may remain problems with poor micro-distribution between different cell types (vessels and surrounding tracheids vs fibres, parenchyma, etc.).

3. In this study, limited evidence for cell wall penetration by the CNSL resin was found so it would be interesting to undertake a study of cell wall pore accessibility of the resin modified wood by using the probes of various sizes through the process called solute exclusion techniques (Forster, 1998; Hill *et al.*, 2005 Ormondroyd, 2007) or by using modern gas porosimetry (Kwon *et al*., 2007). This could be important from the decay protection point of view as it is suggested that the cell wall micropore blocking is an important decay resistant mechanism of chemically and impregnated modified wood (Hill *et al*., 2005).

4. Testing needs to be done to look at the properties of compounds like propyl gallate, including its permanence, resistance to leaching, ecotoxological properties.

5. The work done in this thesis was restricted to testing with selected Basidiomycete decay fungi in pure culture systems. Exposure to a wider range of risks and studies done in wetter decay systems could prove revealing.

6. Determination of mechanical properties of the CNSL resin modified wood was carried out by DMTA which mainly provided the viscoelastic properties and by zero-span tensile test which provided the tensile strength at zero-span mode. These do not cover the general strength properties of wood i.e. static bending strength (MOE, MOR), compression strength, hardness, shear strength etc. So, a similar study could be undertaken to determine these mechanical properties of the resin modified wood.

7. In this study, no convincing evidence of cell wall penetration by CNSL resin has been found by the light microscopic study. A similar study could be undertaken by using Raman confocal microscopy which may reveal whether the resin has entered in the wood cell wall and to what extent.

8. In this study, small wood blocks were used for even penetration of the resin into wood and thus determine the physical and decay resistance properties of the resin modified wood. A similar study could be undertaken by using standard wood blocks e.g. EN 113 for decay resistance test, BS 373 for testing the physical and mechanical properties.

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