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Oxidative Activation of Wood Fibers for the Manufacture of Medium-Density Fiberboard (MDF)

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ABSTRACT

The aim of this thesis work was to study the possibilities of radical formation in wood fiber surfaces to enable direct fiber-to-fiber adhesion by radical-based reactions in the manufacture of fibreboard, particularly medium-density fiberboard (MDF). The fibers were produced by defibration at high temperatures. Radical formation was achieved by treatment with laccase, treatment with Fenton's reagent, or gamma-irradiation.

High-temperature defibration was found to cause cleavage of interunit beta-aryl ether linkages of lignin, resulting in formation of mechanoradicals and phenolic hydroxyl groups. The proportion of water-extractable low-molecular weight lignin and hemicelluloses present in the fibers increased with an increase in defibration temperature.

In the laccase treatment of fibers in water suspension, much more radicals were formed in hardwood than in softwood fibers for fibers produced at equal temperature. Radical formation increased with increasing defibration temperature. The treatment of fibers with Fenton's reagent in water suspension resulted in the formation of similar numbers of radicals in hardwood and softwood fibers. Radical formation increased with increasing defibration temperature but not so drastically as with laccase treatments. Also the gamma-irradiated fibers contained large numbers of radicals. Their content increased as a function of increasing defibration temperature and was higher for hardwood than for softwood fibers.

The internal bond strength (IB) of fiberboards made from fibers treated with laccase or Fenton's reagent in the defibrator blowline improved with increasing defibration temperature. The IB of boards made from laccase-treated fibers correlated with the number of radicals formed in the fibers on laccase treatment in water suspension, indicating that adhesion in the boards was largely due to reactions of radicals on the fiber surfaces. The IB of boards made from fibers treated with Fenton's reagent also correlated with the radical content of the fibers, but this relationship was not as strong as with the laccase treatments. This suggests that bonding mechanisms other than radical coupling may have contributed significantly to adhesion. Gamma-irradiation of fibers before their fabrication into boards resulted in a marked increase in board IB, indicating that radicals play a significant role in the adhesion of boards made from gamma-irradiated fibers.

PREFACE

The work for this thesis was carried out at the Helsinki University of Technology at the Laboratory of Paper Technology and at the University of Helsinki at the Laboratory of Organic Chemistry during the years 1996-2001.

I want to express my gratitude to Prof. Jaakko E. Laine, my thesis advisor and co-author of my publications, for all of his advise and support over the years. Thanks are also due to my other co-authors, Pia Qvintus-Leino and Simo Tuominen for their valuable contribution to the research work.

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Financial support from Neste Research Foundation is gratefully acknowledged.

Finally, warmest thanks to my wife Rosita for her love and support during the preparation of this thesis.

~ Si hoc legere scis nimium eruditionis habes ~

LIST OF PAPERS

This thesis is based on the following publications, which are referred to in the text by their Roman numerals.

I Widsten, P., Laine, J.E., Qvintus-Leino, P. and Tuominen, S. Effect of high-temperature fiberization on the chemical structure of softwood. *Journal of Wood Chemistry and Technology* 21(3), 227-245 (2001).

II Widsten, P., Laine, J.E., Qvintus-Leino, P. and Tuominen, S. Effect of high-temperature defibration on the chemical structure of hardwood. *Holzforschung* 56(1), 51-59, 2002.

III Widsten, P., Laine, J.E. and Tuominen, S. Radical formation on laccase treatment of wood defibrated at high temperatures. Part 1. Studies with hardwood fibers. *Nordic Pulp and Paper Research Journal* 17(2), 139-146, 2002.

IV Widsten, P., Laine, J.E. and Tuominen, S. Radical formation on laccase treatment of wood defibrated at high temperatures. Part 2. Studies with softwood fibers. *Cellulose Chemistry and Technology* 36(1-2), 2002.

V Widsten, P., Laine, J.E., Qvintus-Leino, P. and Tuominen, S. Effect of high defibration temperature on the properties of medium-density fiberboard (MDF) made from laccase-treated hardwood fibers. Accepted for publication in *Journal of Adhesion Science and Technology*.

VI Widsten, P., Laine, J.E., Qvintus-Leino, P. and Tuominen, S. The influence of high defibration temperature on the properties of fiberboard made from laccase-treated softwood fibers. Submitted to *Wood Science and Technology*.

VII Widsten, P., Laine, J.E., Qvintus-Leino, P. and Tuominen, S. Manufacture of fiberboard from wood fibers treated with Fenton's reagent (H_2O_2/$FeSO_4$). Accepted for publication in *Holzforschung*.

VIII Widsten, P., Qvintus-Leino, P., Tuominen, S. and Laine, J.E. Studies on the use of Fenton's reagent (H_2O_2-$FeSO_4$) and gamma-irradiation to produce radicals in wood fibers to be used for fiberboard. Submitted to *Paperi ja Puu*.

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AUTHOR'S CONTRIBUTION

The author wrote all the articles and planned and carried out most of the experimental work.

LIST OF ABBREVIATIONS

^{13}C NMR	carbon-13 nuclear magnetic resonance
CP/MAS	cross polarization/magic angle spinning
CTMP	chemithermomechanical pulp(ing)
CV	*Coriolus versicolor* laccase
DPPH	α,α'-diphenyl-β-picrylhydrazyl
EPR	electron paramagnetic resonance
ESCA	electron spectroscopy for chemical analysis
ESR	electron spin resonance
g	spectroscopic splitting factor (g-value)
h	Planck's constant
G	guaiacyl
H	*p*-hydroxyphenyl
I	nuclear spin
H_0	applied magnetic field
IB	internal bond (strength)
MDF	medium-density fiberboard
ML	middle lamella
MOE	modulus of elasticity
MOR	modulus of rupture
MT	*Myceliophtora thermophila* laccase
O/C	oxygen/carbon
P	primary wall
RMP	refiner mechanical pulp(ing)
S	electron spin vector
S	syringyl
S_1, S_2 and S_3	outer, middle and inner secondary wall
TH	*Trametes hirsuta* laccase
TMP	thermomechanical pulp(ing)
TS	thickness swell
UF	urea-formaldehyde
W	warty layer
β	Bohr magneton
β-O-4	β-aryl ether
ΔE	separation of energy states
μ	magnetic moment

CONTENTS

1 INTRODUCTION

Wood composite boards such as particleboard and fiberboard are traditionally manufactured by mechanical disintegration of the wood raw material followed by gluing of the resulting particles with thermosetting resins to the desired composite products. For example, medium-density fiberboard (MDF), which is used in the manufacture of furniture and sidings, is usually made by a process involving the following steps: 1) mechanical disintegration (defibration) of chips under elevated steam pressure, 2) application of urea-formaldehyde (UF) resin and sizing agents onto the fibers, 3) formation of the fibers to sheets 4) hot-pressing of the sheets into boards. MDF of high mechanical strength and good water resistance is produced by this process.

Several methods for bonding of wood particles without synthetic resins have been developed but are not used commercially. An exception is the manufacture of wet-formed hardboard by the Masonite process based on steam-explosion which, however, suffers from low yield and high energy requirements [1]. Many of these methods are two-component adhesive solutions for the production of particleboards, involving the use of an added bonding agent such as lignin or lignin together with non-phenolic monomers, which are polymerized enzymatically [2-4]. One-component processes, applied to the production of fiberboard, are based on the generation of radicals on the surfaces of fibers so that they may be pressed into boards without any additional bonding agents. The adhesion effect thus obtained is believed to be largely due to radicals formed in the polymeric fiber surface lignin and in low-molecular weight lignin and other phenolic substances present in the fibers [5-9].

One of the one-component processes is the treatment of wood fibers with phenoloxidases such as laccases and peroxidases so that radicals are formed in the lignin and phenolic extractives on the surfaces of the fibres. When enzymatically treated fibers are pressed into boards, an adhesion effect apparently connected to formation and interaction of radicals is observed [7,8,10]. Significant improvements in mechanical strength and thickness swell properties of fiberboards relative to those of control boards made without enzymes have been reached by using laccase [2-5,7,8,10] and peroxidase [11]. Mechanisms accounting for the enhanced adhesion may involve formation of interfiber covalent bonds *via* coupling of radicals on adjacent fibers, reactions of fiber radicals with other reactive groups present in the fibers, or an increased interfiber bonding area resulting either from reactions of fiber radicals with low-molecular weight lignin fragments and other phenolic compounds or a loosening of the lignin structure by the action of the enzyme [7,8]. A more even surface topography could increase the extent of interfiber hydrogen bonding and other secondary forces binding fibers together.

Activation of wood particle surfaces by treating them with inorganic oxidizing agents is another way to achieve direct interparticle bonding [12-17]. Fenton's reagent ($H_2O_2/FeSO_4$) is one of these oxidative activators. Reactive oxygen radicals formed as peroxide decomposition products are able to generate radicals on the surfaces of wood particles. Treatment of wood particles with $H_2O_2/FeSO_4$ or other oxidants has enabled direct surface-to-surface bonding in the fabrication of wood composites [14,16,17]. The improved adhesion observed may be associated with interaction of free radicals formed on the surfaces of the particles [12,14,15] with each other or other reactive groups. The contribution of other bonding mechanisms involving e.g. carboxyl groups cannot be dismissed [12,18,19]. However, these methods suffer from various drawbacks including the harmful long-term effect on the board

properties of the high peroxide charges needed, high variability of product quality and the high board densities needed to obtain boards of sufficient mechanical strength.

γ-Irradiation of wood particles causes oxidative changes in the lignin and carbohydrate components [20-23] whereby radicals are formed [20,21]. The use of γ-irradiation to generate radicals on the surfaces of wood fibers before pressing them into fiberboards seems an interesting avenue of research.

In addition to coupling or other crosslinking reactions of radicals located on wood particle surfaces, also other bonding processes may enhance interfiber adhesion. The extent to which this so-called autoadhesion takes place depends on the pressing conditions and the chemical and physical properties of the fibers.

This thesis deals with the generation of radicals on fiber surfaces by treatment with laccase or Fenton's reagent or γ-irradiation, and use of the resulting activated fibers for the manufacture of fiberboard, particularly MDF. It is plausible to assume that adhesion improves as the number of radicals on the fiber surfaces increases and that the choice of wood species and defibration conditions may exert an appreciable effect on radical formation. The objective of the studies in the articles I-IV and VIII was to produce fibers that are reactive enough in order that large numbers of radicals may be generated on their surfaces and to develop effective methods for radical formation. I and II are concerned with the changes in the chemical structure of wood occurring on high-temperature defibration. III and IV focus on the use of laccases for the generation of radicals in the wood fibers characterized in I and II. V and VI deal with manufacture of fiberboard from laccase-treated fibers while in VII and VIII, Fenton's reagent ($H_2O_2/FeSO_4$) and γ-irradiation were used for radical formation and fiberboard was fabricated from the activated fibers. Based on their density, the fiberboards made in V are classified as MDF while most of those made in VI-VIII have densities in the hardboard range (> 0.9 g/cm^3). The differences in the extents to which radicals were formed in fibers refined at different temperatures (III, IV, VIII) are explained by their different chemical structures (I, II). The relationship between the frequency of radicals in the fibers and properties of fiberboards made from the activated fibres is discussed in V-VIII.

2 STRUCTURE OF NATIVE AND DEFIBRATED WOOD

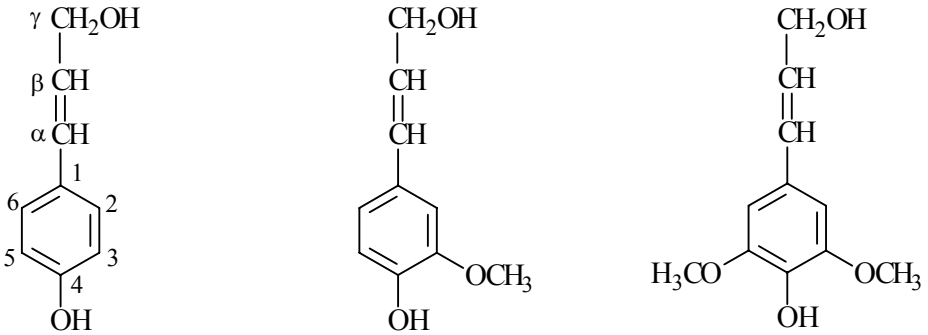
2.1 Structure of native wood

2.1.1 Wood types and principal wood constituents

Trees are classified into two major categories, gymnosperms and angiosperms [24]. Softwood (coniferous) trees such as spruce and pine belong to the first category and hardwood (deciduous) trees such as birch, aspen and eucalypt to the second. The main constituents of a wood cell (or a wood fiber) are cellulose, hemicellulose and lignin. The term "wood fiber" is used to denote a mature, fully developed wood cell.

Cellulose is a linear, crystalline homopolysaccharide composed of glucose units while hemicelluloses are branched, amorphous heteropolysaccharides built up of different monomeric sugars such as xylose, mannose and glucose. The structure and monomeric sugar composition of hardwood hemicelluloses differ from those of softwood hemicelluloses. Wood typically contains about 40-45% cellulose and 20-30% hemicelluloses. These polysaccharides mainly serve as supporting material in the wood cell walls.

Lignin is an amorphous macromolecule functioning as a cementing material in the wood cells and imparting mechanical strength to the tree [24]. It is formed as a result of radical coupling between phenylpropane units called lignin precursors (Fig. 2.1). On the basis of the number of methoxyl groups attached to the aromatic ring, the phenylpropane units are divided into *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units. Softwood lignin is mainly composed of G units while hardwood lignin is a copolymer of G and S units. The G:S ratio varies from 4:1 to 1:2. H units constitute only a small part of wood lignin and are more common in nonwood plants. The phenylpropane units are linked together by carbon-carbon and carbon-oxygen (ether) bonds. A proposed structural scheme for softwood lignin is shown in Fig. 2.2. Softwoods usually contain 26-32% and hardwoods 20-25% of lignin.



Lignin precursor:	*p*-Coumaryl alcohol	Coniferyl alcohol	Sinapyl alcohol
Aromatic unit:	*p*-Hydroxyphenyl (H)	Guaiacyl (G)	Syringyl (S)

Figure 2.1. Lignin precursor molecules and the names of their aromatic moieties.

In addition to lignin and carbohydrates, minor amounts of lipophilic and hydrophilic extractives are present in wood [24]. Typical lipophilic extractives are free and esterified fatty acids, terpenoids and resin acids. The hydrophilic extractives are mostly phenolic substances including lignans (phenylpropane dimers), tannins and flavonoids (Fig. 2.3). The extractives usually act as a source of energy for wood cells or protect the wood against microbiological attack. Their amount seldom exceeds 10% of the dry wood weight.

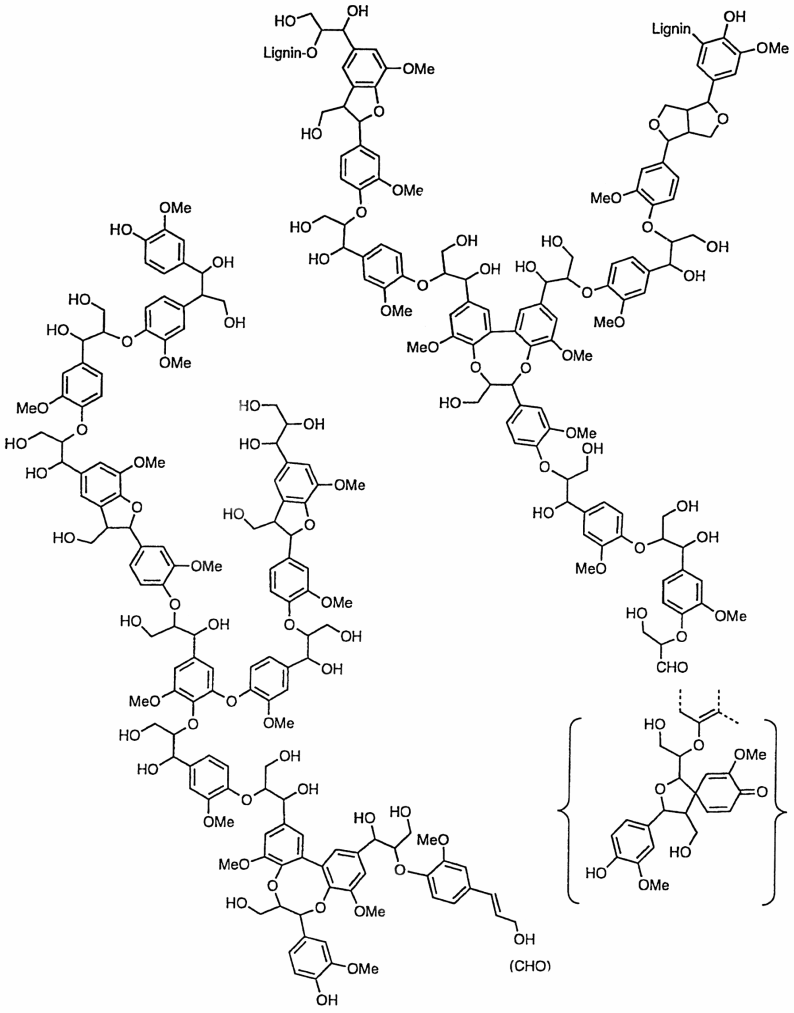


Figure 2.2. Structural scheme of softwood lignin [25].

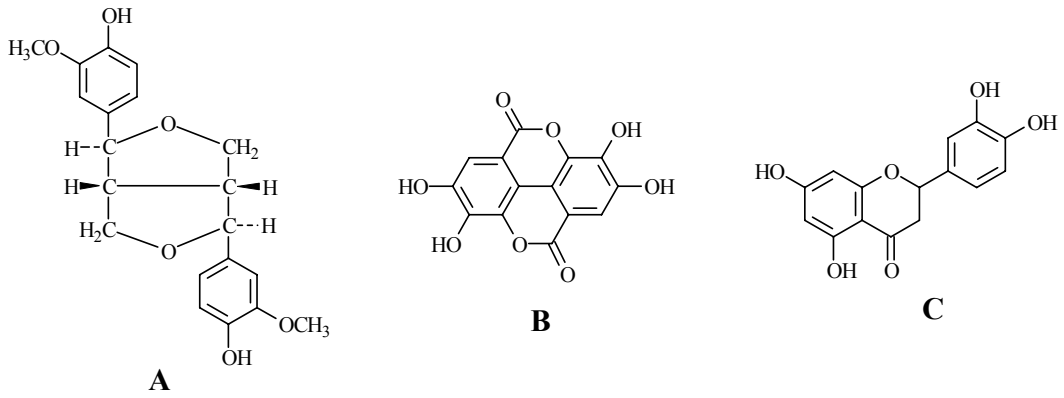


Figure 2.3. Examples of phenolic wood extractives: A) pinoresinol (a lignan), B) ellagic acid (a tannin) and C) chrysin (a flavonoid).

2.1.2 Cellular structure of wood

Wood consists of several types of cells with different sizes and functions [24]. Most of the softwood cells are relatively long tracheids. The main cell types of hardwoods are the large and tubelike vessels and fiber tracheids. The fiber tracheids resemble the softwood tracheids and are much more thick-walled than the vessels. They contribute much more to the wood mass than the voluminous but thin-walled vessels. In addition, parenchyma cells, which are also thin-walled, are found in the resin canals of softwoods and wood rays of both softwoods and hardwoods. The important fiber types from the point of view of fiberboard are the tracheids and fiber tracheids. The vessels and parenchyma cells break readily during mechanical pulping, producing fines (very small fiber fragments).

The main wood constituents are unevenly distributed between the morphological regions of the wood cell, which is composed of various layers (Fig. 2.4). The thickest layer is the secondary wall, which is rich in carbohydrates and low in lignin. The opposite is true for the highly lignified but thin primary wall and middle lamella. The middle lamella, located in the cell corners, binds the cells together. The G and S lignins of hardwoods are not uniformly distributed between the wood cell layers, the secondary wall containing mainly S lignin and the middle lamella mostly G lignin. H lignin in softwoods exists usually as a copolymer with G lignin and is concentrated in certain parts of the tree.

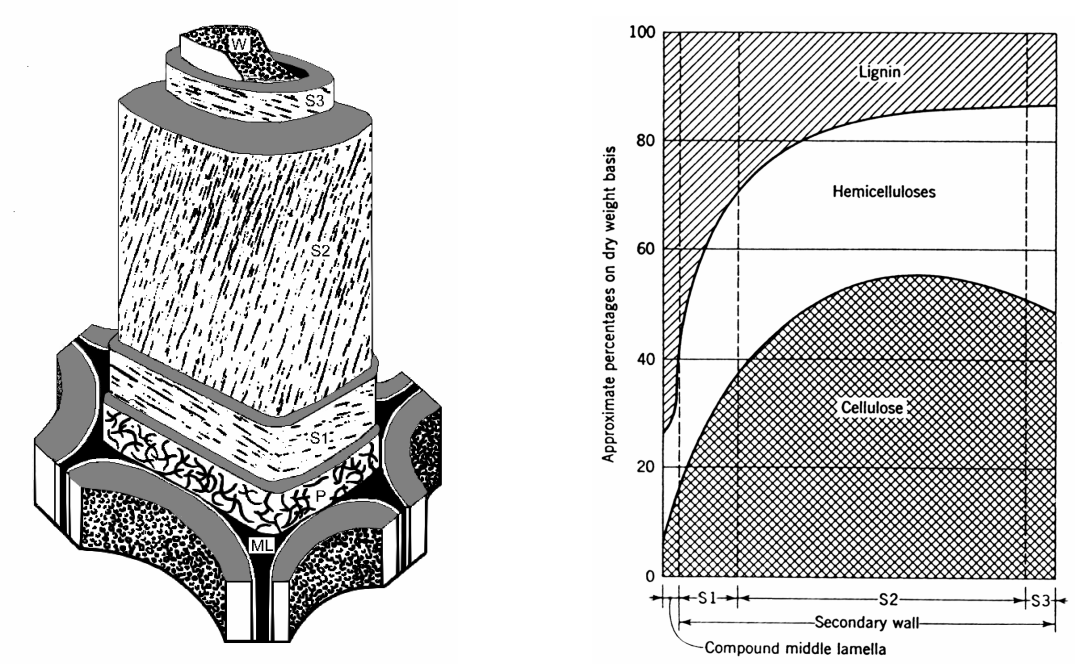


Figure 2.4. Left: Structure of a wood cell (fiber) showing the middle lamella (ML), primary wall (P), the outer, middle and inner secondary wall (S_1, S_2 and S_3) and the warty layer (W) [24]. Right: Approximate distribution in percent of the most important chemical components in the different layers of a softwood tracheid [26]. Compound middle lamella = primary wall + middle lamella.

2.2 Structure of wood defibrated at high temperatures (I, II)

2.2.1 Wood defibration for fiberboard manufacture

The various mechanical pulping processes for paper involve defibration, i.e., the breakdown of the wood matrix resulting in the separation of the fibers [27]. Fiber separation in the mechanical pulping methods, such as refiner mechanical pulping (RMP), thermomechanical pulping (TMP) and chemithermomechanical pulping (CTMP), is achieved by using grinding, refining or a combination of them. Grinding involves the pressing of wood logs against a revolving pulpstone, while in refining (Fig. 2.5) wood chips are disintegrated in a revolving disc refiner. As a result, wood fibers are fractured and the resulting pulp consist of fibers of various lengths, fiber fragments and shives (unseparated fiber bundles).

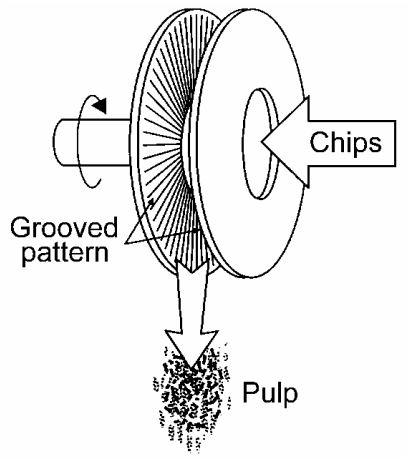


Figure 2.5. Principle of mechanical refining. Wood chips are fed into the refining zone where they are disintegrated between two grooved discs of which at least one is revolving. [28].

Thermomechanical pulping (the Asplund process) [1] is also used for the production of fiberboard. In this process, debarked and chipped wood is preheated with pressurized steam at elevated temperatures to soften the wood after which the chips are refined. The method used for fiberboard production is basically the same as that used in modern TMP pulping for paper, except for the fact that the refining is conducted at about 170°C/0.8 MPa. The fibers for this thesis work (Table 2.1) were produced at 171-202°C (0.8-1.6 MPa) using a pilot-scale Asplund refiner (Sunds Defibrator®). The board manufacturing process is described in more detail in chapter 5. In TMP pulping for paper, the steam temperature is typically in the range 120-130°C [29].

Table 2.1. Description of wood fibers referred to in the text.

Designation	Wood species	Wood type	Preheating conditions	
			Temperature, °C	Pressure, MPa
A-171	European aspen (*Populus tremula*)	Hardwood	171	0.8
A-188	European aspen (*Populus tremula*)	Hardwood	188	1.2
B-Wood	Silver birch (*Betula verrucosa*)	Hardwood	Non-defibrated	Non-defibrated
B-171	Silver birch (*Betula verrucosa*)	Hardwood	171	0.8
B-188	Silver birch (*Betula verrucosa*)	Hardwood	188	1.2
B-196	Silver birch (*Betula verrucosa*)	Hardwood	196	1.4
E-Wood	Eucalypt (*Eucalyptus* sp.)	Hardwood	Non-defibrated	Non-defibrated
E-171	Eucalypt (*Eucalyptus* sp.)	Hardwood	171	0.8
S-Wood	Norway spruce (*Picea abies*)	Softwood	Non-defibrated	Non-defibrated
S-171	Norway spruce (*Picea abies*)	Softwood	171	0.8
S-180	Norway spruce (*Picea abies*)	Softwood	180	1.0
S-188	Norway spruce (*Picea abies*)	Softwood	188	1.2
S-196	Norway spruce (*Picea abies*)	Softwood	196	1.4
S-202	Norway spruce (*Picea abies*)	Softwood	202	1.6
P-Wood	Scots pine (*Pinus sylvestris*)	Softwood	Non-defibrated	Non-defibrated
P-171	Scots pine (*Pinus sylvestris*)	Softwood	171	0.8
P-188	Scots pine (*Pinus sylvestris*)	Softwood	188	1.2

2.2.2 Changes in wood fiber chemical structure occurring during defibration

Wood is a viscoelastic polymer whose components under the conditions of refiner pulping undergo thermal softening as the temperature exceeds their glass transition point [19,29]. In the softened state, the fibers are fractured and separated. The softening temperature depends on the conditions of defibration, wood moisture content and wood type. Wood moisture content plays an important role in softening. In dry state, native lignin, amorphous cellulose and hemicelluloses show glass transition at 170-220°C. An increase in moisture content lowers the softening temperature up to a certain equilibrium point, which is different for the different wood constituents. Cellulose and hemicelluloses are hydrophilic and absorb more water than native lignin that is hydrophobic. As a result, the hemicelluloses and the amorphous part of cellulose in wet wood are softened at temperatures around 0°C. The thermal softening of wood under the water-saturated conditions used in pulping is therefore mainly determined by the behavior of lignin, the stiffest wood component. Native lignin, containing a few percent of moisture, is softened at approximately 115°C, but the glass transition temperature of dry lignin may exceed 200°C. For optimum separation of wet wood fibers along the middle lamella, a refining temperature of 165-170°C is required.

As can be seen from Fig. 2.4, the location of the fracture zone affects the chemical composition of the resulting pulp fiber surfaces [26]. Fig. 2.6 shows the location of the fracture zone in various refiner pulping processes. The fracture zone varies with the pulping process. In mechanical pulping [27,30] and TMP pulping for paper [27,30,31] it is located in the primary, S_1 and S_2 walls. However, under the conditions of thermomechanical pulping for the fabrication of fiberboard, the fracture zone is shifted to the highly lignified middle lamella [30,31]. A lignin-rich crust, formed as the softened middle lamella lignin solidifies, covers the separated fibers. This is the case also for CTMP pulping [32] in which a chemical pretreatment is applied to soften the wood before its mechanical disintegration.

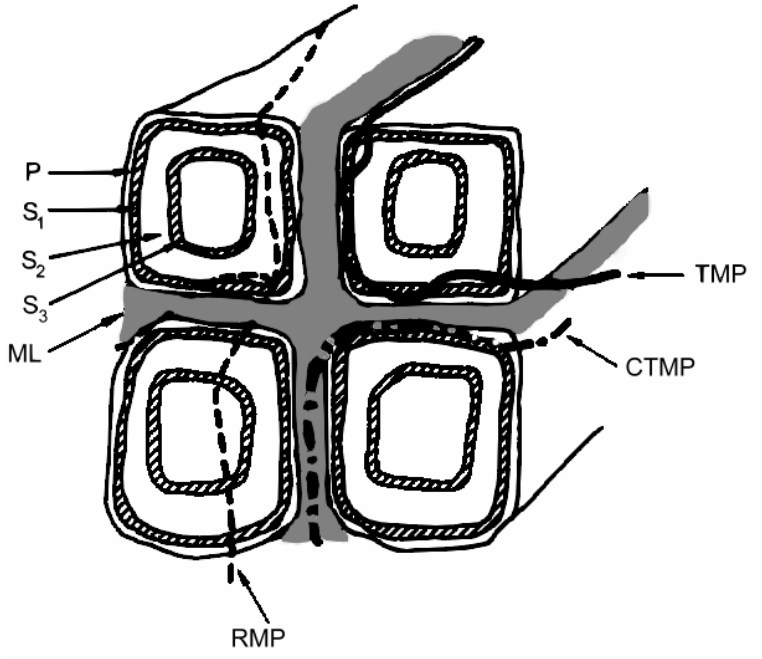


Figure 2.6. Schematic diagram of fracture zones in softwood during refiner mechanical pulping (RMP), thermomechanical pulping (TMP) and chemithermomechanical pulping (CTMP) processes [27].

When lignocellulosic material is subjected to mechanical stress, stress concentrations in parts of the molecule exceed bond strengths and the polymer starts to degrade by cleavage of covalent bonds *via* free radical mechanisms [33]. The mechanoradicals thus generated may be stabilized in the polymeric matrix or decay by coupling or disproportionation reactions. Cellulose is degraded by rupture of glycosidic bonds between the glucose units whereby labile alkoxy-type radicals are formed. Lignin is more severely modified than cellulose and the stability of lignin radicals is higher than that of cellulosic radicals. Homolytic cleavage of lignin carbon-carbon and carbon-oxygen bonds produces carbon and phenoxy radicals, respectively [33,34]. Phenoxy radicals are strongly stabilized by delocalisation of the unpaired electron over the aromatic ring, whereas carbon radicals are more labile. Another factor affecting radical stability is the mobility of a radical within its immediate environment. Steric hindrance or polymer rigidity may restrict its mobility, preventing it from reacting with another radical. The stability of mechanoradicals is heat-dependent, and most of the surviving radicals detected in wood at room temperature are phenoxy radicals.

The depolymerization of wood lignin by free radical mechanisms is to some extent counteracted by radical recombination reactions. Nevertheless, the net effect is usually degradation of the lignin macromolecule. The end products of termination and recombination reactions of lignin radicals include chromophoric quinonoid structures, carbonyl groups and double bonds. Such reactions often proceed *via* peroxy-intermediates formed by combination of a phenoxy radical and oxygen. The phenolic hydroxyl content of lignin is increased by hydrogen atom abstraction reactions of phenoxy radicals.

Also heterolytic cleavage of interunit bonds in wood polymers takes place during defibration. Acetic acid and formic acid, formed from wood carbohydrates [1,14], catalyze the hydrolysis of polysaccharides to water-soluble fragments. Hemicellulose degradation products, particularly pentoses, can be converted into furfural and other furan-type compounds.

Further information on the changes in the chemical structure of wood occurring on high-temperature defibration was gained in I and II, in which the chemical structure of fibers was found to vary depending on the defibration temperature and wood species used. Fiberboard properties (V-VIII) were found to depend on the chemical structure of the fibers, which correlated with the number of radicals formed in the fibers (III, IV).

After defibration, the bulk lignin contents of the fibers were similar to those of the corresponding non-defibrated wood samples (Table 2.2). However, defibration increased the proportion of water-extractable material containing aromatic substances, whose presence turned out to be crucial for the laccase reactivity of wood fibers. Each wood species shows an increase in water extract content as defibration temperature increases (Fig. 2.7). Similar results have been reported for spruce fibers produced at 140-180°C [35] and red maple fibers produced at 148-170°C [36]. At equal temperature, more water extract was formed from hardwoods (II) than from softwoods (I).

The water extracts (I, II) were largely composed of hemicelluloses (Table 2.2), their cellulose content being very low. This result agrees with those reported for *Pinus radiata* MDF pulp [37]. The predominant hemicelluloses in hardwood and softwood water extracts were xylose and mannose, respectively. Aromatic substances, accounting for 16-27% of the hardwood water extracts and 10-13% of the softwood water extracts, were rich in phenolic hydroxyl groups and comprised both substances originally present in wood (Fig. 2.3) and low-molecular weight lignin and oligomeric lignin fragments formed during defibration [38-41]. The results of solid-state ^{13}C cross-polarization/magic angle spinning nuclear magnetic resonance spectroscopy (CP/MAS ^{13}C NMR) studies support this result, showing that the aromatic substances were low in alkyl-aryl β-O-4 ether linkages (I, II). Aside from aromatic substances and carbohydrates, the water extracts contained material probably composed of colloidal lipophilic extractives [39] and ash (inorganic wood constituents) enriched in the water extracts.

Table 2.2. Analytical data on wood fibers and fiber water extracts.

Sample	Unextracted fiber		Fiber water extract		
	Lignin, %	Lignin, % (lit. value) [24]	Lignin and other aromatic substances	Carbohydrates (mainly hemicelluloses)	Phenolic hydroxyl groups, mmol/g
A-171	21.3		27.2	43.2	0.35
A-188	18.8		25.3	47.9	0.33
B-Wood	21.8	22.0	-	-	
B-171	22.3		17.5	56.8	0.48
B-188	21.2		15.8	60.4	0.31
B-196	20.6		18.2	68.2	0.36
E-171	22.4		20.7	42.1	0.96
S-Wood	27.7	27.4	-	-	
S-171	29.9		13.2	65.9	0.25
S-188	28.7		11.7	66.1	0.17
S-196	28.5		11.7	74.6	0.16
S-202	27.2		9.8	71.7	0.14
P-Wood	27.6	27.7	-	-	
P-171	25.3		12.0	66.7	0.25
P-188	27.1		10.2	78.4	0.17

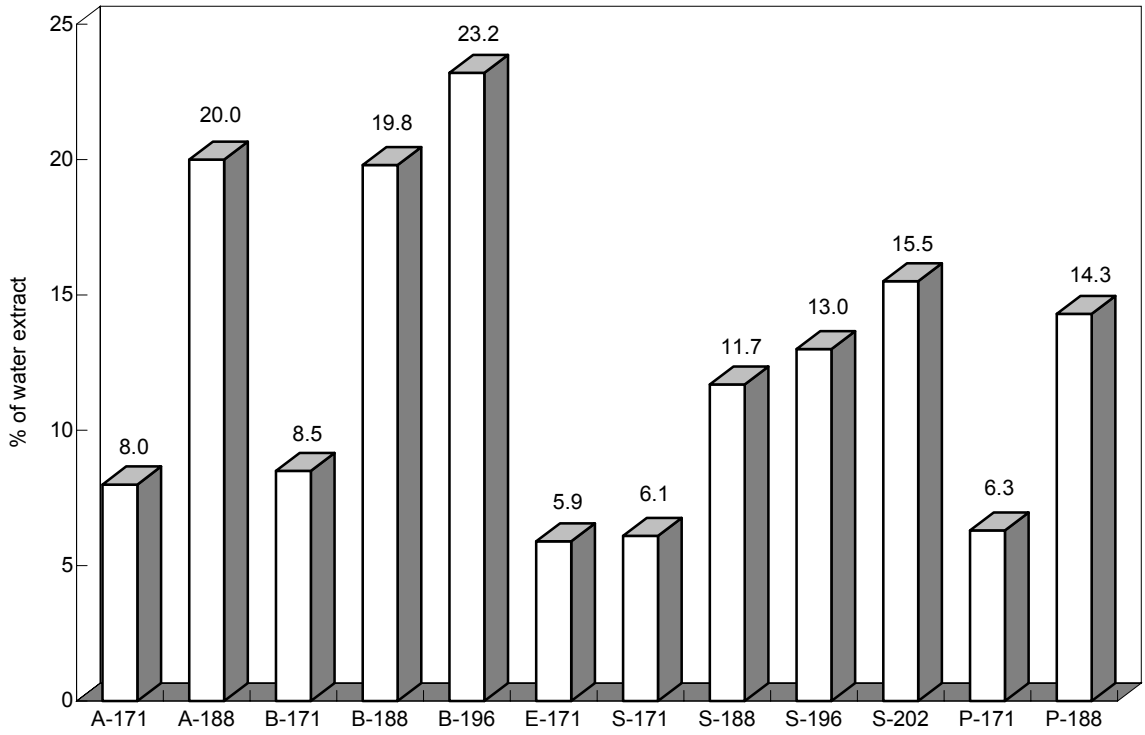


Figure 2.7. Water extract content of wood fibers.

CP/MAS ^{13}C NMR studies [42-45] on unextracted fibers (Figs. 2.8 - 2.11) showed that a progressive cleavage of β-O-4 linkages [46,47] in fiber lignin occurred as defibration temperature increased, accompanied by a concurrent increase in the number of phenolic hydroxyl groups as determined by periodate oxidation [48] (Fig. 2.10). Adams and Ede [49] compared the structure of *Pinus radiata* MDF pulp produced at 170°C to that of *Pinus radiata* wood. The MDF pulp was found to contain only slightly fewer uncondensed β-O-4 linkages than the wood. This is in line with the results on S-171 and P-171 fibers (Fig. 2.10). A comparison of S-171 and P-171 fibers to S-Wood and P-Wood shows that the former contain almost as many β-O-4 linkages as the latter. The phenolic hydroxyl contents of these four samples are in the same range (10-19/100 lignin phenylpropane units) as those of the *Pinus radiata* MDF pulp and wood. For hardwoods, mainly β-O-4 linkages involving an S unit were ruptured, which is consistent with the known higher thermolability of β-O-4 linkages of S units when compared with those of G units [50]. Compared to the non-defibrated wood samples, the MDF fibers were also darker and showed a higher mechanoradical concentration as determined by electron spin resonance (ESR) spectrometry. The g-values of the radicals were consistent with those of phenoxy radicals [33]. The darker color of MDF fibers is explained by an increase in the content of chromophoric structures such as quinones formed *via* radical intermediates. Taken together, the above findings are indicative of depolymerization of lignin occurring by cleavage of β-O-4 linkages. This produces phenoxy radicals, part of which are converted into phenolic hydroxyls and chromophoric structures.

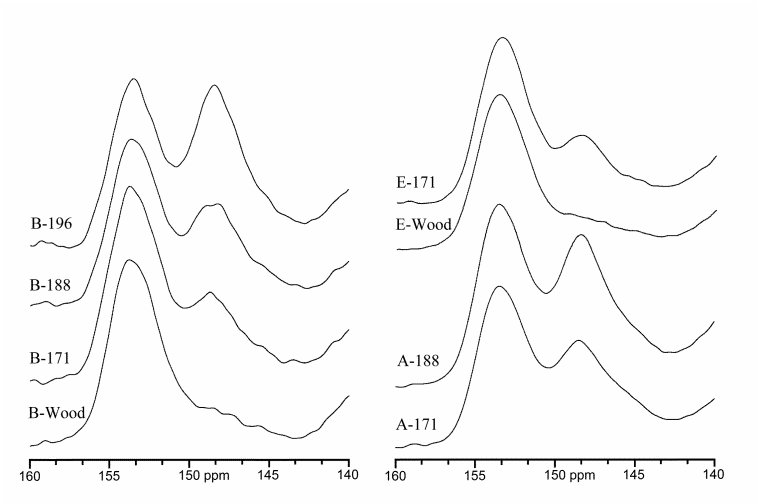


Figure 2.8. Partial CP/MAS ^{13}C NMR spectra of hardwood fibers showing that the ratio of β-O-4 etherified S units (C-3 and C-5 at 153 ppm) to phenolic S units (C-3 and C-5 at 148 ppm) declines as defibration temperature increases.

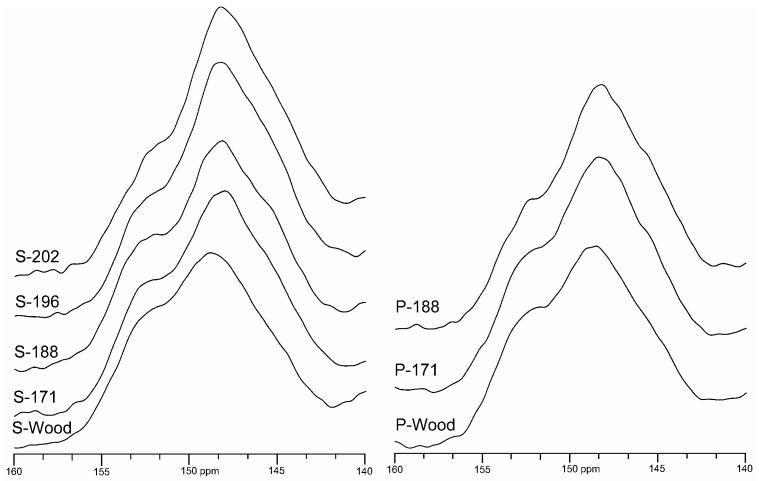


Figure 2.9. Partial CP/MAS ^{13}C NMR spectra of softwood fibers showing that the ratio of β-O-4 etherified G units (C-4 at 153 ppm and C-3 at 148 ppm) to phenolic G units (C-3 at 148 ppm and C-4 at 145 ppm) declines with an increase in defibration temperature.

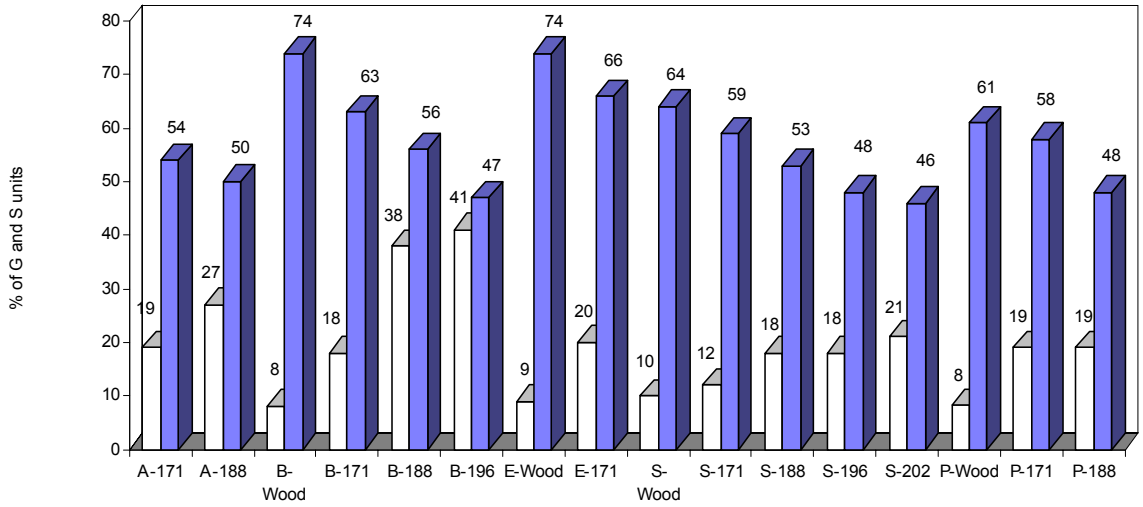


Figure 2.10. Percentages of unetherified (phenolic) G and S units (white bars) and β-O-4 etherified S units (dark bars) in wood fibers.

To study the chemical composition of fiber surfaces, their oxygen/carbon (O/C) atomic ratios were determined (I, II) by means of ESCA (Electron Spectroscopy for Chemical Analysis). The O/C ratio is 0.83 for pure cellulose, about 0.80-0.81 for hemicelluloses and 0.33 for softwood lignin [51], whereas lipophilic extractives such as fatty and resin acids show considerably lower O/C ratios. For oleic acid, a typical wood fatty acid, the O/C ratio is 0.11. For a pure lignocellulosic softwood sample containing, say, 28% lignin and 72% carbohydrates, the bulk O/C ratio is 0.68 if the O/C ratio is 0.33 for lignin and 0.82 for carbohydrates. For a corresponding hardwood sample with a lignin content of 21%, the O/C ratio is 0.72. O/C ratios for extractives-free fibers well below 0.7 thus indicate an enrichment of lignin on the fiber surface, while very low O/C ratios for unextracted fiber surfaces indicate the presence of lipophilic surface extractives.

The O-1s and C-1s spectra of the fibers were recorded to calculate the O/C ratios before and after acetone extraction (Fig. 2.11). Acetone extraction removed the lipophilic surface extractives and the observed increase in O/C ratio indicates that the unextracted fiber surfaces were largely covered by lipophilic extractives. The extractives-free fiber surfaces had O/C ratios of 0.5-0.6, showing that the fiber surfaces were enriched in lignin.

The C-1s spectrum consists of four components representing different types of carbons: C-1 (C-C), C-2 (C-O), C-3 (C=O or O-C-O) and C-4 (O-C=O) [30]. Lipophilic extractives contain mostly unoxidized C-1 carbon and carbohydrates C-2 carbon bound to one oxygen, while lignin contains both C-1 and C-2 carbon. Typical C-1s spectra of unextracted and acetone-extracted fibers are shown in Fig. 2.12. The ratio of C-1 to C-2 declined as a result of extraction. This is consistent with the removal of lipophilic extractives, leaving a pure lignocellulosic surface. As calculated from the C-1s spectra [52,53], the surfaces of unextracted hardwood and softwoods fibers contained 28-93% and 63-80% of lipophilic extractives, respectively, while the lignin contents of the corresponding acetone-extracted fiber surfaces were 43-60% and 46-59%. These results resemble those reported for *Pinus radiata* MDF pulp [54]. The bulk lignin contents of the fibers were appreciably lower (Table 2.2).

The presence of lipophilic surface extractives is explained by deposition of extractives migrating from inner fiber domains to the surface during or after surface formation [12,53]. The paraffin wax added to the fibers during their production to reduce water absorption is another source of lipophilic extractives. The high surface lignin coverage of the extracted fibers is consistent with the mechanism of fiber separation occurring at high temperatures at which thermal softening of lignin takes place and the fiber separation occurs mainly along the lignin-rich middle lamella rather than along the carbohydrate-rich secondary wall (Fig. 2.6). However, no relationship was observed between the surface extractive or lignin content and defibration temperature.

The O/C ratios in the range 0.5-0.6 for extractives-free fiber surfaces (I, II) are similar to those reported for a Norway spruce CTMP after the refining and washing stages (0.53) [32] and for solvent-extracted Norway spruce Asplund fibers refined at 170°C (0.49) [31]. The similarity of the O/C ratios is consistent with the location of the fracture zone in the fiber middle lamella region in CTMP and high-temperature TMP pulping. However, the O/C ratio of 0.66 reported [31] for solvent-extracted Norway spruce TMP refined at 127°C is close to the bulk O/C ratio of extractives-free spruce. This indicates that fiber separation occurs mainly along the secondary wall in TMP pulping for paper.

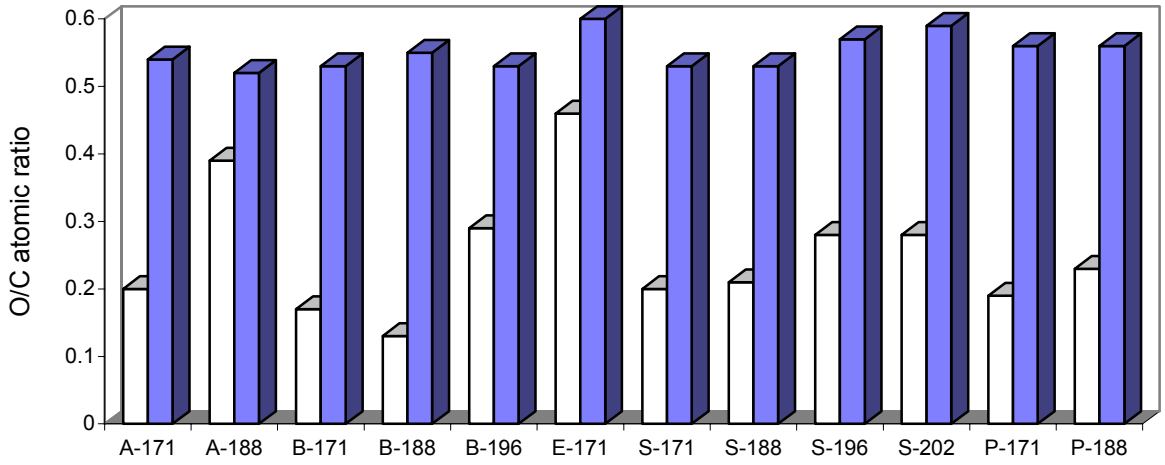


Figure 2.11. O/C atomic ratio of unextracted (white bars) and acetone-extracted (dark bars) wood fiber surfaces as determined by ESCA.

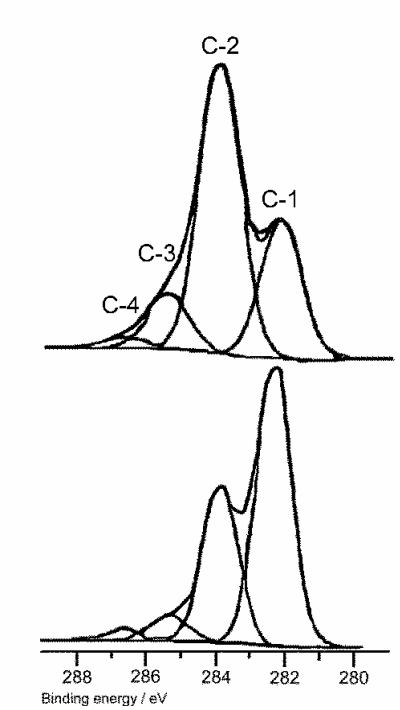


Figure 2.12. Curve-fitted ESCA C-1s signals of unextracted (below) and acetone-extracted (top) B-196 fiber. C-1 = carbon with no bond to oxygen (C-C), C-2 = carbon with one bond to oxygen (C-O), C-3 = carbon with two bonds to oxygen (C=O or O-C-O) and C-4 = carbon with three bonds to oxygen (O-C=O).

3 GENERATION OF RADICALS IN WOOD FIBERS (III, IV, VIII)

3.1 Objectives of the studies on radical formation

In order to elucidate how properties of fiberboards made from laccase-treated fibers (V, VI) depend on the radical concentration of the fibers, various aspects of laccase-catalyzed oxidation of hardwood (III) and softwood (IV) fibers affecting radical formation were studied. The results were interpreted with the aid of data on the fiber chemical structure (I, II). Since the radicals should survive until the oxidatively activated fibers are fabricated into boards, it was also considered worthwhile to study the stability of radicals under different conditions. In the same manner, radical formation by means of $H_2O_2/FeSO_4$ (Fenton's reagent) treatment and γ-irradiation of fibers (VIII) was carried out to shed light on the effect of these activation methods on the properties of corresponding boards (VII, VIII). Answers to the following questions related to radical formation and stability were sought:

- What type of radicals are formed?
- What are the reactive fiber components?
- How does radical formation depend on:
  - wood type?
  - defibration temperature?
  - laccase type and pH in laccase treatment and peroxide dose in $H_2O_2/FeSO_4$ treatment?
- What is the stability of the radicals at room temperature and on heat treatment?
- What is the relationship between radical formation and fiberboard properties?

3.2 Methods used in radical formation studies

3.2.1 Electron spin resonance (ESR) spectroscopy

Electron spin resonance (ESR) spectroscopy [55], also called electron paramagnetic resonance (EPR) spectroscopy, is based on the behavior of the unpaired electron of a radical when the radical is subjected to a magnetic field. The unpaired electron is paramagnetic as it possesses a magnetic moment μ, which is given by

$$\mu = -g\beta S, \quad (1)$$

where g is the spectroscopic splitting factor (g-value), β (= 9.2740 x 10^{-21} erg/gauss) is the Bohr magneton and S the spin vector of the electron. In a magnetic field, the electron is in one of two possible energy states, depending of whether the magnetic moment is oriented with or against the applied field. The separation of the energy states, ΔE, increases with the applied field H_0:

$$\Delta E = g\beta H_0 \quad (2)$$

A certain frequency of electromagnetic radiation induces transitions between these energy states, satisfying the following equation:

(3) $$h\nu = g\beta H_0,$$

where h is Planck's constant (6.626×10^{-27} erg s).

The experiment is carried out at a fixed frequency and the intensity of the static magnetic field is varied to find the resonance condition. An X-band ESR spectrometer such as the one used in this thesis operates with microwave fields at 9.5 GHz and its resonant field is near 3400 gauss. ESR experiments are performed by irradiating a sample in a magnetic field with microwave radiation of a constant frequency while H_0 is varied. Once the conditions of eq. 3 are satisfied, energy is absorbed and detected electronically. The spectrometer then produces the first derivative of the absorption spectrum. Solid-state and solution first derivative ESR spectra are exemplified in Fig. 3.2.

The spectral parameters from which information can be obtained include the g-value, intensity and hyperfine structure. The g-value is characteristic of the electronic environment of a molecule with unpaired electrons and can help to identify a ESR signal. g-Values reported for phenoxy radicals in lignocellulosic materials are typically in the range 2.002-2.004. However, the usefulness of the g-value is rather limited for the complex lignocellulosic samples. The intensity of a derivative signal is proportional to the number of unpaired spins in the sample and can be measured by integration. Radical concentrations may be given as absolute spin concentrations, obtained by a comparison to standards of known radical concentration, or as relative values for similar samples recorded under identical conditions. The hyperfine splitting of spectral peaks is due to the interaction of the electron spins and nuclear spins, "I". There are 2I + 1 orientations of a nuclear spin each with a different energy, and the unpaired electron spin recognizes them whereby the ESR spectrum is split into 2I + 1 lines of equal intensity. The magnitude of the splitting (in gauss) is called the hyperfine splitting constant. The ESR spectrum consists of 2nI + 1 lines, where n is the number of equivalent nuclei. The binomial coefficients in the expansion $(1 + x)^n$ provide the intensities of the lines. Further line splitting may occur if there is more than one set of equivalent nuclei. For solid-state lignocellulosic samples, the ESR spectra consist generally of one singlet signal showing no hyperfine structure. The information derived from these spectra consists mainly of the peak intensities, which allow the radicals to be quantified but not identified.

3.2.2 Oxygen consumption measurements

Radical formation on laccase treatment is a measure of O_2 consumption (eq. 4). For laccase treatments in aqueous solution, the O_2 uptake can be monitored with an O_2 electrode inserted in the reaction vessel. The starting O_2 concentration is recorded after which the measurement is initiated by adding laccase. The O_2 concentration is recorded at suitable intervals. Wood fibers show an initial burst in O_2 consumption, corresponding to the oxidation of laccase-accessible phenolic hydroxyl groups. The slower linear consumption that follows is reported to be due to long-range electron-transfer from phenolic hydroxyls in the inner fiber domains to phenoxy radicals on the fiber surface [9,56]. After proton abstraction from the solvent, the laccase-accessible phenolic hydroxyls are regenerated and then reoxidized. According to this cyclic mechanism, radicals are generated also in parts of fiber not directly accessible to laccase. For the purpose of this thesis, however, these radicals are unimportant as only those radicals located on the fiber surface may contribute to interfiber bonding when laccase-treated

fibers are pressed into boards. The fiber O_2 uptake due to the oxidation of the directly accessible phenolic hydroxyls is estimated by extrapolation of the linear region of the O_2 uptake curve to the concentration axis. Typical O_2 uptake curves are shown in Fig. 3.1.

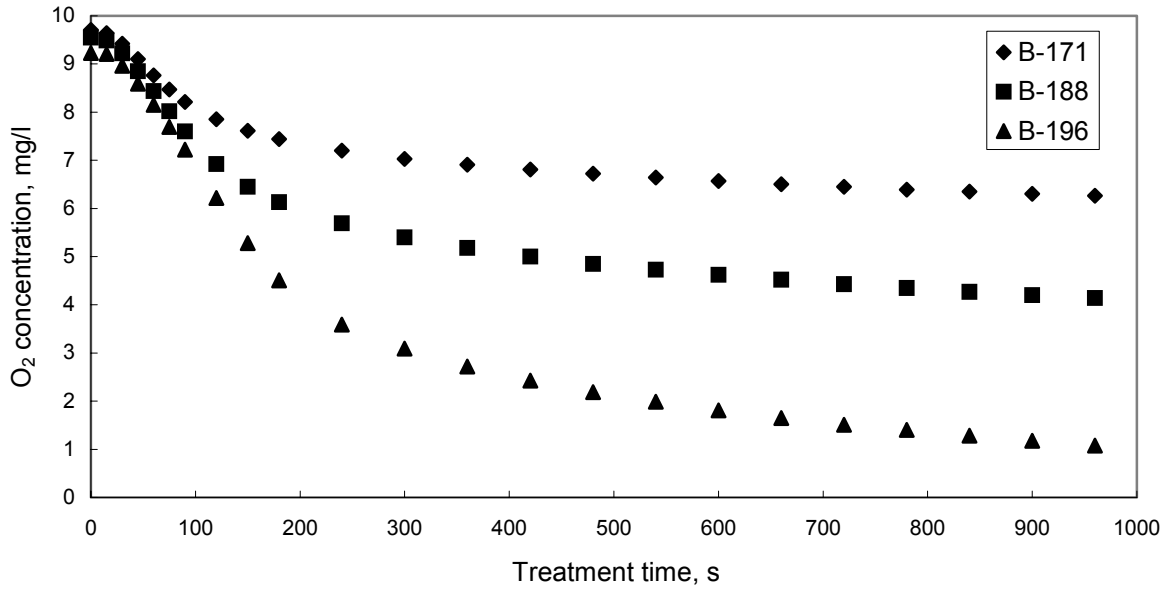


Figure 3.1. O_2 uptake of birch fibers on treatment with MT as a function of time.

3.3 Laccases and their action on phenolic substrates

Laccase enzymes are glycoproteins found in nature in white-rot fungi, trees and other higher plants, bacteria and insects [57,58]. These multicopper oxidases are able to catalyze the one-electron oxidation by molecular oxygen (O_2) of phenols and polyphenols such as lignin to phenoxy radicals while O_2 is reduced to water. The laccase-catalyzed oxidation of phenols may be written as:

(4) $$4\ PhOH + O_2 \xrightleftharpoons{\text{Laccase}} 4\ PhO^{\bullet} + 2\ H_2O$$

The laccases used in the work described in this thesis are extracellular fungal laccases including those from *Trametes hirsuta* (TH) and *Myceliophthora thermophila* (MT), which were used in most studies. Some work was also conducted with laccases from *Trametes villosa, Coriolus versicolor* (CV) and other fungi. In nature, laccases are involved in lignin biodegradation occurring by cleavage of ether bonds linking phenylpropane units and side-chain carbon-carbon bonds. Laccase alone is unable to catalyze the oxidation of etherified (non-phenolic) aromatic units, acting only on phenolic lignin moieties. Since the phenoxy radicals may recombine, the initial depolymerization of lignin may be followed by repolymerization.

The catalytic effect of laccases depends on the pH of the treatment medium [57]. The pH optima vary between different laccases and depend also on the substrate. The optimum pH of fungal laccases is usually in the range 3-7. The rate of laccase-catalyzed oxidation of phenolic

substrates depends also on the chemical and physical properties of the substrate. Substrate reactivity is increased by electron-donating functional groups that in the case of lignin (Fig. 2.2) and phenolic extractives (Fig. 2.3) are phenolic hydroxyl and methoxyl groups attached to the aromatic ring [58,59]. The oxidation rates of phenolic lignin units (Fig. 2.1) thus increase in the order H < G < S. By contrast, electron-attracting carbonyl substituents, usually in the α-carbon of the phenylpropane side chain, decrease the oxidation rate. Because of the large size of the laccase molecule (typically 40-70 kDa), substrate accessibility is often an important factor affecting its reactivity. With simple phenols, the steric hindrance caused by substituents on the aromatic rings is negligible. However, in the case of lignin the spatial structure of the lignin polymer becomes an important factor affecting its laccase accessibility. As for the lignocellulosic fibers of this thesis, only phenolic hydroxyls located on the fiber surfaces or those of dissolved or colloidal low-molecular weight phenolic compounds in the reaction medium are directly accessible to the bulky laccase molecule.

3.4 Generation of radicals in fibers by laccase treatment (III, IV)

3.4.1 Effect of laccase treatment time, laccase type and pH on radical formation

To study the effect of treatment time on radical formation, fibers were treated with laccase in aqueous suspension for 10-120 min after which they were freeze-dried and the radicals quantified by ESR spectroscopy (III, IV). As the maximum radical concentration was obtained after about 1 h of treatment, the 1-h treatment time was used in other ESR experiments involving quantification of radicals. Studies on the effects of laccase type and pH on radical formation (III) revealed distinct differences in the ability of laccases to generate radicals and showed that they had different pH optima.

3.4.2 Identification of radicals formed in fibers on laccase treatment

Solid-state first derivative ESR spectra of laccase-treated fibers (Fig. 3.2) show no hyperfine structure and the analytical information that can be obtained from them consists of peak intensities and g-values. The g-values of all solid-state ESR spectra of laccase-treated fibers were consistent with phenoxy radicals. To find out more about the nature of the radicals that are produced on laccase treatment of hardwoods, the water extract of B-196 fiber was treated with laccase and solution ESR spectra were run during the treatment. One of the spectra (Fig. 3.2), assigned to S radicals, has a 7-peak framework arising from strong coupling of S radicals to the six methoxyl protons of S units. The coupling constant is about 1.5 gauss. Treatment with phenol-oxidizing enzymes of S-type lignin model compounds [60] or the aqueous suspension liquid of hardwood (beech) fibers [6] has yielded similar spectra. While it is evident that S radicals were formed as a result of the laccase treatments, the fate of phenolic G units remains unclear. The fact that G radicals were not detected could be due to the different redox potentials of S and G units as a result of which phenolic G units are only oxidized after the oxidation of S units is complete. An alternative explanation is that if G radicals were formed, they escaped detection because of rapid recombination reactions involving G units unsubstituted at the C-5 position [33,61,62].

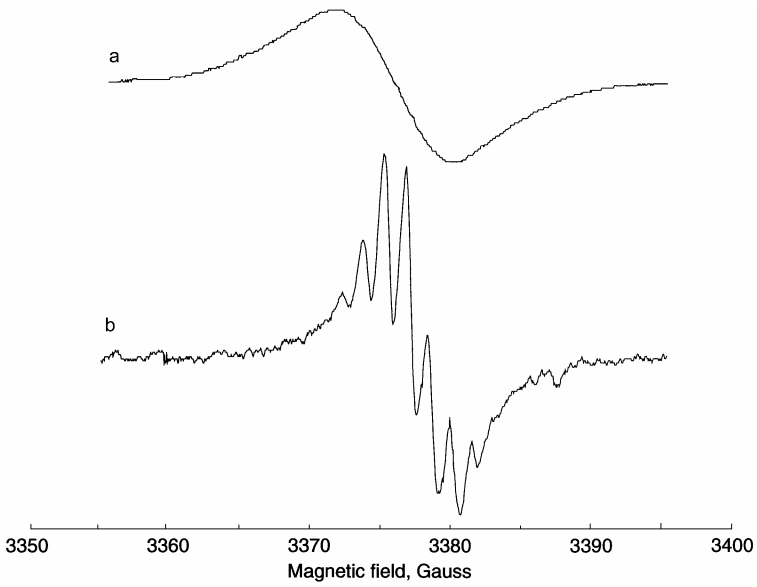


Figure 3.2. First-derivative ESR-spectra of laccase-treated samples. a) Typical solid-state spectrum (B-196 fibers, MT). b) Solution spectrum of the water extract from untreated B-196 fibers run after 5 min from the start of treatment with MT.

3.4.3 Effect of wood type and defibration temperature on radical formation

The effect of wood type and species as well as defibration temperature on radical formation as studied by ESR-spectroscopy is illustrated in Fig. 3.3, showing the radical concentration of untreated and laccase-treated fibers (III, IV). The absolute spin concentrations range from 0.1 x 10^{17} to 9.6 x 10^{17} spins/g of fiber as measured against DPPH (1.5 x 10^{21} spins/g) in toluene [V, VI]. While radicals were formed in all fibers, the radical concentration of hardwood fibers in general is much higher than that of softwood fibers. The enhanced reactivity resulting from defibration is seen from the low radical content of B-Wood as compared to those of the other birch fibers. This can be attributed to an increase in laccase-accessible material rich in phenolic hydroxyl groups formed during defibration. Moreover, a comparison of fibers representing the same species also shows that radical formation increases with an increase in defibration temperature. For fibers produced at equal temperature, approximately equal numbers of radicals are formed in birch and eucalypt fibers and spruce and pine fibers, respectively. However, the aspen fibers have a lower radical content than the corresponding birch and eucalypt fibers.

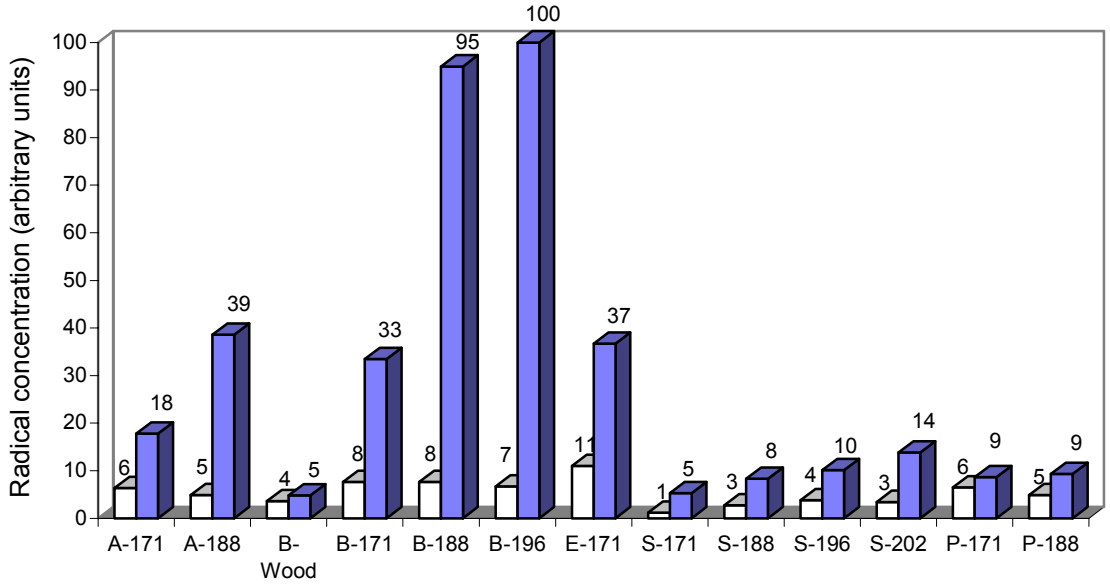


Figure 3.3. Relative radical concentrations of fibers before (white bars) and after (dark bars) treatment with MT as determined by ESR spectrometry (III, IV). The absolute spin concentrations range from 0.1 x 10^{17} to 9.6 x 10^{17} spins/g (V, VI).

The results of studies on radical formation by means of O_2 uptake measurements (Fig. 3.4) using TH were mostly in good agreement with the results of the ESR measurements, supporting the earlier conclusions regarding the effect of wood type and defibration temperature on radical formation. The O_2 uptake of hardwood fibers was somewhat higher with MT but the relative reactivities of the samples were as with the TH. The results obtained with the two methods differed mainly in that the aspen fibers consumed about as much O_2 as the birch and eucalypt fibers produced at equal temperature, whereas the radical content of the laccase-treated aspen fibers was lower than that of other hardwood fibers from defibration at equal temperature. The aspen radicals thus decayed more readily than those of other hardwoods.

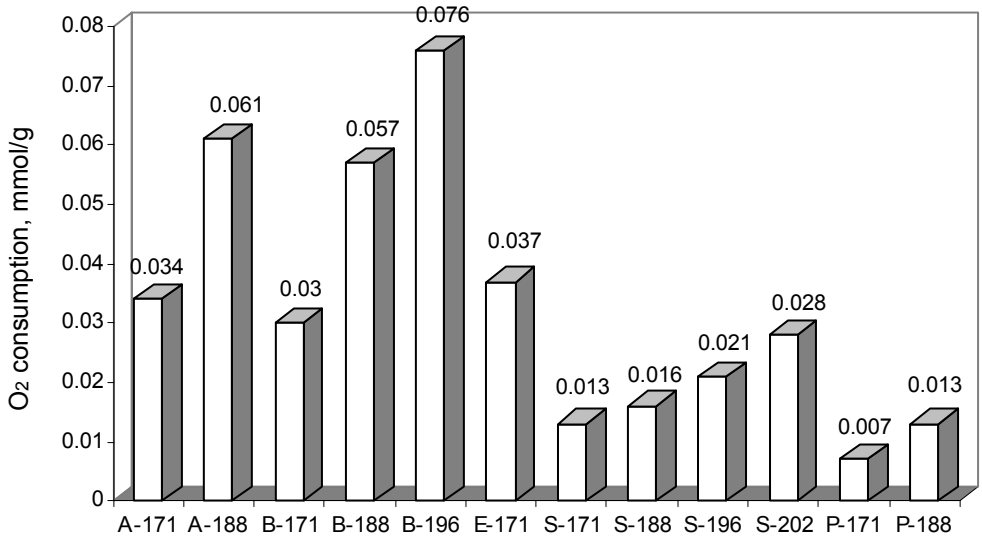


Figure 3.4. O_2 consumption of fibers on treatment with TH.

3.4.4 Identification of reactive fiber components

As the fiber surface lignin contents are similar and there is no relationship between the amount of lipophilic surface extractives and laccase reactivity, the different laccase reactivities of the fibers cannot be ascribed to different chemical compositions of the fiber surfaces. To account for the different laccase reactivities of the fibers, they were fractionated (I, II) into two fractions by water extraction: water extract and water-extracted fibers. The fractions were then treated with laccase to determine their relative reactivities. Since radicals formed in water extracts by laccase treatment decayed too rapidly for quantification by ESR spectrometry, the reactivities of the water extracts were studied by O_2 consumption measurements only. According to the ESR studies on unextracted and water-extracted fibers (Fig. 3.5) water extraction removed most of the material reactive toward laccase-catalyzed oxidation from the fibers. This agrees with the results of O_2 consumption measurements (Fig. 3.6), showing that the water extracts were far more reactive toward laccase than the unextracted fibers, which were, however, appreciably more reactive than the water-extracted fibers. These results are consistent with those of earlier studies on radical formation in beech [6,9] and spruce [9] fibers on laccase treatment. Mechanical properties of spruce fiberboards deteriorated when the fibers were water extracted before they were fabricated into boards [9].

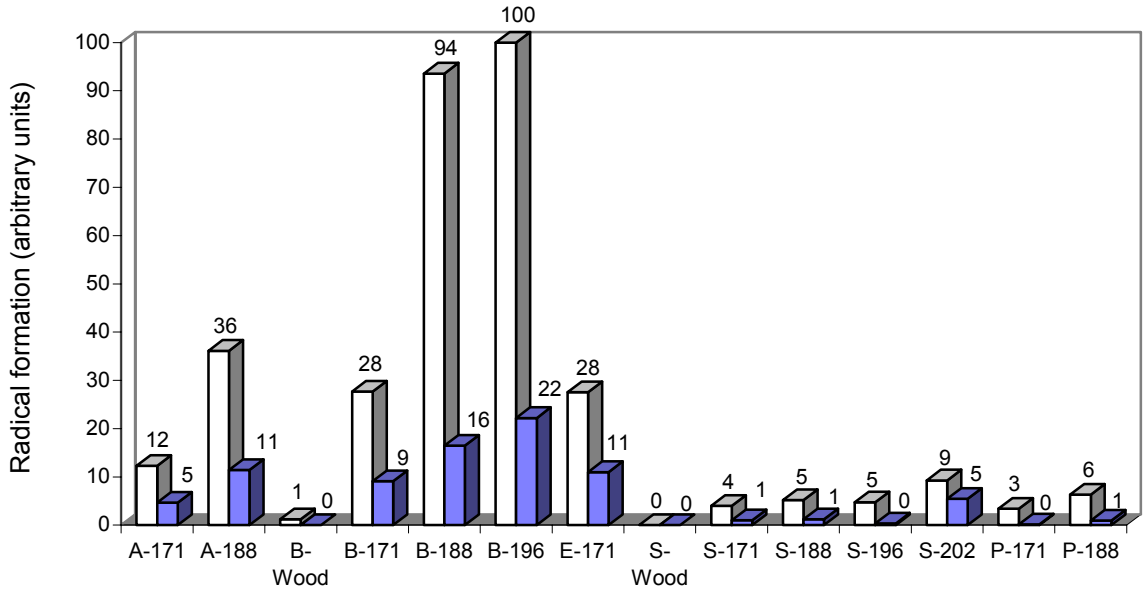


Figure 3.5. Radical formation in unextracted (white bars) and water-extracted (dark bars) wood fibers on laccase treatment as determined by ESR spectrometry. The values represent the difference between the radical concentrations of the untreated and laccase-treated fibers. MT was used for hardwood and TH for softwood samples.

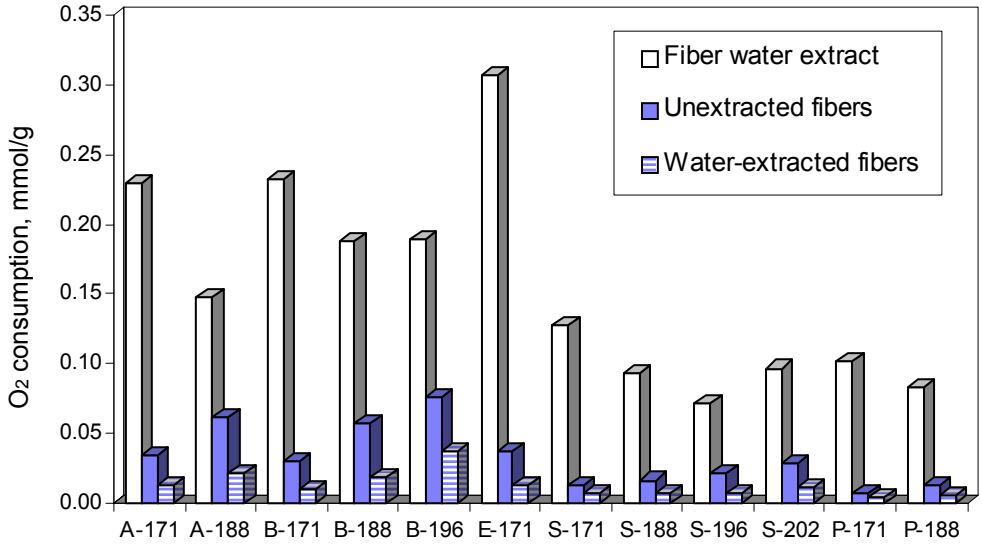


Figure 3.6. O_2 consumption of unextracted wood fibers, fiber water extracts, and water-extracted fibers on treatment with TH.

The above results demonstrate the importance of the water-extractable fiber fraction for radical formation by laccase treatment. The water extracts were responsible for 50-80% of the fiber O_2 uptake while composing only 6-23% of the fibers. Their reactive components are probably lignin fragments formed during defibration [6,9] and native phenolic wood extractives [38,39,41] (Fig. 2.3) enriched in the water extracts and highly accessible to laccase. According to CP/MAS ^{13}C NMR spectra of the water extract from B-171 fibers run before and after treatment with MT (Fig. 3.7), the only change in the chemical structure of the water extract resulting from the laccase treatment is the virtual disappearance of the peak assigned mainly to phenolic syringyl units at 148 ppm, indicating that these units were extensively oxidized to phenoxy radicals. A similar change of a lesser magnitude took place on treatment of unextracted B-196 fibers (Fig. 3.7).

The reactivity of the water-extracted fibers, increasing as defibration temperature increases, can be explained by a presence of laccase-accessible phenolic hydroxyls on the fiber surfaces. As mentioned earlier, the water extract content of the fibers increased with an increase in defibration temperature (Fig. 2.7), while fibers of the same wood species had similar lignin contents (I, II). The increased amount of reactive lignin and other phenolic substances in fiber water extracts and on the fiber surface thus accounts for the increase in reactivity of unextracted fibers with an increase in defibration temperature.

The higher reactivity of hardwood fibers when compared with softwood fibers can be partly explained by the fact that more water extract was formed at a given defibration temperature from hardwood than from softwood. In addition, phenolic substances (and phenolic hydroxyl groups) were more abundant in water extracts of hardwoods than in those of softwoods (I, II). Hardwood water extracts were also more reactive in terms of O_2 uptake than softwood water extracts (Fig. 3.6). Moreover, water-extracted hardwood fibers were more reactive than water-extracted softwood fibers. A higher reactivity of the mixed G:S lignin present in hardwoods when compared with the G lignin of softwoods may contribute to the reactivity difference between hardwood and softwood fibers.

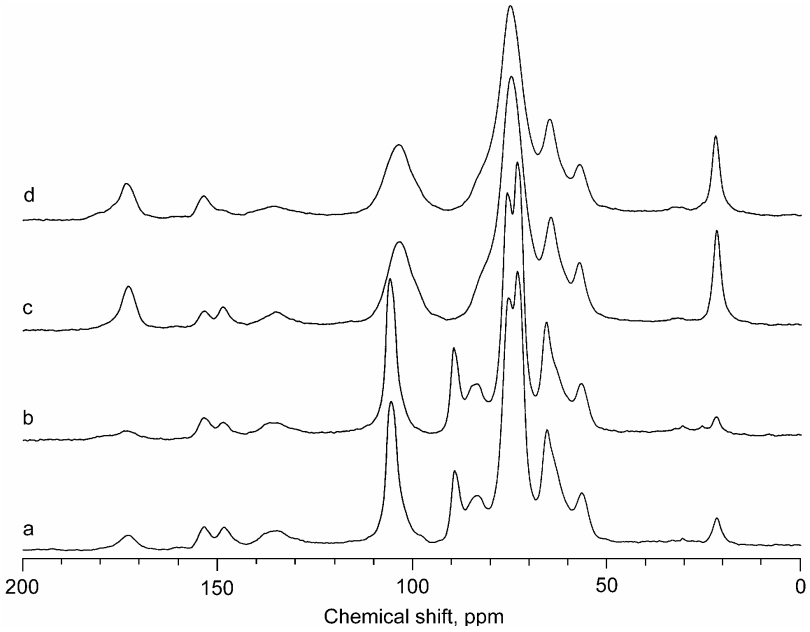


Figure 3.7. CP/MAS ^{13}C NMR spectra of a) untreated B-196 fibers, b) B-196 fibers treated with MT, c) untreated water extract from B-171 fibers, and d) water extract from B-171 fibers treated with MT. The peak at 148 ppm is assigned mainly to the C-3 and C-5 of phenolic S units.

3.4.5 Decay of radicals in laccase-treated wood fibers

The stability of radicals in laccase-treated B-188 fibers was studied by aging the fibers at room temperature and *in vacuo* (Fig. 3.8). It is seen that most of the radicals survived for several days at room temperature and even longer *in vacuo*. The higher radical stability *in vacuo* can be attributed to lower aging temperature or to absence of air oxygen. Oxygen may react with phenoxy radicals to form relatively unstable organic peroxides decaying to non-radical products. The plot of aging time vs reciprocal radical concentration indicates, however, that the rate of radical decay is governed by second-order kinetics. The radicals thus decayed by reacting with each other, probably by coupling or disproportionation reactions.

The stability of radicals in laccase-treated fibers varied considerably between fibers (III, IV). Radical decay in B-196 and S-202 fibers proceeds slowly at 50°C and much faster at 100°C (Fig. 3.9). After 40 min of heat treatment at 100°C, the surviving radicals in S-202 were stable within the experimental time frame. Radicals in B-196 fibers decayed at a steady rate for 60 min. For both fibers, radical decay followed second-order kinetics (Fig. 3.10) as was the case also at room temperature, indicating that heat treatment accelerates the rate of radical decay but may not alter the decay mechanism.

The above results indicate that after radicals have been generated in the fibers for the fabrication of fiberboard, the fibers should be maintained at low temperature to preserve the radicals until pressing into boards in order to maximize the extent of interfiber bonding.

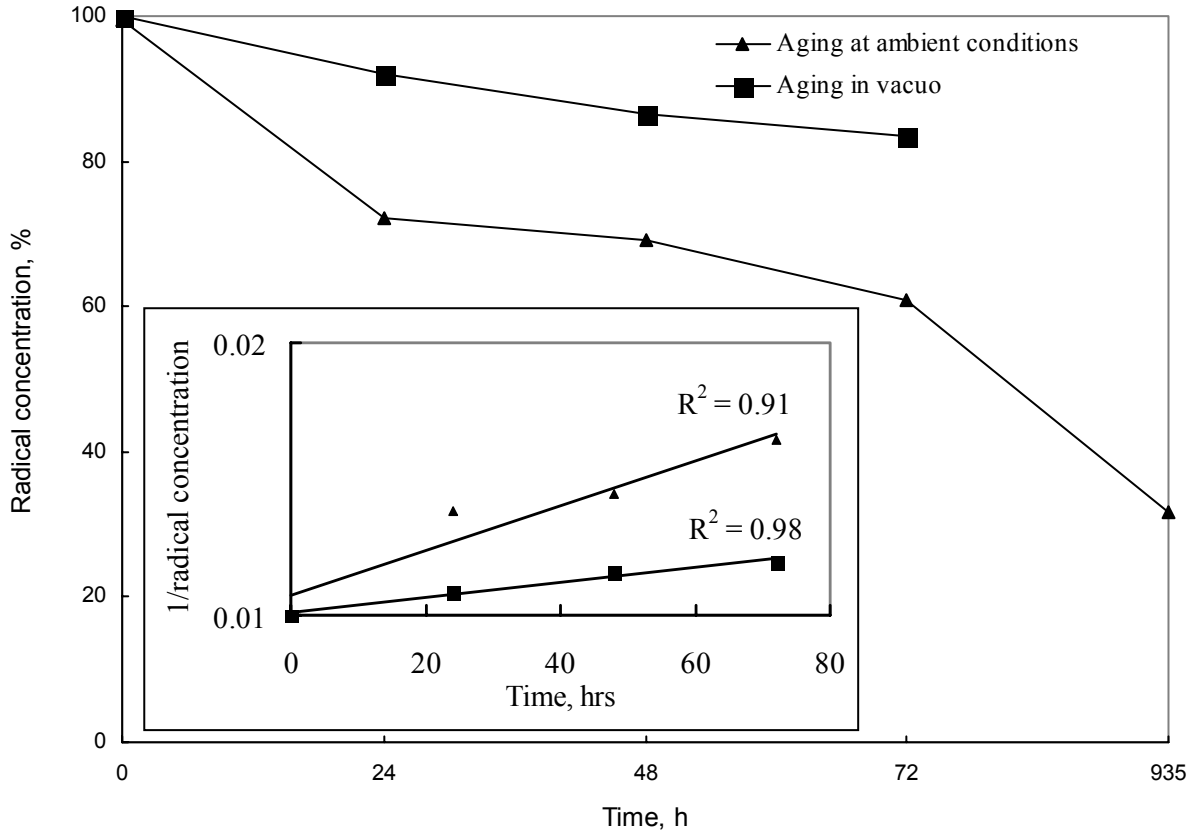


Figure 3.8. Stability at ambient conditions and *in vacuo* of radicals in B-188 fibers treated with MT. Insert: reciprocal radical concentration as a function of time indicating that radical decay follows second-order kinetics.

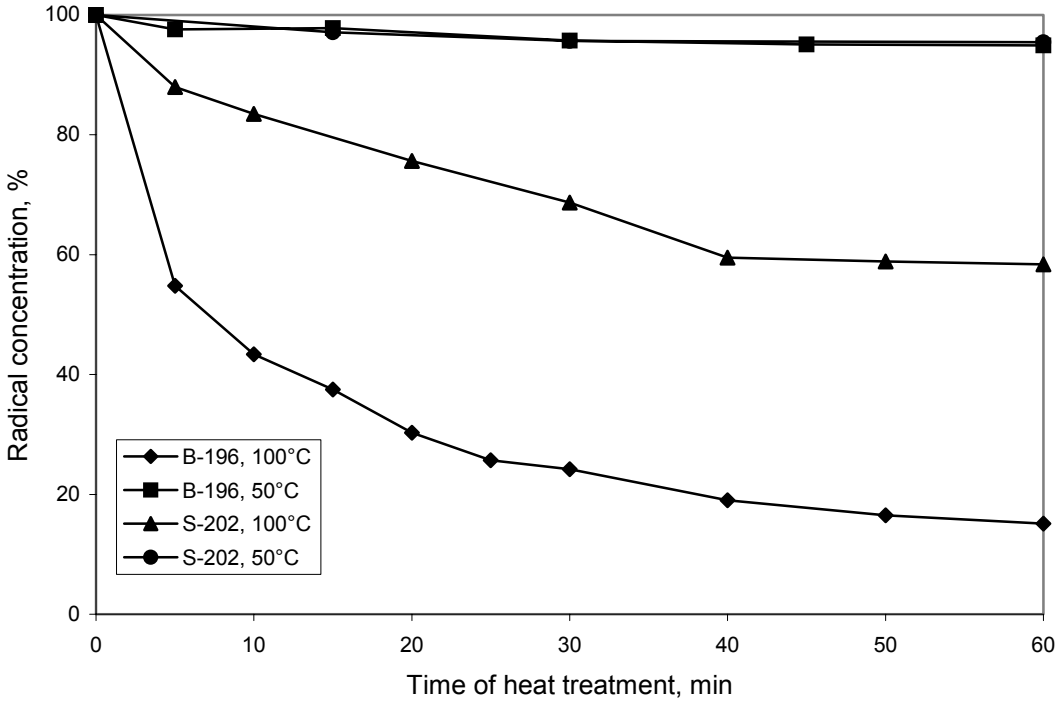


Figure 3.9. Decay of radicals in MT-treated wood fibers on heat treatment.

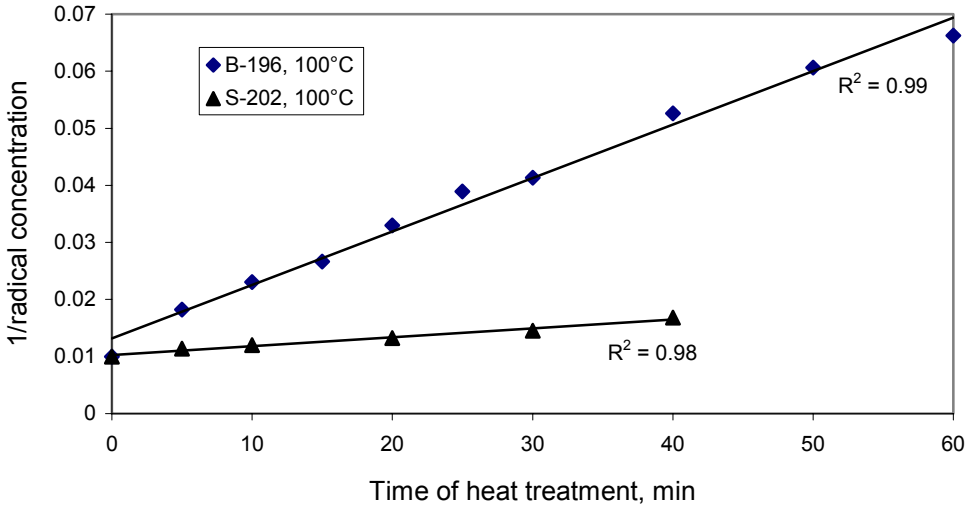


Figure 3.10. Reciprocal radical concentration of MT-treated wood fibers vs time of heat treatment.

3.5 Generation of radicals in wood fibers with Fenton's reagent (VIII)

3.5.1 Fenton's reagent

The solution of hydrogen peroxide and dissolved iron (e.g. ferrous ions) is called Fenton's reagent. Ferrous ions catalyze the decomposition of hydrogen peroxide to hydroxyl radicals ($HO^{\cdot}$) while being oxidized to ferric ions according to eq. 5:

(5) $$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + HO^{\bullet}$$

The decomposition reaction is catalytic as the ferric ions are reduced back to ferrous ions by e.g. superoxide anion or other radicals.

3.5.2 Reactions of hydroxyl radicals with lignin

Hydroxyl radicals are powerful electrophiles, which in contact with wood fibers preferably attack the electron-rich lignin [15] although carbohydrates are affected as well. In addition to radicals, peroxide decomposition products generate carboxyl and other functional groups in wood lignin and carbohydrates [19]. Lignin model compound studies have shown that hydroxyl radicals dehydrogenate phenolic hydroxyl groups whereby phenoxy radicals are formed [63]. Other reactions between lignin and hydroxyl radicals include aromatic ring hydroxylation and demethoxylation. Simplified reactions of lignin and hydroxyl radicals are illustrated in Fig. 3.11. Phenoxy radicals are formed by hydrogen abstraction from phenolic hydroxyl groups and by demethoxylation of phenolic or non-phenolic aromatic units. The hydrogen abstraction may involve phenolic hydroxyls either originally present or formed by aromatic ring hydroxylation.

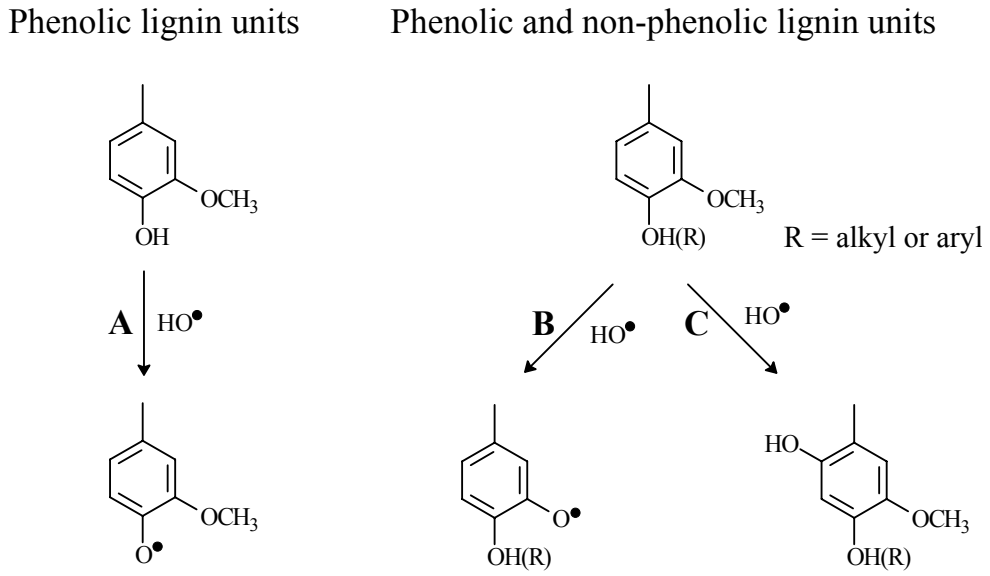


Figure 3.11. Reactions between lignin aromatic units and hydroxyl radicals [63]. A: formation of phenoxy radicals by hydrogen abstraction from phenolic lignin units; B: formation of phenoxy radicals by demethoxylation; C: aromatic ring hydroxylation. The first step of the reactions is the addition of hydroxyl radical to the aromatic unit.

3.5.3 Effect of peroxide dose on radical formation

An increase in peroxide dose from 0.3% to 4.8% increased radical formation by 25% in such a way that radical formation was proportional to the square root of peroxide dose (Fig. 3.12). Based on this result, 3% was considered to be an appropriate peroxide dose for other studies involving tretment of fibers with Fenton's reagent in water suspension.

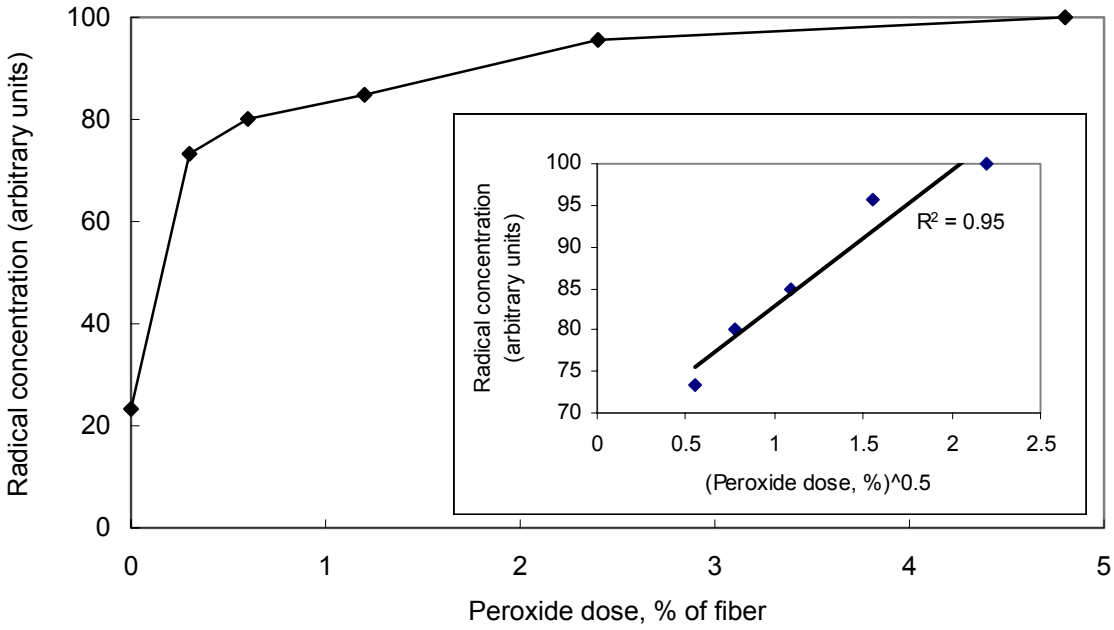


Figure 3.12. Effect of H_2O_2 dose on radical concentration of B-188 fibers on treatment with Fenton's reagent (VIII). Insert: Radical concentration of treated fibers vs the square root of peroxide dose.

3.5.4 Effect of wood type and defibration temperature on radical formation

Radical contents of wood fibers treated with Fenton's reagent (3% H_2O_2, 0.3% $FeSO_4$) are shown in Fig. 3.13 (VIII). The absolute numbers of radicals formed (VII), measured against DPPH (1.5×10^{21} spins/g), ranged from 0.5×10^{17} to 1.9×10^{17} spins/g. Oniki and Takahama [64] report that various wood and nonwood dioxane lignins treated with Fenton's reagent ($H_2O_2/K_3[Fe(CN)_6]$) or $K_3[Fe(CN)_6]$ contained $0.3 - 21 \times 10^{17}$ spins/g. The results in VIII differ from those obtained with laccase treatments (VI, VII) in that for hardwood fibers there is no clear relationship between radical formation and defibration pressure. In addition, the large difference in radical formation between hardwood and softwood fibers observed with laccase treatments is not seen for the Fenton's reagent treatments. The fact that phenolic hydroxyls need not be present in untreated fibers for the formation of phenoxy radicals because of the aromatic hydroxylation reactions occurring during Fenton's reagent treatment may account for this. Moreover, the difference between the reactivity of S and G units toward hydroxyl radicals, if any, is probably small because of the high reactivity of hydroxyl radicals.

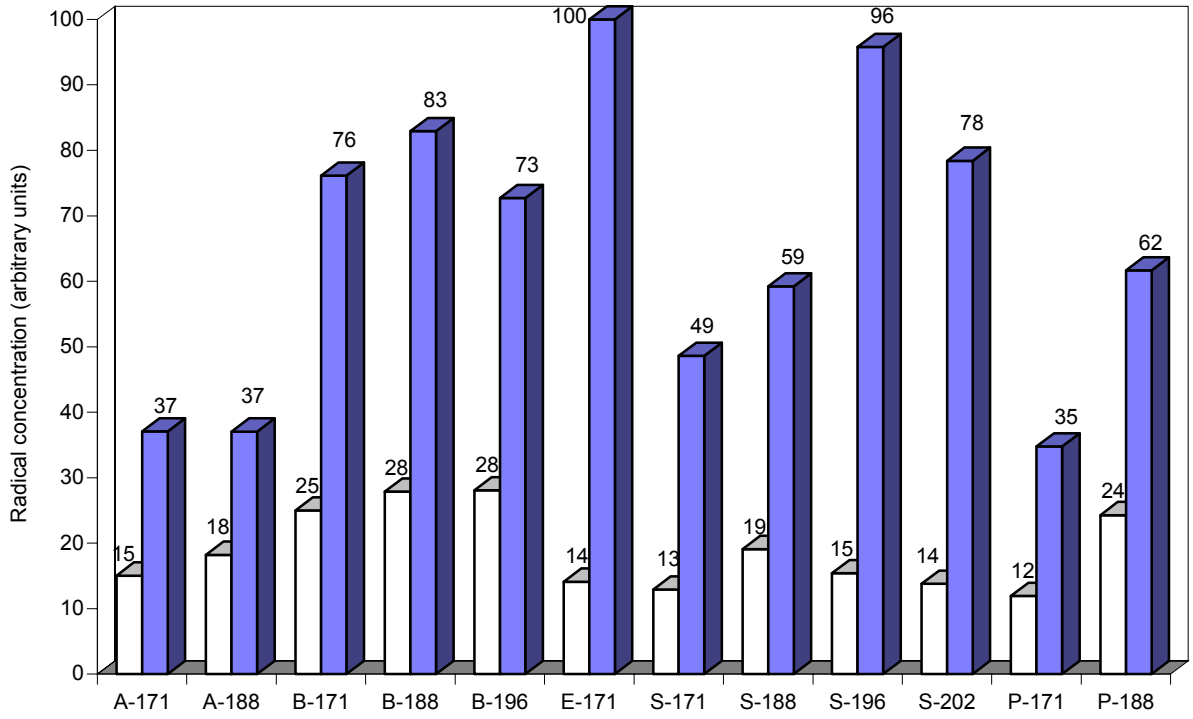


Figure 3.13. Relative radical contents of wood fibers before (white bars) and after (dark bars) treatment with Fenton's reagent (VIII). The absolute number of radicals formed ranged from 0.5 x 10^{17} to 1.9 x 10^{17} spins/g (VII).

3.6 Generation of radicals in wood fibers by γ-irradiation

γ-Irradiation of wood causes oxidative changes in the lignin and carbohydrate components resulting in depolymerization and condensation reactions [20-23,65] which are associated with a generation and decay of various types of carbon and oxygen radicals [20,21]. Only a minor part of the radicals generated are stable at ambient conditions as most radicals undergo recombination reactions. About 10% of the radicals detected in irradiated beech milled wood lignin at 77K survived at room temperature [20]. Radicals generated in cellulose are partly stable in dry pulp at room temperature but are decayed rapidly in wet pulp or when the temperature increases [20,21]. In the light of these results it seems that by means of γ-irradiation it may be possible to generate enough radicals stable at room temperature on the surfaces of air-dry wood fibers to achieve interfiber bonding when irradiated fibers are pressed into fiberboards. Fig. 3.14 shows the absolute radical content of wood fibers or fiber water extracts before and after γ-irradiation measured against DPPH containing 1.5 x 10^{21} spins/g. The values are based on the relative radical contents published in VIII. The radical contents of the irradiated fibers (11 – 78 x 10^{17} spins/g) are much higher than those of fibers treated with laccase (0.5 – 9.6 x 10^{17} spins/g) or Fenton's reagent (0.5 – 2.9 x 10^{17} spins/g). This is explained by the ability of γ-rays to penetrate the fibers, resulting in radical formation in all morphological regions of fibers.

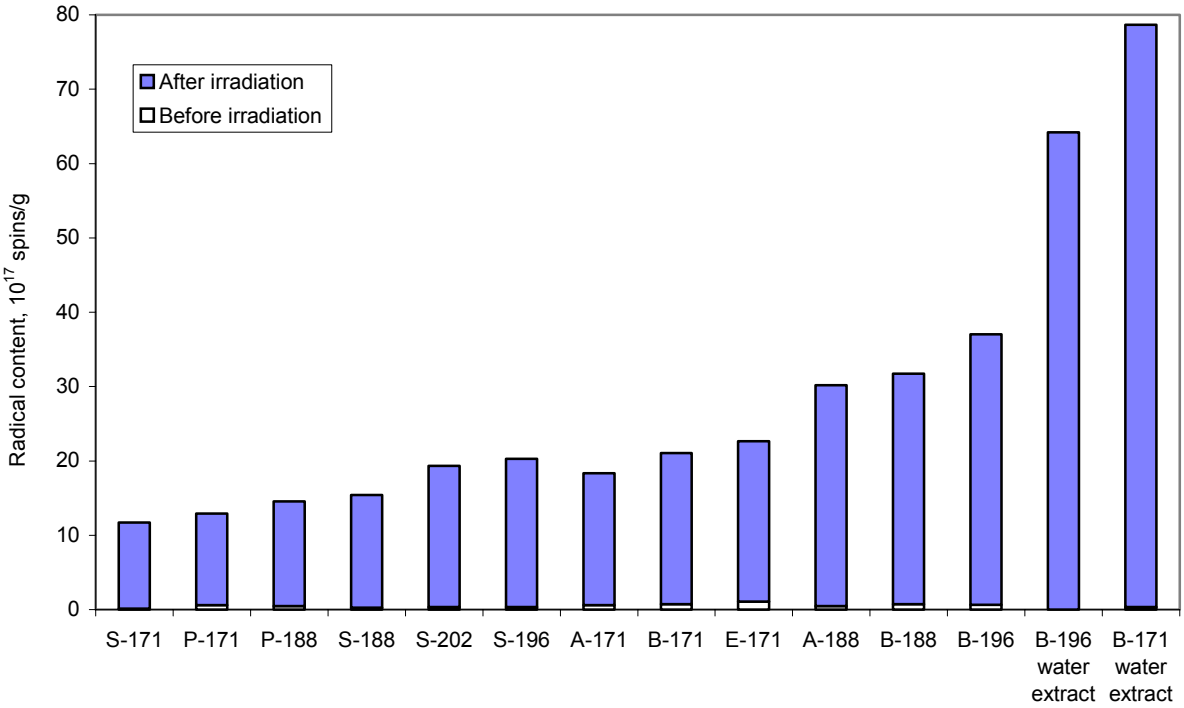


Figure 3.14. Radical content of wood fibers and fiber water extracts before and after γ-irradiation.

4 MANUFACTURE OF WOOD COMPOSITE BOARDS BY CONVENTIONAL METHODS

4.1 Wood composite board types

Medium-density fiberboard (MDF) is one of the many different composite boards produced by the forest products industry [66]. Wood composite boards are made from wood particles of various shapes and dimensions that are glued or otherwise bonded together. In addition to wood, also nonwood lignocellulosic materials such as straw and bagasse are used in the composite board industry. Typical wood composites are particleboard made from discrete pieces or particles, fiberboard made from defibrated wood, waferboard manufactured from large flakes (wafers), and oriented strandboard made from compressed strands lined up and arranged in layers. The manufacturing processes usually involve combining the wood particles with a synthetic thermosetting resin such as urea-formaldehyde (UF) or phenol-formaldehyde (PF) and sizing agent followed by hot pressing during which interparticle bonds are formed.

4.2 Manufacture and standard properties of commercial dry-process fiberboard made by the Asplund process

Fiberboards are roughly classified into three groups according to their density: insulation board is low-density fiberboard (density < 0.5 g/cm^3) and high-density fiberboard (density usually > 0.9 g/cm^3) is called hardboard [1]. The density of MDF varies over a wide range but lies usually in the range 0.5-0.8 g/cm^3 [66,67]. MDF can be made from different types of wood-based furnish such as pulp chips, planer shavings, plywood trims and sawdust or nonwood materials such as sugar-cane bagasse. The MDF manufacturing process is called wet or dry, depending on the distributing medium used in fiber transportation and forming. In wet-process forming the watery fiber suspension flows onto a wire screen which retains the fibers, whereas in the dry process air is used for fiber transportation and bringing the fibers together to produce a mat. Refining is conducted at atmospheric or pressurized conditions. One of the most commonly used pressurized defibrators is the Asplund defibrator, in which the whole preheating and refining process is carried out under pressurized conditions. The Asplund process involves preheating of furnish with pressurized steam usually at 170°C or higher to achieve the thermal softening of lignin necessary for the actual refining stage. From the preheater the furnish is conveyed to a pressurized refining zone between two discs, one or both of which are rotating and have a grooved pattern, where fibers are mechanically separated along the middle lamella. After refining, the fibers are discharged to atmospheric pressure to a cyclone that separates fibers from steam. In the manufacture of dry-process MDF, the moisture content is then adjusted to the desired level in a tube suspension or flash dryer. This is followed by application of thermosetting adhesive, sizing agent (e.g. rosin) and other additives such as preservatives to the fibers, which are then air-formed into mats. Finally, the mats are hot-pressed into boards, typically in a continuous press. The predominant thermosetting resin used in dry-process MDF manufacture is UF. Thick (≥ 10 mm) dry-process MDF boards are typically used as core material in furniture panels while thinner wet-process MDF is generally used as siding material.

Composite panels have to conform to standards regarding their mechanical strength and water resistance properties. The standards depend on the type of composite product and also vary from country to country. Medium-density fiberboards are generally tested for internal bond strength (IB), which is the tensile strength perpendicular to the board surface, and thickness swell (TS) on 24-h cold soak in water. Also the static bending strength is often tested, involving the measurement of modulus of rupture (MOR) and modulus of elasticity (MOE). Typical European MDF standards (Europe-Norm No. CIN DIN 622-5) specify that for 12 mm MDF boards the IB should be > 0.6-0.65 MPa, MOR > 30-35 MPa and MOE > 2,500 MPa while TS should not exceed 10-15%.

5 MANUFACTURE OF FIBERBOARDS FROM ACTIVATED FIBERS (V-VIII)

5.1 Production and oxidative treatment of fibers for fiberboard manufacture

The wood raw material used for the manufacture of fiberboards in V-VIII consisted of debarked chips of fresh wood. The fibers were produced using a Asplund defibrator (chap. 2.2.1) and sprayed with laccase solution or Fenton's reagent at the wax addition point located at the beginning of the blowline when the fibers are in a fluffed state. Other researchers [68] report that attempts to make fiberboard on a pilot-scale by adding laccase in the blowline were unsuccessful because the enzyme was inactivated under the harsh conditions prevailing in the blowline. However, as the properties of boards made from fibers activated by adding laccase in the blowline (V-VII) were far better than those of control boards made without laccase, it seems that a sufficient number of radicals is formed in the fibers to enable manufacture of boards of high mechanical strength.

5.2 Board pressing

The fibers were formed into mats and hot-pressed into 12-mm thick MDF boards in a daylight press. The press temperature was set at 170°C or 190°C and the press time varied from 169 s to 375 s. Panel densities were in the range 0.65-0.95 g/cm^3.

5.3 Board testing

The fiberboards in V-VIII were tested for internal bond strength (IB) and thickness swell (TS) on 24-h cold soak in water. Most boards were also tested for static bending strength, involving the measurement of modulus of rupture (MOR) and modulus of elasticity (MOE). The IB in particular is good for evaluation of the frequency of interfiber bonds formed in fiberboards provided that the panels to be compared have approximately equal densities. Board strength is governed by bonding area, which increases as board density increases [19]. As MOR and MOE depend largely on factors such as fiber length distribution and board density profile, they are less reliable measures of the extent of interfiber bonding than IB.

5.4 Bonding mechanisms in fiberboard manufacture

The objective of the work discussed in this thesis was to achieve bonding of wood fibers by coupling of phenoxy radicals generated on the fiber surfaces on pressing the fibers into boards. However, the exact nature of the adhesion occurring when fibers activated by oxidative treatment are pressed into fiberboards is not known. This is due to the complexity of the chemical structure of oxidized fiber surfaces. When activated wood fibers are compressed under heat, different types of interfiber covalent and secondary bonds are formed *via* interaction of reactive groups on the fiber surfaces. Some of these potential interfiber bonding mechanisms are illustrated in Fig. 5.1. Phenoxy radicals and other functional groups on oxidized fiber surfaces may contribute to adhesion in various ways. Felby *et al.* [8] suggested

that phenoxy radicals on fiber surfaces interact with radicals or other reactive groups on the surfaces of other fibers. An increase in interfiber bonding area due either to bonding of low-molecular weight lignin or lignans to the fiber surfaces or to a loosening of lignin structure by the action of laccase resulting in a more even surface topography was also considered as a possible explanation for enhanced adhesion. Bonding area could be increased also by an increase in polymer mobility due to homolytic or heterolytic breakdown of lignin and carbohydrate bonds during refining (chap. 2.2.2). An increased bonding area would result in a higher frequency of hydrogen bonds and other secondary forces binding fibers together [19].

The treatment of fibers with Fenton's reagent generates not only radicals but also carboxyl and other carbonyl groups in fibers. [12,19,69]. Carboxyl groups may esterify with hydroxyl groups on other fibers while carbonyl groups may crosslink by forming acetal, hemi-acetal and ether bonds. Also self-condensation of furfural or other furan-type carbohydrate degradation products (chap. 2.2.2) may take place, or these compounds may condense with lignin or other phenolic compounds. There are several reported studies of attempts to make particleboard and other wood composite boards except fiberboard by activation of particle surfaces with hydrogen peroxide and other inorganic oxidants [12-17; 70-83]. In these studies adhesion was attributed to bonding processes such as those described in Fig. 5.1. Usually added copolymerizing substances with gap-filling properties such as lignosulfonate spent sulfite liquor, tannin or furfuryl alcohol were included in the bonding solutions.

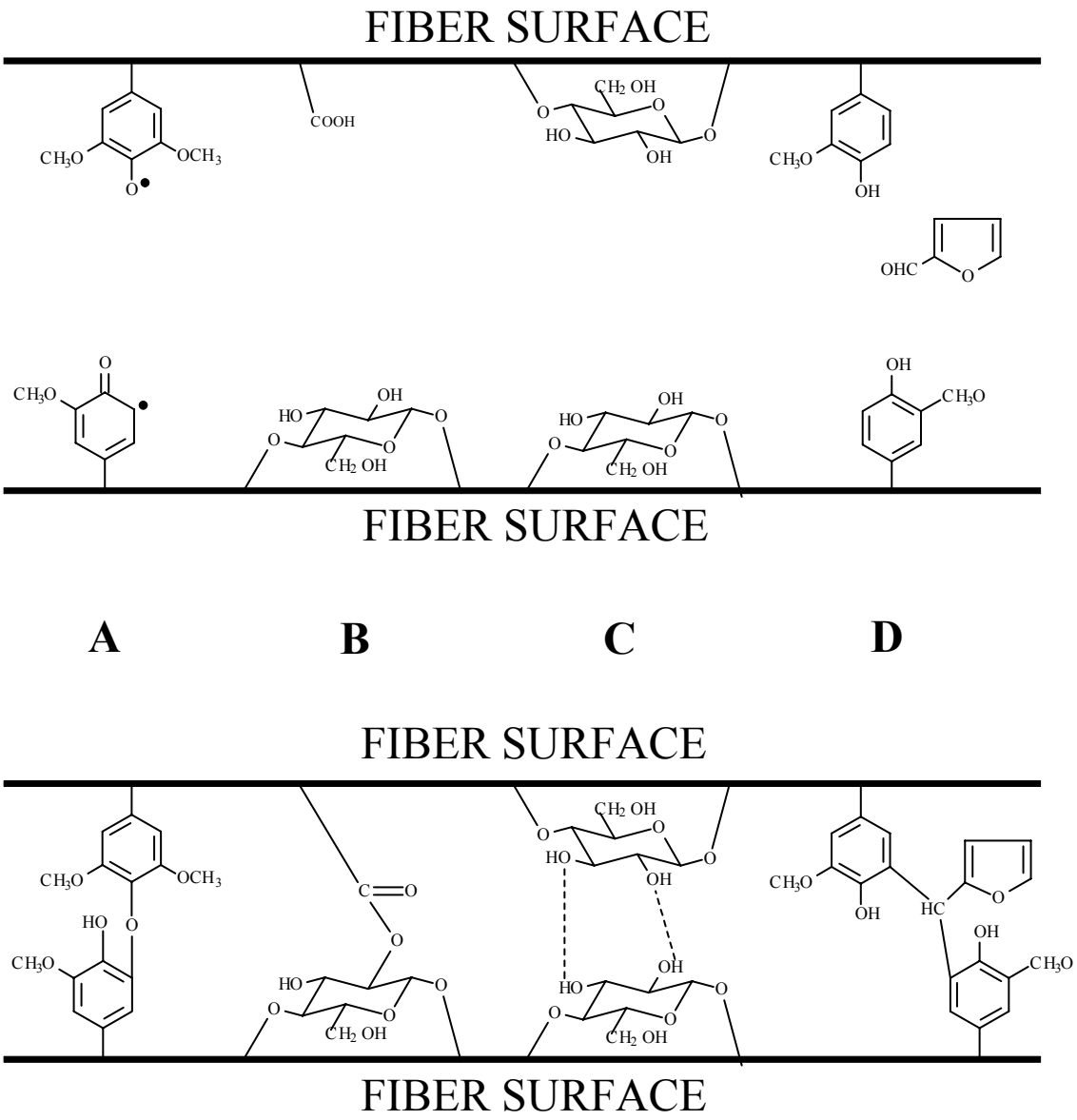


Figure 5.1. Adhesion processes possibly occurring by interaction of oxidized wood fiber surfaces (top) as they are brought together by hot-pressing (below). A) Coupling of phenoxy radicals, B) esterification, C) hydrogen bonding, D) condensation of lignin and furfural.

5.5 Fiberboards made from laccase-treated fibers (V, VI)

Laccase-treated fibers were used in V and VI for the manufacture of 12-mm thick dry-process fiberboards. According to their densities, the boards in V (density < 0.8 g/cm^3) were MDF while those in VI were mainly hardboard (density > 0.9 g/cm^3) The internal bond strength (IB) and thickness swell (TS) properties of the boards were found to improve with an increase in defibration temperature. Fig. 5.2 shows the relationship between the IB of birch MDF boards and the reactivity of the fibers in water suspension in terms of radical formation and oxygen consumption during laccase treatment (V). The results obtained with MT show that the increase in IB is accompanied by an increase in the oxygen consumption and radical content of the fibers when the defibration temperature is elevated from 171°C to 196°C. The

data on spruce fiberboards and fibers (Fig. 5.3) show a similar pattern (VI). The radical content and oxygen consumption were considerably higher for birch fibers than for spruce fibers. As discussed in the previous chapter, this is readily explained by the presence of larger amounts of reactive material in hardwood fibers as compared to softwood fibers, whereas there is no correlation between fiber reactivity and surface chemical composition. Birch boards show better IB than spruce boards when boards from fibers produced at equal temperature are compared. However, the data on birch and spruce boards are not directly comparable due to the different board densities and the different pressing parameters and laccase types and doses used in their fabrication. The data on aspen boards and fibers (V) and pine boards and fibers (VI) resemble the data on birch and spruce boards and fibers, respectively.

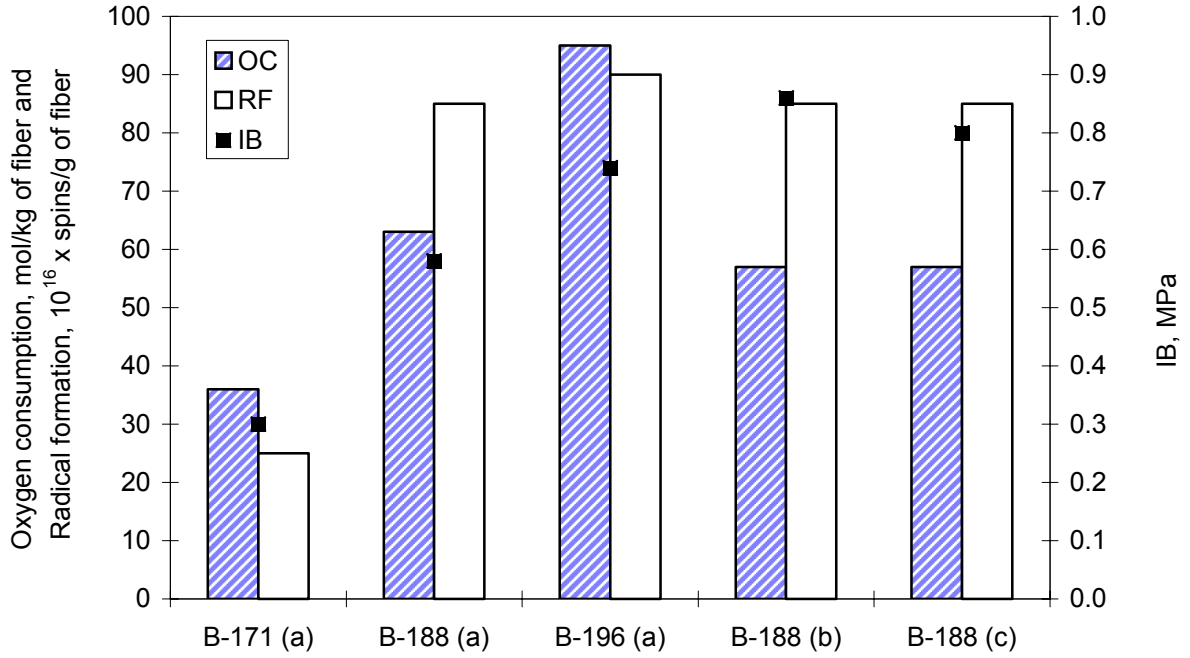


Figure 5.2. Relationship between IB strength of 12 mm birch MDF boards bonded with laccase and fiber radical formation (RF) and oxygen consumption (OC) in water suspension on laccase treatment (1 nkat/g of fiber). The OC measurements were carried out using the same laccases that were used for making the corresponding MDF boards. The laccase types and doses used in board manufacture were (a) = 400 nkat/g of MT, (b) = 100 nkat/g of TH, (c) = 400 nkat/g of TH.

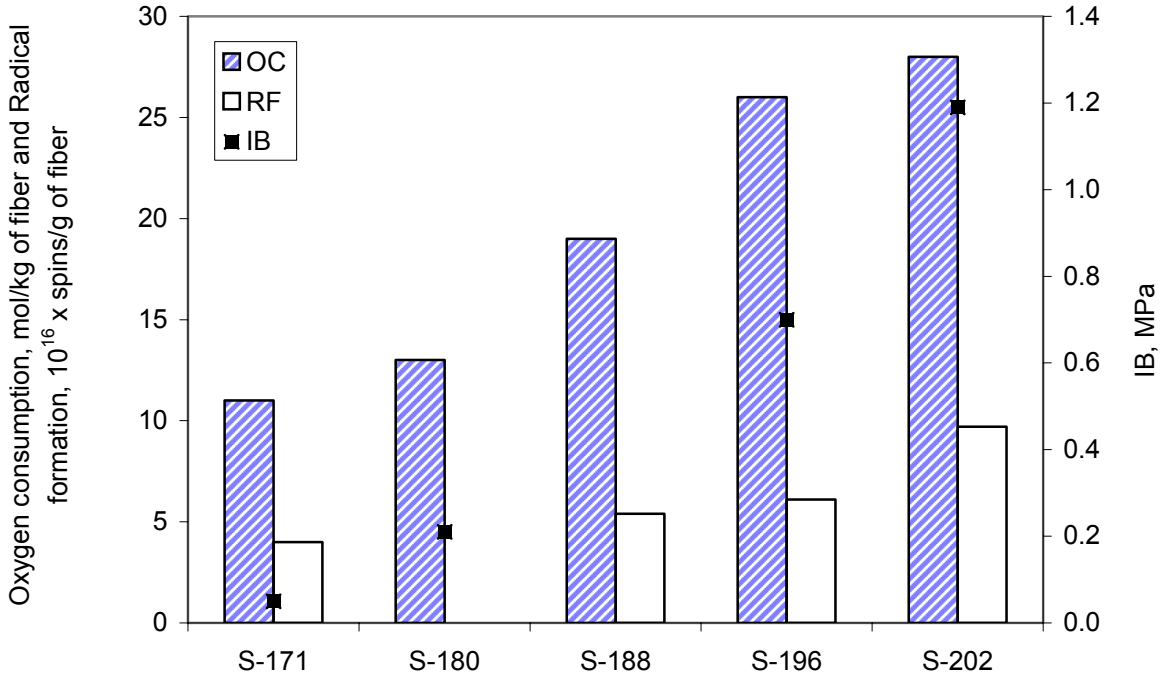


Figure 5.3. Relationship between IB strength of 12 mm spruce fiberboards bonded with laccase and fiber radical formation (RF) and oxygen consumption (OC) in water suspension on treatment with CV. The OC measurements were carried out using the same laccases that were used for making the corresponding fiberboards.

Other researchers have used laccases [2,3,5,7,8,10] and peroxidase [11] to manufacture thin (3-6 mm) MDF boards, so the results are not directly comparable with the properties of the 12 mm boards discussed above. Test results from these studies are presented in Table 5.1. The data show that laccase treatment of fibers gives boards with mechanical properties and TS superior to those of control boards made by using untreated fibers or deactivated laccase. This indicates that the improvement in board properties with an increase in defibration temperature is largely due to increased formation and reactions of fiber radicals rather than to an increase in other crosslinking reactions.

Table 5.1. Properties of fiberboards bonded with phenoloxidases.

Wood furnish	Enzyme type	Defibration temperature, °C	Thickness, mm	Density, g/cm^3	IB, MPa	MOR, MPa	MOE, GPa	TS, %	Ref.
Beech	None		3	0.906	0.95	27.4	3.61	37	[8]
Beech	Laccase		3	0.895	1.57	41.7	4.02	19	[8]
Beech	None		3	1.050	1.04	38.3	3.09	59	[8]
Beech	Laccase		3	1.037	1.55	44.6	3.36	57	[8]
Spruce/ pine/beech	Denatured laccase	180	5	0.8	0.1			Disintegrated	[10]
Spruce/ pine/beech	Laccase	180	5	0.8	0.52			23	[10]
Spruce/ pine/beech	Laccase	180	5	0.78	0.95			23	[10]
Spruce/ pine/beech	None	180	5	0.75	-			>70	[11]
Spruce/ pine/beech	Peroxidase	180	5	0.76	0.42			28	[11]
Spruce/ pine/beech	Peroxidase	180	5	0.8	0.60			25	[11]
Spruce/ pine/beech	Peroxidase	180	5	0.9	0.76			20	[11]
Spruce/ pine/beech	Peroxidase	180	5	1.0	1.00			15	[11]

5.6 Fiberboards made from fibers treated with Fenton's reagent (VII)

Mechanical properties of 12-mm thick dry-process fiberboards of equal density made from S-171, S-188 and S-202 fibers and bonded with Fenton's reagent ($H_2O_2/FeSO_4$) are shown in Fig. 5.4. The same figure shows the number of radicals detected in the freeze-dried fibers after treatment with Fenton's reagent (3% H_2O_2, 0.3% $FeSO_4$). It is seen that all the mechanical properties improve as a function of increasing defibration pressure, as does the number of radicals present in the fibers. While there is a clear difference between the IB of the panels, the difference between radical formation in S-171 and S-188 fibers seems too small to fully account for the large IB difference. The possibly significant role of carboxyl or other reactive functional groups formed on the fiber surfaces by the Fenton's reagent in fiber crosslinking cannot be excluded [12,18,19]. The TS of Fenton's reagent-bonded boards was found to depend on the amount of paraffin wax (0.5% or 1%) or $CaCl_2$ added to the fibers and the defibration temperature. The lowest TS (17%) reached was that of S-202 boards made with 1% wax or 0.5% wax and 2% $CaCl_2$. Boards made with 0.5% wax but without $CaCl_2$ showed TS higher than 100% regardless of defibration temperature.

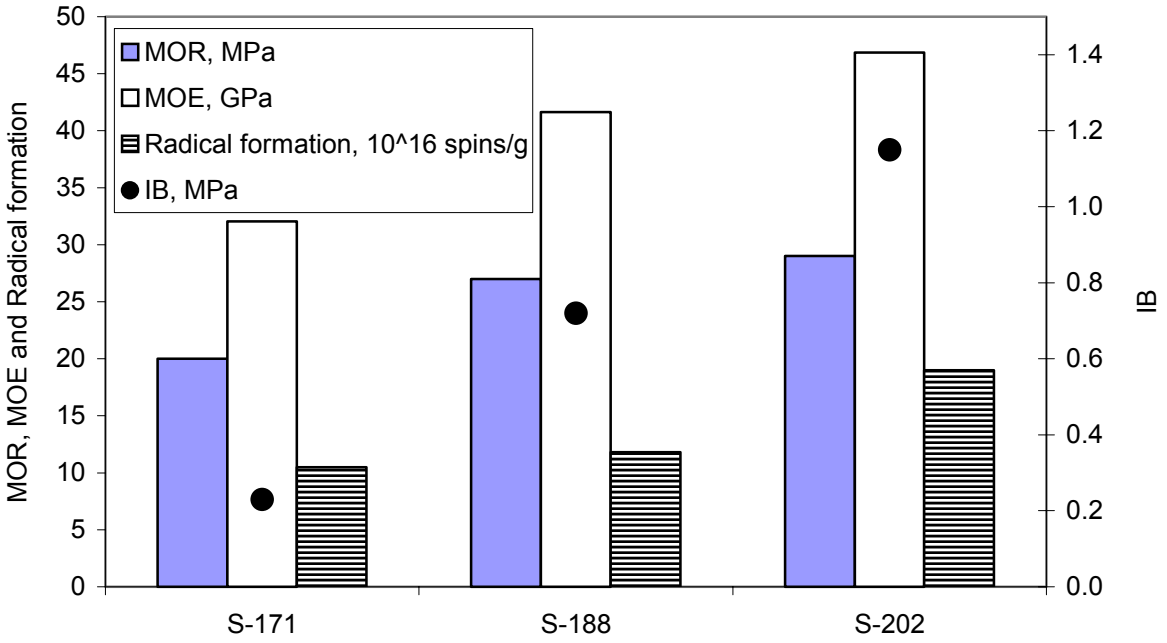


Figure 5.4. Mechanical properties of 12 mm dry-process spruce fiberboards with 0.96-g/cm^3 density bonded with Fenton's reagent and number of radicals formed in the fibers on treatment with Fenton's reagent in water suspension.

5.7 Fiberboard made from γ-irradiated fibers (VIII)

A fiberboard with a density of 0.85 g/cm^3 was manufactured from γ-irradiated S-202 fibers (VIII), found to contain a large number of radicals (chap. 3.6). The board showed higher IB (0.60 MPa) and lower TS (40%) than a control panel at a density of 0.91 g/cm^3 from unirradiated S-202 fibers (IB 0.41 MPa, TS 125%). As in the case of boards made from fibers activated by laccase or Fenton's reagent treatment, the improved board properties may be due to surface-to-surface bonds formed by radical coupling but the contribution of other modifications in fiber structure caused by irradiation cannot be ruled out.

6 CONCLUSIONS

Pressurized high-temperature defibration of wood at temperatures exceeding 170°C leads to breakdown of wood macromolecules, resulting in formation of large amounts of water-extractable carbohydrates and low-molecular weight lignin. Acetone-extracted fiber surfaces are enriched in lignin or other phenolic compounds as compared to the original wood. The amount of water-extractable material increases as defibration temperature increases and is higher for hardwoods than for softwoods, whereas the fiber surface lignin coverage is always 43-60%. The fibers can be activated for bonding by treatment with laccase or Fenton's reagent ($H_2O_2/FeSO_4$) or by γ-irradiation.

Laccase treatment of wood fibers produced at high defibration temperatures results in the formation of phenoxy radicals. Radical formation depends on the amount of reactive water-extractable low-molecular weight lignin and other phenolic material present in the fibers and thus increases with an increase in defibration temperature, being much higher for hardwood fibers than for softwood fibers. Also pH and laccase type affect the extent of radical formation. The radicals are relatively persistent at ambient conditions but decay rapidly on heat treatment. Fibers produced at 188°C or higher and activated by laccase treatment can be used to manufacture fiberboards exhibiting high internal bond strength (IB). Board thickness swell properties improve with an increase in defibration temperature. The adhesion is probably largely connected to interfiber bonds formed by reactions of phenoxy radicals on the fiber surfaces.

Treatment of wood fibers with Fenton's reagent increases the phenoxy radical content in fibers. Compared to laccase treatment, more radicals are formed in softwood fibers and fewer in hardwood fibers. Radical formation increases with defibration temperature but not as strongly as with laccase, and differences between hardwood and softwood are small. An increase in peroxide dose results in increased radical formation, being proportional to the square root of peroxide dose. The relationship between radical formation and board properties is not as clear as with laccase activation. Adhesion of fiberboards made from fibers activated with Fenton's reagent improves as a function of defibration temperature and boards of acceptable IB can be manufactured from fibers produced at 188°C or higher. The thickness swell of the boards depends largely on the amount of sizing agent and the defibration temperature used.

Fibers activated by γ-irradiation show a very high phenoxy radical content but obviously only radicals located on fiber surfaces may contribute to adhesion. Radical formation increases as defibration temperature increases and is higher for hardwood fibers than for softwood fibers. The amount of board data is limited but the existing data indicate that board properties improve as a result of fiber irradiation.

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