Structural Changes to *Pinus radiata* Wood Lignin During Kraft Pulping and Bleaching

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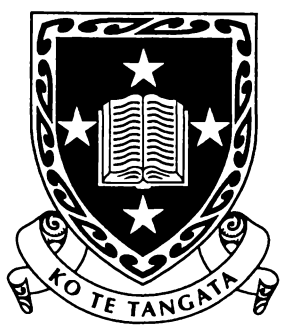

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Abstract

The aim of this thesis was to improve the quality of the information available concerning the structure of native (within wood) and residual (within pulps) lignins. A better understanding of the structure of lignin will assist the development of improved pulping and bleaching sequences. However, before this can be done, it is necessary to develop approaches to lignin isolation which provide more representative lignin samples without resorting to what are often very tedious extraction procedures. A key requirement for such isolation methods would be to isolate lignins in high yield with a structure as unmodified as possible. This would allow meaningful comparisons to be drawn with respect to structural changes occurring during the pulping and bleaching processes.

An improved method for the isolation of lignin from *Pinus radiata* wood was developed. The method involves a three day enzymatic hydrolysis of ball milled wood, followed by a second ball milling stage. A combination of extraction and purification steps enables isolated yields of approximately 75% of the lignin present in the wood to be obtained. The key step is the second ball milling treatment, which allows essentially all the lignin in the enzyme digest residue to be extracted. The resulting cellulolytic enzyme lignins (CEL) contain approximately 5–8% carbohydrates and less than 2% protein.

The CEL lignins, which were soluble in common lignin solvents, were characterised chemically and spectroscopically. They were shown to be remarkably similar to milled wood lignin (MWL) in terms of the total phenolic hydroxyl content, uncondensed β-O-4 ether content, and the distribution of interunit linkages, although there was some indication that the CEL lignins had a higher molecular weight than MWL. This shows that MWL, as far as a soluble lignin is concerned, is a reasonable representation of native lignin. However, the CEL method developed in this study is superior to the MWL method in that much higher yields of lignin can be obtained, thus less effort is required to obtain the same quantities of lignin from wood by the CEL method as compared to the standard MWL method.

Unfortunately, when this CEL method (and modifications of) were applied to *Pinus radiata* kraft pulps no significant improvement, in terms of yield or protein contamination, were achieved compared to other reported CEL methods. However, as part of this work it was shown that an enzyme hydrolysis treatment to enrich the residual lignin content in kraft and kraft-oxygen pulps had the potential to make the analysis of *in situ* residual lignin by degradation studies easier.

In order to study the structure of the residual lignin in pulps bleached with elemental chlorine free sequences residual lignins were isolated by dioxane acidolysis instead. The approach taken in this thesis was to compare residual lignin from pulps bleached to the same kappa number. Such a comparison should remove the variables associated with the different oxidising powers of each reagent and yield a more comparable residual lignin, thus a kraft-oxygen pulp was bleached to approximately kappa number 10 with cyanamide activated hydrogen peroxide (KOQPn), ozone (KOQZE), and chlorine dioxide (KODE). For comparison a hydrogen peroxide bleached pulp (KOQP) was generated using the same peroxide charge as KOQPn; and unextracted ozone and chlorine dioxide bleached pulps (KOQZ and KOD, respectively) were also studied.

The residual lignins from the bleached pulps were very similar in structure and resembled the kraft pulp residual lignin in many aspects except in having slightly elevated levels of oxidised groups. Additionally, many of the structural elements expected to be highly reactive during bleaching (for example phenolic and catechol type structures) were detected in the residual lignins. These results support the view that the removal of lignin during bleaching is controlled to a significant extent by the accessibility of the lignin and penetration of the bleaching reagents rather than just by the nature of the functional groups present in the lignin.

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Chapter One
INTRODUCTION

1.1 Papermaking – Past, Present, Future

Paper is recorded as being invented in China by Tsai Lun in 105AD. The art of papermaking was kept a secret in China for 700 years until it was introduced to Japan in 807AD. After this point in time, the art spread through Europe and finally reached England in 1495. Nearly 200 years later, in 1690, the craft was brought to America (Clark 1985).

Paper was made by hand from materials; such as, rags, hemp, and nets. Pulp was prepared from these materials by fermenting and then boiling with wood ash. Subsequently, small batches of well-washed pulped material were heavily beaten on wooden blocks. Paper sheets were formed by immersing rectangular sieves, made out of fine threads of bamboo or horsehair, into a vat containing dilute suspensions of the beaten fibres (Clark 1985).

Paper continued to be made in this manner until the invention of the Fourdrinier and cylinder paper machines during the period 1798–1809. These machines allowed large quantities of paper to be made and it was found there was insufficient rag or cotton fibre raw materials available to keep up with demand. This lead to intensive research into finding other sources of fibre and ultimately to the invention of modern wood pulping processes (1840–1884).

Groundwood pulping was the first pulping process to utilise wood as the source of fibre. Fifteen years later the first chemical pulping process was invented with soda pulping (1851). This was closely followed by the sulfite process (1857) and finally the kraft process (1884), completing the development of the popular types of pulping used today (Clark 1985). Kraft pulping remains the dominant process in industry due to its reliability in producing strong bleachable fibres for papermaking. However, environmental and economical limitations associated with the process are stimulating a search for alternatives (Aziz & Sarkanen 1989). Sulfur-free organosolv processes are potential alternatives to kraft pulping and many of these have reached pilot scale.

The bleaching of pulp for the production of white paper is also common practice. The early bleaching of pulp was performed with potash in sunlight and much later with hypochlorite (Fengel & Wegener 1984). A new period of bleaching history began at the end of the nineteenth century with the industrial bleaching of wood pulp. Initially, hypochlorite was the sole bleaching agent, however, with the commercialisation of chlorine bleaching in 1930, hypochlorite lost its position as the sole bleaching agent and became part of multi-stage sequences. Sulfite and mechanical pulps were easily bleached with 2 or 3 stage processes, however, only a few kraft mills produced bleached pulp before the advent of chlorine dioxide in 1949. With the introduction of chlorine dioxide the kraft industry was then able to compete with companies producing high brightness sulfite pulps. Chlorine, chlorine dioxide and hypochlorite remained the dominant bleaching reagents until recently when the environmental impact of the bleach plant effluents became a concern.

The interest in developing chlorine-free bleaching processes has steadily increased due to restrictions placed on the pulp and paper industry by environmental protection legislation. One of the driving forces for these changes is the public concern over the emission of chlorinated organic material into the environment since many of the chlorinated compounds are toxic to aquatic life. The carcinogenic tetra-chlorinated dibenzodioxin and dibenzofuran compounds found in the effluents are of particular concern (Axegard et al. 1993). These concerns emphasise the need to reduce or cease the discharge of chlorinated effluents from chemical pulp mills. Therefore, in industry today the use of elemental chlorine for bleaching is declining and being replaced mainly by elemental chlorine free (ECF) bleaching, although totally chlorine free (TCF) processes are in commercial operation in Europe. Other measures have also been taken in order to reduce the discharge of chlorinated organic matter. These include: reducing the residual lignin content of the incoming pulp, improving pulp washing, and improving process control (Hise et al. 1992).

Effluents are also a source of oxygen demand; that is, the discharged organic matter consumes oxygen from the aquatic environment. Effluents, particularly from kraft mills, are also highly coloured. Therefore, the ultimate goal of industry is to completely eliminate discharge with the complete closure of the bleach plant cycle. The main obstacle to closure in conventional and ECF sequences is the presence of the corrosive chloride ion (from the chlorine containing bleaching agents). The elimination of chlorine-based chemicals (chlorine, hypochlorite, and chlorine dioxide) by substitution with oxygen-based reagents (oxygen, hydrogen peroxide, and ozone) in TCF bleaching is a subject of ongoing interest.

Production of fully bleached TCF pulp from hardwood sulfite pulps, hardwood kraft pulps, and softwood sulfite pulps has become a reality (Chirat & Lachenal 1994), however, it remains a challenge to produce fully bleached TCF softwood kraft pulps. The two main difficulties are that softwood kraft pulps usually have a higher lignin content and the residual lignin is harder to remove.

The reasons why the last fraction of residual lignin is difficult to remove by extended pulping or bleaching is unclear. One impediment to the complete understanding of delignification is that the structure of the residual lignin remaining in the fibre after kraft pulping and subsequent chlorine-free bleaching is not well understood. Therefore, by gaining an understanding of the structure of residual lignin, it should be possible to gain better understanding of the mechanisms involved in delignification, thereby permitting optimisation of the bleaching processes.

The pulping of wood, the bleaching of pulp, and the methods of lignin analysis are reviewed in the next chapter. Emphasis is given to kraft pulping, chemical pulp bleaching, and the reactions of lignin taking place during these processes. Additionally, methods for the isolation and characterisation of lignin are discussed, with particular emphasis on current literature concerning the characterisation of residual lignin.

1.2 Aims

As has already been mentioned, the nature and extent of changes to the residual lignin of kraft pulps during oxygen-based bleaching processes is still not very well understood. By gaining a better understanding of the structure of the residual lignin in bleached pulps, it should be possible to gain insight into the optimisation of such bleaching processes. However, before this can be done, it is necessary to develop a consistent approach to lignin isolation. This would allow meaningful comparisons to be drawn with respect to structural changes occurring during the pulping and bleaching processes. The aim of this research was to improve the quality of the information available concerning the structure of native (within wood) and residual (within pulps) lignins, by developing improved methods for isolating lignin from the wood and fibre matrix.

An improved enzyme-based lignin isolation technique for the isolation of lignin from *Pinus radiata* wood was developed. Ball-milled wood was subjected to sequential enzyme treatment and solvent extraction to recover the released wood lignin. The methodology was based on that recently reported by Lachenal et al. (1995). The effects of ball milling, duration of the enzyme treatment, and alkali pretreatments of both wood powder and insoluble wood residues were investigated. The released wood lignin was then characterised by chemical and spectroscopic techniques and the structural characteristics were compared to milled wood lignin (MWL). The results from these studies are discussed in Chapter 4.

Attempts were made to apply the improved method to the isolation of residual lignin from unbleached and bleached kraft pulps. The effects of ball milling, duration of the enzyme treatment, and alkali pretreatments were investigated as a means to obtain residual lignin in high yield with low contamination from kraft pulps. These are discussed in Chapter 5.

In the last part of this study the effects of oxidative treatments (oxygen, ozone, hydrogen peroxide, and chlorine dioxide) on the structure of residual lignin were investigated. Residual lignin was isolated from the individual pulps using the acidolysis method of Gellerstedt et al. (1994). The lignins were characterised by a range of techniques and the structural changes occurring in the residual lignins were identified and quantified. The effect of the acidolysis treatment was also studied by comparing the structural characteristics of lignin isolated from *Pinus radiata* wood using the enzyme method with the lignin isolated using acidolysis. The isolation and characterisation of the residual lignins is discussed in Chapter 6, while the comparison between acidolysis wood lignins and enzyme lignins is made in Chapter 4.

1.3 Abbreviations and Technical Terms

Kappa number	Permanganate oxidation number – residual lignin content
Consistency	Moisture content – dry weight of pulp/total weight × 100%
odg	Oven dry weight of pulp in grams
Charge	Amount of chemical used for bleaching (% on od pulp)
Active chlorine	Equivalent weight of substance equal to equivalent weight of chlorine
Chlorine multiple	Active chlorine charge/kappa number of pulp
ECF	Elemental chlorine free
TCF	Totally chlorine free
C	Chlorine bleaching stage
D	Chlorine dioxide bleaching stage
O	Oxygen bleaching stage
P	Hydrogen peroxide bleaching stage
Pn	Cyanamide activated, hydrogen peroxide bleaching stage
Q	Chelation stage
Z	Ozone bleaching stage
E	Alkali extraction stage
KP	Unbleached kraft pulp (obtained from NZ mills refer Table 5.1)
KOP	Oxygen bleached kraft pulp (obtained from NZ mills refer Table 5.1)
DCM	Dichloromethane
DMF	Dimethyl formamide
EDTA	Ethylenediaminetetra-acetic acid
DTPA	Diethylenetriaminepenta-acetic acid
GC/MS	Gas chromatography mass spectroscopy
GC/FID	Gas chromatography flame ionisation detection
NMR	Nuclear magnetic resonance spectroscopy
Chapter Four	
DIR	Dioxane insoluble residue
CEL	Cellulase enzyme lignin
MWL	Milled wood lignin
BMWAL	Acidolysis lignin isolated from ball milled wood
WAL	Acidolysis lignin isolated from Wiley milled wood
WNAL	Acidolysis lignin isolated from Wiley milled wood with additional neutralisation step
Chapter Six	
K	Acidolysis lignin isolated from KP
KO	Acidolysis lignin isolated from KOP
K1-F1	First acidolysis lignin fraction isolated from KP
K1-F2	Second acidolysis lignin fraction isolated from KP
K4	Acidolysis lignin isolated from KP4 (refer Table 6.1)
K-dig	Acidolysis lignin isolated from enzyme treated KP
KO-dig	Acidolysis lignin isolated from enzyme treated KOP
KOQP-dig	Acidolysis lignin isolated from enzyme treated peroxide bleached pulp
KOQPs	Spent liquor lignin from hydrogen peroxide bleaching
KOQPns	Spent liquor lignin from cyanamide/peroxide bleaching
KOQZEs	Spent liquor lignin extracted from ozone bleached pulps

Chapter Two
PULPING, BLEACHING, AND LIGNIN ANALYSIS

This chapter reviews the relevant literature concerning pulping, bleaching and lignin analysis. The first section describes the raw material for pulpwood. In this section the structure and composition of wood is discussed with particular reference to the features that influence the behaviour of a particular wood species during pulping. The second section discusses the pulping processes with emphasis given to kraft pulping due to its relevance to this thesis. The bleaching of chemical pulps is the topic of the third section; the processes, chemistry, and recent developments concerning each particular bleaching agent are briefly discussed. The final sections discuss the isolation and characterisation of lignin giving particular emphasis to the recent literature concerning the characterisation of residual lignin.

2.1 The Raw Material for Pulpwood

Wood is the main source of fibre for papermaking. The development of successful methods for preparing pulp from wood, both mechanically and chemically, in the late 19th century paved the way for the rapid growth and diversification of the paper industry in the 20th century. Reasons for using wood for pulping include the relative availability, the low cost, the convenience of handling and storage, and the versatility of the fibre properties from different wood species. The behaviour of a particular species during pulping and the final pulp properties are strongly influenced by the physical and chemical properties of the wood. Hence, it is important for a pulping chemist to have a running knowledge of the cellular structure and chemical composition of wood.

2.1.1 Anatomical structure of wood

The trees harvested for pulpwood are identified botanically as gymnosperms or angiosperms. Gymnosperms, commonly referred to as conifers or softwoods, are the source of the long-fibre pulp, whereas angiosperms (commonly referred to as hardwoods) are the source of the short-fibre pulp. The differences in the structure between hardwoods and softwoods are illustrated in Figure (2.1) and some of the common cell types are illustrated in Figure (2.2). Softwoods have a relatively simple structure mainly consisting of *tracheids*, which are long, slender cells with a tapered end (Figure 2.2a). Tracheids give softwood the mechanical strength and provide for water transport. They have an average length of 3–5 mm and a diameter of 0.03 mm and this makes them an excellent source of fibre for papermaking. At the beginning of the growth cycle tracheids are thin-walled and have a large cavity for efficient water transport. This tissue is known as *earlywood*. Later, the rate of growth decreases and the *latewood* is produced. It consists of thick-walled tracheids and gives mechanical strength to the stem. The abrupt changes from earlywood to latewood are visible as annual rings.

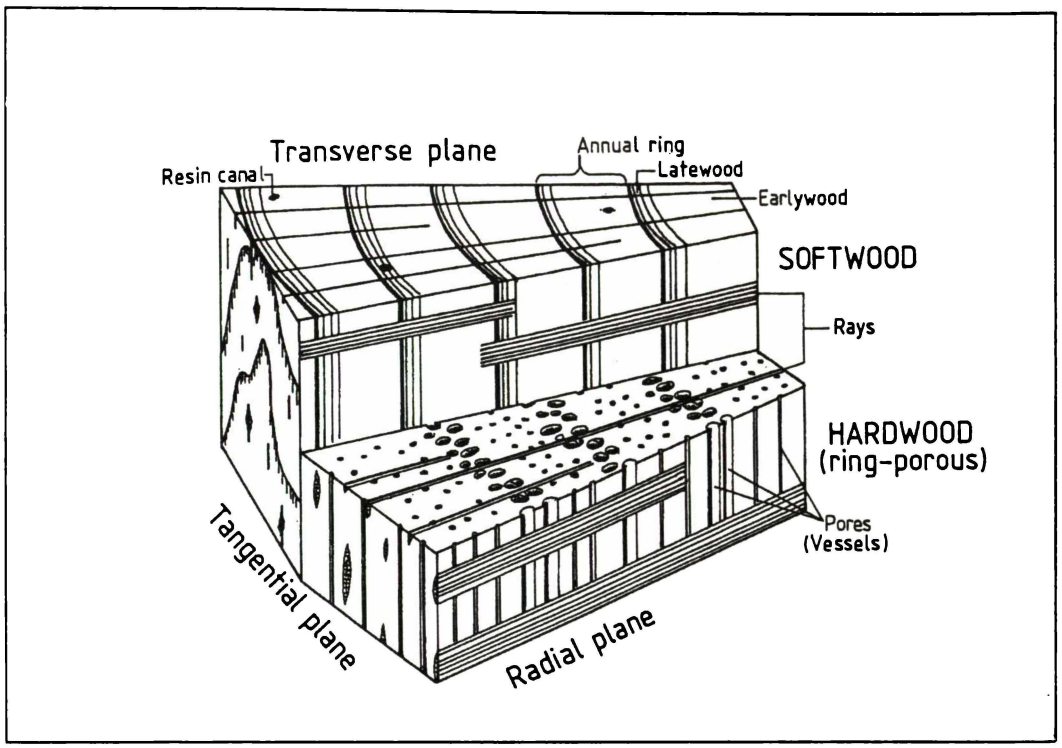

Figure (2.1). Cross section of a wood stem

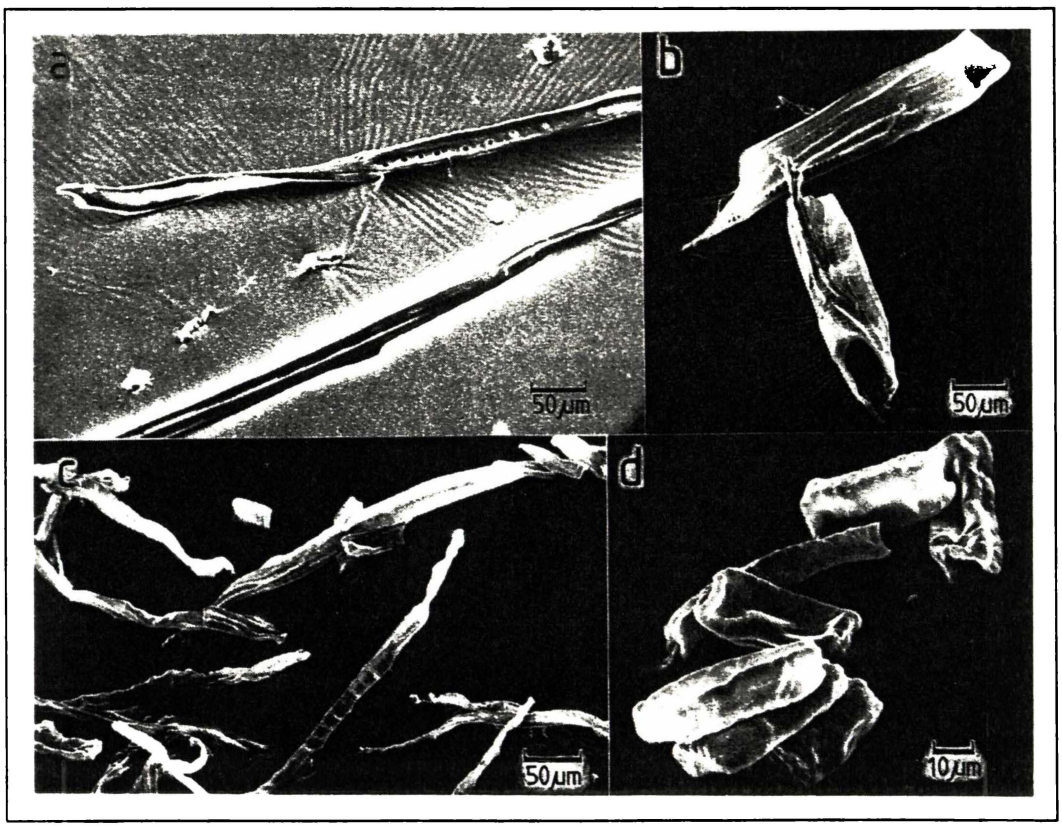

Figure (2.2). Scanning electron microscope photographs of some softwood and hardwood cells; a) Softwood tracheid, b) Hardwood vessel element, c) Hardwood fibres, d) Hardwood parenchyma cells

In contrast, hardwoods are more complex and consist of several cells specialised for different functions. Supporting tissue consists of both *libriform fibres* and *fibre tracheids*. These fibres are on average 1 mm in length and 0.02 mm diameter (Figure 2.2c) and account for more than 50% of the volume of the wood. Within this strengthening tissue are distributed *vessels* (Figure 2.2b), which have a large central cavity and perforated ends. Vessels are aligned end to end permitting fluids to rise vertically.

Storage and transport of nutrients takes place within *Parenchyma cells*, which are short compact cells with stubby ends (Figure 2.2d). In softwoods, the parenchyma cells are predominantly arranged radially (*ray parenchyma*), whereas in hardwoods they are aligned both radially and longitudinal. *Resin canals* are intercellular spaces building up a uniform channel network throughout the wood of conifers. The resin canals are lined with *epithelial cells*, which secrete resins into the canals. The majority of cell walls in wood are pitted and this enables the lateral movement of fluids.

The porous nature of the wood structure is important for the impregnation of chemicals during pulping. Most of the cell types are either destroyed and/or mechanically lost during the papermaking process. Softwood tracheids and hardwood fibres are the main constituents of paper.

2.1.2 Cell wall ultrastructure

The wood cell wall consists mainly of cellulose, hemicellulose, and lignin (these are discussed in Section 2.1.3). The cellulose chains form microfibrils which are embedded in a matrix of hemicellulose and lignin. An analogy with reinforced concrete is often given, where the cellulose microfibrils are the reinforcing rods while the hemicellulose and lignin form the concrete matrix.

The cell wall is built up of different layers that can be seen with an electron microscope (Figure 2.3). The visible differences of the cell wall layers arise from differences in the chemical composition and by differences in the orientation of the structural elements. The *middle lamella* (M) is the thin layer between individual cells which glues the cells together to form the tissue. This layer is generally free of cellulose. The *primary wall* (P) is the first true cell wall layer consisting mainly of cellulose. The primary wall is flexible and allows the cell to grow. Once growth is complete the *secondary wall* is laid down. The secondary wall contains more hemicelluloses than the primary wall and once these layers are placed the cell is no longer able to grow. The main difference between the S1, S2, and S3 layers is the orientation of cellulose fibres.

The predominant layer of the cell wall is the S2 layer. Its thickness varies between cell type and between early and late wood cells of softwoods. The characteristics of the S2 layer (thickness and orientation of microfibrils) has a strong influence on the fibre stiffness and other papermaking properties (Sjostrom 1981).

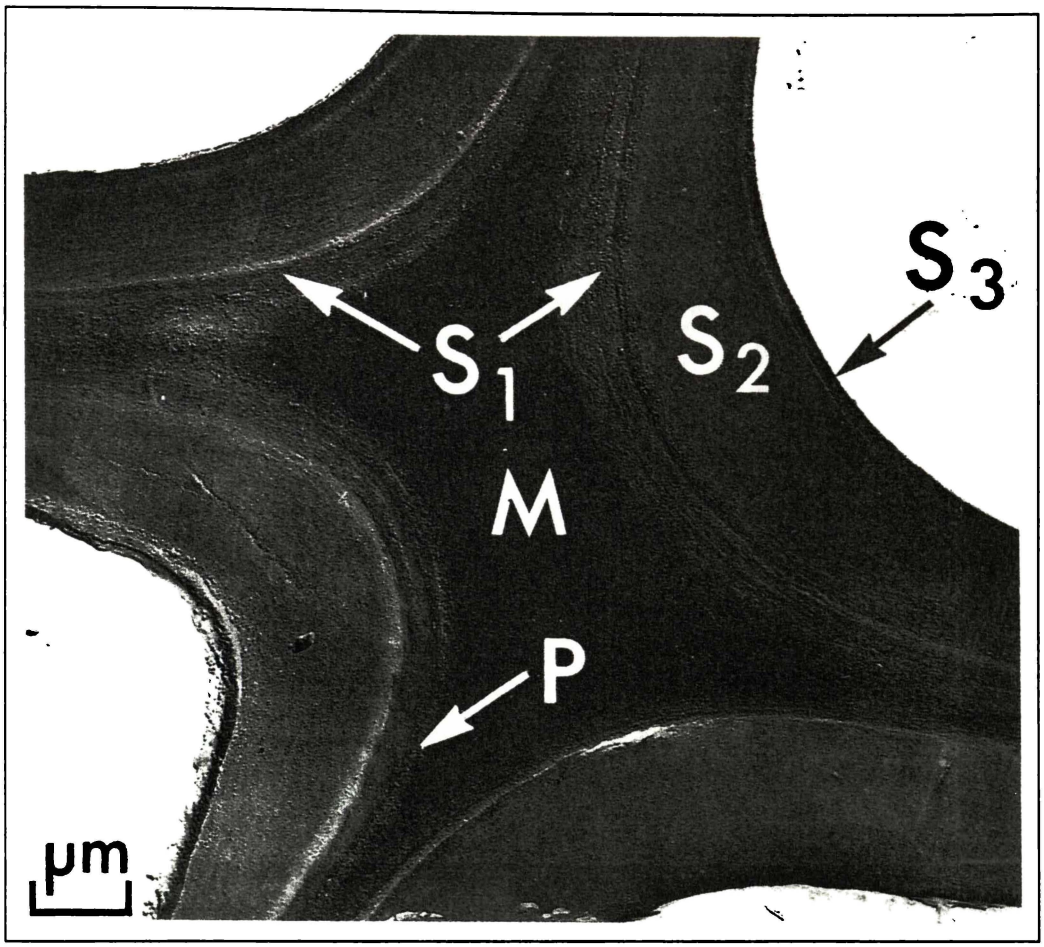

Figure (2.3). Transverse section of earlywood tracheids in tamarack (Sjostrom 1981)

2.1.3 Chemical composition of wood

The chemical composition of wood is another important characteristic for determining the properties of the final pulp product. Cellulose, hemicellulose, and lignin are the three main constituents of wood (Figure 2.4), however, pectin substances, extractives, and inorganic material are also present in small quantities. The chemical composition of wood is important mainly with respect to the usable cellulose content (the main constituent of paper) and whether the extractive material has an adverse effect on the pulping process. However, hemicelluloses are also important in papermaking since they promote fibre-to-fibre bonding and lignin is also important since its characteristics influence both delignification and the fibre properties.

2.1.3.1 Low molecular weight material

The low molecular weight material makes up only a small percentage of the dry weight of wood. The ash is the residue remaining after burning of the organic matter and consists entirely of inorganic material.

The low molecular weight organic material is classed as extractives (ie the material extracted from wood by polar and non-polar solvents). The extractives consist of a large number of different compounds including terpenes, terpenoids, fats, oils, waxes, lignols, tannins, and flavanoids (Fengel & Wegener 1984).

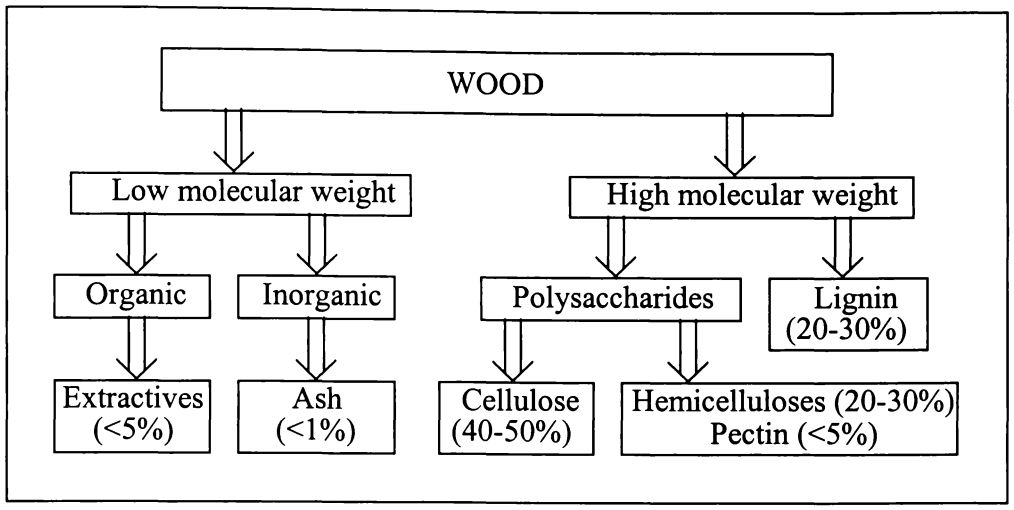

Figure (2.4). Chemical composition of wood (Fengel & Wegener 1984)

2.1.3.2 High molecular weight material

Cellulose is the main component of wood, making up 40–50% of the dry weight. It consists exclusively of glucose units linked together by β(1,4)-glycosidic bonds. Cellulose molecules are completely linear and have a strong tendency to form hydrogen bonds between the hydroxyl groups. Bundles of cellulose chains aggregate together in the form of microfibrils, in which highly ordered crystalline regions alternate with less ordered amorphous regions. These microfibrils in turn aggregate to form fibrils, which then aggregate further to give the cellulose fibres that are visible under the electron microscope.

Hemicelluloses are the second major constituent of wood and are the group of polysaccharides that can be extracted from delignified wood (termed *holocellulose*) with cold alkali. Hemicelluloses differ substantially from cellulose in that they are composed of combinations of five-carbon and six-carbon sugars, possess side groups on the chain molecule, and are amorphous in the fibre wall. They also tend to be much shorter then cellulose chains. The main constituents are: glucose, mannose, galactose, xylose, and arabinose. Hardwoods and softwoods differ not only in the amounts of hemicellulose but also in composition. Softwood hemicelluloses are largely galactoglucomannans while those in hardwoods are mostly xylans. In both cases, they serve as a supportive matrix for the cellulose microfibrils.

Pectin compounds form a minor component of wood and comprise of galacturonans, galactans, and arabinans. Pectins are situated in the middle lamella, and along with lignin serve to hold the cells together. Galacturonic acid is a major constituent of these polymers making them acidic polysaccharides. They have a strong tendency to hold onto water; thus they also function like a clotting agent when the plant is wounded.

Lignin is the third major component in the plant cell wall. The incorporation of lignin into the cell wall of plants increases the mechanical strength of the wall and this ultimately enabled the evolution of the vascular plants. Lignin is unlike any other biopolymer in that its structure can not be described as a simple combination of one or more types of units connected by a few types of linkages. Lignin is a complex, three dimensional, branched phenolic polymer consisting of phenylpropane units.

Lignin is located at high concentration in the cell corners and middle lamella along with pectin but the majority is found in the secondary wall. That is; 20–30% of the total lignin is found in the cell corners and middle lamella, while 70–80% of the total lignin is found in the secondary wall (Fengel & Wegener 1984). Lignification proceeds in three distinct stages, preceded by the deposition of carbohydrates (Terashima 1993). The first stage of lignification occurs at the cell corners and middle lamella after the deposition of pectin has finished and the formation of the S1 layer has started. The second stage is slow and occurs during the formation of the S2 layer. The third stage, following the start of the S3 layer formation, is the main lignification stage and the majority of lignin is deposited during this stage. The lignin replaces the water in the cell wall causing the fibres to become more tightly bound together and making the cell wall stronger.

The building blocks for the lignin molecule are coniferyl alcohol, sinapyl alcohol and, *p*-coumaryl alcohol (Figure 2.5). Each is incorporated into lignin in different amounts depending on the wood species, the age of the cell, and the morphological position within the cell wall (Terashima & Fukushima 1988, Fukushima & Terashima 1990). For example, radiotracer studies have shown that H units are incorporated into the cell wall at an early stage of cell development. This leads to the lignin that is found in the cell corners and middle lamella being enriched in H-units (Terashima & Fukushima 1988). A number of studies also show the middle lamella lignin contains more condensed type linkages (Figure 2.6) than the secondary wall lignin (Eom et al. 1987, Fukuda & Terashima 1988, Fukushima & Terashima 1990).

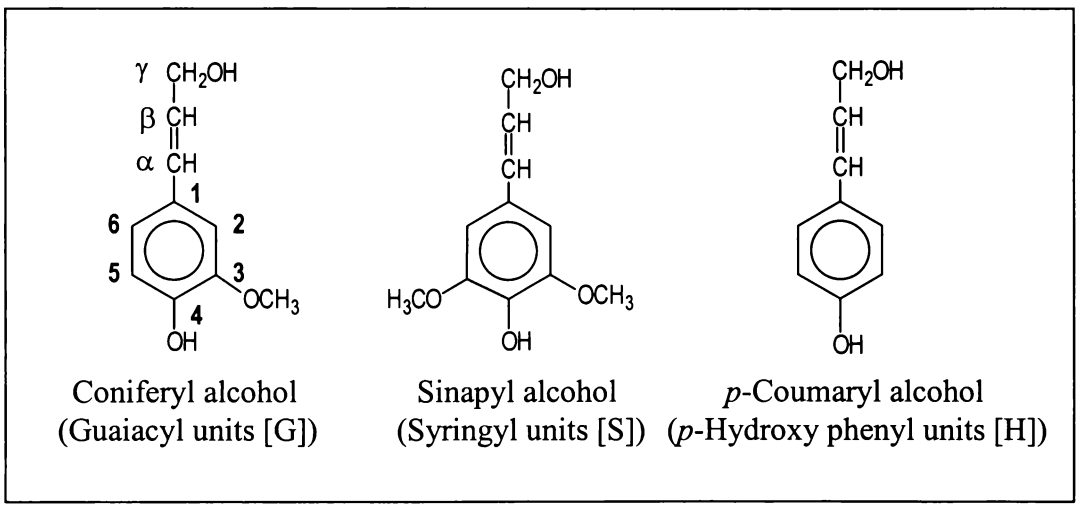

Figure (2.5). Building units of lignin

Most softwood lignins are classed as G-lignins since they are mainly made up of G units with a small amount of H units (<5%) (Fengel & Wegener 1984). However, there is much debate concerning the existence of S units in softwood lignin. Based on results from degradation studies estimates of 2–5% have been made (Freudenberg 1964, Adler 1977, Glasser & Glasser 1981). However, more recent results suggest that if S units are present their levels are extremely low (<0.1%)(Obst & Landucci 1986).

Hardwoods, on the other hand, are generally classed as SG-lignins since they contain significant amounts of both S and G units (Fengel & Wegener 1984). The syringyl content of typical hardwood SG-lignins varies between 20 and 60%, and the range is even wider if herbaceous plants are also included (10-65%). Hardwood lignins also contain a small quantity of H units.

Lignin polymerisation is initiated by hydrogen abstraction from the phenol precursors forming phenoxy radicals, which exist in a number of mesomeric forms. The lignin polymer is formed through the coupling of the different radicals. Electron densities determine the frequency of the different sites involved in the coupling reactions and steric factors determine the stability, and thus the frequency, of each possible combination. For example; the highest electron density resides on the phenolic oxygen atom, thereby, favouring the formation of aryl ether linkages, such as the β-O-4 linkage (Fengel & Wegener 1984, Figure 2.6). The structures can be classed as uncondensed or condensed. The uncondensed structures contain easily hydrolysable ether bonds (eg β-O-4, α-O-4), whereas, the condensed structures contain linkages that are not easily hydrolysed (eg. β-1, β-β, 5-5, 4-O-5). Condensed structures are important in terms of pulping and bleaching because they are not easily removed in these processes.

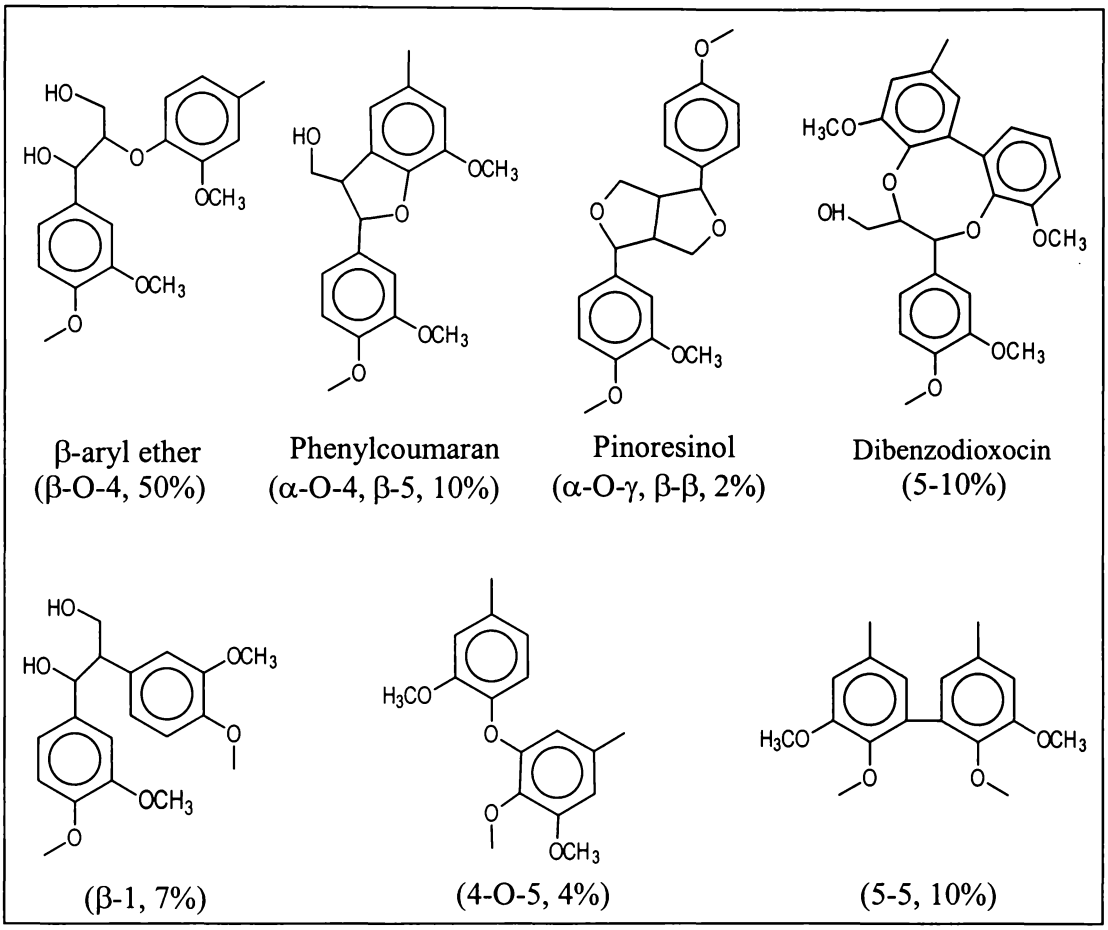

Figure (2.6). Frequencies of some typical linkages in softwood lignin (Adler 1977), Note the dibenzodioxocin linkage was more recently identified (Karhunen et al. 1995), (Uncondensed structures: β-O-4, α-O-4; Condensed structures: β-1, 4-O-5, 5-5)

Based on results from degradation studies many statistical models for the lignin structure have been proposed. The most well known is the model for spruce lignin derived by Adler (1977). This is shown in Figure (2.7). Although this model was proposed 20 years ago it is still considered to be a reasonable representation of the lignin polymer, however, it cannot be considered as gospel. As techniques for the analysis of lignin improve new structural elements maybe discovered. One example of this is the identification of a novel structure containing an 8-membered ring in softwood lignin using 2D NMR (Karhunen et al. 1995). This structure (dibenzodioxocin) is shown in Figure (2.6).

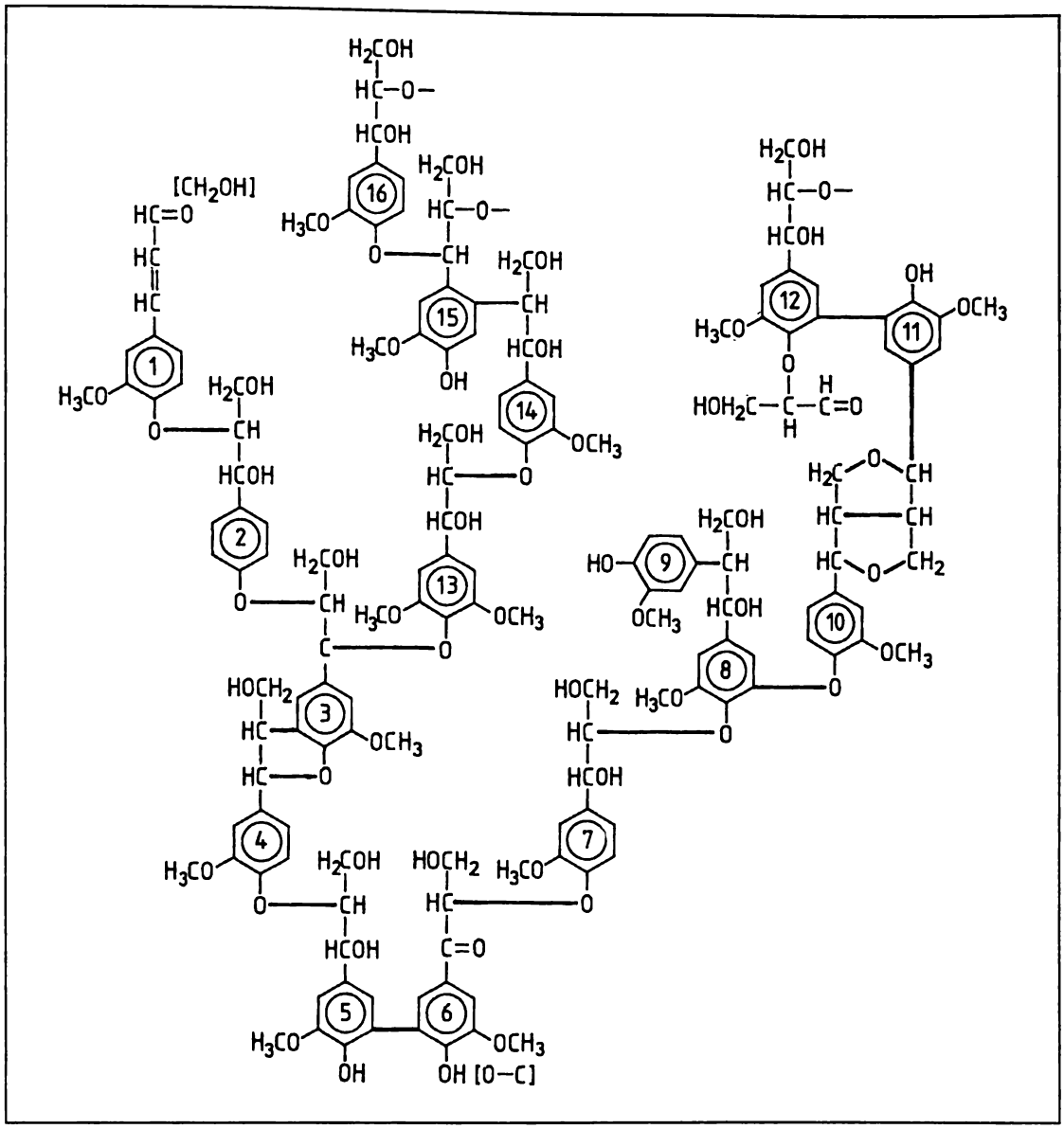

Figure (2.7). Model representing the structure of softwood lignin (Adler 1977)

In its natural state in the wood cell wall, lignin is referred to as either *protolignin* or *native lignin* and is remarkably thermoplastic. It is also very much less hydrophilic than either cellulose or hemicellulose, almost to the point of being hydrophobic. Lignin in the pulp fibre has the general effect of inhibiting water absorption and fibre swelling, and can render the fibre less responsive to mechanical treatment. However, lignin is thermoplastic and this characteristic is utilised during mechanical pulping when high temperatures are used. Chemical pulping processes are based on reactions that degrade, solubilise, and remove the lignin from the cell fibre. Thus, the structure of lignin and its association with the other components of the cell wall are important since they influence the behaviour of wood during pulping and bleaching. These are discussed in the next sections.

2.2 Chemical Pulping of Wood

The production of pulp for papermaking is the most important chemical conversion of wood in industry, over one third of harvested wood is used for the production of paper. A large variety of paper types and paper grades are produced globally (Table 2.1, Reeve 1996a).

Table (2.1). Pulp and paper production in 1994 (million tons)

	Total paper and board	**Chemical pulp**[1]	**Mechanical pulp**
USA	80.6	49.9	5.3
Japan	28.5	8.5	1.6
Canada	18.3	12.8	10.9
Germany	14.4	0.7	1.2
Finland	10.9	5.8	4.1
Sweden	9.3	7.6	2.9
France	8.6	1.8	0.9
Brazil	5.7	5.4	–
New Zealand[2]	0.8	0.7	0.6

1. Chemical pulp includes; bleached and unbleached kraft pulp, and bleached sulfite pulp.
2. From: New Zealand Forestry Statistics 1995 (Ministry of Forestry). Chemical pulp includes; bleached and unbleached kraft and unbleached semichemical pulp.

Pulping processes can be grouped into three categories (mechanical, semichemical, and chemical) according to the method used to release the fibre (Table 2.2). Mechanical pulping liberates fibres by application of mechanical energy with little or no chemical treatment. The two main examples are *stone groundwood* and *refiner mechanical.* Chemical processes liberate fibres by reacting with the lignin. As a result, lignin is either dissolved or softened to the point that fibres fall apart with only minor mechanical treatment. Semichemical processes combine both mechanical and chemical processes to liberate the fibres. They are mainly used for production of corrugating medium and linerboard.

General trends in the pulping industry over the past 10–20 years have been the increased use of hardwood species, utilisation of the whole tree, and the use of both non-wood fibres and secondary fibres from recycled paper. Technologies have been (and still are) aimed at improving pulp quality, bleachability, and yield while reducing energy consumption, chemical consumption, pollution, costs, and demand on the raw material. One major area is the development of sulfur-free pulping and chlorine-free bleaching processes in the attempt to reduce air and water pollution.

These and other aims form the background to an increasing diversification of pulping processes (Table 2.2). Important modifications are alkaline pulping with non-sulfur additives (eg soda-anthraquinone), modified refiner mechanical pulping (eg CTMP), or sulfur-free pulping (eg soda-oxygen pulping, organosolv pulping). However, at present the fully chemical processes remain dominant. Chemical pulping processes are discussed further below with the main emphasis on kraft pulping.

Table (2.2). Pulping processes (Fengel & Wegener 1984)

Pulp type		Treatment	Mechanical treatment	Wood[1]	Yield (%)
Mechanical pulping					
Stone grinding:	Ground wood (SGW)	None	Grindstone	S	93-99
	Steamed groundwood	Steam	Grindstone	S	80-90
Refiner pulping	Refiner mechanical (RMP)	None	Disk refiner	S	93-98
	Thermomechanical (TMP)	Steam	Disk refiner (pressure)	S	91-98
	Asplund	Steam	Disk refiner	S	80-90
Chemimechanical and Chemi-thermomechanical					
Stone grinding	Chemigroundwood	Neutral sulfite or acid sulfite or Na_2s + NaOH	Grindstone	S/H	80-92
Refiner pulping	Chemi-refiner (CRMP)	NaOH or $NaHSO_3$ or alkaline sulfite or acid sulfite	Disk refiner	S/H	80-90
	Chemi-thermo (CTMP)	Steam + Na_2SO_3 or steam + NaOH	Disk refiner (pressure)	S/H	65-97
Biomechanical					
Refiner	Biomechanical	Fungal	Disk refiner	S/H	85-90
Semichemical					
	Neutral sulfite (NSSC)	Na_2SO_3 + Na_2CO_3 or $NaHCO_3$	Disk refiner	H	65-90
	Cold Soda	NaOH	Disk refiner	H	85-92
	Alkaline sulfite	Na_2CO_3 + Na_2S+ NaOH	Disk refiner	H/S	80-90
	Sulfate	NaOH + Na_2S	Disk refiner	H/S	75-85
	Soda	NaOH	Disk refiner	H	65-85
	Green liquor	Na_2S + Na_2CO_3	Disk refiner	H	65-85
	Nonsulfur	Na_2CO_3 + NaOH	Disk refiner	H	65-85
High yield chemical					
	Kraft	Na_2S + NaOH	Disk refiner	S/H	55-65
	Sulfite	Acidic sulfite (Ca, Na, Mg) or bisulfite (Na, Mg)	Disk refiner	S	55-70
Full chemical					
Alkaline pulping	Kraft (+ AQ)	NaOH + Na_2S (+AQ)	Mild to none	S/H	40-55
	Kraft (polysulfide)	(NaOH + Na_2S_x)	None	S/H	45-65
	Soda	NaOH	None	H	40-55
	Soda + AQ	NaOH + AQ	Mild to none	H	45-55
	Soda-oxygen, 2-stage	NaOH, oxygen	Disk refiner	H	45-60
Sulfite pulping	Acid sulfite	Acidic sulfite (Ca, Na, Mg)	Mild to none	S	45-55
	Bisulfite	Bisulfite (Na, Mg, NH_3)	Mild to none	S/H	45-60
	Neutral sulfite	Neutral sulfite	Mild to none	S/H	45-60
	Magnefite	Mg-bisulfite	Mild to none	S/H	45-60
	Multi-stage	Na_2SO_4 + $NaHSO_3/SO_2$ or $NaHSO_3$ + SO_2/Na_2CO_3	None	S/H	45-55
Organosolv pulping	Alcell	50% ethanol	None	H	
	Organocell	MeOH + NaOH + AQ	None	S/H	
	ASAM	MeOH + NaOH + Na_2SO_3, + AQ	None	S/H	
Dissolving pulping	Acid sulfite	Acid sulfite (Ca, Na)	None	H/S	35-42
	Prehydrolysis kraft	NaOH + Na_2S after prehydrolysis	None	H/S	30-35

2.2.1 Chemical pulping processes

2.2.1.1 Sulfite pulping

The sulfite process has had a significant role in the production of chemical pulps for over 100 years. However, its role has declined in recent years with the growth of the kraft process. Although, the sulfite process has gone into decline it has certain advantages over the kraft process. Sulfite pulps are brighter so can be used in many grades without bleaching. They are also easier to bleach so shorter bleaching sequences can be used. High yields and the low cost of chemicals are also advantages. Major disadvantages include: the limited number of wood species that can be pulped; the poorer strength properties compared to kraft pulps; and the difficulty of recovering the bleaching chemicals (Bryce 1980a).

Sulfur dioxide is the essential chemical in all the sulfite processes. In solution it exists in different forms (Figure 2.8). The aqueous sulfur dioxide solutions are combined with a base to form the pulping liquor.

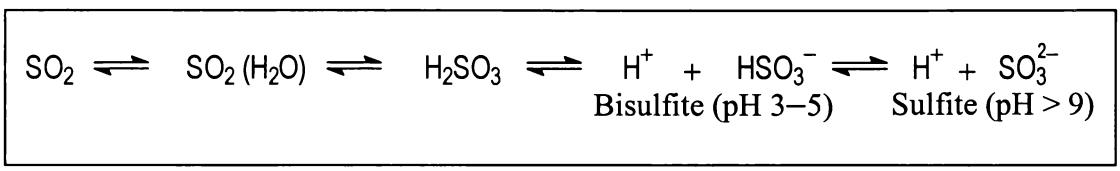

Figure (2.8). Forms of sulfur dioxide in solution

Acid bisulfite pulping is carried out within a pH range of 1.2–1.5 and the liquor contains an excess amount of SO_2 which is not consumed during pulping. *Bisulfite* pulping is carried out within a pH range of 3–5 and the liquor contains equal amounts of free and combined SO_2. *Neutral sulfite* liquors contain a small excess of alkali and are mainly used for the production of semimechanical pulps. The *Alkaline sulfite* process uses a combination of sodium sulfite and sodium hydroxide at pH 13.

Aqueous sulfur dioxide is the effective pulping chemical in acidic sulfite pulping, while in neutral sulfite pulping; bisulfite and sulfite promote delignification. Under acidic conditions initial cleavage occurs at the α-carbon, of both phenolic and non-phenolic units, followed by sulfonation of the resultant carbonium ion. Sulfonation increases the hydrophilicity of the lignin, thus, increasing the solubility in the pulping liquor (Fengel & Wegener 1984). In neutral sulfite pulping the initial reaction is the addition of sulfite or bisulfite ions to the quinone methide intermediates of phenolic units. The resulting α-sulfonic acid structures undergo sulfitolytic cleavage of β-aryl ether linkages (Fengel & Wegener 1984).

2.2.1.2 Alkaline pulping

The *kraft* process and the *soda* process are the two main alkaline pulping techniques. Sodium hydroxide is the main cooking chemical in both processes, while sodium sulfide is an additional component of kraft pulping liquors.

The kraft process is by far the most important process in industry today (Figure 2.9). This is mainly due to the higher yields and superior strength properties of the pulps. The kraft process also dominates over the sulfite process due to lower sulfur charges and increased recoverability of the chemicals involved.

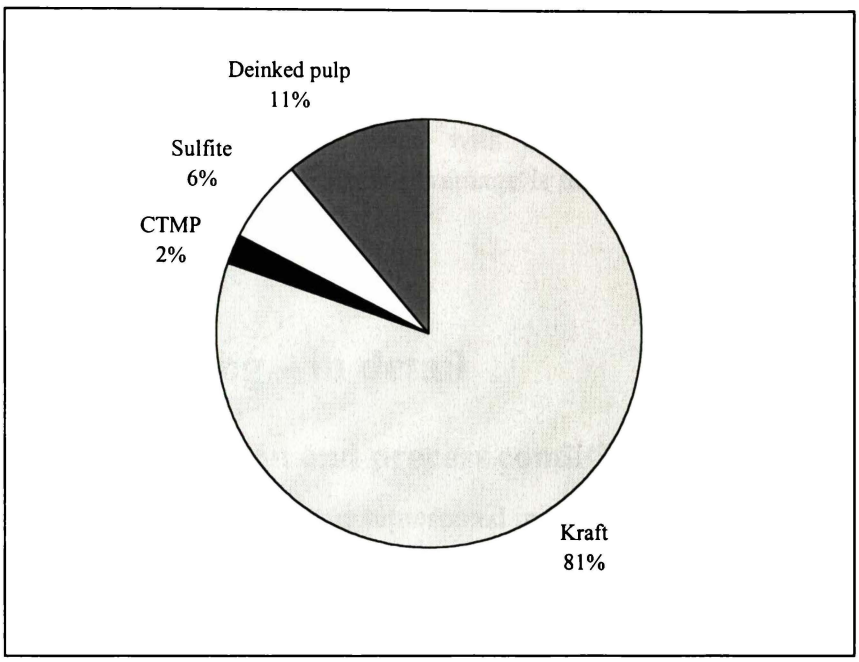

Figure (2.9). Global bleached fibre capacity in 1994 (Reeve 1996a)

Kraft pulping has rapidly grown in popularity since the early 1930s. The reasons for this include the wide range of wood types that can be pulped; the high tolerance for large amounts of extractives, wood decay, and bark; the short cooking times; the excellent pulp strength; and the well established spent liquor recovery procedures (Bryce 1980b).

The main drawbacks are the odour problems; the low yields compared to sulfite pulping; and the dark colour of the unbleached pulp and effluents.

2.1.2.3 Organosolv pulping

The global dominance of kraft pulping is due to its reliability in producing strong, bleachable fibres for papermaking. However, environmental and economical limitations associated with the process are stimulating a search for alternatives (Aziz & Sarkanen 1989). Sulfur-free organosolv processes are potential alternatives to kraft pulping.

Organosolv pulping methods are based on the use of organic solvents as part of the pulping liquors to aid the dissolution of lignin. They can be divided into either *acid-catalysed* or *base-catalysed* processes. The Alcell process is an example of acid-catalysed organsolv pulping (Pye & Lora 1991). A typical process is run in 50% aqueous ethanol at 195°C and acetic acid, which is released from the wood during pulping, functions as the catalyst. A novel organosolv process based on the use of peroxyformic acid has also recently been proposed (Poppius et al. 1986). Peroxyformic acid is generated from the reaction between formic acid and hydrogen peroxide. Acid-catalysed organosolv processes are more suited for hardwoods than softwoods.

The ASAM process and the Organocell process are examples of based-catalysed organosolv pulping (Stockburger 1993). The former utilises NaOH, Na_2SO_3, and anthraquinone in aqueous methanol (30%) as the pulping liquor, whereas the latter uses NaOH and anthraquinone in methanol (30%). These processes are suitable for both hardwoods and softwoods; giving pulps with strength properties comparable to corresponding kraft pulps. An additional advantage is that the pulps are easier to bleach than kraft pulps.

2.2.2 Kraft pulping – in detail

2.2.2.1 Pulping operation and process conditions

An outline of the kraft process flow is represented in Figure (2.10). Chips and liquor are charged to the digester at a temperature of 70–80°C. The digester is then heated to within the range of 160–180°C, and held at that temperature until the desired delignification has been achieved. The cooking process can either be batch or continuous. In batch cooking, the chips are cooked in individual digesters with loading, cooking, and dumping done in sequence. In the continuous process, the chips and liquor are fed into the top of the digester and the chips move downward in a continuous manner discharging at the bottom. The total cooking time is determined by the flow rate.

At the end of the cook, the cooked chips and spent liquor are discharged to a blow tank under pressure, causing the wood chips to break up into individual fibres. The larger incompletely cooked particles (knots) are removed on perforated screens and sent back to the digester for re-cooking. The spent liquor is removed by countercurrent washing and sent to the recovery system. The pulp is diluted to low consistency and further screened. The cleaned stock is then thickened and stored for further processing.

The filtrate from the pulp washers is evaporated to about 60% solids. To minimise odour problems caused by loss of hydrogen sulfide, the black liquor is often oxidised before the evaporation step. Makeup sodium sulfate is added to the *strong black liquor* to compensate for the loss of sulfidity. The liquor is then incinerated in the recovery furnace. The organic material is burned generating heat, which is utilised by other parts of the mill. The inorganic material is recovered as a molten smelt and dissolved in water generating the *green liquor*. After clarifying, by removal of insoluble material, the green liquor is causticised by reaction with calcium hydroxide (slaked lime) to convert the sodium carbonate to sodium hydroxide. After clarifying, the resulting *white liquor* is ready for use as fresh liquor in the digester. The lime is regenerated by incineration.

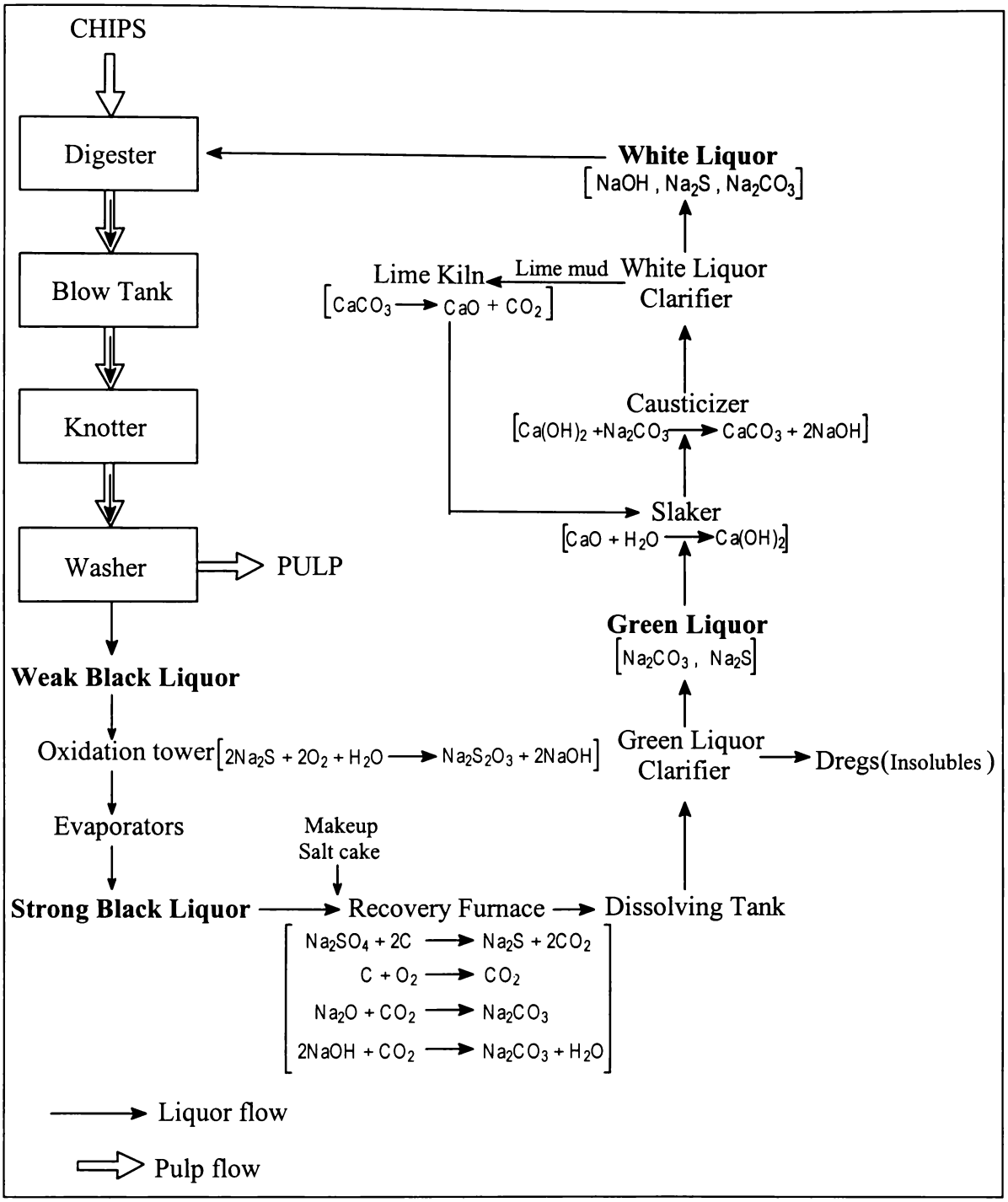

Figure (2.10). Schematic flow diagram of the kraft process

Pulp properties such as yield, lignin content, tensile strength, and opacity, are dependent on both the wood composition and process conditions. The fibre dimensions, chemical composition, and wood quality are the main variables associated with the wood. Process variables include liquor-to-wood ratio; composition of the liquor; charge and concentration of cooking chemicals; time and temperature profile of the cook; chip dimension; and impregnation. These parameters are highly dependent on each other and only an optimum coordination between them yields the desired pulp (Bryce 1980b).

2.2.2.2 Kinetics of delignification

Delignification during kraft pulping can be divided into three phases depending on the rate of lignin dissolution (Figure 2.11, Axegard et al. 1978).

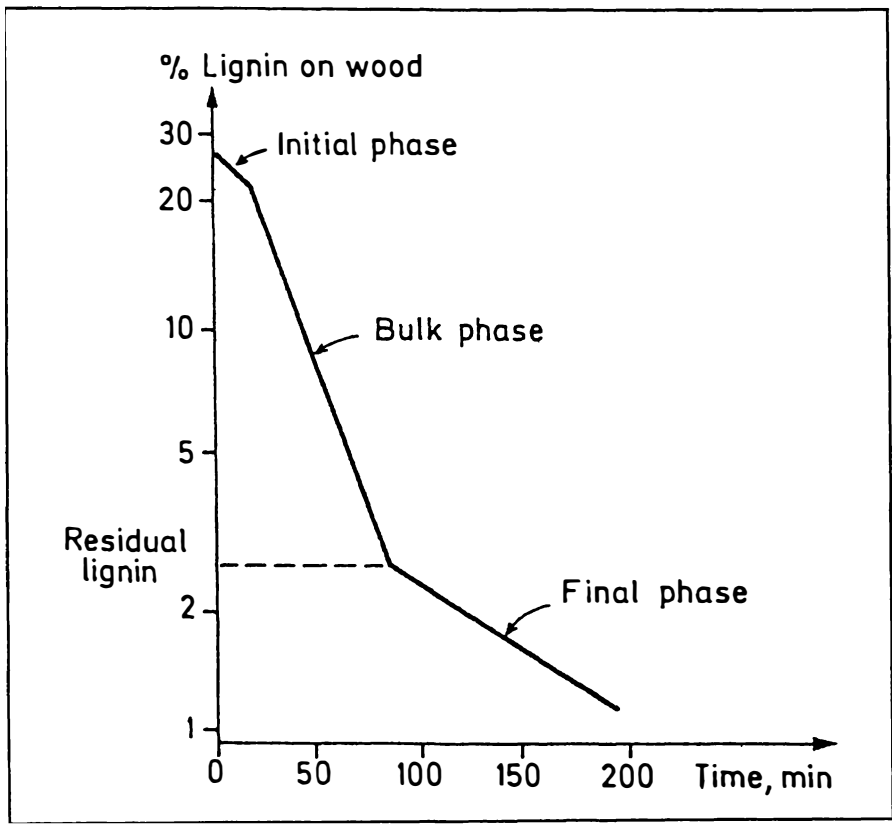

Figure (2.11). Kinetics of delignification during kraft pulping (dotted line indicates lignin content at transition point between bulk and residual delignification phases)

The *initial phase* comprises the pulping treatment up to a temperature of about 150°C and results in dissolution of 20–25% of the total lignin present in the wood (Wilder & Daleski 1965). The delignification rate is first-order with respect to lignin concentration and independent of both hydroxide and hydrosulfide ion concentrations. The reaction is believed to be diffusion controlled rather than chemically controlled due to the low activation energy (Olm & Tistad 1979). The *bulk phase* includes the final heating period and the cooking treatment at the maximum temperature. The main proportion of lignin present in the wood is dissolved during this phase. The reaction is pseudo-first order with respect to residual lignin at constant hydroxide and sulfide concentrations. However, the rate is dependent on both the hydroxide and sulfide ion concentrations. The rate of bulk delignification appears to be chemically controlled (Wilder & Daleski 1965). The *residual phase* is characterised by a much slower rate of delignification and leads to the dissolution of the final 10–15% of the lignin. The rate is dependent on the hydroxide ion concentration and the temperature, but almost independent of the sulfide ion concentration (Lindgren & Lindstrom 1996).

2.2.2.3 Lignin reactions

Model compound experiments have been applied extensively to the study of the delignification reactions that occur during pulping (Gierer & Noren 1962, Gierer & Kunze 1961, Gierer et al. 1972, Gierer & Smedman 1965, Gierer & Smedman 1971, Miksche 1972, Adler et al. 1964). Compounds containing only one, or a few, of the many types of linkages involved in the lignin molecule have been subjected to appropriate treatments. Such procedures remove the complexity involved in the real system, allowing investigation of a single reaction. Individual reactions are pieced together to give an overall picture of what is occurring during pulping. However, studies such as these are oversimplified in that certain interactions between different reactive groups occurring in the real system are omitted. Also, the influence of the physical association between the different polymers within the cell wall is neglected. Model systems have, on the other hand, played an important role in the elucidation of delignification reactions. Other evidence, such as the behaviour of milled wood lignin and its modified forms (Gierer et al. 1964, Gierer et al. 1965, Gierer & Noren 1980) and the structure of spent liquor lignins (Gierer & Lindeberg 1980, Gellerstedt & Gustafasson 1987) also give support to the proposed reaction mechanisms.

The various reactions during kraft pulping can be divided into two major groups. The first are degradation reactions, which lead to the liberation of lignin fragments and ultimately to their dissolution. The second are condensation reactions between lignin fragments leading to an increased molecular weight and reduced solubility of the fragments.

Degradation

During kraft pulping the dissolution of lignin is achieved mainly by breaking the lignin macromolecule into smaller soluble fragments. All types of aryl ether bonds are cleaved in the alkaline medium. Aryl-alkyl and alkyl-alkyl, carbon-to-carbon bonds are cleaved to some extent, however, diaryl ether and diaryl carbon-to-carbon bonds are stable. Since β-aryl ether linkages are the dominant linkage in lignin, the cleavage of these bonds predominantly contributes to lignin degradation.

By comparing the kinetics at each phase with those of individual model compounds a tentative correlation has been made between the individual reactions and the overall course of delignification that occurs during pulping (Gierer 1980).

The initial phase of delignification involves the rapid cleavage of both α- and β-aryl ether bonds in phenolic lignin units. Bonds of the α-aryl ether type are cleaved by a base-catalysed elimination reaction via a quinone methide intermediate (Figure 2.12A) (Gierer & Noren 1962).

Phenolic units containing β-aryl ether bonds are cleaved through the action of the hydrosulfide ions present during kraft pulping. Units with an eliminatable group in the α-position (hydroxyl or alcohol) will form quinone methide intermediates equivalent to the above reaction. This step is followed by the reversible addition of hydrosulfide ions (Figure 2.12B) giving benzyl mercaptan structures, which eliminate the β-substituent via an episulfide intermediate (Gierer & Smedman 1965). The thiarane type structures produced eliminate sulfur yielding the corresponding *p*-hydroxystyrene structures (Brunow & Miksche 1969).

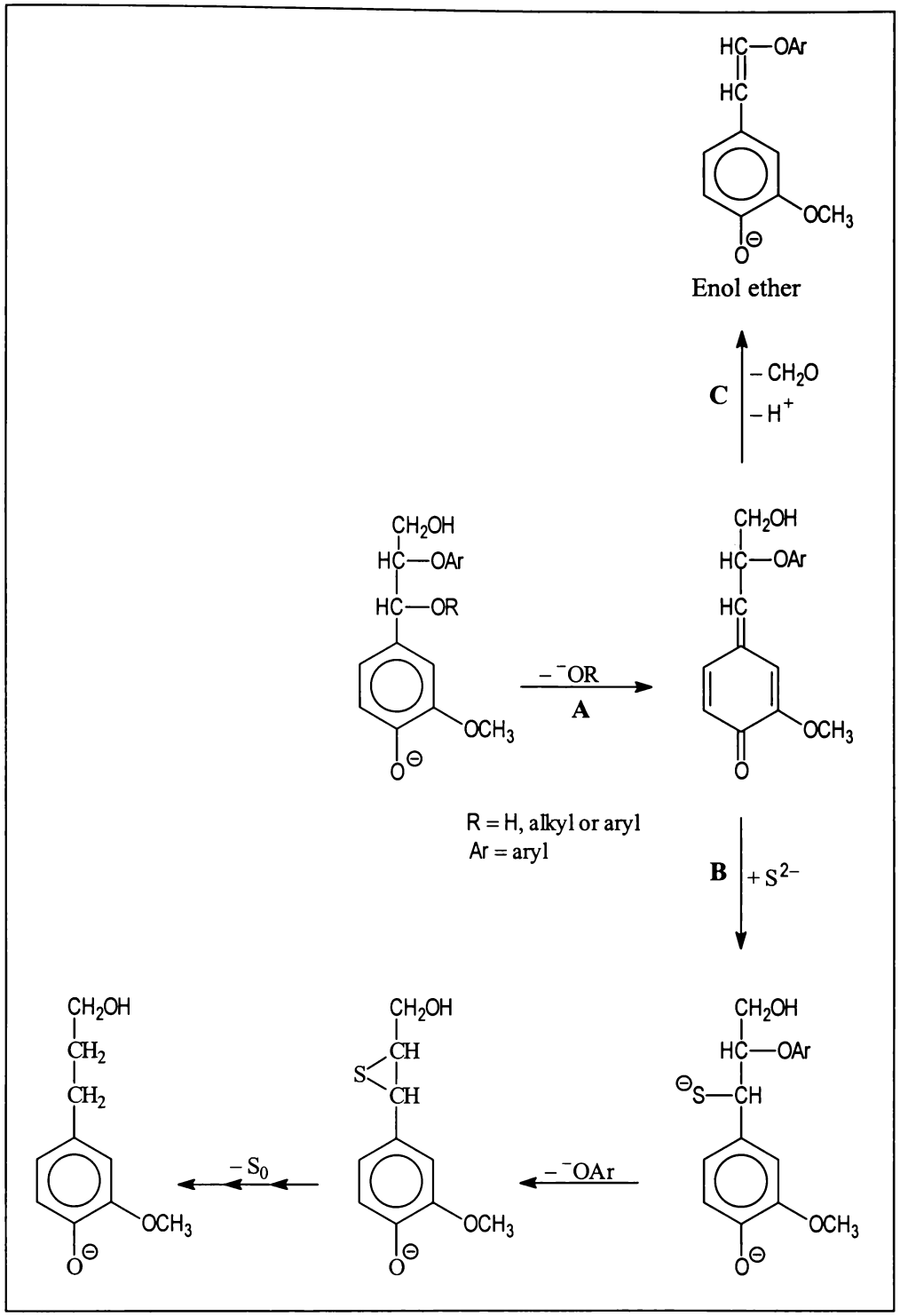

Figure (2.12). Reaction of phenolic β-aryl ether structures during kraft pulping

A competing reaction can also take place. The quinone methide intermediate formed from the above α-elimination may also eliminate the terminal hydroxymethyl group (as formaldehyde) yielding an alkali stable enol ether type structure (Figure 2.12C, Gierer & Noren 1962).

The alkali cleavage of α-aryl ethers and the sulfidolytic cleavage of β-aryl ethers in phenolic units generate new phenolic units, which can then undergo the same types of cleavage reactions. Therefore, degradation of lignin during the initial phase may continue until it reaches units which are not of the α- or β-aryl ether type.

The rate determining reaction in the bulk phase has been attributed to the cleavage of β-aryl ether groups in non-phenolic units (Gierer 1980). The conversion into the quinone methide intermediate, and thus the cleavage of the β-aryl ether bond is precluded in non-phenolic units. However, β-aryl ether bonds in non-phenolic units with a free hydroxyl group on one of the adjacent carbons are cleaved (Gierer & Noren 1962). Internal nucleophilic attack by one of these hydroxyl anions at the β-carbon facilitates the elimination of the β-aroxy group via an epoxide intermediate. A similar reaction occurs in non-phenolic β-hydroxy, α-aryl ether structures with the elimination of the α-group (Gierer & Noren 1962).

Cleavage of β-aryl ether linkages in non-phenolic units liberates new phenolic units, which may then undergo elimination reactions as in the initial phase. As a result, lignin degradation in the bulk phase, initiated by the rate determining cleavage of β-aryl ether linkages, is extended to the point where dissolution of the lignin takes place.

The possible reactions contributing to the final phase are not known, nor the reasons for the reduced delignification rate. However, the residual phase delignification could be attributed to alkali promoted fragmentation of carbon-to-carbon bonds (Gierer 1980) or solvolytic cleavage of ether linkages not assisted by neighbouring groups. The drop in rate of these reactions could be due to the reduced alkalinity at the end of the cook. Additionally, competing condensation reactions become more important, thereby retarding lignin dissolution further.

Cleavage of carbon-to-carbon bonds only makes a minor contribution to lignin fragmentation. One example is the elimination of the γ-carbon as formaldehyde. Additionally, cleavage of Cα–Cβ and α-aryl bonds can occur via addition of water across conjugated double bonds. This is followed by a base-catalysed reverse-aldol reaction and ultimately results in bond cleavage (Gierer 1980).

Methyl aryl ether bonds are also cleaved extensively during kraft pulping. The strong sulfur containing nucleophiles present in the liquor partake in S_N2 displacement reactions affording odorous mercaptan and dimethyl sulfide (Mckean et al. 1965).

Condensation

Various condensation reactions of the partly degraded lignin also take place during pulping. Carbanions formed in the degraded lignin compete with the cooking liquor nucleophiles for reactive sites in the lignin (Gierer 1970). Condensation reactions can be split into *primary* or *secondary* depending on the site of attack.

Primary condensations involve quinone methide intermediates formed from elimination of the α-substituent from phenolic units (Figure 2.13A). Secondary condensations (Figure 2.13B) involve extended quinone methide intermediates formed from conjugated phenolic units (eg. coniferyl alcohol type structures). The reversible addition of a carbanion to the quinone methide is followed by an irreversible proton or proton and formaldehyde elimination resulting in the re-aromatisation of the added unit. Carbanions derived from various phenolic units can also condense with formaldehyde to produce diaryl methane type structures (Figure 2.13C).

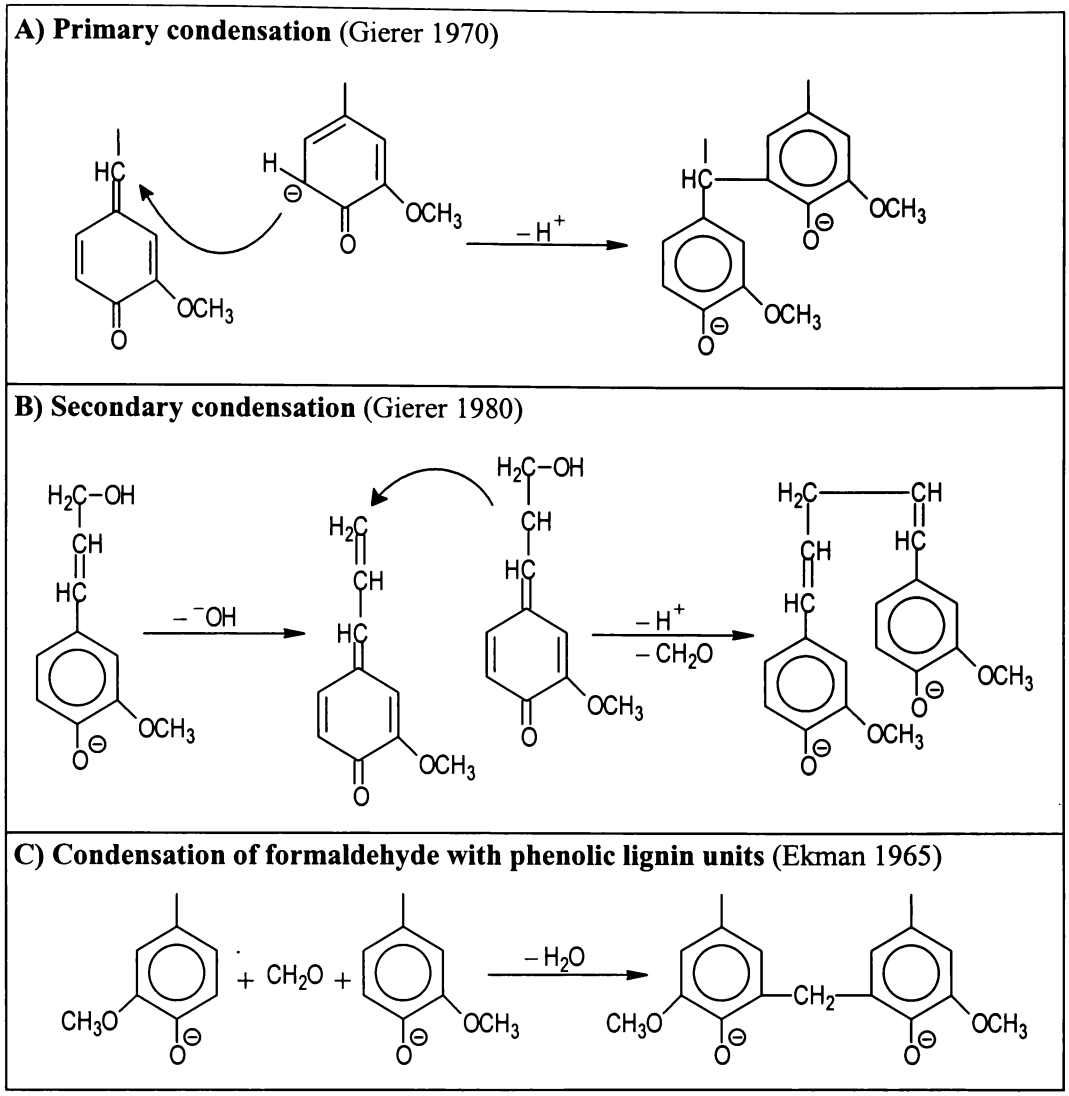

Figure (2.13). Examples of lignin condensation reactions

The relative importance of the various competing condensation reactions is determined by the alkalinity and nucleophilicity of the reactants involved (Gierer 1980). High alkalinity promotes degradation and suppresses condensation of the quinone methide by favouring formaldehyde or proton elimination. High sulfidity also suppresses condensation reactions by protecting the quinone methide intermediates from attack by carbanions in the lignin fragments. Therefore, condensation reactions become increasingly more important towards the end of the cook where the rate of delignification decreases, the concentration of lignin fragments increases, and the concentration of cooking chemicals decreases.

2.2.2.4 Process modifications

In the conventional kraft process, the cook is stopped before reaching the residual phase to prevent severe losses in pulp yield and strength. Softwoods are pulped to kappa numbers of 32–28 and hardwoods to 18–22. This is equivalent to a lignin content of 4–5% for softwoods and 2–3% for hardwoods. The residual lignin is then removed by bleaching. Several methods have been investigated to improve pulp yield and reduce residual lignin content. Reducing the residual lignin content reduces the demand of the bleach plant and consequently reduces the environmental impact of bleaching.

Additives

One method for improving yield is to reduce carbohydrate degradation by addition of chemicals that stabilise the carbohydrates to alkali degradation. The reducing end group plays an important role in the degradation of carbohydrates during alkaline pulping. The majority of treatments aimed at reducing carbohydrate degradation are centred on the modification of this group. The reagents used for this purpose include polysulfide, hydrogen sulfide, anthraquinone, and sodium borohydride.

Polysulfide is formed in the liquor by addition of elemental sulfur. Reactive end groups are oxidised to alkali stable aldonic acid. Under optimal conditions a yield increase of 6% on wood can be achieved (Bryce 1980b). However, the trend towards lower sulfidity and reduced odour emissions has limited its application. Treatment of the chips with hydrogen sulfide prior to cooking has a similar effect on yield (Bryce 1980b). The reactive carbohydrates are stabilised by a reductive thiolation of the reducing end. The major problem is the handling of hydrogen sulfide at elevated temperatures and pressures without causing air pollution. Sodium borohydride is also an effective reducing agent, however, cost has prevented its commercial use.

The additive with the greatest potential is anthraquinone and its derivatives. Anthraquinone increases both yield and delignification rate at very low concentrations (0.05–1.0% on wood), leading to reduced lignin levels for equivalent cooking conditions. Carbohydrates are stabilised by oxidation of the reducing end groups to aldonic acids. Anthraquinone increases delignification by catalysing the cleavage of ether linkages in lignin and also by suppressing the condensation reactions.

Low lignin pulping

In the manufacture of bleached kraft pulp, it is advantageous to remove as much of the lignin as possible in the pulping stage. This leads to a reduced demand on the subsequent bleaching stages in terms of chemical consumption and effluent load. Benefits also arise from the increased heat generation from incineration of the lignin in the recovery furnace.

Studies (Heimburger et al. 1988a, Sjoblom et al. 1983a, Sjoblom et al. 1988, Backstrom et al. 1996) have shown that the following conditions are essential for improved selectivity. The concentration of effective alkali ($NaOH + \frac{1}{2}Na_2S$) should be lowered initially and kept more uniform over the whole cook. The sulfidity should be kept as high as practical, especially during the initial phase and the beginning of the bulk phase. The content of dissolved lignin and the ionic strength should be kept low, especially at the end of the cook. A decrease in cooking temperature is also advantageous.

Batch processes achieve this by dividing the cook into two distinct periods between which the old liquor is replaced with liquor containing less lignin. Mill trials (Sjoblom et al. 1983b) at the ASSI mill in Karlsong (Sweden) showed that pulps with a kappa number of 25 could be obtained without affecting pulp properties. A 20% reduction in chlorine and a 24.9% reduction in total organic chlorine (TOCl) was achieved. One problem with this technology is the increased steam consumption and cooking times. These drawbacks have been overcome by coupling this technology with processes utilising heat transfer.

For example, in cold blow cooking, the hot black liquor is displaced with cold wash liquor at temperatures below 100°C. The displaced black liquor is used to heat white liquor for a subsequent cook. Mill trials showed that kappa numbers could be reduced to 25 while achieving a 30–35% decrease in steam consumption and a 24.9% reduction in TOCl (Heimburger et al. 1988a).

Concurrent with this, was the development of another extended delignification process by Beloit Corporation called rapid displacement heating (Heimburger et al. 1988a). This process involves the recovery of digester heat by displacement and the accumulation of the black liquor with the wash liquor. Special heat exchangers are employed to heat the white liquor, which is stored in another accumulator. The pulp is also blown with compressed air for a faster blow and additional savings on steam. Results using RDH indicate a kappa number reduction from 33.5 to 18.9 on softwood kraft.

More recently another process has been developed; that is the Super Batch process (Kovasin & Tikka 1993). Kappa numbers as low as 10–15 can be reached for softwoods without significant loss of pulp strength. The process begins with two successive black liquor treatments; warm black liquor impregnation followed by a hot black liquor treatment. Part way through the cook hot white liquor is introduced along with black liquor, displacing the previously added black liquor. The high initial temperature and pretreated wood chips results in rapid delignification.

The principles of extending delignification in a continuous digester are implemented by adding the alkali at more than one place and partial countercurrent flow to reduce the dissolved lignins at the end of the cook (Johansson et al. 1984). The process is known as modified continuous cook (MCC). Reductions of 8 kappa units for softwoods and 4–5 kappa units for hardwoods can be achieved.

2.3 Bleaching Chemical Pulps

The principal aim of bleaching is to increase the brightness and colour stability of the final paper product. The majority of colour in pulps arises from chromophoric groups present in the residual lignin. Bleaching can be performed either by converting the chromophoric groups without loss of material (*lignin-preserving bleaching*), or by further delignification (*lignin-removing bleaching*).

Industrial bleaching processes involve multistage sequences adapted to the specific pulps for which they are used. They utilise the different oxidising or reducing properties of the different bleaching chemicals. To preserve the advantage of high yields, mechanical pulps are generally bleached by lignin-preserving bleaching. Peroxide and dithionite are most commonly used, giving a final brightness of about 80% (Fengel & Wegener 1984). More recently, ozone has been investigated as an alternative bleaching reagent for mechanical pulp (Kibblewhite et al. 1980). However, ozone is more expensive and less specific. Similar sequences can be used for high yield semichemical pulps (Loras 1980) but these are more often used in unbleached products such as corrugating material.

Chemical pulps are bleached using sequences which completely remove the residual lignin achieving brightness levels greater than 90% (Fengel & Wegener 1984). Industrial processes generally consist of several oxidative steps including at least one alkali extraction stage to remove oxidised residual lignin. Sulfite and bisulfite pulps are easier to bleach than kraft pulps. Furthermore, hardwood pulps usually require fewer bleaching stages than softwood pulps.

In earlier days of paper production chlorine was the major bleaching chemical used. However, public concern with the environmental impact of chlorine has prompted the removal of chlorine-based bleaching procedures. Ongoing research into the development of elemental chlorine free (chlorine dioxide in place of chlorine) and totally chlorine free, oxygen-based (peroxide, oxygen and ozone) procedures is widespread.

Chlorine dioxide has replaced chlorine in the first stage for the majority of industrial processes reducing the environmental impact of bleach plant effluents. The use of chlorine dioxide has the added advantage of improving pulp strength while lowering chemical consumption. Oxygen is the most widely studied alternative because it is a cheap bleaching agent. However, during oxygen bleaching the pulp carbohydrates are also degraded by a certain extent so delignification is limited to about 50%, thus oxygen must be used in conjunction with other bleaching reagents to achieve high brightness. Oxygen delignification is generally used as a pre-bleaching stage followed by additional bleaching stages. Hydrogen peroxide and ozone are other bleaching reagents important for totally chlorine free bleaching.

2.3.1 Bleaching reactions

Pulps can be bleached by a large number of processes, which comprise of a combination of different steps. Studying the chemistry that occurs within pulps during bleaching is difficult for several reasons. First, the number of possible combinations of each reagent into the final bleaching sequence is large. Second, the structure of the residual lignin remaining in the pulp after each stage is not well understood, and finally, the other oxidative species arising during each individual bleaching step can also partake in the course of reactions (Table 2.3).

For the majority of studies simplifications have been made. For example model compounds representing certain structures thought to be present in the pulps have been extensively used in the study of bleaching reactions. Additionally, the bleaching reagents are generally studied individually. These simplifications remove the complexity associated with the heterogeneity of the lignin structure and the interaction of the bleaching reagents, respectively.

Bleaching chemicals can be classed into three categories with respect to their initial reaction; that is cationic, radical or anionic as shown in Table (2.3) (Gierer 1990a, 1990b).

Table (2.3). Reactive chemical species during bleaching (Dence 1996a)

Species	Type		pH	Initial reaction
Present during bleaching with chlorine, hypochlorite and chlorine dioxide				
Cl^+	Cationic	Electrophile	Acidic	Oxidising
$Cl\cdot$	Radical	Electrophile	Acidic	Oxidising
OCl^-	Anionic	Nucleophile	Alkali	Reducing
ClO_2	Radical	Electrophile	Acidic	Oxidising
Present during oxygen based bleaching stages				
O_2	Radical	Electrophile	All	Oxidising
$O_2^{-}\cdot$	Radical	Electrophile	Alkali/Neutral	Oxidising
$HOO\cdot$	Radical	Electrophile	Acidic	Oxidising
HOO^-	Anionic	Nucleophile	Alkali	Reducing
$HO\cdot$	Radical	Electrophile	All	Oxidising
HO^+	Cationic	Electrophile	Acidic	Oxidising
O_3	Cationic	Electrophile	Acidic	Oxidising
Present during dithionite bleaching				
$SO_2^{-}\cdot$	Radical/anion	Nucleophile	All	Reducing
HSO_3^-	Anionic	Nucleophile	Acidic	Reducing

Cationic and oxidative radical processes are generally electrophilic in nature. They attack electron rich, aromatic and olefinic, sites in the lignin and usually constitute the early stages of the lignin-removing type bleaching sequences. This is in contrast to anionic and reductive radical reagents, which are nucleophilic and attack sites such as carbonyl or conjugated carbonyl groups. These reagents constitute the lignin-preserving type bleaching and also the latter stages of a multistage bleaching sequence.

2.3.1.1 Cationic processes

Reaction of cationic reagents with the aromatic ring is initiated by electrophilic aromatic substitution. Electron donating oxygen containing substituents greatly enhance the attack, directing the substitution ortho or para to their position. There are two possible pathways by which the substituted intermediate can further react depending on where the substitution occurs (Figure 2.14).

If the substitution occurs at a carbon carrying an eliminatable side group, re-aromatisation occurs through the elimination of that group (Figure 2.14A, B). The substitution products are subject to further attack by cations. Hydrophilicity is increased by OH substitution and reduced by Cl substitution (although, Cl substituted products are susceptible to alkali extraction).

Substitution at a carbon carrying an oxygen containing substituent (eg. OCH_3) affords ortho quinoid structures following the hydrolysis of the ether bond (Figure 2.14C, D). This is the most important cationic process because lignin fragmentation is achieved by cleavage of aryl-alkyl and aryl-aryl ether linkages. The quinoid structures undergo further degradation and polymerisation reactions with nucleophiles. Cationic reagents do not partake in ring opening reactions (Gierer 1990a).

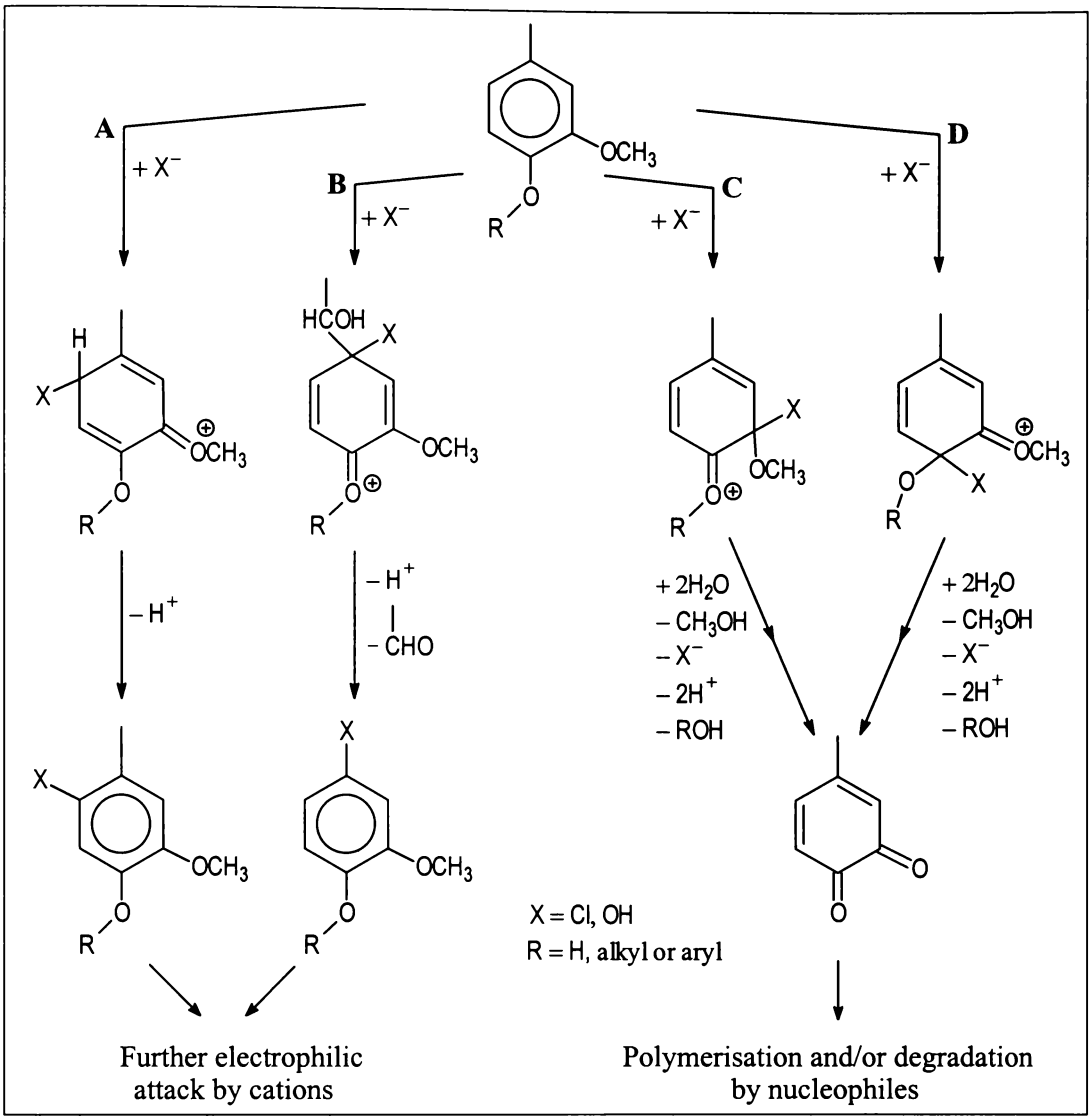

Figure (2.14). Initial reactions of aromatic structures with cationic species (Gierer 1986)

Reactions of cationic species with olefinic structures are initiated by addition across the double bond forming either a carbonium ion or cyclic onium ion (Figure 2.15). These then react with weak nucleophiles (eg Cl^- or OH^-) giving disubstituted products. This reaction proceeds with particular ease if the double bond is conjugated to the aromatic ring.

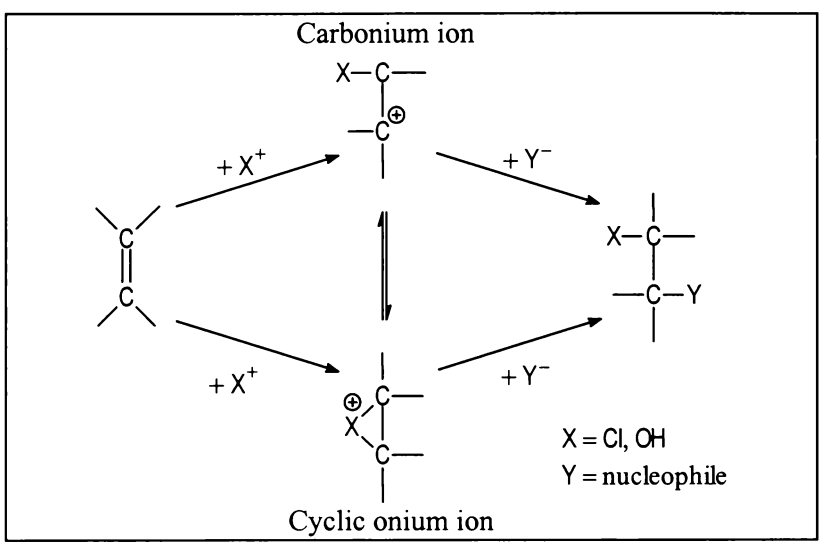

Figure (2.15). Reaction of olefinic structures with cationic reagents

2.3.1.2 Radical processes

Oxidative radical processes are electrophilic. Initiation of the reaction with aromatic sites is similar to the cationic processes. However, reaction of the initial cyclohexadienyl intermediate is different. A complex set of reactions is possible depending on the initial intermediate and the pH (Figure 2.16).

In acidic media (Figure 2.16A) the radical intermediates are readily protonated which then lead to the elimination of HX (X pertains to the bleaching species) forming cationic radical intermediates. Phenolic units will eliminate another proton giving phenoxy radicals, whereas, non-phenolic units exist in various mesomeric radical forms of the oxonium ion type.

In alkaline media (Figure 2.16C) the bleaching reagent (X) is exchanged with hydroxy anions giving hydroxy-cyclohexadienyl radicals. Elimination of water from phenolic structures gives phenoxy radicals while the ether linked structures remain as hydroxy-cyclohexadienyl radicals in different mesomeric forms.

Reactions in neutral media (Figure 2.16B) are similar to the alkali reactions and differ only in that no exchange with hydroxy anions occurs. Phenolic forms eliminate HX to form phenoxy radicals and non-phenolic radicals exist in ortho and para isomeric forms.

These intermediate radicals are highly unstable and react further in a number of ways. The main pathway is the coupling with another bleaching agent radical (Figure 2.17) leading to a range of degradation products depending on the site of attack. For example, coupling at ether linked positions (Figure 2.17A) ultimately leads to hydrolysis of the aryl ether linkage producing quinoid structures or the heterolytic cleavage of the aromatic ring giving muconic acid type products. Coupling at other sites leads to either proton or side chain elimination yielding substitution products (Figure 2.17B). In certain situations the substitution products will undergo secondary reactions (Figure 2.17B) and certain intermediates can rearrange to give epoxide type structures (Figure 2.17C). Non-phenolic radical intermediates react in a similar manner to phenolic radicals yielding similar products.

In addition to coupling with bleaching reagent radicals, these radical intermediates may undergo a number of other reactions (Gierer 1990a). These include the coupling of radical intermediates to give lignin dimerisation products; or the cleavage of the Cα–Cβ bond in hydroxy-cyclohexadienyl and cationic radical intermediates containing an α-hydroxyl group. A competing reaction to the latter homolytic rearrangement involves hydrogen abstraction from the Cα resulting in oxidation to α-carbinol structures.

The initial reaction of radical species with ring conjugated double bonds are analogous to those of aromatic double bonds (Figure 2.18) and leads predominantly to the β-radical intermediate shown. Subsequent reactions involve attack of a nucleophile (external or internal) at the α-carbon of the quinonium intermediate giving rise to a wide range of oxidation products (Brage et al. 1991). Under acidic conditions no appreciable cleavage of the double bond is observed (Brage et al. 1991), however, Cα=Cβ bond cleavage is observed for phenolic units under alkali conditions (Gierer 1993).

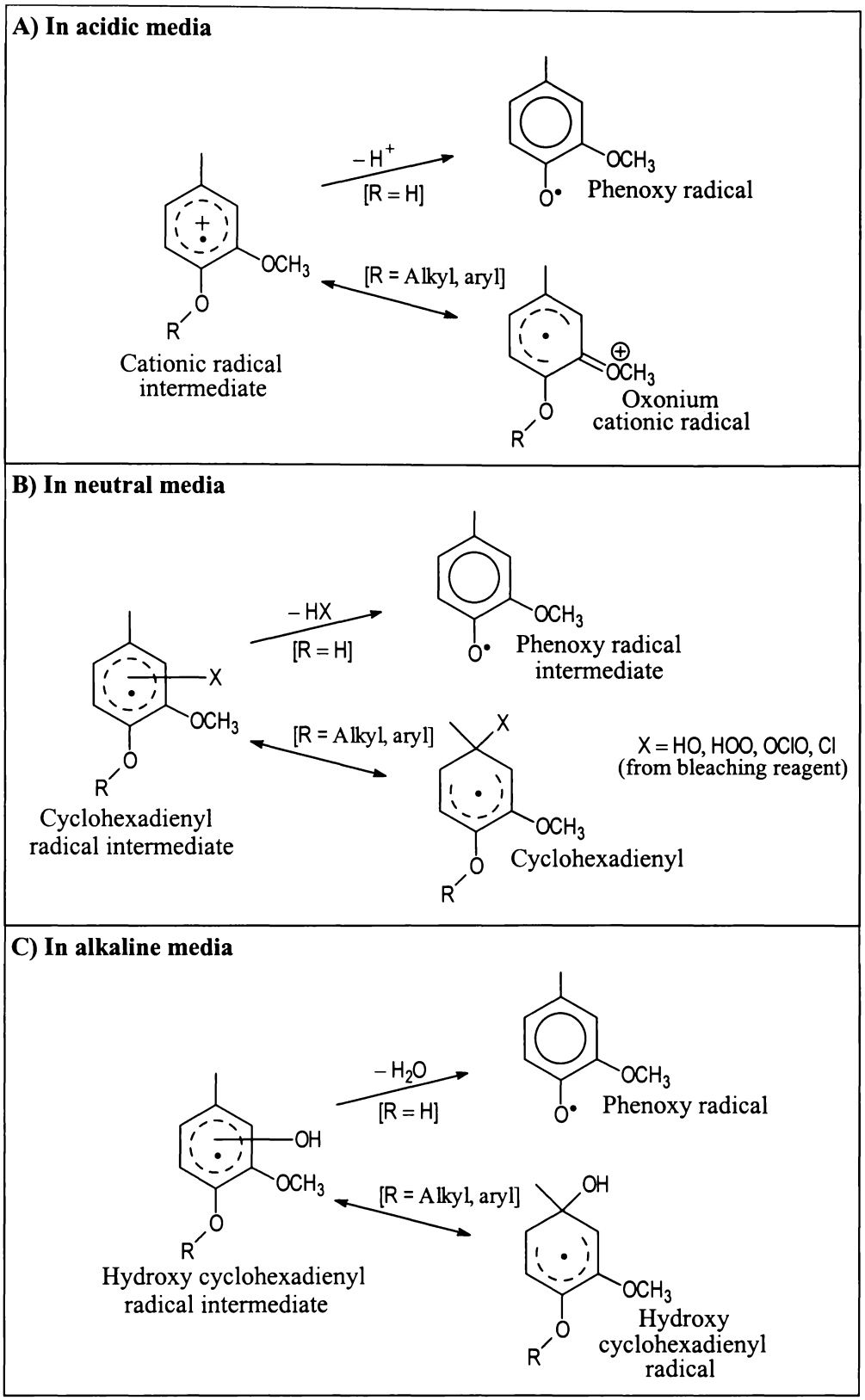

Figure (2.16). Initial reactions of aromatic structures with oxidative radical species

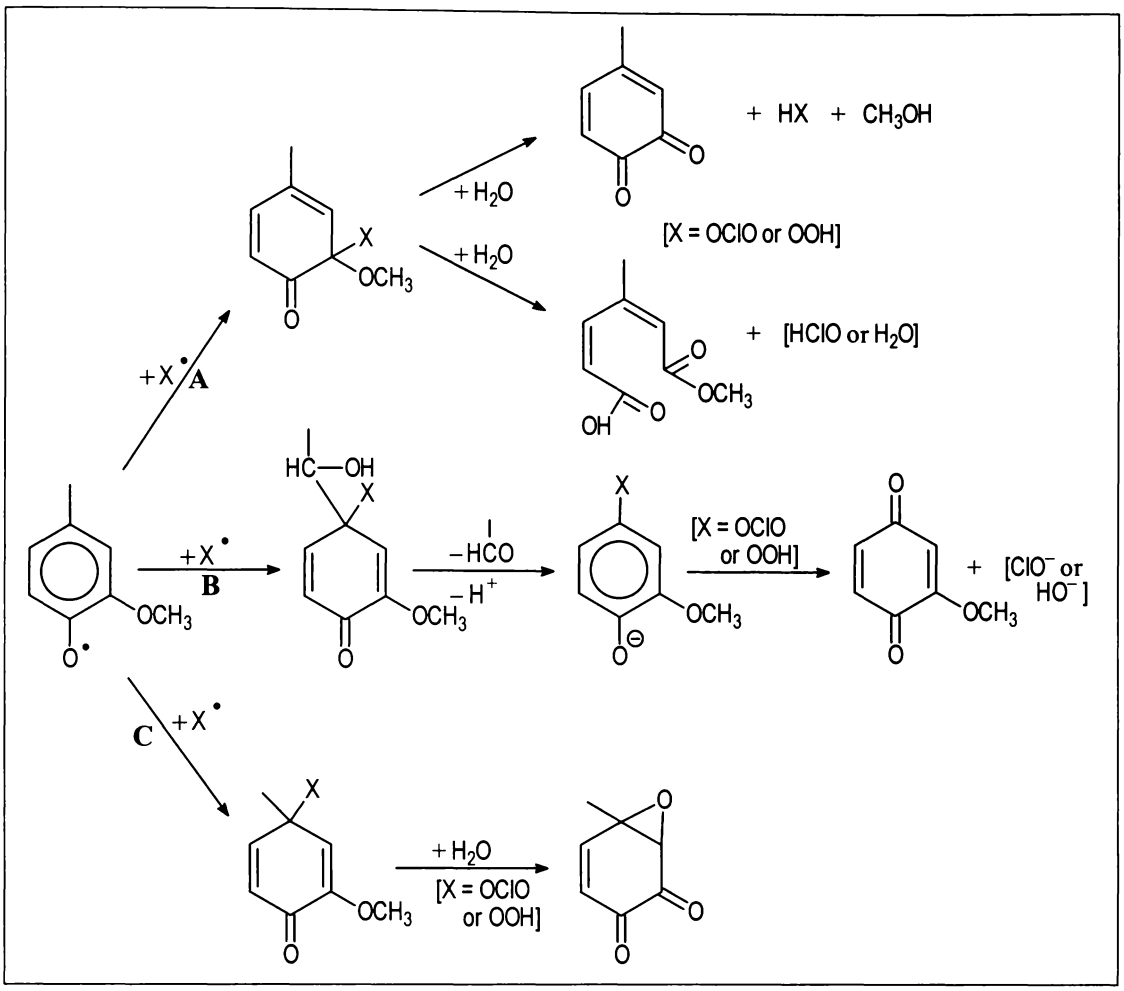

Figure (2.17). Reaction of phenoxy radicals with bleaching radicals (X•)

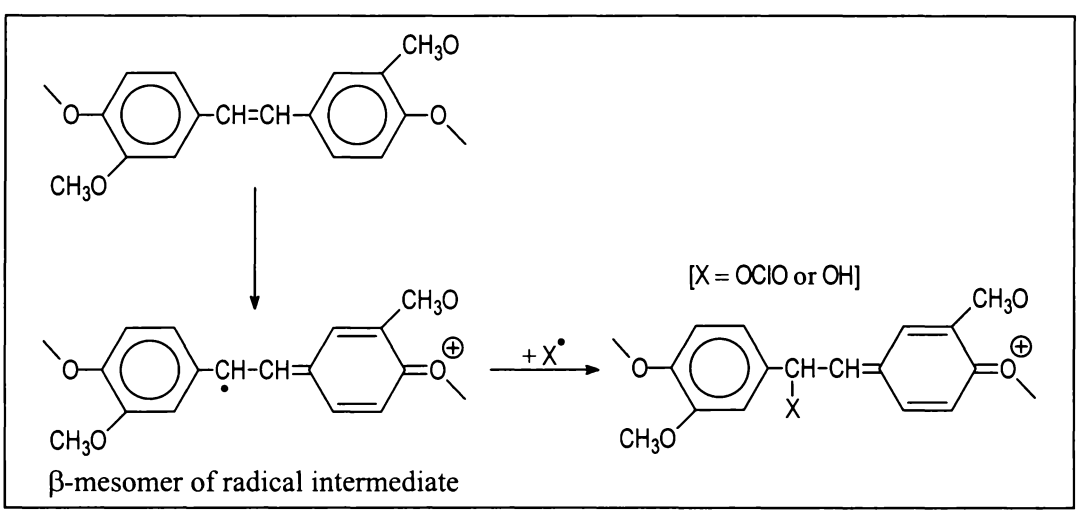

Figure (2.18). Initial reactions of olefinic structures with radical reagents in acidic media

2.3.1.3 Anionic processes

The substrates for anionic reagents are carbonyl and conjugated carbonyl structures that are either present originally in native lignins or, more importantly, arise during pulping and pre-bleaching steps.

Reaction of anionic reagents is illustrated in Figure (2.19) for coniferaldehyde. The reaction is initiated by nucleophilic attack directly at the carbonyl carbon or at sites vinalogous to the carbonyl carbon. The enolic intermediates rearrange with the elimination of X^- affording highly reactive oxirane intermediates. The latter readily undergo further nucleophilic attack resulting in the fragmentation of the original double bond. The carbonyl containing fragments may then be further oxidised.

Reactions with quinoid structures are of particular importance because they are chromophoric groups. Addition to ortho quinones is followed by ring cleavage yielding muconic acid type structures and their cyclisation products.

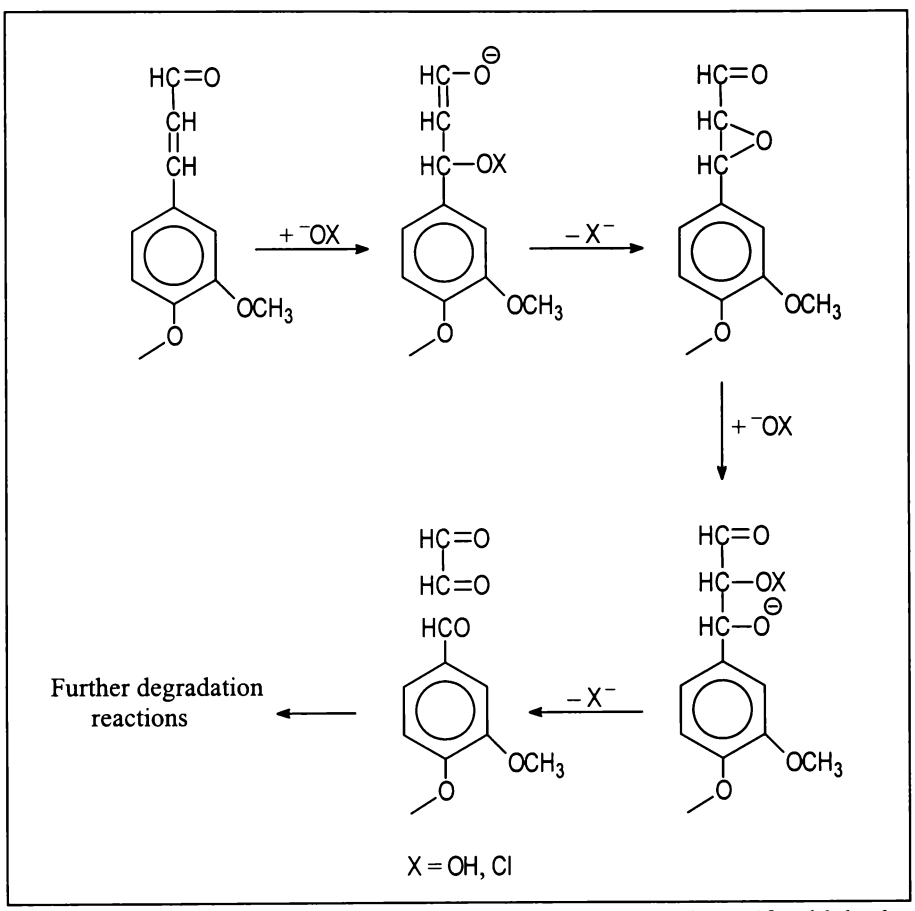

Figure (2.19). Reaction of anionic bleaching reagents with coniferaldehyde

2.3.2 The bleaching chemicals

Table (2.4) summarises the chemicals used in pulp bleaching. The main functions of each chemical and the symbol representing the bleaching stage are summarised.

Table (2.4). Chemicals used in pulp bleaching sequences

Reagent	Symbol[1]	Pulp type[2]	Function
Oxidants			
Chlorine	C	C	Oxidise and chlorinate lignin
Hypochlorite	H	C	Oxidise and decolourise lignin
Chlorine dioxide	D	C	Oxidise, decolourise, and solubilise
Oxygen	O	C	Oxidise and solubilise lignin
Hydrogen peroxide	P	C + M	Oxidise and decolourise lignin
Ozone	Z	C	Oxidise, decolourise and solubilise
Reductants			
Dithionite	Y	M	Reduce and decolourise lignin
Alkali			
Sodium hydroxide	E	C	Extract modified lignin
Chelators			
EDTA, DTPA	Q	C	Remove metal ions

1. Symbol used to denote bleaching stage
2. C = chemical pulp bleaching, M = mechanical pulp bleaching

Dithionite is the only reducing agent used for pulp bleaching and is solely used for bleaching mechanical pulps while hydrogen peroxide is used for bleaching both mechanical and chemical pulps. The agents used for bleaching of chemical pulps are discussed below.

2.3.2.1 Chlorine

Traditionally, chlorine was the bleach of choice in the pulp and paper industry because of its versatility. A chlorination stage followed by alkaline extraction can selectively remove 75–90% of the residual lignin in the fibre after pulping (Hise 1996). The remaining lignin is then easily removed or decolourised in one or more additional steps. Chlorine has been described as "very user friendly" (Hise 1996), because it has the ability to remove lignin rapidly and selectively over a broad pH range, over a wide range of temperatures, and over a wide range of consistencies. However, chlorination is usually carried out at low consistency (3–4%) and ambient temperatures (20–40°C), for a duration of between 30 to 60 minutes. It is important to keep the pH low during bleaching to minimise the formation of chlorite, which does not react with lignin to an appreciable extent. The dosage of chlorine is determined by the incoming kappa number and is often expressed as chlorine multiple:

$$\text{Chlorine multiple} = \frac{\text{Applied chlorine (\% on pulp)}}{\text{Kappa number}}$$

Chemistry

In solution molecular chlorine exists in equilibrium with hypochlorous acid and both forms are present during chlorine bleaching. Additionally, chlorine radicals may also be present.

The initial reacting species during chlorination is the chloronium ion (Cl^+) formed from the heterolytic cleavage of the Cl–Cl bond in elemental chlorine or the O–Cl bond in hypochlorous acid (Gierer 1986). Therefore, the initial reactions during chlorination are cationic (Section 2.3.1.1); that is, electrophilic substitution, electrophilic side chain displacement and oxidative cleavage of aryl-alkyl ether linkages (Figure 2.20). Oxidative ring opening reactions also occur during chlorine bleaching, however, Gierer (1990a) suggests that the chloronium ion is not responsible.

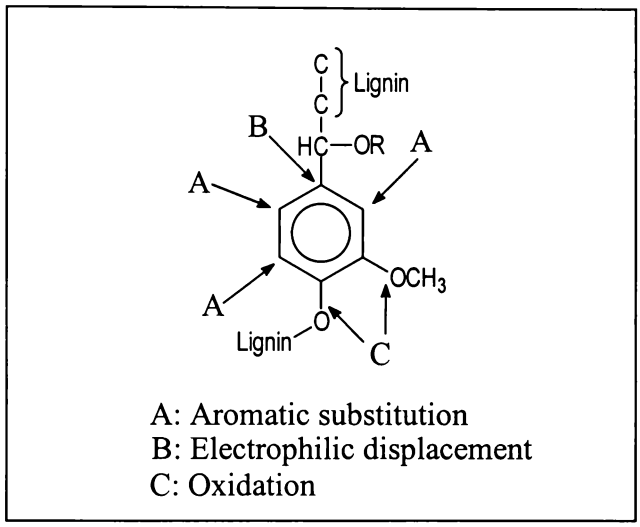

Figure (2.20). Reactions of lignin during chlorination

The low molecular weight oxidised fragments are dissolved during the C stage. Chlorine substitution decreases the solubility of the lignin in water but in the following alkaline extraction stage the majority of the chlorinated lignin is removed by alkaline hydrolysis reactions (Section 2.3.2.4).

Developments

The major concern with chlorine bleaching is the environmental impact of the effluents; a conventional mill could produce as much as 4kg organic chlorine per tonne of pulp (Rajan et al. 1996). The majority of chlorinated organic compounds in the effluent are high molecular weight. These fragments are not themselves toxic but the concern is the possible breakdown of these compounds to low molecular weight fragments and their contribution to pollution. Effluents also contain polychlorinated dibenzodioxins, dibenzofurans, and phenolic compounds, which are toxic to aquatic life (Rajan et al. 1994). The chlorine substituents also increase the lipophilicity of the molecules so they tend to bioaccumulate.

A large effort has been made to reduce or eliminate chlorination. The formation of chlorinated organic compounds during bleaching was found to decrease proportionately with a decrease in applied chlorine. It has been shown that a chlorine multiple of 0.15–0.17 is sufficient to reduce the toxic chlorinated dibenzodioxins and dibenzofurans to negligible amounts. Split addition of the chlorine into smaller doses or by addition of the dose continuously over a period of time also significantly decreases the amount of chlorination. Another method for reducing chlorination is to replace chlorine with chlorine dioxide in the first pre-bleaching stage. Additionally, introducing more effective brown stock washing to reduce the carry over from the pulp mill is another effective way of reducing organically bound chlorine (Hise et al. 1992, Heimburger et al. 1988b).

At present, the use of chlorine is in decline and elemental chlorine free (ECF) or totally chlorine free (TCF) bleaching sequences are becoming predominant as emission standards for pulp mills become more stringent (Hise 1996).

2.3.2.2 Chlorine dioxide

Chlorine dioxide is a versatile bleaching reagent. It is used in both the first pre-bleaching step (with or without chlorine) as a delignifying agent and in the final bleaching stages as a brightening agent. In final bleaching, two chlorine dioxide steps are often used in sequence separated by an extraction stage to improve bleaching efficiency.

Traditionally, all mills generating high brightness kraft pulps used chlorine dioxide in the final stages of bleaching, following the first chlorination and extraction stages. (Reeve 1996b). However, as concerns about the environmental impact of chlorinated effluents grew the use of chlorine dioxide in place of chlorine also grew. Chlorine dioxide is now an integral part of elemental chlorine free bleaching.

Chlorine dioxide is generated at the mill and absorbed into chilled water to form a dilute solution of about 1% at 5–10°C. Chlorine is often produced as a side product (the amounts depending on the method of ClO_2 generation) so is often present in small amounts. Bleaching is generally performed at low to medium consistency, at low temperature in the first stage and about 70°C for the following stages, and the retention times are usually between 3–5 hours (Fengel & Wegener 1984). The initial pH is set to achieve a final pH between 3–4 to optimise delignification. Additionally, it is important to have residual chlorine dioxide at the end of the stage to prevent pulp darkening (Reeve 1996c).

Chemistry

Chlorine dioxide is an oxidant that accepts five electrons when being reduced to a chloride ion. The reduction proceeds through a series of steps involving a number of chlorine containing intermediates (Figure 2.21). The first step is the production of a chlorite ion (ClO_2^-) by one electron transfer to the substrate (1). Additionally, chlorine dioxide also reacts with the residual lignin to give hypochlorous acid (HClO) (2), which is, in part, converted to Cl_2 by hydrolysis (3). Hypochlorous acid and chlorine react with pulp lignin producing chloride ions and chlorinated organic matter (4 to 7).

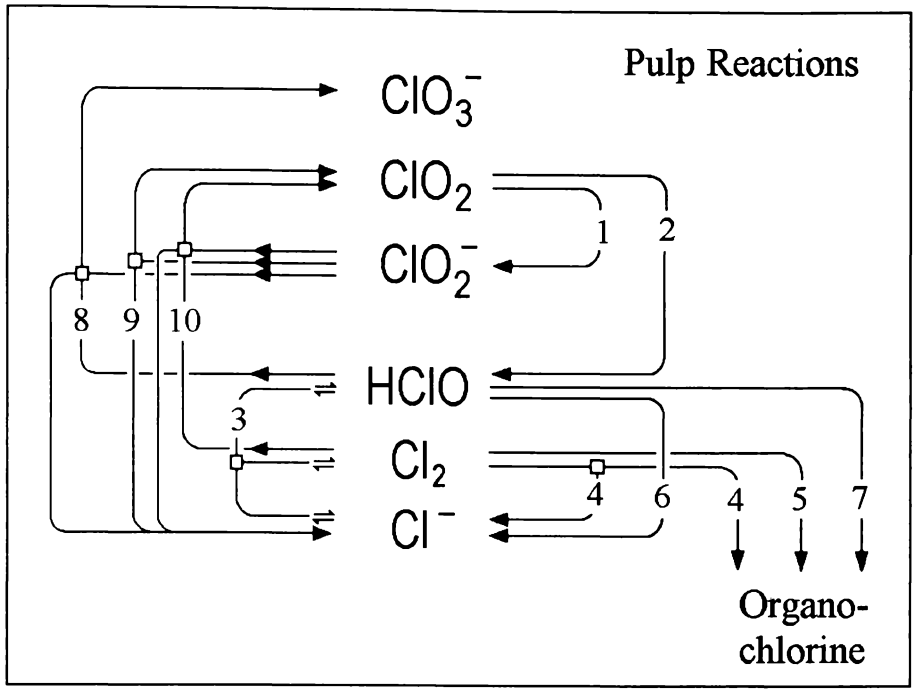

Figure (2.21). Reactions of ClO_2 during bleaching (Reeve 1996c)

Chlorine also reacts with chlorite to regenerate chlorine dioxide (10), while hypochlorous acid reacts with chlorite to form chlorate (ClO_3^-) (8). Neither chlorite nor chlorate react with lignin during bleaching, however, under acidic conditions chlorite decomposes to chlorine dioxide and chloride ion (9).

Chlorine dioxide reacts with both phenolic and non-phenolic structures yielding similar degradation products. However, non-phenolic structures react at a much slower rate and under normal bleaching conditions chlorine dioxide mainly (if not solely) reacts with the phenolic structures (Dence 1996a). Chlorine dioxide bleaching is classed as an oxidative radical process (Gierer 1990a). Radical intermediates (Figure 2.16), formed after the initial reaction with chlorine dioxide, add another molecule of the oxidant to give chlorous acid esters of the quinol type or undergo homolytic fragmentation of the Cα–Cβ bond. The quinol esters may react in various ways, including the hydrolytic cleavage of aryl-alkyl and aryl-aryl ether bonds to give quinone type structures; and heterolytic fragmentation of the quinol esters affording muconic acid structures. Side chain oxidation also occurs, yielding benzyl alcohol or α-carbonyl structures. Oxidative degradation of alkyl-aryl ether and diaryl ether structures, as well as homolytic fragmentation of β-aryl ether structures, appear to constitute the main fragmentation modes during chlorine dioxide bleaching. Demethylation is also a predominant reaction as evidenced by recovery of methanol from the product mixtures (Ni et al. 1994).

2.3.2.3 Hypochlorite

Calcium hypochlorite was the first agent used for bleaching chemical pulps. Initially, sequences containing only hypochlorite and alkaline extraction steps were used (Histed et al. 1996). However, with the advent of chlorine bleaching (which is more selective) the role of hypochlorite was reduced and hypochlorite became part of multistage sequences, such as CEH. These sequences were able to bleach soda and sulfite pulps to

high brightness. Kraft pulps, on the other hand, could not be bleached to high brightness until the introduction of chlorine dioxide bleaching. At which point, sequences such as CEHED, CEHD, and CEHDED were developed. Hypochlorite has also been used to reinforce delignification during alkaline extraction stages. However, hypochlorite use is now being phased out of industry for environmental reasons. The most important concern is that hypochlorite is the largest source of chloroform generation in a bleach plant (Dallons et al. 1990).

Hypochlorite has a severe degrading effect on cellulose at neutral pH and low kappa numbers. It is, therefore, always applied under alkaline conditions and strict control of the pH is necessary during bleaching to ensure the pH does not fall below 9 (Loras 1980). Usually, sodium hydroxide is added to sodium hypochlorite and the bleaching is performed at medium consistency, at about 40°C, with retention times between 1–3 hr.

Chemistry

During hypochlorite bleaching lignin is both decolourised and removed. Hypochlorite in alkaline media acts as a strong nucleophile reacting with positively charged sites, such as quinone structures. These reactions are probably the main decolourisation reactions. Partial decomposition to electrophilic radicals (ClO· and Cl·) may also occur. These species may be responsible, in part, for the delignification and chlorination reactions.

2.3.2.4 Alkaline extraction

Extraction of pulp with alkali is not a bleaching step in the sense of pulp whitening but serves several useful purposes in the multistage bleaching of chemical pulps (Loras 1980). An extraction stage is usually used after an acid stage. The main role is to neutralise acidic groups formed in the preceding stage, thus enhancing the solubility of the lignin in the aqueous liquor. Acidic groups in residual lignin include phenols, carboxylic acids, and enols.

About 60–70% of the organically bound chlorine in a chlorinated pulp is removed in the subsequent alkaline extraction stage (Hise 1996). Hydroxyl groups replace the chlorine substituents by nucleophilic displacement (Gierer 1986, Figure 2.22). This reaction enhances the alkali solubility of the lignin.

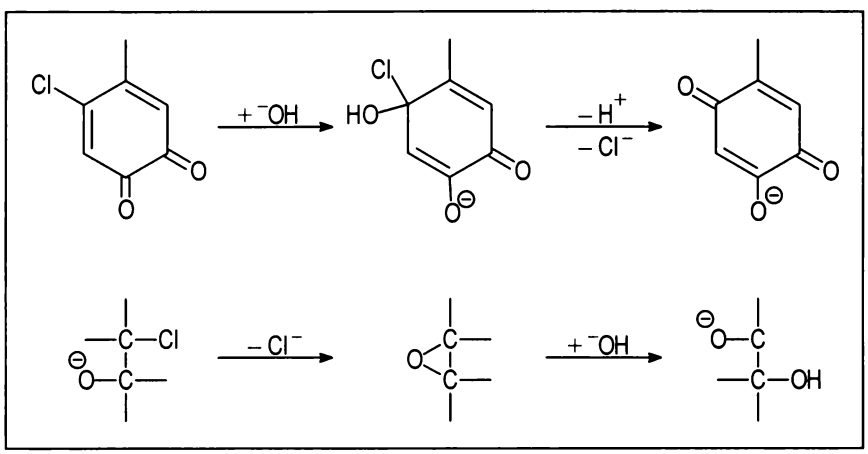

Figure (2.22). Base-catalysed elimination of organically bound chlorine

The concomitant alkali darkening effect is attributed to the reactions of quinone structures (Simson et al. 1978). Depending on the number of ring substituents, quinones display varying degrees of instability in aqueous media. Typical structural changes include condensation to biphenyl structures, decrease in carbonyl groups, and an increase in phenolic hydroxyl groups. Brightness loss is attributed to the formation of quinone-substituted di- or tri-phenolic structures.

The extraction stage is usually carried out at medium consistency with 1–2% alkali (on pulp), at 50–60°C for 30–60 minutes (Fengel & Wegener 1984). Reinforcing the alkaline extraction with oxygen (0.5% on pulp), hydrogen peroxide (0.1–0.5%) or both, improves delignification without significantly affecting pulp strength (Helming et al. 1989). Extraction is enhanced by partial oxidation of the lignin. The increased effectiveness of the reinforced extraction can be utilised in a number of ways. One application of the technology is to reduce the chlorine multiple in the first bleaching stage. This benefits the plant by reducing emissions of chlorinated organics. Another benefit is that consumption of bleaching chemicals in the final steps can be decreased leading to reductions in cost. The conversion of conventional sequences to ECF sequences is often hampered by the chlorine dioxide production capacity of the mill, however, with the use of reinforced extraction the chlorine dioxide requirements can be reduced. Reinforced extraction has also allowed the elimination of hypochlorite from many sequences.

2.3.2.5 Oxygen

Oxygen delignification was developed as a commercially feasible process in the late 1960s and early 1970s in Sweden and South Africa (Johnson 1993) and is now growing in importance globally. Oxygen is mainly applied as a pre-bleaching stage (after pulping) to reduce the residual lignin before bleaching. The main technical obstacle to full commercialisation of the oxygen process was that severe degradation of pulp carbohydrates occurred during treatment yielding pulps with poor strength properties. The breakthrough came with the discovery that magnesium salts (eg $MgSO_4$) inhibit cellulose degradation (Robert et al. 1963).

The interest in oxygen delignification stemmed from the need for mills to reduce pollution. Significant reductions in the biological oxygen demand (BOD) and colour of plant effluents, as well as, the amount of chlorinated organic compounds can be achieved with the introduction of oxygen bleaching (McDonough 1996). The main reason for this is that the amount of residual lignin in the pulp is reduced before bleaching. Additionally, the effluents from the oxygen stage can be recycled because they do not contain any chloride ions. Cost savings arise mainly from the reduced consumption of bleaching chemicals in the subsequent bleaching steps. Other savings arise from the possibility of using shorter bleaching sequences and reduced effluent treatment. Extra heat is also produced from the incineration of the dissolved organics during recovery.

Oxygen bleaching is a gas phase process at pressures between 390 and 780kPa in alkaline media, performed at temperatures ranging from 90 to 140°C (depending on the alkali used). Common sources of alkali for kraft pulps are sodium hydroxide, sodium carbonate, and oxidised white liquor. Commercial bleaching is carried out at medium or

high consistency. The mass transfer problems at medium consistency are overcome by intense mixing of pulp and gas. Retention times are in the order of 30 to 60 minutes, and are usually longer for medium consistency processes (Fengel & Wegener 1984).

Chemistry

Oxygen delignification is classed under oxidative radical processes (Gierer 1990a); the initial reaction being a one electron transfer from ionised phenolic units in lignin to molecular oxygen forming a superoxide anion radical and a phenoxy radical (Figure 2.23A, Gierer et al. 1994). These radicals then combine to form intermediates of the peroxide anion type which undergo further degradation reactions described earlier (Figure 2.17). Aromatic ring opening is a predominant reaction. Molecular oxygen is weakly oxidising and will only react with ionised phenolic units, thus, oxygen bleaching is carried out at high alkalinity. Molecular oxygen is also unreactive towards non-phenolic units for the same reason.

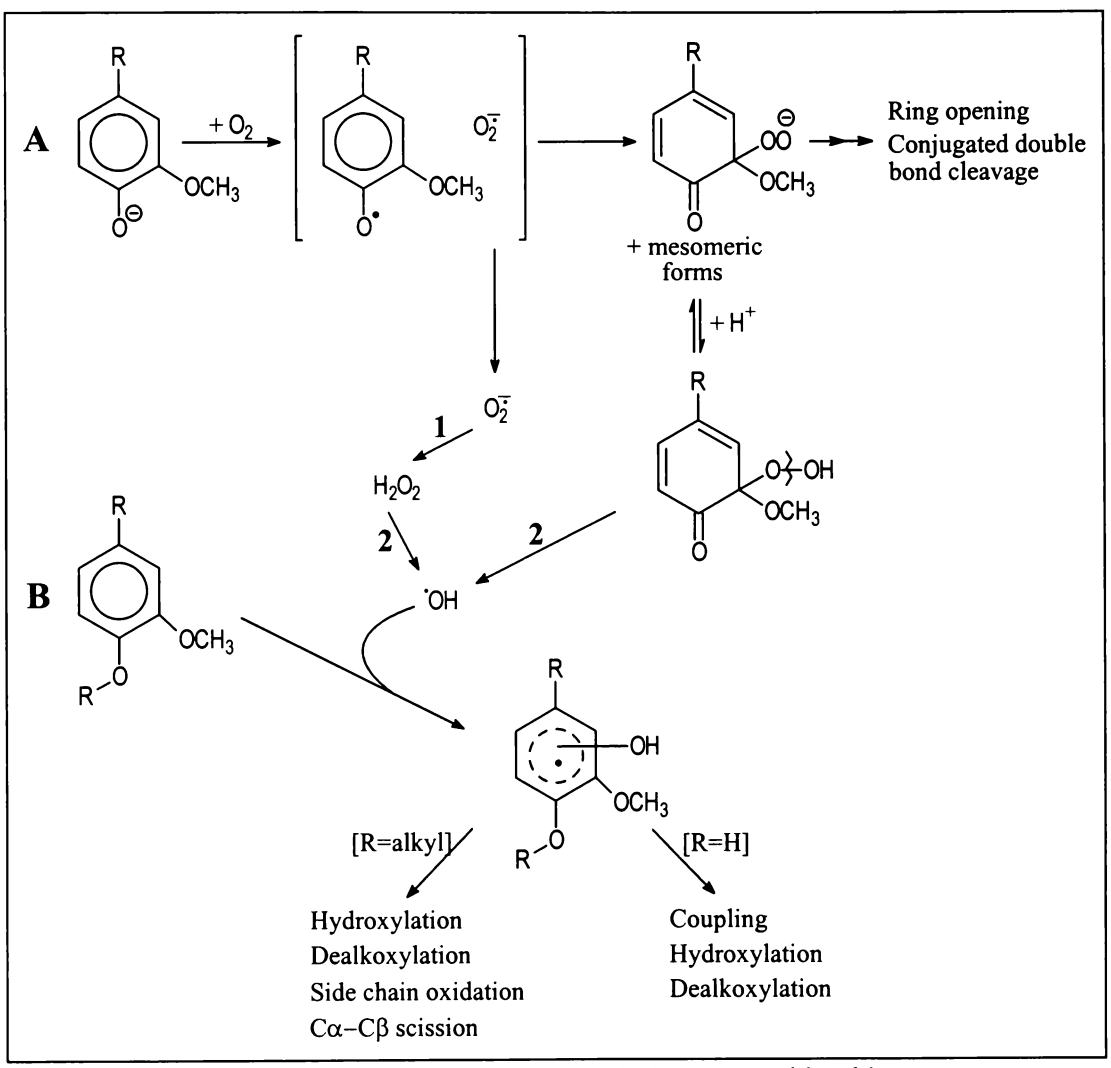

Figure (2.23). Reactions of lignin during oxygen bleaching

Superoxide anion radicals may also dismutate yielding oxygen and hydrogen peroxide (1). The latter, together with organic hydroperoxides, may be homolytically cleaved (2) giving rise to hydroxyl radicals (Yang 1995). Hydroxyl radicals are more reactive than the superoxide anion radical and react rapidly with both non-phenolic and phenolic structures in lignin by oxidative radical processes. Hence, they also contribute to lignin degradation during oxygen bleaching (Figure 2.23B, Gierer 1993). However, hydroxyl radicals are the main cause of carbohydrate degradation during oxygen bleaching (Ek et al. 1989). The reactivity of the hydroxyl radical is at least five times larger with lignin model compounds than with carbohydrate models (Ek et al. 1989), thus during the early stages of delignification, at high kappa numbers, carbohydrates are protected from degradation by competition from the lignin reactions.

Hydrogen peroxide also reacts with lignin (Section 2.3.2.6). Quinone structures generated by the oxidation of lignin are susceptible to nucleophilic attack by the hydroperoxide anion. These reactions lead to the opening of the quinone ring forming muconic acid and other acidic structures which are soluble in the alkaline medium (Gierer 1993). The brightness improvement accompanying oxygen delignification has been attributed to hydroperoxide anions.

Condensation reactions may also take place during bleaching. The phenoxy radicals may couple together generating new carbon-to-carbon bonds (Kratzl et al. 1966). These reactions are undesirable since they reduce the solubility of the lignin fragments. Furthermore, condensed structures are resistant to further degradation (Gellerstedt et al. 1986, Johansson & Ljunggren 1994).

Developments

Research on improving oxygen delignification has mainly focused on improving the selectivity of oxygen delignification. Generally an oxygen stage can only remove about 50% of the residual lignin before severe carbohydrate degradation occurs. To further extend oxygen delignification a number of modifications have been proposed.

Acidic pretreatments with nitrogen dioxide, chlorine, chlorine dioxide, hydrogen peroxide, or ozone have been investigated as a means to improve selectivity (Fossum & Marklund 1988). Pretreatment with nitrogen dioxide showed the most promise and has been extensively studied (Samuelson & Ojteg 1990, 1991, Andersson et al. 1984, Ku et al. 1991, 1992). Pulps with kappa numbers as low as 6 have been produced by pretreatment with nitrogen dioxide with no significant change in their pulp strength properties (Samuelson & Ojteg 1991). These pulps can be bleached to high brightness with a three stage sequence (eg $DE_{OP}D$).

The removal of transition metal ions is another method proposed to increase selectivity (Fossum & Marklund 1988). Transition metals catalyse the decomposition of oxygen to radical species that cause the degradation of carbohydrates (Yang 1995). An acid washing stage prior to oxygen delignification to remove transition metal ions allows kappa numbers to be reduced by 1 or 2 units (Johnson 1993). The main disadvantage is the large pH change required between each step. A pretreatment with chelating agents, such as DTPA or EDTA, under neutral conditions followed by washing also improves selectivity in a subsequent oxygen stage (Johnson 1993).

2.3.2.6 Hydrogen peroxide

Hydrogen peroxide has been used commercially as a pulp bleaching agent since 1940 when it was first introduced as a bleaching agent for mechanical pulps (Anderson & Amini 1996). In order to utilise alkaline peroxide as an efficient lignin-preserving bleaching agent for mechanical pulps precautions must be taken to avoid a decomposition of hydrogen peroxide to more reactive radical species (Andrews & Singh 1979). In practice, this is achieved by choosing mild alkaline bleaching conditions (1% alkali and temperatures below 70°C), adding sodium silicate and magnesium sulfate to stabilise the hydrogen peroxide, and removing transition metal ions from the pulp by treatment with a chelating agent prior to bleaching (Loras 1980).

The use of peroxide for bleaching of chemical pulps was traditionally limited to a final hydrogen peroxide stage to improve final brightness and brightness stability. However, as demands for reducing bleach plant emissions increase so does the use of hydrogen peroxide. Hydrogen peroxide has two functions (Lachenal 1996). The first, is the removal of chromophores leading to brightening of the pulp. In alkaline media hydrogen peroxide reacts with carbonyl and conjugated carbonyl structures, which are the main source of colour in pulps. Hydrogen peroxide can be added to the alkaline extraction stage, at charges of 0.05–2%, depending on the stage (more is used in the first extraction stage). Hydrogen peroxide can also be used at the end of the bleaching sequence. Peroxide bleaching is usually carried out at consistencies of 10-20%, at temperatures of 60–80°C for 2-4 hours (Loras 1980).

The second function is as a delignifying agent. At temperatures above 80°C, with stringent control of hydrogen peroxide decomposition, hydrogen peroxide can also effectively function as a delignifying agent (Gellerstedt & Pettersson 1982). Hydrogen peroxide bleaching is improved using pressurised reactors allowing temperatures greater than 100°C to be used, however, this is at the expense of pulp strength (Anderson & Amini 1996). Hydrogen peroxide stages are an integral part of TCF sequences.

Chemistry

The action of hydrogen peroxide in alkaline media has been attributed to the hydroperoxide anion (Andrews & Singh 1979). The hydroperoxide anion (HOO^-) is a nucleophile and reacts with carbonyl and conjugated carbonyl structures as described earlier for anionic reagents (Gierer & Imsgard 1977). The bleaching of mechanical pulp has essentially been explained by this mechanism and very little delignification takes place (Dence 1996b).

However, bleaching of chemical pulps at temperatures greater than 80°C results in a significant amount of delignification which cannot be solely accounted for by reaction of the hydroperoxide anion. Delignification has, in part, been attributed to reactions of the decomposition products of hydrogen peroxide ($O_2^{\cdot-}$, $OH^{\cdot}$) with the lignin (Agnemo & Gellerstedt 1979).

2.3.2.7 Ozone

Ozone is a powerful oxidising agent, which reacts readily with organic material; including lignocelluloses. Ozone is used commercially for the bleaching of textiles, waxes, starch, and for disinfecting air (Lierop et al. 1996). Ozone is strongly electrophilic and preferentially reacts with lignin in lignocellulosic materials (Eriksson & Gierer 1985) and so has great potential in the pulp and paper industry. However, radicals formed during the ozone reaction with lignin as well as from its decomposition in water promote the unwanted degradation of carbohydrates (Ek et al. 1989). This selectivity problem has limited the commercialisation of ozone, until recently. Interest in ozone in the 1990s as a replacement for chlorine has been accelerated by the trend towards minimising discharge of chlorinated compounds and market demands for TCF bleaching.

Ozone is generated by sparking a flow of oxygen or air and is applied to the pulp as a gas mixture with oxygen (or air). Ozone bleaching can be carried out at low (1%), medium (10%) or high (>25%) consistency. At low consistency the ozone is dissolved in water and the diffusion resistance is reduced by intensive stirring, while at high consistency the pulp must be fluffed to ensure even delignification. Medium consistency oxygen technology has recently been applied to ozone bleaching (Sixta et al. 1991). To overcome problems associated with large gas volumes the gas is compressed before being introduced to the pulp.

Ozone is very good at delignifying and brightening but tends to cause damage to the fibre as well. Selectivity of ozone bleaching decreases with increasing temperature so ozone bleaching is usually carried out at ambient temperatures (Patt et al. 1991). The best results have been obtained when ozone is added in multiple stages, at low charges, with washing in between to remove lignin fragments and resins, which also consume ozone (Byrd Jr. et al. 1992).

A large number of sequences incorporating ozone are now in existence (Lierop et al. 1996, Byrd Jr et al. 1992, Liebergott 1992b). Sulfite pulps respond well to ozone especially after an oxygen stage (Byrd Jr. et al. 1992). Hardwood kraft pulps can be bleached with OZEP or OZED sequences without significant damage to strength properties (Nutt et al. 1993, Liebergott et al. 1992b), however, softwood pulps remain more difficult to bleach.

Chemistry

Ozone can be viewed as a resonance hybrid of four mesomeric forms. The dipole character of the mesoforms suggests that ozone may react as a nucleophile or an electrophile (Dence 1996a). However, it is generally believed that during reaction with lignin structures ozone acts as an electrophile reacting with electron rich sites, such as, aromatic rings and conjugated double bonds (Eriksson & Gierer 1985, Gierer 1986).

Ozone reacts with the aromatic ring and conjugated double bonds by a 1,3-dipolar cycloaddition reaction leading to ring opening (Figure 2.24A) and double bond scission (Figure 2.24B), respectively. Both non-phenolic and phenolic units are degraded by ozone, however, ozone shows a preference for phenolic units (Eriksson & Gierer 1985).

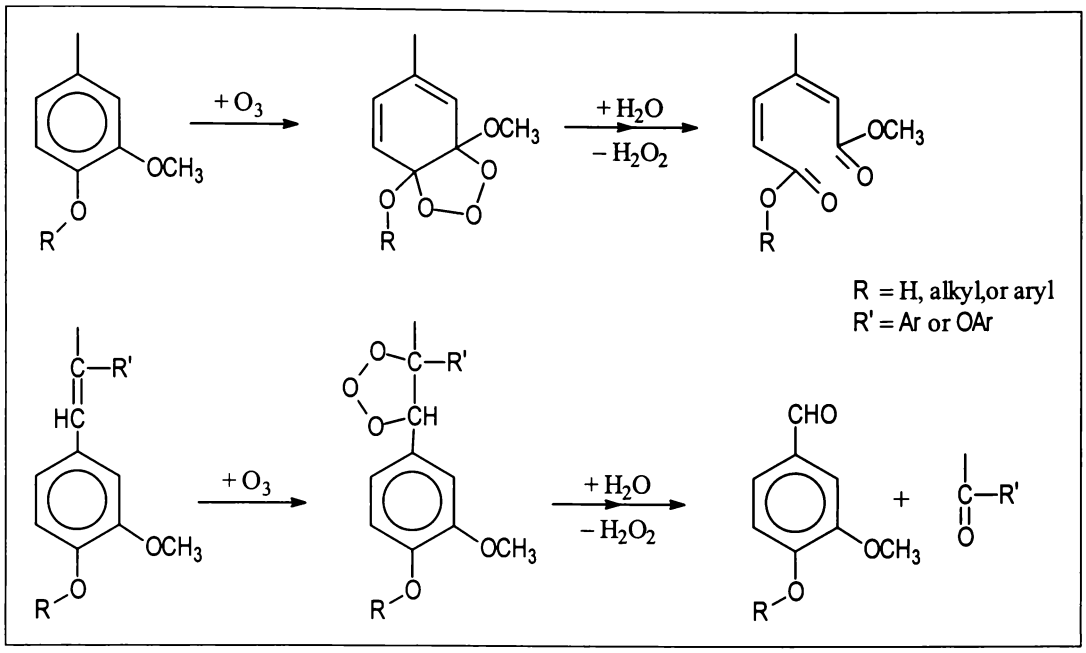

Figure (2.24). 1,3-dipolar cycloaddition of ozone to double bonds

Electrophilic aromatic substitution also occurs during ozone bleaching, resulting in ring hydroxylation and oxidative dealkylation. Insertion into C–H bonds appears to be the reaction mechanism of ozone with aldehydes, ethers, and alcohols leading to bond cleavage (Gierer 1986).

Developments

The major drawback with ozone bleaching is low selectivity. Considerable research has been directed towards identifying an additive or pretreatment capable of preventing carbohydrate degradation (Liebergott et al. 1992a, 1992b).

Some organic additives such as methanol, urea-methanol, and dimethyl formamide help retain pulp viscosity to varying degrees. These additives act as radical scavengers suppressing the radical reactions with cellulose. The quantities required, however, exceed practical limitations. Inorganic additives that protect cellulose during oxygen bleaching have no effect during ozone bleaching. Dissolved lignin fragments may protect carbohydrates in low consistency bleaching and acid pretreatments in the presence of chelators or nitrogen dioxide also improve selectivity slightly.

Ozone introduces carbonyl and carboxylic acid groups into carbohydrates, which destabilise the carbohydrates in subsequent alkali stages. A reduction treatment with sodium borohydride effectively stabilises pulp viscosity and improves brightness stability (Chirat & Lachenal 1994). However, the high cost of sodium borohydride prevents its commercialisation. The problem of finding a cheap effective commercial inhibitor of carbohydrate degradation is still to be solved.

2.3.2.8 Other bleaching reagents

For many years industry has been searching for alternative bleaching agents to replace the conventional chlorine-based systems. Some of the potential alternatives are discussed below.

Chemical

Nitrilamine (cyanamide) significantly improves the performance of peroxide delignification (Smit 1995). The peroxide and cyanamide combine together forming an amino-imino peracid, which is significantly more reactive with lignin than the original peroxide. The peracid reacts with carbonyl and conjugated carbonyl structures via the same mechanisms as peroxide and the improved rate is attributed to the different leaving groups (OH^- for peroxide cf urea for cyanamide activated). However, more recently Kadla et al. (1998a, 1998b) has proposed that radical degradation products of the peracid intermediate play an important role in delignification.

Polyoxometalates have been identified as possible bleaching alternatives. Compounds such as α-$K_5[SiVW_{11}O_{40}]$ and $H_5[PV_2Mo_{10}O_{40}]$ can delignify kraft pulps, resulting in well delignified pulps with properties comparable to those of chlorination pulps (Weinstock et al. 1993, 1996). During reaction phenolic units in lignin are oxidised to quinones via two 1-electron transfers between the phenol and the polyoxometalate. The reduced polyoxometalates are then reoxidised with air, oxygen, peroxide, or ozone. However, to be economically viable an extremely high recovery of the catalyst would be necessary.

Peroxy acids contain a perhydroxyl group in place of the hydroxyl group. They are generated from reaction between the parent acid and hydrogen peroxide and can effectively delignify and brighten pulp (Allison & McGrouther 1995, Amini & Webster 1995, Springer & McSweeney 1993). Reaction is initiated by the hydroxide cation leading to hydroxylation of phenolic structures, epoxidation of olefinic bonds and oxidation of ketone structures (Lawrence et al. 1980). The main disadvantage of this technology is that peracids are unstable making them difficult to transport and store. However, on-site generation of the peracid would overcome these problems.

Dioxiranes are cyclic peroxides prepared from the oxidation of ketones by peracids, (Murray 1989) and are very effective oxidising agents. One common example is dimethyldioxirane formed by reaction between acetone and peroxymonosulfate. It can effectively delignify kraft pulps without resulting in significant damage to the fibre (Ragauskas 1993).

Biological

Research into the utilisation of biological processes for pulp bleaching has centred on two different approaches. The first is the use of white rot fungi that attack lignin in wood. Many different fungal species have been screened as potential delignifiers of pulp (Paice et al. 1989, Fujita et al. 1991, Addleman & Archibald 1993). Hardwood kraft pulps respond well to fungal treatment (Paice et al. 1989, Addleman & Archibald 1993), whereas softwood pulps are more difficult to delignify. However, promising results have been obtained when the fungal step is followed by alkali extraction (Reid et al. 1990).

The main limitation of this type of technology is the very long treatment times; at least five days are required to obtain significant delignification. More attention has, therefore, been paid to isolated enzymes.

Xylanases are now used commercially (Farrell et al. 1996). Treatment of kraft pulp with xylanases significantly improves bleachability and, thus reduces the bleaching chemical requirements in subsequent steps (Yang et al. 1992, Senior et al. 1992). The precise mechanism of these enzymes is unclear. One theory is that the degradation of reprecipitated xylan makes the removal of lignin fragments in and on the fibre easier (Kantelinen et al. 1993). Alternatively, xylanases could hydrolyse the xylan within the inner fibre allowing entrapped residual lignin to diffuse out more easily (Hortling et al. 1994).

Certain enzymes, such as *lignin peroxidase* and *laccase* are produced by white rot fungi and have been implicated in the oxidative degradation of lignin (Kirk & Farrell 1987). Both types have been investigated as potential *biobleaching* agents for kraft pulp.

Most studies with laccase for pulp bleaching have been with isozymes from *Trametes versicolor*. These enzymes contain four copper atoms at the active site, and oxidise a variety of aromatic amines and phenols while reducing oxygen to water (Ferrar et al. 1995). Laccases are too large to penetrate into the pulp fibre but in the presence of a mediator (Figure 2.25) significant reductions in kappa number have been obtained in only a few hours (Bourbonnais & Paice 1996). In the presence of oxygen, laccase oxidises the mediator to stable radical intermediates (Potthast et al. 1997). These intermediates are able to penetrate the fibre and selectively oxidise lignin structures. The laccase-mediated oxidation of lignin is more selective than an oxygen stage and the oxygen requirement is significantly lower (Bourbonnais & Paice 1996).

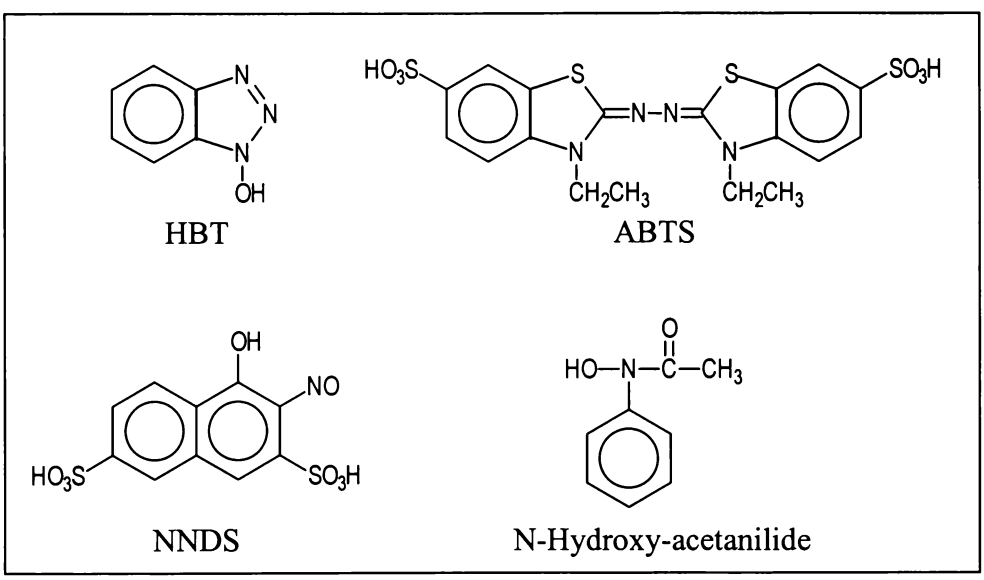

Figure (2.25). Laccase mediators (Amann 1997)

White rot fungi produce a variety of lignin peroxidases, many of which have been implicated in the degradation of lignin (Kirk & Farrell 1987). Mixed success has been obtained in the study of these enzymes for delignification of kraft pulps, however, a manganese dependent peroxidase has shown some promise (Paice et al. 1993). With the aid of hydrogen peroxide this enzyme oxidises Mn(II) to Mn(III), and chelated Mn(III) oxidises lignin in the fibre (Paice et al. 1997). The peroxide concentration must be kept low (to prevent enzyme denaturation) but if the peroxide generation is optimised a significant decrease in kappa number can be achieved within four hours (Paice et al. 1995). The resulting pulp also shows improved bleachability, especially in a subsequent peroxide stage compared to convention pulps. Hardwood pulps respond well to the Mn-peroxidase treatment but softwoods are more resistant (Ehara et al. 1997). Promising results have been obtained if additional manganese is added and the peroxidase step is repeated with an alkali extraction or wash stage in between.

The commercial viability of these processes will largely depend on their cost effectiveness and on how compatible they are with existing processes.

2.4 Isolation and Characterisation of Lignin

The development of wood pulping processes generated much interest in the structure and reactivity of lignin. The principal structural elements of lignin have been largely clarified (Section 2.1.3.2), as a result of detailed studies on isolated samples and using degradation techniques based on oxidation, reduction, and hydrolysis. However, many aspects in the chemistry of lignin still remain unclear; such as, the variation in lignin structure with age and morphological region; the nature of the association between lignin and the other cell wall components; and of particular interest, the exact nature of residual lignin. Hence, the study of lignin structure and reactivity is ongoing. Methods for the isolation and characterisation of lignin are discussed in the following sections. The literature concerning the isolation and characterisation of residual lignin from bleached and unbleached kraft pulps is relevant to the work in this thesis so it is discussed in a separate section (Section 2.4.3).

2.4.1 Isolation methods for lignin

Due to the properties of lignin resulting from its complex molecular structure and its intimate association with the other cell wall components, the isolation of lignin in an unchanged form and its exact determination have not yet proved possible. The common methods of lignin isolation are summarised in Table (2.5). All the methods have the disadvantage of either fundamentally changing the native structure of lignin or releasing only parts of it in a relatively unchanged form.

The first group, the so-called acid lignins, are extensively modified during isolation so are not suitable for investigating structure but are mainly applied to estimating lignin content; for example, Klason lignin. The preparation considered to be the most appropriate for the study of wood lignin is MWL, while the CEL, dioxane, and alcohol lignins have been extensively used for the study of residual lignin.

Table (2.5). Methods for the isolation of lignin (Fengel & Wegener 1984)

Method	Treatment	Remarks
A) Lignin obtained as a residue		
Lignin is obtained as a residue after acid hydrolysis of polysaccharides	H_2SO_4 (Klason lignin) HCl (Willstatter lignin) HF CF_3COOH	Condensation and substitution reactions observed
Oxidation of polysaccharides to water soluble components leaving lignin as insoluble residue	$NaIO_4$ (periodate lignin)	Lignin is oxidised to a certain degree
Hydrolysis of polysaccharides using milder conditions	H_2SO_4 and $Cu(NH_3)_4(OH)_2$	Very tedious
B) Lignin obtained by extraction		
Modification of the lignin during extraction		
Partial hydrolysis of polysaccharides and lignin with concomitant solubilisation of the lignin	Alcohol/HCl (alcohol lignin) Dioxane/HCl (dioxane lignin) $CH_3COOH/MgCl_2$ (acetic acid lignin)	Reaction between solvent or acid, and lignin
Derivatisation or chemical degradation of lignin into aqueous soluble form	$HSCH_2COOH$/HCl – NaOH (TGA lignin)	Good yields Lignin converted to lignothioglycolic acids
	NaOH (alkali lignin) $NaOH/Na_2S$ (kraft lignin)	Degradation and condensation reactions take place in the alkali
	Sulfite/Bisulfite	Lignin converted to lignosulfonates
Extraction of lignin with limited modification		
Brauns native lignin	Alcohol extraction of ground wood	Low molecular weight
Bjorkman or milled wood lignin (MWL)	Ball milling and dioxane-water extraction	Most common method
Cellulolytic enzyme lignin (CEL)	Ball milling/enzyme treatment/solvent extraction	Better yields than MWL Long enzyme treatment
Enzymatically liberated lignin (ELL)	Brown rot fungi treatment/solvent extraction	Lignin is partially oxidised by the fungi

2.4.1.1 Isolation of lignin as a residue

Acid lignin preparations were once exclusively used in the chemical characterisation of lignin. Lignin is extracted by treating wood with strong sulfuric acid (72%), concentrated hydrochloric acid, or some other strong mineral acid. The strong acids hydrolyse the carbohydrates to water soluble monomers and the lignin is obtained as a brown insoluble residue when water is added. However, these lignins were later found to be highly changed in structure and properties (Figure 2.26, Lai & Sarkanen 1971).

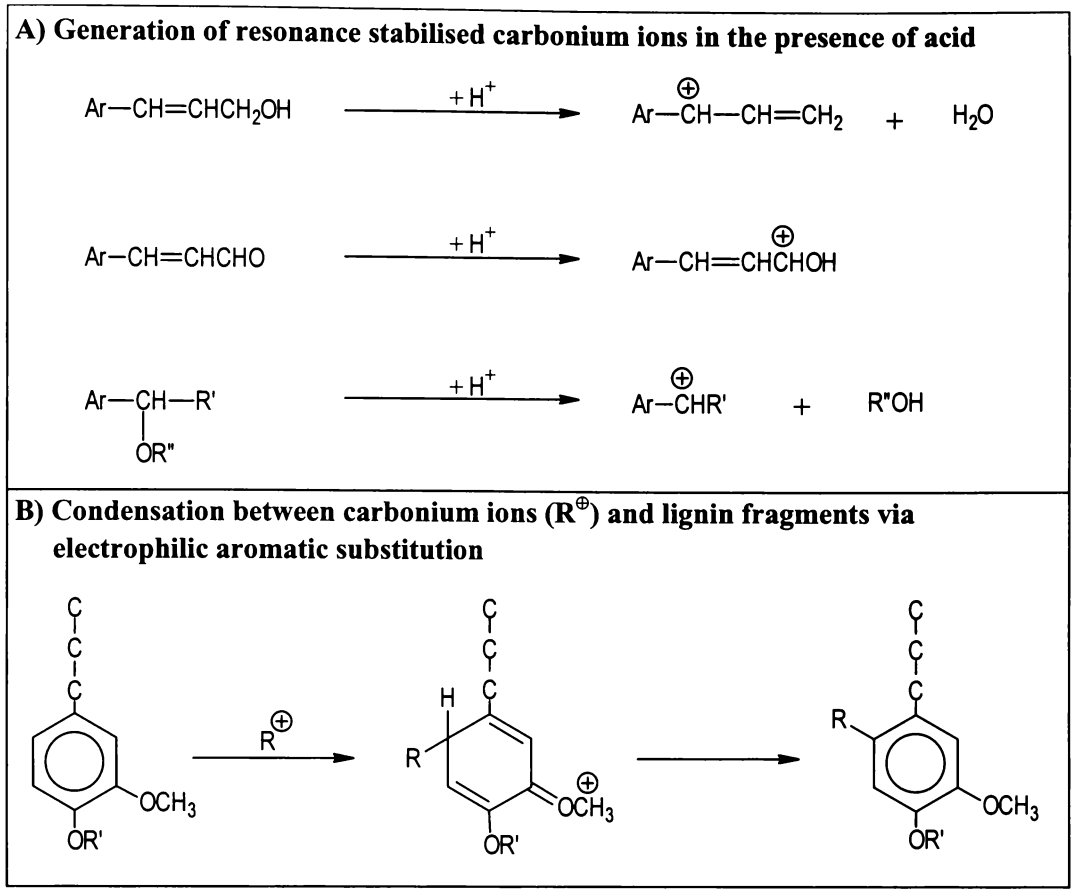

Figure (2.26). The effect of strong mineral acids on the structure of lignin

Lignin contains structures that can be converted to resonance stabilised carbonium ions (Figure 2.26A) in the presence of acid. These ions undergo electrophilic aromatic substitution (Figure 2.26B) forming a number of condensation products with other lignin fragments or combine with anions (mainly arising from the acid) forming substitution products. The degree of reaction depends on the acid type and strength (for example, HCl lignin is less condensed than H_2SO_4 lignin). Condensation reactions are minimised by reducing the exposure to the acid and lowering the temperature.

Acid lignin preparations are no longer used as sources of lignin for structural studies due to the extensive modification of the lignin that occurs during isolation. However, these preparations are used to estimate the lignin content of lignocellulosic materials.

The *cuoxam lignin* preparation was developed to reduce the degree of condensation in acid lignins. Carbohydrates are extracted into cuprammonium hydroxide after being partially hydrolysed with dilute sulphuric acid (1%). The degree of condensation in the isolated lignin is reduced due to the less severe acid treatment. However, this method is extremely tedious requiring several repeat treatments. Yields are approximately 80% of the total lignin and the repeated exposures to acid and base are not considered desirable. For these reasons this method has fallen into disfavour.

Periodate lignin is another preparation that is isolated as a residue (Ritchie & Purves 1947). Pre-extracted wood meal is treated with periodate solution for 24hr at 20°C, filtered, washed, and then extracted with boiling water for 3hr. For best results this cycle is repeated six times. Monosaccharide units of the polysaccharide are gradually converted to dialdehydes, which are susceptible to hydrolysis by boiling water (Figure 2.27A).

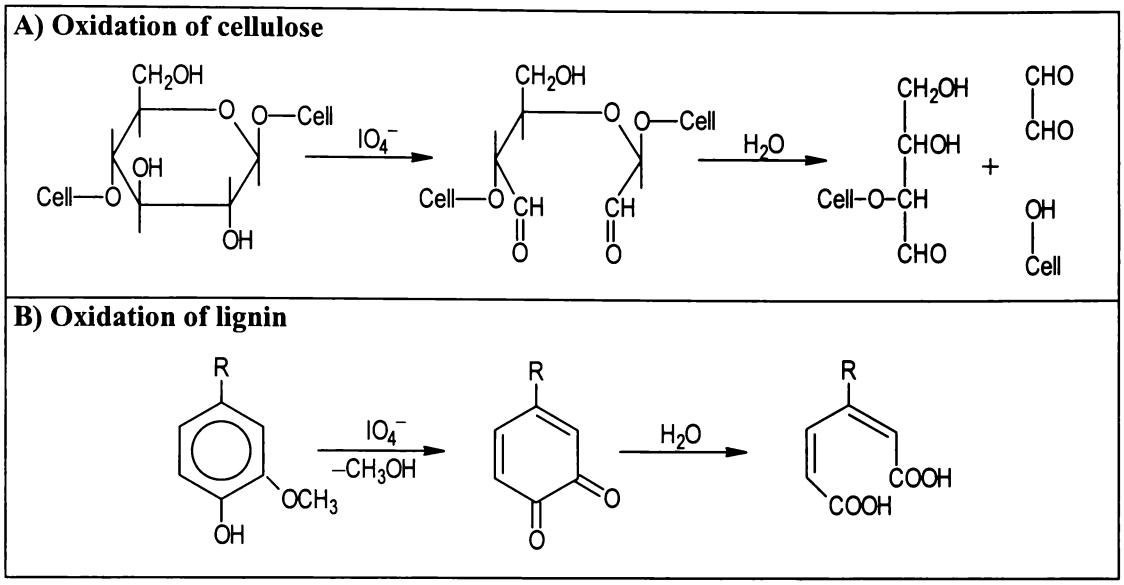

Figure (2.27). Oxidation of lignin and cellulose by periodate

The condensation reactions of the acid lignins are avoided by elimination of the acid, however, the lignin is partially oxidised by periodate, especially units containing a free phenolic OH group (Figure 2.27B, Lai & Sarkanen 1971). The degree of oxidation is dependent on the duration of the periodate treatment and generally about 80% of the periodate lignin remains unoxidised (Lai & Sarkanen 1971).

2.4.1.2 Isolation of lignin by extraction with structural modification

Organosolv lignin (or acidolysis lignin) is extracted into an organic solvent after partial degradation of the carbohydrates and lignin by acid. A wide range of solvents and acids can be used, giving different types of lignin in varying yields. For example, alcohol lignins are isolated from wood meal using alcoholic hydrochloric acid at elevated temperatures. Ethanol, methanol, isobutyl alcohol, ethylene glycol, and phenol are just some of the solvents that have been used (Brauns 1952). One disadvantage of this method is that during isolation, the lignin is alkoxylated (or phenolated) to a certain extent. For this reason dioxane is more commonly used for the isolation of organosolv lignin because it is an inert solvent and does not react with the lignin.

In addition to the substitution reactions with the solvent, the isolated lignin is altered in a number of other ways. An increase in phenolic hydroxyl groups and a decrease in the methoxyl content is observed in lignins isolated after prolonged acidolysis (Curvelo et al. 1993). Condensation between dissolved fragments also occurs and condensation between dissolved fragments and carbohydrates is also possible. The severity of the modifications depends on the strength of the acid, the duration of the extraction, and the isolation

temperature (Solar & Kacik 1995, Curvelo et al. 1993). To reduce the degree of condensation continuous flow-through methods have been developed. For example, Pan et al. (1992) developed a continuous delignification method based on ethanol acidolysis, while Jiang & Argyropoulos (1997) isolated residual lignin from kraft pulps using a continuous dioxane acidolysis method based on the batch process used by Gellerstedt et al. (1994).

Alkali lignin is extracted into aqueous or alcohol solutions of alkali hydroxides (Brauns 1952). Alkali extraction is not well suited for wood lignins because strong alkali and high temperatures are necessary to solubilise sufficient quantities of lignin. However, lignin from grass species (such as wheat, rye, and rice straw) can be obtained in reasonable yields under mild conditions (Lai & Sarkanen 1971). The mild alkali is not prone to cause much chemical modification to the grass lignins beyond saponification of *p*-coumaric and ferulic ester structures. Alkali lignins are often contaminated with protein and carbohydrates but these can be reduced by appropriate solvent treatments and also by enzyme treatment prior to alkali extraction (Lai & Sarkanen 1971).

TGA lignin is isolated from wood or pulp in almost 100% yield by derivatisation with thioglycolic acid. The procedure involves heating pre-extracted lignocellulose material with thioglycolic acid in 2M HCl for several hours followed by a subsequent extraction of the insoluble residue with 2% NaOH. The derivatisation is based on the reaction of benzyl alcohol groups with thioglycolic acid. Yields of TGA lignin from pulps are increased by treatment with formaldehyde in alkali prior to derivatisation. Lignin thioglycolic acids are rarely contaminated with polyphenols or protein impurities and do not appear to undergo any condensation reactions (Lai & Sarkanen 1971). TGA lignin preparations can also used to estimate the total lignin content of lignocellulosic materials.

2.4.1.3 Isolation of lignin by extraction with limited modification

Isolated lignin samples, which are to be used for structural and reactivity studies, need to be fully representative of lignin in its native state. This is to ensure that a true picture of lignin structure and behaviour is elucidated. The development of an isolation procedure that produces a soluble lignin in high yield with the least amount of modification is of ongoing interest to the wood chemist.

The *Brauns lignin* preparation was the earliest attempt at obtaining lignin in an unaltered form. Lignin is extracted from finely ground plant material with 95% ethanol and then precipitated with ether in yields of about 3–6% (Brauns 1939). Brauns lignin is quite similar to MWL in its chemical and physical properties; that is, there is no significant differences in the elemental composition; the Hibbert ketones and vanillin yields are the same; and only minor differences are observed in the infrared spectra (Lai & Sarkanen 1971). However, Brauns lignin has a significantly lower molecular weight and higher phenolic hydroxyl content than MWL.

Originally much structural information was inferred from Brauns lignin, however, the very low molecular weight and high phenolic hydoxyl content of the lignin suggests that Brauns lignin is not a good representative of native lignin. Lai & Sarkanen (1971) suggest that Brauns lignin consists of lignin degradation products.

Milled wood lignin (MWL) is the most commonly used lignin preparation. MWL is extracted from vibratory milled wood with aqueous dioxane (Lai & Sarkanen 1971). Yields of MWL are generally low and are highly dependent on the duration and efficiency of milling (Wegener & Fengel 1977, Polcin & Bezuch 1978). Yields of purified MWL from softwoods are generally well below 25% but yields as high as 50% have been reported, although 20 days of ball milling was necessary (Lai & Sarkanen 1971). The yields of MWL from hardwoods are usually higher than from softwoods and yields as high as 80% have been reported (Lai & Sarkanen 1971).

Milled wood lignin has been used widely in the study of lignin, however, there are two main disadvantages of using this procedure. The first is associated with the low yields and the concern that MWL may not be fully representative of the protolignin within the cell wall. The second is that lignin is depolymerised by ball milling (Lai & Sarkanen 1971) and the nature and extent of changes to the lignin structure are not completely known. Chang et al. (1975) observed that ball milling increases the phenolic hydroxyl and α-carbonyl content of the lignin and these changes may be due to homolytic cleavage of β-O-4 linkages in the lignin (Lee & Sumimoto 1990, Wu & Sumimoto 1992).

The structure of protolignin in the cell wall is heterogeneous. Differences in structure occur depending on the location within the cell wall and the age of the cell (Terashima 1993). There are two proposals on the origin of MWL. The first is that MWL is mainly derived from the middle lamella lignin (Bjorkman 1957). This proposal is supported by the residual carbohydrate composition of MWL (Lai & Sarkanen 1971) and the variation in the ratio between G and S units in different fractions of MWL isolated from birch wood (Lee et al. 1981).

The second proposal is that MWL originates from the secondary wall. Whiting & Goring (1981) have shown that separated fractions of middle lamella and secondary wall yield different quantities of MWL and concluded that the secondary wall is the main source of MWL. Maurer & Fengel (1992a) showed that the middle lamella is more resistant to ball milling than the secondary wall and that the secondary wall lignin is more easily released from the cell wall matrix. Radio tracer studies also agree with these findings (Terashima et al. 1992).

Differences in the origin of MWL between hardwoods and softwoods have also been observed (Maurer & Fengel 1992b). Ultrathin sections of spruce and beech wood samples embedded in water soluble melamine resins were extracted with dioxane under various conditions. After delignification the sections were stained with potassium permanganate and observed under an electron microscope. The results showed that MWL of spruce wood tracheids was derived predominately from the secondary wall whereas MWL of beech wood fibres was derived from both the middle lamella and secondary wall.

A lignin isolated in a higher yield should be more representative of the lignin present in wood. Other methods have thus been developed with the objective of increasing yields. Pew (1957) treated ball milled wood with a commercial enzyme to hydrolyse the polysaccharides and increase the lignin accessibility. Nearly all the lignin from aspen and spruce was isolated as an insoluble residue after 4 to 6 days of enzyme treatment. However, these lignin preparations, call milled wood enzyme lignin (MWEL), contained about 10% carbohydrate and were not very soluble in the common lignin solvents.

Fractionation of MWEL, based on solubility in aqueous dioxane, is a means of preparing a soluble lignin that can be purified in the same manner as MWL (Polcin & Bezuch 1978, Chang et al. 1975). These preparations, known as cellulase enzyme lignin (CEL), are obtained in slightly higher yields than MWL. Additionally, cellulase enzyme lignin has a higher average molecular weight and a lower phenolic hydroxyl content than MWL (Chang et al. 1975). However, the long enzyme treatments are a disadvantage to this method.

Enzymatically liberated lignin (ELL) is obtained from brown-rotted wood. Fungi that cause the so-call brown-rot type of wood decay inhabit the lumen of cells and secrete enzymes that selectively digest the wood polysaccharides. Brown rot fungi almost completely digest the carbohydrates leaving a residue consisting mainly of lignin (Nord & Schubert 1951, Brown et al. 1968). As with MWEL, the residue includes essentially all the lignin present in the original wood. However, the severe physical pretreatment required for the isolation of MWEL is not required and thus the ELL should be more representative of protolignin. Similarly, the ELL is not very soluble in typical lignin solvents (Kirk 1975). Nord & Schubert (1951) extracted about 10% of the ELL into 95% ethanol and showed that it was very similar to Brauns lignin.

ELL is modified by the brown-rot fungi to a certain extent (Kirk 1971, Kirk 1975). Modifications are mainly oxidative and include demethylation of methoxyl groups, formation of carboxylic groups, and aromatic hydroxylation. Demethylation results in the formation of new phenolic OH groups in both phenolic and non-phenolic units, and occurs in about a third of the phenylpropane units. Aromatic hydroxylation also introduces new OH groups but only occurs in a small percentage of the units. ELL contains more aromatic hydroxyl groups, aliphatic hydroxyl groups, and carboxyl groups than MWL, however, depolymerisation of the lignin is limited (Jin et al. 1990).

2.4.2 Lignin characterisation

Lignin is a highly complex heterogeneous aromatic polymer consisting of phenylpropane units connected by both ether and carbon-to-carbon bonds (Section 2.1.3.2). It is intimately associated with, and may be chemically bonded, to the carbohydrates within the cell wall. Because of these structural features, lignin is difficult to characterise and as a consequence there are a large number of techniques used to study its structure. In most cases, a combination of several analytical techniques is used.

To overcome the problems associated with interference from the carbohydrate components of the cell wall the majority of methods require isolated lignin samples. The exception is the degradation methods, which incorporate a chromatographic step to separate the carbohydrate products from the lignin products.

2.4.2.1 Elemental analysis

Elemental analysis and methoxyl content determination can be used as an initial step in the characterisation of lignin. The results can be used to generate an average molecular formula (Adler 1977) for the phenylpropane unit allowing easier comparison between lignin samples. Any differences in the elemental composition of the lignin indicate structural differences; for example, an increased oxygen content is an indication of a more oxidised lignin.

2.4.2.2 Molecular weight distribution

The majority of lignin isolation procedures (Section 2.4.1) involve the degradation of the lignin macromolecule into soluble fragments. These have a wide range of molecular weights (that is, soluble lignin fractions are polydisperse), thus it is difficult to speculate on the molecular weight of protolignin from the molecular weight of isolated samples. However, changes to the average molecular weight or the molecular weight distribution of similar lignin samples can give indications of changes to the extent of degradation or condensation of the lignin. Additionally, the molecular weight of lignin is considered to be an important factor in determining its dissolution during pulping and bleaching processes.

There are a number of methods for determining the average molecular weight of polymers (Seymour & Carraher 1988), of which osmometry, ultrafiltration, sedimentation, and light scattering are the most common for lignin (Pla 1992, Lin 1992a, Fengel & Wegener 1984). However, the high polydispersity of isolated lignin samples generates problems when estimating the average molecular weight by these methods, thus measuring the molecular weight distribution of lignin by size exclusion chromatography is more common, although, this method also has disadvantages (Gellerstedt 1992a). For example, differences in the molecular weight distribution can arise when using different solvents and different gels so care must be taken when comparing distributions obtained using different methods. In most studies polystyrene fractions of known molecular weight are used to calibrate the chromatographic system. These standards show good correlation with low molecular weight lignin fractions but differences can be expected at higher molecular weight due to branching and cross-linking in the lignin. However, a molecular weight scale based on polystyrene standards can facilitate comparisons between different systems to a limited extent.

2.4.2.3 Degradation studies

The complexity and size of the lignin macromolecule makes the characterisation of lignin as a whole very difficult. The techniques that overcome this problem are the chemical degradation methods where the lignin macromolecule is degraded into smaller fragments that are more easily characterised. Characteristics of the fragments give information regarding interunit linkages and side chain structures of the lignin macromolecule. One advantage of these techniques is that lignin can be studied *in situ*, since the lignin degradation products are easily separated from the carbohydrate degradation products by chromatography.

Hydrolysis methods

Alkyl-aryl ether bonds are the main interunit linkages in lignin and hydrolysis of these leads to considerable depolymerisation of the lignin into fragments. Organic extraction of the hydrolysis media yields a mixture of monomers, dimers, trimers and other small oligomers, which are easily characterised. The monomers and other small volatile fragments can be characterised by GC techniques and the larger fragments can be separated using TLC or HPLC and then characterised using NMR or FTIR spectroscopy.

A *mild hydrolysis* of lignocellulosic material in boiling water or acidified boiling water cleaves benzyl ether bonds releasing a low yield (10% for spruce) of products into solution (Nimz 1993). From the product mixtures several β-O-4, β-β, and β-1 type structures have been identified giving the first direct evidence for their existence in lignin.

The degree of hydrolysis can be increased under more severe conditions, such as by *acidolysis*, which is generally carried out by refluxing lignin or lignocellulosic material with hydrochloric acid (0.2M) in aqueous dioxane for four hours (Kirk & Obst 1988). Under these conditions both α-aryl and β-aryl ether linkages are cleaved. Phenolic β-aryl ether structures yield a series of low molecular weight compounds (Figure 2.28, Lundquist 1992a). These can be quantified by GC and used to estimate of the frequency of phenylpropane-β-aryl ether linkages. Other types of β-aryl ether structures can be determined in a similar manner using appropriate model compounds. This method has been used to analyse changes in the amounts of enol ether, β-O-4 and α-carbonyl β-aryl ether structures in kraft residual lignin during pulping (Gellerstedt & Lindfors 1984a).

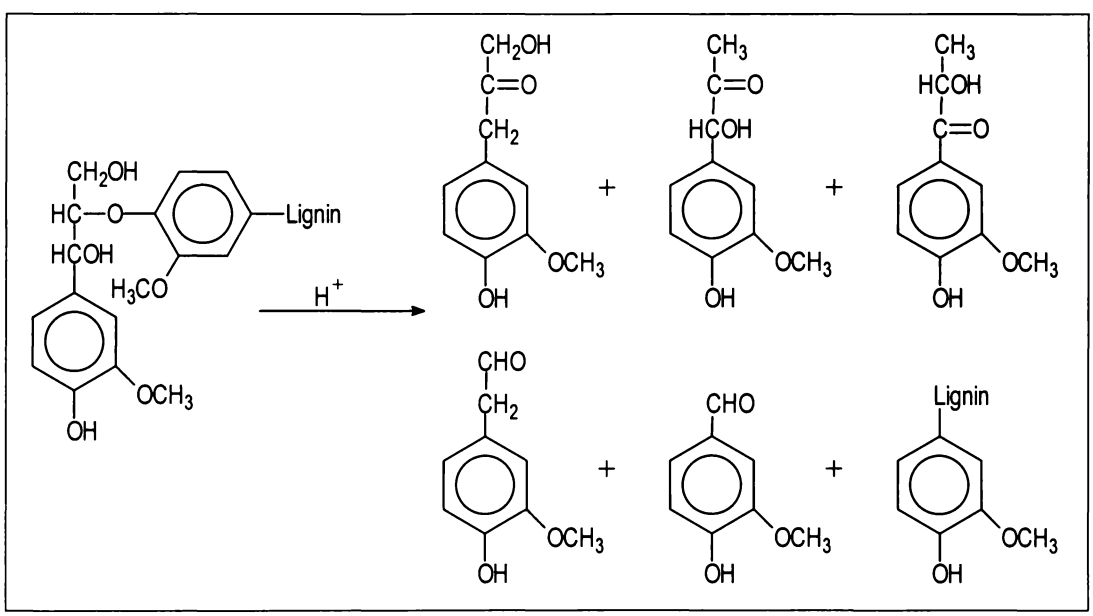

Figure (2.28). Reaction products from acidolysis of guaiacylpropane-β-aryl ether

One drawback to acidoloysis is that condensation reactions between degradation products occur in the acidic medium causing a decrease in monomer yields. These secondary reactions can be eliminated if degradation is carried out using *thioacidolysis*. Thioacidolysis involves the cleavage of β-aryl ether linkages by heating lignocellulosic material with ethanethiol and BF_3-etherate in aqueous dioxane (Figure 2.29, Rolando et al. 1992). The condensation reactions are avoided by conversion of the benzylic cation intermediates to acid stable thiol-benzyl ether derivatives and consequently the monomer yield is generally higher compared to acidolysis (Tanahashi & Higuchi 1988). Monomers, which are used to quantify uncondensed β-O-4 linkages, are analysed by GC after trimethylsilyl derivatisation and the dimers relating to β-5, 5-5, β-1, 4-O-5, and β-β structures are analysed in a similar way after Raney-Nickel desulfurisation (Lapierre et al. 1990).

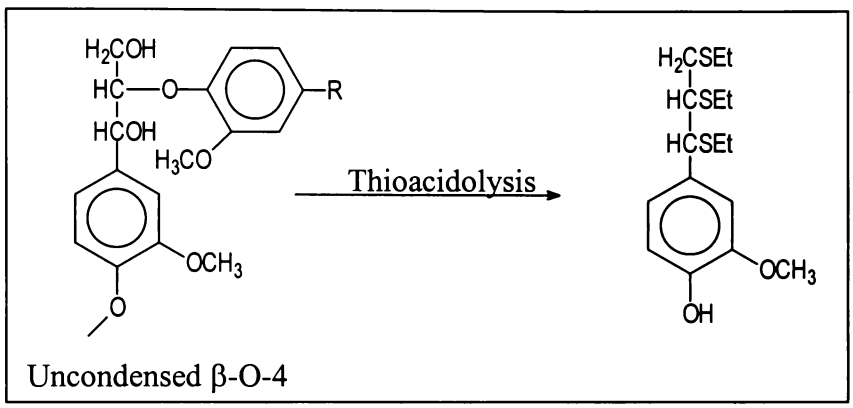

Figure (2.29). Thioacidolysis degradation of β-aryl ether bonds

Derivatisation Followed by Reductive Cleavage (DFRC) is a new method for the structural analysis of wood (Lu & Ralph 1997). It involves the cleavage of α-aryl and β-aryl ether linkages in lignin and provides data analogous to thioacidolysis without the need for the odorous ethanethiol. Three steps are involved (Figure 2.30), an initial reaction with acetyl bromide (1) followed by reductive cleavage of the β-bromoether linkage with zinc in acidic media (2), and finally an acetylation step (3). The degradation monomers are then quantified by GC-analysis.

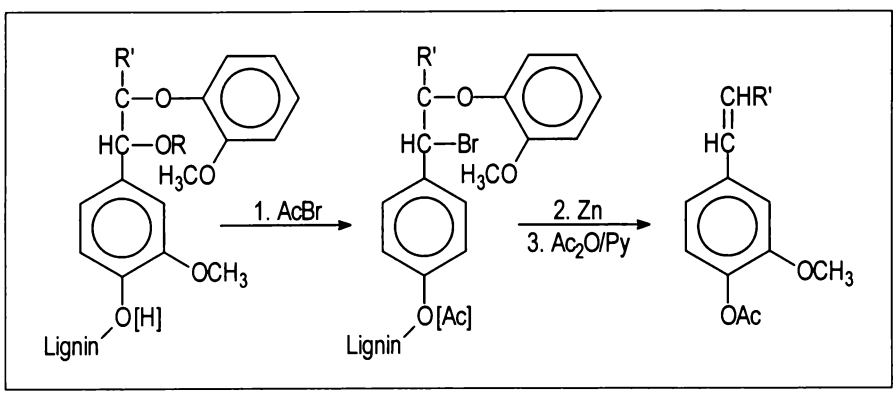

Figure (2.30). Ether cleavage in lignin using DFRC method

Hydrogenolysis is another hydrolysis degradation method that avoids secondary condensation reactions (Sakakibara 1992). The main reaction during hydrogenolysis of lignin is cleavage of ether linkages connecting the α, β, and γ carbon atoms of the side chain to position 4 of an adjacent phenolic ring. In general, alkyl-O-alkyl linkages are more stable to hydrogenolysis than alkyl-O-aryl linkages so they often survive hydrogenolysis, although their stability strongly depends on the conditions used. Carbon-to-carbon bonds also survive. Therefore, characterisation of the monomers, dimers, trimers, and other small fragments reveals the nature of some of the structural groups in lignin and the manner in which they are linked.

Hydrogenolysis is accompanied by hydrogenation; that is, reduction of the side chain and aromatic ring. For example, side chain structures such as coniferaldehyde and coniferyl alcohol are readily reduced to dihydroconiferyl alcohol and if the catalyst is sufficiently active or the reaction conditions are severe enough the aromatic ring can also be hydrogenated to a cyclohexyl ring. However, the latter is undesirable for lignin structural studies.

Degradation of the lignin macromolecule can also be achieved thermally, as in the *pyrolysis-GC* method (Meier & Faix 1992). During pyrolysis, lignocellulosic material is rapidly heated to over 400°C resulting in thermal degradation of the components. Rapid heating of the sample to the maximum temperature is of paramount importance to obtain optimal thermal degradation and to avoid secondary reactions. To further reduce the chance of secondary reaction the volatile degradation products should be removed quickly from the hot zone of the pyrolyser.

Under appropriate conditions, the pyrolysis of lignin gives rise to a characteristic pattern of substituted monomeric phenols in which the propanoid side chain has been split off completely or shortened to one or two carbons. The pyrolysis technique can, therefore, be used to classify lignins in terms of their G, S, and H content. Pyrolysis has also been used to fingerprint and identify residual lignins.

The final hydrolysis degradation technique to be discussed is the *nucleus exchange* (NE) method (Funaoka et al. 1992). This method involves the cleavage of the α-aryl ether bond in uncondensed phenolic units, following phenolation at the Cα position (Figure 2.31). This leads to quantitative release of the phenyl nuclei (guaiacyl unit or syringyl unit) which may then be subsequently demethylated. Therefore, the sum of the phenyl nuclei released from lignocellulosic material can be used to estimate the total uncondensed phenolic units in lignin. This method can also be used to estimate the ratio between syringyl and guaiacyl units in hardwood lignin. The disadvantage of this method is that a wide range of products are obtained from lignocellulosic materials making analysis of the gas chromatograms quite difficult (Chan et al. 1995).

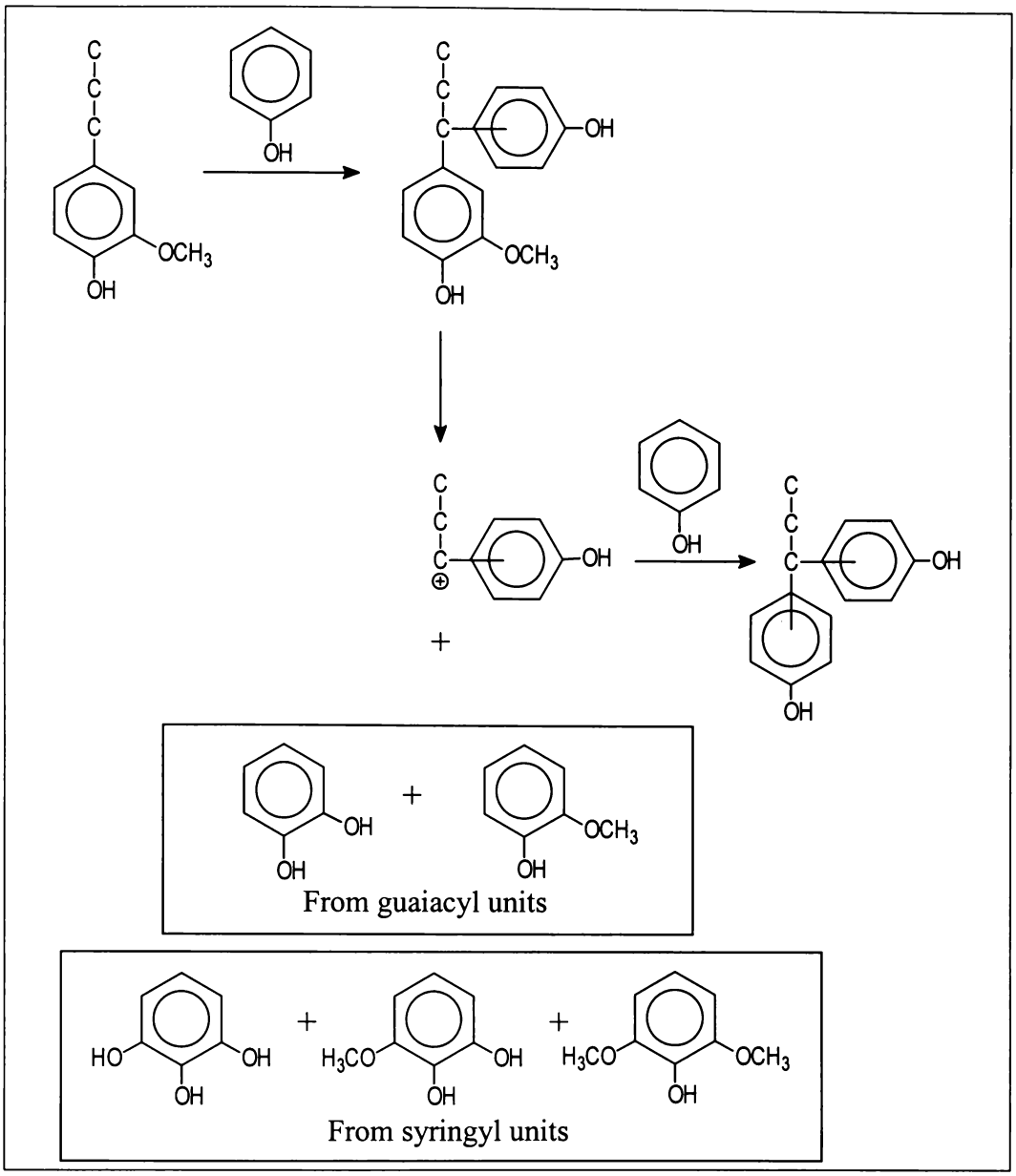

Figure (2.31). Degradation of uncondensed phenolic units in lignin by the NE method

Oxidative degradation

Oxidative degradation of lignin can be used to study lignin structure if the aromatic ring is preserved during the oxidation. The two most common oxidative methods, namely nitrobenzene oxidation and permanganate oxidation, oxidise the lignin to aromatic aldehydes and carboxylic acids. These are used to quantify certain structural elements in the lignin. The major drawback to oxidative degradation is that only structures carrying a free phenolic unit are analysed. Therefore, the information gained accounts for only a fraction of the lignin, however, such information can still be used to gain insight into the lignin structure. A pretreatment, which cleaves aryl ether linkages, can be incorporated to increase the phenolic content of the lignin sample thereby increasing the yield of degradation products (Adler 1977).

Nitrobenzene oxidation (Chen 1992a, Kirk & Obst 1988) of lignin in alkaline solution at elevated temperatures (170–180°C) degrades lignin to a mixture of aromatic aldehydes (Figure 2.32). The low molecular weight products are extracted into an appropriate organic solvent (such as chloroform) and analysed by HPLC or GC. In general, softwood lignins give rise to vanillin (2) as the major product with minor amounts of *p*-hydroxybenzaldehyde (1), syringaldehyde (3), and other minor degradation products (4, 5). In contrast, lignin from typical hardwoods gives rise to vanillin and syringaldehyde as the major products. The yield and molar ratio of the phenolic aldehydes, (1–3), depend on the plant species being investigated, so nitrobenzene oxidation can be used for the classification of lignin. The total yield of monomeric degradation products is also an indication of the minimal quantity of uncondensed phenolic units in the lignin and can provide a relative measure of the extent of condensation within the lignin.

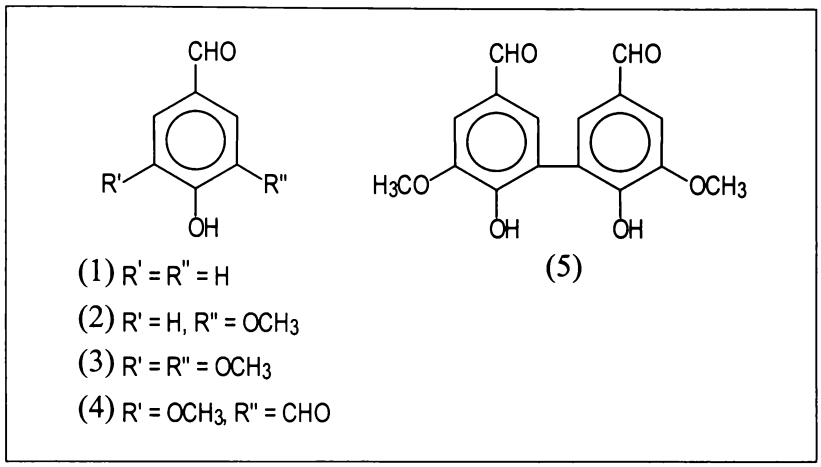

Figure (2.32). Common nitrobenzene oxidation products of lignin

The range of degradation products obtained from lignin by *metal oxide oxidation* is the same as the products obtained by nitrobenzene oxidation, however, the yields are slightly different. A range of metals can be used; for example Cu, Ag, Hg, and Co, each of which gives a slightly different distribution of products (Fengel & Wegener 1984). Cupric oxide gives the most comparable results to nitrobenzene, however cupric oxide has a greater tendency to further oxidise the aldehydes to the corresponding acids (Chen 1992a).

Much more structural information can be obtained from p*ermanganate oxidation* of lignin or lignocellulosic material (Gellerstedt 1992b, Kirk & Obst 1988). The general procedure for permanganate oxidation involves several steps. First, the lignin is methylated or ethylated to protect the free phenolic hydroxyl groups from oxidation. This step is followed by permanganate oxidation using a mixture sodium periodate and potassium permanganate in alkali at 100°C. The sodium periodate re-oxidises any manganate back to permanganate and allows the potassium permanganate levels to be kept low, thus minimising the risks of over-oxidising the lignin. Under these conditions a mixture of phenylglyoxylic and aromatic acids is obtained. A second oxidation step under mild conditions with alkaline hydrogen peroxide converts all the intermediate degradation products into aromatic acids. In the last steps the mixture of acids is methylated with diazomethane and characterised by GC/MS.

The major degradation products (Figure 2.33) reflect the distribution of various types of phenylpropane units in lignin. Structure (1) originates from uncondensed lignin end groups in which the propanoid side chain has been oxidised to a carboxylic acid. Phenypropane units which have, in addition to the normal Cα side chain linkage, a carbon-to-carbon linkage to either the 5 or 6 position of an adjacent aromatic unit give rise to degradation products (2) and (3), respectively. Additionally, diaryl ether and diaryl structures give rise to structures (4) and (5), respectively.

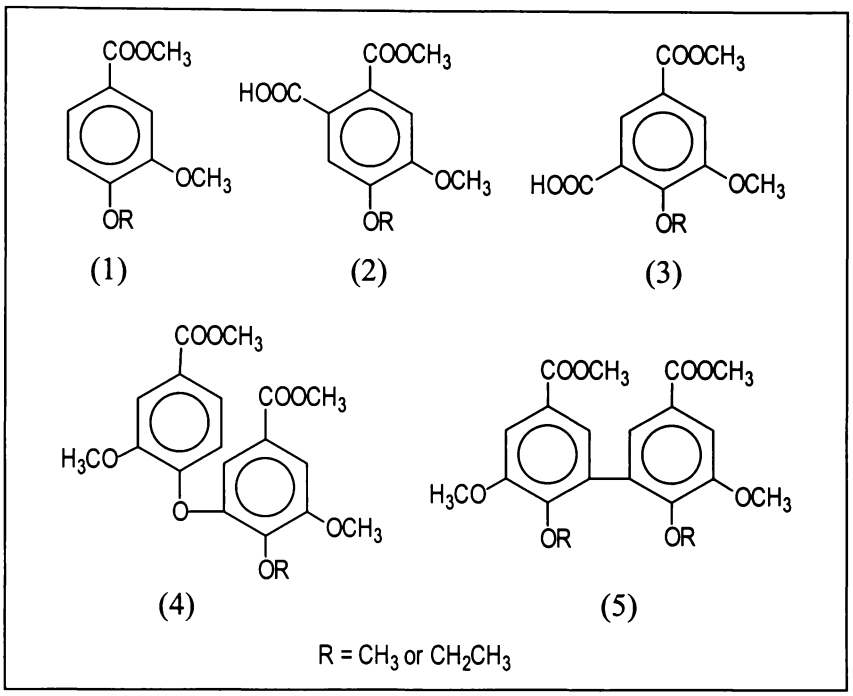

Figure (2.33). Main potassium permanganate oxidation products from softwood lignin

2.4.2.4 Functional group analysis

Certain functional groups are important when considering the reactivity of lignin, particularly during pulping and bleaching. The phenolic hydroxyl group is an important functional group, affecting both physical and chemical properties of the lignin. It plays a prominent role in pulping and bleaching reactions promoting base-catalysed cleavage of aryl ether linkages and oxidative degradation. On the other hand, it contributes to the poor brightness stability of lignin rich pulps. Therefore, the quantitative determination of the phenolic hydroxyl group provides information relating to structure and reactivity of the lignin as well as to the extent of lignin degradation.

Aliphatic hydroxyl groups also influence the reactivity of lignin towards pulping and bleaching, although not to the extent of the phenolic hydroxyl group. For example, aliphatic hydroxyl groups assist in the cleavage of β-O-4 linkages in non-phenolic units during kraft pulping (Gierer & Noren 1962) and an α-OH is detrimental to the degradation of phenolic units during oxygen bleaching (Johansson & Ljunggren 1994)

Carbonyl structures, particularly conjugated carbonyls, ortho quinoid, and para quinoid structures are important since they contribute or potentially contribute to the colour of lignin and play an important role in brightness reversion in pulps.

Carboxyl groups are introduced when lignin is subjected to oxidative degradation (either chemical or biological). Carboxyl groups are present in muconic acid structures arising from degradation of the aromatic ring or as aliphatic acids when carbonyl groups are oxidised. Thus, the quantitative measure of the carboxyl content of lignin provides information on the degree of oxidation.

Hydroxyl groups, carbonyl groups, and carboxylic acids also increase the hydrophilicity of the lignin and thus increase its solubility during pulping and bleaching. Due to the importance of these groups, several techniques are available to quantify them in lignin (Table 2.6).

2.4.2.5 Spectral techniques

The spectroscopic techniques commonly used for the study of lignin are UV, FTIR, and NMR. The advantage of spectroscopic techniques is that the whole lignin macromolecule contributes to the spectra, so a more complete picture of the lignin structure is observed. This is in contrast to the degradation studies, which involve only small fragments of the lignin. Also, no severe modification of the lignin macromolecule, other than what occurs during isolation, is necessary for analysis.

UV spectroscopy

Ultraviolet absorption is a commonly used tool for lignin analysis (Goldschmid 1971). The distinct absorption of lignin in the ultraviolet range (Figure 2.34) is based on its aromatic character and on several chromophoric structural elements, such as conjugated double bonds and conjugated carbonyl structures.

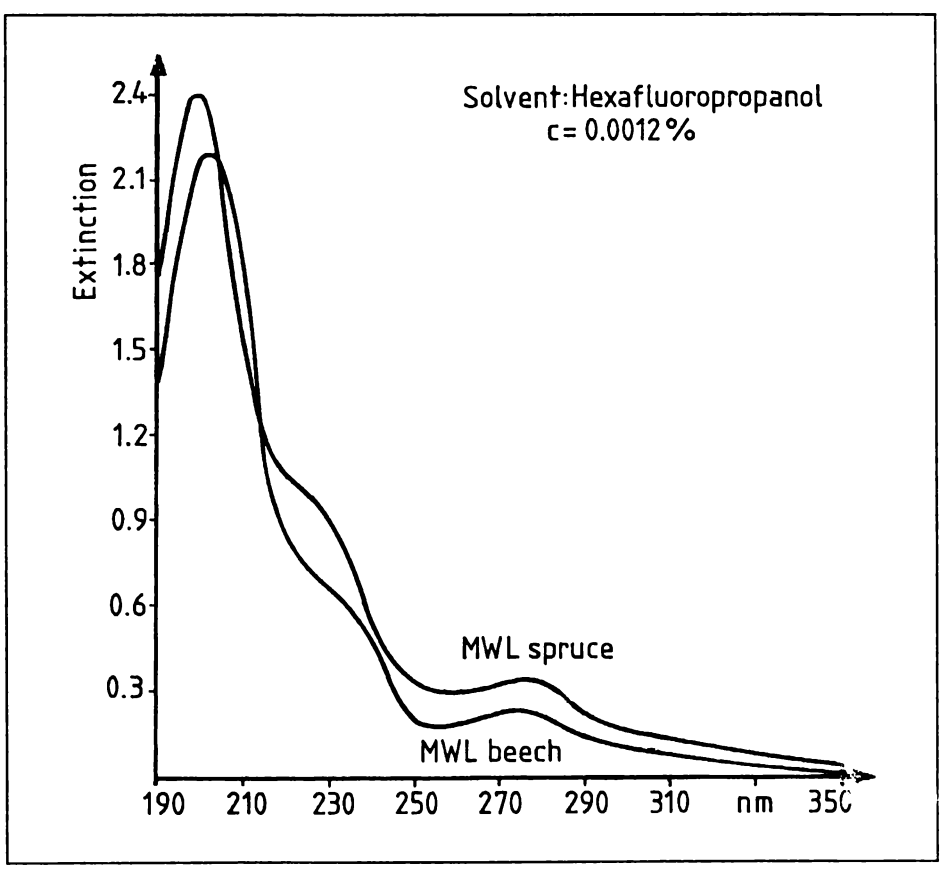

Figure (2.34). UV spectra of milled wood lignin (Fengel & Wegener 1984)

Table (2.6). Methods for the analysis of functional groups in lignin

Method	Groups	Methodology
Diazomethane[1]	Phenolic OH and carboxyl	Lignin is methylated with diazomethane and the methoxyl content is determined. The increase in methoxyl content is a measure of the acidic hydroxyl (carboxyl, phenolic) content of lignin. The carboxylic acids can then be determined from the methanol released upon saponification of the resulting methyl esters.
Acetylation[2]	Total OH	This method is used to determine the total hydroxyl content of lignin. Lignin is acetylated in acetic anhydride and pyridine and the acetic acid produced from the saponification of the acetate is used to estimate the total hydroxyl content. Carbohydrates interfere with this method but if the carbohydrate content of the sample is known a correction can be made. The aliphatic hydroxyl content is usually determined by subtracting the phenolic content of the lignin from the total hydroxyl content.
Hydroxylamine hydrochloride[3]	Carbonyl	Reaction between $NH_2OH{\cdot}HCl$ and the C=O of carbonyl groups yields quantitatively a mole of HCl per carbonyl group. The liberated HCl is determined by titration at intervals until the reaction is complete.
Aminolysis[4]	Phenolic OH	This method is based on the fact that phenolic acetyl groups are deacetylated by pyrrolidine at a much faster rate than aliphatic acetyl groups. The phenolic OH content of the lignin is estimated from the amount of 1-acetyl-pyrrolidine (determined by GC) formed in the early stages of the reaction. This method can be applied to *in situ* lignin if the carbohydrates are reduced with $NaBH_4$ to prevent their interference.
Periodate oxidation[5]	Phenolic OH	The periodate oxidation method is based on the finding that guaiacyl and substituted guaiacyl units are oxidised by aqueous $NaIO_4$ to ortho quinone structures. In the process a mole of methanol per phenolic hydroxyl group is released. The liberation of methanol is followed by GC until the levels cease to change. The main disadvantage of this method is that units without a methoxyl group (catechol and *p*-hydroxy phenol units) are not detected.
Conductometric or potentiometric titration[6]	COOH and PhOH	The analysis involves neutralisation of the acid groups in lignin using potentiometry (pH) or conductivity to detect the endpoints. The endpoints of both the more acidic carboxylic groups and the weakly acidic phenolic OH groups can be distinguished from the conductance titration curve. However, in the case of pH titration the inflections corresponding to the various acidic groups are often indistinct, making the detection of endpoints for the weaker acids difficult. This problem can be overcome by addition of an internal standard, such as *p*-hydroxybenzoic acid, which enhances the instrumental response and allows the individual inflections to be resolved.
Spectral techniques	All groups	A number of different spectral methods (UV, FTIR and NMR) have been used to estimate the quantities of different functional groups in lignin and these are discussed in the following sections.

References: **1**. Yamasaki et al. (1981); **2**. Chen (1992b); **3**. Chen (1992c); **4**. Mansson (1983), Gellerstedt & Lindfors (1984b); **5**. Lai et al. (1990); **6**. Katz et al. (1984), Dence (1992)

Absorption at any one of the three maxima can be used to estimate lignin content. For example, during Klason lignin determinations a small fraction of the lignin remains in solution and UV absorption at about 205nm is used to estimate the amount of acid soluble lignin. Sjostrom and Enstrom (1966) used UV spectroscopy to estimate the residual lignin content of a series of sulfite and kraft pulps. Pulps were dissolved in cadoxen and the lignin content was estimated from the UV absorption maxima at 280nm. The accuracy of these measurements largely depends on the estimate used for the extinction coefficient of the lignin sample but additional errors may also arise from interference associated with solvent, carbohydrate degradation products, or phenolic impurities.

Chemical modification of lignin affects its light absorption properties. As a general rule, modifications that result in the introduction of conjugated double bonds or additional aromatic substitution cause a shift of absorption maxima to longer wavelengths. Conversely, a reduction in the chromophoric structures produces a shift of the absorption maxima to shorter wavelengths. These spectral changes are useful for determining the extent to which lignin has been modified. For example, one of the most significant changes to the UV spectra of lignin arises from the loss in aromatic character caused by chlorination. As the degradation of the lignin proceeds a dramatic reduction of the absorption at 280nm is observed.

Free and etherified phenolic units contribute significantly to the absorption maxima near 280nm (Lin 1992b). In neutral solution, the spectra of free and etherified phenols are the same. However, in alkali, the ionisation of the hydroxyl group shifts the absorption band of free phenolic units towards longer wavelengths while having no effect on the absorption of etherified units. Therefore, the difference spectrum, resulting from the subtraction of the neutral UV spectrum from the alkali UV spectrum, essentially represents the absorption of the ionisable phenolic units. With the aid of appropriate model compounds, to identify the difference maxima and estimate the extinction coefficients, the phenolic hydroxyl content of different structural elements can be estimated (Goldschmid 1971, Lin 1992b). Lignin structures containing α-carbonyl groups can be determined in a similar way by $NaBH_4$ reduction difference spectra. This approach is based on the spectral changes occurring on borohydride reduction of the α-keto group.

Although, the UV technique for estimating the phenolic and conjugated carbonyl content of lignin is a convenient and simple method, there are a number of intrinsic errors. The major error is associated with the use of model compounds to estimate the extinction coefficients of different lignin structures. The structural variation and steric restraints typifying lignin, which are absent in model compounds, probably influence the UV absorption of the lignin. Therefore, the extinction coefficients of the model compounds will only approximate the true absorptivities of the lignin. Additionally, difference spectroscopy utilises certain spectral modifications arising from chemical treatment of the lignin. Therefore, the accuracy of the UV method is reliant on whether the chemical modifications are fully quantitative and whether any other modifications occur as a result of the chemical treatment. Overlapping peaks in the UV difference spectra are another source of error.

Infrared spectroscopy

FTIR spectroscopy is also a widely used technique for the study of isolated lignin. One advantage of FTIR is that soluble lignin samples are not necessary since routine FTIR analysis is carried out on solid samples in the form of KBr disks. The infrared spectra are, however, strongly influenced by carbohydrate contaminants and protein (in the case of enzymatically isolated lignins).

The most common use of FTIR is for qualitative analysis of structural changes in lignin, which are visualised as changes in specific peak intensities. FTIR has been useful in comparing lignins from different species as well as comparing lignin isolated by different techniques (Hergert 1971). FTIR has also been used for the study of residual lignins (Hortling et al. 1990, Hortling et al. 1992).

FTIR spectra of lignin show several major absorption bands, which can be assigned to specific structural groups in lignin (Table 2.7).

Table (2.7). Assignments of some infrared adsorption bands of lignin (Hergert 1971)

Position (cm^{-1})	Band origin
3450 – 3400	OH stretching
2940 – 2820	CH stretching in methyl and methylene groups
1760 – 1680	C=O stretching; nonconjugated to the aromatic ring
1675 – 1640	C=O stretching; conjugated to the aromatic ring
1605 – 1600	Aromatic skeletal vibrations
1515 – 1500	Aromatic skeletal vibrations
1470 – 1460	C–H bending
1430 – 1425	Aromatic skeletal vibrations
1370 – 1365	C–H bending
1330 – 1325	Syringyl ring breathing
1270 – 1275	Guaiacyl ring breathing
1140	Aromatic C–H bending; guaiacyl unit
1128 – 1125	Aromatic C–H bending; syringyl unit

Spectral changes associated with differences in the ratios of G, S, and H units can be used to classify lignins (Faix 1991). For example, a composite band system between $1175 cm^{-1}$ and $1065 cm^{-1}$ shows a maximum at $1140 cm^{-1}$ in G-type lignins, whereas in lignins containing S-units (GS and HGS lignins) the maximum is shifted to between $1128 cm^{-1}$ and $1125 cm^{-1}$. An additional band at $1167 cm^{-1}$ in lignins containing a larger proportion of H-units can be used to distinguish between GS and HGS lignins. A number of other differences are also apparent (Faix 1991).

The carbonyl stretching region, between $1640 cm^{-1}$ to $1760 cm^{-1}$, is the most useful region of a lignin FTIR spectrum. Since this region is reasonably isolated, changes in the carbonyl content of the lignin sample are easily visible. A semiquantitative FTIR method to determine the carboxyl content of lignin was reported by Hortling et al. (1997), in which, the carbonyl band at $1740 cm^{-1}$ and aromatic band at $1510 cm^{-1}$ were used to estimate the carboxylic acid content of MWL and kraft residual lignin.

The difference spectrum between acetylated and non-acetylated lignin can be used to estimate the relative amounts of phenolic and aliphatic hydroxyl groups (Faix et al. 1992). Acetylated lignin shows a strong band between 1710 cm^{-1} and 1790 cm^{-1} arising from the acetoxy carbonyl vibrations. The band is a combination of the phenolic acetoxy signal at around 1765 cm^{-1} and the aliphatic acetoxy signals at 1744 cm^{-1}, thus the ratio between the two acetoxy signals can be used to estimate the ratio between the phenolic and aliphatic hydroxyl groups.

Nuclear magnetic resonance

NMR techniques have been extensively used for the study of lignin due to the amount of information obtained. As with the other spectral techniques, the whole lignin macromolecule is contributing to the signal, so a more complete picture of the lignin is observed. However, compared with the other spectral techniques discussed above, NMR has the added advantage that more detailed information concerning the bonding pattern within the lignin macromolecule can be elucidated.

One limitation common to most NMR experiments (except for solid state ^{13}C) is that lignin samples must be dissolved in an appropriate NMR solvent. The two most common NMR solvents for underivatised lignin samples are deuterated-acetone/D_2O or deuterated-DMSO/D_2O mixtures. However, in many cases the solubility of the underivatised lignin sample is limited causing a reduction in sensitivity. For this reason, acetylation of the lignin is often recommended (Lundquist 1992b, Chen & Robert 1988) to improve solubility ($CDCl_3$ or acetone as the solvent). Additionally, extra structural information can obtained due to an increase in spectral dispersion.

^{1}H-NMR spectra are both complex and not well resolved. These disadvantages are attributed to the complex nature of the lignin structure as well as the narrow range of ^1H chemical shifts. ^1H nuclei reside in a number of similar, but not identical environments, resulting in broad signals that are not well resolved. Additionally, the presence of carbohydrates in isolated lignin samples further complicates the spectra. In spite of these problems, certain structural elements can be identified (Lundquist 1980, Chen & Robert 1988).

The major regions in a ^1H-NMR spectrum of acetylated lignin include; the aldehyde protons (9–10 ppm), the aromatic protons (6.25–7.90 ppm), the methoxyl protons (3.55–3.95 ppm), and the acetate methyl protons (1.60–2.50 ppm). Integration of these regions can give information on the levels of lignin end groups (such as coniferaldehyde and vanillin), the degree of aromatic substitution, and the hydroxyl content of the lignin. Calibration of the methoxyl signal with the methoxyl content of the acetylated lignin allows quantitative analysis. Alternatively, an internal standard, such as docosane (Lundquist 1980), can be added for calibration.

The H_α signal of β-O-4 structures (6.06 ppm) is also well resolved and can be used to estimate the levels of both erythro and threo β-O-4 structures in lignin. Spectral characteristics which are attributed to β-5 and β-β structures have also been identified (Lundquist 1980).

^{13}C-NMR spectroscopy is one of the most powerful techniques for lignin analysis. Since carbon atoms make up the backbone of organic molecules, ^{13}C chemical shifts are much more dependent on the structural environments and substitution patterns than those in ^{1}H-NMR. For this reason ^{13}C-NMR spectra contain much more structural information over a much wider range of chemical shifts. An additional advantage of the wide chemical shift range is that the ^{13}C-NMR spectra are more well resolved (Chen & Robert 1988). The assignment of ^{13}C signals is almost exclusively based on the information obtained from model compounds. The use of model compounds may not be totally accurate in representing the behaviour of the lignin macromolecule because structural features present in the lignin, which are absent in the model compound, may cause slight differences in chemical shifts. The accuracy of assignments is also limited by the availability of relevant model compounds.

Due to the complexity and heterogeneity of the lignin structure, many of the ^{13}C signals remain unresolved. This problem can be overcome, in part by spectral editing techniques based on the number of attached protons. The DEPT sequence is considered a suitable technique since it is the least dependent on CH coupling constants (Landucci 1991). This 1D sequence edits the ^{13}C signals into three separate subspectra for the CH, CH_2 and CH_3 signals, respectively. By separating the different types of carbon atoms into separate subspectra many of the overlapping signals become resolved. Another advantage of this type of spectral editing is that information regarding carbon multiplicity, which is lost during proton decoupling, is regained. Furthermore, the signal intensities are enhanced by polarisation transfer.

Functional groups and interunit linkages that give reasonably well resolved signals at a good signal to noise ratio can be analysed reliably using ^{13}C-NMR. For example, changes in carboxylic acids, quaternary aromatic carbons, and β-O-4 linkages are easily identifiable and quantified (Nimz 1993, Chen et al. 1995a, Pan et al. 1994). Additionally, primary, secondary, and phenolic hydroxyl groups can be estimated using the acetate carbonyl carbon signals of acetylated lignin samples (Robert & Brunow 1984).

Solid state ^{13}C-NMR has also been applied to the characterisation of lignin (Newman 1989). Potentially, this technique provides direct evidence of the *in situ* structure of lignin. However, broad line widths and poor resolution of the spectra limits the structural information that is obtained. Signals from non-protonated aromatic carbons are most commonly used for the characterisation of lignin by solid state ^{13}C-NMR. These signals can be isolated from other overlapping signals by using an interrupted decoupling sequence which results in the removal of the CH and the CH_2 signals from the spectra (Leary & Newman 1992).

The interrupted-decoupled spectra of softwoods show a broad band of signals between 140 and 156ppm. Variations in the shape of this band are attributed to variations in the abundance of β-O-4 linkages in lignin. By curve fitting with a series of Gaussian lineshapes estimates of the degree of etherification can be made (Leary & Newman 1992). Estimation is more difficult for hardwoods since the presence of both guaiacyl and syringyl units adds to the number lineshapes required. Signals centred at 153ppm and 149ppm for hardwood lignins are assigned primarily to syringyl units (C3 and C5) and guaiacyl units (C3 and C4), respectively and can be used to estimate the S/G ratio of *in situ* lignin.

The main drawback with 1D techniques is the complexity of the spectra leading to a number of overlapping signals. Peak assignments are made by comparison of a single, identifiable, chemical shift from a model compound with a signal observed for the lignin. In the majority of cases this is sufficient for identifying structural elements, however, there are certain structures (eg. β-1, α-O-4) that cannot be unambiguously assigned using a single resonance (Ede & Kilpelainen 1995) because they overlap with other stronger signals. Spreading the NMR signals into a second dimension potentially overcomes these problems, since the chance of overlap is reduced and assignments can be made using more than one resonance.

Proton-proton correlations for lignin are best determined using the TOCSY sequence (Ede & Brunow 1992). The TOCSY sequence has two main advantages over COSY. First the TOCSY sequence is inherently more sensitive than COSY and second using the TOCSY sequence it is possible to observe correlations between all the protons (H_α, H_β, 2 H_γ) in the side chain.

Once the side chain protons have been assigned, the HMQC sequence can be used to assign the carbons directly bonded to each proton (Ede & Kilpelainen 1995). The combined use of TOCSY and HMQC techniques not only allows the assignment of the protons attached to a particular carbon but also the assignment of other carbons and protons belonging to the same spin system. This provides a much better basis for structural elucidation than the 1D techniques and is particularly useful for obtaining detailed information about the side-chain interunit structures (Ede & Kilpelainen 1995, Ede & Brunow 1992, Ede & Ralph 1996). Evidence for new unidentified side chain structures, has been obtained using the 2D techniques (Ede & Ralph 1996) and recently a novel dibenzodioxocin structure was discovered that correlates with some of the unassigned signals in softwood lignins (Karhunen et al. 1995).

The HMBC sequence is a 2D NMR technique that can detect long range proton-carbon correlations. However, during the long delay times, which are required to observe these long range correlations, there is a significant loss in signal for large polymers. This results in very poor signal to noise in HMBC spectra of lignins. An alternative experiment, which provides similar information to HMBC, is the 3D HMQC-TOCSY experiment. This sequence can correlate a carbon with its own protons as well as all the other protons in the spin system (Ede & Kilpelainen 1995). It can also provide important information about signals that overlap in both the 1D and 2D experiments. However, the low sensitivity of this experiment requires ^{13}C labelled samples.

Functional group analysis by NMR

The need for routine methods for the quantification of functional groups in lignin using NMR lead to the development of two techniques that involve the derivatisation of lignin with NMR active nuclei, other than ^{1}H and ^{13}C (Figure 2.35). Calibration against an appropriate internal standard allows the functional groups to be quantified.

The advantages of these two methods are that both ^{31}P and ^{19}F nuclei are NMR sensitive and have a wide range of chemical shifts, which are dependent on the structural groups in the lignin. Another advantage is that ^{31}P or ^{19}F signals are singlets so no decoupling is necessary.

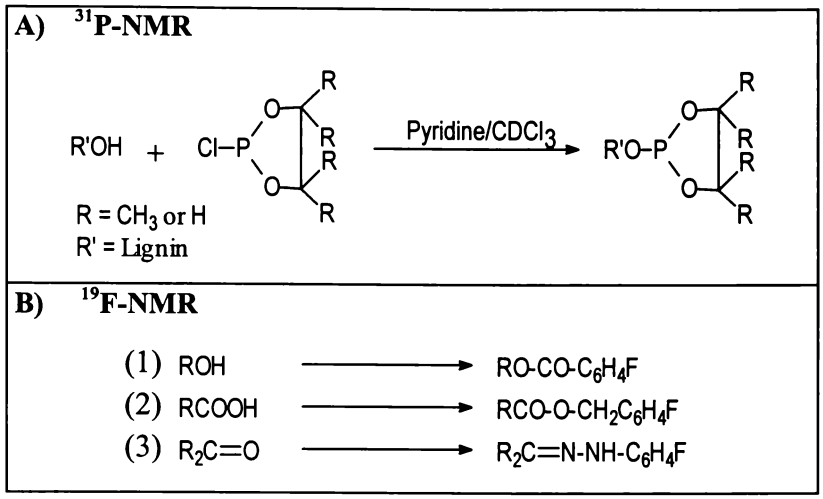

Figure (2.35). Derivatisation of lignin for functional group analysis by NMR

The ^{31}P-NMR method (Figure 2.35A) involves the derivatisation of alcohol groups (both phenolic and aliphatic) and carboxylic acids in lignin with 4,4,5,5-tetramethyl-1,3,2-dioxaphospholanyl chloride (R=CH_3) or 1,3,2-dioxphospholanyl chloride. This method is able to distinguish between syringyl and guaiacyl units, as well as between unsubstituted and C5-condensed phenolic units in lignin (Granata & Argyropoulos 1995). It is also possible to differentiate between primary and secondary alcohols (including both threo and erythro forms of the β-O-4 structure) if the non-methylated reagent is used (Argyropoulos 1993).

Smit et al. (1997) developed a method combining thioacidolysis degradation with quantitative ^{31}P-NMR analysis of the total thioacidolysis products. This method enables quantification of over 90% of the C5-condensed (5-5, 4-O-5, and β-5) and non-condensed phenolic units in wood lignin.

The ^{19}F-NMR method (Figure 2.35B) involves tagging the lignin with fluorine containing reagents. Alcohol groups and carboxylic acid groups can be analysed in the same experiment by derivatisation with 4-fluorobenzoic acid anhydride (1) and 4-fluorophenyldiazomethane (2), respectively (Barrelle 1993, Barrelle et al. 1992). Carbonyl groups, which take longer to react, are analysed in a separate experiment by derivatisation with fluorophenyldiazomethane (3, Barrelle et al. 1992).

2.4.3 Characterisation of kraft pulp residual lignin

Although, research in developing more environmentally friendly and economical chemical pulping processes is widespread, kraft pulping remains the dominant process throughout the world due to its reliability in producing strong, bleachable fibres for papermaking. However, one significant drawback to kraft pulping is the relatively high residual lignin content, which can not be easily removed by extended cooking without damaging the pulp fibre. The physical and chemical characteristics of residual lignin, not only influence its reactivity during kraft pulping, particularly at the latter stages of the kraft cook, but also strongly influence its removal in subsequent bleaching steps.

Kraft cooking proceeds in three phases with respect to lignin dissolution (Figure 2.11, Axegard et al. 1978). The final phase is extremely slow and shows poor selectivity. In conventional pulping the cook is terminated before this phase, at lignin contents of about 5% for softwoods. Reasons for this dramatic change in selectivity are not fully understood, however several possibilities have been proposed. Condensation reactions, giving rise to unreactive carbon-to-carbon bonds, become more frequent at the end of the cook. These structures are less soluble and are resistant to further reaction (Gierer 1980). The existence of alkali stable linkages between lignin and carbohydrates (either native or formed during the cook) inhibit the dissolution of the lignin fragments (Yamasaki et al. 1981, Iversen & Wannstrom 1986). The relatively low concentration of reactive β-aryl ethers and the high concentration of condensed and other unreactive structures may also contribute to the low reactivity (Gierer 1980).

For the production of high-grade papers, residual lignin remaining in the kraft pulp is removed by bleaching. Traditionally chlorine was used as the main delignifying agent but due to recent concern about the environmental impact of chlorinated organic compounds in mill effluents the switch to oxygen-based systems is prevalent. Pulp and residual lignin behave differently with the various reagents; for example, only 50% of the residual lignin can be removed during an oxygen stage before severe pulp degradation occurs. Reasons for these differences are not understood. The lack of knowledge on the structure of residual lignin present in kraft and bleached kraft pulps makes it difficult to thoroughly understand which chemical and physical properties are important for the further delignification and brightening of pulp. A better understanding of the nature of residual lignin would be a key step in improving current pulping and bleaching processes.

2.4.3.1 Analysis of *in situ* residual lignin

Several methods have been used to analyse the residual lignin without first isolating it from the pulp. Periodate oxidation and aminolysis have been used to estimate the phenolic hydroxyl contents of *in situ* kraft and kraft-oxygen residual lignin (Lai et al. 1995, Francis et al. 1991, Gellerstedt & Lindfors 1984b, 1987). In these studies changes to the phenolic hydroxyl content of the lignin during kraft pulping, extended kraft pulping, and oxygen delignification under different conditions was investigated.

Residual lignin has also been studied by acidolysis (Gellerstedt & Lindfors 1984a) and thioacidolysis (Rolando et al. 1992, Lapierre et al. 1990) to determine the effects of pulping on the lignin aryl ether content. Gel permeation chromatography of the total thioacidolysis products has been used as a gauge of lignin condensation (Pasco & Suckling 1994). Additionally, oxidative degradation has been used to study the effect of pulping on the distribution of interunit linkages in lignin (Gellerstedt & Lindfors 1984c, Gellerstedt et al. 1988) and nucleus exchange coupled with nitrobenzene oxidation has been used to measure condensation of the residual lignin (Chiang & Funaoka 1988).

The major problem with analysing *in situ* residual lignins is their low concentration, particularly in the highly delignified pulps, thus the majority of studies have been carried out on isolated lignin samples.

2.4.3.2 Isolation of residual lignin

Theoretically, any of the isolation methods discussed earlier (Section 2.4.1) can be applied to pulps. However, the low concentrations and the nature of residual lignin makes its isolation more difficult than the isolation of native lignin.

Dioxane acidolysis and CEL isolation are the two main methods used for the isolation of residual lignin. (Froass et al. 1996, Gellerstedt & Heuts 1997, Pekkala 1985, Gellerstedt et al. 1994, Jiang & Argyropoulos 1997, Yamasaki et al. 1981, Hortling et al. 1990, Johansson 1997, Chen et al. 1995b, Lachenal et al. 1995).

The enzyme method involves complete digestion of the pulp carbohydrates followed by extraction of the lignin into aqueous dioxane. Yields of CEL generally range between 20–70% of the total pulp lignin; being highest for unbleached kraft pulps and decreasing with the extent of residual lignin oxidation. In most cases residual lignin from bleached pulps is recovered from buffer solution by acidification and as a consequence is highly contaminated with protein.

Many attempts have been made to reduce the protein contamination of enzyme lignin isolated from pulps. Johansson (1997) found that by reducing the incubation time with the enzyme it was possible to reduce the amount of protein contamination, regrettably this was at the expense of yield. Purification of isolated lignin with dimethyl acetamide and NaOH is also effective at reducing protein contamination (Hortling et al. 1992), as is a protease treatment (Tamminen et al. 1994).

A small percentage (5–10%) of carbohydrates remains in the CEL after purification. It was concluded (Yamasaki et al.1981) that this residual carbohydrate is chemically bound to the lignin and was taken as proof of the existence of linkages between lignin and carbohydrates. These linkages are either present originally in the wood and survive the cook (Minor 1986), or are formed during pulping (Iversen & Wannstrom 1986).

The other commonly used method for the isolation of residual lignin is the acidolysis method. Pulp samples are refluxed in a mixture of dioxane and aqueous hydrochloric acid for 1–2 hours. The lignin is precipitated from solution by removal of the dioxane in yields of 30–60% of the total residual lignin. The yields depend on the wood type as well as the pulp. The main disadvantage of this method is that structural changes occur in the lignin during isolation. Aryl ether linkages sensitive to acid hydrolysis are expected to be cleaved introducing new phenolic hydroxyl groups. Acid-catalysed condensation reactions may also occur.

2.4.3.3 Structure of kraft pulp residual lignin

After pulping, residual lignin retains many of the structural characteristics of native lignin, however, there are a number of differences. Residual lignin has a greater number of phenolic hydroxyl groups compared to native lignin (Jiang & Argyropoulos 1997, Yamasaki et al. 1981, Francis et al. 1991). This result is consistent with the cleavage of aryl ether bonds, which introduces new phenolic hydroxyl groups into the residual lignin. The cleavage of aryl ether groups is also evidenced by a decrease in the levels of these bonds in residual lignin; however, they are not completely removed even after

extended delignification (Gellerstedt & Lindfors 1984c, Froass et al. 1996). About 10% of the original uncondensed β-O-4 linkages remain after pulping, as determined by thioacidolysis (Johansson 1997, Gellerstedt & Al-Dajana 1996).

A difference in the removal of the erythro and threo forms of β-O-4 structures is also apparent, in that residual lignin has a higher proportion of threo linkages compared to erythro linkages (Froass et al. 1996, Jiang & Argyropoulos 1994). Carboxylic acid groups in residual lignin increase after pulping (Gellerstedt et al. 1989, Jiang & Argyropoulos 1997), which is presumably a consequence of enrichment rather than formation. The expected demethylation reactions are evidenced by the reduced methoxyl content of residual lignin compared to native lignin (Froass et al. 1996, Jiang & Argyropoulos 1997) and a small amount of sulfur is also incorporated into the lignin (Yamasaki et al. 1981, Johansson 1997). Furthermore, residual lignin is more condensed than MWL (Pasco & Suckling 1994, Froass et al. 1996, Chiang & Funaoka 1988, Yamasaki et al. 1981, Leary et al. 1988), and has a higher average molecular weight (Yamasaki et al. 1981, Jiang et al. 1987, Pekkala 1985, Hortling et al. 1990).

Residual lignin from modified cooks has fewer β-O-4 linkages, a larger sulfur content, a lower methoxyl content, and a slightly lower molecular weight compared to lignin from conventional pulps (Froass et al. 1996, Hortling et al. 1993). However, the phenolic OH content is similar (Hortling et al. 1993, Froass et al. 1996), and there is some evidence to suggest the lignin is slightly more condensed (Hortling et al. 1993, Hortling et al. 1995a).

Spent liquor lignin has more phenolic hydroxyl groups, more carboxylic acids, and a lower molecular weight compared to residual lignin (Yamasaki et al. 1981). This suggests that lignin needs to be modified to a certain extent before it can be dissolved (Gellerstedt & Lindfors 1984b, 1984c). However, the sulfur content of residual and dissolved lignins is similar indicating residual lignin in the fibre has been in intimate contact with the cooking liquor. This suggests the accessibility of the lignin is not a significant cause for its reduced reactivity during pulping (Yamasaki et al. 1981).

One reason for the reduced delignification rate at the end of a kraft cook may be the presence of stable linkages between lignin and carbohydrates (Yamasaki et al. 1981, Hortling et al. 1990). Compared to wood, the proportion of glucose units in isolated residual lignin samples is greater suggesting that bonds are formed between lignin and glucose during pulping (Hortling et al. 1990, Iversen & Wannstrom 1986). On the other hand, xylose and arabinose contents are similar suggesting linkages between these sugars and lignin are stable. Furthermore, mannose and galactose residues are lower in residual lignin suggesting linkages between these sugars and lignin are unstable during pulping (Hortling et al. 1990). The exact nature of the association between lignin and carbohydrate remains unclear, however, glycosidic bonds linking carbohydrates to the Cα in residual lignin have been detected (Fukagawa et al. 1992).

There are several features of residual lignin considered important for the decrease in lignin reactivity and selectivity towards the end of the cook. These include the reduction in reactive groups (particularly the β-O-4 structures); the increase in carbon-to-carbon bonds resulting in reduced reactivity and solubility of the lignin; an increase in molecular weight which inhibits its dissolution; and the possible existence of alkali stable linkages between lignin and carbohydrates.

2.4.3.4 Structure of bleached kraft pulp residual lignin

The structure of bleached residual lignins is remarkably similar to kraft pulp residual lignin; much less structural modification is observed between all the residual lignins than occurs to the native lignin on pulping.

Chlorine has the greatest influence on the structure of residual lignin (Lachenal et al. 1995), whereas, over the course of TCF and ECF bleaching sequences residual lignin is not significantly changed. However, slight differences are apparent between the different lignins. Elemental analysis of lignins isolated from different pulps show variations in the oxygen contents indicating differences in the extent of oxidation. Demethylation is also apparent from variation in the methoxyl content (Johansson 1997, Lachenal et al. 1995). The largest observed change in bleached residual lignins is an increase in carboxylic acids; increasing in the order Z>D>P≥O>C (Lachenal et al. 1995).

The various bleaching reagents influence the molecular weight of residual lignin differently (Lachenal et al. 1995). For example, very little depolymerisation is observed during ozonation (Lachenal et al. 1995), whereas a significant decrease in molecular weight is observed during chlorine bleaching (Lachenal et al. 1995). Additionally, the average molecular weight of residual lignin tends to decrease with the degree of oxidation (Tamminen et al. 1997).

The effectiveness of chlorine as a bleaching agent for pulp can be seen by its effect on residual lignin (Lachenal et al. 1995). Chlorine has the capability of depolymerising lignin while forming new phenolic groups, and as a consequence a residual lignin is generated that is easy to remove in subsequent bleaching stages.

The predominant substrate for chlorine dioxide and oxygen is unetherified phenolic units and as expected a reduction in phenolic hydroxyl groups in residual lignin is observed after treatment with oxygen and chlorine dioxide (Lachenal et al. 1995). Selectivity of oxygen delignification towards uncondensed structures is also evident, in that the ratio of condensed to uncondensed structures increases after oxygen treatment (Gellerstedt & Heuts 1997, Jiang & Argyropoulos 1997).

Residual lignin from hydrogen peroxide and oxygen bleached pulps are also very similar. Phenolic structures are degraded by both with an accompanied increase in carboxyl groups (Gellerstedt & Heuts 1997, Lachenal et al. 1995). This is in agreement with the idea that the decomposition products of hydrogen peroxide (O_2^{-}; $OH^{\bullet}$) are responsible for lignin degradation during hydrogen peroxide bleaching, while the role of the perhydroxyl anion is restricted to the destruction of coloured groups. This explains the improved brightness development during a peroxide stage compared with an oxygen stage and the reduced carbonyl content after a peroxide stage (Lachenal et al. 1995).

The strong resemblance between all the residual lignins and the fact that many of the structural elements expected to be extremely reactive during bleaching (particularly, unetherified phenolic structures) still remain in the bleached residual lignins suggests the accessibility of the lignin is a limiting factor during bleaching (Johansson 1997). Furthermore, the dissolved lignins are more significantly modified than the corresponding residual lignin. This suggests the lignin which is accessible to the bleaching agents is oxidised and removed, leaving behind a residual lignin that is less modified compared to the lignin that is removed (Gellerstedt et al. 1989).

Chapter Three
EXPERIMENTAL METHODS

3.1 Isolation of Lignin From Wood

3.1.1 Preparation of wood

Pinus radiata wood chips (which were obtained from a New Zealand pulp mill) were ground to coarse sawdust using a Wiley mill. The milled wood was extracted with dichloromethane (DCM) for 20 hours and then for another 20 hours with 90% ethanol in a Soxhlet extractor. The air-dried wood was Wiley milled through a 60 mesh screen, and then milled for three days in a vibratory ball mill (Siebtechnik Vibratom SM 0.6) equipped with a porcelain pot (1L capacity) filled with porcelain balls.

After ball milling the pot was emptied into a large Buchner type funnel, which was placed over a conical flask. The funnel and conical flask were lightly shaken to dislodge particles adhered to the porcelain balls and any remaining powder adhered to the walls of the pot were brushed off with a soft brush.

3.1.2 Enzyme lignin

3.1.2.1 Initial isolation method

The initial isolation method was based on the method of Lachenal et al. (1995).

Enzyme digestion

Ball milled wood (5g) was suspended in acetate buffer (100mL, 0.5M, pH 4.6) containing *Onozuka R-10* cellulase enzyme (1.25g/5g wood) and incubated in a shaking incubator at 37°C for a total period of up to 2 weeks. Every 3 days the undigested residue was recovered by centrifugation and resuspended in fresh buffer (100mL) containing fresh enzyme (1.25g).

Lignin extraction

At the end of the incubation period the undigested residue was recovered by centrifugation and washed several times with distilled water. The residue was suspended in 90% aqueous dioxane (50mL) and stirred for 16–20hr. The residue was recovered by centrifugation and the extraction was repeated. The supernatants from the two extracts were combined, rotary evaporated at 40°C to remove the dioxane, and freeze dried from a water suspension.

Purification

The crude enzyme lignin was dissolved in a minimum volume of dimethyl formamide (DMF) and precipitated with diethyl ether. The precipitate was washed several times with fresh ether and finally freeze dried.

Recovery of lignin from the buffer

Buffer solutions were collected after each step, combined and acidified to pH 2 using dilute HCl. The precipitate was left to settle and most of the buffer was decanted off. The precipitate was recovered by centrifugation and washed several times with distilled water. The crude material was then purified in DMF, as above.

3.1.2.2 Optimisation trials

Ball milling and incubation time

Several experiments were conducted to determine the effect of the ball milling and enzyme digest times on the isolation procedure.

The general isolation procedure outlined above (Section 3.1.2.1) was used but the duration of the ball milling and the enzyme digestion were changed. That is, the ball milling was reduced to 1.5 days and the enzyme incubation was varied between 3 and 14 days.

The enzyme

The efficiency of a [*Celluclast* (1.5L) and *Novozyme* (sp342)] system was compared to the Onozuka cellulase. Ball milled wood (5g) was suspended in acetate buffer (100mL, 0.5M, pH 4.6) containing one of the following:

1. 1.5g Onozuka
2. 2mL Novozyme and 1mL Celluclast.

After incubation for 3 days, enzyme lignins were recovered as above (Section 3.1.2.1).

Table (3.1). Properties of the enzymes used in this study

	Source	Form	Activity[1]	Recommended conditions
Onozuka R10	Yakult Pharmaceutical Industries, Japan.	Powder	Unknown	–
Celluclast 1.5L	Novo Nordisk A/S, Denmark	Solution (1.2g/mL)	1500 NCU/g	Temp: 50–60°C, pH: 4.5–6.0
Novozyme sp342	Novo Nordisk A/S, Denmark	Solution	Unknown	Temp: 50°C, pH: 5.0–6.0

NCU = 1μmol glucose released from carboxymethyl cellulose per minute (40°C, pH 4.8).

Solvent swelling of cellulose

Ball milled wood was solvent swelled using the method of Chen et al. (1995b). That is, ball milled wood powder (10g) was shaken at room temperature in the swelling solvent, consisting of dimethylacetamide (100mL) and LiCl (7g), for 48hr. The residue was then washed with distilled water and freeze dried.

The swelled wood residue was treated with Onozuka cellulase (1g) in acetate buffer (200mL, 0.5M, pH 4.6) at 50°C for 24hr. For comparison an unswelled sample (4.2g) was treated with enzyme (420mg) in acetate buffer (85mL, 0.5M, pH 4.6) at 50°C for 24hr.

The enzyme lignins were isolated as above (Section 3.1.2.1).

NaOH/HCl treatment

Insoluble wood residues were suspended in NaOH (10mM or 100mM). The suspensions were then acidified to pH 2 with dilute HCl and the precipitate was recovered by centrifugation and washed until neutral.

Acetone purification method

Crude residual lignin was dissolved in NaOH (100mM) and precipitated with dilute HCl. The lignin was recovered by centrifugation, washed until neutral, and then dissolved in 90% aqueous acetone. Any undissolved material was removed by centrifugation, the solution was then rotary evaporated and the resulting water suspension was freeze dried.

3.1.2.3 The improved method

Enzyme digestion

Ball milled wood (5g) was suspended in acetate buffer (100mL, 0.5M, pH 4.6) containing *Onozuka R-10* cellulase enzyme (1.25g/5g wood) and incubated in a shaking incubator at 37°C for 3 days.

Lignin extraction

Extraction 1

At the end of the incubation period the undigested residue was collected by filtration. The residue was suspended in NaOH (10mM, 10mL/g residue) and stirred for 10min. The suspension was acidified to pH 2 with dilute HCl and then centrifuged to recover the precipitate, which was then washed several times with distilled water before being extracted with 90% aqueous dioxane (50mL/g residue) for 16–20hr.

The soluble and insoluble fractions were separated by centrifugation. The insoluble residue was washed several times with distilled water before being freeze dried. The washings were combined with the soluble fraction and the dioxane was removed by rotary evaporation at 40°C. The water suspension was then freeze dried to yield crude CELF1 lignin.

Extraction 2

The insoluble residue from Extraction 1 was combined with the well washed, DMF insoluble material from the purification step (Purification step 1, following) and ball milled for three days (in a similar manner used for wood preparation; Section 3.1.1). After ball milling, the residue was suspended in NaOH (100mM, 10mL/g residue) and stirred for 10 minutes. The suspension was acidified to pH 2 with dilute HCl. The precipitate was washed several times with distilled water, until the washings became neutral, and then extracted with 90% aqueous dioxane for 16–20hr. About 50mL of solvent was used per gram of residue. The dioxane was removed by rotary evaporation at 40°C and the water suspension was freeze dried to yield crude CELF2 lignin.

For the isolation of CEL2, the enzyme digest residue was ball milled for 3 days without applying the first extraction step. The crude lignin was extracted from the ball milled residue into aqueous dioxane in same manner as CELF2.

All crude lignin samples were purified as follows.

Purification

Purification step 1

The crude lignin was dissolved in a minimum volume of DMF and any insoluble material was removed by centrifugation. The DMF solution was added dropwise to diethyl ether with stirring (the volume of ether was approximately ten fold in excess). The precipitated lignin was recovered by centrifugation and washed several times with fresh diethyl ether. The lignin was then air dried, washed several times with distilled water, and freeze dried.

Purification step 2

In order to reduce nitrogen contamination, samples were dissolved in NaOH (100mM) and precipitated at pH 2 with dilute HCl. The precipitated lignin was collected by centrifugation, washed with distilled water until neutral and dissolved in 90% aqueous acetone. The acetone was removed by rotary evaporation and the lignin was freeze dried from a water suspension.

3.1.3 Dioxane acidolysis lignin

Wood

Wiley milled (60 mesh) or ball milled extractive free wood (1g wood/35mL) was refluxed in 4:1 (v/v) dioxane, aqueous HCl (0.1M) solution for 2hr. The dioxane was dried over sodium metal and benzophenone and distilled before use. The acidolysis solvent was flushed with nitrogen for at least 15min before the wood sample was added.

After refluxing, the suspension was cooled slightly and filtered. The residue was washed with 90% aqueous dioxane and then with distilled water. The filtrate and washings were combined and rotary evaporated at 40°C to remove the dioxane. To prevent the solution from becoming too acidic the volume of liquid was not reduced below half of the original volume used for refluxing. More distilled water was added if necessary.

The acidolysis lignin was recovered by centrifugation, washed with distilled water until neutral, and then freeze dried.

Variation

The dioxane solution was neutralised with saturated $NaHCO_3$ before rotary evaporation. The rest of the work-up was the same as above.

CELF2 treatment

The cellulase enzyme lignin fraction CELF2 (2g/mL) was refluxed in 4:1 (v/v) dioxane, aqueous HCl (0.1M) solution for 1.5hr. The treated lignin was recovered as above.

3.2 Isolation of Lignin from Kraft Pulps

3.2.1 Method 1

Pulp treatment

Pulp samples were freeze dried, Wiley milled through a 40 mesh screen and ball milled for 3 days.

Enzyme treatment

The pulp powder (20 g) was suspended in acetate buffer (500 mL, 0.5 M, pH 4.6) containing *Onozuka R-10* cellulase enzyme (5 g) and incubated in a shaking incubator at 37°C for 3 days.

Lignin extraction

At the end of the incubation period the undigested residue was recovered by centrifugation and washed several times with distilled water. The residue was suspended in 90% aqueous dioxane (100 mL) and stirred for 16–20hr.

The soluble fraction was recovered by centrifugation and the dioxane was removed by rotary evaporation at 40°C. The water suspension was then freeze dried to recover the crude lignin

The crude lignin was purified using at least one of the following methods.

Purification

DMF purification

The crude lignin was dissolved in a minimal volume of DMF. Insoluble material was removed by centrifugation and the lignin was precipitated by adding the DMF solution (dropwise) to diethyl ether. The precipitate was recovered by centrifugation, washed several times with fresh ether, and freeze dried.

Acetone purification

The residual lignin was dissolved in 0.1 M NaOH and precipitated at pH 2 with dilute HCl. The precipitated lignin was collected by centrifugation, washed with distilled water until neutral and dissolved in 90% aqueous acetone. The supernatant was recovered after centrifuging and the acetone was removed by rotary evaporation. The water suspension was freeze dried to recover the purified lignin.

3.2.2 Method 2

Enzyme digest

Pulps were digested at 5% consistency in acetate buffer (0.5 M, pH 4.6) with *Onozuka R-10* cellulase enzyme (1 g/4 g pulp) in a shaking incubator at 37°C for 15–24hr.

Lignin extraction

The undigested residue was recovered by centrifugation, washed several times with distilled water, and then freeze dried. The dried residue was then ball milled for 3 days and then extracted with 90% aqueous dioxane (5 g residue/20 mL) for 16–20hr. The soluble fraction was recovered by centrifugation and the dioxane was removed by rotary evaporation at 40°C. The water suspension was then freeze dried to recover the crude lignin.

The crude residual lignin was purified using the acetone method (Section 3.2.1).

Variation

The dioxane insoluble residue remaining after the first lignin extraction was suspended in 0.1 M NaOH (5 g/20 mL) for 10 min. The suspension was then acidified at pH 2 with dilute HCl. The precipitate was recovered by centrifugation, washed until neutral, and extracted for 20 hr with 90% aqueous dioxane. The soluble fraction was recovered by centrifugation and the dioxane was removed by rotary evaporation at 40°C. The water suspension was then freeze dried to recover the extracted lignin, which was then purified using the acetone purification method (Section 3.2.1).

3.2.3 Method 3

Enzyme digest

Pulps were digested at 5% consistency in acetate buffer (0.5 M, pH 4.6) with *Onozuka R-10* cellulase enzyme (1 g/4 g pulp) in a shaking incubator at 37°C for 2–3 days.

Lignin extraction

The undigested residue was recovered by centrifugation, washed several times with distilled water and extracted with 90% aqueous dioxane (5 g residue/20 mL) for 16–20 hr. The soluble fraction was recovered by centrifugation and the dioxane was removed by rotary evaporation at 40°C. The water suspension was then freeze dried to recover the crude lignin

The crude residual lignins were purified using the acetone purification method (Section 3.2.1).

Variation

The enzyme charge was reduced to 1 g/8 g pulp and the duration of the enzyme treatment was reduced to 24 hr. The first lignin fraction was recovered and purified as above (Section 3.2.1). The dioxane insoluble residue was also recovered, extensively washed with distilled water, freeze dried, and ball milled for 3 days. The resulting powder was extracted with 90% aqueous dioxane for 20 hr and the crude lignin was recovered and purified using the acetone method (Section 3.2.1).

3.2.4 Recovery of lignin from the buffer

Buffer solutions were acidified to pH 2 using dilute HCl. The precipitate was left to settle and most of the buffer was decanted off. The precipitate was recovered by centrifugation, washed several times, and then extracted with 90% aqueous dioxane (50mL) for 16–20 hr. The extracted lignin was purified using the acetone purification method (Section 3.2.1).

3.2.5 Dioxane acidolysis lignin

Residual lignin was isolated from the pulps following the method of Gellerstedt et al. (1994).

Pulp samples were refluxed at 3% consistency in 4:1(v/v) dioxane, aqueous HCl(0.1M) solution under an atmosphere of nitrogen. The dioxane was dried over sodium metal and benzophenone and distilled before use. The acidolysis solvent was flushed with nitrogen for at least 15min before the pulp was added.

After refluxing, the suspension was cooled slightly and filtered. The pulp residue was washed with 90% aqueous dioxane and then with distilled water. The filtrate and washings were combined and rotary evaporated at 40°C to remove the dioxane. To prevent the solution from becoming too acidic the volume of liquid was not reduced below half of the original volume used for refluxing. More distilled water was added if necessary. The acidolysis lignin was recovered by centrifugation, washed with distilled water until neutral, and freeze dried.

The insoluble residue remaining after acidolysis was thoroughly washed and dewatered to about 20% consistency. The kappa number of the residue was then determined using the Micro-Kappa method of PAPRO.

3.2.5 Isolation of spent liquor lignin

Spent liquor lignin from the peroxide (KOQPs) and cyanamide activated peroxide (KOQPns) bleaching was recovered by precipitation. Enough sulfurous acid was added to the spent liquor to neutralise the pH. The liquor was then acidified to pH 2 with dilute HCl to precipitate the lignin. The lignin was left to settle and most of the liquor decanted off. The lignin was then recovered by centrifugation, washed with distilled until neutral, and freeze dried.

The spent liquor lignin from the alkaline extraction of the ozonated pulp (KOQZEs) was recovered by acidifying the liquor to pH 2 with dilute HCl. Once the precipitate had settled most of the liquor was decanted off. The lignin was recovered by centrifugation, washed with distilled water until neutral, and freeze dried.

3.3 Analytical Procedures

3.3.1 Carbohydrate monomer analysis

Lignin (20 mg) was reacted with trifluoroacetic acid (2 mL, 2 M) in a screw capped vial at 100°C for 4 hr. After cooling, about 3 mL of distilled water was added and the suspension filtered. The residue was washed with water several times. The filtrate and washings were combined, rotary evaporated, and freeze dried. Carbohydrates were analysed using the method given by Pettersen & Schwandt (1991). The analysis was carried out by the Wood Materials Test Centre, Forest Research, Rotorua, New Zealand.

3.3.2 Consistency

The consistency of all pulps was determined in triplicate. Samples (1–3 g) were dried at 105°C overnight. The consistencies were determined from the ratio of the dry weight to the wet weight.

3.3.3 Kappa number

Kappa numbers were determined using the Micro-Kappa method of PAPRO (Method 1.107).

3.3.4 Klason lignin

Klason lignin and acid soluble lignin were determined by the Wood Materials Test Centre, Forest Research, Rotorua, New Zealand.

3.3.4 Elemental analysis

The elemental analysis, along with the determination of methoxyl content and moisture content was carried out by Campbell Microanalytical Laboratory, University of Otago, Dunedin, New Zealand. The methoxyl group determinations involved cleavage of the methyl ether with hydroiodic acid producing volatile methyl iodide. This was then condensed in glacial acetic acid containing excess bromine producing iodate on dilution with water. The iodate was then estimated iodimetrically.

3.3.5 Periodate oxidation

The procedure used for the periodate oxidation followed the method used by Lai et al. (1990). Lignin (50 mg), residual lignin (20 mg), or wood (200 mg) samples, in a 50 mL teflon centrifuge tube, were treated with sodium periodate (6 mL, 130 mg/mL) in distilled water and acetonitrile (1 mL, 3 mg/mL) in distilled water. The solutions were cooled to 4°C before addition. The suspensions were shaken in the dark at 4°C for the duration of the reaction (generally about 7 days).

The rate of methanol formation was followed by gas chromatography by periodically injecting 2 μL aliquots of the reaction supernatant. The mixture was centrifuged to obtain a clear solution prior to sampling. A Shimadzu-14B GC equipped with a glass column (1.5 m × 3.3 mm), packed with Porapak Q, was used for analysis. The injector and detector were held at 250°C and the column at 130°C with a nitrogen flow of 2.3 mL/min.

3.3.6 Phosphorus NMR

The procedure used for ^{31}P-NMR analysis followed the method of Granata & Argyropoulos (1995) with some modifications. The wood lignin and residual lignin were analysed using different modifications due to differences in solubility.

Reagents

Pyridine:	Analytical grade pyridine was dried over KOH pellets and distilled. Stored in a dark bottle over molecular sieves.
$CDCl_3$:	Analytical grade. Stored over molecular sieves.
Phosphitylating agent:	Obtained from Aldrich.
Cholesterol:	Laboratory grade cholesterol was recrystalised twice from ethanol. Stored over P_2O_5 in a desiccator.
$Cr(acac)_3$:	Stored over P_2O_5 in a desiccator.

Wood lignins

Lignin (30–40 mg) was dissolved in pyridine (800 µL) in a 3 mL volumetric flask. $CDCl_3$ (800 µL) was added followed by the internal standard and relaxation agent solutions (100 µL each). The lignin was then phosphitylated with 2-chloro-4,4,5,5-tetramethyl-1,2,3-dioxaphospholane (100µL) and the solution was then made up to 3 mL with a 1.2:1 (v/v) mixture of pyridine and $CDCl_3$.

Cholesterol (50 mg/mL) was used as the internal standard and chromium(III) acetylacetonate (5 mg/mL) as the relaxation agent. Both solutions were made up in pyridine.

Residual and spent liquor lignins

Vacuum dried lignin samples (20–30 mg) were dissolved in pyridine (325 µL) in a 1mL volumetric flask. To aid dissolution samples were sonicated. $CDCl_3$ (300µL) was added followed by the internal standard (74 µL) and relaxation agent (100 µL).

The lignin was then phosphitylated by slowly adding 2-chloro-4,4,5,5-tetramethyl-1,2,3-dioxaphospholane (100 µL) and the solution was then made up to 1 mL with a 1:1 (v/v) mixture of pyridine and $CDCl_3$.

Cholesterol (40–50 mg/mL in $CDCl_3$) was used as the internal standard and chromium(III) acetylacetonate (5 mg/mL in pyridine) as the relaxation agent.

NMR

All spectra were run on a Bruker AC300 spectrometer using an inverse gated decoupling pulse sequence with a 90° tip angle and a 25 s delay. The spectra were acquired for 12–15hr. Chemical shifts were measured relative to hypothetical internal orthophosphoric acid (H_3PO_4).

3.3.7 Nuclear magnetic resonance spectroscopy

Acetylation

Lignin was acetylated with a 1:1 (v/v) mixture of pyridine and acetic anhydride (5 mg/mL) for 16–20 hr. The solvent was removed by azeotroping with toluene and then with 90% ethanol under reduced pressure. The acetylated samples were freeze dried from a water suspension.

HMQC acquisition

Acetylated lignin (30 mg) samples were dissolved in $CDCl_3$ (400 µL) for analysis.

HMQC spectra were obtained at 9.4 T and 300 K on a Bruker DRX400 spectrometer, operating at 400.13 MHz for ^1H and 100.6 MHz for ^{13}C. Spectra were acquired on a dual $^1H/^{13}C$ inverse probe with z-gradients. Chemical shifts were measured relative to hypothetical internal TMS.

The standard Bruker pulse sequence *inv4gstp* was used for the acquisition, with z-gradient suppression of artefacts and GARP ^{13}C-decoupling, using standard instrumental settings.

The spectra were acquired over an F2 spectral window of 4000 Hz, and an F1 window of 20,000 Hz, with a 2 K × 256 matrix (zero filled to 512 W in F1), and from 96 to 256 scans per increment. Squared cosine bell apodisation was applied in both dimensions.

The spectra were processed using standard Bruker software.

3.3.8 Thioacidolysis

The method used for thioacidolysis was based on the method described in Roland et al. (1992).

Reagents

Dioxane:	Analytical grade dioxane was dried by refluxing with sodium metal in the presence of benzophenone and then distilled.
BF_3-etherate:	Analytical grade; obtained from Sigma.
Ethanethiol:	Analytical grade.
Hexacosane:	Dissolved in CCl_4. Concentrations 0.7–0.8 mg/mL.

Thioacidolysis

Lignin samples were vacuum dried overnight prior to analysis.

Soluble lignins (10 mg) or Wiley milled wood (10 mg) samples were placed in a teflon lined bomb with dioxane (9 mL), ethanethiol (1 mL), and BF_3-etherate (250 µL) under a nitrogen atmosphere. The bombs were tightly screwed shut and immersed in an oil bath at 110°C for 2–3 hr. The bombs were shaken 2 or 3 times over the duration.

The reaction vessels were cooled under cold tap water and the internal standard, hexacosane, was then added to the reaction mixture. For the isolated wood lignin samples 1 mL of internal standard was used, while 0.5 mL was used for the wood and residual lignin samples. The reaction mixture was then poured into a separating funnel containing approximately 30 mL of analytical grade dichloromethane (DCM).

The mixture was shaken with aliquots of 0.4M $NaHCO_3$ to neutralise the acid and the organic phase was then separated. The aqueous phase was extracted with 20mL of DCM. The two organic fractions were combined, dried with anhydrous sodium sulfate, and then rotary evaporated at 40°C. The oily residue was redissolved in Na_2SO_4 dried DCM (2mL) and transferred to a clean, dry, screw capped vial. The samples were blown dry with nitrogen at 40°C, the vials were sealed under the nitrogen atmosphere and stored over silica gel in a refrigerator.

GC analysis of monomeric compounds

The thioacidolysis residues were redissolved in 250μL of dry DCM.

Silylation

Aliquots (Table 3.2) of the thioacidolysis mixtures were placed in 200μL glass inserts inside GC autoinjector vials and silylated with N-O-bis(trimethylsilyl)acetamide (BSA).

Table (3.2). Reagents and volumes used for silylation of thioacidolysis products

Lignin sample	Thioacidolysis solution	DCM	Pyridine	BSA
Isolated wood lignins	20μL	30μL	50μL	50μL
Pulp lignins and wood	50μL	–	50μL	50μL

GC/FID analysis

The main thioacidolysis product was quantified by GC/FID.

GC analysis was performed using a 5890 Series II, Hewlett Packard GC. The Column was a HP-1, crosslinked methyl siloxane, capillary column (25m, 0.2mm). Hydrogen was used as the carrier gas at a head pressure of 10psi. The temperature program consisted of 3 steps. Initially, the temperature was ramped from 140°C to 210°C at 10°/min. Then from 210°C to 290°C at 3°/min and finally held at 290°C for 15min. Volume injected was 3μL.

The response factor was determined using the trithioether β-O-4 monomer compound isolated from the thioacidolysis of guaiacylglycerol-β-guaiacyl ether. The trithioether monomer compound was kindly provided by Tracy Adams (Forest Research, Rotorua).

GC/MS analysis

GC/MS was used to identify the other thioacidolysis monomers. The amounts of all the identified degradation products were quantified using the response factor determined from the trithioether β-O-4 model compound discussed above.

GC/MS analysis was performed using a 5890 Series, Hewlett Packard GC with a 5970 Series Mass Selective Detector. The column was a HP-1, crosslinked methyl siloxane, capillary column (25m, 0.2mm). The carrier gas was helium at a head pressure of 7psi. Injection volumes were either 1μL or 3μL. The temperature program was the same as used for the GC/FID analysis.

Chapter Four
AN IMPROVED METHOD FOR THE ISOLATION OF LIGNIN FROM *PINUS RADIATA* WOOD

4.1 Introduction

There is a wide range of methods available for the isolation of lignin from wood (Section 2.4.1). The majority, however, either modify the native structure of lignin, or release only a small fraction of the total lignin in a relatively unchanged form. For structural and reactivity studies, a lignin is required which can be isolated in good yield and which represents the lignin in its native state.

The most important method for obtaining lignin in a relatively unchanged form is Bjorkman's procedure (Bjorkman 1957). Milled wood lignin (MWL) is extracted from ball milled wood with aqueous dioxane. However, the yields after purification are generally somewhat less than 20% of the total lignin and are dependent on both the duration and efficiency of the milling. Also, the lignin is depolymerised during ball milling and the nature and extent of changes that occur during milling are not completely understood. Therefore, MWL may not be fully representative of the total lignin structure within the cell wall.

A lignin isolated in a higher yield should be more representative of the lignin present in wood. Other methods have thus been developed with the objective of increasing yields. Chang et al. (1975) treated ball milled wood with a commercial cellulase enzyme to solubilise the carbohydrates, allowing higher yields of lignin to be extracted as cellulolytic enzyme lignin (CEL). This procedure requires an enzyme treatment of 10 days or more and gives a higher yield of lignin compared to MWL, but with contamination from carbohydrates and protein from the enzyme.

The first part of this chapter describes the development of an improved CEL method for the isolation of lignin from *Pinus radiata* wood. Trials based on existing methods (Lachenal et al. 1995, Chen et al. 1995b, Chang et al. 1975) were carried out with the aim of developing an optimised isolation procedure; giving a soluble lignin in high yield with minimal contamination. The effects of ball milling, enzyme digestion, and alkali pretreatments of the wood powder and insoluble wood residues were investigated. Based on the results and observations from these studies an improved CEL method for the isolation of lignin from wood was developed.

The CEL lignins obtained using the improved method were characterised using a number of chemical and spectroscopic techniques and the results were compared to those for MWL isolated from *Pinus radiata* wood.

As a prelude to Chapter 6 (which discusses the isolation and characterisation of dioxane acidolysis lignin from *Pinus radiata* kraft pulps) lignin was isolated from wood by dioxane acidolysis. The effects of the acidolysis treatment on native lignin structure are discussed in the final section of this chapter.

4.2 Development of Improved CEL Method

4.2.1 The initial isolation procedure

The method (Figure 4.1) initially used in this study was loosely based on the method of Lachenal et al. (1995). Extractive free, ball milled wood was treated with a cellulase/hemicellulase enzyme complex to remove the carbohydrates. The carbohydrate digestion was carried out in a series of 3 day enzyme treatments. After each treatment the undigested residue was recovered by centrifugation and re-suspended in fresh buffer containing fresh enzyme. After a total time of about two weeks the residue was recovered, washed several times with distilled water, and then extracted with aqueous dioxane (90%). The crude lignin was freeze dried from a water suspension after removing the dioxane by rotary evaporation.

The DMF purification procedure involved the dissolution of the crude lignin in dimethyl formamide and re-precipitation of the purified lignin by adding the solution to diethyl ether. The buffer soluble lignin was recovered by acidifying the buffer solutions using dilute hydrochloric acid and the recovered precipitate was purified using the DMF procedure. These methods are described in detail in the Chapter 3 (Section 3.1).

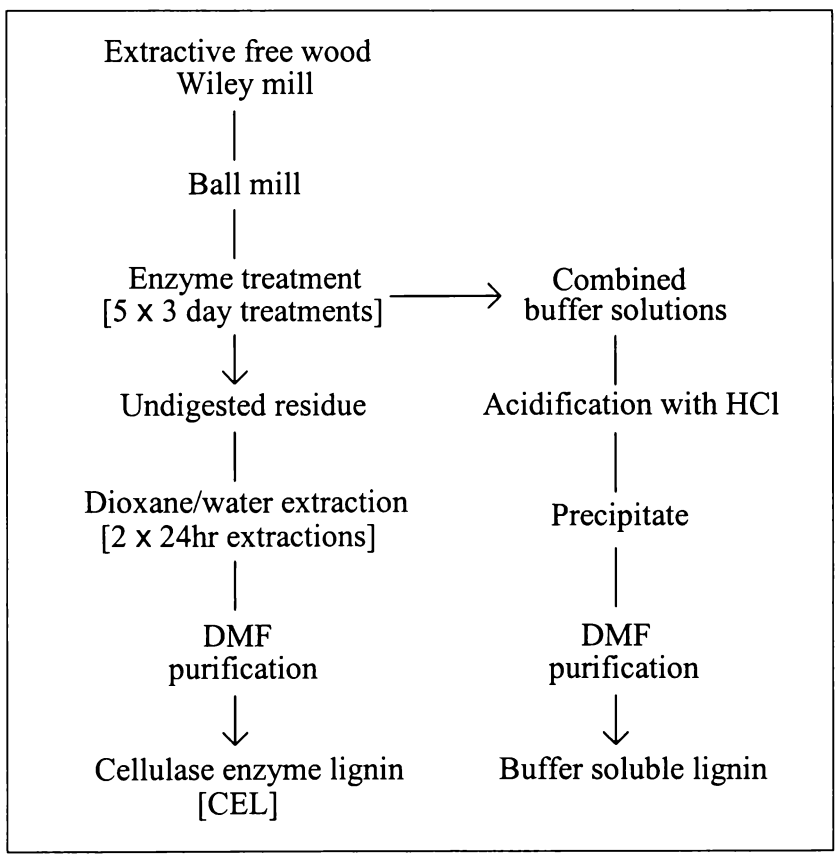

Figure (4.1). Cellulase enzyme isolation of lignin from wood; based on the method of Lachenal et al. (1995)

Johansson (1997) isolated residual lignin from *Pinus radiata* kraft and bleached kraft pulps using the method of Lachenal et al. (1995). Yields of dioxane extracted lignin ranged between 30 and 70%, and were highest for the unbleached kraft pulp. A proportion of residual lignin was also recovered from the buffer (3–50%); the smallest yield of buffer soluble lignin was obtained from the kraft pulp and were highest for pulps bleached to lower kappa number. Both fractions were highly contaminated with protein (as determined by the nitrogen content). The protein content of individual lignin fractions ranged between 7% and 47% and the buffer soluble fractions had a higher protein content than the dioxane extracted lignin. The protein content also increased in the lignin fractions obtained from the pulps that were bleached to a lower kappa number.

In this investigation the behaviour of *Pinus radiata* wood lignin during isolation using the modified cellulolytic enzyme method (Figure 4.1) was found to differ from the behaviour of *Pinus radiata* kraft pulp residual lignin found by Johansson (1997). The yield and protein contamination of the CELs from *Pinus radiata* kraft pulp and wood are compared in Table (4.1). The yield of CEL from the wood is significantly lower than from the kraft pulp and the protein content of the wood CEL is also lower. These differences between residual lignin and wood lignin during isolation are likely to be due to differences in their structure or accessibility. That is, residual lignin contains more phenolic hydroxyl groups and carboxylic acids than wood lignin so will tend to be more soluble in the extracting solvent. Another important factor is that the cell wall structure and tight association between polymers in the cell wall are disrupted to a certain extent during pulping so the residual lignin will be more accessible to the extracting solvent than the wood lignin.

Table (4.1). Yield and protein content of lignin isolated from *Pinus radiata* wood and kraft pulp by the method of Lachenal et al. (1995)

Sample	**CEL**		**Buffer soluble material**			
	Yield (%)	Protein (%)[1]	Yield (%)	Fraction	OCH_3 (%)	Protein (%)[1]
Kraft pulp[2]	69[3]	16	3[3]	DMF soluble	–	20
Wood[4]	20[5]	5	< 0.5[5]	DMF soluble	6.64	5
				DMF insoluble	1.06	80

1. Determined from nitrogen content (6.25 × %N)
2. From Johansson (1997)
3. Percentage of total lignin determined from the kappa number (0.15 × kappa number)
4. From this study
5. Percentage of total lignin content of the wood

The yield of material recovered from the buffer is also lower for the wood compared to the kraft pulp (Table 4.1). Much less than 0.5% of the wood lignin was recovered from the buffer and only about a third of this was soluble in DMF. The methoxyl content of the DMF soluble lignin suggests a lignin content of approximately 50%; the remaining 50% is probably largely made up of carbohydrate. The DMF insoluble fraction consists predominantly of protein, as determined by its nitrogen content and contains very little lignin (about 5%) as indicated by the low methoxyl content. These results show that only minor amounts of wood lignin are recoverable from the buffer during the isolation of CEL. They are also consistent with the trend of increasing buffer solubility of the residual lignin with increasing lignin modification reported by Johansson (1997). That is, the lowest yield of buffer soluble material is recovered from the least modified of the lignin samples (ie the wood lignin).

A CEL yield of 20% was obtained from *Pinus radiata* wood using the modified method of Lachenal et al. (1995) and only a very minor amount of the wood lignin was recovered from the buffer. This suggests that a large proportion of the wood lignin is not being extracted into the aqueous dioxane. The CEL from wood is also contaminated with 5% protein and possibly carbohydrates. These impurities may interfere with structural characterisation of the lignin. Therefore, several experiments were performed to optimise the CEL procedure for *Pinus radiata* wood and improve both the yield and purity of the isolated lignin. The results are discussed below.

4.2.2 Optimisation studies

4.2.2.1 Ball milling and extraction time

Pew (1957) demonstrated that a commercial cellulase preparation (Rohn & Haas No. 19) was unable to degrade spruce wood meal unless it was ball milled. Ring milling is an alternative means of grinding wood meal to small particles. It was of interest to determine whether ring milling could be an efficient grinding method to allow enzyme degradation. The effect of ring milling and ball milling wood meal on the isolation of lignin was investigated. Table (4.2) shows the effect of milling on the enzyme treatment and isolation of lignin.

Table (4.2). The effect of ring milling, ball milling, and incubation time on the isolation of CEL from *Pinus radiata* wood

Weight of aqueous dioxane insoluble residue and yield of CEL						
Incubation time	Ring milled		Ball milled 1.5 days		Ball milled 3 days	
	Residue[1]	**Yield**[2]	**Residue**[1]	**Yield**[2]	**Residue**[1]	**Yield**[2]
14 days	–	1	–	–	22	18
9 days	–	–	28	5	–	–
6-7 days	80	0.3	24	1.5	19	18.5
3 days	–	–	–	–	18	20

1. Percentage of the initial weight of wood
2. Percentage of the total lignin in *Pinus radiata* wood

When a ring mill was used for grinding only about 20% of the initial weight of wood was lost in 7 days of enzyme treatment. This is compared to about 80% weight loss if the wood had been ball milled and demonstrates that ball milling has a major effect on the efficiency of carbohydrate removal during the enzyme treatment. There are likely two reasons for this difference. The first is that ball milling generates smaller particles (Lai & Sarkanen 1971) which have a greater surface area to volume ratio, so overall, there are more surfaces for the enzyme to work on. The second is that ball milling also disrupts the crystallinity of the cellulose matrix (Maurer & Fengel 1992a). This improves the digestion because cellulases degrade amorphous cellulose more easily than crystalline cellulose (Wood 1989).

The very low yield of lignin isolated from the ring milled wood may be a consequence of the small extent of carbohydrate degradation by the enzyme; that is, no significant improvement in the accessibility of the lignin was achieved because very little carbohydrate was removed. Additionally, the cell wall structure may not be sufficiently disrupted by ring milling to allow the release of the lignin.

The amount of carbohydrate degradation in the 3 day ball milled wood is very similar to the amount of degradation in the 1.5 day milled wood, however, the lignin yields are significantly different. The insoluble residue remaining after aqueous dioxane extraction of the 1.5 day milled wood (after 7 days of enzyme treatment) is similar to the residue remaining after extraction of the 3 day milled wood. Increasing the enzyme treatment of the 1.5 day ball milled wood to 9 days did not further increase the amount of digestion. Ball milling for 1.5 days is apparently enough to damage the wood structure sufficiently to allow enzyme action, however, much lower yields of lignin were released from the 1.5 day ball milled wood as compared to the 3 day milled wood.

The yield of lignin from ball milled wood (MWL) increases with milling time (Lai & Sarkanen 1971). Ball milling not only disrupts the cell wall matrix but also breaks the lignin macromolecule into smaller fragments which can then be extracted (Lai & Sarkanen 1971). Therefore, with longer milling more fragmentation occurs and more lignin can be released. Similar trends in yield, as seen for MWL, are seen here with the improved lignin yield after 3 days of milling as compared to 1.5 days. More lignin may be released if the milling is extended further, however, this would result in a greater amount of damage to the lignin.

The yields of CEL from the 3 day milled wood are all approximately 20%, irrespective of the enzyme treatment. Extending the enzyme treatment beyond 3 days has no significant effect on the amount of weight removal nor in yields of lignin.

Two different factors associated with lignin dissolution are perhaps apparent from the above discussion; that is, *lignin accessibility* and *lignin extractability*. The accessibility of the lignin to the solvent is improved by the disruption of the cell wall matrix by ball milling and by the removal of carbohydrates by enzyme degradation. Lignin extractability into appropriate lignin solvent systems is improved by ball milling and may, therefore, be highly dependent on the size of lignin fragments generated.

4.2.2.2 Nitrogen contamination

The presence of nitrogen in the lignin samples indicates the presence of either unremoved DMF used in the clean-up procedure or protein contamination. Both of these are undesirable; ideally, a totally uncontaminated lignin is required.

Nitrogen contamination can be reduced by extensively washing the extracted lignin. After the lignin is recovered from the DMF solution, washed several times with ether (which should remove residual DMF) and air dried, further washing with distilled water further reduces the level of nitrogen contamination (Table 4.3). This suggests that protein is the source of the nitrogen contamination. Residual protein, which is not likely to be soluble in ether, can be removed by washing samples with water.

Table (4.3). The effects of washing the CEL with water and the length of enzyme treatment on nitrogen contamination

Lignin sample	Elemental composition (% by weight)				
	C	H	O	N	OCH_3
14 day digest[1]	56.5	6.03	36.6	0.9	12.4
Washed 14 day digest[2]	57.9	5.90	35.6	0.7	12.9
Washed 3 day digest[2]	56.7	5.58	37.7	Trace[3]	12.9

1. The lignin sample was only washed with ether
2. These samples were washed extensively with water following the ether washing step
3. Trace nitrogen is detectable levels below 0.2%

A more dramatic effect on nitrogen content is seen by the shorter (3 day) enzyme treatment. The isolated lignin after a 14 day digest still contains 0.7% nitrogen after washing, whereas, the washed 3 day lignin has only trace (< 0.2%) nitrogen. Johansson (1997) also observed this when isolating residual lignin from kraft pulps. This result agrees with the hypothesis that the nitrogen contamination is arising from protein, since a shortened contact time between the enzyme and the lignin reduces the nitrogen contamination.

The formation of chemical bonds between lignin and proteins may be the reason for the occurrence of protein impurities in the isolated lignin samples. Preliminary studies on the effect of enzyme treatment on milled wood lignin by NMR indicated differences in the levels of lignin carbonyl groups after enzyme treatment. The formation of imine type linkages between aldehyde groups in the lignin and amino groups in the protein could be a possibility. This hypothesis was investigated by treating milled wood lignin (MWL) and reduced milled wood lignin (R-MWL) samples under the same conditions used for the isolation of enzyme lignin (milled wood lignin was reduced using the method of Torr 1994). The results are shown in Table (4.4).

Table (4.4). The effect of a 3 day enzyme treatment on MWL samples

Sample	MWL	R-MWL
Recovered from dioxane[1]	90	99
Recovered from DMF[1]	83	97
Nitrogen content of washed lignin	Trace[2]	Trace[2]

1. Percentage of initial weight of MWL or R-MWL
2. Trace nitrogen is detectable at levels below 0.2%

It can be seen that no significant increase in protein in either sample, as measured by their nitrogen content, occurred during the enzyme treatment. Similar results were obtained by Hortling et al. (1992) who treated MWL and kraft lignin samples with a cellulase and detected only trace nitrogen in the treated samples. It was suggested that the interaction between lignin and protein was physical adsorption rather than covalent attachment.

4.2.2.3 The enzyme

The Onozuka enzyme preparation, which is used in this study, is highly impure and accordingly, it will have a very low specific activity. The filter paper assay method described in Ghose (1987) was used to try and measure the activity of the cellulase component of the enzyme. Reducing end groups released from filter paper cellulose by the enzyme are quantified by reaction with dinitrosalicylic acid, producing a bright red colour that can be quantified colorimetrically. However, the enzyme blank produced such a significant colour change that no activity measurements could be taken. This result suggests the Onozuka enzyme preparation contains a large quantity of carbohydrates. These carbohydrates could interfere with the digestion or could possibly contaminate the extracted lignin.

Hortling et al. (1992) obtained better yields of enzyme lignin from kraft pulps when a cellulase of high activity was used. Therefore, the efficiency of a different cellulase/hemicellulase enzyme system was studied. A combination of Celluclast and Novozyme enzymes was compared to the Onozuka preparation. Celluclast is a multienzyme cellulase from *Trichoderma reesei* and catalyses the degradation of cellulose into glucose, cellobiose and higher oligomers, whereas Novozyme exhibits cellulase, hemicellulase, glucanase, and pentosanase activity. The Celluclast and Novozyme enzymes are synergistic; that is, dissolution of the cell wall material is improved if both enzymes are used.

The efficiency of digestion of each enzyme system (as measured by the amount of residue remaining after lignin extraction) as well as the lignin yields were essentially the same (Table 4.5).

Table (4.5). Comparison between two different cellulase enzyme systems with respect to the isolation of CEL from wood

Weight of dioxane insoluble residue and CEL yield					
Incubation time	Onozuka		Cell/Nov		
	Residue[1]	**Yield**[2]	**Residue**[1]	**Yield**[2]	
3 days	19	20	21	21	
6 days	19	–	23	–	
Elemental composition of extracted lignin (% by weight)					
	C	H	O	N[3]	OCH_3
Onozuka	56.7	5.58	37.7	Trace	12.9
Cell/Nov	56.4	6.10	37.5	Trace	12.8

1. Percentage of initial weight of wood
2. Percent of total lignin in *Pinus radiata* wood
3. Trace nitrogen is detectable below 0.2%

The elemental compositions of the lignins are also very similar. The methoxyl content is a good measure of purity and indicates that both isolated lignin samples have similar purities. Pine MWL has a methoxyl content of 14–15% (Kininmonth & Whitehouse 1991). If this is used as the content of a pure lignin, then the CEL lignin samples contain approximately 10–15% impurity. The impurities are probably carbohydrate, protein or moisture. The low nitrogen content indicates that only small amounts of protein (< 2%)

are present in the samples. In later studies it is shown that lignin samples retain a moisture content of 5 to 10% after freeze drying (Appendix 2). This will lead to a reduction of between 0.7 and 1.2% in the methoxyl content compared to a completely dry sample. The carbohydrate content will, therefore, be in the range of 5–10%.

4.2.2.4 Solvent swelling

Chen et al. (1995b) treated Wiley milled Todomatsu wood meal with LiCl in dimethylacetamide to swell the cellulose before enzyme digestion. An increase in the efficiency of digestion was reported. After 12 days of enzyme treatment, the residue was 82% of the original weight for the Wiley milled wood meal, and 40% for the sample that had been ball milled. This was compared to 28.5% for the pretreated wood meal after an enzyme treatment of only one day. The yield of lignin extracted from the swelled wood with 90% aqueous dioxane was about 40%.

The effect of this treatment on the isolation of lignin from 3 day ball milled *Pinus radiata* wood was investigated. No significant differences in the degree of digestion or in the yields of extracted lignin were found between treated and untreated *Pinus radiata* wood powder (Table 4.6).

Table (4.6). The effect of solvent swelling ball milled wood on enzyme digestion and lignin yield

Weight of dioxane insoluble residue and CEL yield					
	Residue[1]			Yield[2]	
Swelled	25			18	
Unswelled	27			20	
Elemental composition of extracted lignin (% by weight)					
	C	H	O	N[3]	OCH_3
Swelled	57.9	5.54	36.6	Trace	13.1
Unswelled	58.3	5.81	35.9	Trace	13.0

1. Percentage of initial weight of wood
2. Percentage of total lignin in *Pinus radiata* wood
3. Trace nitrogen is detectable below 0.2%

It should be noted that the enzyme treatment in this experiment was 24hr and the incubation temperature was 50°C, in accordance with the Chen et al. (1995b) method. The degree of digestion is slightly less than achieved in 3 days at the lower temperature but the lignin yields are essentially the same (cf Table 4.5). Comparing these result with the results of Chen et al. (1995b) suggest that solvent swelling and ball milling of Wiley milled wood have the same effect in terms of increasing the accessibility of the cellulose to enzyme degradation.

The elemental compositions of the lignin samples in Table (4.5) and Table (4.6) suggest there is no significant difference in the composition of the CELs isolated using the slightly different methods.

4.2.2.5 Sodium hydroxide treatment

Protein adsorbed onto the surface of the undigested material could be hindering the lignin extraction. Washing the digested residue with sodium hydroxide prior to dioxane extraction was investigated as a means to remove any protein and perhaps enhance the lignin extraction. Johansson (1997) and Hortling et al. (1992) found that protein contamination of cellulolytic enzyme lignin from kraft pulps was reduced after a sodium hydroxide treatment followed by an acid treatment to precipitate the lignin. Additionally, the alkali pretreatment may disrupt certain interactions between the wood polysaccharides and lignin allowing more lignin to be released from the wood (Bland & Menshun 1967).

The effect of treating the residue remaining after digestion with NaOH/HCl was investigated in several experiments. During the NaOH/HCl treatment, the residue remaining after enzyme digestion was suspended in NaOH solution and then acidified to pH 2 with dilute HCl. The precipitate was recovered by centrifugation and washed several times until neutral. The results from the trials are discussed below.

Trial 1

The enzyme digest residue from 20g of wood powder was treated as outlined in Figure (4.2). Half of the residue was washed with water (Route 1) and the other half was NaOH/HCl treated with 100mM NaOH (Route 2). The DMF lignins were recovered after aqueous dioxane (90%) extraction and DMF purification. The results are summarised in Table (4.7).

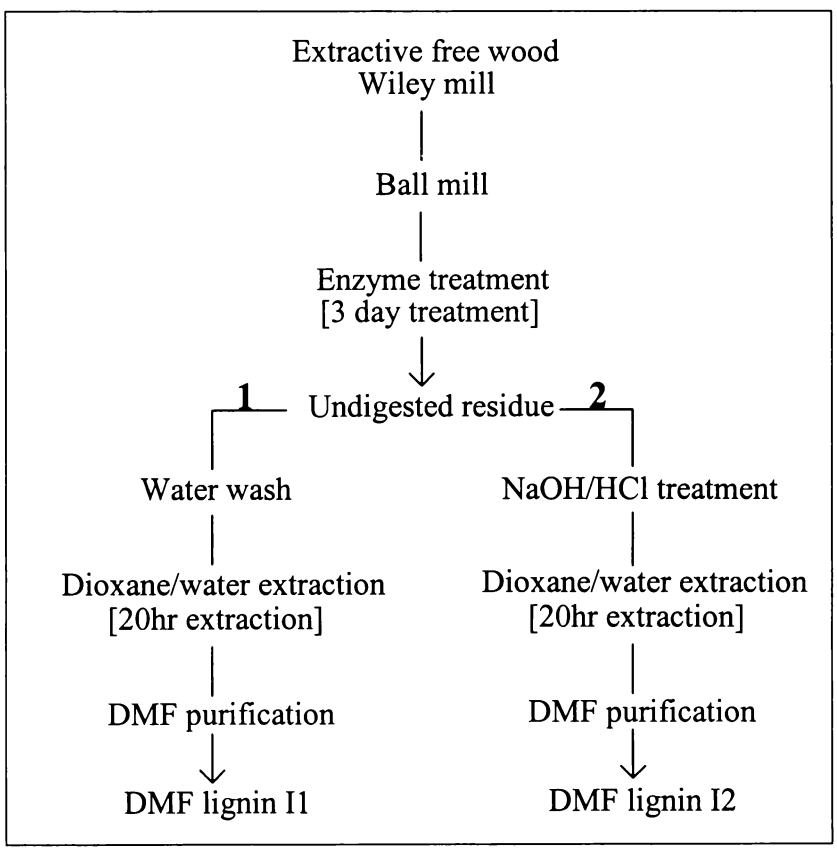

Figure (4.2). Scheme for NaOH trials 1 & 2

Table (4.7). The effect of treating the enzyme digest residue with 100mM NaOH prior to lignin extraction

Weight of dioxane insoluble residue and CEL yield from 10g of wood					
	I1		I2		
Residue after extraction[1]	14		19		
Crude lignin[2]	17		26		
DMF lignin[2]	10		3		
DMF insoluble residue[3]	–		65		
Elemental composition of extracted lignin (% by weight)					
	C	H	O	N	OCH_3
DMF lignin I1	54.6	6.51	38.7	0.16	11.95
DMF lignin I2	54.6	6.34	38.9	0.19	11.92
DMF insol; NaOH insol (I2)	55.1	6.36	37.6	0.97	11.10
DMF insol; NaOH sol, acid ppted (I2)	51.5	6.15	39.1	3.20	8.74

1. Percentage of initial weight of wood
2. Percentage of total lignin in *Pinus radiata* wood
3. Percentage of crude lignin

The NaOH/HCl treatment had a marked effect on the extractability of the lignin from the enzyme digest residue. The yield of crude lignin after the treatment is almost 1.5 times greater than when no treatment is carried out. However, a large proportion of the crude I2 lignin is insoluble in DMF resulting in a very low yield of purified lignin.

The DMF insoluble material from I2 was suspended in 100mM NaOH and the NaOH insoluble fraction was recovered by centrifugation, washed extensively with water, and then freeze dried. The supernatant was acidified to pH 2 with dilute HCl to recover the NaOH soluble material. The precipitate was recovered, extensively washed with water, and freeze dried. Approximately 20% of the DMF insoluble material was insoluble in 100mM NaOH and approximately 55% of the DMF insoluble material was precipitated from the alkali solution. This leaves approximately 25% unrecovered. The elemental composition of the DMF lignins from the wood and the fractions from the DMF insoluble material are also shown in Table (4.7).

The elemental analysis shows that the fraction of material insoluble in both DMF and NaOH contains a large quantity of nitrogen; almost five times as much as the purified CEL. Therefore, the reason for the low solubility of the crude I2 lignin in DMF may be due to high protein contamination. Furthermore, the fraction of the DMF insoluble material precipitated from alkali solution contains an even larger amount of nitrogen, which could indicate the protein is being preferentially removed by the alkali. The protein content (estimated from the nitrogen content) of this fraction is approximately 20%.

Trial 2

The effect of a 10mM NaOH/HCl treatment was investigated in this trial. The enzyme digest residue from 10g of wood powder was NaOH/HCl treated with 10mM NaOH and extracted with 90% aqueous dioxane using the same method as above (Figure 4.2, Route 2). The results are shown in Table (4.8).

Table (4.8). The effect of treating the enzyme digest residue with 10mM NaOH prior to the extraction step

Weight of dioxane insoluble residue and CEL yield from 10g of wood	
Dioxane insoluble residue[1]	24
Crude lignin[2]	59
Purified lignin[2]	29

1. Percentage of initial weight of wood
2. Percentage of total lignin in *Pinus radiata* wood

The 10mM NaOH/HCl treatment affected the lignin extraction in a similar way to the 100mM NaOH/HCl treatment, however, the yields of both the crude and purified lignin are significantly improved. A large proportion of the crude lignin (around 50%) was not recovered following the DMF purification step.

Trial 3

In this trial the effects of the NaOH/HCl treatments were re-evaluated. Wood powder (20g) was treated as outlined in Figure (4.3A). The undigested residue remaining after the enzyme treatment was split into three equal proportions. One portion was extensively washed with water (III1). The other two were NaOH/HCl treated with either 10mM NaOH (III2) or 100mM NaOH (III3). The treated residues were then extensively washed with water and freeze dried.

The dried residues weighed 2.13, 2.36, and 2.38g, respectively. In total this is about 35% of the initial weight of wood, which means that approximately 65% of the weight has been solubilised during the enzyme treatment. This is almost equivalent to the amount of polysaccharides in the cell wall which suggests that during the enzyme treatment, most of the carbohydrates are removed leaving an enzyme digest residue which is almost exclusively made up of lignin.

Only a small amount of lignin was extracted from the freeze dried residues into aqueous dioxane. The only difference in this experiment compared to Trials 1 and 2, was that the residues were freeze dried prior to the aqueous dioxane extraction. It was concluded that freeze drying the digest residue prior to extraction is detrimental to lignin yields.

With the aim of improving the extractability of the lignin, the above residues (III1, III2, III3) were NaOH/HCl treated with 100mM NaOH and extensively washed with water, before being extracted with 90% aqueous dioxane (Figure 4.3B). The yields of crude lignin and the dioxane insoluble material from the NaOH/HCl treated residues are shown in Table (4.9). This NaOH/HCl treatment has rendered the previously insoluble lignin, from residues III1 and III2, soluble in aqueous dioxane. The highest yield of crude lignin was obtained from treatment III2. However, the lignin in residue III3 remained insoluble in aqueous dioxane. This residue was then extracted with 100mM NaOH. The extracted lignin was recovered by precipitation at pH 2, washed extensively with water, and freeze dried. The yield of NaOH extracted lignin from residue III3 is also shown in Table (4.9).

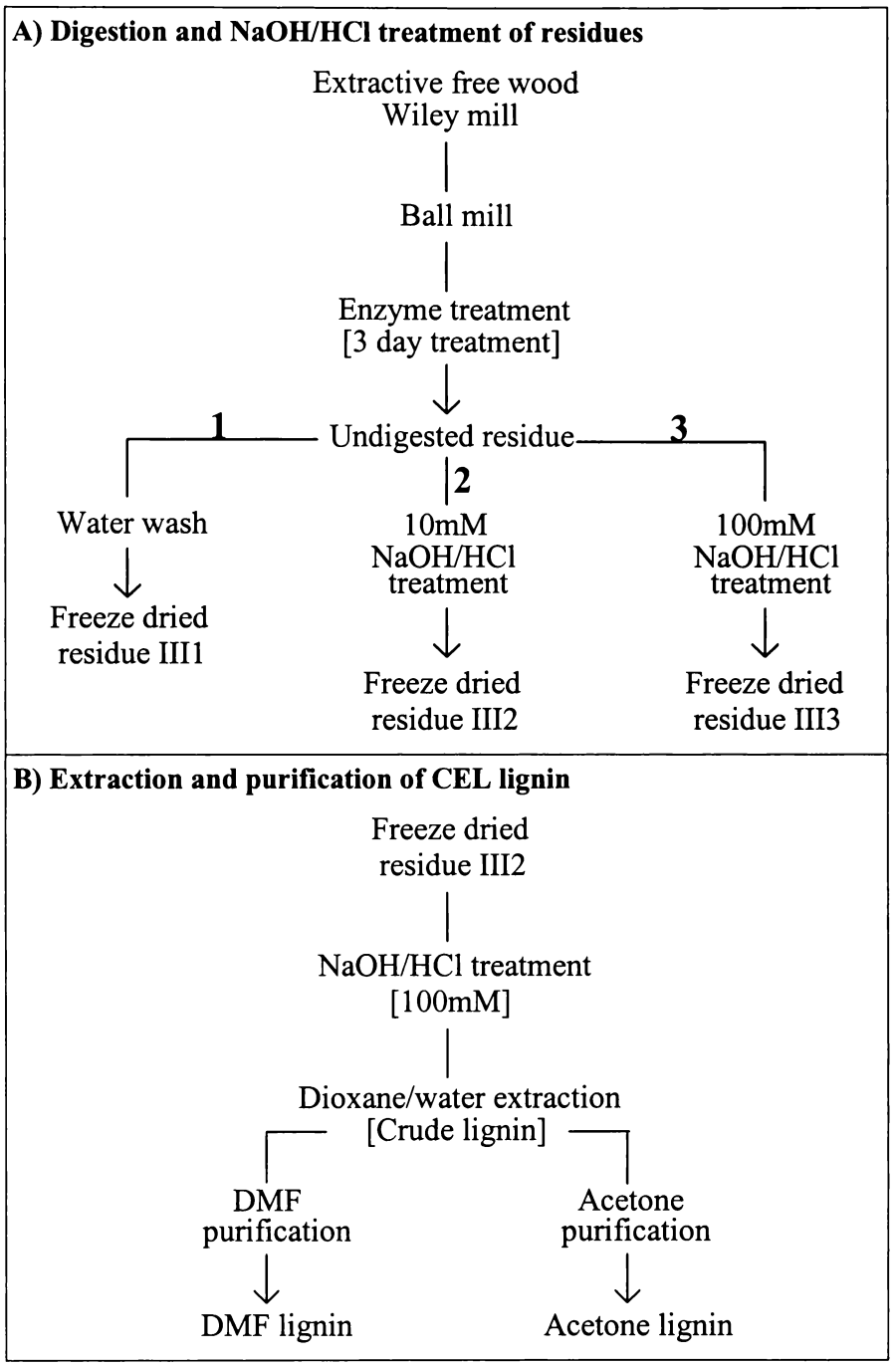

Figure (4.3). Scheme for NaOH trial 3

Table (4.9). The effect of treating the enzyme digest residue with NaOH prior to the extraction step

Weight of dioxane insoluble residue and yield of crude CEL[1]						
Sample	Residue[2]		Crude lignin[3]		NaOH ext.[3]	
III1	29		26		–	
III2	22		90		–	
III3	19		–		10	
Elemental composition of isolated lignin (% by weight)						
	Yield[4]	C	H	O	N	OCH_3
DMF lignin [III2]	5	54.5	6.48	39.0	Trace[5]	11.83
Acetone lignin [III2]	30	53.9	6.24	39.6	0.31	11.65
DMF insol [III2]	–	53.2	5.75	40.4	0.60	10.49
NaOH ext. [III3]	–	54.2	5.96	39.6	0.29	11.66

1. From NaOH (100mM)/HCl treated residues (Figure 4.3B)
2. Percentage of initial weight of wood
3. Percentage of total lignin in *Pinus radiata* wood
4. Yield of purified lignin from residue III2 (Figure 4.3B)
5. Trace nitrogen is detectable below 0.2%

An alternative purification procedure was also investigated in this trial (Figure 4.3B; Acetone purification). During acetone purification the crude lignin is suspended in 100 mM NaOH, the suspension is acidified to pH 2 with dilute HCl and the precipitate is recovered and extensively washed with water. The fraction that is soluble in 90% aqueous acetone is collected as purified acetone lignin.

The crude lignin fraction from residue III2 was used to compare the acetone purification method with the DMF procedure. The yield and elemental composition of the purified lignins are shown in Table (4.9). The DMF lignin yield was only 4%, however, about 30% of the crude lignin was not soluble in DMF. On the other hand, the acetone lignin yield was 30%.

The composition of both the DMF lignin and the acetone lignin are similar, although the nitrogen content of the acetone lignin is slightly higher. As seen earlier (Table 4.7), the DMF insoluble fraction has a high nitrogen content suggesting high protein contamination. Additionally, the elemental composition of the NaOH extracted lignin from residue III3 does not seem very different from the purified lignins from residue III2 so the reasons for the differences in solubility are unclear.

4.2.2.6 Acetone as an alternative solvent

One limitation of using dioxane and DMF is that they are very difficult solvents to work with. It was found that if the dioxane was not totally removed by rotary evaporation before freeze drying, the crude lignin samples ended up being totally insoluble when re-dissolution in aqueous dioxane or aqueous acetone was attempted. During the freeze drying step any residual dioxane seems to bind irreversibly to the lignin causing problems with solubility.

Additionally, during DMF purification, a large proportion of the lignin is not recovered from the DMF solution, particularly if a large volume is used. Another problem with DMF is that the solvent may contribute to nitrogen contamination.

Aqueous acetone is another good lignin solvent (Bland & Menshun 1967). Acetone has a lower boiling point than dioxane so is easier to remove. Therefore, 90% aqueous acetone was investigated as an alternative solvent for lignin extraction. The preliminary results from above (Section 4.2.2.5, Trial 3) also suggest that the acetone method is a viable purification step. The use of aqueous acetone for lignin extraction and the acetone purification procedure were evaluated, as discussed in the following.

The digest residue from 20g of wood powder was split into six and treated as outlined in Figure (4.4). Three fractions were washed extensively with water (path A) and three were NaOH/HCl treated with 10mM NaOH (path B) prior to the extraction step. Aqueous acetone (90%) was used for the extraction of lignins A3 and B3, in place of 90% aqueous dioxane. These lignins were purified using the acetone purification method. For comparison, samples were extracted with 90% aqueous dioxane and purified using either the DMF method (DMF lignin A1, B1) or the acetone method (Acetone lignin A2, B2). The yield and elemental composition of the isolated lignins are shown in Table (4.10).

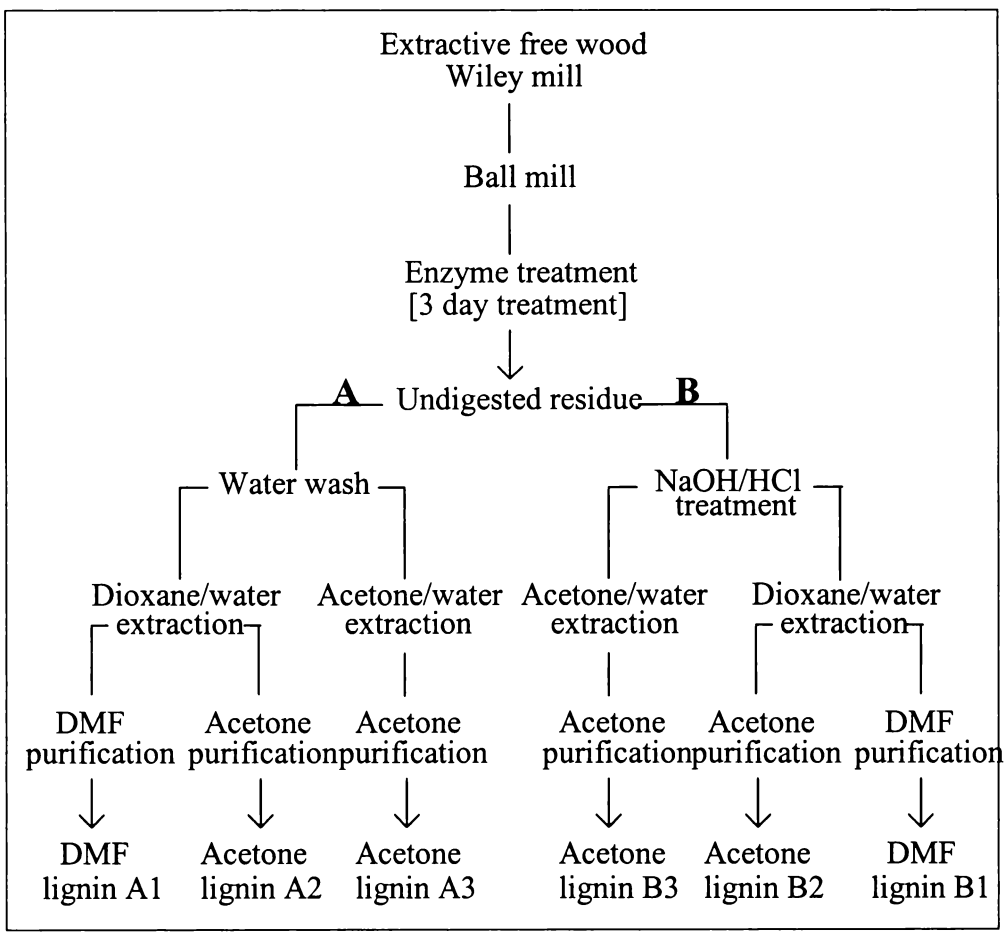

Figure (4.4). Scheme for acetone trials

Table (4.10). Yields of lignin extracted from enzyme digested wood into 90% acetone or 90% dioxane

	Crude lignin[1]		Purified lignin[1]		
DMF lignin A1	22		8		
Acetone lignin A2	22		17		
Acetone lignin A3	21		15		
DMF lignin B1	22		7		
Acetone lignin B2	22		16		
Acetone lignin B3	25		19		
Elemental composition (% by weight)					
	C	H	O	N	OCH_3
DMF lignin A1	54.6	6.51	38.7	0.16	11.95
Acetone lignin A2	56.7	5.62	37.4	0.33	12.28
Acetone lignin A3	59.0	5.72	35.0	0.27	13.27
Acetone lignin B2	57.9	5.58	36.2	0.35	12.76

1. Percentage of total lignin in *Pinus radiata* wood

The amount of lignin extracted into aqueous acetone or aqueous dioxane are the same, and although the yield results indicate the acetone purification procedure is superior to the DMF procedure, the samples cleaned up in this way contain approximately twice as much nitrogen (and therefore protein) as the DMF lignin. The methoxyl contents of the acetone lignins are higher than the DMF lignins which suggests the purities of the acetone lignins are higher. Assuming the moisture contents of the lignin samples after drying are approximately the same then it can be assumed that the acetone lignins have a lower carbohydrate content. This is consistent with the results of Johansson (1997) who reported that CEL lignin extracted from kraft pulps into aqueous dioxane and purified with DMF had a higher carbohydrate content than the lignin extracted into aqueous acetone and purified with NaOH/HCl-acetone.

4.2.2.7 The residue after lignin extraction

During the enzyme digestion, approximately 65% of the weight of wood is removed. This is almost equivalent to the amount of carbohydrate in the cell wall and would suggest that the undigested material contains the majority of lignin from the wood, of which only 20% is soluble in 90% dioxane or acetone. The insoluble residue remaining after aqueous dioxane extraction of the enzyme digest residue was shown to contain approximately 70% Klason lignin. Further investigations were carried out with the aim of extracting the remaining lignin from this residue.

Trial 1

Chang et al. (1975) obtained a higher yield of cellulase enzyme lignin from spruce and sweetgum by extracting the residue remaining after enzyme treatment and lignin extraction with 50% aqueous dioxane. This was investigated as a means of isolating the lignin that remained in the aqueous dioxane insoluble residue (DIR). That is, 10g of the DIR was extracted with 50% aqueous dioxane for 24 hours and the crude lignin was purified using the DMF purification method. The results in Table (4.11) show that only a very low yield of lignin could be extracted from the DIR with 50% aqueous dioxane.

Table (4.11). Yield of lignin extracted from 10g of the dioxane insoluble residue with 50% aqueous dioxane

Sample	Yield (%)
Crude lignin[1]	6
DMF lignin[1]	0.7
DMF insol. residue[2]	38
Insoluble residue[3]	86

1. Percentage of Klason lignin
2. Percentage of crude lignin
3. Percentage of initial weight of DIR

Trial 2

Protein adsorbed onto the surface of the DIR could be a possible reason for the insolubility of the lignin in the DIR. Alkali was shown earlier (Section 4.2.2.5, Trial 1) to have a preference for protein contaminated lignin. To test this hypothesis the DIR was extracted with 100mM and 1M NaOH. Lignin was recovered from solution by precipitation at pH 2. The precipitate was extensively washed with water before being extracted with 90% aqueous acetone. The acetone lignin was freeze dried from a water suspension after the acetone was removed by rotary evaporation. The yields are shown in Table (4.12).

Table (4.12). Yield of lignin extracted from 1g of the dioxane insoluble residue with NaOH

	100mM NaOH	1M NaOH
Acetone lignin[1]	8	8
NaOH insol. residue[2]	74	50

1. Percentage of Klason lignin
2. Percentage of initial weight of the DIR

A larger proportion of the DIR was soluble in alkali than in 50% dioxane (compare Table 4.11). Also, the percentage of the DIR that dissolved in alkali increased with the strength of alkali. However, only 8% of the Klason lignin was recovered as acetone lignin. The reasons for these differences are unclear. However, protein contamination could have a strong influence on the solubility of the lignin.

Trial 3

Chang et al. (1975) found that lignin extracted into 50% aqueous dioxane was insoluble in 96% aqueous dioxane. Additionally, the lignin extracted into 50% aqueous dioxane had a higher molecular weight than the lignin that was soluble in 96% aqueous dioxane. Chang et al. (1975) proposed that this difference in molecular weight was the reason for the differences in solubility of the lignins, thus a higher molecular weight of the lignin in the DIR may be the reason for its insolubility. Ball milling is known to fragment lignin and enhance its extractability, therefore, ball milling the DIR may enhance the lignin solubility. This was investigated in the following experiment. The DIR was ball milled for 3 days and then treated as outlined in Figure (4.5).

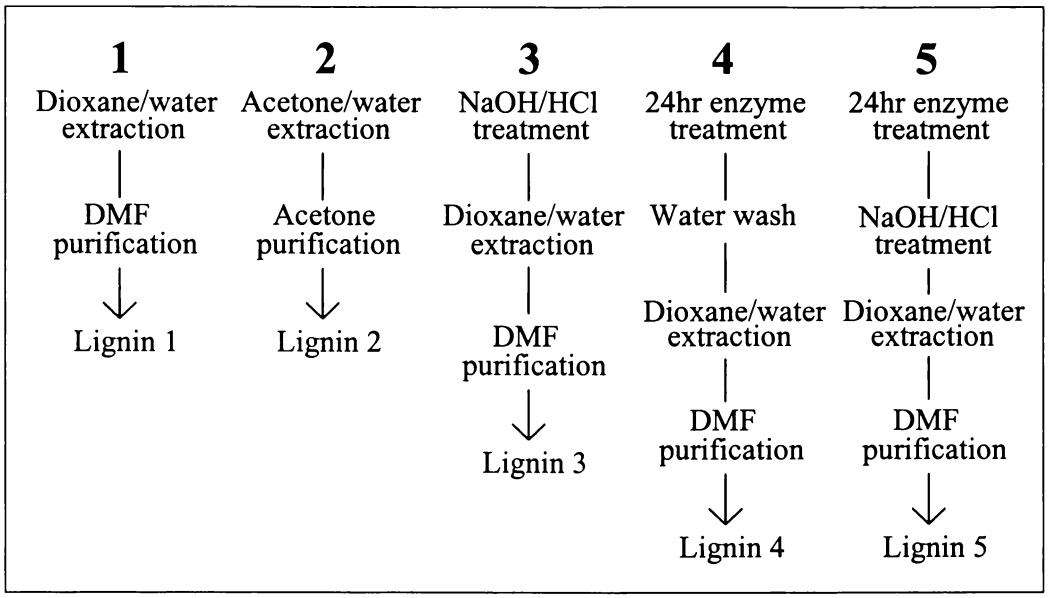

Figure (4.5). Treatments of the ball milled dioxane insoluble residue (DIR)

Lignin 1 was extracted from the ball milled DIR with 90% aqueous dioxane and purified using the DMF method. Lignin 2 was extracted with 90% aqueous acetone and purified using the acetone procedure. Lignin 3 was extracted with 90% aqueous dioxane from ball milled DIR after a NaOH/HCl treatment with 100mM NaOH. Treatments 4 and 5 involved a 24hr enzyme digestion of the ball milled DIR prior to lignin extraction. For treatment 5 the resulting enzyme digest residue was NaOH/HCl treated with 10mM NaOH before the extraction step, whereas, for treatment 4 the digest residue was washed with water before the extraction step. Lignin 3, 4, and 5 were purified using the DMF method.

The aqueous dioxane or acetone insoluble material was also extracted with 100mM NaOH. The extracted lignin was recovered by precipitation at pH 2. The precipitate was washed extensively with water and then extracted with 90% aqueous acetone. The acetone soluble material was freeze dried from a water suspension after the acetone was removed by rotary evaporation. Yields and elemental composition of the extracted lignins are shown in Table (4.13).

Over 80% of the DIR became soluble in 90% aqueous dioxane or acetone after the ball milling treatment. This result is in agreement with the idea that lignin in the enzyme digest residue has a high molecular weight which prevents its extraction. During ball milling the lignin in the DIR has been fragmented into smaller molecules which are soluble in aqueous dioxane or acetone.

For all treatments the total yield (Crude + NaOH) accounts for all the lignin in the DIR. This indicates that no additional treatment after the ball milling step is necessary to achieve the maximum yields (Crude + NaOH) of lignin from the DIR. However, NaOH/HCl treating the ball milled DIR with 100mM NaOH prior to the extraction step (Lignin 3) improved the yield of lignin after purification by about 10%. The nitrogen content of purified lignin 3 is also slightly lower than lignin 1, which has not been NaOH/HCl treated.

Table (4.13). Yield and composition of lignins isolated from the dioxane insoluble residue (DIR) after ball milled

Yields of lignin extracted from the ball milled DIR					
Lignin sample	Crude lignin[1]	Purified lignin[1]	NaOH lignin[1]	Crude + NaOH[1]	Insoluble residue[2]
1	92	46	13	105	14
1	90	56	15	105	16
2	128	56	30	160	19
3	84	64	9	93	12
4	75	46	–	–	–
4	86	67	6	92	7
5	71	55	–	–	–

Elemental composition (% by weight)					
	C	H	O	N	OCH_3
Lignin 1	56.2	5.77	37.9	0.15	12.81
Lignin 2	49.8	5.02	44.2	0.88	10.68
Lignin 3	56.9	6.04	36.9	0.12	13.25
Lignin 4	55.8	6.00	38.0	0.20	12.22
Lignin 5	55.5	5.69	38.6	0.18	12.35
NaOH lignin (5)	55.5	5.84	37.9	0.80	11.47

1. Percentage of Klason lignin
2. Percentage of initial weight

The very high yield of the crude acetone lignin (lignin 2) can be explained through high protein contamination. The purified acetone lignin (2) still contains a high percentage of nitrogen and it is expected the contamination will be even higher before the purification step. The presence of a large quantity of protein will lead to an overestimation of the yield since the yields were determined from the weight of dry material.

4.2.3 The improved method

The NaOH/HCl treatments and the second ball milling step were amalgamated to form an extended CEL method (Figure 4.6). To determine the overall improvement in yield of CEL using this method, 40g of wood was treated following this improved method.

One half of residue remaining after enzyme digestion was NaOH/HCl treated with 10mM NaOH and then extracted with 90% aqueous dioxane (Route 1). The crude lignin was purified using the DMF procedure to give CELF1. The residue insoluble in aqueous dioxane and the crude lignin that was insoluble in DMF were extensively washed with water and freeze dried. The aqueous dioxane and DMF insoluble fractions were then combined and ball milled for 3 days. The resultant powder was washed out of the ball mill with water. The insoluble residue was recovered by filtration and was NaOH/HCl treated with 100mM NaOH before being extracted with 90% aqueous dioxane. The crude lignin was purified using the DMF procedure to give CELF2. The filtrate was freeze dried to recover the water soluble material.

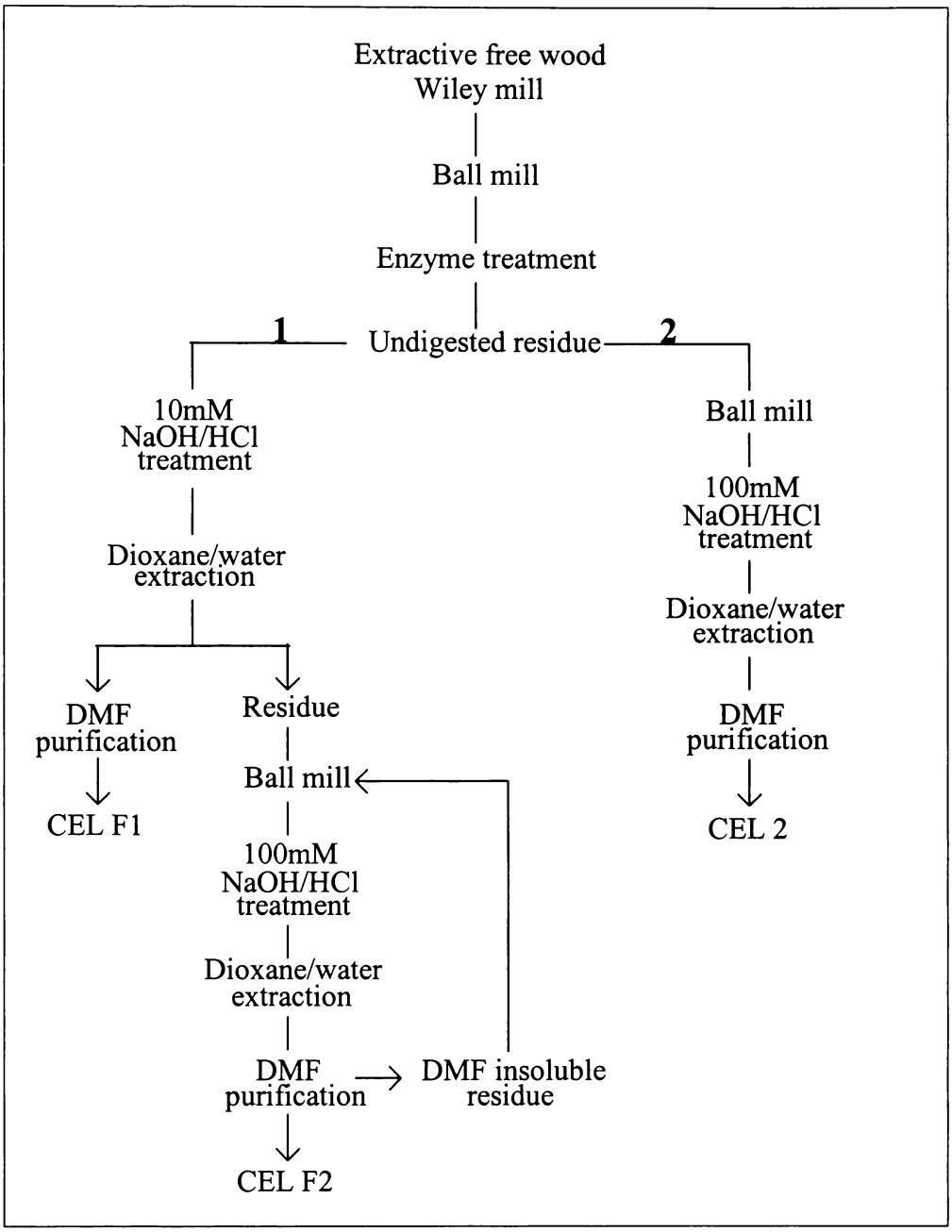

Figure (4.6). The improved CEL isolation method

The second half of the enzyme digest residue was extensively washed with water, freeze dried, and then ball milled for 3 days (Route 2). The resultant powder was washed out of the ball mill with water and the insoluble residue was recovered by filtration. The residue was then NaOH/HCl treated with 100mM NaOH before being extracted with 90% aqueous dioxane. The crude lignin was purified using the DMF procedure to give CEL2. The filtrate was freeze dried to recover the water soluble material.

The yields of extracted lignin are shown in Table (4.14). Nearly 80% of the lignin from *Pinus radiata* wood has been isolated using Route 1 (Figure 4.6). The yield of CELF1 is only slightly lower than the yield of CEL from spruce wood obtained by Chang et al. (1975). The second ball milling step has released nearly all of the lignin remaining insoluble after the first extraction step. If the first extraction step is omitted (CEL2) a loss in yield of 10% is incurred. The CELF1 fraction, which is isolated after one ball milling treatment (Route 1), is not removed in Route 2 before the second ball milling treatment. Therefore, when Route 2 is used, this CELF1 fraction of lignin is possibly being fragmented by the second ball milling treatment to an extent that a part of it remains in solution during the isolation procedure.

Table (4.14) Yields (%) of lignin extracted from *Pinus radiata* wood using the improved CEL method

From 20g of wood		
1	**CELF1**	**CELF2**
Water soluble[1]	–	4
Crude lignin[2]	90	60
Dioxane insoluble residue[2]	15	–
DMF insoluble residue[3]	35	–
Purified DMF lignin[2]	22	54
Total CEL1 yield[2]	76	
2	**CEL2**	
Water soluble[1]	4	
Crude lignin[2]	105	
Dioxane insoluble residue[1]	3	
Purified DMF lignin[2]	66	

1. Percentage of initial weight of wood
2. Percentage of total lignin in 20g wood
3. Percentage of crude lignin

The elemental composition of the lignin fractions is shown in Table (4.15). The (P2) indicates the DMF lignin has been purified again using the acetone purification method. The nitrogen content of the three DMF lignins fractions is high compared to that which was achieved earlier (compare Table 4.9 and Table 4.13) and this may be due to the difficulty of washing the large amounts of lignin obtained. However, the nitrogen content of the isolated lignins was significantly reduced after a subsequent acetone purification step (P2 lignins).

Table (4.15). Elemental composition (% by weight) of lignin extracted from *Pinus radiata* wood using the improved CEL method

	C	H	O	N	OCH_3
CELF1	59.3	5.77	34.6	0.27	13.23
CELF1 (P2)	56.2	5.88	38.0	Trace	12.89
CELF2	59.2	5.60	34.8	0.42	12.27
CELF2 (P2)	55.3	5.89	38.6	0.26	12.68
CEL2	59.0	5.55	34.9	0.44	13.59
CEL (P2)	56.6	5.81	37.3	0.26	12.95
Dioxane insol. residue (2)	25.2	3.22	71.3	0.15	1.87
Water soluble (2)	45.9	5.70	47.8	0.63	5.30

(P2): DMF lignins purified again using the Acetone method
(2): Fractions from Route 2
Trace: Nitrogen is detectable below 0.2%

The material remaining insoluble after the extraction step (Route 2) has a very low methoxyl content which suggests a very low lignin content. This fraction is perhaps mainly made up of residual carbohydrates that are not solubilised during the isolation procedure.

The water soluble fraction, on the other hand, (from Route 2) contains approximately 40% lignin as determined by its methoxyl content (and accounts for approximately 5% of the total lignin in the wood). This lignin fraction may be made up of small fragments that are water soluble. Additionally, adsorbed protein could also be enhancing the water solubility of these lignin fragments. The low methoxyl content of this fraction also suggests a high carbohydrate content (over 50%). Carbohydrate bonds or lignin-carbohydrate bonds of the residual polysaccharides in the enzyme digest residue (Route 2) could be breaking during the second milling step sufficiently to solubilise the sugars.

4.2.4 Summary

In the above sections the development of an improved CEL method for the isolation of lignin from wood was discussed. The key step of this new method is the introduction of the second ball milling step. The majority of the lignin remaining in the enzyme digest residue becomes extractable after a second ball milling treatment. Additionally, an alkali/acid treatment prior to lignin extraction improves the yield of both the crude and purified lignin. The yields of CEL isolated from wood using this method are far greater than those previously reported; particularly, compared to the commonly used MWL method.

Several points were made in the above discussion concerning the methodology and these are reiterated in the following. The first point is that it is important to ensure that all the organic solvent (dioxane or acetone) has been completely removed from the extracted sample before freeze drying. The solvent (especially dioxane) seems to irreversibly bind to the sample when freeze dried resulting in the lignin becoming insoluble in 90% aqueous dioxane or acetone.

The NaOH/HCl treatment of the enzyme digest residue before the first extraction results in a larger fraction of the crude lignin being insoluble in DMF compared to when no treatment is carried out, however, the yield and purity of the purified lignin are slightly improved. Protein contamination is possibly the cause for the low solubility of the crude lignin in DMF since this fraction has a reasonably high protein content. Additionally, the fraction of the DMF insoluble material which is soluble in NaOH contains an even higher percentage of nitrogen suggesting the NaOH has a preference for protein contaminated lignin. The dioxane insoluble and DMF insoluble fractions can be combined, before the second ball milling stage to maximise the overall CEL yield.

Aqueous acetone can be used as an alternative to aqueous dioxane in the extraction steps. Similar yields of crude lignin were extracted into both solvents. The acetone purification procedure for the clean-up of the crude lignin was found to have varied success with respect to nitrogen contamination; often nitrogen content remained high, even after considerable washing. More consistent results were obtained using the DMF method, with nitrogen contamination consistently kept below 0.5% (which is equivalent to about 3% protein). By combining the DMF and acetone purification methods, in sequence, further improvements in purity were gained.

The main point is that ball milling plays an important role in the isolation procedure. The first ball milling step disrupts the structure of the cell wall sufficiently to allow the action of the enzymes and release of the first fraction of lignin (CELF1). The accessibility of the lignin, compared to ball milled wood, is increased by the removal of the carbohydrates during the enzyme treatment. The solubility of the remaining lignin is likely to be largely dependent on its molecular weight and the main function of the second ball milling step is to fragment the lignin sufficiently to allow its dissolution. The expected increase in phenolic hydroxyl groups by ball milling will also play a role in the increased extractability of the lignin.

4.3 Characterisation of CELs

Due to the complexity of the lignin structure a large number of methods for the study of lignin are available (Section 2.4.2). Several of these methods were used in this study. Elemental, methoxyl, and carbohydrate monomer analyses were used to determine the overall composition and purity of the lignin samples. The content of phenolic hydroxyl groups in the lignin was determined by periodate oxidation and ^{31}P-NMR analysis. Thioacidolysis was used to quantify uncondensed β-O-4 linkages in the lignin and ^{1}H-^{13}C correlation NMR spectroscopy was used to obtain an overall picture of the side chain structures present in the CELs. A review of these methods is given in the following section.

4.3.1 Review of methods

4.3.1.1 Determination of phenolic hydroxyl groups

Reaction of lignin with periodate under mild conditions results in the release of quantitative amounts of methanol from unetherified guaiacyl and substituted guaiacyl units in lignin (Figure 4.7A) The released methanol, therefore, can be used to estimate the phenolic hydroxyl content of lignin. The method involves reacting lignin with sodium periodate at 0°C for at least a week, periodically removing samples and analysing for methanol by GC. The major disadvantage of this method is that units which do not have a methoxyl group ortho to the hydroxyl group are not measured (ie catechol and *p*-hydroxy phenyl units). However, since softwood lignins only contain minor amounts of these types of units a reasonable estimate of the phenolic hydroxyl content can be made using this method.

^{31}P-NMR spectroscopy of phosphitylated lignin (Figure 4.7B) can also be used to characterise the phenolic hydroxyl groups in lignin. One advantage of this method is that, in addition to phenolic hydroxyl groups, aliphatic alcohols and carboxylic acid groups can also be distinguished. Additionally, C5-condensed (β-5, 4-O-5, 5-5) and uncondensed phenolic units can be distinguished. Table (4.16) shows the chemical shift range for each group. Cholesterol, whose phosphitylated signal appears between the aliphatic and condensed regions, was used as the internal standard. The main disadvantage of this method is that the NMR signals are broad resulting in considerable overlap, particularly in the phenolic region, thus, limiting the accuracy of this method. Additionally, a soluble lignin sample is required.

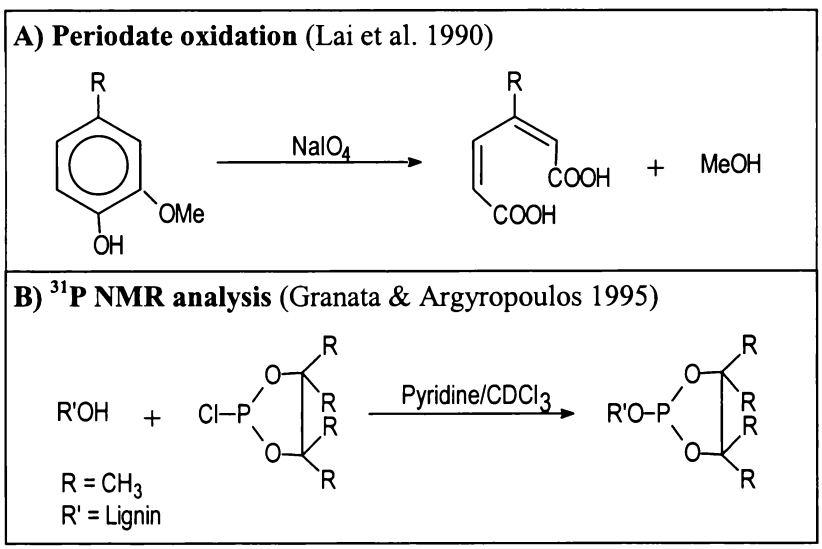

Figure (4.7). Hydroxyl group determination by NMR methods

Table (4.16). ^{31}P-NMR peak assignments for phosphitylated lignin

Peak (ppm)	Assignments
134–136	Carboxylic acids
137–138	*p*-hydroxy phenyl units
138–140	Guaiacyl units
140–144.5	C5-condenced guaiacyl units
145–150	Aliphatic hydroxyl groups

4.3.1.2 Determination of β-O-4 content

Thioacidolysis (Figure 4.8) involves the cleavage of α- and β-aryl ether bonds followed by thioethylation of the phenylpropane side chain to give the trithioether (1) as the major product. Analysis of this monomer by GC/FID enables the estimation of the levels of uncondensed β-O-4 aryl ether linkages in lignin. Other minor monomeric products can also be identified using GC/MS analysis enabling the quantification of certain end groups in the lignin (Table 4.17). The response factors are determined from appropriate model compounds (Roland et al. 1992). However, in this study, for simplification, the response factor for compound (1) was used for all the degradation products. This will lead to inaccuracies in the quantification but since all the samples were analysed in the same way comparisons between samples can still be made.

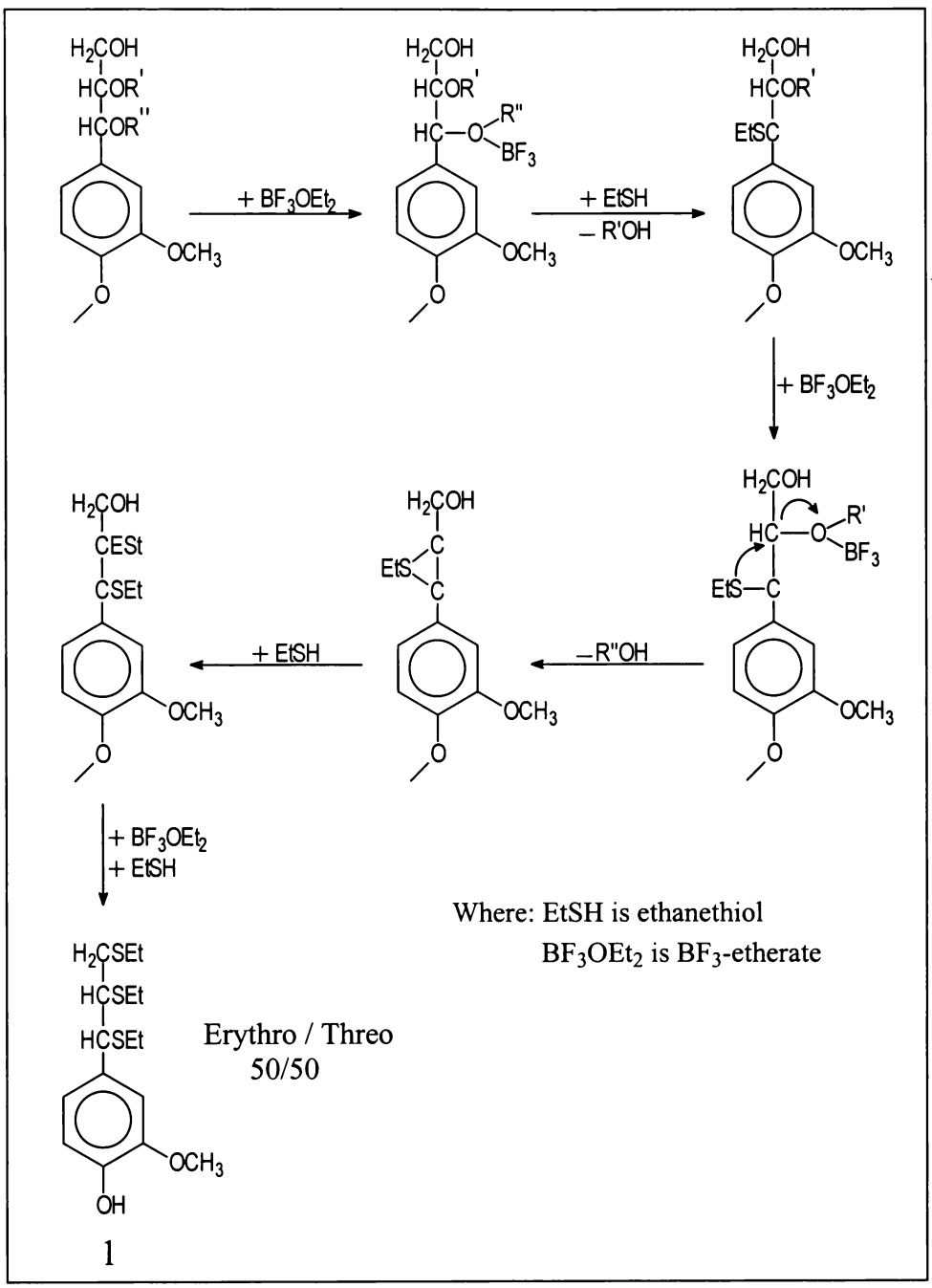

Figure (4.8). Reaction β-aryl ether structures during thioacidolysis

Table (4.17). Main thioacidolysis degradation products from softwood lignin

	Monomer product[1]	Lignin structure
1	G-CHRCHRCH$_2$R	β-O-4; guaiacyl unit
2	G-CR=CHR	β-O-4 with Cα=O; guaiacyl
3	H-CHRCHRCH$_2$R	β-O-4; *p*-hydroxy phenyl unit
4	C-CHRCHRCH$_2$R	β-O-4; catechol unit
5	G-CHR$_2$	Vanillin
6	G-COOH	Vanillic acid
7	G-CH$_2$CH$_2$CH$_2$OH	Dihydroconiferyl alcohol
8	G-CH=CHCH$_2$R	Coniferyl alcohol
9	G-CHRCH$_2$CH$_2$R	Coniferyl alcohol
10	G-CHRCH$_2$CHR$_2$	Coniferaldehyde
11	G-CH$_2$CHR$_2$	Enol ether; guaiacyl unit
12	G-CH=CHR	Enol ether; guaiacyl unit
13	G-CHRCH$_2$-G	Stilbene or β1; guaiacyl units

1. R = SCH$_2$CH$_3$

4.3.1.3 Two dimensional NMR spectroscopy

One dimensional NMR techniques have long been used as tools for the characterisation of soluble lignins. In spite of severe line broadening in ^{1}H-NMR spectra and overlap in ^{13}C-NMR spectra a surprising amount of structural information has been obtained. Further difficulties in interpreting 1D NMR spectra arise from the degeneracy of chemical shifts of the different nuclei in lignin. Two dimensional NMR techniques partially overcome these problems by spreading the NMR signals into a second dimension, thereby reducing the chance of overlapping signals. It also becomes possible to base interpretations on more than one chemical shift. Of the number of 2D techniques available, the homonuclear Hartmann-Hahn ^{1}H–^{1}H correlation (or TOCSY) experiment and the ^{13}C-decoupled ^{1}H-detected multiple quantum ^{1}H–^{13}C correlation (HMQC) experiment are considered the most useful for the study of lignin (Ede & Brunow 1992)

The structurally most useful region of either a ^{1}H or ^{13}C-NMR spectrum of lignin is the region in which the side chain signals appear. For underivatised lignins this region is from approximately 50–90ppm in the ^{13}C-NMR spectrum and 2.8–5.5ppm in the ^{1}H-NMR spectrum. To improve spectral dispersion of the ^{1}H signals acetylated lignin samples can be used, such that side chain ^{1}H signals appear in the region 3–6.2ppm. Lignin model compounds have played a key role in assigning the chemical shifts to facilitate the interpretation of the spectra. Some of the more important ^{1}H and ^{13}C chemical shifts for acetylated model compounds are given in Table (4.18). Information about the side chain interunit structures (examples shown in Figure 4.9) can be gained using NMR particularly with the aid of 2D techniques.

Table (4.18). ^{1}H and ^{13}C chemical shifts of side chain structures in acetylated lignin model compounds (Kilpelainen et al. 1994)

Structure[1]	α	β	γ
1	6.02–6.12; 73.9–74.5	4.63–4.65; 80.2	4.01–4.46; 62.6–63.1
2	5.51; 87.6	3.76; 50.7	4.29, 4.45; 65.5
3	4.80; 85.5	3.10; 54.5	3.94, 4.28; 72.0
4	6.05; 75.2	3.35; 50.4	4.11, 4.28; 63.9
5	5.92–6.14; 72.8–73.5	5.41–5.44; 72.3–72.5	3.81, 4.24; 61.7–62.1
6	– ; 194.4	5.45; 81.7	4.56; 64.6
7[2]	4.86; 84.0	4.16; 82.4	4.05,4.51; 63.5

1. Numbers refer to structures given in Figure (4.9)
2. From Karhunen et al. (1995)

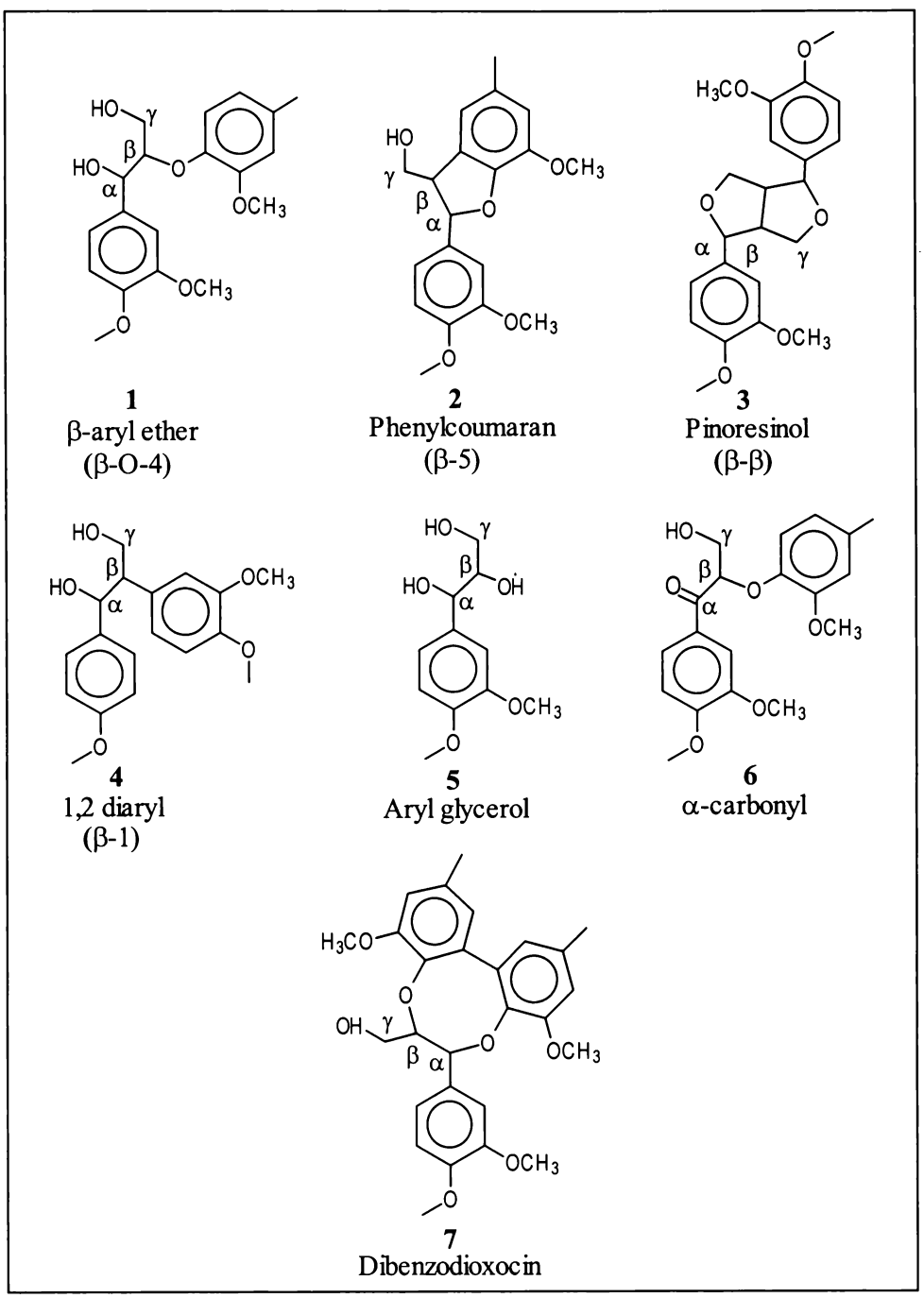

Figure (4.9). Some important structures in softwood lignin

4.3.2 Composition of CEL lignins

The analytical composition of the three enzyme lignins is summarised in Table (4.19), along with the calculated average compositions per C_9 unit which have been corrected for the protein, carbohydrate, and moisture contents (Appendix 2 contains the raw data). For comparison the data for *Pinus radiata* MWL are included (Adams & Ede 1997).

Table (4.19). Analytical composition of enzyme lignins and MWL isolated from *Pinus radiata* wood

	MWL[1]	CEL F1	CEL F2	CEL 2
Elemental composition				
Carbon	62.5	56.2	55.3	56.6
Hydrogen	6.12	5.88	5.89	5.81
Oxygen	31.4	38.0	38.6	37.3
Methoxyl	14.12	12.89	12.68	12.95
Nitrogen	0	Trace[2]	0.26	0.26
Moisture	–	7.2	9.3	7.6
Carbohydrates[3]	–	7.2	5.4	5.0
Klason lignin[4]	–	88	87	88
C_9 Formula[5]				
Carbon	9	9	9	9
Hydrogen	9.00	7.50	7.24	7.19
Oxygen	2.90	2.95	2.94	2.86
Methoxyl	0.87	0.93	0.93	0.93

1. From Adams & Ede (1997)
2. Trace = Detectable nitrogen below 0.2%
3. g/100g oven dry sample
4. Klason lignin + acid soluble lignin (g/100g oven dry sample)
5. Corrected for protein, carbohydrate, and moisture

The average C_9 unit compositions of the three CEL lignins are very similar, the most significant difference being the slightly lower hydrogen content of the CELF2 and CEL2 lignins compared to CELF1. This could indicate that these two lignin fractions contain a higher proportion of condensed structures than CELF1. A more significant difference is observed between MWL and the CEL lignins; that is, MWL has a lower methoxyl content and a higher hydrogen content. The higher hydrogen content can be explained in terms of the number interunit linkages. Chang et al. (1975) showed that MWL has a lower molecular weight and higher phenolic hydroxyl content than an enzyme lignin isolated from the same species. Consequently, this will lead to the higher hydrogen content for MWL since there are fewer interunit linkages. The lower methoxyl content of MWL may be due to demethylation caused by ball milling, however, this is not consistent with the similarity in the methoxyl contents of CELF1 and CELF2 which have been milled by different extents. The lower methoxyl content of the MWL may, therefore, be due to a higher proportion of *p*-hydroxy phenyl units. This would support the Bjorkman's theory that MWL arises from the middle lamella since this lignin generally contains a higher proportion of *p*-hydroxy phenyl units. The greater

accessibility of the lignin after removal of carbohydrates by enzyme treatment could result in a less selective removal of the lignin resulting in a higher concentration of guaiacyl units in the isolated sample and thus a larger methoxyl content. The similarity between all the CEL fractions would also support this idea.

As stated earlier, the nitrogen in the CEL lignins most likely originates from the enzyme used during the isolation procedure. After the two purification steps it is possible to reduce the protein content to below 2% (as indicated from the nitrogen content), however, the trace protein resists removal. This suggests that a strong association between the protein and the lignin (or residual carbohydrates) is involved. The exact nature of the association is unclear but Hortling et al. (1992) suggest the association is a strong physical attraction rather than covalent bonding between the isolated lignin and protein.

The CEL lignins also contain a small amount of carbohydrates (5–8%) of mainly hemicellulose origin (Appendix 2) which is normal for cellulase enzyme lignins (Yamasaki et al. 1981, Hortling et al. 1990, Johansson 1997). The lower carbohydrate content of CELF2 and CEL2 compared to CELF1 may be a result of the second ball milling treatment. The residual carbohydrates present during the second ball milling treatment could be partially broken to water soluble fragments and thus removed during isolation of the lignin.

The carbohydrate content of the CEL lignins is slightly higher compared to spruce CEL obtained by Chang et al. (1975), however, 96% aqueous dioxane was used for the extraction (compared to 90% aqueous dioxane). Chang et al. (1975) also reported an increase in carbohydrate content when 50% aqueous dioxane was used for extraction. Similar trends of increasing lignin purity (determined from Klason lignin content) of purified CEL lignins with solvent strength was observed by Chen et al. (1995b). Additionally, the Klason lignin contents of the CEL lignins extracted into 90% aqueous dioxane by Chen et al. (1995b) are similar to the Klason lignin contents reported here.

4.3.3 Phenolic hydroxyl groups

The distribution of hydroxyl groups in the CEL lignins was determined by quantitative ^{31}P-NMR analysis as described by Granata & Argyropoulos (1995). The results are shown in Table (4.20), along with the data for *Pinus radiata* MWL (Smit et al. 1997), and the total phenolic hydroxyl content determined using periodate oxidation. The total phenolic hydroxyl content determined by both methods are comparable, however, the estimates obtained using the periodate method for CELF2 and CEL2 are slightly lower compared to ^{31}P-NMR analysis. This indicates the periodate method slightly underestimates the levels free phenolic units in the lignin.

The effect of ball milling on the total phenolic hydroxyl content of lignin is apparent in the larger phenolic content of the CEL lignins that have been exposed to ball milling twice (CELF1 compared to CELF2 and CEL2). Interestingly, the MWL, which has only been exposed to ball milling once, has a similar phenolic content to CELF2 and CEL2 lignins.

Table (4.20). Distribution of hydroxyl groups in CELs and MWL isolated from *Pinus radiata* wood (mmol OH/ g lignin)[1]

Functional group	MWL[2]	CELF1[3]	CELF2[3]	CEL2[3]
Carboxylic acid	0.11	0.06 (1)	0.15 (3)	0.16 (4)
Aliphatic-OH	3.6	4.4 (2)	5.1 (3)	5.0 (2)
Guaiacyl	0.61	0.41 (3)	0.57 (6)	0.70 (3)
Condensed units	0.36	0.24 (2)	0.37 (6)	0.38 (5)
p-hydroxy phenyl	0.08	0.04 (1)	0.04 (1)	0.04
Condensed / Uncondensed	0.52	0.53 (4)	0.54 (4)	0.51 (6)
Total phenolic	1.05	0.68 (4)	1.05 (5)	1.13 (5)
Total phenolic by periodate oxidation[4]	–	0.77	0.90	0.99

1. Determined by phosphorus NMR method of Granata & Argyropoulos (1995)
2. From Smit et al. (1997)
3. Numbers in parenthesis indicate the variability of last significant figure determined from the standard deviation of all readings in Appendix 2
4. β-5, 4-O-5, and 5-5 phenolic structures
5. Determined using method of Lai et al. (1990)

The similarities between MWL, CELF2, and CEL2 fractions suggests that ball milling is a crucial step in the isolation of lignin leading to increased phenolic groups and reduced molecular weight allowing dissolution of the lignin. The results also suggest that for MWL, the solvent is preferentially removing the lignin that has undergone a greater degree of mechanical modification.

The CELF1 fraction has considerably fewer phenolic hydroxyl groups than the other lignins. This fraction may, therefore, consist of a less modified lignin, which has been made accessible to the extracting solvent by removal of the carbohydrates. The lignin remaining insoluble after this first extraction requires further milling to break it up into smaller soluble fragments. Therefore, the CELF2 fraction may include the lignin that was protected from the first milling treatment by the carbohydrates, whereas CELF1 includes the lignin that was more exposed. A fraction of CELF1 can be removed as MWL before an enzyme treatment and an additional less modified amount becomes accessible after enzyme treatment. The CEL2 fraction, on the other hand, contains both CELF1 and CELF2 lignin fractions, however, the more exposed CELF1 lignin can be expected to be further modified by the second milling treatment. This explains the larger phenolic hydroxyl content of CEL2. Additionally, these results suggest the duration of the second milling treatment could be reduced. That is, if the lignin remaining insoluble after the first extraction is modified by ball milling to levels similar to CELF1 it may be possible to dissolve it.

The carboxylic acid content of the lignins show a similar trend to the phenolic content; that is, CELF2 and CEL2 which have been ball milled longer, have higher levels of carboxylic acids. The level of carboxylic acids in MWL are similar to CELF2 and CEL2 fractions; consistent with the idea that for MWL the solvent is removing the lignin which has been modified by a greater extent. The greater amount *p*-hydroxy phenyl units in the MWL is consistent with its lower methoxyl content. However, the amount of *p*-hydroxy phenyl units does not fully compensate for the lower methoxyl content and suggests that demethylation is a more important cause for the decrease in methoxyl groups.

4.3.4 Thioacidolysis

Thioacidolysis cleaves β-aryl ether linkages in lignin by reaction with ethanethiol and BF_3-etherate. The yield of the resultant trithioether product (1, Figure 4.8) can be used to estimate the levels of uncondensed β-O-4 aryl ether linkages in lignin. The monomer yields for the guaiacyl units (G) and *p*-hydroxy phenyl units (H) from the CEL lignins are shown in Table (4.21), along with the yields from Wiley milled wood and corresponding MWL (Adams & Ede 1997) for comparison.

Table (4.21). β-O-4 aryl ether content of CELs and MWL isolated from *Pinus radiata* wood as determined by thioacidolysis-GC/FID

Lignin Sample	**β-O-4 (G)**[1]		**β-O-4 (H)**[2]	
	μmol/g lignin	Error[3]	μmol/g lignin	Error[3]
Wood	1070	70	11	2
MWL[4]	930	–	–	–
CELF1	1120	70	16	2
CELF2	980	30	12	1
CEL2	940	40	13	1

1. Guaiacyl units
2. *p*-hydroxy phenyl units
3. Error in β-O-4 content determined from standard deviation of all readings shown in Appendix 2
4. From Adams & Ede (1997)

The β-O-4 monomer yields are consistent with the changes in total phenolic hydroxyl groups discussed above. The uncondensed β-O-4 content of MWL is comparable to CELF2 and CEL2 as was the content of phenolic hydroxyl groups, suggesting these lignins have been modified to the same extent. The CELF1 fraction has slightly more β-O-4 linkages than the other lignin fractions, which is consistent with its lower phenolic hydroxyl content. These results provide evidence to support the idea that ball milling fragments the lignin by cleaving β-aryl ether linkages. The monomer yields for CELF1 are also remarkably similar to the wood sample.

Analysis of the monomer degradation products by GC/MS allows some of the end groups in lignin to be characterised. To simplify the method, the response factor for compound (1) was used to quantify the amounts of all the degradation products. This means that the quantification is not totally accurate, however, comparison of the same degradation product between samples is possible because all the samples were analysed in same way. The results are summarised in Table (4.22) and the raw data can be found in Appendix 2.

Table (4.22). Levels of thioacidolysis monomeric degradation products of the CELs isolated from *Pinus radiata* wood as determined by GC/MS

Monomer product[1]	Wood[2]		CELF1[2]		CELF2[2]		CEL2[2]	
	μmol/g lignin	Error[3]	μmol/g lignin	Error[3]	μmol/g lignin	Error[3]	μmol/g lignin	Error[3]
Vanillic acid (6)	16	3	4	1	5	1	7	3
Vanillin (5)	32	13	53	10	69	9	75	9
Dihydroconiferyl alcohol (7)	64	12	37	4	25	4	24	5
Coniferaldehyde (10)	20	8	24	8	42	9	38	5
Coniferyl alcohol (8,9)	52	3	26	7	14	4	13	3
β-O-4 (G) with Cα =O (2)	Trace[3]	–	7	1	11	2	11	2
β-O-4 (H) (3)	16	4	16	7	14	9	11	5
β-O-4 (G) (1, 11, 12)	1250	100	1200	130	1000	100	990	100
β-O-4 (C) (4)	Trace[3]	–	11	5	13	4	13	4
Stilbene (13)	23	8	41	10	34	8	28	8
Total	1473	–	1419	–	1227	–	1211	–

1. Number in parentheses refers to degradation product numbers in Table (4.18)
2. Error in monomer yields determined from standard deviation of all readings shown in Appendix 2
3. Trace = 0–1μmol

The products corresponding to enol ether structures (11 and 12, Table 4.18) were observed in the product mixtures, however, Rolando et al. (1992) reported that approximately 10% of the uncondensed β-O-4 structures give rise to these structures during thioacidolysis. Therefore, since the levels of these products did not exceed 10% of the β-O-4 monomer (1) it was assumed they arose from the degradation of uncondensed β-O-4 structures and were added to the β-O-4 monomer yield to give the tabulated values for uncondensed β-O-4 linkages in Table (4.22). As expected, the same trend in the levels of uncondensed β-O-4 linkages observed with the GC/FID analysis is seen with the GC/MS analysis. That is, the reduced levels of uncondensed β-O-4 linkages in the samples that have been ball milled longer (CELF2 and CEL2).

The results indicate that ball milling also damages the side chain. This is apparent from the increase in vanillin and decrease in dihydroconiferyl alcohol end groups with the extent of milling (ie wood → CELF1 → CELF2 & CEL2). Additionally, the trend of increased coniferaldehyde end groups at the expense of the coniferyl alcohol end group suggests the isolation procedure is slightly oxidative. This can be expected since the lignin isolation was carried out in the presence of oxygen.

The isolated lignins also have slightly more catechol structures. These probably arise from demethylation during ball milling. If it assumed that the ratio between yields of thioacidolysis products β-O-4 (G) and the sum of yields of β-O-4 (H) and β-O-4 (C) represents the ratio of G units to non-methylated units in the whole lignin, then the thioacidolysis results suggest a methoxyl content (per phenylpropane unit) of 0.97–0.98 (compared to 0.93 for elemental analysis). Additionally, the results suggest that approximately 1% of phenylpropane units are H units. Therefore, it could be concluded that 6% of the phenylpropane units are demethylated to catechol structures by milling and that 80–90% of these catechols are oxidised further to quinone type structures at some point during the isolation. This is plausible since catechol structures are very reactive under oxidative conditions (Johansson & Ljunggren 1994).

Apart from these obvious differences no other significant differences in the monomer yields between the three CEL lignins are apparent. These results again show the similarities between the isolated CEL lignins.

4.3.5 NMR

The CEL lignin samples were acetylated before analysis to improve the solubility of the lignin and the spectral dispersion in the proton dimension. ^{1}H, ^{13}C, TOCSY, and HMQC spectra were recorded for all three CEL lignins. Each spectrum contained similar information concerning the structural elements present but the HMQC spectra contained the most concerning the side chain structures; these are included for comparison. The HMQC experiments were recorded using the same parameters for each sample and the spectra are standardised to the methoxyl signal. This allows for a more reliable comparison between samples. The HMQC spectrum for MWL and CELF1 are shown in Figures (4.10) and those for CELF2 and CEL2 in Figure (4.11).

The correlations relating to some of the important structures are labelled. The spectra show the presence of β-O-4, β-β, and β-1 type structures consistent for softwood lignins (Kilpelainen et al. 1994, Ede & Brunow 1992). The signals centred around δ4.7–85ppm and δ4.2–82ppm match well with the Cα–Hα and Cβ–Hβ correlations, respectively, of the newly discovered dibenzodioxocin structure (Karhunen et al. 1995).

The most striking observation is the remarkable similarity between each sample. This indicates all the CEL lignin fractions and MWL are structurally similar, in terms of side chain structures, which is consistent with the other results discussed above. The NMR signals for MWL are all sharper than the CEL lignins suggesting that MWL has a lower molecular weight than the CELs. This was also apparent in both the ^{1}H and ^{13}C-NMR spectra and is consistent with the findings of Chang et al. (1975).

The only other significant difference between MWL and the CEL lignins is the absence of carbohydrate signals in the MWL spectra. This is expected since MWL does not generally contain significant quantities of carbohydrate.

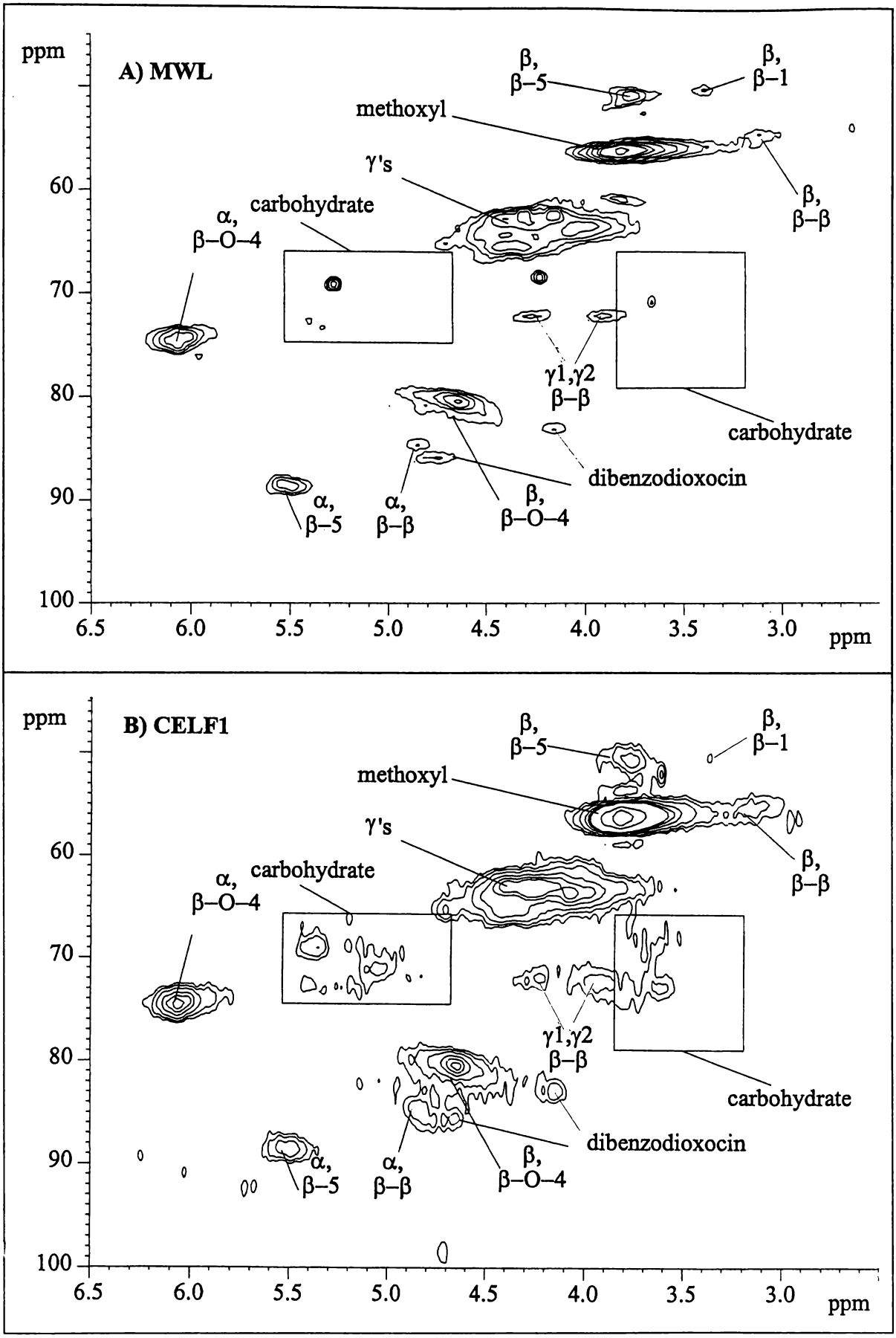

Figure (4.10). Expansion of the side chain region of HMQC spectra of acetylated MWL (A) and acetylated CELF1 (B); some important $^{1}H-^{13}C$ correlations are labelled; the α, β, γ referring to the three side chain carbons of the particular structures outlined in Figure (4.9)

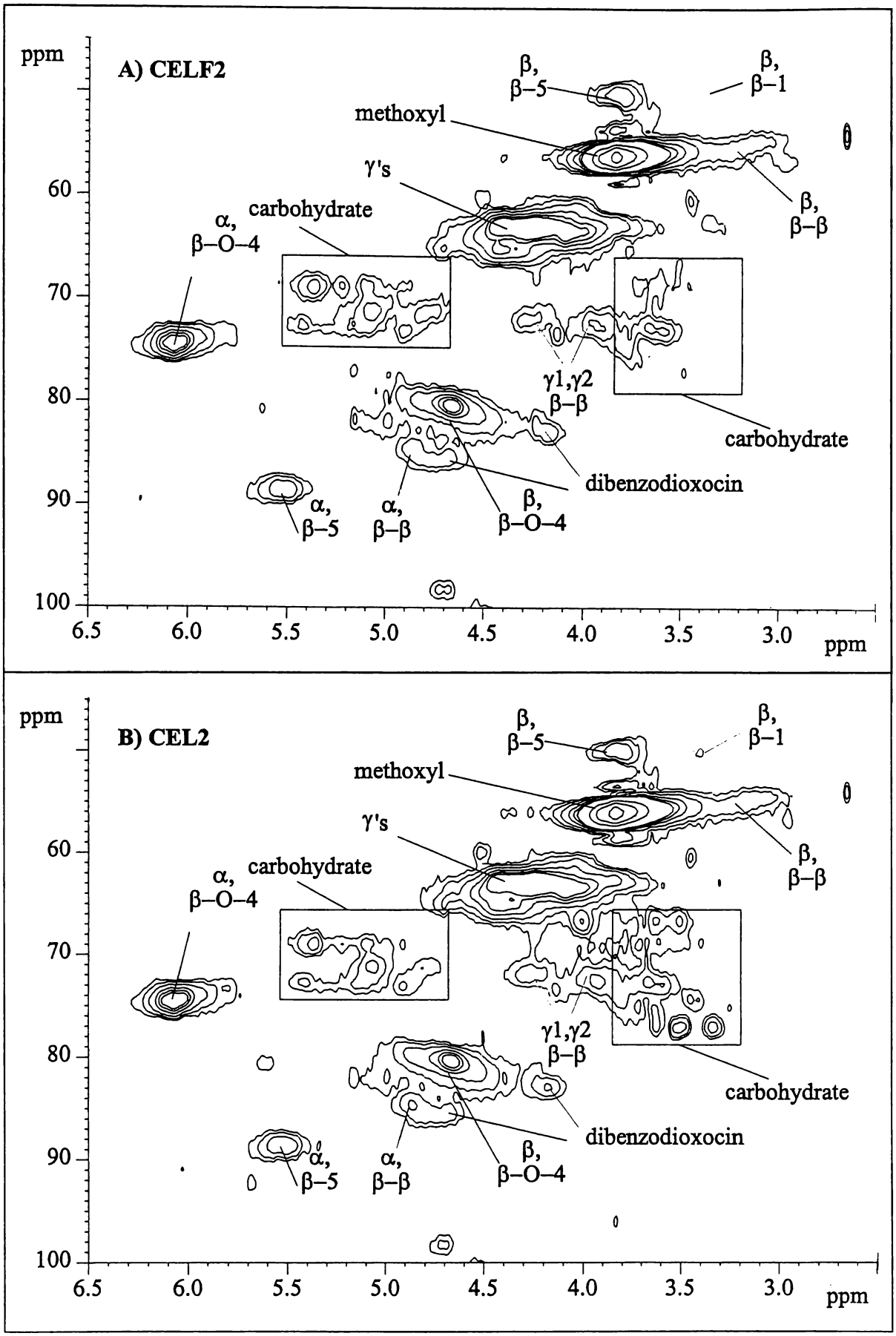

Figure (4.11). Expansion of the side chain region of HMQC spectra of acetylated CELF2 (A) and acetylated CEL2 (B); some important $^{1}H-^{13}C$ correlations are labelled; the α, β, γ labels refer to the three side chain carbons of the particular structures outlined in Figure (4.9)

4.3.6 Summary

The results discussed in the above section demonstrate the similarities between the CEL lignins and MWL. The CELF1 fraction is the least modified of the CEL lignins having the lowest phenolic hydroxyl content and highest uncondensed β-O-4 content. The CELF2 and CEL2 lignins are remarkably similar to MWL; the main difference being the larger hydrogen content of MWL. This shows that MWL, as far as a soluble lignin is concerned, is a reasonable representation of native lignin.

The similarities between the different lignin fractions also suggest that a certain level of modification is necessary for lignin isolation. A reasonable surmise is that lignin, in its native state, is not soluble due to a high molecular weight and that ball milling is crucial to fragment the lignin and allow it to dissolve. This is probably achieved by cleavage of β-aryl ether linkages and possibly involves radical processes (Lee & Sumimoto 1990, Wu & Sumimoto 1992). The concomitant increase in phenolic hydroxyl groups will also aid the dissolution.

The major limitation of both MWL and CEL methods is that the isolated lignins have been exposed to considerable mechanical treatment, resulting in heightened levels of oxidised groups, ether-cleavage products, and side chain damage. However, there is possibly a minimum level of modification required for lignin isolation.

4.4 Dioxane Acidolysis Lignin from Wood

In Chapter 6 the isolation and characterisation of acidolysis lignin from kraft pulps is discussed. Comparing the structure of acidolysis lignin from the pulps with CEL wood lignins to investigate changes in lignin structure is not totally satisfactory since differences in lignin structure may arise as a result of the different isolation procedures. Therefore, an investigation into the effects of the acidolysis treatment on the native lignin structure was carried out. Acidolysis lignin was isolated from *Pinus radiata* wood under the same conditions used for the pulps (Section 6.4). The isolated lignins were structurally characterised and compared to the enzyme lignins discussed earlier.

The acidolysis was carried out following the method of Gellerstedt et al. (1994). *Wiley acid lignin* (WAL) was isolated from Wiley milled wood using this method; that is, Wiley milled wood was refluxed in 4:1 dioxane/aqueous HCl (0.1M) for 2 hours and the acidolysis lignin was recovered from solution by removal of the dioxane. To minimise the acid-catalysed changes to lignin, which may occur during isolation, Jiang & Argyropoulos (1997) used a modification of this method to isolate acidolysis lignin from pulps. The modification involved a neutralisation step of the acidic dioxane solution prior to removal of the dioxane. *Wiley/neutral acid lignin* (WNAL) was isolated following this modified method; thus, Wiley milled wood was refluxed in acidic aqueous-dioxane for 2 hours in the same manner as for the WAL extraction. However, before the dioxane was removed the acid solution was neutralised using saturated $NaHCO_3$. *Ball milled wood acid lignin* (BMWAL) was isolated from ball milled wood following the same procedure as WAL.

To further investigate the effects of the acid treatment on lignin structure CELF2 was acid treated using similar conditions. CELF2 was refluxed for 1.5hr in acidic aqueous-dioxane solution and CELF2A was recovered in the same manner as WAL.

4.4.1 Lignin yield and composition

As can be seen from Table (4.23), only very small yields of lignin were isolated from wood by acidolysis under the conditions used. The neutralisation step did not significantly affect the yield (WNAL), nor did ball milling the wood prior to acidolysis (BMWAL). Higher yields of acidolysis lignin have been isolated from other softwood species (Pepper et al. 1959, Curvelo et al. 1993, Solar & Kacik 1995), using longer cooking times or higher acid concentrations. Under these more severe conditions it can be expected that the isolated lignin will be more highly modified. The yields of acidolysis lignin are similar to typical yields of MWL from *Pinus radiata* wood indicating a similar lignin fraction is isolated by both methods.

Approximately 30% of CELF2 was not recovered from solution after acidolysis treatment. This could suggest the lignin is undergoing a certain amount of acid-catalysed degradation leading to the generation of low molecular weight fragments which do not precipitate in the work-up stages.

Table (4.23) Yields and analytical composition of acidolysis lignins isolated from *Pinus radiata* wood

	MWL	CEL F2	CELF2A[1]	WAL[2]	WNAL[3]	BMWAL[4]
Yield[5]	4–8	–	68	6.5	6.2	10
Composition						
Carbon	62.5	55.3	57.7	60.1	58.2	60.2
Hydrogen	6.12	5.89	5.35	6.00	5.86	5.89
Oxygen	31.4	38.6	36.6	33.9	35.9	33.9
Methoxyl	14.12	12.68	13.13	14.71	14.09	13.74
Nitrogen	nil	0.26	0.35	nil	nil	nil
Moisture	–	9.3	7.0	4.31	5.84	4.39
Carbohydrates	–	5.4	2.6	–	–	–
Formula[6]						
Carbon	9	9	9	9	9	9
Hydrogen	9.0	7.2	6.4	7.9	8.1	8.0
Oxygen	2.9	2.9	2.9	3.0	2.8	2.8
Methoxyl	0.87	0.93	0.91	0.93	0.94	0.87

1. CELF2A: acidolysis lignin isolated from CELF2 lignin
2. WAL: acidolysis isolated from Wiley milled wood
3. WNAL: acidolysis lignin isolated from Wiley milled wood with an additional neutralisation step
4. BMWAL: acidolysis lignin isolated from ball milled wood
5. Wood: percentage of lignin content of wood; CELF2A: percentage of initial weight of CELF2
6. Formulae are corrected for protein content and moisture, as well as for carbohydrates, where appropriate (CELF2, CELF2A).

BMWAL, which was isolated from ball milled wood, has a slightly lower methoxyl content than the other two acidolysis lignins and is comparable to MWL. This result is a another indication that ball milling is causing the reduction in methoxyl content rather than the isolation of *p*-hydroxy phenyl rich lignin, as suggested earlier, since all the acidolysis lignins should originate from the same fraction of protolignin. The only other significant difference in the lignin composition is the hydrogen content. The slightly lower hydrogen content of BMWAL compared to MWL may suggest the acidolysis lignin is more condensed since the acidolysis treatment is known to cause a certain amount of condensation (Solar & Kacik 1995). This is also consistent with the lower hydrogen content of CELF2A compared to CELF2. Other than these differences the acidolysis lignins are similar in composition to MWL and CELF2.

The acid treatment of CELF2 did not significantly affect the protein content and only halved the carbohydrate content. This suggests the protein and part of the residual carbohydrate are connected to the lignin by acid stable bonds. Kosikova et al. (1979) showed that during acidolysis with 0.1M HCl at 75°C, benzyl ether carbohydrate linkages in etherified phenylpropane model compounds are more stable than those in non-etherified model compounds. Therefore, the carbohydrates may be linked to the CEL lignin via an α-aryl ether bond and the residual carbohydrates in CELF2A are those that remain linked to etherified lignin units.

The nature of the interaction between the lignin and protein remains unclear but this result suggests the interaction is reasonably strong and perhaps involves covalent bonding. This is not consistent with results discussed in Section (4.2.2.2) or the results of Hortling et al. (1992), concerning the interaction of cellulase enzymes with isolated lignin samples. These suggest the interaction between lignin and protein is physical adsorption rather than covalent attachment, however, it is possible the interaction between enzyme and isolated lignin samples is different from the interaction with lignin embedded in the cell wall and could be strongly influenced by the presence of carbohydrates. One possibility is that the interaction only occurs when the enzyme is actively degrading the carbohydrates.

4.4.2 Phenolic hydroxyl groups

The distribution of hydroxyl groups in the acidolysis wood lignins and CELF2A was determined by ^{31}P-NMR analysis using the method of Granata & Argyropoulos (1995). The results are tabulated in Table (4.24) along with the results for MWL (Smit et al. 1997) and CELF2 for comparison.

Comparison between the acid treated and untreated CELF2 lignins shows the acid treatment generates new phenolic OH groups which is presumably through the cleavage of aryl ether linkages in the lignin. The acid treatment did not significantly alter the ratio of condensed to uncondensed units in CELF2 suggesting the acid treatment did not cause an increase in condensed structures. However, this method only detects C5-condensed structures and condensation at C6 is considered to be more important during acidolysis (Gellerstedt et al. 1994).

Table (4.24). Distribution of hydroxyl groups (mmol/g lignin) in acidolysis lignins isolated from *Pinus radiata* wood

	Aliphatic	**Phenolic**					**COOH**
		Condensed[1]	Guaiacyl	*p*-Hydroxy phenyl	Total	**Cond. / Uncond.**	
MWL	3.61	0.36	0.61	0.08	1.05	0.52	0.11
CELF2[2]	5.1 (3)	0.32 (6)	0.57 (7)	0.04 (1)	1.06 (5)	0.54 (4)	0.15 (3)
CELF2A[2, 3]	4.97	0.45 (3)	0.77 (6)	0.04 (1)	1.23 (3)	0.56 (2)	0.10 (1)
WAL[4]	4.51	0.33	1.02	0.08	1.42	0.30	0.07
WNAL[5]	4.50	0.27	0.93	0.05	1.25	0.27	0.03
BMWAL[6]	4.6 (1)	0.35 (3)	0.87 (2)	0.07 (1)	1.29 (5)	0.37	0.07 (1)

1. Condensed phenolic structures (β-5, 4-O-5, 5-5)
2. Number in parentheses indicates the variation of the last significant figure as determined from the standard deviation of all readings shown in Appendix 2
3. CELF2A: acidolysis lignin isolated from CELF2 lignin
4. WAL: acidolysis isolated from Wiley milled wood
5. WNAL: acidolysis lignin isolated from Wiley milled wood with an additional neutralisation step
6. BMWAL: acidolysis lignin isolated from ball milled wood

All three lignins isolated from wood by acidolysis have a considerably higher total phenolic hydroxyl content compared to MWL. Cleavage of aryl ether linkages in lignin is necessary to allow dissolution of the lignin during both isolation procedures. However, the higher phenolic content of the acidolysis lignins compared to MWL suggests a lower molecular weight lignin is being released by acidolysis under the conditions used.

One surprising observation is that the higher total phenolic content in the acidolysis wood lignins is mainly due to a larger guaiacyl content without any significant change in the levels of condensed structures. This indicates the acidolysis treatment is generating a low molecular weight highly uncondensed type of lignin and that aryl ether linkages in uncondensed lignin units are preferentially cleaved during acidolysis. The acidolysis lignin isolated from ball milled wood also has a relatively high guaiacyl content. This supports the hypothesis that uncondensed units are preferentially cleaved since an acidolysis lignin more like MWL would be expected from ball milled wood. However, compared to CELF2, the CELF2A lignin does not show this trend which suggests that either the accessibility of the lignin or the presence of carbohydrates has an influence on the behaviour of lignin during acidolysis.

Comparison between WAL and WNAL shows that during recovery of the lignin from an acid solution (WAL) further acid-catalysed modification takes place in the lignin. That is, WAL has slightly more condensed structures relative to uncondensed and a higher phenolic OH content compared to WNAL.

4.4.3 Thioacidolysis

Thioacidolysis was used to determine the levels of uncondensed β-O-4 linkages in the acidolysis lignins and the results are summarised in Table (4.25). There is no significant difference in the uncondensed β-O-4 content between samples, except for the slightly lower amounts in the samples that have been exposed to ball milling (MWL, CEL2, CELF2, and BMWAL).

Table (4.25). β-O-4 aryl ether content of acidolysis lignins isolated from *Pinus radiata* wood as determined by thioacidolysis-GC/FID

Sample	Uncondensed β-O-4 aryl ether linkages (μmol/g lignin)
Wood	1070
MWL[1]	930
CELF2	980
CELF2A	950
WAL	1020
WNAL	1060
BMWAL	940

1. From Adams & Ede (1997)

The monomeric degradation products were also analysed by GC/MS and the results are summarised in Table (4.26).

Table (4.26). Yields (μmol/g lignin) of thioacidolysis monomer degradation products from acidolysis lignins isolated from *Pinus radiata* wood as determined by GC/MS

Monomer product[1]	Wood	WAL[2]	WNAL[3]	BMWAL[4]	CELF2	CELF2A[5]
Vanillic acid (6)	16	4	4	Trace[6]	5	5
Vanillin (5)	32	33	33	29	69	51
Dihydroconiferyl alc (7)	64	31	28	19	25	25
Coniferaldehyde (10)	20	26	37	32	42	30
Coniferyl alcohol (8,9)	52	13	16	16	14	11
β-O-4 (G) with Cα =O (2)	Trace[6]	4	4	2	11	10
β-O-4 (H) (3)	16	22	20	13	14	11
β-O-4 (G) (1, 11, 12)	1250	1040	1030	960	1000	1060
β-O-4 (C) (4)	Trace[6]	11	5	3	13	11
Stilbene (13)	23	64	42	55	34	17
Total	1473	1248	1219	1129	1227	1240

1. Number in parentheses refers to degradation product numbers in Table (4.18)
2. WAL: acidolysis isolated from Wiley milled wood
3. WNAL: acidolysis lignin isolated from Wiley milled wood with an additional neutralisation step
4. BMWAL: acidolysis lignin isolated from ball milled wood
5. CELF2A: acidolysis lignin isolated from CELF2 lignin
6. Trace = 0–1μmol/g lignin

The distribution of monomeric thioacidolysis products from all three acidolysis wood lignins is very similar indicating that in terms of these types of structures, the ball milling step or the neutralisation step did cause (or prevent) any additional modification to the lignin. The distribution of products from these lignins is also similar to the wood except for two minor differences. The first is the lower levels of dihydroconiferyl alcohol and coniferyl alcohol end groups in the acid lignins suggesting these types of structures are degraded under the acid conditions during isolation. However, an equivalent decrease in these structures in CELF2 after the acid treatment is not observed. The second difference is the increase in the stilbene type degradation product (arising from the β-1 structures illustrated in Figure 4.12) and the slight increase in coniferaldehyde degradation products. This result suggests either a lignin rich in these types of structures is preferentially isolated or that these structures are formed during the isolation.

The differences between CELF2 and CELF2A are not as significant as the differences between the other acidolysis lignins and wood, which perhaps suggests the observed changes result from preferentially isolating a certain type of lignin rather than from acid-catalysed modification during isolation. However, it is likely that both factors are involved to some extent.

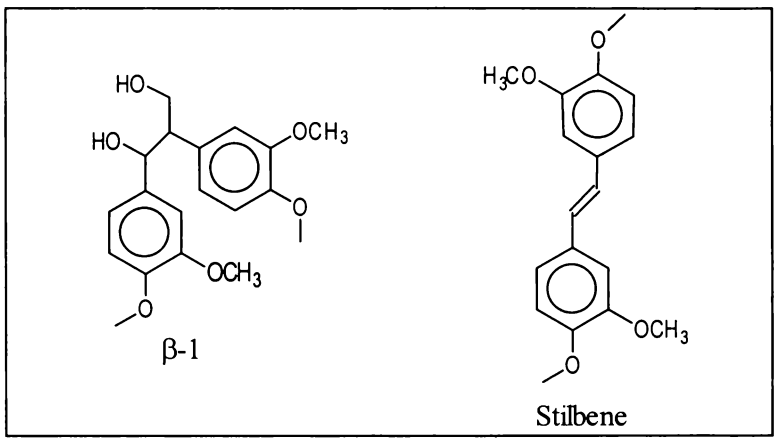

Figure (4.12). Lignin structures which degrade to product (13) during thioacidolysis

4.4.4 NMR

The HMQC spectra of acetylated CELF2A and BMWAL are shown in Figure (4.13). Comparing CELF2A (Figure 4.13A) with CELF2 (Figure 4.11A) no significant changes are apparent except for the reduction of carbohydrate signals in CELF2A. This is consistent with the results discussed above, which showed that the acidolysis treatment did not significantly alter the structure of CELF2 except for a slight increase in phenolic hydroxyl groups. The BMWAL (Figure 4.13B) and MWL (Figure 4.10A) spectra are also very similar in terms of both the side chain structures that are present and in the size of the individual signals. This indicates that both procedures (MWL and acidolysis) are isolating a similar lignin fraction in terms of the distribution of side chain structures and molecular weight. The BMWAL spectrum also indicates the acidolysis lignin is not contaminated with carbohydrates to a significant extent.

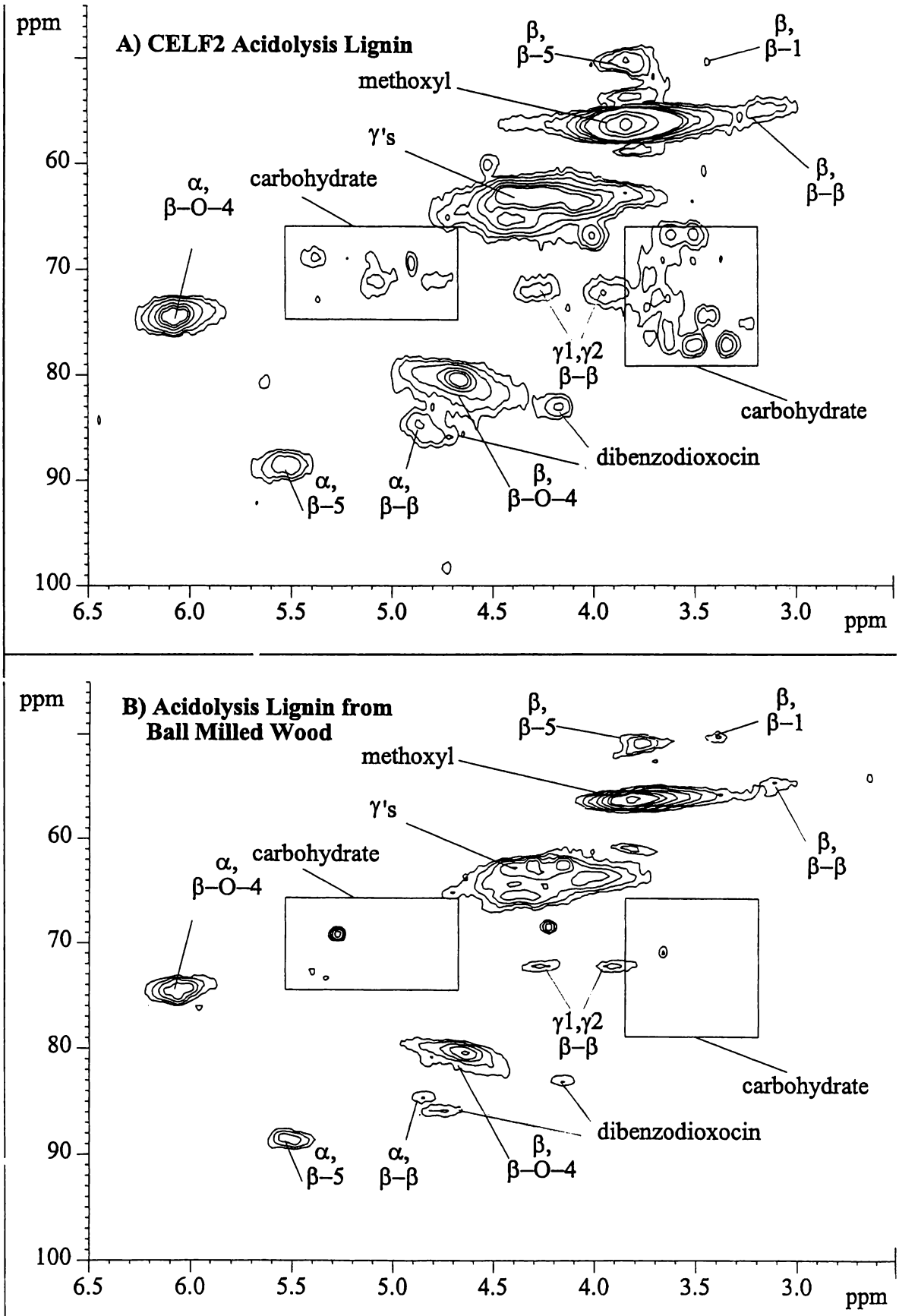

Figure (4.13). Expansion of side chain region of HMQC spectra of acetylated CELF2A (A) and acetylated BMWAL (B); some important $^{1}H-^{13}C$ correlations are labelled; the α, β, γ referring to the three side chain carbons of the particular structures outlined in Figure (4.9)

4.4.5 Summary

Under mild conditions only low yields of lignin could be isolated from wood by acidolysis. The lignin that is released has a significantly higher uncondensed phenolic content compared to MWL suggesting that a low molecular weight, uncondensed type of lignin is being generated by acidolysis. However, the NMR results seem to suggest that MWL and the acidolysis lignin are quite similar. The thioacidolysis results show that side chain modification occurs during the isolation and stilbene (or β-1) type structures increase.

The trends observed between the wood and acidolysis lignins, in terms of both the phosphorus results and the variation in thioacidolysis products, were not observed between CELF2 and CELF2A lignins. This suggests that either the accessibility of the lignin or the presence of carbohydrates has an influence on the behaviour of the lignin during acidolysis.

4.5 Concluding Remarks

In this chapter the development of a new cellulolytic enzyme method for the isolation of lignin from *Pinus radiata* wood was described. This method has several advantages over previously developed methods. That is, the yield of lignin is high (ca. 75%); the lignin has a reasonably low carbohydrate content (5–8%); the protein content is below 2%; and the lignins are reasonably soluble in the common lignin solvents (although over time, the solubility tends to decrease).

The CEL lignins are remarkably similar to MWL in terms of the total phenolic hydroxyl content, uncondensed β-O-4 ether content, and the distribution of interunit linkages. This shows that MWL, as far as a soluble lignin is concerned, is a reasonable representation of native lignin. However, this CEL method is superior to the MWL method in that significantly higher yields of lignin can be obtained, thus it is easier to extract reasonable quantities of lignin from wood using the developed CEL method as compared to the standard MWL method.

The major limitation of both MWL and CEL methods is that the isolated lignins have been exposed to considerable mechanical treatment, leading to increased levels of oxidation, ether-cleavage products, and side chain damage. However, there is probably a minimum level of modification necessary to dissolve the lignin.

In comparison, dioxane acidolysis under mild conditions is not effective enough in disrupting the cell wall matrix to allow the release of significant amounts of lignin. The lignin that is released is probably the most exposed and consists of low molecular weight and highly uncondensed fragments.

Chapter Five
CELLULOLYTIC ENZYME ISOLATION OF RESIDUAL LIGNIN FROM KRAFT PULPS

5.1 Introduction

Determination of the structure of residual lignin is important in the development of new pulping and bleaching methods, the aim of which is to produce fully bleached pulps without the use of chlorine-based chemicals. A better understanding of the structure of residual lignin remaining after kraft pulping and bleaching is vital to the understanding of the reactions taking place during these processes.

Residual lignin in kraft pulps has been subject to many investigations over the last several years. These studies have been mainly based on three different approaches (and these are reviewed in Section 2.4.3). The first approach is the analysis of *in situ* residual lignin by chemical degradation techniques. The main difficulty with the use of these is the low concentration of residual lignin in the pulps, particularly in pulps bleached to low kappa number.

The other two methods involve the characterisation of isolated fractions of residual lignin. The isolation of residual lignin has mainly been achieved by acid hydrolysis or by enzyme degradation of the carbohydrates and subsequent dissolution of the lignin into a lignin selective solvent. Both of these approaches have limitations. The residual lignin isolated by acidolysis is modified to a certain extent. Although the exact nature of the changes is not completely known, increases in the phenolic content and amount of condensed structures do occur.

Although, the enzyme method has a greater potential to allow the isolation of a lignin that is unmodified, the isolated lignin is highly contaminated with protein from the enzyme. The drawback to this contamination is that it may interfere with the characterisation of lignin structure and it also appears to render the lignin rather insoluble in subsequent purification steps. A number of studies have been carried out with the aim of reducing the protein contamination (Johansson 1997, Hortling et al. 1992, Tamminen et al. 1994). The work discussed in this chapter has the same aim. Modifications of the improved CEL method developed in the previous chapter were investigated as a means to isolate residual lignin from kraft pulps with less contamination and improved yield.

The work discussed in this chapter has been divided into three distinct methods. The first method follows the improved CEL isolation procedure except that the NaOH/HCl treatments were omitted and steps necessary to recover lignin from the buffer were added. The effects of incubation time and purification method on yield and purity of the lignin were investigated. In the second method the effect of ball milling the pulp after the enzyme treatment was investigated. This was performed to allow a larger volume of pulp to be extracted and hopefully allow higher yields to be gained. The third method was used to ascertain whether the initial ball milling step was necessary and whether ball milling the residue after extraction could improve the lignin yield.

These methods are then compared to the existing enzyme methods (Hortling et al. 1992, Lachenal et al. 1995, Johansson 1997), in terms of yield and protein content of the isolated lignins. Additionally, an enzyme treatment was investigated as a means of enriching the lignin within a pulp, to enable the analysis of *in situ* residual lignin by degradation studies.

5.2 Commercial Pulps

Several different commercial *Pinus radiata* kraft pulps were used throughout this work (Chapters 5 & 6). These are shown in Table (5.1) along with the experiments for which they were used.

Table (5.1). Properties of commercial *Pinus radiata* pulps used in this study

Pulp[1]	Kappa number	Lignin content[2]	Experiment[3]
Kraft pulps			
KP1	26.2	3.93	Enzyme isolation (5.3.1)
KP2	30.0	4.50	Enzyme digest trials (5.4)
KP3	28.0	4.20	Enzyme digest trials (5.4), Acidolysis lignin (5)
KP4	22.5	3.37	Acidolysis lignin (5)
Kraft-oxygen pulps			
KOP1	18.0	2.70	Enzyme isolation (5.3.1)
KOP2	18.4	2.76	Enzyme isolation (5.3.2, 5.3.3), Enzyme digest trials (5.4), Bleaching experiments (A1), Acidolysis lignin (6)
Kraft-oxygen-oxygen-chlorine dioxide pulp			
KOOD	2.3	0.35	Enzyme isolation (5.3.2), Enzyme digest trials (5.4)

1. All pulps were obtained from New Zealand mills
2. Percent of oven dry pulp (estimated from kappa number (kappa × 0.15))
3. Enzyme isolation: Section 5.3; Enzyme digest trials: Section 5.4; Acidolysis lignin: Chapter 6; Bleaching experiments: Appendix 1

5.3 Method Trials

5.3.1 Method 1 – Enzyme treatment post-ball milling

The first method to be investigated followed the scheme outlined in Figure (5.1). Kraft pulp (KP1; kappa 26) and kraft-oxygen pulp (KOP1; kappa 18) samples were freeze dried, Wiley milled, and then ball milled for 3 days. The residue remaining after the subsequent enzyme treatment was recovered and extensively washed with water before aqueous dioxane extraction. The isolated lignin samples were purified using either the DMF procedure or the acetone procedure previously described in Chapter 4. For details of the methodology refer to Section (3.2.1).

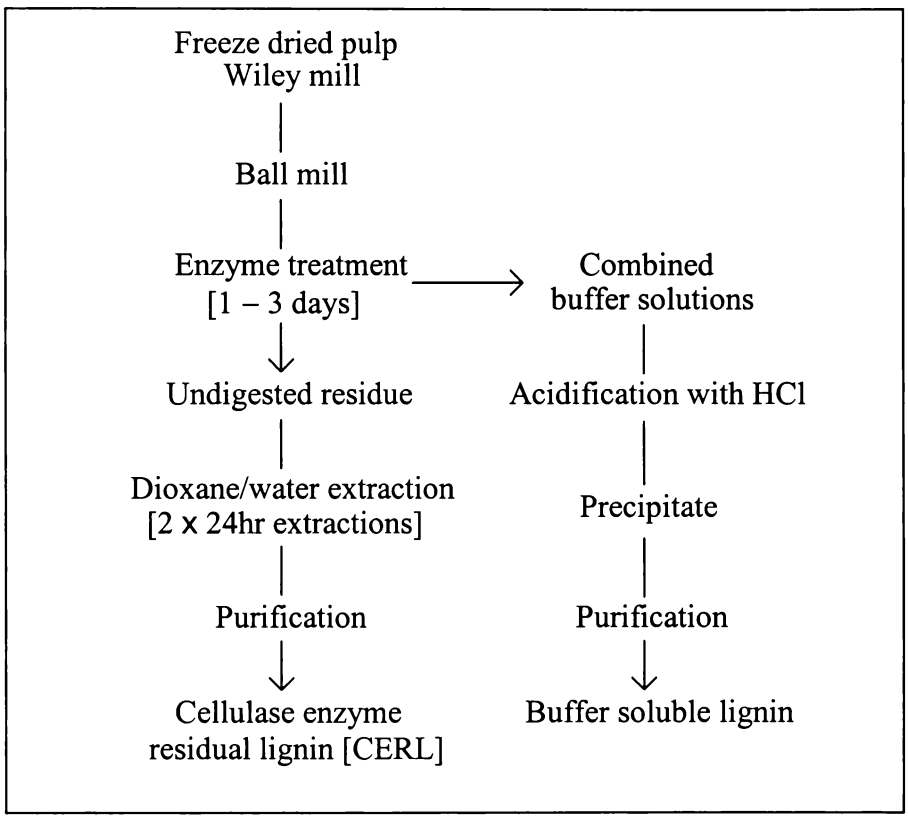

Figure (5.1). Cellulase enzyme isolation of residual lignin; Method 1

Trial 1

The influence of the enzyme incubation period on yield and nitrogen content of the isolated residual lignin was investigated in this trial. KOP1 pulp samples were enzyme treated for 1 or 3 days and CERL lignin was isolated as outlined in Figure (5.1). The isolated lignins were purified using the DMF procedure. The DMF lignins were then further purified using the acetone method. Table (5.2) shows the yield and nitrogen content of the 1 day and 3 day CERLs.

Table (5.2). Yield of residual lignin isolated from 20g of KO pulp (KOP1) using Method 1 after a 3 day or 1 day enzyme treatment

	Yield (%)	N (%)	Protein (%)[1]	OCH_3[2]
KO: 3 day enzyme digest				
Dioxane insoluble residue[3]	4	–	–	–
Crude CERL[4]	84	–	–	–
Purified DMF CERL[4]	45	1.84	12	9.2
Acetone purified DMF CERL[4]	8	1.80	11	–
KO: 1 day enzyme digest				
Dioxane insoluble residue[3]	27	–	–	–
Crude CERL[4]	59	–	–	–
Purified DMF CERL[4]	20	2.20	14	8.9
Acetone purified DMF CERL[4]	8	1.70	11	–

1. Determined from nitrogen content (%N × 6.25)
2. Percent by weight
3. Percentage of initial weight of pulp
4. Percentage of lignin content determined from the kappa number (0.15 × kappa)

A relatively high yield of crude lignin was isolated from the KO pulp after an enzyme treatment of only 24 hours, however, over half of this crude material was not recovered after DMF purification. Both the DMF purified CERL samples contain approximately 10–15% protein (as determined from their nitrogen contents) which shows the enzyme treatment duration did not have a significant effect on protein contamination. This result is not consistent with previous findings (Section 4.2.2.2, Johansson 1997) and may indicate that DMF is contributing to the nitrogen content. As was expected, more lignin was released from the more highly digested pulp, although there is no significant difference in the yields of purified lignin.

The DMF lignins were further purified using the acetone method to further reduce the nitrogen. However, most of the DMF lignin did not precipitate from the alkali solution on acidification (Table 5.2). Also, the nitrogen content of the acetone purified lignin is only slightly lower compared to the DMF purified lignin.

Buffer soluble material was recovered from the 1 day enzyme treatment by precipitation (Figure 5.1). The buffer solutions were combined and acidified to pH 2 with dilute HCl. The precipitate was recovered, extensively washed with water, and then extracted with DMF. The DMF soluble material was recovered by precipitation with ether and washed several times with fresh ether. The air-dried precipitate was then dissolved in 90% aqueous acetone and the soluble and insoluble fractions separated by centrifugation. The insoluble material was washed several times with water and freeze dried. The supernatant was rotary evaporated and the water suspension freeze dried to recover the aqueous acetone soluble lignin. The yields, protein content, and methoxyl content of the fractions recovered from the buffer are shown in Table (5.3).

Table (5.3). Buffer soluble material isolated from 20g of KO pulp (KOP1) after a 24hr enzyme treatment

	Yield (%)[1]	N (%)	Protein (%)[2]	OCH_3[3]
DMF soluble/acetone soluble	5	8.5	53	3.4
DMF soluble/acetone insoluble	11	12.9	81	1.3
DMF insoluble	54	12.6	79	1.3

1. Percentage of lignin content of pulp (determined from kappa number).
2. Determined from nitrogen content (%N × 6.25)
3. Percent by weight

The nitrogen content of the buffer extracts indicates they are mainly made up of protein, particularly the DMF and acetone insoluble fractions. The acetone soluble fraction is less contaminated with nitrogen and has a higher methoxyl content than the other two fractions suggesting it contains a higher proportion of lignin, however, it is still very crude.

Trial 2

In this trial the DMF was totally eliminated from the work-up due to the problems associated with the recovery of lignin from the DMF and the possibility of DMF contamination. A kraft pulp (KP1) sample was treated in the same manner as the KO pulp (Figure 5.1), except that the pulp was digested for 2 days. The crude CERL was purified using the acetone method while the buffer precipitate was extracted with 50% aqueous dioxane and the extracted material was purified using the acetone method. The results are summarised in Table (5.4).

Table (5.4). Yield of residual lignin isolated from 20g of kraft pulp (KP1) using Method 1 after a 48hr enzyme treatment

	Yield (%)	N (%)	Protein (%)[1]
CERL			
Dioxane insoluble residue[2]	5	–	–
Crude CERL[3]	123	–	–
Acetone purified CERL[3]	40	1.5	9
Acetone insoluble residue[3]	33	6.4	40
Buffer soluble material			
Crude lignin[3]	47	–	–
Acetone lignin[3]	1	–	–

1. Determined from the nitrogen content (%N × 6.25)
2. Percentage of initial weight of pulp
3. Percentage of lignin content of pulp (determined from kappa number)

The yield of crude lignin would suggest that all the pulp residual lignin has been extracted into aqueous dioxane, however, only one third of this crude fraction was recovered as acetone purified lignin. The acetone purified CERL has a lower nitrogen content than the acetone insoluble fraction suggesting the aqueous acetone is preferentially extracting lignin with the lowest protein content. The fraction of crude material not recovered after purification may be low molecular weight lignin and/or carbohydrates that do not precipitate from solution.

A large amount of crude material was also extracted from the buffer precipitate into 50% aqueous dioxane but only a very small amount was recovered after purification. It is likely that a large proportion of protein is extracted from the buffer precipitate as seen above (Table 5.3), and therefore, only a very small fraction of the crude lignin is recovered after purification. This result is in accordance with other enzyme extraction methods (Lachenal et al. 1995, Hortling et al. 1992) which report that only a small fraction of unbleached kraft pulp residual lignin is recovered from the buffer.

Comments

CERL was isolated from a kraft pulp in 40% yield using Method 1. However, the purified lignin still contained approximately 10% protein, which was difficult to remove.

A similar yield was obtained from the KO pulp after a 3 day enzyme treatment, however, the purified DMF lignin had a higher nitrogen content than the CERL isolated from the kraft pulp. Reducing the enzyme digestion to 24hr halved the yield of CERL from the KO pulp but did not reduce the nitrogen content of the purified lignin. The high nitrogen content of the DMF lignins indicates a high contamination with either protein or DMF. The dramatic loss of yield of the CERLs from the KO pulp after acetone purification may be associated with DMF contamination.

The main drawback to Method 1 is that the ball milling step limits the amount of pulp that can be treated. Therefore, Method 1 would not be well suited for low kappa number pulps, in which, a large volume of pulp would be needed to obtain a reasonable amount of lignin.

5.3.2 Method 2–Ball milling of enzyme digest residue

Method 2 was designed with lower kappa number pulps in mind. The residual lignin is concentrated within the pulps by a partial enzyme digestion prior to ball milling (Figure 5.2). The first fraction of lignin (CERL2a) is extracted from the ball milled with aqueous dioxane and purified using the acetone method. A second fraction of lignin (CERL2b) is extracted from the dioxane insoluble residue after a NaOH/HCl treatment.

Trial 1

A KOP2 pulp sample was digested for 16 hours and approximately 50% of the initial weight was dissolved. After ball milling, the residue was extracted with 90% aqueous dioxane following Path A (Figure 5.2) and purified using the acetone method. The buffer soluble fraction was recovered in the same manner as for the kraft pulp in Method 1. The yields are shown in Table (5.5).

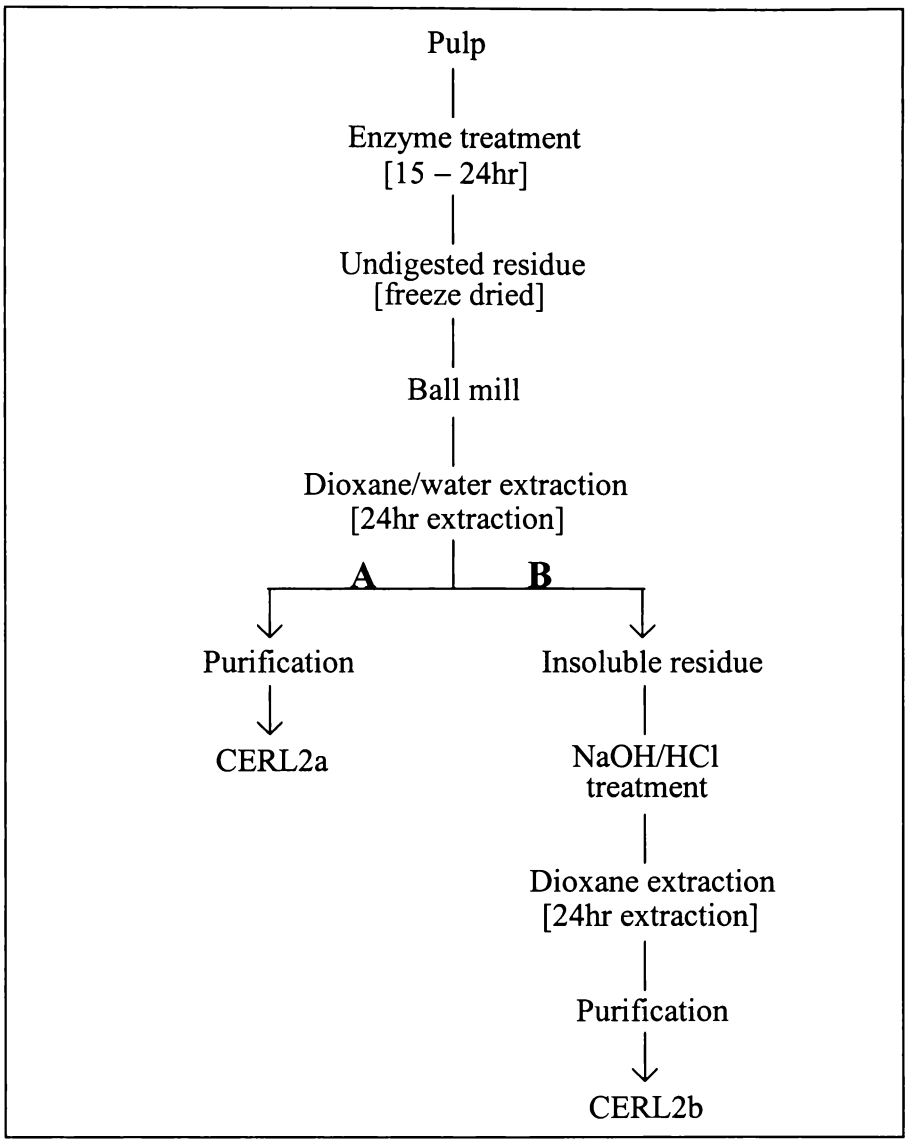

Figure (5.2). Cellulase enzyme isolation of residual lignin; Method 2

Table (5.5). Lignin isolated from 60 g of KO pulp using Method 2 after a 16 hr enzyme treatment

	Yield[1] (%)	**N** (%)	**Protein** (%)[2]	**OCH_3**[3]
Crude lignin	63	–	–	–
Purified CERL2a	13	1.3	8	8.8
Recovered from buffer	8	–	–	–

1. As a percentage of lignin content of pulp (determined from kappa number)
2. Determined from nitrogen content (%N × 6.25)
3. Percent by weight

A large amount of crude material was extracted from the ball milled residue with aqueous dioxane. Ball milling may fragment a proportion of the carbohydrates in the pulp to give aqueous dioxane soluble fragments. However, only about 20% of the crude material was recovered after purification. Compared with the kraft pulp from Method 1 (Table 5.4), a smaller fraction of the crude lignin is recovered after purification from the KO pulp, however, the protein content of both purified lignins are similar. Additionally, the nitrogen content of the purified CERL2a in Table (5.5) is slightly lower compared to the DMF purified CERLs in Table (5.2). This indicates the acetone purification method produces a lignin that is slightly less contaminated with protein as compared to the DMF purification method.

A larger fraction of lignin was recovered from the buffer compared to the kraft pulp (Table 5.5 cf Table 5.4). Hortling et al. (1992), Johansson (1997), and Lachenal et al. (1995) reported similar differences in the behaviour of unbleached and oxygen bleached residual lignin.

Trial 2

Method 2 was tested on a hydrogen peroxide (KOQP1) and a cyanamide activated hydrogen peroxide (KOQPn1) bleached KO pulp, which have lower residual lignin contents than the KOP2 pulp. These two pulps were generated in the laboratory as described in Appendix 1.

The KOQP pulp sample (kappa 13.9) and the KOQPn pulp sample (kappa 9.6) were treated following the scheme outlined in Figure (5.2). The pulps were digested for 22 hr and about 30% of the pulp weight was dissolved during the treatment.

CERL2a was extracted from the ball milled residue into 90% aqueous dioxane following Path A (Figure 5.2). The insoluble fraction was then treated following Path B. That is, the residue was NaOH/HCl treated with 100 mM NaOH and extracted with 90% aqueous dioxane to recover CERL2b. The aim of this modification was to improve the overall yield of isolated lignin. The results are shown in Table (5.6).

Table (5.6). Yields of purified lignin isolated from 100 g of hydrogen peroxide bleached KO pulps using Method 2 after a 24 hr enzyme treatment

	Yield[1] (%)	**N** (%)	**Protein** (%)[2]	**OCH_3**
KOQP				
CERL2a	8	–	–	–
CERL2b	4	–	–	–
Total CERL2	**12**	0.9	6	10.5
Recovered from buffer	6	8.9	55	3.3
KOQPn				
CERL2a	4	–	–	–
CERL2b	2	–	–	–
Total CERL2	**6**	1.2	8	8.3
Recovered from buffer	9	1.6	10	8.0

1. Percentage of lignin content of pulp (determined from kappa number)
2. Determined from nitrogen content (%N × 6.25)

The yield of CERL2a isolated from both bleached pulps is considerably lower than the yield from the KO pulp (Table 5.5). This could be due to the lower degree of digestion that was achieved for these two pulps, as measured by the weight loss during the enzyme treatment (30% for KOQP and KOQPn compared to 50% for KO). A similar trend of reduced yield with lower digestion was observed for the KO pulp in Method 1. The NaOH/HCl treatment of the aqueous dioxane insoluble residue and subsequent extraction did not significantly improve the yield of lignin isolated from these pulps.

The total yield of CELR2 from KOQPn is lower than from KOQP and this may be due to differences in the residual lignin structure. Cyanamide activates the hydrogen peroxide (Smit 1995), and as a result the residual lignin from the KOQPn pulp may be more oxidised and thus more hydrophilic than the residual lignin from the KOQP pulp. Therefore, the residual lignin from the KOQPn pulp is perhaps remaining in solution during the isolation procedure.

A higher proportion of KOQPn residual lignin is recovered from the buffer compared to KOQP residual lignin. This result is also in accordance with the hypothesis that the KOQPn residual lignin is more hydrophilic. Similar behaviour has been observed during enzyme isolation of bleached pulps (Lachenal et al. 1995), where the more highly oxidised pulp residual lignin is recovered from the buffer in greater yields.

The protein contamination of the buffer soluble material from the KOQP pulp is much higher than the KOQPn buffer soluble material. This result is not consistent with the results of Johansson (1997) who found that protein contamination of lignin recovered from the buffer increased with the degree of oxidation. The reasons for this difference are not known.

Trial 3

Method 2 was also tried on a very low kappa number pulp. A KOOD pulp sample (kappa 2.3) was digested for 24hr. The pulp was digested from 240g down to 95g, thus approximately 60% of the original weight was solubilised. The size of the ball mill used limited the amount of residue that could be milled, thus 50g of the digest residue was ball milled and extracted with 90% aqueous dioxane (Figure 5.2, Path A). The results are summarised in Table (5.7).

Table (5.7). Residual lignin extracted from KOOD pulp using Method 2 after a 24hr enzyme treatment

	Yield (%)
CERL2a from 50g residue	
Crude lignin[1]	375
Purified CERL2a[1]	10
Acetone insoluble residue[2]	8
From 240g pulp	
Recovered from buffer[1]	4

1. Percentage of lignin content of pulp (determined from kappa)
2. Percentage of weight of crude lignin

A very high amount of crude material was extracted from the ball milled KOOD residue, however, only a 10% yield of lignin was recovered after purification. The crude material was very hygroscopic which could suggest the crude fraction contains a large amount of small carbohydrate fragments. Additionally, only a low yield of lignin was recovered from the buffer.

Comments

Method 2 did not improve on Method 1 in terms of yield or protein contamination for the KO pulp. Ball milling the residue remaining after the enzyme treatment had no effect on the yield of extracted lignin after purification. Additionally, ball milling may fragment a proportion of the carbohydrates to aqueous dioxane soluble fragments, thus, increasing the apparent crude lignin yield relative to the purified lignin yield.

Only low yields of lignin were isolated from the low kappa number pulps using this method and again this is possibly due to the lower degree of digestion achieved during the enzyme treatment.

In Section 4.2.2.7 it was shown that ball milling the enzyme digest residue significantly improves the CEL yield from wood, however, this is not observed for the pulp residues. One of the main differences between the wood digest residues and the pulp digest residues is the level of carbohydrates; that is, the level of carbohydrates is significantly higher in the pulp digest residues. The carbohydrates in the pulp digest residues could be protecting the lignin from being fragmented by the ball milling treatment, whereas, in the wood digest residues the lignin is more exposed and becomes extractable after ball milling.

Ball milling the pulp is not necessary for the enzyme digestion and seems ineffectual at improving the yield of lignin from the enzyme digest residues so perhaps the ball milling step could be omitted altogether. This was investigated using Method 3 as discussed below.

5.3.3 Method 3–Ball milling post-lignin extraction

Method 3 was used to determine whether the ball milling step was necessary for lignin isolation. Pulps were enzyme treated and extracted with aqueous dioxane following the scheme outlined in Figure (5.3).

Trial 1

A KOP2 pulp sample was digested for 55hr and the residue was extracted with 90% aqueous dioxane (Figure 5.3, Path A). The crude material was purified using the acetone method to give CERL3a (P1). The CERL3a (P1) was purified again using the same method to give CERL3a (P2). The results are shown in Table (5.8).

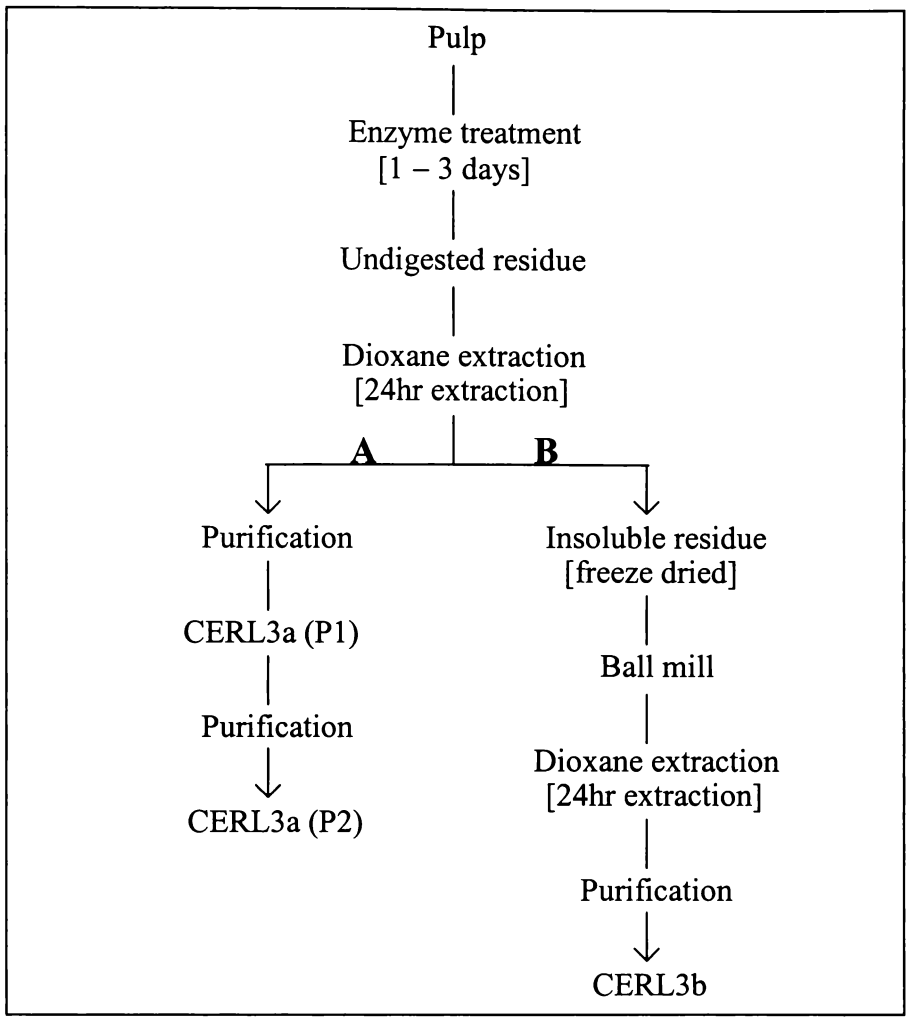

Figure (5.3). Cellulase enzyme isolation of residual lignin; Method 3

Table (5.8). Yield of residual lignin isolated from 100g of KO (KOP2) pulp by Method 3 after a 55hr treatment

	Yield (%)	**N** (%)	**Protein** (%)[1]	**OCH_3**[2]
Dioxane insoluble residue[3]	3	–	–	–
CERL3a (P1)[4]	42	1.9	12	10.6
CERL3a (P2)[4]	34	1.7	11	–
Recovered from buffer[4]	9	–	–	–

1. Determined from nitrogen content (%N × 6.25)
2. Percent by weight
3. Percentage of initial weight of pulp
4. Percentage of lignin content of pulp (determined from kappa number)

The yield of CERL3a after a single purification step is almost the same as the 3 day treated KO pulp in Method 1 (Table 5.2). This shows that ball milling the pulp before the enzyme treatment has no significant effect on the lignin yield. The difference between the DMF purification method and the acetone purification method can be evaluated by comparing the yields of CERL3a with the purified CERL lignins in Table (5.2). Only about 10% of the CERL3a (P1) is lost in the subsequent purification step, whereas, over 60% of the DMF purified CERL (Table 5.2) is lost in the subsequent purification step. This suggests that DMF contamination influences the recoverability of the lignin in a subsequent purification step since both CERL and CERL3a should be the same in terms of structure, and their nitrogen (and thus protein) content is similar. However, the protein content of the isolated CERL3a still remains at around 10% even after the two purification steps.

Trial 2

Johansson (1997) showed that protein contamination of isolated residual lignin could be reduced if the duration of the enzyme treatment was reduced. This same behaviour is observed when isolating lignin from wood (Section 4.2.2.2). Therefore, an investigation of the effects of reducing both the enzyme concentration and incubation time on nitrogen contamination was carried out.

A KOP2 pulp sample was digested for 24hr using 1g enzyme per 7g pulp (compared to 1g enzyme/4g pulp). The residue was extracted with 90% aqueous dioxane, as above (Figure 5.3, Path A). The dioxane insoluble residue was then ball milled and extracted following Path B with the aim of further improving yields. The results are shown in Table (5.9).

Table (5.9). Yield of residual lignin isolated from 100g of KO (KOP2) pulp using Method 3 after a 24hr enzyme treatment

	Yield[1] (%)	**N** (%)	**Protein** (%)[2]	**OCH_3**[3]
CERL3a				
Crude lignin	21	–	–	–
Purified lignin	14	0.4	3	10.33
CERL3b				
Crude lignin	15	–	–	–
Purified lignin	12	2.5	16	5.85

1. Percentage of pulp residual lignin determined from kappa number
2. Determined from nitrogen content (%N × 6.25)
3. Percent by weight

The protein content of CERL3a is reduced to below 5% in this trial (compared to 11% in trial 1). This shows the duration of the enzyme treatment has a significant effect on protein contamination, however, a significant reduction in yield is incurred through the reduced enzyme treatment (14% cf 34% (Table 5.8)).

A further 12%, of the total residual pulp lignin was isolated from the dioxane insoluble residue after ball milling (CERL3b), however, the lower methoxyl content of this fraction compared to CERL3a suggests a lignin content of only about 60%. CERL3b not only has a higher protein content, but may also contain a larger proportion of carbohydrates than CERL3a.

Comments

The results of Method 3 show that omitting the ball milling step has no significant effect on the yield of purified lignin after the first extraction. Ball milling the insoluble residue after extraction releases an additional fraction of the remaining lignin, however, the isolated lignin is considerably more contaminated. Ball milling may be fragmenting a proportion of the carbohydrates in the insoluble residue and releasing a small amount of the lignin as lignin-carbohydrate complexes.

The three methods discussed in this section are compared to the existing methods in the next section. Comparisons are made with regards to the yield and nitrogen contents of the isolated lignins

5.4 Comparison Between CEL Methods

The three common CEL methods for the isolation of residual lignin from pulps are outlined in Figure (5.4).

The Lachenal method (Figure 5.4A) involves a set of 72 hr enzyme treatments using the Onozuka enzyme preparation. The digest residue is recovered between each 72 hr treatment and re-suspended in fresh buffer and enzyme for the next period. At the completion of the enzyme treatment the insoluble residue is extracted twice with 90% aqueous dioxane. The extracted lignin is then purified by dissolving in DMF and precipitating with ether. The buffer soluble fraction is recovered by precipitation at pH 2 and purified in the same manner as the CEL fraction.

The Johansson method (Figure 5.4B) is a modified version of the Lachenal method. The enzyme treatment is reduced to approximately 18 hours and the undigested residue is extracted with 90% aqueous acetone. The purification process involves dissolving the crude lignin in NaOH solution at pH 12 and precipitating the lignin at pH 2.5 with dilute HCl. The fraction of lignin soluble in 90% aqueous acetone is recovered as the purified CEL lignin. This process can be repeated using the pulp residue remaining after the extraction step to recover subsequent lignin fractions.

The Hortling method (Figure 5.4C) involves a 48 hr enzyme treatment at 50°C using an Econase enzyme preparation. The residual lignin is recovered from the undigested residue as CEL or recovered from the buffer in two different fractions. In certain cases a precipitate is recovered from the buffer by addition of saturated NaCl (BS-NaCl). An additional fraction is obtained by acidification to pH 2.5 (BS-pH 2.5). All three lignin fractions are purified using either a chemical or biochemical purification step (or both).

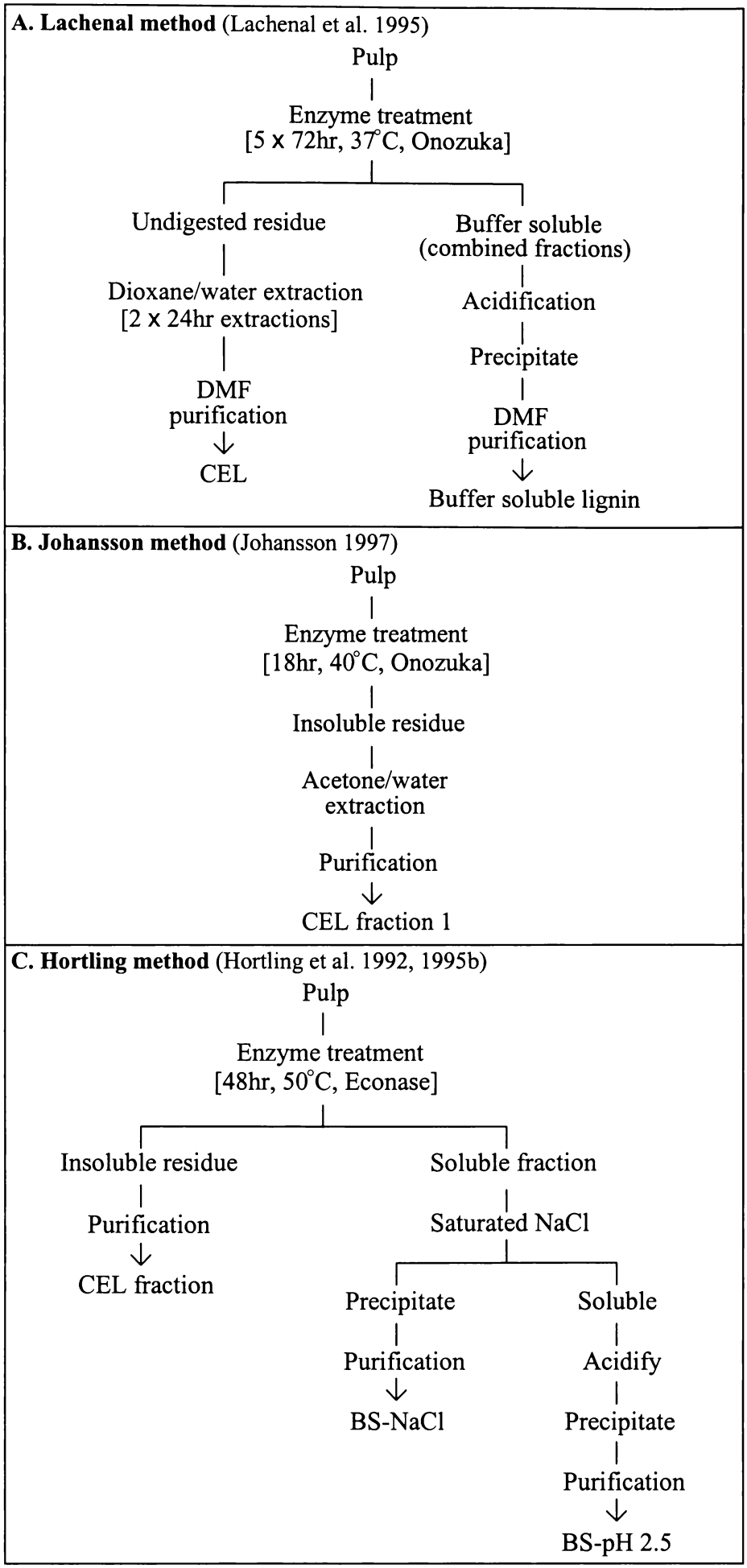

Figure (5.4). Outline of different CEL methods for the isolation of lignin from pulps

The chemical process involves two steps. First, the crude lignin is dissolved in dimethyl acetamide at 60°C and the soluble fraction is recovered by precipitation with ether. The precipitate is then dissolved in NaOH for 1hr and the purified lignin is recovered by precipitation at pH 2.5 (Hortling et al. 1992). The biochemical process involves a protease subtilisin enzyme treatment at pH 9.5 to reduce the protein contamination (Tamminen et al. 1994). The distribution of lignin in each fraction depends on the pulp type and kappa number (Hortling et al. 1995b) as well as the activity of the enzyme (Hortling et al. 1992).

These methods are compared below, in terms of yield and nitrogen contamination, with the methods used in this study. Table (5.10) shows the results for the kraft and kraft-oxygen pulps and Table (5.11) shows the results for the lower kappa number bleached pulps.

Comparing the results from Methods 1, 2, and 3 (Table 5.10) it can be seen that an enzyme treatment (with an enzyme charge of 1g/4g pulp) at 37°C of greater than 48hr does not significantly improve the yield of CEL extracted from the enzyme digest residue. Nor, does incorporating a ball milling step into the isolation procedure. However, reducing the enzyme treatment below 48hr significantly reduces the yield of CEL extracted from the digest residue. The yields of CEL extracted from the digest residue of the KO pulp after a 16–24hr enzyme treatment (M1, M2, M3II) are comparable to the yields obtained using the Johansson method.

Both the enzyme treatment time and the enzyme charge have an influence on the nitrogen content of the isolated lignin. The nitrogen content of the CELs increases slightly with the duration of the enzyme treatment. This is easily seen by comparing the Lachenal CEL and the Johansson CEL which have significantly different enzyme treatment times (Lachenal method is 20 times longer). The same trend was observed in this study (M2 compared to M3I), although the increase in nitrogen between a 16hr and 55hr enzyme treatment is less significant. A more pronounced effect on the nitrogen content of the CELs is observed when enzyme concentration is reduced (M2 cf M3II).

Only a small fraction of the total residual lignin from both K and KO pulps was recovered from the buffer after the reasonably short enzyme treatments (< 72hr) used in this study, although the amount of material was slightly higher for the KO pulp. The yield of KO pulp lignin recovered from the buffer using the Lachenal method is larger than the yields obtained in this study and this is most likely due to the significantly longer enzyme treatment time.

The results of this study concerning the K and KO pulps are comparable with the results of Johansson (1997), however the results of Hortling et al. (1992) are slightly different. The yield of CEL from the kraft pulp was higher, while the residual lignin from the KO pulp was recovered from the buffer. These differences can be explained by variations in the methods used. Both the enzyme system and the treatment temperature used by Hortling et al. (1992) were different than those used here. These will have a significant effect on the degree of digestion taking place during the enzyme treatment and, therefore, have a significant effect on the yields of CEL and the amount of lignin dissolved in the buffer. Hortling et al. (1992) also used different work-up procedures which also leads to differences in yield and nitrogen contamination.

Table (5.10). Yields (%) and nitrogen content (in parentheses) of lignin isolated from kraft and kraft-oxygen pulps by different CEL methods

	Method 1 (M1)[1]			**Method 1**[2]			**Method 2** (M2)[3]			**Method 3I** (M3)[4]		
	CERL[5]		Buffer[6]	CERL[5]		Buffer[6]	CERL2[5]		Buffer[6]	CERL3a[5]		Buffer[6]
	Yield (%)	**N (%)**		**Yield (%)**	**N (%)**		**Yield (%)**	**N (%)**		**Yield (%)**	**N (%)**	
K	40	1.5	1	–	–	–	–	–	–	–	–	–
KO	40	0.8	–	20	2.2	5 (8)	13	1.3	8	42	1.9	9

	Method 3II[7]			**Lachenal**[8]			**Johansson**[9]			**Hortling**[10]		
	CERL3a[5]		Buffer[6]	CEL[5]		Buffer[6]	CEL[5]		Buffer[6]	CEL[5]		BS-pH 2.5[6]
	Yield (%)	**N (%)**		**Yield (%)**	**N (%)**		**Yield (%)**	**N (%)**		**Yield (%)**	**N (%)**	
K	–	–	–	69	2.6	3 (3.4)	30	0.4	–	67	1.2	–
KO	14	0.4	–	54	2.0	17 (5.7)	21	0.9	–	–	–	46 (1.0)

1. Enzyme treatment at 37 °C; 48hr for K, 72hr for KO; pulp ball milled before enzyme treatment
2. 24hr enzyme treatment at 37 °C; pulp ball milled before enzyme treatment
3. 16hr enzyme treatment at 37 °C; pulp ball milled after enzyme treatment
4. 55hr enzyme treatment at 37 °C; pulp not ball milled
5. Yield of lignin extracted from enzyme digest residue; percent of pulp lignin content
6. Yield of lignin precipitated from buffer solution; percent of pulp lignin content
7. 16hr enzyme treatment at 37 °C; enzyme charge ≈ 14g/100 g pulp cf M1–M3I[11]; pulp not ball milled
8. From Johansson (1997); 5 × 72hr enzyme treatment at 37 °C; pulp not ball milled
9. Johansson (1997); 18hr enzyme treatment at 40 °C; pulp not ball milled
10. Hortling et al. (1992); 48hr enzyme treatment at 50 °C; pulp not ball milled
11. Enzyme charges: M1–M3I: 25g/100 g pulp; Lachenal/Johansson: 10g/100 g pulp

The residual lignins isolated from bleached kraft pulps are compared in Table (5.11). The variation in pulp samples (especially in kappa number) makes the comparison difficult, however, a number of trends are apparent.

Table (5.11). Yield (%) and nitrogen content (in parentheses) of lignin isolated from bleached kraft pulps by different CEL methods

CEL method	Pulp	Kappa number	CEL[1]	Buffer lignin[2]
Method 2[3]	KOQP	13.9	12 (0.9)	6 (8.9)
	KOQPn	9.6	6 (1.2)	9 (1.6)
	KOOD	2.3	10	4
Lachenal[4]	KOQZ	13.2	58 (1.1)	6 (5.4)
	KOQZE	10.3	38 (4.7)	31 (5.8)
	KOD	7.0	35 (4.3)	73 (6.0)
	KODE	3.1	–	11 (7.5)
Johansson[5]	KOO	6.8	22 (1.0)	4 (1.0)
Hortling[6]	KOP	7.3	–	24 (2.4)
	KOZ	6.2	–	46 (4.1)
	KOD	5.6	–	37 (4.5)
	KODE	4.3	–	21 (3.2)

1. Yield of CEL extracted from enzyme digest residue; percent of pulp lignin content
2. Yield of lignin recovered from the buffer; percent of pulp lignin content
3. 24hr enzyme treatment at 37 °C; ball milled after enzyme treatment
4. From Johansson (1997); 5 × 72hr enzyme treatment at 37 °C; pulp not ball milled
5. Johansson (1997); 18hr enzyme treatment at 40 °C; pulp not ball milled
6. Hortling et al. (1995b); 48hr enzyme treatment at 50 °C; pulp not ball milled; buffer soluble recovered after acidification (BS-pH 2.5)

Lower yields of lignin were isolated from the bleached pulps using Method 2 compared with other studies. The low yields are most likely due to the low degree of digestion achieved during the enzyme treatment. Since these pulps contain a higher proportion of carbohydrates, a longer enzyme treatment would be necessary to remove sufficient carbohydrates to improve the accessibility of the lignin. However, increasing the enzyme treatment would not only increase the protein contamination but also increase the proportion of residual lignin dissolved in the buffer (as is seen in the Lachenal lignins).

Method 2 is similar to the Johansson method in terms of nitrogen contamination. This is expected, since the enzyme treatment and work-up are essentially the same. However, the yield of CEL from the KOO pulp is twice the yield obtained from the pulps by Method 2. The ball milling treatment in Method 2 may be fragmenting a fraction of the lignin to water soluble fragments or the difference in yield may just be due to the variation between the pulps.

A significant difference in the distribution of lignin (buffer insoluble verses soluble) is apparent between the Lachenal method and the Hortling method. That is, a proportion of lignin from the bleached pulps is recovered from the insoluble residue with the Lachenal method, whereas all of the residual lignin is recovered from the buffer with the Hortling method. This is probably due to the differences in the enzyme treatment. Hortling et al. (1995b) used a different enzyme system which had a higher specific activity than the enzyme preparation used for the isolation lignin by the Lachenal method (Johansson 1997). Additionally, a higher temperature was used by Hortling et al. (1995b). The ultimate result of these more severe conditions is that complete solubilisation of the pulp occurs. The Lachenal method, on the other hand, leads to a greater proportion of the pulp remaining unsolubilised at the end of the enzyme treatment. Hortling et al. (1995b) contributed this to differences in the enzyme activities and substrate specificities.

A higher yield of lignin is recovered from the buffer by the Lachenal and Hortling methods compared to Method 2 and the Johansson method. This is probably due to the higher degree of digestion of the former methods. The lignin recovered from the buffer using the Lachenal method has a slightly higher nitrogen content than the Hortling lignin. This would suggest the purification methods of Hortling et al. (1995b) are slightly better at reducing protein contamination than the DMF method of Lachenal et al. (1995).

Overall, the Hortling method seems the best method for the isolation of residual lignin from pulps. The greatest yields of lignin with the lowest protein contamination were isolated from pulps using this method of isolation, however, the purification procedure is quite long.

5.5 Concentration of Residual Lignin

One of the general methods used to characterise residual lignin is the study of *in situ* lignin by chemical degradation (Lai et al. 1995, Francis et al. 1991, Gellerstedt & Lindfors 1984a, 1984b, 1987, Gellerstedt et al. 1988). The main difficulty with the use of these techniques is the low concentration of residual lignin in the pulps, particularly in pulps bleached to low kappa numbers.

Degradation studies are easily carried out on wood samples, which have a lignin content of between 20–30%. In theory, if residual lignin content of pulps were at these levels, analysis by degradation could be made easier. Partial removal of the carbohydrates from a pulp would be an effective way of increasing the concentration of the residual lignin.

Degradation of the carbohydrates by cellulase enzymes could be a means for increasing the relative amount of residual lignin within a pulp to allow analysis of low kappa number pulps by degradation studies. The practicality of such a method would largely depend on the amount of lignin solubilised during the enzyme treatment and the influence of adsorbed protein on the degradation of the lignin. The feasibility of using enzymes to concentrate the residual lignin in pulps was investigated by studying the effect of enzyme digestion on the Klason lignin content of the resulting residue.

A series of pulp digest residues were generated from unbleached kraft pulps (KP2 and KP3) and bleached kraft pulps (KOP2, KOQP2, KOQPn2, and KOOD). Pulp samples were treated with varied amounts of cellulase enzyme for varying times to obtain pulp residues digested by different extents. The lignin content of the residues was then determined as Klason lignin and the presence of adsorbed protein was determined by analysing for nitrogen.

Figure (5.5) shows the sequence of treatments carried out on the kraft pulp samples. KP2 samples were incubated for 16, 27, and 48 hr with either 1 g of enzyme per 4 g of pulp (1 A, B, C) or 1 g of enzyme per 8 g of pulp (2 F, G, H). A proportion of residue 1 was then digested for a further 16 hrs to generate residue 7 (1 D). Similarly, a proportion of residue 7 was further digested to obtain residue 9 (1 E). Residue 5 was also treated in this manner to give residues 8 and 10, respectively (2 I, J). The KP3 sample was digested for 36 h to give residue 11 (3).

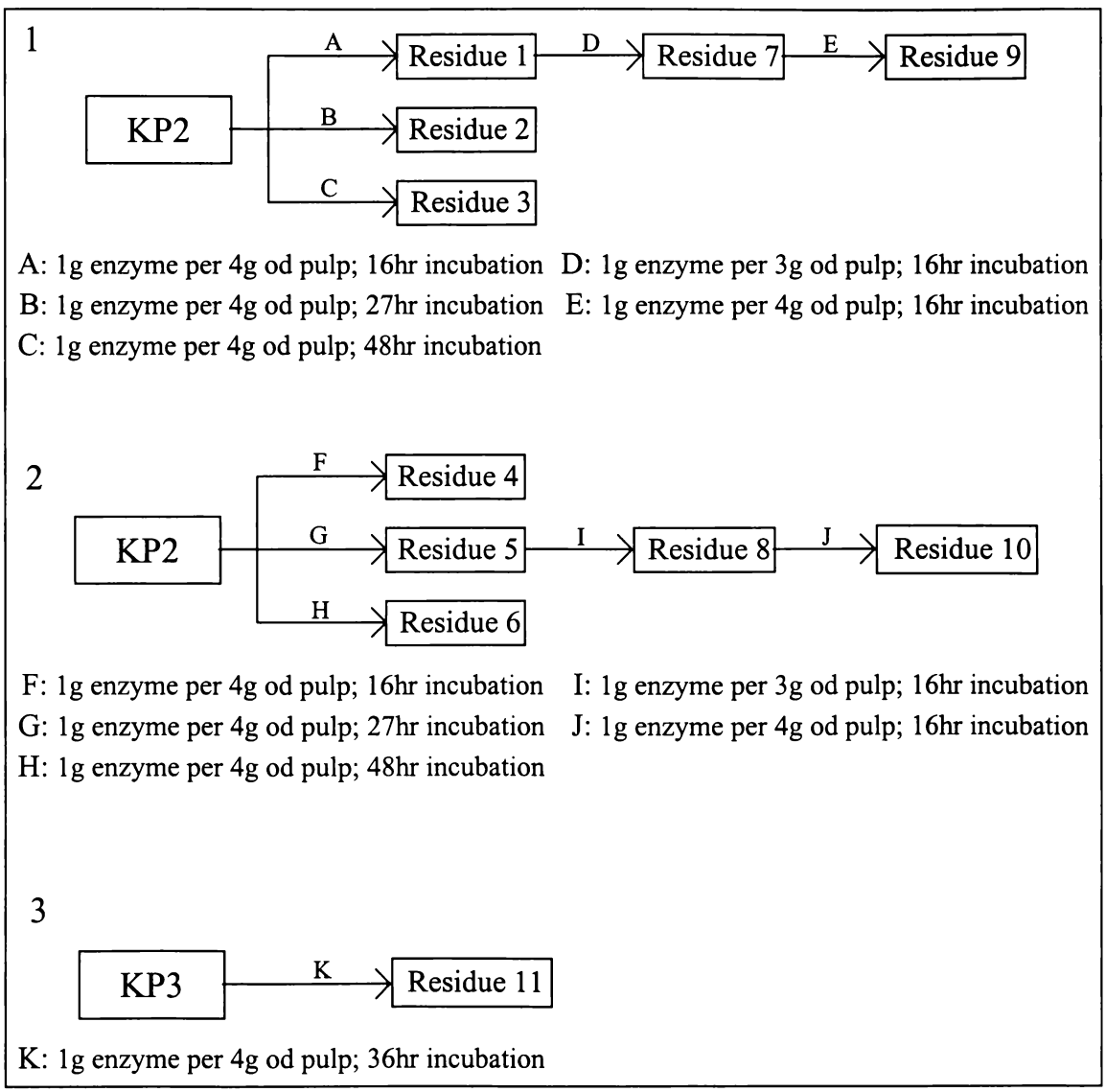

Figure (5.5). Enzyme treatments carried out on the kraft pulps

The results for the unbleached kraft pulps are summarised in Table (5.12). It can be seen from the first six treatments that the protein content of the residues increases with an increase in the duration of the enzyme treatment and is significantly greater when a higher concentration of enzyme is used. A similar trend was seen earlier (Section 5.4) for the nitrogen content of enzymatically isolated residual lignin from pulps.

The results suggest that a possible way of minimising adsorbed protein during enzyme treatment of pulps may be to digest the pulps with several short enzyme treatments with washing stages between each treatment. For example; residue 9, which has been digested for a total of 48hr in three 16hr enzyme treatments, has a slightly lower protein content than residue 3. The total amount of weight removed during the enzyme treatment is also similar for each; that is, 86% for residue 3 compared to 83% for residue 9. However, the Klason lignin content of residue 9 is slightly lower indicating that slightly more lignin is solubilised over the duration of the three treatments.

The weight removed from KP2 samples during enzyme treatment is plotted against time in Figure (5.6). The plot shows that after 48hr of enzyme treatment at the enzyme concentration of 1g/4g pulp, essentially all the carbohydrates have been removed and extending the enzyme treatment further has no significant effect on the weight loss. This result is consistent with the finding that at this enzyme concentration no significant improvement in the yield of CEL is achieved by extending the enzyme treatment beyond 48hr (Table 5.10). The effect of halving the enzyme concentration is also demonstrated in the plot; that is, when the enzyme concentration is halved the expected reduction in reaction rate is observed.

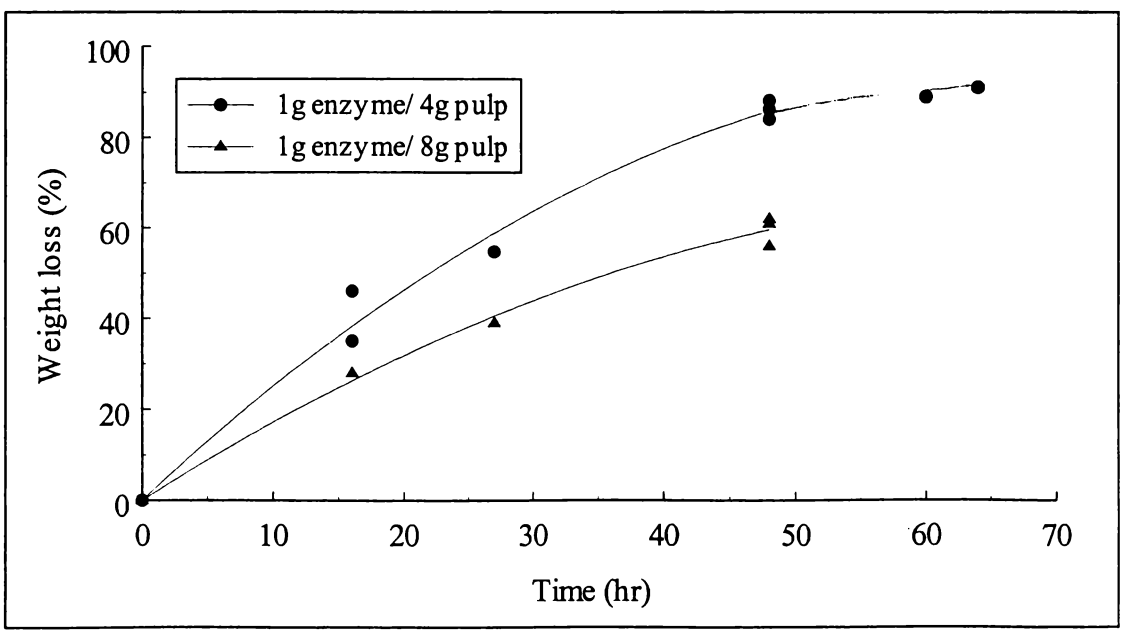

Figure (5.6). Weight removed from KP2 samples by the Onozuka enzyme preparation

Table (5.12). Effect of enzyme concentration and incubation time on the digestion, lignin content, and protein contamination of kraft pulps

Residue no.	Pulp	Enzyme concentration	Time (hr)	Figure reference	Initial		Residue weight [2]	Percent digestion[3]	Lignin content		Protein (%)[4]
					Weight (g)	Lignin[1] (%)			Expected	Klason	
1	KP2	1g enzyme/ 4g pulp	16	1a	25	4.5	54	46	8.3	7.2	3
2	KP2	1g enzyme/ 4g pulp	27	1b	25	4.5	45	55	10.0	6.8	3
3	KP2	1g enzyme/ 4g pulp	48	1c	9.4	4.5	14	86	32.5	21.9	9
4	KP2	1g enzyme/ 8g pulp	16	2f	25	4.5	72	28	6.3	5.05	0.6
5	KP2	1g enzyme/ 8g pulp	27	2g	25	4.5	61	39	7.4	5.77	0.6
6	KP2	1g enzyme/ 8g pulp	48	2h	9.4	4.5	44	56	10.3	7.95	2
7	Residue 1	1g enzyme/ 3g residue	16	1d	4.02	8.3	45	76	18.6	11.8	5
8	Residue 5	1g enzyme/ 3g residue	16	2i	4.02	7.4	52	68	14.1	10.1	5.6
9	Residue 7	1g enzyme/ 4g residue	16	1e	1.08	18.6	66	83	28.3	18.2	6
10	Residue 8	1g enzyme/ 8g residue	16	2j	1.09	14.1	68	78	20.7	12.1	4
11	KP3	1g enzyme/ 4g pulp	36	3k	30	4.2	14	86	30.7	15.9	–

1. Determined from kappa number (kappa × 0.15) for pulps; expected content for digestion residues ((Initial lignin content / residue weight) × 100)
2. Percentage of initial weight.
3. Equivalent to the total weight removed from the undigested pulp
4. %N × 6.25

The results from the enzyme treatments of the bleached kraft pulps are shown in Table (5.13). The same activities, with respect to weight removed with time, compared to the unbleached kraft pulps are observed. Significantly less protein was adsorbed onto the KOOD pulp compared to the kraft and KO pulps which could suggest the protein is interacting with the lignin rather than the carbohydrates, since the lignin content of the KOOD pulp is significantly less than the other pulps.

Table (5.13). Effect of enzyme concentration and incubation time on the digestion, lignin content, and protein contamination of bleached kraft pulps

Pulp	Enzyme[1]	Time (hr)	Initial weight (g)	Initial lignin (%)[2]	Digestion[3] (%)	Lignin content		Protein[5] (%)
						Expected[4]	Klason	
KOP2								
1	1/4	48	10	2.7	12	22.5	19.9	10
2	1/4	64	30	2.7	9	28.9	20.3	–
3	1/8	48	10	2.7	38	7.1	6.55	3
KOQP2								
	1/4	64	30	2.1	17	12.0	15.8	–
KOQPn2								
	1/4	64	30	1.4	15	9.4	2.11	–
KOOD								
1	1/4	48	10	0.35	16	2.2	1.1	3
2	1/8	48	10	0.35	39	0.9	1.04	2

1. Enzyme concentration; 1g enzyme/ 4g or 8g pulp
2. Initial lignin content of pulp estimated from kappa number
3. Weight of residue as a percentage of initial weight
4. Expected lignin content if no lignin is lost from residue
5. Determined from nitrogen content (%N × 6.25)

The Klason lignin content of all residues relative to the initial lignin content of the pulps (enrichment factor) is plotted against the residue weight in Figure (5.7). The solid line indicates the theoretical values if no lignin was lost from the residues during the enzyme treatment. The dotted line is a best-fit line through the data obtained from the treatments of KP2 so represents the behaviour of this pulp during enzyme digestion. The results indicate that only a small amount of the residual lignin is lost to the buffer at the early stages of digestion but as the digestion proceeds beyond about 60% (ie 40% of initial pulp remaining) the amount of lignin lost from the residue progressively increases. The results show that when 10% of the original weight remains approximately one third of the Klason lignin has been lost from the KP2 residue. The second kraft pulp sample (KP3, +) also followed this trend.

The behaviour of the KO pulp (solid squares) follows the same trends as the KP2 pulp indicating the residual lignins from the kraft and kraft oxygen pulp behave in a similar way during digestion with the Onozuka cellulase/hemicellulase preparation. This result is not consistent with the findings of Lachenal et al. (1995) or Hortling et al. (1992) who reported differences in the behaviour of the residual lignin from kraft and kraft oxygen pulps. That is, after almost complete digestion the residual lignin from the kraft pulp remained insoluble, whereas, the KO residual lignin was essentially all dissolved.

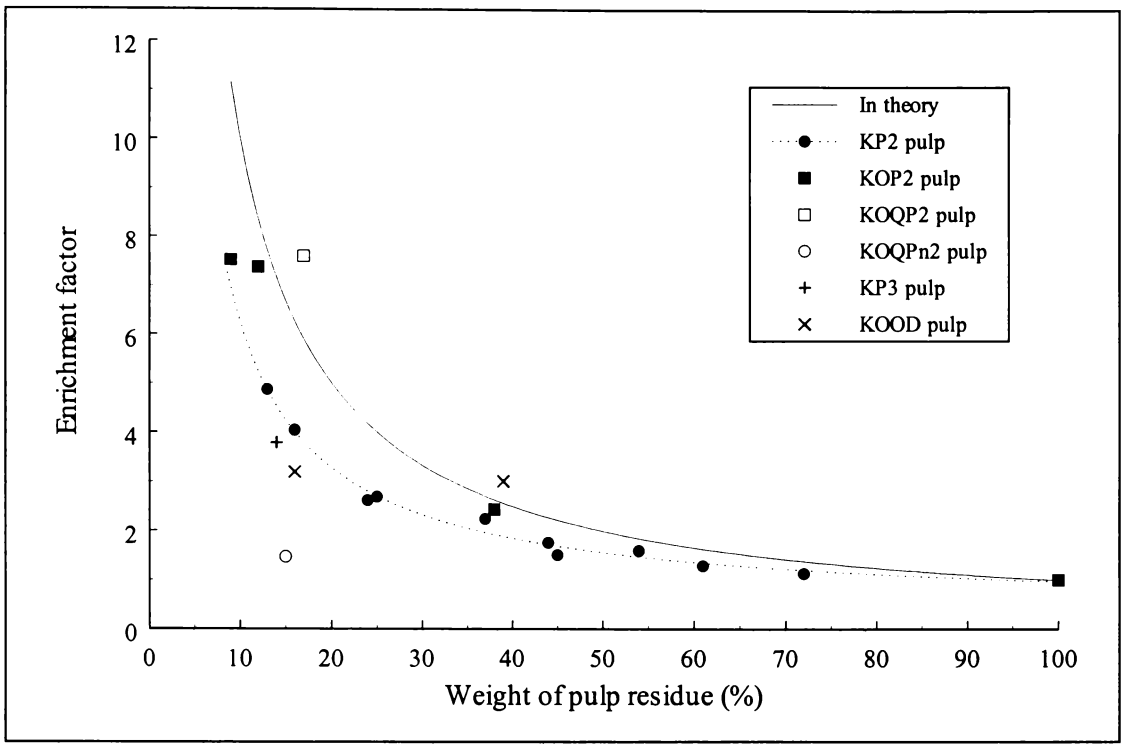

Figure (5.7). Effect of enzyme digestion on the lignin content of pulps

The main difference between these studies is that the KO pulps used by Lachenal et al. (1995) and Hortling et al. (1992) had a lower kappa number than the KO pulp used in this study (15 cf 18.4). This may lead to differences in the behaviour of the residual since the former residual lignins are likely to be more oxidised, and thus be more hydrophilic, compared to the latter.

The results obtained from the other bleached pulps are not consistent. The Klason lignin content of the two KOOD residues are not significantly different, however, the levels are probably too low to be accurately determined by Klason analysis. The Klason lignin content of the KOQPn pulp residue is significantly lower than the theoretical lignin content and is significantly lower than an equivalent kraft pulp digest residue. This result suggests that at 85% digestion a large proportion of lignin has been lost to the buffer from the KOQPn pulp. It is also consistent with other findings (Johansson 1997, Hortling et al. 1992, Lachenal et al. 1995) that residual lignin from more highly oxidised pulps are solubilised during prolonged enzyme treatment. The Klason lignin content of the KOQP pulp residue is larger than the theoretical value suggesting the Klason lignin analysis has over estimated the level of lignin in the residue. The expected result is that the behaviour of the residual lignin from the KOQPn and KOQP pulps be alike.

These preliminary results suggest that an enzyme treatment, as a means of concentrating the residual lignin, is only viable for the higher kappa number pulps. The extremely low kappa number pulps such as the KOOD pulp require a more extensive level of digestion than carried out here to significantly increase the lignin concentrations in the pulps. However, if the behaviour of the KOQPn pulp is true for the majority of low kappa number bleached pulps then most of the lignin will be lost from the residue before a reasonable level of digestion can been reached.

The results for the kraft and kraft oxygen pulps look promising; showing that it is possible to increase the levels of lignin in these pulps to levels similar to those found in wood without significantly losing much of the residual lignin to the buffer. Preliminary studies involving thioacidolysis of the kraft and KO pulp residues indicated that the concept of enrichment was potentially a viable method.

5.6 Concluding Remarks

The studies concerning the isolation of residual lignin by enzyme methods discussed in this chapter were not very successful in that no significant improvements, in terms of yield or protein contamination, were achieved compared to earlier studies (Hortling et al. 1992, Lachenal et al.1995, Johansson 1997).

The main problem with the enzyme isolation methods investigated is that only very low yields (<15%) of lignin could be isolated with low protein contamination from the low lignin pulps. Reducing the contact time between the enzyme and lignin significantly reduces the protein contamination, however, this also significantly reduces the yield. If the enzyme treatment is extended, a higher proportion of the residual lignin becomes dissolved in the buffer. Part of this lignin can be recovered by precipitation via acidification of the buffer, however, the recovered lignin has a significantly higher protein content than the lignin which is extracted from the undigested residue.

The tendency of the lignin to dissolve into the buffer during the enzyme treatment could be largely due to the adsorption of protein onto the lignin. Since lignin is generally not significantly water soluble the adsorption of protein onto the surface of the lignin could result in the lignin becoming water soluble. The differences in the behaviour of different lignins and differences arising from the use of different enzymes could be explained in terms of the degree of protein adsorption. For example, the more highly modified lignin is perhaps more likely to adsorb protein and thus more likely to be solubilised during the enzyme treatment. Additionally, the properties of the enzyme, in terms of its tendency to adsorb and its hydrophilic nature, would also have an influence. Therefore, to improve the enzyme methods a way of minimising protein adsorption needs to be found.

The results obtained from the digestion trials showed that the concept of carbohydrate degradation as a means of enriching the residual lignin in pulps had some potential and that this area is worthy of further investigation.

Chapter Six
PINUS RADIATA KRAFT PULP RESIDUAL LIGNIN

6.1 Introduction

In the past several years kraft pulping and bleaching processes have continued to advance; the aim being to produce fully bleached pulps with good strength properties using environmentally friendly methods. This includes pulping to lower kappa numbers by means of modified kraft pulping followed by oxygen delignification and chlorine (Cl_2) free bleaching.

Research aimed at increasing the understanding of the delignification processes that occur during pulping and bleaching is ongoing. Ultimately, this knowledge can be used to further improve existing processes.

One prominent area of research has been the study of pulping and bleaching reactions using model compounds that represent certain structural elements thought to exist in the residual lignin. Although, much of today's understanding concerning pulping and bleaching reactions (which are discussed in Section 2.3) has been obtained by such studies, the behaviour of model compounds can go only so far in representing the behaviour of the residual lignin embedded in the cellulosic matrix of the pulp.

The lack of knowledge concerning the structure of residual lignin present in kraft and bleached kraft pulps makes it difficult to thoroughly understand which chemical and physical properties are important for the further delignification and brightening of the pulp. A better understanding of the nature of residual lignin would be a key step in improving current pulping and bleaching processes.

In order to gain better insight into the actual structures present in pulps and how the residual lignin behaves under different pulping and bleaching processes more recent research has centred on the isolation and characterisation of the residual lignin remaining in pulps. Residual lignin in unbleached kraft pulps has been extensively studied (Yamasaki et al. 1981, Hortling et al. 1992, Froass et al. 1996, Pekkala 1985). However, studies on bleached residual lignins are less extensive and in the majority of cases comparisons are made between residual lignins isolated from pulps bleached to different kappa numbers using conditions which replicate those in industrial-scale bleaching operations (Lachenal et al. 1995, Johansson 1997, Hortling et al. 1995b, Gellerstedt & Heuts 1997). As a result, the residual lignin from each pulp will probably be oxidised to different extents. Therefore, a better approach would be to compare residual lignin from pulps bleached to the same kappa number. Such a comparison would remove the variables associated with the different oxidising powers of each reagent and yield a more comparable residual lignin.

In this study a kraft-oxygen (KO) pulp was bleached to approximately the same kappa number using cynamide-activted hydrogen peroxide, ozone, and chlorine dioxide (including an extraction stage for the Z and D treatments). The residual lignins were isolated by dioxane acidolysis and characterised using a number of chemical and spectral techniques.

Structural characteristics of the residual lignin from the different pulps are compared and discussed in terms of the reactions taking place during delignification. Comparisons are also made with the spent liquor lignins from the peroxide and ozone bleaching processes and also with the residual lignin isolated from the enzyme treated kraft, KO, and KOQP pulps discussed in the previous chapter (Section 5.5).

6.2 Pulping and Bleaching Reactions

A brief overview of the reactions that occur during kraft pulping and the oxygen-based bleaching processes used in this study is given below. A more detailed discussion was given in Chapter 2.

The behaviour of lignin during kraft pulping can be explained as a competition between degradation reactions, mainly involving the cleavage of β-aryl ether linkages; and condensation reactions, such as those involving addition of carbanions to quinone methide intermediates. During the initial and bulk phases of delignification extensive cleavage of β-aryl ether linkages occurs with the assistance of the sulphide ion and neighbouring groups in the lignin (Section 2.2.2.3). In the final phase of delignification, the rate of delignification drops dramatically. This has been attributed to a number of factors, including; a decrease in the number of reactive groups in the lignin, an increase in condensation reactions, and the presence of carbohydrate-lignin linkages that inhibit the dissolution of degraded lignin fragments. Hence, the residual lignin can be expected to be enriched in unreactive structures (such as condensed structures), and to have lower levels of the reactive structures (such as aryl ether linkages) compared to native lignin.

The interpretation of bleaching processes in chemical terms is considerably more difficult than the description of pulping chemistry. This is due to the fact that much less is known about the structure of the substrate of bleaching compared to the substrate of pulping (ie residual lignin vs native lignin). Further, bleaching is usually performed in a number of steps and in various sequences. Additionally, a number of different reactive species derived from the bleaching reagent contribute to lignin reactions. However, through extensive work with model compounds, a general outline of the reactions of lignin with the different bleaching reagents has been reported (Gierer 1990a, 1990b).

The reactions of chlorine dioxide are classed as oxidative radical processes. The initial step of reaction involves electrophilic attack at electron rich aromatic and olefinic sites. This oxidant abstracts hydrogen from phenolic hydroxyl groups generating phenoxy and mesomeric cyclohexadienyl radicals. These then combine with another bleaching reagent radical affording unstable chlorite esters which are subsequently hydrolysed or undergo elimination to give quinoid and muconic acid type structures. Chlorine dioxide reacts with olefinic structures in a similar manner, ultimately leading to side chain oxidation without appreciable bond cleavage. Nonphenolic units also react with chlorine dioxide but at a much slower rate. During these reactions, chlorine dioxide becomes reduced to either chlorate or hypochlorous acid. The latter, along with its equilibrium partner, elemental chlorine, are considered to cause the chlorination reactions which take place during chlorine dioxide bleaching.

Oxygen and hydrogen peroxide bleaching have common features because in both cases the medium is alkaline, and the same reactive species are present, although in different proportions. The initial reactions during oxygen bleaching are very similar to the reactions taking place during chlorine dioxide bleaching. However, molecular oxygen is a weaker electrophile compared to chlorine dioxide and will only react with ionised phenolic units. The initial step is the abstraction of an electron from the phenolate ion forming a phenoxy radical and superoxide anion radical which in turn combine to form various hydroperoxide intermediates. These intermediates decompose in a similar fashion to the chlorite esters formed during chlorine dioxide bleaching, however, in the alkali media scission of ring-conjugated double bonds is a predominant reaction.

During oxygen bleaching hydrogen peroxide and hydroxyl radicals are also formed, both of which also contribute to lignin oxidation. Hydrogen peroxide reacts with carbonyl and conjugated carbonyl structures resulting in pulp brightening. Hydroxyl radicals react rapidly with both non-phenolic and phenolic structures in lignin by oxidative radical processes; thus, they also contribute to lignin degradation (Gierer 1993). However, hydroxyl radicals are the main cause of carbohydrate degradation during oxygen bleaching (Ek et al. 1989).

On the other hand, the main reactive species during hydrogen peroxide bleaching is the nucleophilic hydroperoxide anion (HOO^-) which reacts with carbonyl and conjugated carbonyl structures. Alkaline and/or oxidative degradation of the initially formed hydroperoxide intermediates afford mainly carboxylic acid type degradation products. Under lignin-preserving hydrogen peroxide bleaching very little dissolution of lignin takes place and the main effect is the removal of chromophoric groups in the lignin. In contrast, hydrogen peroxide bleaching under more severe conditions can result in significant delignification and this has been attributed to reactions involving the decomposition products of hydrogen peroxide (namely $OH^{\bullet}$ and $O_2^{\bullet-}$). Therefore, many aspects of delignification during hydrogen peroxide bleaching are the same as oxygen bleaching.

Recently, selective degradation of pulp lignin with cyanamide activated hydrogen peroxide has been shown (Smit 1995). The reactions are proposed to proceed via a peroxyimidic acid intermediate (1, Figure 6.1) which is a stronger oxidant than hydrogen peroxide. Smit et al. (1996) showed that a wide range of model compounds (phenolic, non-phenolic, and condensed) reacted extensively with cyanamide activated hydrogen peroxide without any apparent selectivity. However, more recently (Kadla et al. 1998a 1998b) showed that non-phenolic structures were unreactive during cyanamide activated bleaching. The main difference between these two studies is that different model compounds were used. Therefore, the exact nature of the reactions taking place during cyanamide activated hydrogen peroxide bleaching are unclear. However, Kadla et al. (1998b) proposes that a variety of radicals, arising from the decomposition of the peroxyimidic acid, play an important role in the delignification. Furthermore, it was suggested that either superoxide anion radicals or hydroperoxide radicals are the predominant reactive species and that the involvement of hydroxyl radicals is insignificant. Hence, many of the chemical aspects of cyanamide activated hydrogen peroxide bleaching should resemble those of oxygen bleaching.

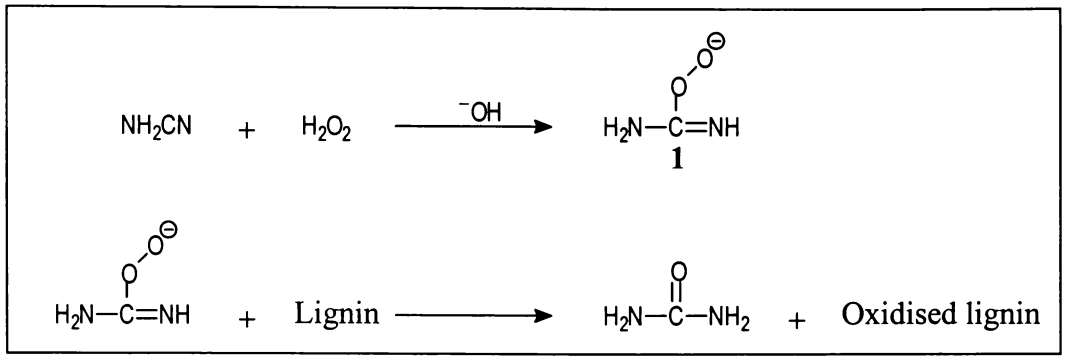

Figure (6.1). Pathway of cyanamide activated hydrogen peroxide bleaching

Ozone is generally believed to react with lignin structures as an electrophile, reacting with electron rich sites such as aromatic and olefinic double bonds. Reaction is initiated by a 1,3-dipolar cycloaddition reaction leading to ring opening and double bond cleavage, respectively. Ozone also reacts with olefinic double bonds via a 1,1-cycloaddition reaction forming oxirane type structures and inserts into C–H bonds of alcohols, aldehydes, and ester type structures. Cleavage of aryl ether bonds during ozonation occurs via the latter mechanism. Ozonation of aromatic and olefinic structures is accompanied by the generation of molecular oxygen or hydrogen peroxide. Decomposition of the latter as well as the starting oxidant forms hydroxy and hydroperoxy radicals, which are responsible for the carbohydrate degradation taking place during ozone bleaching.

From the above discussion it can be seen that the reactions taking place during each specific bleaching stage are quite similar, therefore, the residual lignin within pulps bleached with chlorine dioxide, hydrogen peroxide or ozone should be similar.

6.3 Pulp Properties

The properties of the commercial and laboratory bleached pulps used in this study are summarised in Table (6.1).

The laboratory bleached pulps were generated using the methods of PAPRO. Bleaching trials were carried out to determine the conditions necessary to achieve the target kappa number of 10. The results of these trials and the pulp generation are discussed in Appendix 1. The KOQP and KOQPn pulps were bleached under the same conditions and using the same hydrogen peroxide charge so any differences between these two pulps will arise from the presence of cyanamide. To study the effects of an alkali extraction stage on residual lignin, unextracted ozone and chlorine dioxide pulps samples were kept for analysis (KOQZ and KOD, respectively).

Table (6.1). Properties of unbleached and bleached kraft pulps discussed in this chapter

Pulps	Kappa number	Lignin content (%)[1]
Kraft pulps		
KP3	28.0	4.20
KP4	22.5	3.37
Kraft-oxygen pulp		
KOP2	18.4	2.76
Laboratory bleached pulps		
KOQP	14.7	2.21
KOQPn	9.9	1.49
KOQZ	14.0	2.10
KOQZE	10.6	1.59
KOD	14.3	2.15
KODE	11.0	1.65

Estimated from kappa number (0.15 × kappa number)

6.4 Isolation of Residual Lignin

Dioxane acidolysis lignin (acid lignin 1) was isolated from all pulp samples as outlined in Figure (6.2), following the general method of Gellerstedt et al. (1994). Pulp samples were heated at reflux in 4:1 dioxane/aqueous HCl (0.1 M) solution for two hours under a nitrogen atmosphere. The solubilised lignin was precipitated by removal of the dioxane and then recovered and washed by centrifugation. A second acid lignin fraction (acid lignin 2) was isolated from the kraft and KO pulp samples by re-extracting the residue remaining after the first acidolysis treatment.

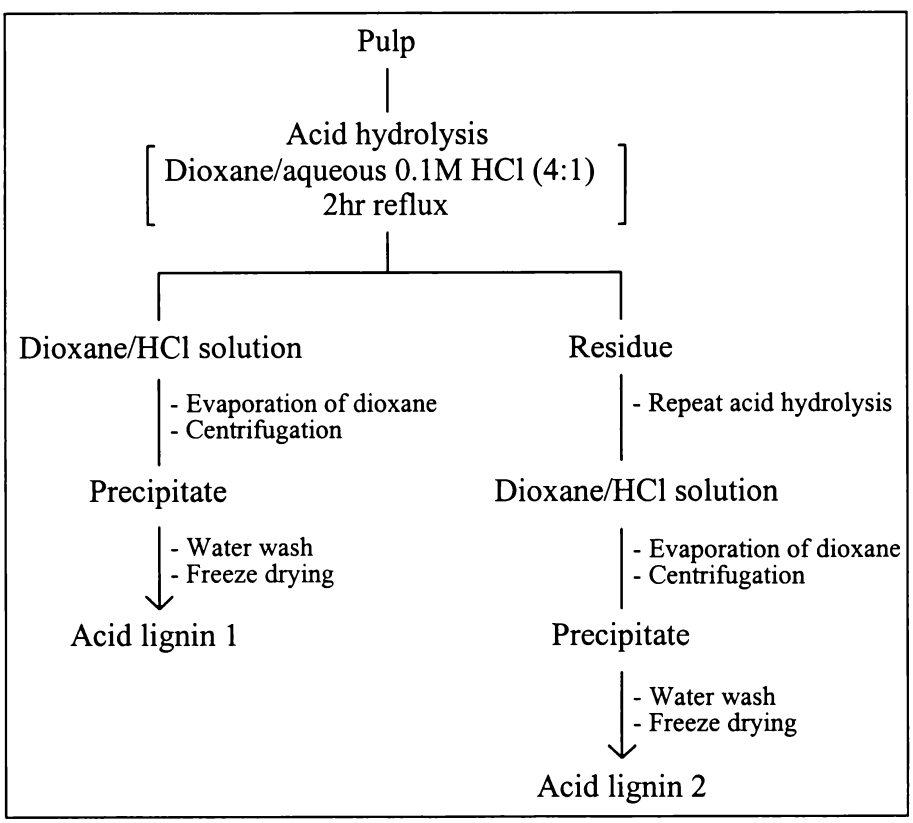

Figure (6.2). Scheme for the isolation of dioxane acidolysis from kraft pulps

6.4.1 Preliminary experiments

A series of preliminary experiments were carried out on KP3 and KOP2 pulp samples to determine the effects of extraction consistency, drying the pulp prior to extraction, and Wiley milling the pulp prior to extraction, on lignin yield. The experimental conditions are summarised in Table (6.2), along with the yields of acidolysis lignin. For the kraft pulp the effect of air drying (K2) and Wiley milling (K3) prior to acidolysis were investigated. This was compared with an untreated pulp sample (K1). K2 was heated using an oil bath (110°C) and was continuously stirred using a magnetic stirrer to prevent bumping. The other samples were heated using a heating mantle (under the conditions used the samples were refluxing at approximately 90°C). The influence of solvent volume was also investigated by changing the consistency. The KO pulp samples were cooked without pretreatment at either 3% (KO1) or 4% (KO2) consistency.

The acidolysis pulp residues from K1, K3, and KO2 were extracted for a further two hours at approximately 3% consistency. The acid lignin fraction 2 was recovered from solution using the same procedure as the first acid lignin fraction.

Table (6.2). Experimental conditions and yields of acidolysis lignin from KP3 and KOP2 pulp samples

Sample	**Experimental conditions**		**Acidolysis lignin yield (%)**[1]		
	Pretreatment	Consistency (%)	Acid lignin 1[2]	Acid lignin 2[3]	Total
KP3					
K1	None	3	35	5	40
K2	Air dry	4	27	–	27
K3	Freeze dry, Wiley mill	5	22	16	38
KOP2					
KO1	None	3	39	–	39
KO2	None	4	31	7	38

1. Percentage of theoretical pulp residual lignin content (kappa number × 0.15)
2. First acidolysis lignin fraction isolated from pulp
3. Acidolysis lignin isolated from the residues remaining after the first extraction (at 3% consistency)

The first fraction of acidolysis lignin isolated from both the kraft and kraft-oxygen pulp samples seem to follow a trend of increasing yield with increasing solvent volume (ie decreasing consistency). Therefore, since both the kraft and kraft-oxygen pulps follow this trend, it can be concluded that drying or Wiley milling the pulp prior to acidolysis has no significant effect on yield. Additionally, only low yields of acidolysis lignin (acid lignin 2) were released from the residue after the second cook. Poor accessibility or poor solubility of the remaining lignin are the possible causes for these low yields.

Since the conditions used for K1 and KO1 gave the best yields of acidolysis lignin these were used for further studies.

6.4.2 Yields of acidolysis lignin

Residual lignin was isolated from a number of bleached and unbleached kraft pulp samples using the same conditions as for K1 and KO1 (Table 6.2). Kappa number determinations were used to estimate the lignin remaining in the acidolysis residues. The residues were extensively washed to remove any residual acid or dioxane before analysis. The balance of residual lignin for each pulp is shown in Table (6.3).

Table (6.3). Yields of residual lignin isolated from kraft pulps by acidolysis and amounts of residual lignin remaining unextracted

Sample	**Original Pulp** (P)	**Acidolysis lignin** (A)	**Acidolysis Residue** (R)	**Total lignin** (A + R)/P	**Lignin lost** 1–((A+R)/P)
	Kappa no. (mg lignin)	Percent yield (mg lignin)	Kappa no. (mg lignin)	Percent of original lignin	Percent of original lignin
KP3[3]					
K2[4]	28.0 (430)	29 (125)	16.4 (235)	84	16
K3[5]	28.0 (430)	38 (165)	12.3 (159)	75	25
KP4[3]	22.0 (432)	21 (92)	–	–	–
	22.0 (432)	25 (110)	–	–	–
KOP2[3]					
KO2[4]	18.4 (414)	31 (129)	13.8 (266)	95	5
KO2[5]	18.4 (414)	38 (159)	11.6 (210)	89	11
KOQP	14.7 (437)	32 (139)	9.9 (304)	101	-0.1
	14.7 (437)	37 (162)	8.9 (261)	96	4
KOQPn	9.9 (370)	42 (157)	5.7 (214)	100	-0.3
KOD	14.3 (374)	35 (129)	9.5 (241)	99	1
	14.3 (374)	34 (125)	8.9 (228)	95	5
KODE	11.0 (335)	31 (103)	6.7 (205)	92	8
	11.0 (335)	32 (106)	6.9 (203)	92	8
	11.0 (248)	28 (69)	6.7 (166)	95	5
KOQZ	14.0 (367)	39 (143)	8.0 (169)	92	8
	14.0 (367)	43 (160)	7.9 (169)	97	3
KOQZE	10.6 (278)	26 (71)	7.4 (189)	94	6
	10.6 (278)	28 (76)	7.0 (177)	91	9
	10.6 (278)	25 (70)	7.0 (179)	89	11

1. Determined from kappa number of pulp (0.15 × kappa number)
2. Determined from kappa number of residue (0.15 × kappa number)
3. Refer to Table (6.1) for pulp description
4. After first extraction
5. Total after two extractions

The highest yields of acidolysis lignin were obtained from the KOQZ and KOQPn pulps which could indicate a more oxidised residual lignin that is either more soluble in the extracting solvent or more accessible. The decrease in yield between the KOD and KODE pulps, as well as the KOQZ and KOQZE pulps, would also be consistent with this hypothesis. The alkali extraction stage removes more highly oxidised or accessible residual lignin from the KOD and KOQZ pulps which leads to the reduced yields observed for the KODE and KOQZE pulps. The trend of increased yield of acidolysis lignin with the degree of oxidation is also observed with the slight increases in yield of acidolysis lignins from the pulps in the order of K< KO< KOx (where x pertains to the third bleaching step).

The balance of acidolysis lignin and the lignin remaining in the acidolysis residue shows that the majority of lignin dissolved during the acidolysis cook is recovered from solution. Less than 10% of the initial residual lignin is lost during the isolation. However, KP3 was the exception; a slightly higher percentage of the acidolysis lignin from this pulp was not recovered from solution. The kraft pulp acidolysis lignin may contain a larger proportion of low molecular weight fragments that do not precipitate from solution. These fragments could arise from acid-catalysed degradation of the kraft residual lignin during isolation or be present initially in the kraft pulp. Two possible reasons for the difference in behaviour of the bleached and unbleached pulp residual lignins are that any low molecular weight material present in the kraft pulp would most likely be removed during bleaching (and thus not be present in the bleached pulps); or the residual lignin from the bleached pulps is more resistant to acid-catalysed degradation.

Alternatively, these variations in the balance of lignin between the bleached and unbleached pulps may result from errors associated with estimating lignin content from the kappa number (lignin = kappa × 0.15). One problem is that hexeneuronic acids may be present initially in the pulps. These acids contribute to kappa number and will lead to an over estimated lignin content. Further, these hexeneuronic acids are removed from the pulp residue during acidolysis and this would ultimately lead to an overestimation of the amount of lignin lost during the isolation. Additionally, for the bleached pulps, the relationship between lignin content and kappa number may alter if the lignin becomes more oxidised and consumes less permanganate per unit of lignin.

During isolation, the residual lignin from the second kraft pulp sample (KP4) behaved in a slightly different manner to the residual lignin from KP3. The yield of acidolysis lignin from KP4 was slightly lower than from KP3. Additionally, the acid solution remaining after removal of the precipitated lignin from KP4 was less coloured compared to KP3 suggesting a lower amount of the acidolysis lignin from KP4 was lost compared to KP3. These variations may be due to differences in the structure of the residual lignin since KP4 was cooked to a lower lignin content than KP3. During the final phase of kraft pulping lignin condensation reactions increase (Gierer 1980), thus the residual lignin in KP4 can be expected to be slightly more condensed than the lignin from KP3. Additionally, Pekkala (1985) showed that molecular weight of the residual lignin also increases during the final phase of kraft pulping, therefore the residual lignin from KP4 could also be expected to have a slightly higher molecular weight. Differences in molecular weight and degree of condensation could explain the differences in behaviour of the residual lignins from these two kraft pulps.

The yields of acidolysis lignin from the pulps are considerably larger than the yield obtained from wood (Section 4.4.1) using similar conditions illustrating inherent differences in the accessibility of the lignin. In wood, the lignin is tightly associated with carbohydrates in the cell wall matrix and is difficult to access, whereas in the pulp fibre, the cell wall matrix has been disrupted to a certain degree so the lignin is more accessible. Differences in the structure of the lignin will also play a role in determining its extractability; for example, residual lignin is considered to be more hydrophilic than wood lignin so will tend to be more soluble.

The acid lignins isolated from each pulp in separate experiments were combined and characterised as discussed in Section (6.5). For KP3, the first acidolysis lignin fractions of K1, K2, and K3 were combined for further analysis, as were the second acidolysis fractions of K2 and K3. The first acid fraction will be referred to as K1-F1, the second as K1-F2, and the lignin from KP4 as K4.

6.4.3 Isolation of residual lignin from digested pulps

For comparison, the residues remaining after enzyme digestion of the kraft, KO, and KOQP pulps (Chapter 5) were extracted using acidolysis. During the enzyme treatment a fraction of residual lignin is lost from the pulp residues to the buffer solution (Figure 5.7). Therefore, the acidolysis lignin isolated from the digested pulp residues may originate from a slightly different fraction of residual lignin compared to the acidolysis lignin isolated from an untreated pulp. The properties of the pulp residues and the yields of acidolysis lignin are summarised in Table (6.4).

Table (6.4). Yields of residual lignin isolated from enzyme treated pulps by acidolysis

Pulp sample	**Klason lignin**[1]	**Lignin lost**[2]	**Yield of acidolysis lignin (as a percentage of)**	
			Klason lignin	Initial lignin
KP3	15.9	14	48	25
KOP2	20.3	9	47	33
KOQP2	15.8	0	19	25

1. Klason lignin content of digested pulp residues (g/100 g)
2. Lignin lost from residue during enzyme treatment (% initial lignin)

Approximately half of the Klason lignin remaining in the digested kraft and KO pulps was isolated as acidolysis lignin. This is almost equivalent to the yields of acidolysis lignin isolated from the untreated pulps (ie K3 and KO1 in Table 6.2) with respect to the initial lignin content. Another interesting observation is that the quantities of lignin lost to the buffer during enzyme treatment of the pulps are essentially the same as the quantities of lignin not recovered after acidolysis of the untreated pulps. This result could suggest that the lignin isolated from both the enzyme treated and untreated pulps is the same. Additionally, the fraction of residual lignin that is lost to the buffer during the enzyme treatment may be the same as the lignin that is not recovered after acidolysis of the untreated pulps.

A lower yield of acidolysis lignin was isolated from the KOQP pulp digest relative to its Klason lignin content, although, it was shown that there was an error in the Klason lignin content of this residue (Section 5.5).

6.5 Characterisation of Residual Lignins

6.5.1 Elemental composition

6.5.1.1 Residual lignin

From the elemental analysis and methoxyl content (Appendix 2) the average elemental compositions per 100 carbon atoms (not including the methoxyl carbons) for the various residual lignins were calculated. These are shown in Table (6.5) along with the composition of an enzyme isolated wood lignin (CELF1, Chapter 4) for comparison.

Table (6.5).[1]Elemental composition (per C100) of residual lignins isolated from kraft pulps by acidolysis

	C	H	O	S	Cl	OCH_3	DBE[2]
CELF1	100	83	33	0	0	10.3	5.8
K1-F1[3]	100	91	29	1.3	0	7.0	5.6
K1-F2[4]	100	90	33	1.1	0	7.1	5.6
K4[5]	100	97	25	1.0	0	7.4	5.3
KO	100	91	22	0.6	0	6.0	5.6
KOQP	100	96	31	0.7	0	7.6	5.3
KOQPn	100	87	33	0.6	0	7.8	5.7
KOQZ	100	90	33	0.7	0	6.9	5.6
KOQZE	100	96	31	0.7	0	7.0	5.4
KOD	100	94	37	0.7	0.6	6.5	5.5
KODE	100	97	32	0.6	0.2	6.2	5.4

1. H, O and S vary by 2%; Cl, OCH_3 do not vary significantly
2. DBE: Double bond equivalents; error (determined from σ_2) is ± 0.1
3. Combined acidolysis lignin 1 fractions from KP3
4. Combined acidolysis lignin 2 fractions from KP3
5. Acidolysis lignin from KP4

The double bond equivalents (DBE) shown in Table (6.5) is a measure of the degree of unsaturation in the residual lignin (Robert et al. 1984). It is calculated according to the following equation, where a and b are the C and H contents (in moles per C9; including methoxyl atoms), respectively.

$$DBE = \frac{(2a + 2) - b}{2}$$

Changes in DBE between samples can be used to indicate changes in the levels of double bonds, ring structures and/or interunit linkages within the lignin (Robert et al. 1984).

It can be seen from Table (6.5) that the composition of all residual lignins are remarkably similar and the main difference is the increase in oxygen content in the bleached residual lignins. This is consistent with the idea that the lignin, which has been exposed to the bleaching chemicals, is removed from the fibre, leaving an essentially unmodified residual lignin. In contrast, the differences between the kraft lignins and CELF1 are more significant. This shows that pulping affects the lignin structure more dramatically than the subsequent bleaching stages.

Changes in the lignin compositions and DBE are discussed in the following, with respect to the chemistry of pulping and bleaching. The results are also compared to those found in literature.

Unbleached residual lignin

Comparison between the composition of CELF1 and K1-F1 shows that a decrease in the methoxyl content, an increase in sulfur, and a slight decrease in the DBE occurs in residual lignin after pulping. The decrease in methoxyl content is consistent with the expected demethylation reactions that occur during pulping (Mckean et al. 1965). The incorporation of sulfur indicates the residual lignin has been in contact with the pulping chemicals and that perhaps the lignin accessibility is not a limiting factor during pulping. The reduction in DBE could be due to the cleavage of aryl ether linkages, as suggested by Robert et al. (1984), which results in an increase in phenolic hydroxyl groups and thus a reduction in DBE. Additionally, side chain reduction will also cause a decrease in DBE.

There is no significant difference in the composition of the two acidolysis lignin fractions (K1-F1 and K1-F2) from the kraft pulp, which could suggest a lack of heterogeneity in the residual lignin. This is not consistent with the results of Johansson (1997) who reported variations in the composition of different CEL fractions from *Pinus radiata* kraft pulp. Jiang & Argyropoulos (1997) obtained lower yields of acidolysis lignin from hemlock kraft pulps when using a batch process as compared to a continuous flow process and concluded that re-deposition of lignin fragments onto the pulp fibres during the batch process was a likely cause. Therefore, it is possible that re-deposited lignin is the source of the second acidolysis fraction (K1-F2) and this would explain the similarities in the composition of the two fractions. The composition of K4 is also similar to the K1-F1, even though the K4 lignin was obtained from a kraft pulp of a significantly lower kappa number.

The compositions of residual lignin isolated from different kraft pulps are compared in Table (6.6). Similar trends in terms of decreasing methoxyl content and incorporation of sulfur during pulping are observed in each case. However, there is quite a significant variation in the behaviour of the hydrogen content (and consequently DBE), as well as the oxygen content, between wood lignin and kraft pulp residual lignin. The hydrogen and oxygen contents are more strongly influenced by contaminants such as moisture, protein, and carbohydrates (particularly for the enzyme isolated samples). Differences will also arise due to the different wood species, pulping processes, and isolation procedures involved. As would be expected the results of Johansson (1997) are the most comparable, since the wood species is the same and the pulps were obtained from the same mills.

Table (6.6). Elemental compositions of kraft pulp residual lignins reported in literature

Reference	Sample (Kappa)[1]	Yield (%)[2]	Elemental composition[3] (C100)					DBE
			C	H	O	S	OCH_3	
This study; Radiata pine; acidolysis isolation	CELF1	–	100	83	33	0	10.3	5.8
	K1-F1 (28.0)	30	100	91	29	1.3	7.0	5.6
	K1-F2	10	100	90	33	1.1	7.1	5.6
	K4 (22.0)	23	100	97	25	1.0	7.4	5.3
Johansson (1997); Radiata pine; CEL method	Kraft (26.2)	69	100	98	37	1.0	8.8	5.2
Yamasaki et al. (1981); Loblolly pine; CEL method	MWL	–	100	75	28	0	12.1	6.1
	Kraft (35.6)	23	100	72	18	1.1	10.2	6.3
Pekkala (1985); Scots pine; acidolysis isolation	Wood	–	100	92	32	0.1	10.8	5.4
	Kraft (33.9)	64	100	84	35	0.7	8.2	5.9
Froass et al. (1996); Southern pine; acidolysis	Wood	–	100	106	34	0	9.0	4.8
	Kraft (27.4)	–	100	90	38	0.8	8.1	5.6
Jiang & Argyropoulos (1997); Spruce; acidolysis	MWL	–	100	113	40	0	9.7	4.5
	Kraft (29.4)	30.9	100	110	32	0.6	7.3	4.7

1. Kappa number of original pulp
2. Yield of isolated as a percentage of total pulp residual lignin (estimated from kappa number)
3. Where necessary, literature compositions were converted to compositions per C100 and corrected for carbohydrate and protein content where possible

Oxygen bleached residual lignin

The composition of the KO residual lignin (Table 6.5) is not significantly different from the kraft pulp residual lignin (K1-F1) except for a slightly lower methoxyl content and a lower sulfur content. The trend of decreasing methoxyl content following an oxygen stage is consistent with other reports (Table 6.7). However, in most of these studies no significant difference in the sulfur content of the kraft and kraft-oxygen residual lignins was reported. The variation observed here, may therefore, be a result of variations in the particular pulp samples analysed since the kraft and kraft-oxygen pulps were not obtained from the same sequence. Another possibility is that the higher sulfur content in the kraft residual lignin is due to the presence of elemental sulfur, which is then removed in the subsequent oxygen bleaching stage. Sun & Argyropoulos (1995) also reported a decrease in sulfur content after oxygen bleaching (Table 6.7).

The apparent decrease in oxygen content that is observed in this study is not consistent with other reports and is difficult to explain since an increase in oxygen content would be expected. This result also shows that comparison between the kraft and kraft-oxygen pulps is not entirely satisfactory due to the fact that they are not from the same sequence.

In this study no significant change in the DBE between a kraft residual lignin (K1-F1) and a KO residual lignin was reported. This further demonstrates the similarity between the residual lignin before and after the oxygen stage and indicates that most of the lignin which is modified during the oxygen stage is removed leaving behind a lignin which is essentially the same as the kraft residual lignin.

Table (6.7). Elemental compositions reported in literature of residual lignins isolated from kraft and kraft-oxygen pulps

Reference	Sample (Kappa)[1]	Yield (%)[2]	Elemental composition (C100)[3]					DBE
			C	H	O	S	OCH_3	
This study; Radiata pine;	K1-F1 (28.0)	30	100	91	29	1.3	7.0	5.6
acidolysis	KO (18.4)	35	100	91	22	0.6	6.0	5.6
Johansson (1997);	Kraft (26.2)	69	100	98	37	1.0	8.8	5.2
Radiata pine; CEL	KO (18.9)	54	100	90	38	1.1	9.1	5.5
Jiang & Argyropoulos	Kraft (29.4)	30.9	100	110	32	0.6	7.3	4.7
(1997); Spruce; acidolysis	KO (17.0)	28.9	100	116	35	0.7	5.5	4.5
Gellerstedt & Heuts (1997);	Kraft (22.7)	–	100	88	30	0.9	8.8	5.6
Softwood; acidolysis	KO (14.7)	–	100	86	30	0.9	7.8	5.8
Hortling et al. (1991)	Kraft (31.4)	82	100	91	38	1.1	9.5	5.5
CEL method	KO (16.2)	55	100	123	44	1.2	7.1	4.1
Sun & Argyropoulos (1995);	Kraft (34.3)	–	100	96	27	0.7	7.8	5.3
Black spruce; acidolysis	KO (17.9)	–	100	106	32	0.4	6.3	4.9

1. Kappa number of original pulp
2. Yield of lignin as percentage of total pulp residual lignin (estimated from kappa number)
3. Where necessary literature compositions were converted to compositions per C100 and corrected for carbohydrate and protein content where possible.

Bleached KO residual lignin

In subsequent bleaching stages a slight increase in the oxygen content of the residual lignins is observed (Table 6.5) indicating a slight increase in the degree of oxidation of the residual lignin has occurred. Apart from this change the compositions are quite similar demonstrating the similarity between the residual lignins. The lack of chlorine in all the isolated lignins except those from chlorine dioxide bleached pulps shows that the lignin is not being chlorinated to a significant extent during isolation. The incorporation of chlorine during chlorine dioxide bleaching is believed to be due to hypochlorous acid which is produced during bleaching (Kolar et al. 1983) and, as expected, the majority of chlorinated lignin is removed in the subsequent extraction stage.

Table (6.8) compares the elemental composition of the bleached lignins with other compositions reported in literature. The results are consistent in showing the similarities between the residual lignin compositions and the general trend of increased oxygen content. The most significant difference between the results in this study and the other results is the difference between the methoxyl content of the lignin isolated from the bleached pulps compared to the KO residual lignin. This is probably due to the pulps being bleached under different conditions to different kappa numbers. This is especially the case for the KOD and KODE pulps, which have a significantly lower kappa number compared to the original KO pulp.

The hydrogen and oxygen contents reported by Johansson (1997) are higher compared to those found in this study. The KOD and KODE pulps studied by Johansson (1997) have a significantly lower kappa number than those in this study, so differences in the hydrogen and oxygen contents of the residual could be expected. However, the residual lignins isolated from the ozonated pulps also have higher hydrogen and oxygen contents, even though the kappa numbers of these pulps are similar to those in this study.

Table (6.8). Elemental compositions reported in literature of residual lignins isolated from bleached kraft pulps

Reference	Sample (Kappa)[1]	Yield (%)[2]	Elemental composition (per C100)[3]						DBE
			C	H	O	S	Cl	OCH_3	
This study; Radiata pine; acidolysis isolation	KO (18.4)	35	100	91	22	0.6	0	6.0	5.6
	KOQP (14.7)	35	100	96	31	0.7	0	7.6	5.3
	KOQPn (9.9)	42	100	87	33	0.6	0	7.8	5.7
	KOQZ (14.0)	41	100	90	33	0.7	0	6.9	5.6
	KOQZE (10.6)	26	100	96	31	0.7	0	7.0	5.4
	KOD (14.3)	35	100	94	37	0.7	0.6	6.5	5.5
	KODE (11.0)	30	100	97	32	0.6	0.2	6.2	5.4
Johansson (1997); Radiata pine; CEL isolation	KO (18.9)	15	100	90	38	1.1	–	9.1	5.5
	KOQZ (13.2)	9	100	107	51	0.8	–	8.3	4.8
	KOQZE (10:3)	11	100	102	52	0.9	–	7.6	5.1
	KOD (7.0)	4	100	117	56	0.9	–	6.4	4.4
	KODE (3.1)	1	100	107	67	1.0	–	5.6	4.9
Gellerstedt & Heuts (1997); Softwood pulp; acidolysis	KO (14.7)	–	100	86	30	0.9	0	7.8	5.8
	KOQP (6.2)	–	100	96	43	0.6	0	7.1	5.4
	KODE (2.1)	–	100	104	48	0.9	1.1	4.9	5.1

1. Kappa number of original pulp
2. Yield as a percentage of total pulp residual lignin (estimated from kappa number)
3. Where necessary literature compositions were converted to compositions per C100 and corrected for carbohydrate and protein content where possible

The differences between these two studies may, therefore, be associated with the different isolation procedures. Johansson (1997) used an enzyme-based isolation procedure and the resulting lignins contain significant amounts of protein (8–20%, lowest for KO and highest for KODE). Protein and other impurities influence the hydrogen and oxygen contents (although a correction has been made). Another difference is that lower yields of lignin were isolated by Johansson (1997), particularly from the chlorine dioxide bleached pulps.

The residual lignin from the KOQP has a higher oxygen content and lower DBE compared to the KO residual and is consistent with the reactions taking place during a P-stage. Degradation of ortho quinones to muconic acid type structures and reaction of conjugated carbonyls leading to lignin fragmentation are predominant reacts taking place during a P-stage. These types of reactions lead to the incorporation of oxygen and a reduction in DBE. Most of the oxidised fragments can be expected to be removed from the fibre during bleaching, however, the fact that the KOQP lignin has a higher oxygen content and lower DBE than the KO lignin suggests not all the oxidised lignin is being removed.

In comparison, the KOQPn residual lignin has a slightly higher oxygen content than the KOQP residual lignin indicating that it is slightly more oxidised. Additionally, KOQPn has a higher DBE value. Kadla et al. (1998a, 1998b) showed that phenolic lignin model compounds are extensively degraded during cyanamide activated peroxide bleaching, whereas nonphenolic compounds do not react. This sequence of reactions would lead to enrichment of nonphenolic structures in the KOQPn residual lignin resulting in a larger DBE compared to KOQP (where less extensive degradation has taken place). The DBE of the KOQPn residual lignin is not significantly different from the KO residual lignin indicating that, in terms of undegraded aromatic structures and interunit linkages, the lignins are reasonably alike.

The composition of KOQZ residual lignin is also very similar to the KO residual lignin except for the increased oxygen content. Molecular weight determinations reveal that residual lignin is not significantly depolymerised during an ozone bleaching stage (Lachenal et al. 1995), although a significant amount of oxidation is observed. Therefore, the residual lignin in the KOQZ pulp can be expected to contain a number of oxidised groups and thus have a higher oxygen content. However, the relative contents of carbon and hydrogen in the residual lignin should be essentially unchanged compared to KO residual lignin since very little fragmentation has occurred.

In comparison, the KOQZE residual lignin has a slightly lower oxygen content and a slightly lower DBE compared to KOQZ lignin. In the subsequent extraction stage saponification of muconic acid esters and ionisation of oxidised groups, results in fragmentation and dissolution of the oxidised lignin (Dence 1996a). The decrease in DBE will most likely be a result of the fragmentation reactions. However, only a slight decrease in the oxygen content of the residual lignin between KOQZ and KOQZE is observed suggesting a significant amount of oxidised groups still remain in the residual lignin after the extraction stage.

The KOD residual lignin has a higher oxygen content and a slightly lower DBE value compared to the KO residual lignin. Chlorine dioxide reacts predominantly with phenolic structures giving rise to muconic acid esters, ortho or para quinones, and side

chain oxidation products. The oxidised lignin will not be very soluble in the acidic media so only small fragments will be removed from the pulp fibre during the D-stage and hence the KOD pulp residual lignin has a higher oxygen content and lower DBE value compared to the KO residual lignin. The oxygen content of the KOD residual lignin is higher than the KOQZ pulp residual lignin suggesting that chlorine dioxide oxidised the residual lignin remaining in the fibre more than ozone under the conditions used for bleaching. The lower DBE value could be a result of differences in the degree of lignin fragmentation or the demethylation that occurs during chlorine dioxide bleaching. In the subsequent extraction stage the majority of the oxidised and/or chlorinated lignin is removed resulting in similar effects to those observed in the ozone pulps. This is apparent from the lower oxygen and chlorine contents of the KODE residual lignin compared to the KOD residual lignin.

6.5.1.2 Spent liquor lignin

The compositions of the spent liquor lignins from the peroxide bleaching and alkali extraction of the ozonated pulp are compared to the corresponding residual lignins in Table (6.9). The spent liquor lignins have a higher oxygen content than the corresponding residual lignin suggesting they are more oxidised. This result is consistent with other findings (Gellerstedt & Heuts 1997) and also consistent with the hypothesis that the oxidised lignin is removed from the fibre during bleaching leaving behind a less altered residual lignin. The lower methoxyl content of the KOQZE spent liquor lignin could be a result of aromatic ring degradation reactions during ozonation and the subsequent saponification of the muconic acid methyl esters in alkali.

Table (6.9). Elemental composition (C100) of the spent liquor lignins

	C	H	O	S	OCH_3	%N
KOQP	100	97	31	0.7	7.6	0.23
KOQPs[1]	100	82	32	0.7	7.2	0.46
KOQPn	100	88	33	0.6	7.8	0.22
KOQPns[2]	100	78	37	0.9	7.4	0.48
KOQZ	100	90	33	0.6	7.1	0
KOQZE	100	95	30	0.7	7.0	0.16
KOQZEs[3]	100	92	39	0.6	5.8	0.48

1. Spent liquor lignin from hydrogen peroxide bleaching
2. Spent liquor lignin from cyanamide activated peroxide bleaching
3. Spent liquor lignin from alkali extraction of ozonated pulp

Nitrogen is present in nearly all the lignin samples. The nitrogen contents of the residual lignins are similar to the nitrogen contents of the residual lignin isolated from KP4 and KOP2 (K4 and KO-F2, Appendix 2). This nitrogen probably arises from small amounts of protein present in the samples and presumably originates from the wood. The higher amount of nitrogen in the spent liquor lignins suggests the alkali is removing protein from the pulps. Additionally, the similarity in the nitrogen contents of the KOQP and KOQPn lignin indicates there is no significant incorporation of nitrogen from the cyanamide during the Pn-stage.

6.5.1.3 Residual lignin from enzyme treated pulps

The elemental compositions of the lignins isolated from the digested pulps are compared to the corresponding lignins isolated from the untreated pulps in Table (6.10).

Table (6.10). Elemental composition (C100) of the acidolysis lignins isolated from the enzyme treated pulps

	C	H	O	S	OCH_3	%N
K1-F1	100	91	29	1.3	7.0	–
K-dig[1]	100	90	29	1.3	7.4	2.15
KO	100	91	22	0.6	6.0	–
KO-dig[1]	100	84	32	0.8	8.1	1.40
KOQP	100	96	31	0.7	7.6	–
KOQP-dig[1]	100	92	29	0.7	7.9	1.39

1. Compositions are corrected for protein

The residual lignin isolated from the enzyme treated kraft and KOQP pulps do not show any significant differences in composition compared to the acidolysis lignins from the untreated pulps. However, residual lignin isolated from the enzyme treated KO pulp has a higher oxygen and lower methoxyl content than the residual lignin from the untreated KO pulp which could indicate a heterogeneity in the KO residual lignin. In fact, the composition of the lignin from the digested KO pulp could be considered to be more like the kraft residual lignin in terms of the oxygen and methoxyl content suggesting this lignin fraction is less modified than the acidolysis lignin from the untreated KO pulp. Johansson (1997) also showed that a subsequent CEL fraction isolated from a KO pulp showed closer similarity, in composition, to a kraft residual lignin.

6.5.2 Phosphorus NMR results

Two major functional groups in lignin that are important during pulping and bleaching are phenolic hydroxyl groups and carboxylic acids. Cleavage of aryl ether linkages during pulping releases new phenolic hydroxyl groups, which are important sites of reactivity in subsequent bleaching stages. Therefore, changes in the phenolic hydroxyl content can be used to gauge the degree of degradation that has occurred during pulping and bleaching. Carboxylic acid groups are introduced into the lignin during oxidative degradation, so these groups can also be used as a measure of the degree of lignin degradation.

The distribution of hydroxyl groups in the residual lignins and spent liquor lignins was determined using the method of Granata & Argyropoulos (1995). This method allows quantification of both phenolic and aliphatic hydroxyl groups, as well as, carboxylic acids in lignin. Additionally, C5-condensed phenolic units (β-5, 4-O-5, and 5-5) can be distinguished from uncondensed phenolic structures allowing possible differences in selectivity of each bleaching reagent to be observed.

The ^{31}P-NMR spectra for kraft, KO, KOQPn, KOQZ, and KOQZE are compared to an isolated wood lignin (CELF2) in Figure (6.3). The spectra for the other residual lignin samples are similar to the shown residual lignins. It can be seen that the ^{31}P-NMR signals for the residual lignins are broader than CELF2. This indicates that the residual lignin either has a greater heterogeneity in its structure or a higher molecular weight compared to wood lignin. The broadness of the peaks also resulted in considerable overlap of the individual signals, consequently the peaks were not completely resolved leading to inaccuracies in the quantification. Additionally, several sharp peaks appeared in the residual lignin spectra (Figure 6.3 (S)), which were regarded as not being due to the lignin. Although these were subtracted from the integrals during quantification they will introduce further errors. However, since all signals were integrated in the same manner comparisons between the samples can still be made. The results are shown in Table (6.11).

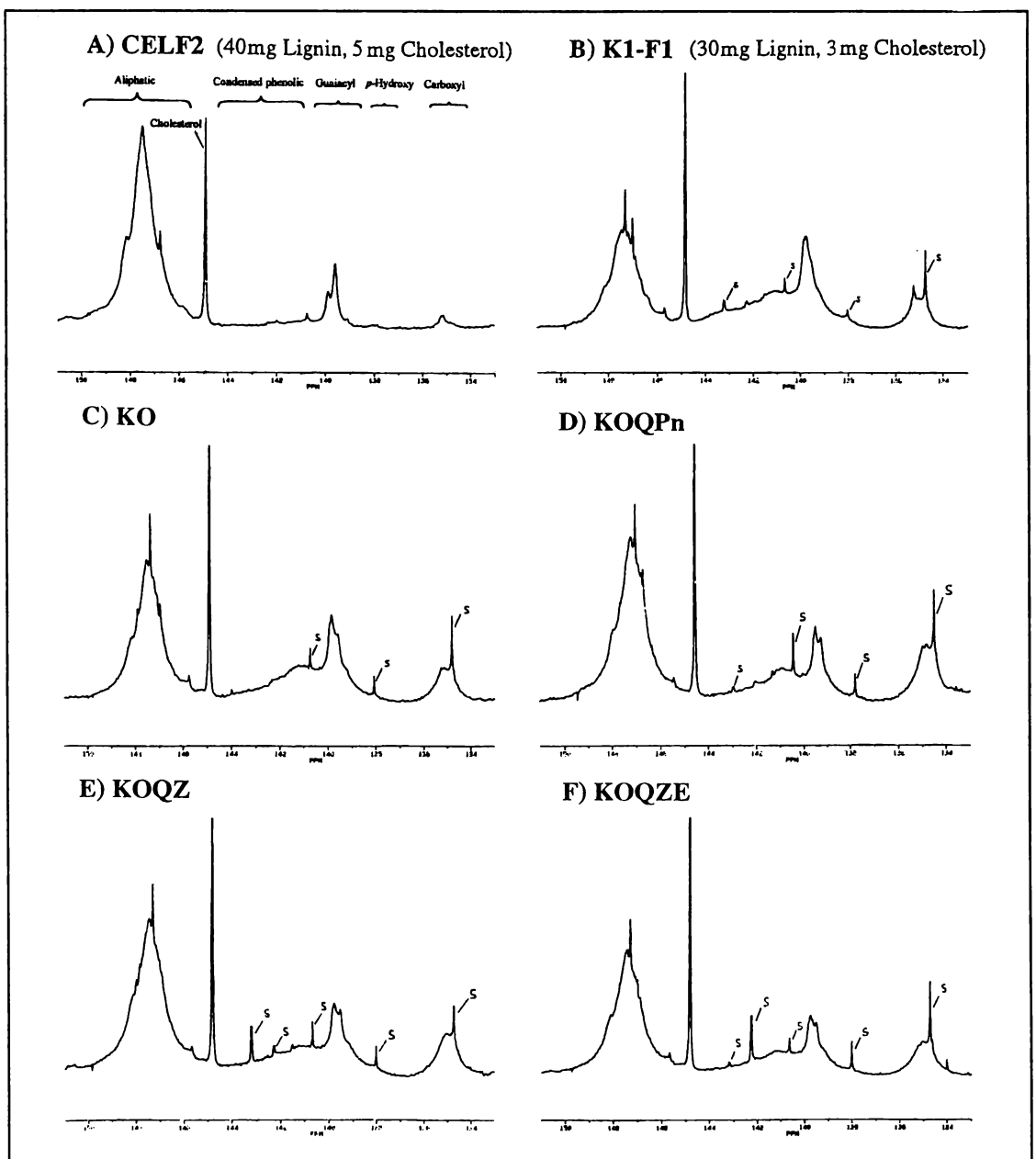

Figure (6.3). Quantitative ^{31}P-NMR spectra, and signal assignment of wood lignin (A) along with spectra of residual lignins isolated from kraft (B), kraft-oxygen (C), KOQPn (D) and ozonated pulps (E, F). The sharp peaks regarded as not arising from lignin are labelled (S). Residual lignins were analysed at the approximate quantities shown in parentheses for K1-F1.

Table (6.11). Hydroxyl content (mmol/g lignin) of acidolysis lignins isolated from kraft pulps

	Aliphatic	Phenolic					COOH
		Cond.[2]	G	H	Total	Cond. / Uncond.	
CELF1	4.39	0.24 (2)	0.41 (3)	0.04 (1)	0.68 (4)	0.53	0.06 (1)
CELF2	5.10	0.37 (6)	0.57 (6)	0.04 (1)	1.05 (5)	0.54	0.15 (3)
K1-F1	1.45 (8)	0.78 (5)	0.74 (2)	0.06 (1)	1.58 (8)	0.98	0.3 (1)
K1-F2	2.21	0.80	0.73	0.09	1.62	0.98	0.29
K-dig	1.98	0.76	0.69	0.13	1.59	0.93	0.23
K4	1.98	0.97	0.88	0.05	1.90	1.04	0.18
KO	1.71	0.63	0.55	0.06	1.24	1.03	0.30
KO-dig	2.58	0.71	0.65	0.09	1.45	0.96	0.29
KOQP	2.3 (1)	0.61 (2)	0.57 (4)	0.05 (1)	1.20 (3)	0.98	0.39 (6)
KOQPn	2.12	0.58	0.44	0.03	1.05	1.23	0.52
KOQZ	1.96	0.56	0.45	0.05	1.07	1.12	0.34
KOQZE	2.07	0.54	0.52	0.04	1.10	0.96	0.35
KOD	2.1 (2)	0.61 (6)	0.47 (3)	0.06 (1)	1.1 (1)	1.14	0.43 (3)
KODE	2.4 (3)	0.53 (3)	0.46 (4)	0.04 (1)	1.02 (5)	1.06	0.40 (8)
KOQPs[3]	2.20	0.53	0.46	0.03	1.02	1.08	0.64
KOQPns[3]	2.17	0.49	0.34	0.05	0.88	1.26	0.69
KOQZEs[3]	2.06	0.30	0.26	0.03	0.59	1.03	0.41

1. Numbers in parentheses indicate the variation of last significant figure as determined from the standard deviation between duplicate experiments
2. Condensed phenolic structures (β-5, 4-O-5, and 5-5)
3. Spent liquor lignins from bleaching sequence.

Comparing CELF1 with kraft residual lignin (K1-F1) it can be seen that the total phenolic hydroxyl content of the lignin is more than doubled during pulping. This has been reported by others (Yamasaki et al. 1981, Jiang & Argyropoulos 1997) and is consistent with the cleavage of aryl ether linkages during pulping which generates new phenolic hydroxyl groups in the residual lignin. The slight increase in carboxylic acids is likely to be due to enrichment of existing acid groups since the conditions during pulping are not likely to be oxidative. The lower amounts of aliphatic hydroxyl groups in the kraft residual lignin is consistent with the loss of the γ-OH by elimination and side chain reduction reactions that take place during kraft pulping. Similar trends were observed by Yamasaki et al. (1981), Froass et al. (1996), and Jiang & Argyropoulos (1997).

There is no significant difference in the total phenolic hydroxyl content between K1-F1, K1-F2 and K-dig. This is consistent with the elemental compositions and gives further evidence for the lack of heterogeneity in the structure of the kraft residual lignin. The most significant difference between these lignins is the variation in the aliphatic OH groups. The reasons for this are unclear but may be due to the presence of impurities in the K1-F2 and K-dig lignins. The H-phenolic content of K1-F2 and K-dig lignins is also larger than K1-F1, however, due to the large error associated with integration of this peak, the observed difference is not considered significant. The increased total phenolic OH content and slightly higher amount of condensed structures of K4 is consistent with a residual lignin isolated from a pulp that has been cooked to a lower kappa number. That is, a greater degree of β-O-4 cleavage resulting in a higher phenolic OH content and an increase in the amount of condensation with the extension of delignification into the residual phase during kraft pulping (Gierer 1980).

In the subsequent oxygen stage the free phenolic units are the predominant sites for reaction and this is consistent with the reduction in phenolic OH content in the KO residual lignin. The acidolysis lignin isolated from the enzyme digested KO pulp (KO-dig) has a higher phenolic OH content compared to the KO lignin and shows some similarities to the kraft residual lignin. Part of the residual lignin from the KO pulp is lost during the enzyme treatment, therefore, both KO and KO-dig may originate from a slightly different fraction of lignin. The phosphorus results indicate the lignin isolated from the KO-dig is less modified compared to KO. A possible conclusion is that the lignin which is solubilised during enzyme treatment originates from more exposed parts of the residual lignin and is perhaps more modified compared to the lignin that remains in the residue after the enzyme treatment. Additionally, the lignin isolated from the untreated KO pulp may contain a fraction of the lignin that is solubilised during the enzyme treatment. This could explain the observed differences in the composition and content of hydroxyl groups between these two lignin fractions. It would also be consistent with the idea that during oxygen delignification, the exposed lignin in the fibre is oxidised and removed leaving behind a residual lignin which is oxidised at the surface but relatively untouched at the inner parts of the lignin.

The phosphorus results complement the elemental composition data in showing the bleached residual lignins are all remarkably similar and that the lignin which is more heavily degraded is dissolved during bleaching. The slight differences in the residual lignin can be explained in terms of the chemistry that is taking place in each step.

The KOQP lignin has only a slightly lower phenolic hydroxyl content than the KO lignin indicating that either the phenolic structures are not being degraded or that both phenolic and nonphenolic structures are being removed by the same extent. During hydrogen peroxide bleaching the hydroperoxide anion (HOO^-) is a major reactive species. Since it is a nucleophile, the hydroperoxide anion does not react with the aromatic ring but mainly reacts with carbonyl and conjugated carbonyl structures. Degradation of aromatic structures will largely be through the action of radical species (such as, $OH^{\bullet}$) arising from the degradation of the hydrogen peroxide, thus a non-selective degradation of aromatic structures could be expected. The increase in carboxylic acids will mainly arise from the degradation of ortho quinoid structures by the hydroperoxide anion and/or from the degradation of phenolic units by radical species.

The KOQPn lignin has a significantly lower phenolic content and a significantly higher carboxylic acid content than the KO lignin. This is consistent with the results of Kadla et al. (1998a, 1998b) rather than the results of Smit et al. (1996). Kadla et al. (1998a) showed that lignin model compounds with a free phenolic hydroxyl group are extensively degraded during cyanamide activated hydrogen peroxide bleaching but etherified model compounds are not. This will lead to the observed decrease in the phenolic hydroxyl content of the KOQPn residual lignin. Kadla et al. (1998b) also suggest the superoxide anion radical plays a predominant role in the delignification during cyanamide bleaching. Therefore, the carboxylic acids in the KOQPn residual lignin may be of the muconic acid ester type arising from extensive degradation of phenolic units by the superoxide anion radical.

Both the KOQP and KOQPn spent liquor lignins have a lower phenolic content and higher carboxylic acid content than the corresponding residual lignin. This is consistent with the elemental composition results and suggests the dissolved lignins are more extensively degraded than the corresponding residual lignins.

The KOQZ residual lignin has a lower phenolic content than the KO residual lignin suggesting ozone also has a preference for unetherified phenolic units since if both free and etherified phenolic units were reacting at the same rate there would be no change in the relative amounts of phenolic units. This result is consistent with the results of Eriksson & Gierer (1985) that report differences in the reactivity of free and etherified model compounds with ozone. After the subsequent extraction stage a slight increase in the total phenolic structures is observed in the residual lignin. This is possibly a result of enrichment of undegraded units via the removal of the degraded lignin structures. However, the carboxylic acid contents of the KOQZ and KOQZE lignins are essentially the same, which is not consistent with the idea that the oxidised lignin is removed from the fibre, since a decrease would be expected. The spent liquor lignin is, however, enriched in carboxylic acids indicating that perhaps a lignin enriched with these structures is being preferentially removed from the fibre.

Similarly, the lower phenolic OH content of the KOD residual compared to the KO residual lignin is consistent with the expected preferential degradation of phenolic structures during chlorine dioxide bleaching. The phosphorus results also show that the residual lignin from the KOD and KODE pulps are quite similar and only a slight decrease in the total phenolic hydroxyl groups after extraction stage is observed. This suggests that the lignin fragments, which are removed from the KOD pulp during the extraction stage, have a slightly higher concentration of phenolic structures compared to the residual lignin left in the fibre. It would also explain the apparent decrease of phenolic structures in the KODE residual lignin compared to the KOD residual lignin.

By comparing the ratio of condensed phenolic units to uncondensed phenolic units between different residual lignins, an indication of the selectivity of a particular reagent can be obtained. For example, an increase in the ratio indicates a preference for uncondensed structures. The kraft pulp residual lignins have a higher proportion of condensed structures compared to CELF1. This increase is either a result of the enrichment of existing condensed structures that are unreactive during the pulping process and/or a result of their formation during pulping. Froass et al. (1996) and Jiang & Argyropoulos (1997) have reported similar findings.

Oxygen has been shown to react preferentially with uncondensed phenolic units (Johansson & Lundggren 1994). This will lead to enrichment of condensed phenolic structures in kraft-oxygen residual lignin. However, only a very small increase in C5-condensed phenolic units relative to uncondensed phenolic units was observed in the KO lignin using phosphorus analysis (Table 6.11). This result is consistent with the results of Gellerstedt et al. (1986) who bleached a pine kraft pulp (kappa 35.6) with oxygen to generate four KO pulps, having kappa numbers ranging from 22 to 11. The residual lignin was then analysed by permanganate oxidation. Their results (Table 6.12) showed that the KO pulp of kappa number 22, which has been delignified by the same extent as the KO pulp in this study (ie ca. 35%), had only a slightly higher content of C5-condensed structures relative to uncondensed than the original kraft pulp. As the delignification was extended to lower kappa number the relative proportions of

condensed structures increased. However, both Jiang & Argyropoulos (1997) and Gellerstedt & Heuts (1997) reported a more significant increase in C5-condensed structures in residual lignin isolated from a kraft-oxygen pulp (Table 6.12). The reasons for these differences in the relative amounts of condensed structures are unclear but may be due to the different wood species being analysed. Jiang & Argyropoulos (1997) also reported a similar increase in structures condensed at C6.

Table (6.12). The effect of an oxygen stage on the levels of condensed structures in kraft pulps

Reference	Pulp	Kappa number	Percent [1] delignification	Cond. / Uncond. [2]	KO / Kraft [3]
This study: Radiata pine	Kraft	28.0	–	0.98	–
^{31}P analysis.	KO	18.4	34	1.03	1.05
Gellerstedt et al. 1986: Pine,	Kraft	35.6	–	1.11	–
permanganate oxidation,	KO	22.0	38	1.15	1.04
in situ lignin.		17.2	52	1.74	1.57
		14.8	58	1.77	1.60
		11.4	68	1.79	1.61
Jiang & Argyropoulos (1997):	Kraft	29.4	–	1.54	–
Spruce, ^{31}P analysis.	KO	17.0	42	4.45	2.89
Gellerstedt & Heuts (1997):	Kraft	22.7	–	1.35	–
softwood, permanganate.	KO	14.7	35	1.88	1.39

1. Percent of kraft pulp residual lignin removed during oxygen stage
2. Ratio of C5-condensed phenolic units to uncondensed
3. Relative increase in cond./uncond. ratio in pulp after oxygen stage

Of the subsequent bleaching stages, the cyanamide activated peroxide stage shows the greatest selectivity difference; with preference for uncondensed structures (ie the condensed/uncondensed ratio is larger compared to KO lignin). This is not consistent with the results of Smit et al. (1996) that demonstrated cyanamide activated hydrogen peroxide reacted at an indistinguishable rate with both condensed and uncondensed phenolic model compounds. Kadla et al. (1998a, 1998b) in their model compound study, did not compare the reactivity of condensed and uncondensed models with cyanamide. Both chlorine dioxide and ozone show a slight preference for uncondensed structures, although the ratio of condensed to uncondensed structures decreases following the alkali extraction stage.

It should be pointed out that the acidolysis lignin might not be fully representative of the whole lignin. The results in Chapter 4 concerning the structural differences between the CEL lignin and acidolysis lignin from wood suggest that, at least in this case, the acidolysis method is isolating a less condensed type of lignin. A similar situation may be occurring for the isolation of residual lignin; that is, the acidolysis method is perhaps isolating the more soluble parts of the residual lignin and leaving behind a less soluble more condensed type of lignin. Additionally, a certain level of acid-catalysed modification to the lignin during isolation can be expected; for example, an increase in phenolic hydroxyl groups (Section 4.4). Therefore, the variation in condensed structures of the isolated residual lignins may not be fully representative of the lignin as a whole. Further, the residual lignin that is not isolated may be enriched in condensed structures so that the true selectivities in terms of condensed to uncondensed structures is perhaps are not being observed.

6.5.3 Periodate oxidation

Periodate oxidation is another method used to estimate the phenolic hydroxyl content of lignin (Lai et al. 1990). On reaction with sodium periodate, one mole of methanol is released per guaiacyl or substituted guaiacyl unit. The methanol is then quantified by GC and used to estimate the phenolic hydroxyl content of the lignin.

This method was applied to the acidolysis lignins isolated from kraft pulps. The behaviour of some of the lignins upon reaction with sodium periodate is illustrated in Figure (6.4) and the other lignin samples behaved in a similar manner to either the KOQPn or the KOD lignin.

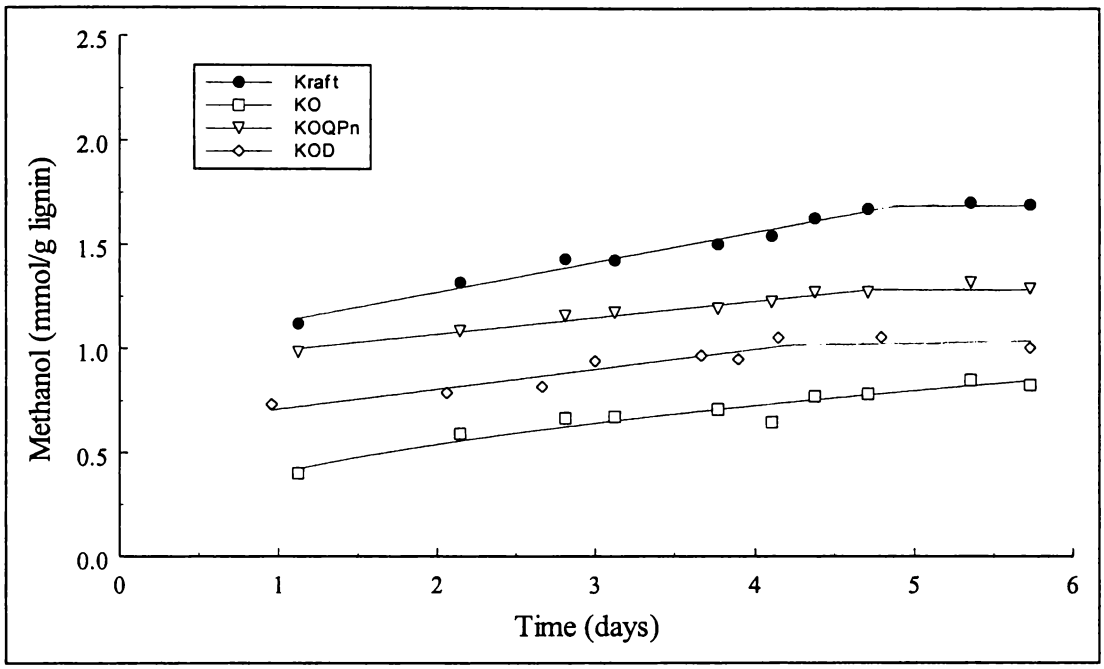

Figure (6.4). Methanol released from K-F1, KO, KOQP, and KOD lignins during periodate oxidation

The phenolic hydroxyl content of the samples was estimated from the average of the last four methanol determinations. These are shown in Table (6.13) along with the estimates obtained by phosphorus analysis. The KO, KOQPn, and KOD lignin samples were analysed twice and the estimate of the phenolic content of the duplicates differed, at most, by 5%.

The estimates of the total phenolic hydroxyl content obtained by either method are reasonably comparable in the majority of cases. The lignin samples that show the best comparability are the kraft lignin, the KOQP lignin, and the lignins from the ozone treated pulps (KOQZ and KOQZE). The phenolic content of the KOD residual lignin estimated by periodate oxidation is lower compared to that obtained by phosphorus analysis. This can be explained if the KOD residual lignin has significant levels of catechol type structures, which are measured as phenols by the phosphorus method but not by the periodate method.

Table (6.13). Total phenolic hydroxyl content of acidolysis lignins estimated by periodate oxidation and phosphorus NMR

Sample	Periodate oxidation[1] (mmol/g lignin)	^{31}P-NMR analysis[1] (mmol/g lignin)
K1-F1	1.67	1.58 (8)
KO	0.84 (4)	1.24
KOQP	1.23	1.20 (3)
KOQPn	1.24 (6)	1.05
KOD	1.05 (2)	1.1 (1)
KODE	1.17	1.02 (5)
KOQZ	1.09	1.07
KOQZE	1.14	1.10

1. The number in parenthesis indicates the variation of the last significant figure (determined from standard deviation)

The estimates obtained using periodate oxidation are larger than the phosphorus estimates for the KODE and KOQPn pulp residual lignins. The reasons for this are unclear but shows that either the phosphorus method is underestimating the phenolic content or the periodate method is overestimating the phenolic content of these lignin samples.

The KO lignin sample stands out the most, in that the estimate obtained from periodate oxidation is considerably lower than the estimate obtained by phosphorus analysis. This is most likely due to the fact that the periodate reaction is not complete after reaction for 6 days as shown in Figure (6.5). This plot demonstrates that even after 20 days there is no indication the reaction is complete.

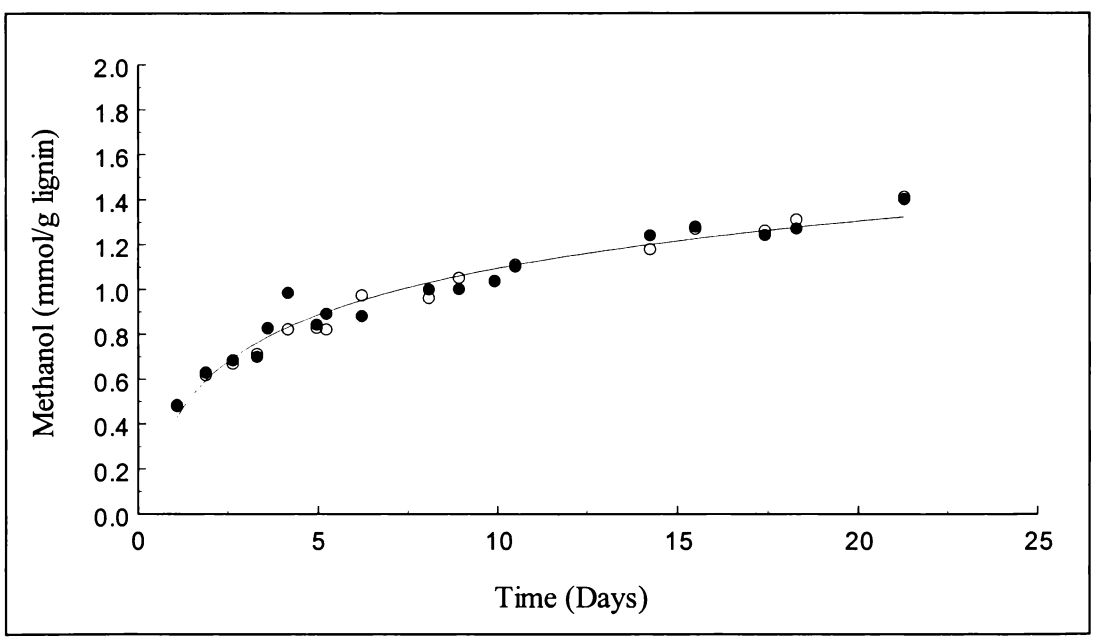

Figure (6.5). Methanol formation upon periodate oxidation of KO residual lignin (open and closed circles refer to individual KO lignin samples analysed)

6.5.4 Thioacidolysis

The isolated residual lignins were analysed by thioacidolysis following the method of Roland et al. (1992). The levels of uncondensed β-O-4 linkages in the lignin samples were evaluated from the yields of trithioether product 1 (Figure 6.6) by GC/FID analysis of the degradation products. Additionally, GC/MS analysis was used to identify other degradation products relating to certain end groups in the lignin (Table 6.14, Roland et al. 1992).

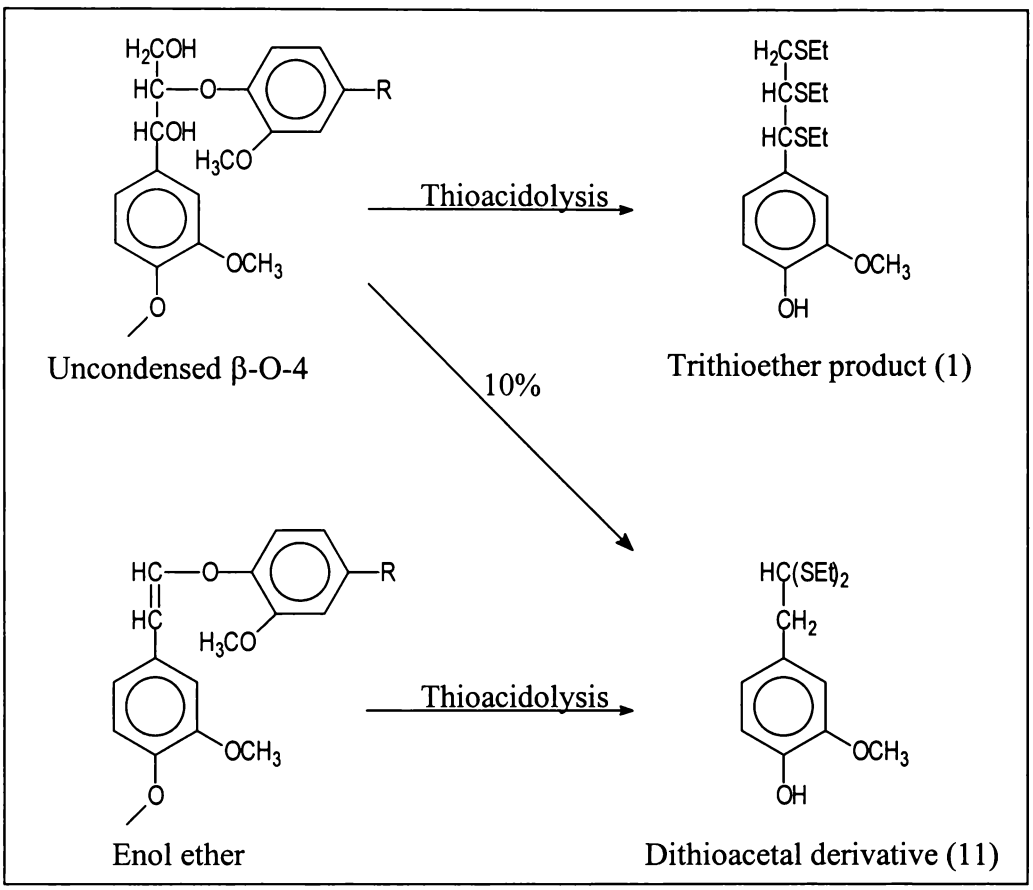

Figure (6.6). Thioacidolysis reactions of β-aryl ethers and enol ethers

Table (6.14). Main thioacidolysis degradation products from softwood lignin

	Monomer product[1]	**Lignin structure**
1	G-CHRCHRCH$_2$R	β-O-4; guaiacyl unit
2	G-CR=CHR	β-O-4 with Cα=O; guaiacyl
3	H-CHRCHRCH$_2$R	β-O-4; *p*-hydroxy phenyl unit
4	C-CHRCHRCH$_2$R	β-O-4; catechol unit
5	G-CHR$_2$	Vanillin
6	G-COOH	Vanillic acid
7	G-CH$_2$CH$_2$CH$_2$OH	Dihydroconiferyl alcohol
8	G-CH=CHCH$_2$R	Coniferyl alcohol
9	G-CHRCH$_2$CH$_2$R	Coniferyl alcohol
10	G-CHRCH$_2$CHR$_2$	Coniferaldehyde
11	G-CH$_2$CHR$_2$	Enol ether; guaiacyl unit
12	G-CH=CHR	Enol ether; guaiacyl unit
13	G-CHRCH$_2$-G	Stilbene; guaiacyl unit

1. R = SCH_2CH_3

The results from GC/FID analysis are shown in Table (6.15) while the yields of monomer degradation products determined by GC/MS analysis are shown in Table (6.16).

Table (6.15). Uncondensed β-O-4 content of acidolysis lignins isolated from kraft pulps as determined by GC/FID analysis

Lignin sample	**β-O-4 monomer yield** (μmol/g lignin)
Wood lignins	
Wood	1070
CELF1	1120
Residual lignins	
K1-F1	120
KO	220
KOQP	240
KOQPn	250
KOQZ	300
KOQZE	220
KOD	200
KODE	200
Spent liquor lignins	
KOQPs	170
KOQPns	130
KOQZEs	150

The results in Table (6.15) show that a certain amount of β-O-4 linkages survive the kraft cook and is consistent with earlier findings (Gellerstedt & Al-Dajana 1996, Johansson 1997). The increased level of uncondensed β-O-4 linkages in the residual lignin after oxygen bleaching is difficult to explain but may be due to the enrichment of these structures in the lignin. As suggested above, oxygen may only be reacting with the exposed parts of the residual lignin in the fibre. This would mainly result in the outer surfaces of the residual lignin being oxidised and removed and could lead to an apparent increase in β-O-4 linkages if they are relatively stable under oxygen bleaching conditions. Alternatively, the apparent increase may be due to variation in the particular pulp samples analysed since the kraft and kraft-oxygen pulps were not obtained from the same sequence.

During the subsequent bleaching steps the β-O-4 content remains essentially unchanged indicating there is no preference for (or against) these ether linkages during TCF and ECF bleaching sequences. The spent liquor lignins contain fewer β-O-4 linkages than the corresponding residual lignins suggesting a certain amount of aryl ether cleavage is occurring during bleaching.

The yields of monomer degradation products determined by GC/MS are shown in Table (6.16) The low yields make it difficult to draw many conclusions from the results, however, the similarities in the distribution of products are apparent again illustrating the similarities in the residual lignins.

Table (6.16). Yields of monomer degradation products from thioacidolysis of the acidolysis lignins isolated from kraft pulps

	Wood	CELF1	K1-F1	KO	KOQP
Vanillic acid	16	4	4	5	3
Vanillin	32	53	8	19	22
Dihydroconiferyl alcohol	64	37	9	24	9
Coniferaldehyde	20	24	ND	ND	ND
Coniferyl alcohol	52	26	ND	ND	ND
β-O-4 (G) with Cα=O	Trace	7	Trace	Trace	Trace
β-O-4 (H)	15	16	ND	ND	ND
β-O-4 (G)	1250	1200	153	332	293
β-O-4 (C)	Trace	11	21	27	27
Stilbene (G)	23	41	ND	ND	ND
	KOQPn	**KOQZ**	**KOQZE**	**KOD**	**KODE**
Vanillic acid	3	3	3	3	3
Vanillin	10	29	23	15	16
Dihydroconiferyl alcohol	9	12	9	7	11
Coniferaldehyde	ND	ND	Trace	Trace	Trace
Coniferyl alcohol	ND	ND	ND	ND	ND
β-O-4 (G) with Cα=O	Trace	Trace	Trace	Trace	Trace
β-O-4 (H)	ND	ND	ND	ND	ND
β-O-4 (G)	298	319	253	219	239
β-O-4 (C)	20	25	18	14	11
Stilbene (G)	ND	ND	ND	ND	ND

G: Guaiacyl
H: *p*-hydroxy phenyl
C: Catechol
ND: Not detected; Trace = 0–1 μmol/g lignin

The main degradation product arising from enol ether structures is the dithioacetal derivative (11) shown in Figure (6.6). However, this product also arises from the degradation of β-O-4 aryl ether structures in the lignin, so a correction needs to be made. Pasco & Suckling (1994) determined the correction factor from the yields of (11) obtained from wood lignin since it is assumed that enol ether structures are not present in wood. The same correction was made in this study, however, in all cases the amounts of compound (11) relative to compound (1) did not exceed that of the wood lignins. This indicates that enol ether structures in uncondensed phenylpropane units are not detectable in any of the kraft pulp residual lignins. However, they would not be expected since any enol ether structures present in the pulp would have been hydrolysed during the isolation of the lignin under the acid conditions.

It is also interesting to note that catechol type structures can be identified in the residual lignins, particularly in the KO residual lignin since these groups are expected to be quite reactive during bleaching. The presence of two hydroxyl groups on the aromatic ring increases the rate of degradation during oxygen bleaching considerably. For example, methylcatechol is extremely reactive under oxygen bleaching conditions even at ambient temperatures (Johansson & Ljunggren 1994). The first order rate constant for the disappearance of methylcatechol is approximately $0.2\,min^{-1}$ at 0°C, giving a half life of

four minutes (Johansson & Ljunggren 1994). Therefore, during oxygen bleaching the catechol structures should be degraded rapidly. The finding of catechol units in the residual lignin after oxygen delignification supports the hypothesis that the accessibility of lignin has a strong influence on delignification and that only the exposed lignin is removed leaving behind a residual lignin in the fibre that is less modified.

6.6 Concluding Remarks

The residual lignins isolated from all the bleached pulps were rather similar in structure. They also resembled the kraft pulp residual lignin in many aspects except in the levels of oxidised groups. This and the fact that many of the structural elements expected to be highly reactive during bleaching (eg phenolic units and catechol units) are still present in the residual lignins suggests the accessibility of the lignin or reagent penetration is a limiting factor during bleaching. However, the nature of the functional groups in the residual lignin will also play a part in determining the extent of lignin reaction since the different reagents show preferences for certain types of structures (eg phenolic units).

The spent liquor lignins (P, Pn and ZE) are more highly modified compared to the corresponding residual lignins. They contain more carboxylic acids, have a lower phenolic hydroxyl content, and have fewer uncondensed β-O-4 linkages. These results are similar to other findings (Gellerstedt & Heuts 1997, Gellerstedt & Lindfors 1991) and suggest the lignin which has been exposed to the bleaching reagents is oxidised and removed from the pulp fibre leaving behind a residual lignin that is less modified.

A picture can perhaps be formed in terms of penetration of the bleaching reagents and accessibility of the lignin. That is, the bleaching reagents penetrate to a certain extent into the pulp fibre oxidising the exposed surfaces of the residual lignin. The oxidised fragments are then dissolved out of the pulp fibre leaving pockets of residual lignin in the fibre, which have been oxidised at the surface but remain unmodified at the centre. A similar suggestion was made by Gellerstedt & Lindfors (1991).

The findings of Ljunggren et al. (1991) also support the idea that accessibility of the lignin plays an important role in determining lignin reactivity, at least during oxygen delignification. Residual lignin isolated from a softwood kraft pulp was treated with oxygen in alkali solution at various temperatures and the resulting material was analysed by oxidative degradation and ^{13}C-NMR spectroscopy. The permanganate oxidation results showed the lignin did not undergo as comprehensive a degradation at higher temperatures as the lignin model compound reactions predict. Additionally, phenolic structures still remain in the lignin even after one hour at 150°C. Further, the ^{13}C-NMR spectra of treated and untreated residual lignin were quite similar. It was suggested that the polymeric lignin formed micelle type structures in the alkaline solution protecting many of the reactive groups from being degraded.

Furthermore, the studies of Laine et al. (1996) concerning the effect of ECF and TCF bleaching on the surface composition of kraft pulps indicate that lignin on the surface of the pulp fibres retards the bleachability of kraft pulps during these processes. The surface lignin either arises from the wood fibre (remnants of middle lamella lignin) or is formed

during pulping (reprecipitated lignin) and is quite difficult to remove particularly in the early stages of bleaching. There was some indication that the poor dissolution of the surface lignin in early stages of bleaching was due to a low amount of free phenolic lignin units or to a high amount of condensed type structures in the lignin. Alternatively, the outer layers of the fibre may contain a high amount of lignin-carbohydrate complexes. This lignin could therefore play an important role in determining the accessibility of the bulk residual lignin in the fibre by inhibiting the penetration of the bleaching reagents.

There are two major drawbacks with using acidolysis to isolate lignin. The first is that less than 50% of the residual lignin could be isolated from the pulps and there is a possibility that the isolated samples may not be fully representative of the total residual lignin in the pulps. The results discussed in Chapter 4 concerning the acidolysis lignins from wood suggest a less condensed type of lignin is being isolated from the wood by acidolysis. A similar situation may be occurring in the isolation of residual lignin from the pulps. A possibility is that the fraction of residual lignin isolated by acidolysis is the fraction that has been more extensively modified since this lignin would be more soluble and more exposed. Additionally, a more highly condensed type of lignin may be left unextracted so that the full extent of lignin modification is not being observed. The second drawback is that the acidic conditions that prevail during isolation are expected to modify the lignin to a certain extent but the exact nature of the changes are unclear.

In regards to cyanamide activated hydrogen peroxide bleaching. In this study it was shown that KOQPn residual lignin contains fewer phenolic hydroxyl groups compared to KO residual lignin. Additionally, the ratio of condensed to uncondensed phenolic structures was shown to increase after the Pn-stage. This suggests phenolic phenylpropane units are preferentially degraded during a Pn-stage and is more consistent with the results of Kadla et al. (1998a) rather than the results of Smit et al. (1996). Kadla et al. (1998a) report that nonphenolic model compounds are unreactive towards cyanamide activated hydrogen peroxide even under extreme reaction conditions (90°C), while phenolic compounds undergo rapid oxidation (although, condensed phenolic structures were not analysed). On the other hand, Smit et al. (1996) report that all model compounds reacted at an indistinguishable rate regardless of their structure (ie condensed phenolic, uncondensed phenolic, and nonphenolic compounds were all degraded to a similar extent after 2hr at 60°C). Further study into the reactions of cyanamide activated hydrogen peroxide is necessary to fully understand the mechanisms involved and to find explanations for these conflicting results.

Chapter Seven
FINAL CONCLUSIONS

7.1 CEL Isolation Method

One aim of this thesis was to develop an improved lignin isolation method that produces a soluble lignin in high yield and high purity from both wood and pulp fibre. This aim was partially achieved in that an effective method for the isolation lignin from wood was successfully developed.

The method, which is outlined in Figure (4.6), yields approximately 75% of the lignin from *Pinus radiata* wood (and slightly less if the first extraction step is omitted). The key step is the second ball milling treatment, which allows essentially all the lignin in the enzyme digest residue to be extracted. The resulting CEL lignins contain 5–8% carbohydrates and less than 2% protein, which is reasonable for enzyme isolated lignin.

The CEL lignins are remarkably similar to MWL in terms of the total phenolic hydroxyl content, uncondensed β-O-4 ether content, and the distribution of interunit linkages, although, there was some indication that the CEL lignins have a higher molecular weight than MWL. This shows that MWL, as far as a soluble lignin is concerned, is a reasonable representation of native lignin. However, this CEL method is superior to the MWL method in that significantly higher yields of lignin can be obtained, thus making it easier to isolate high amounts of lignin from wood as compared to the standard MWL method.

The first fraction of enzyme lignin isolated from wood (CELF1) has fewer phenolic hydroxyl groups, fewer carboxylic acid groups, and a higher uncondensed β-O-4 content (which is similar to that found for wood) compared to the second fraction of lignin (CELF2). This fraction could consist of a less modified lignin, which has been made accessible to the extracting solvent by removal of the carbohydrates. The lignin that remains insoluble after the extraction of CELF1 requires further milling to enable it to dissolve.

The other CEL lignin fractions (CELF2 and CEL2) are exposed to ball milling twice and have more phenolic hydroxyl groups, more carboxylic acids, and fewer uncondensed β-O-4 linkages compared to CELF1. These results support the idea that ball milling fragments the lignin by cleaving β-aryl ether linkages. Interestingly, the MWL, which has only been exposed to ball milling once, has a similar phenolic content, carboxylic acid content, and uncondensed β-O-4 content to CELF2 and CEL2 lignins. This indicates that in the case of MWL the solvent is preferentially extracting the lignin that has undergone the greater degree of mechanical modification.

The phenylpropane side chain is also damaged to a certain extent by ball milling. This was apparent from an increase in vanillin and decrease in dihydroconiferyl alcohol end groups with the extent of milling (ie wood → CELF1 → CELF2 & CEL2). Additionally, an increase in coniferaldehyde end groups at the expense of the coniferyl alcohol end group was observed suggesting the isolation procedure is slightly oxidative.

A key point is that ball milling plays an important role in the isolation procedure. The first ball milling step disrupts the structure of the cell wall sufficiently to allow the action of the enzymes and release of the first fraction of lignin (CELF1) and the second ball milling step fragments the remaining insoluble lignin fraction allowing it to dissolve. The concomitant increase in phenolic hydroxyl groups will also play an important role in the increased solubility of the lignin.

The major limitation of both MWL and CEL methods is that the isolated lignins have been exposed to considerable mechanical treatment, leading to increased levels of oxidation, ether-cleavage products and side chain damage. However, the fact that CELF1 is less modified than CELF2 (ie has fewer phenolic OH groups, fewer carboxylic acids and a higher β-O-4 content) could suggest there is a minimum level of modification required to dissolve the lignin.

7.2 Structure of Residual Lignin

The residual lignin remaining in the fibre after kraft pulping has a significantly lower uncondensed β-O-4 content and a higher phenolic hydroxyl content compared to isolated wood lignin. This is consistent with the cleavage of β-O-4 linkages in lignin that take place during pulping. An increase in condensed structures relative to uncondensed structures was also observed. After oxygen bleaching, the residual lignin has fewer phenolic hydroxyl groups and slightly more carboxylic acids. This is consistent with the oxidative degradation of phenolic structures taking place during oxygen delignification. However, the expected enrichment of condensed structures was not observed.

In the subsequent bleaching stages the residual lignins become more oxidised as indicated by an increase in their oxygen and carboxylic acid contents. In all cases, except hydrogen peroxide bleaching, a decrease in the total phenolic hydroxyl groups within the residual lignin compared to KO residual lignin was observed. This indicates that oxygen, chlorine dioxide, cyanamide activated hydrogen peroxide, and ozone have a certain preference for phenolic structures in lignin. Additionally, cyanamide activated hydrogen peroxide was the only reagent that showed a preference for uncondensed structures. In comparison, the spent liquor lignins (P, Pn and ZE) were more highly modified compared to the corresponding residual lignins. They contain more carboxylic acids, have a lower phenolic hydroxyl content, and have fewer uncondensed β-O-4 linkages. This suggests the lignin that has reacted with the bleaching reagents is removed from the pulp fibre leaving behind a residual lignin that is substantially less modified than the removed lignin.

These results support the view that the removal of lignin during bleaching is controlled to a certain extent by the accessibility of the lignin and penetration of the bleaching reagents rather than just by the nature of the functional groups present in the lignin. That is, the bleaching reagents penetrate to a certain extent into the pulp fibre oxidising the exposed surfaces of the residual lignin. The oxidised fragments are then dissolved out of the pulp fibre leaving pockets of residual lignin in the fibre, which have been oxidised at the surface but remain unmodified at the centre. Additionally, the extent of residual lignin oxidation varied between reagents as indicated by the variation in oxygen and carboxylic acid contents. This suggests the extent of penetration of the each reagent also differs.

It should be pointed out that the structural characteristics of the residual lignin will still be an important factor during bleaching, since under normal bleaching conditions the effectiveness of the different reagents generally varies, in terms of consumption per kappa unit decrease. Hence, different doses of each reagent are required to achieve a similar degree of delignification, however, the charge of reagent used and the degree of delignification that can be achieved during a particular bleaching stage is generally limited by the amount of carbohydrate degradation that takes place. These factors are associated with the differing chemistry of each reagent and will be partly dependent on the structural characteristics of the residual lignin.

The major limitation of this study is that the isolated samples may not be fully representative of the total residual lignin in the pulps since less than 50% of the residual lignin was isolated from the pulps. Additionally, some modification of the lignin under the acidic conditions during isolation can be expected. The results discussed in Chapter 4 concerning the acidolysis lignins from wood suggest, that at least in this case, a less condensed type of lignin is being generated by acidolysis. A similar situation may be occurring during the isolation of residual lignin from the pulps. A possibility is that the fraction of residual lignin isolated by acidolysis is the fraction that has been more extensively modified since this lignin would be more soluble and more exposed. Additionally, a more highly condensed type of lignin may be left unextracted so that the full extent of lignin modification is not being observed. For these reasons, the structure of residual lignin can not be conclusively determined until a method is available which allows complete isolation of the lignin with a minimal amount of alteration.

7.3 Future Work

The ideal procedure for the isolation of residual lignin from pulps still remains elusive. The structure of residual lignin can not be conclusively determined until a method is available which allows complete isolation of the lignin with a minimal amount of alteration or contamination. The acidolysis method generally yields less than half of the total residual lignin and the isolated lignin may not be fully representative. In contrast, the enzyme method yields a slightly less modified lignin but the lignin is highly contaminated.

The studies concerning the optimisation of the CEL method for pulps (Chapter 5) were unfortunately not successful in that no significant improvements, in terms of yield or protein contamination, were achieved compared to earlier studies (Hortling et al. 1992, Lachenal et al.1995, Johansson 1997).

However, one suggestion was made in light of the results presented. That is, the differences in the behaviour of different lignins and differences arising from the use of different enzymes, in terms of buffer solubility of the lignin, could be related to the degree of protein adsorption rather than directly to the lignin structure. For example, the more highly modified lignin is perhaps more likely to adsorb protein and thus more likely to be solubilised during the enzyme treatment. Additionally, the properties of the enzyme, in terms of its tendency to adsorb and its hydrophilic nature, would also have an

influence. Therefore, to improve the enzyme methods a way of minimising protein adsorption needs to be found. An appropriate study may be to find an enzyme preparation which is less likely to adsorb to oxidised residual lignin leading to the majority of lignin remaining buffer insoluble after the enzyme treatment. Consequently, a less contaminated lignin could then be extracted. Additionally, if the solubility of the residue in typical lignin solvents is limited a ball milling treatment would possibly enhance the extractability as was observed for the digest residue from wood.

Further, the exact nature of the interaction between the CEL lignins and both carbohydrate and protein contaminants remains unclear. The association is very stable resisting both acid and base treatments. This means that covalent linkages are possibly involved since any physical (rather than chemical) association should be disrupted during either the acid or base treatment. It has been suggested (Kosikova et al. 1979) that carbohydrates and lignin are linked via an α-aryl ether type bond, which have been shown to be partially stable towards acid and base degradation. In regards to the association with protein, it was demonstrated in Chapter 4 that protein does not significantly adsorb onto isolated lignin samples during enzyme treatment. This could suggest the interaction between the enzyme and lignin only takes place in the presence of carbohydrates, and perhaps only occurs when the enzyme is actively degrading the carbohydrates.

Furthermore, the results obtained from the digestion trials in Chapter 5 indicated that the concept of carbohydrate degradation as a means of enriching the residual lignin in pulps had some potential and that this area is worthy of further investigation. Another point of interest is the effect of ball milling on lignin structure. In Chapter 4 it was shown that ball milling modifies the lignin structure to a certain extent. Additionally, the fraction of lignin isolated after one milling treatment (CELF1) was less modified than the fraction isolated after two treatments (CELF2). It was concluded that there is possibly a minimal level of modification necessary to solubilise the lignin.

The last comments are in regards to cyanamide activated hydrogen peroxide bleaching. It was shown that KOQPn residual lignin contained fewer phenolic hydroxyl groups compared to KO residual lignin and the ratio of condensed to uncondensed phenolic structures increased following the Pn-stage. This suggests uncondensed phenolic structures with a free phenolic hydroxyl group are preferentially degraded during the Pn-stage and is more consistent with the results of Kadla et al. (1998a, 1998b) rather than a less selective type of mechanism supported by Smit et al. (1996). Further study into the mechanisms of cyanamide activated hydrogen peroxide are needed to find explanations for these conflicting results.

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Appendix One
LABORATORY BLEACHING OF KO PULP

In Chapter 6 the isolation and characterisation of residual lignin isolated from bleached kraft-oxygen pulps was discussed. The aim was to study the structure of residual lignin in KO pulps that were bleached to approximately the same kappa number using chlorine dioxide, hydrogen peroxide, and ozone (including alkali extraction for the D and Z pulps). The chosen target kappa number was 10 to maximise the possible residual lignin modifications while maintaining sufficient quantity of lignin for ease of isolation.

The bleached pulps were generated in the laboratory using the methods of PAPRO (Forest Research, NZ). Bleaching trials were carried out to determine the conditions required to achieve the target kappa number. The results of these trials and the pulp generation are discussed below, but first, some of the technical terms used are defined.

Definitions

Kappa number

Kappa number is a measure of the residual lignin content of a pulp and is determined from the amount of $KMnO_4$ consumed per gram of pulp.

The lignin content of a pulp can be estimated using the equation;

$$\text{Residual lignin content (\%)} \approx (0.15 \times \text{kappa number})$$

Consistency

Consistency is defined by the equation;

$$\text{Consistency} = \frac{\text{Dry weight of pulp}}{\text{Wet weight of pulp}}$$

and is expressed either as a percentage or a decimal. Consistency defines the water content of a pulp. For example, 10% consistency means 10g of dry pulp per 100g of wet pulp (ie at 10% consistency a pulp sample contains 90g of liquid).

Oven dry weight (odg)

Weight of oven dry pulp in grams.

Chemical charge

The amount of chemicals applied to a pulp are generally expressed as grams of chemical per 100 odg of pulp (as a percentage).

Active chlorine

Active chlorine is a common unit of oxidant used in chlorine-based bleaching technology. It defines the equivalent weight of substance equal to the equivalent weight of chlorine (where the equivalent weight is the molecular mass divided by the number of electrons transferred during reaction). For example; during bleaching one weight unit of chlorine dioxide is equal to 2.63 weight units of active chlorine.

Chlorine multiple

Chlorine multiple defines the chemical charge of chlorine-based chemicals on pulp and is determined from the equation;

$$\text{Chlorine multiple} = \frac{\text{Chemical applied (as \% active chlorine on pulp)}}{\text{Kappa number of incoming pulp}}$$

A1.1 Peroxide Bleaching

A1.1.1 Bleaching trials

Small (10g) samples of EDTA pretreated KOP2 pulp (Kappa number 18.4) were bleached using the hydrogen peroxide charges outlined in Table (A1.1). Cyanamide ($NCNH_2$) was added to treatments 4–6 at a molar ratio of 1mole per 2 moles of hydrogen peroxide.

Table (A1.1). [1]Bleaching conditions used for the hydrogen peroxide bleaching trials and the final pulp properties

	Chemical charge (% on pulp)			**pH**		**Kappa**
	H_2O_2	NaOH	$NCNH_2$[2]	Initial	Final	**number**
1	0.5	0.9	–	11.58	11.41	15.2
2	1.5	1.5	–	11.69	11.52	14.7
3	2.5	2.0	–	11.53	11.41	12.6
4	0.5	0.9	0.3	11.68	11.61	15.6
5	1.5	1.5	0.6	11.73	11.62	14.6
6	2.5	2.0	1.5	11.61	10.62	11.3

1. Bleaching at 10% consistency and 80°C for 60min; 2% DTPA added
2. H_2O_2:$NCNH_2$ mole ratio = 2:1

Sample 6, which was bleached at the highest peroxide charge and was cyanamide activated, consumed the most alkali during bleaching, whereas very little pH change was observed for the other treatments. Treatment 6 also achieved the greatest degree of delignification, as measured by the drop in kappa number. Additionally, treatment 6 was the only treatment that gave a significantly greater reduction in the kappa number compared to the equivalent unactivated sample.

Treatments 3 and 6 were closest to the target kappa number (10) so were used for the generation of the KOQP and KOQPn bleached pulps, respectively.

A1.1.2 Pulp bleaching

Samples (100g) of EDTA pretreated KOP2 pulp were bleached using the chemical charges used for treatments 3 and 6 (Table A1.1), except that the DTPA charge was reduced to 0.2% on pulp. During the course of this study it became necessary to carry out the larger scale bleaching twice. The first peroxide bleached samples (1) were used for the enzyme experiments (Chapter 5), while the other peroxide bleached pulps (2) were used for the enzyme digest trials (Chapter 5) and the acidolysis experiments (Chapter 6). The results of the hydrogen peroxide bleaching are summarised in Table (A1.2).

Table (A1.2). [1]Bleaching conditions and pulp properties for the large scale hydrogen peroxide bleached pulps

	pH		**H_2O_2 Consumed**		**Kappa**
	Initial	Final	% of initial	% on od pulp	**number**
KOQP1	11.39	11.25	46	1.15	13.9
KOQP2	11.53	11.21	4	0.11	14.7
KOQPn1[2]	11.42	10.00	89	2.23	9.6
KOQPn2[2]	11.42	10.00	72	1.92	9.9

1. Bleaching at 10% consistency and 80°C for 60 min; 2.5% H_2O_2, 2.0% NaOH, and 0.2% DTPA
2. H_2O_2:$NCNH_2$ mole ratio = 2:1

The KOQP and KOQPn pulps in Table (A1.2) have different kappa numbers compared to the bleaching trials (Table A1.1). The kappa numbers for the KOQP pulps are slightly higher compared to the trial, indicating slightly less delignification occurred in the scale-up experiments. This may be associated with poorer mixing of the chemicals with the larger volume of pulp.

The KOQPn pulps, on the other hand, have a lower kappa number compared to the trial which indicates a higher degree of delignification occurred in the scale-up experiments. This may be a result of the different DTPA charges. Greater stabilisation of the reagent perhaps occurs at the higher DTPA charge (in the trials) so less decomposition to more reactive species takes place and, thus, less delignification.

The reason for the large difference in hydrogen peroxide consumption between KOQP1 and KOQP2 is not clear but may be associated with differences in the Q-stage. Any variation in the metal ion content of a pulp will lead to differences in hydrogen peroxide decomposition and delignification.

A1.2 Chlorine Dioxide Bleaching

The chlorine dioxide used for bleaching was mill generated and contained 7–8% of the active chlorine as Cl_2.

A1.2.1 Bleaching trials

Small (10g) samples of KOP2 were bleached with chlorine dioxide at 10% consistency for 10 minutes at 50°C using the chemical charges in Table (A1.3). After bleaching, the pulps were alkali extracted at 10% consistency, for 10 minutes at 90°C, using a NaOH charge of 2% (on od pulp). The results are shown in Table (A1.3).

Table (A1.3). Bleaching conditions used for the chlorine dioxide bleaching trials and final pulp properties

	Chlorine dioxide charge[1]		**Final**	**Active chlorine consumed**		**KODE**
	Cl multiple	% on pulp	**pH**	% on pulp	% of applied	**Kappa**
1	0.04	0.74	3.53	0.71	95.9	15.0
2	0.06	1.10	3.88	1.04	94.5	12.9
3	0.07	1.29	3.22	1.27	98.4	10.9
4	0.08	1.47	3.03	1.46	99.3	11.3
5	0.10	1.84	2.89	1.84	99.8	9.0

1. Chlorine dioxide charge as active chlorine

Maximum chlorine dioxide bleaching is achieved when the final pH is between 2–4 (Reeve 1996c). At higher chlorine dioxide charges (0.15–0.25 Cl-multiple), which are normally used for bleaching, alkali is usually added to the initial liquor to prevent the final pH dropping below this range. However, in these trials a particularly low chlorine dioxide charge was required to give the target kappa number of 10 and the addition of alkali was not necessary since the final pH was within the required range.

Sample 3 was closest to the target kappa number so a chlorine multiple of 0.07 was used for the scale up experiments.

A1.2.2 Pulp bleaching

KOP2 pulp was bleached with chlorine dioxide at a chlorine multiple of 0.07 at 10% consistency, for 10min at 50°C. The pulp was bleached in 33g lots to ensure even mixing of the chlorine dioxide with the pulp and thus even bleaching throughout the pulp. After bleaching the samples were alkali extracted at 10% consistency with 2% NaOH (on od pulp). The results for the chlorine dioxide bleaching are shown in Table (A1.4).

An unextracted pulp sample (KOD) was collected for comparison. Replicates 4 and 5 were combined and a third of the combined sample was alkali extracted under the same conditions as 1, 2, and 3; the kappa number of the resulting pulp is shown in Table (A1.4). The remaining 2/3 of the combined sample was not alkali extracted giving KOD with a kappa number of 14.3.

Table (A1.4). Bleaching conditions and pulp properties for the large scale chlorine dioxide bleached pulps (bleached at a 0.07 chlorine multiple)

Sample	Final pH	Active Cl consumed		KODE Kappa number
		% on od pulp	% of applied	
1	2.76	1.28	98.8	11.1
2	2.60	1.29	99.5	10.3
3	2.79	1.29	99.3	11.3
4	3.29	1.31	98.5	11.3[1]
5	3.43	1.32	99.0	

1. Kappa number of 4 & 5 combined

The variation in kappa numbers of the KODE pulps is indicative of the complexity of chlorine dioxide bleaching. Since a large number of reactions can take place during chlorine dioxide bleaching a small variation in the chemical environment will possibly result in a variation in the degree of delignification. Additionally, the efficiency of mixing the chemicals with the pulp will be an important factor (and perhaps the more important one).

The alkali extraction decreased the kappa number of the D-stage pulp by 3 units, which shows that about 0.5g of the residual lignin per 100g of KOD pulp is removed by the alkali treatment.

For further studies the KODE pulps were combined and fluffed giving a KODE pulp with an average kappa number of 11.0.

A1.3 Ozone Bleaching

A1.3.1 Bleaching trials

A sample (100g) of EDTA pretreated KOP2 pulp was bleached with an ozone charge of 0.5% on pulp at 1.2% consistency. The total time which the ozone was applied to the pulp determined the ozone charge.

After ozone bleaching the pulp was alkali extracted at 10% consistency and 70°C for one hour with 2% NaOH (on pulp). The results are shown in Table (A1.5).

Table (A1.5). Bleaching conditions used for the ozone bleaching trials and the final pulp properties

Total ozone applied (% on pulp)	0.704
Ozone consumed (% on pulp)	0.405
Ozone consumed (% of applied)	57.5
KOQZE kappa number	10.5

The target kappa number of approximately 10 was reached in the first attempt so the ozone bleaching was repeated using the same conditions.

A1.3.2 Pulp bleaching

Two additional KOP2 pulp samples (100g) were bleached with ozone using the same conditions as the trial. Both ozonated pulp samples were then combined and half was extracted with alkali as earlier to give KOQZE, while the other half was left untreated to give KOQZ. The ozone consumption and final kappa numbers are shown in Table (A1.6).

Table (A1.6). Bleaching conditions and pulp properties for the large scale ozone bleached pulps

Ozone charge	**1**	**2**
Total ozone applied (% on pulp)	0.704	0.703
Ozone consumed (% on pulp)	0.422	0.423
Ozone consumed (% of applied)	58.5	58.6
Kappa number		
KOQZ	14.0	
KOQZE	10.6	

The alkali extraction reduced the kappa number of the Z pulp by about 3.4 units. This is almost the same as the drop which was observed for the D pulp after alkali extraction and could suggest that under the bleaching conditions used both the chlorine dioxide and ozone are oxidising the lignin to similar extents.

For further studies both this KOQZE pulp and that from the trial were combined and fluffed to give a KOQZE pulp with an average kappa number of 10.6.

A1.4 Experimental Methods

All experiments were performed using a laboratory-washed commercial *Pinus radiata* kraft-oxygen pulp (KOP2, Table 5.1). Hydrogen peroxide, cyanamide activated hydrogen peroxide and chlorine dioxide bleaching was carried out inside sealed plastic bags. The sealed plastic bags were incubated at the desired temperature by being immersed in a water bath. Ozonation was carried out in a reactor. Prior to bleaching with hydrogen peroxide and ozone the pulps were treated with a chelating agent. The chlorine dioxide and ozone bleached pulps were extracted with alkali. These treatments were also carried out in plastic bags. Kappa numbers were determined for all bleached pulps using the Micro-Kappa number method of PAPRO (Method 1.107).

A1.4.1 Chelation

Pulp was treated with 1% (on od pulp) EDTA at 80°C and 10% consistency for 1.5hr (the amount of pulp per bag did not exceed 100g). The EDTA solution was kneaded into the pulp and the pH was adjusted to between 5 and 6 with 10% H_2SO_4. The volume was made up to 10% consistency with distilled water.

After incubation the pulp was dewatered to 20% consistency and diluted to 1% consistency with distilled water. The pulp was soaked overnight at room temperature and then dewatered to 20% consistency and fluffed.

A1.4.2 Hydrogen Peroxide bleaching

PAPRO method 1.602

Chelated pulp samples were bleached at 10% consistency for 1hr at 80°C. The amount of pulp per bag did not exceed 100 g.

The hydrogen peroxide, NaOH and DTPA were added to the makeup water before being kneaded into the pulp. The hydrogen peroxide was the last chemical to be added to the liquor. The cyanamide, with half the makeup water, was kneaded into the pulp 15 minutes before the rest of the chemicals.

A sample (10 mL) of the liquor was squeezed out of the pulp and the initial pH was measured. The liquor sample was returned to the bag, which was then sealed and incubated. After incubation the spent liquor was squeezed out of the pulp, the final pH was measured and residual hydrogen peroxide was determined by iodometric titration.

The pulp was diluted to 1% consistency with hot tap water and the pH was adjusted to between 5–6 with sulfurous acid. The pulp was soaked overnight at room temperature and then dewatered to about 20% consistency.

A1.3.3 Chlorine dioxide bleaching

PAPRO method 1.604

The chlorine dioxide used for bleaching was obtained from a New Zealand mill and contained 7–8% of active chlorine as Cl_2.

Pulp samples were bleached at 10% consistency for 10 minutes at 50°C. The amount of pulp per bag did not exceed 35 g. The makeup water and chlorine dioxide were mixed before being kneaded into the pulp. Air was pressed out of the bags which were sealed and incubated.

After incubation the spent liquor was squeezed out of the pulp, the final pH was measured and the residual chlorine dioxide was determined. The pulp was diluted to 1% consistency with hot tap water and soaked for 15 minutes before being dewatered to about 20% consistency.

A1.3.4 Ozone bleaching

PAPRO method 1.606

Ozone was generated using an OREC 03B1-O ozonator. The amount generated was determined as a flow rate by titration.

Chelated pulp samples (100 g) were bleached at 1.2% consistency in a 13 L stirred reactor (the propeller was spun at about 600 rpm). The pH of the pulp suspension was adjusted to between 2–3 with 10% H_2SO_4 prior to ozonation. The ozone/oxygen gas mixture was applied to the reactor at the base and bubbled through the pulp suspension. The duration of ozonation determined the ozone charge. The residual ozone was trapped and the concentration determined by titration.

After the required time the gas flow was turned off and the reactor was flushed with nitrogen for about 5–10 minutes to remove the remaining residual ozone. This residual ozone was also trapped and titrated.

The pulp suspension was removed from the reactor, dewatered, washed at 1% consistency with hot tap water and finally dewatered to about 20% consistency.

A.1.3.5 Alkali extraction

PAPRO method 1.604

Bleached pulps were extracted with 2% (on od pulp) NaOH at 10% consistency. The D-pulp was extracted in approximately 35 g quantities at 90°C for 10 minutes. The Z-pulp was extracted in 100 g quantities at 70°C for 1hr.

After incubation the pulps were dewatered to 20% consistency and then diluted to 1% consistency with hot tap water. After about 15 minutes the pulps were dewatered to about 20% consistency.

A1.4 References

Reeve D. (1996c), "Chlorine dioxide in delignification", In: *Pulp Bleaching - Principles and Practise* (Dence C. and Reeve D. eds), Tappi Press (Atlanta), 261–290.

PAPRO 1.107, "Micro Kappa Number", PAPRO Laboratory Manual Vol 1, Forest Research, Rotorua, NZ.

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PAPRO 1.604, "Laboratory procedures for multichlorination bleaching", PAPRO Laboratory Manual Vol 1, Forest Research, Rotorua, NZ.

PAPRO 1.602, "Laboratory procedures for peroxide bleaching", PAPRO Laboratory Manual Vol 1, Forest Research, Rotorua, NZ.

PAPRO 1.606, "Laboratory procedures for low and medium consistency ozone bleaching", PAPRO Laboratory Manual Vol 1, Forest Research, Rotorua, NZ.

Appendix Two
DATA FROM ANALYTICAL PROCEDURES

A2.1 Elemental Composition

The elemental compositions were converted into molecular formula per C9 or C100 (non methoxyl carbon atoms). The C, H, and O contents were corrected for the presence of methoxyl groups, moisture, carbohydrates and protein as outlined in Table (A2.1) prior to being converted into the formula.

Table (A2.1). Amounts subtracted from C, H and O content to correct for methoxyl, moisture, carbohydrate and protein content of the lignin samples.

	C	H	O
Methoxyl content	$\frac{12 \times \text{Methoxyl}}{31}$	$\frac{3 \times \text{Methoxyl}}{31}$	$\frac{16 \times \text{Methoxyl}}{31}$
Moisture	No correction	$\frac{3 \times \text{Moisture}}{31}$	$\frac{3 \times \text{Moisture}}{31}$
Carbohydrates	$\frac{72 \times \text{Carbo's}}{180}$	$\frac{12 \times \text{Carbo's}}{180}$	$\frac{96 \times \text{Carbo's}}{180}$
Protein	$(6.25 \times N) \times 0.5$	$(6.25 \times N) \times 0.07$	$(6.25 \times N) \times 0.25$

The raw data obtained from elemental analysis of the wood lignins and pulps lignins are shown in Table (A2.2) and Table (A2.3), respectively. The oxygen content was obtained by subtracting the C, H, S, Cl and N contents from 100.

Table (A2.2). Data from elemental analysis of wood lignins

	C	H	OCH_3	N[1]	H_2O
WAAL	60.13	6.00	14.71	0	4.31
WANL	58.22	5.86	14.09	0	5.84
BMWAL	60.22	5.89	13.74	0	4.39
CELF1	55.77	5.53	12.96	Trace	7.20
	55.62	5.42	12.74		
	56.64	6.27	12.97		
	56.62	6.29			
CELF2	55.78	5.98	12.67	0.46	9.27
	55.81	6.13	12.63	0.20	
	54.79	5.72	12.73	0.22	
	54.83	5.74		0.16	
CEL2	56.67	6.11	13.15	0.24	7.65
	56.92	6.20	12.74	0.33	
	56.32	5.45	12.96	0.22	
	56.46	5.47			
CELF2A	57.66	5.35	13.13	0.35	6.97

1. Trace nitrogen detected <0.2%

Table (A2.3). Data from elemental analysis of pulp lignins (samples ending in 's' refer to spent liquor lignins)

	C	H	S	Cl	OCH_3	N	H_2O
K1-F1	59.71	6.00 5.79	1.87	0	10.14	0	6.32
K1-F2	57.60	5.82 5.71	1.59	0	9.85	0	6.78
K-dig	57.65	6.06 6.24	1.62	0	8.99	2.15	7.07
K4	63.38 63.22	6.26 6.17	1.61	0	11.20	0.25 0.27	3.47
KO	65.35 65.15 66.05 65.84	6.08 6.22 6.19 5.92	1.02 1.07	0	9.57 9.68	0	4.19
KO-F2	58.8	5.98	1.44	0	–	0.25	6.44
KO-dig	57.68	5.77 5.74	1.00	0	10.31	1.36 1.47	6.36
KOQP	58.68 58.5	6.23 5.99	1.06	0	10.65	0.23	6.50
KOQP-dig	58.85	6.21 5.95	0.94	0	10.27	1.39	6.10
KOQPs	57.42 57.56	5.44 5.57	1.03	0	9.52	0.47 0.44	6.19
KOQPn	58.21 58.27	5.8 5.6	0.92	0	10.85	0.22	6.45
KOQPns	55.14 55.27	5.23 5.38	1.14 1.19	0	9.47	0.48	6.86
KOQZ	59.06 59.25 59.15 59.12	5.84 5.57 5.53 5.82	0.94 0.99	0	10.14 9.58	0 0	5.31
KOQZE	60.26 60.27 59.03 59.06	5.92 6.06 6.05 6.12	1.05 1.08	0	10.25 9.85	0.16	5.02
KOQZEs	55.52 55.82	5.52 5.38	0.85	0	7.54	0.48	4.26
KOD	57.60 57.46 57.00 57.01	5.69 5.59 5.42 5.76	0.93 0.93 0.97 0.97	0.98	12.49 9.06	0	4.73
KODE	58.10 58.26 58.53 58.75	5.93 6.05 6.03 6.08	0.85 1.01	0.35	8.81 8.76 8.83	0	6.73

A2.2 Carbohydrate and Klason Lignin Content

Table (A2.4). Carbohydrate monomer and Klason lignin contents of isolated lignins

	Carbohydrate monomers (g/100g lignin)					**Klason** (g/100g lignin)	
	Glucose	Galactose	Mannose	Xylose	Arabinose	Lignin	Acid sol.
CELF1	1.34	1.54	2.61	1.69	0.54	86.5	0.99
CELF1	1.27	1.39	2.22	1.44	0.46	–	–
CEL2	0.85	1.50	1.69	0.98	0.43	87.1	0.96
CELF2	0.95	1.74	0.17	1.58	0.56	86.2	0.93
CELF2A	0.67	1.20	1.08	0.65	0.12	–	–
BMWAL	–	–	–	–	–	89.9	1.04
KOQPs	1.02	2.70	0.19	1.24	0.53	–	–
KOQPns	1.17	2.84	0.14	0.55	0.48	–	–
KOQZEs	1.74	2.6	0.34	1.43	0.74	–	–
K4	–	–	–	–	–	94.6	1.14
KOD	–	–	–	–	–	90.5	2.16
K-dig	–	–	–	–	–	86.9	3.38
KO-dig	–	–	–	–	–	88.6	2.54
KOQP-dig	–	–	–	–	–	90.5	3.03

A2.3 Periodate Oxidation Results

The methanol produced during periodate oxidation of the lignin samples are tabulated in the following tables. Table (A2.5) shows the results for the isolated wood lignins, as well as, for ball milled wood (MW) and Wiley milled wood (Wiley). Table (A2.6) shows the results for the residual lignins, and Table (A2.7) shows the results from the extended reaction of the KO lignin.

Table (A2.5). Methanol (mmol/g lignin) formation during periodate oxidation of wood lignins

Time (hr)	**MW**[1]	**Wiley**[2]	**CELF1**		**CELF2**	
48	0.724	0.611	0.646		0.749	
71	0.764	0.623	0.682		0.782	
86	0.785	0.631	0.704		0.806	
93	0.787	0.649	0.717		0.833	
116	0.799	0.662	0.736		0.866	
165	0.815	0.664	0.789		0.891	
189	0.817	0.678	–		–	
213	0.828	0.684	0.836		0.949	
260	0.883	0.708	0.849		0.962	
364	0.922	0.685	0.864		0.956	
412	0.941	0.683	0.891		1.006	
Time (hr)	**MW**[1]	**Wiley**[2]	**CELF1**		**CELF2**	
67	0.869	0.633	0.684	0.626	0.804	0.731
94	0.899	0.613	0.746	0.683	0.826	0.800
117	0.900	0.637	0.781	0.748	0.862	0.873
138	0.908	0.652	0.765	0.761	0.943	0.897
167	0.925	0.669	0.773	0.784	0.918	0.928
167	–	–	–	0.771	0.907	–
Time (hr)	**CEL2**					
66	0.901	0.888				
118	1.001	0.981				
142	0.947	0.993				
172	0.988	0.993				

1. Ball milled wood
2. Wiley milled wood (60 mesh)

Table (A2.6). Methanol (mmol/g lignin) formed upon periodate oxidation of residual lignins

Time (hr)	K	KO	KO	KOQP	KOQPn	KOQPn
27.0	1.119	0.400	0.381	0.952	0.850	0.982
51.5	1.317	0.590	0.597	1.050	0.961	1.085
67.5	1.430	0.665	0.658	1.127	1.002	1.158
75.0	1.425	0.672	0.628	1.157	1.080	1.173
90.5	1.502	0.707	0.695	1.165	1.058	1.190
98.5	1.542	0.645	0.760	1.241	1.067	1.224
105	1.628	0.770	0.827	1.259	1.163	1.271
113	1.674	0.781	0.866	1.285	1.169	1.270
128	1.703	0.849	0.887	1.171	1.233	1.319
137	1.692	0.824	0.906	1.212	1.240	1.288

Time (hr)	KOD	KOD	KODE	KOQZ	KOQZE
23.0	0.732	0.940	0.726	0.734	0.899
49.5	0.789	0.951	0.819	–	0.885
64.0	0.817	0.877	–	0.860	1.015
72.0	0.940	–	1.155	0.945	1.163
88.0	0.966	–	–	0.92	1.128
93.5	0.949	1.063	1.199	1.041	1.158
99.5	1.052	1.075	1.205	1.068	–
115	1.054	1.114	–	1.1	–
137	1.004	1.025	1.116	1.098	1.13

Table (A2.7). Methanol (mmol/g lignin) produced upon periodate oxidation of KO lignin.

	Days	mmol/g	Days	mmol/g	Days	mmol/g	Days	mmol/g
KO	1.08	0.478	5.22	0.914	15.50	1.282	18.29	1.256
	1.90	0.629		0.867	16.29	2.456		1.294
	2.65	0.683	6.21	0.881		2.436		1.253
	3.31	0.699	8.08	0.984		2.521	21.29	1.380
	3.60	0.581		1.031	17.42	1.261		1.411
		0.602	8.92	1.000		1.193		1.402
	4.17	0.984	9.92	1.038		1.275		1.416
	4.96	0.842		1.058				
			10.50	1.101				
	Days	**mmol/g**	**Days**	**mmol/g**	**Days**	**mmol/g**	**Days**	**mmol/g**
KO	1.08	0.484	5.22	0.821	15.50	1.293	18.29	1.309
	1.90	0.617	6.21	0.973		1.238		1.355
	2.65	0.668	8.08	0.961		1.236		1.277
	3.31	0.712	8.92	1.051		1.271	21.29	1.400
	3.60	0.827	9.92	1.035	16.29	2.591		1.423
		0.826	10.50	1.110	17.42	1.240		1.400
	4.17	0.822				1.278		1.475
	4.96	0.828				1.269		

A2.4 Phosphorus NMR

A2.4.1 Wood lignins

The data obtained from the phosphorus analysis of the lignins isolated from wood are shown in Table (A2.8). The data is grouped into the results obtained from individual experiments and each column pertains to the results obtained from different plots of the same experiment. The data shows that the results not only vary between individual experiments but that errors associated with differences in the integration also arise.

Table (A2.8). Data from phosphorus analysis of wood lignins (mmol/g lignin)

CELF1								
Aliphatic	4.20	–	–	4.32	–	–	4.64	–
Total	–	–	0.61	–	0.69	0.72	–	0.69
Cond.	–	–	0.21	–	0.23	0.27	–	0.22
Guaiacyl	0.39	0.39	0.37	0.42	0.41	0.40	0.46	0.43
p-hydrox.	–	0.05	0.03	–	0.05	0.05	–	0.04
COOH	–	0.07	0.05	–	0.07	–	–	0.06

CELF2								
Aliphatic	4.76	–	–	–	–	5.32	5.31	–
Total	–	–	–	–	0.99	–	1.08	1.08
Cond.	–	–	–	–	0.37	–	0.37	0.36
Guaiacyl	0.54	0.57	0.52	0.52	0.57	0.54	0.65	0.66
p-hydrox.	–	0.03	0.03	0.03	0.05		0.06	0.05
COOH	–	0.14	0.10	0.15	0.15	–	0.20	0.18

CEL2							
Aliphatic	5.24	–	4.85	–	–	5.02	–
Total	–	1.20	–	1.05	–	1.15	1.13
Cond.	–	0.30	–	0.34	0.46	0.40	0.37
Guaiacyl	0.74	0.67	0.69	0.66	0.73	0.71	0.70
p-hydrox.	–	0.04	–	0.05	0.04	0.04	0.06
COOH	–	0.19	0.10	–	0.13	0.18	0.18

BMWAL			
Aliphatic	4.68		4.47
Total	1.30	1.34	1.23
Cond.	0.35	0.39	0.32
Guaiacyl	0.88	0.88	0.85
p-hydrox	0.06	0.07	0.06
COOH	0.06	0.07	0.07

CELF2A		
Aliphatic		4.97
Total	1.17	1.30
Cond.	0.43	0.48
Guaiacyl	0.71	0.83
p-hydrox.	0.03	0.04
COOH	0.09	0.10

The data for each sample was averaged and the standard deviation was used to estimate the error in the data.

A2.4.2 Residual lignins

The phosphorus results for the residual lignin samples that were analysed more than once are shown in Table (A2.9). The data is organised in the same manner as the wood lignin samples (Table A2.8).

Table (A2.9). Data from phosphorus NMR analysis of pulp lignins (mmol/g lignin)

K-F1			
Aliphatic	1.35	1.51	1.48
Total	1.56	1.68	1.51
Cond.	0.76	0.84	0.74
Guaiacyl	0.75	0.76	0.71
p-Hydrox.	0.06	0.07	0.06
COOH	0.32	0.33	0.12

KOQP		
Aliphatic	2.19	2.36
Total	1.17	1.23
Cond.	0.62	0.59
Guaiacyl	0.53	0.60
p-Hydrox.	0.05	0.04
COOH	0.44	0.34

KOD			
Aliphatic	2.04	1.97	2.33
Total	1.08	1.03	1.24
Cond.	0.57	0.58	0.68
Guaiacyl	0.45	0.45	0.50
p-Hydrox.	0.06	0.06	0.06
COOH	0.47	0.43	0.40

KODE		
Aliphatic	2.20	2.62
Total	0.98	1.05
Cond.	0.51	0.55
Guaiacyl	0.43	0.48
p-Hydrox.	0.05	0.03
COOH	0.46	0.34

The data for each sample was averaged and the standard deviation was used to estimated the error.

A2.4 Thioacidolysis

A2.4.1 GC/FID analysis

The results from GC/FID analysis of the β-O-4 thioacidolysis degradation products (G: guaiacyl unit, H: *p*-hydroxy phenyl units) are tabulated below (Table A2.10).

Table (A2.10). Data from GC/FID analysis of thioacidolysis degradation products (μmol/g lignin) from all lignins (samples ending in 's' refer to spent liquor lignins)

Wood	G	977.5	1085.5	1153.9			
	H	8.7	13.3	12.8			
CELF1	G	1119.5	1186.4	1113.6	1098.5	997.4	1197.1
	H	17.3	19.1	19.5	14	13	14
CELF2	G	957.5	984.8	966.0	984.0	1000.6	972.0
	H	11.7	13.1	11.7			
CEL2	G	973.7	902.1				
	H	14.5	12.3				
CELF2A	G	955.8	936.3	KOQPn	G	243.8	253.3
WAL	G	993.9	1038.5	KOQZ	G	–	298.3
WANL	G	1057.6		KOQZE	G	210.4	223.4
BMWAL	G	937.4		KOD	G	209.9	183.9
				KODE	G	209.0	188.8
K	G	118.9		KOQPs	G	169.3	
KO	G	209.8	233.8	KOQPns	G	131.5	
KOQP	G	240.2		KOQZEs	G	148.8	

A2.4.2 GC/MS analysis

The GC/MS results are shown in Table (A2.11). All samples were analysed in duplicate; a larger volume was injected for the second run. This was to allow any small peaks which may not be detected in the first run to be detected. The result of increasing the injection volume was that the main β-O-4 monomer peak (1) became overloaded, thus in these runs this peak was not quantified. Also, in a number of cases the peak (G-CHRCH2CHR2) which was situated on the front of the β-O-4 peak was not visible.

Table (A2.11). Data from GC/MS analysis of thioacidolysis degradation products (μmol/g lig) (ND= not detected; T = trace (0-1 μmol/g lignin))

		Normal			Overloaded		
	Wood						
1	G-CHRCHRCH$_2$R	990.9	1216.2	1209.7		–	
2	G-CR=CHR	T	ND	1.9		ND	
3	H-CHRCHRCH$_2$R	18.6	12.3	17.8		–	
4	C-CHRCHRCH$_2$R	1.5	T	6.4		T	
5	G-CHR$_2$	29.6	17.1	30.8		51.5	
6	G-COOH	ND	ND	ND		15.7	
7	G-CH$_2$CH$_2$CH$_2$OH	41.8	62.6	63.5		88.5	
8	G-CH=CHCH$_2$R	ND	ND	ND		ND	
9	G-CHRCH$_2$CH$_2$R	55.8	49.2	50.8		–	
10	G-CHRCH$_2$CHR$_2$	12.4	7.9	27.6		30.6	
11	G-CH$_2$CHR$_2$	73.3	57.5	107.4		109.8	
12	G-CH=CHR	21.5	12.8	18.3		24.4	
13	G-CHRCH$_2$-G	14.6	16.3	32.6		29.5	
	CELF1						
1	G-CHRCHRCH$_2$R	1000	999	1320	1109	–	–
2	G-CR=CHR	5.3	6.7	T	8.6	7.8	4.8
3	H-CHRCHRCH$_2$R	20.2	17.5	5.1	24.7	8.5	18.7
4	C-CHRCHRCH$_2$R	8.0	8.2	T	17.4	16.5	6.3
5	G-CHR$_2$	67.2	44.5	44.0	65.5	37.8	60.2
6	G-COOH	4.2	4.7	ND	4.3	5.4	2.9
7	G-CH$_2$CH$_2$CH$_2$OH	36.5	37.7	35.6	35.1	30.5	44.2
8	G-CH=CHCH$_2$R	ND	ND	ND	ND	ND	ND
9	G-CHRCH$_2$CH$_2$R	ND	21.0	18.4	ND	36.1	29.0
10	G-CHRCH$_2$CHR$_2$	29.9	26.3	19.2	10.3	–	32.1
11	G-CH$_2$CHR$_2$	64.4	66.5	71.2	75.8	65.5	65.0
12	G-CH=CHR	25.3	26.7	19.4	25.9	10.1	27.1
13	G-CHRCH$_2$-G	40.8	41.7	18.4	54.3	47.6	43.5
	CELF2						
1	G-CHRCHRCH$_2$R	866	882	1085	956	–	–
2	G-CR=CHR	10.5	11.1	T	14.1	13.2	7.4
3	H-CHRCHRCH$_2$R	15.3	11.8	3.6	31.1	13.9	8.4
4	C-CHRCHRCH$_2$R	10.1	10.1	T	19.5	16.1	9.4
5	G-CHR$_2$	80.4	64.5	51.7	77.8	67.7	71.0
6	G-COOH	4.5	5.0	ND	5.5	4.8	2.9
7	G-CH$_2$CH$_2$CH$_2$OH	25.0	26.0	17.7	29.3	25.7	26.2
8	G-CH=CHCH$_2$R	ND	ND	ND	ND	ND	ND
9	G-CHRCH$_2$CH$_2$R	ND	13.9	8.4	ND	15.2	19.7
10	G-CHRCH$_2$CHR$_2$	49.9	53.5	29.6	32.3	–	44.8
11	G-CH$_2$CHR$_2$	47.9	49.0	30.2	56.5	49.3	34.9
12	G-CH=CHR	16.0	16.5	4.4	17.1	16.0	10.9
13	G-CHRCH$_2$-G	33.3	35.0	17.8	42.5	39.7	37.1

		Normal			Overloaded		
	CEL2						
1	G-CHRCHRCH$_2$R	868	870	1093	873	–	828
2	G-CR=CHR	9.6	10.1	7.6	13.8	12.3	12.7
3	H-CHRCHRCH$_2$R	ND	12.0	4.6	9.1	18.9	9.9
4	C-CHRCHRCH$_2$R	ND	10.1	5.9	17.6	15.9	13.2
5	G-CHR$_2$	87.8	70.6	62.7	80.4	–	74.0
6	G-COOH	7.4	7.8	T	9.4	8.3	1.1
7	G-CH$_2$CH$_2$CH$_2$OH	T	25.9	14.4	29.1	26.6	22.0
8	G-CH=CHCH$_2$R	ND	ND	ND	ND	ND	ND
9	G-CHRCH$_2$CH$_2$R	T	12.7	9.2	T	11.5	17.9
10	G-CHRCH$_2$CHR$_2$	39.5	39.0	30.6	36.0	–	46.0
11	G-CH$_2$CHR$_2$	51.5	51.7	43.4	71.2	52.9	50.3
12	G-CH=CHR	32.8	33.9	8.6	31.4	28.6	12.5
13	G-CHRCH$_2$-G	18.1	20.0	27.3	32.9	23.6	46.9
	CELF2A						
1	G-CHRCHRCH$_2$R	745.5	690.1	1109.4	–	689.3	901.8
2	G-CR=CHR	10.5	8.1	T	10.2	6.4	16.0
3	pH-CHRCHRCH$_2$R	17.6	10.0	6.6	12.4	ND	8.8
4	C-CHRCHRCH$_2$R	13.2	9.6	T	11.6	2.9	16.6
5	G-CHR$_2$	63.6	48.5	40.9	36.9	59.8	56.6
6	G-COOH	5.4	5.7	ND	6.3	4.5	7.3
7	G-CH$_2$CH$_2$CH$_2$OH	31.4	26.0	14.6	27.3	24.6	24.1
8	G-CH=CHCH$_2$R	ND	ND	ND	ND	ND	ND
9	G-CHRCH$_2$CH$_2$R	11.2	12.2	T	8.8	7.5	12.9
10	G-CHRCH$_2$CHR$_2$	26.3	29.8	24.4	–	29.3	40.4
11	G-CH$_2$CHR$_2$	53.9	42.7	27.4	43.7	42.2	27.6
12	G-CH=CHR	22.5	22.6	7.8	20.6	21.5	7.8
13	G-CHRCH$_2$-G	21.6	14.9	10.6	18.5	12.5	22.4
	WAL						
1	G-CHRCHRCH$_2$R	809	819	1212	905	–	1042
2	G-CR=CHR	5.1	3.5	T	6.7	6.5	2.7
3	H-CHRCHRCH$_2$R	22.4	27.0	6.2	36.1	22.7	19.6
4	C-CHRCHRCH$_2$R	9.1	ND	T	14.8	7.7	T
5	G-CHR$_2$	26.9	39.5	16.0	45.8	41.0	29.3
6	G-COOH	4.1	2.8	ND	4.4	3.6	3.6
7	G-CH$_2$CH$_2$CH$_2$OH	33.9	34.1	17.4	38.9	33.7	29.6
8	G-CH=CHCH$_2$R	ND	ND	ND	ND	ND	ND
9	G-CHRCH$_2$CH$_2$R	11.5	ND	9.9	ND	12.7	17.0
10	G-CHRCH$_2$CHR$_2$	30.6	29.6	17.6	19.9	–	33.4
11	G-CH$_2$CHR$_2$	59.6	58.6	40.0	71.4	66.3	52.9
12	G-CH=CHR	33.5	32.0	8.9	36.6	31.8	14.7
13	G-CHRCH$_2$-G	63.9	62.1	42.3	75.9	66.4	71.6

	WNAL	Normal	Over		**BMWAL**	Normal	Over
1	G-CHRCHRCH$_2$R	976.2	–	1	G-CHRCHRCH$_2$R	918.3	–
2	G-CR=CHR	3.9	3.1	2	G-CR=CHR	1.5	3.3
3	H-CHRCHRCH$_2$R	20.5	18.8	3	H-CHRCHRCH$_2$R	9.3	17.1
4	C-CHRCHRCH$_2$R	T	5.5	4	C-CHRCHRCH$_2$R	1.5	4.9
5	G-CHR$_2$	33.9	31.4	5	G-CHR$_2$	20.1	38.0
6	G-COOH	4.2	3.6	6	G-COOH	T	T
7	G-CH$_2$CH$_2$CH$_2$OH	29.5	25.5	7	G-CH$_2$CH$_2$CH$_2$OH	15.8	22.4
8	G-CH=CHCH$_2$R	ND	ND	8	G-CH=CHCH$_2$R	ND	ND
9	G-CHRCH$_2$CH$_2$R	17.5	15.4	9	G-CHRCH$_2$CH$_2$R	11.1	20.2
10	G-CHRCH$_2$CHR$_2$	39.6	33.9	10	G-CHRCH$_2$CHR$_2$	30.9	33.3
11	G-CH$_2$CHR$_2$	49.7	45.2	11	G-CH$_2$CHR$_2$	27.9	40.3
12	G-CH=CHR	10.6	11.5	12	G-CH=CHR	4.9	12.1
13	G-CHRCH$_2$-G	41.2	42.9	13	G-CHRCH$_2$-G	52.7	57.4

	Kraft	Normal			Overloaded		
1	G-CHRCHRCH$_2$R	139.3	140.2		–	148.5	
2	G-CR=CHR	ND	ND		2.3	ND	
3	H-CHRCHRCH$_2$R	ND	ND		ND	ND	
4	C-CHRCHRCH$_2$R	18.9	18.7		22.3	25.2	
5	G-CHR$_2$	6.5	6.5		6.6	10.8	
6	G-COOH	4.6	4.4		1.4	3.9	
7	G-CH$_2$CH$_2$CH$_2$OH	7.3	7.4		10.2	11.0	
8	G-CH=CHCH$_2$R	ND	ND		ND	ND	
9	G-CHRCH$_2$CH$_2$R	ND	ND		ND	ND	
10	G-CHRCH$_2$CHR$_2$	ND	ND		ND	ND	
11	G-CH$_2$CHR$_2$	5.7	5.4		7.4	8.3	
12	G-CH=CHR	3.4	3.1		4.2	4.5	
13	G-CHRCH$_2$-G	ND	ND		ND	ND	
	KO						
1	G-CHRCHRCH$_2$R	310.7	312.9		–	286.2	
2	G-CR=CHR	ND	ND		2.5	ND	
3	H-CHRCHRCH$_2$R	ND	ND		ND	ND	
4	C-CHRCHRCH$_2$R	22.4	21.3		29.0	33.4	
5	G-CHR$_2$	19.5	19.0		13.6	24.9	
6	G-COOH	6.6	5.3		2.8	4.0	
7	G-CH$_2$CH$_2$CH$_2$OH	21.1	20.6		25.3	29.5	
8	G-CH=CHCH$_2$R	ND	ND		ND	ND	
9	G-CHRCH$_2$CH$_2$R	ND	ND		ND	ND	
10	G-CHRCH$_2$CHR$_2$	ND	ND		ND	ND	
11	G-CH$_2$CHR$_2$	15.8	14.9		18.2	21.3	
12	G-CH=CHR	10.1	9.3		12.4	13.6	
13	G-CHRCH$_2$-G	ND	ND		ND	ND	
	KOQP						
1	G-CHRCHRCH$_2$R	276.0	273.8	253.5	–	284.8	–
2	G-CR=CHR	ND	ND	0.5	2.1	2.2	1.9
3	H-CHRCHRCH$_2$R	ND	ND	ND	ND	ND	ND
4	C-CHRCHRCH$_2$R	26.7	26.1	16.7	32.0	37.9	21.8
5	G-CHR$_2$	16.4	16.2	10.1	16.2	27.3	42.9
6	G-COOH	3.7	3.1	ND	3.3	3.2	3.2
7	G-CH$_2$CH$_2$CH$_2$OH	11.0	11.8	4.4	10.4	11.5	7.0
8	G-CH=CHCH$_2$R	ND	ND	ND	ND	ND	ND
9	G-CHRCH$_2$CH$_2$R	ND	ND	ND	ND	ND	ND
10	G-CHRCH$_2$CHR$_2$	ND	ND	ND	ND	ND	ND
11	G-CH$_2$CHR$_2$	14.2	14.2	9.0	15.4	17.2	16.1
12	G-CH=CHR	6.0	5.6	3.9	6.2	7.1	13.1
13	G-CHRCH$_2$-G	ND	ND	ND	ND	ND	ND
	KOQPn						
1	G-CHRCHRCH$_2$R	268.8	269.7	277.8	–	290.0	–
2	G-CR=CHR	1.4	ND	T	1.6	ND	1.8
3	H-CHRCHRCH$_2$R	ND	ND	ND	ND	ND	ND
4	C-CHRCHRCH$_2$R	19.2	18.0	11.0	24.9	28.0	18.3
5	G-CHR$_2$	9.6	9.6	5.7	6.7	11.0	17.6
6	G-COOH	5.0	3.8	ND	3.4	3.0	1.9
7	G-CH$_2$CH$_2$CH$_2$OH	10.1	9.7	5.8	9.8	10.8	9.4
8	G-CH=CHCH$_2$R	ND	ND	ND	ND	ND	ND
9	G-CHRCH$_2$CH$_2$R	ND	ND	ND	ND	ND	ND
10	G-CHRCH$_2$CHR$_2$	ND	ND	ND	ND	ND	ND
11	G-CH$_2$CHR$_2$	14.8	14.7	9.9	15.6	17.3	16.8
12	G-CH=CHR	7.2	7.2	3.9	7.0	7.7	8.1
13	G-CHRCH$_2$-G	ND	ND	ND	ND	ND	ND

	KOQZ	Normal			Overloaded		
1	G-CHRCHRCH$_2$R	340.5	341.4	220.1	–	317.8	–
2	G-CR=CHR	2.0	ND	0.5	2.7	ND	1.6
3	H-CHRCHRCH$_2$R	ND	ND	ND	ND	ND	ND
4	C-CHRCHRCH$_2$R	28.1	24.1	11.9	35.2	35.9	17.8
5	G-CHR$_2$	36.3	35.6	13.3	32.7	32.8	23.7
6	G-COOH	5.5	3.3	ND	3.5	3.0	1.9
7	G-CH$_2$CH$_2$CH$_2$OH	15.0	14.3	6.7	13.2	13.0	8.0
8	G-CH=CHCH$_2$R	ND	ND	ND	ND	ND	ND
9	G-CHRCH$_2$CH$_2$R	ND	ND	ND	ND	ND	ND
10	G-CHRCH$_2$CHR$_2$	ND	ND	0.4	ND	ND	1.3
11	G-CH$_2$CHR$_2$	12.3	11.6	6.4	13.0	13.1	11.3
12	G-CH=CHR	3.5	2.8	1.2	3.6	3.2	2.9
13	G-CHRCH$_2$-G	ND	ND	ND	ND	ND	ND
	KOQZE						
1	G-CHRCHRCH$_2$R	241.2	235.1	236.4	–	240.6	–
2	G-CR=CHR	1.3	ND	T	2.4	ND	1.3
3	H-CHRCHRCH$_2$R	ND	ND	ND	ND	ND	ND
4	C-CHRCHRCH$_2$R	18.3	16.9	10.5	19.0	22.2	18.5
5	G-CHR$_2$	23.4	22.0	13.5	26.6	30.2	20.2
6	G-COOH	2.4	2.2	ND	5.3	4.3	1.6
7	G-CH$_2$CH$_2$CH$_2$OH	7.6	7.3	7.3	10.8	12.1	7.8
8	G-CH=CHCH$_2$R	ND	ND	ND	ND	ND	ND
9	G-CHRCH$_2$CH$_2$R	ND	ND	ND	ND	ND	ND
10	G-CHRCH$_2$CHR$_2$	ND	ND	ND	ND	ND	2.3
11	G-CH$_2$CHR$_2$	9.9	9.6	8.3	11.1	11.8	12.4
12	G-CH=CHR	4.0	3.5	2.0	4.4	4.6	3.2
13	G-CHRCH$_2$-G	ND	ND	ND	ND	ND	ND
	KOD						
1	G-CHRCHRCH$_2$R	205.5	207.4	203.7	–	202.5	–
2	G-CR=CHR	ND	ND	T	1.5	2.6	1.7
3	H-CHRCHRCH$_2$R	ND	ND	ND	ND	ND	ND
4	C-CHRCHRCH$_2$R	14.2	13.4	8.5	19.3	13.3	16.2
5	G-CHR$_2$	14.7	14.5	6.6	17.2	19.4	17.2
6	G-COOH	3.0	2.0	ND	2.6	2.4	1.9
7	G-CH$_2$CH$_2$CH$_2$OH	6.3	6.2	5.3	7.9	8.9	8.7
8	G-CH=CHCH$_2$R	ND	ND	ND	ND	ND	ND
9	G-CHRCH$_2$CH$_2$R	ND	ND	ND	ND	ND	ND
10	G-CHRCH$_2$CHR$_2$	ND	ND	0.5	ND	ND	1.6
11	G-CH$_2$CHR$_2$	9.4	9.2	6.8	10.9	12.1	13.2
12	G-CH=CHR	4.4	3.8	2.2	4.6	5.1	3.6
13	G-CHRCH$_2$-G	ND	ND	ND	ND	ND	ND
	KODE						
1	G-CHRCHRCH$_2$R	224.1	214.9	246.7	–	215.6	–
2	G-CR=CHR	ND	ND	T	ND	ND	1.4
3	H-CHRCHRCH$_2$R	ND	ND	ND	ND	ND	ND
4	C-CHRCHRCH$_2$R	10.1	8.8	7.5	11.1	14.1	12.4
5	G-CHR$_2$	16.8	16.3	7.9	13.1	22.0	17.8
6	G-COOH	3.6	3.0	ND	2.2	2.5	2.0
7	G-CH$_2$CH$_2$CH$_2$OH	10.7	10.3	9.2	11.1	12.4	11.2
8	G-CH=CHCH$_2$R	ND	ND	ND	ND	ND	ND
9	G-CHRCH$_2$CH$_2$R	ND	ND	ND	ND	ND	ND
10	G-CHRCH$_2$CHR$_2$	ND	ND	0.9	ND	ND	0.8
11	G-CH$_2$CHR$_2$	9.4	8.9	7.7	10.3	11.0	12.1
12	G-CH=CHR	4.2	3.6	2.4	4.3	4.4	3.3
13	G-CHRCH$_2$-G	ND	ND	ND	ND	ND	ND