Ultrastructural Characterization (Morphological and Topochemical) of Wood Pulp Fibres; Effects of Mechanical and Kraft Processes

Dinesh Fernando
Faculty of Forest Sciences
Department of Forest Products/Wood Science
Uppsala

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Abstract

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Different electron microscopy techniques including SEM (scanning electron microscopy), FE-SEM (field emission-scanning electron microscopy), TEM (transmission electron microscopy) and Immuno-gold TEM (immuno-gold transmission electron microscopy) were applied in order to gain a better understanding of the influence of the native softwood fibre cell wall ultrastructure including morphology and topochemistry (*i.e.* lignin and glucomannan distribution) during mechanical pulping. In thermomechanical pulp (TMP) processing, wood fibres undergo structural changes (cell wall delamination and fibrillation) that are regulated by the native fibre micro- and ultrastructure. In addition, novel information was obtained on the fibre cell wall architecture.

In contrast, the stoneground wood (SGW) process inflicted severe damage to the fibre structure resulting in transverse and longitudinal fibre breakage. However, juvenile wood SGW fibres showed improved properties (strength and light scattering) compared to mature wood. Ultrastructural aspects of fibre processing and development explained the differences in physical properties observed. During the SGW process, the native morphological fibre cell wall ultrastructure and microfibrillar organization governed the manner of juvenile wood fibre development similar to TMP fibres.

Ultrastructural studies on Norway spruce and Scots pine TMPs revealed fundamental features that governed the different behaviour exhibited by the two wood species. Specific ultrastructural characteristics of pine TMP fibre cell walls were explored in relation to both morphology and topochemistry and that regulating the different pine fibre development mechanisms compared to spruce. The negative behaviour shown by Scots pine during TMP processing was most likely attributable to the observed fibre development mechanism.

Histochemical techniques were applied to study wood resin associated problems during mechanical and kraft pulping. Studies provided information on the spatial micro-morphological distribution/redistribution of lipophilic extractives that were visualized on single fibre and cell wall fractions. Results from histochemical staining and chemical analysis performed on Norway spruce and Scots pine TMPs showed that there were morphological and chemical differences in the redistribution of extractives between the two species. This may further contribute to the effects of extractives on pulp- and paper properties and processing.

Localization of lipophilic birch wood extractives involved in pitch problems was performed using histochemical techniques. Correlated information from gas chromatography-mass spectrometry and specific staining methods gave details on how extractives are removed during processing as well as information on the mechanisms of removal.

Key words: Mechanical pulps, fibrillation, fibre cell wall ultrastructure, hemicellulose, glucomannan, lignin, *Picea abies* (L.) Karst, *Pinus sylvestris* L., extractives, cytochemical staining, SEM, FE-SEM, TEM, immuno-EM.

Author's address: Dinesh Fernando, Wood Ultrastructure Research Centre, Department of Forest Products/Wood Science, Swedish University of Agricultural Sciences, P.O. Box 7008, SE-750 07 Uppsala, Sweden. E-mail: Dinesh.Fernando@sprod.slu.se

Wood Ultrastructure Research Centre (WURC)

This work was carried out within the framework of the Wood Ultrastructure Research Centre (WURC). WURC is a centre of competence established in co-operation with VINNOVA (NUTEK), six companies from the pulp and paper industry and one company from the chemical industry. The centre's main operations are at the Swedish University of Agricultural Sciences (SLU) in Uppsala. Research on wood fibre ultrastructure is performed in co-operation between SLU, Swedish Pulp and Paper Research Institute (STFI-Packforsk), Royal Institute of Technology (KTH), Chalmers University of Technology (CTH), Uppsala University (UU), Karlstads University, Örebro University and industrial partners (EKA Chemicals, Holmen, Smurtfit Kappa Kraftliner, Korsnäs, M-real, SCA, StoraEnso, Sveaskog and Södra). Website: www-wurc.slu.se.

List of abbreviations and definitions

AFM	Atomic force microscopy
CPD	Critical point drying
EM	Electron microscopy
FE-SEM	Field Emission-scanning electron microscopy
FM	Fluorescence microscopy
GM	Glucomannan
MFA	Microfibril angle. Defined as the angle between the fibre axis and the orientation of cellulose microfibrils
ML	Middle lamella
MP	mechanical pulp
P	Primary wall
SEM	Scanning electron microscopy
SGW	Stone groundwood
S1	Outer layer of the secondary cell wall
S2	Middle dominating layer of the secondary cell wall
S3	Inner layer of the secondary cell wall
TMP	Thermomechanical pulp
TEM	Transmission electron microscopy
Ultrastructure	Defined as features which are rendered visible by methods which are able to resolve structures at higher resolution than light microscopy. Ultrastructural features are measured in nanometers.
XPS	X-ray Photoelectron Spectroscopy

Contents

Appendix

Papers I-V

The present doctoral thesis is based on the following papers, which will be referred to by their Roman numerals.

I. Fernando, D. and Daniel, G. (2004) Micro-morphological observations on spruce TMP fibre fractions with emphasis on fibre cell wall fibrillation and splitting. *Nordic Pulp and Paper Research Journal.* 19:3, 278-285.

II. Fernando, D., Rosenberg, P., Persson, E. and Daniel, G. (2007) Ultrastructural aspects of fibre development during SGW process; A key to insights into pulp property development. *Submitted to Holzforschung*.

III. Fernando, D. and Daniel, G. Exploring pine fibre development mechanisms during TMP processing; Impact of cell wall ultrastructure (morphological and topochemical) on negative behaviour (Manuscript).

IV. Fernando, D., Hafrén, J., Gustafsson, J. and Daniel, G. (2007) Micro-morphology and topochemistry of extractives in pine and spruce TMP; Cytochemical and physical property comparisons. *Submitted to Journal of Wood Science.*

V. Fernando, D., Daniel, G. and Lidén, J. (2005) The state and spatial distribution of extractives during birch kraft pulping, as evaluated by staining techniques. *Nordic Pulp and Paper Research Journal.* 20:4, 383-391.

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Related publications

i. Fernando, D., Hafrén, J., Brändström, J. and Daniel, G. (2004) Influence of native fibre structure on refiner mechanical pulps and final products. 4th Fundamental Mechanical Pulps Seminar, June 7-8, Stockholm, Sweden.

ii. Fernando, D., Lidén, J. and Daniel, G. (2005) The nature and behaviour/redistribution of extractives during birch kraft pulping; microscopical histochemical approach. 6th PRWAC Kyoto2005, December 1-5, Kyoto University, Kyoto, Japan.

iii. Fernando, D., Rosenberg, P., Persson, E. and Daniel, G. (2007) Fibre development during stone grinding; ultrastructural characterization for understanding derived properties. TAAPI International Mechanical Pulping Conference 2007, May 7-9, Minneapolis, Minnesota, USA.

1. Introduction

1.1 Background

Wood fibre-based products have long been catering for the well-being of human lives in many ways ever since the beginning of their life history. Although the pulp and paper industry is one major user today for renewable lignocellulosic wood fibres, they also provide an endless supply for the construction and furniture industries, for chemicals (*e.g.* tar), and in recent years with new innovations lignocellulosic biomass is an attractive feedstock for future supplies of biofuels, *i.e.* ethanol (Gray, Zhao & Emptage, 2006). However, in all these areas of lignocellulosic usage, a better understanding of the structure and in particular the behaviour of wood fibres (*i.e.* response of fibre cell wall) during processing (*e.g.* pulp and paper processing) is fundamental and of importance for its optimal utilization and for improvements in end-products.

Since wood fibres are the building blocks of pulp and paper, the morphological and chemical characteristics of fibre cell walls at the ultrastructural level are fundamental and governing for most physical properties of final pulp and paper products. Intensive studies over several decades on mechanical pulps (MP) particularly of physical characteristics have provided better understanding of process-property relationships of mechanical pulp-based furnishes. However, fundamental knowledge on fibre development leading to pulp and paper property development at the cell wall level (*i.e.* topochemical and morphological ultrastructure of pulp fibres) is still very limited.

Furthermore, the chemical heterogeneity of wood raw materials especially lipophilic wood extractives, although present in minor amounts, strongly influence pulping processes leading to the shutting down of process equipment and the blemishing of final paper products. Although modern techniques in chemical analysis (*e.g.* GC-MC, FT-IR, XPS *etc*) provide improved opportunities for distinguishing between individual extractive components and their quantification, such tools provide little information concerning the spatial distribution and redistribution of extractives and their morphological behaviour during processing. Therefore, new tools should be introduced that render visualization of the micro-morphological behaviour of individual extractive components during processing, that in combination with chemical analysis can provide important clues for effective control measures.

The present study focuses on characterizing the topochemical and morphological ultrastructure of mechanical pulp fibres that are fundamental and govern the development of most pulp and paper properties. In addition, the present work involves techniques that provide information concerning spatial micro-morphological and topochemical aspects of wood resin during pulping.

1.2 Objectives of the study

The main objective of this thesis was to obtain a greater understanding of the ultrastructural characteristics of wood pulp fibres during mechanical pulping, particularly the surface morphological ultrastructure in relation to fibre cell wall fibrillation and cell wall responses to the process. An attempt was made to understand the fundamentals on how the surface morphological ultrastructure and topochemical features of the pulp fibre cell walls govern pulp and paper properties.

Another major objective was to study the nature and behaviour of wood resin (*i.e.* lipophilic extractives) during pulping in relation to topochemical and micro-morphological distribution and redistribution. The application of histochemical microscopy as an analytical tool in wood resin research was conducted for understanding problems associated with extractives (*i.e.* pitch problems) during pulping processes.

1.3 The structure of wood

All tree species are seed-bearing plants (*i.e.* Spermatophytae) that produce wood (*i.e.* secondary xylem) during growth. They are further sub-divided into gymnosperms and angiosperms. Coniferous woods (*i.e.* softwoods; *e.g.* Norway spruce and Scots pine) belong to the gymnosperms and hardwoods (*e.g.* birch) to the angiosperms. In all trees, wood is formed by a thin layer of living cells called vascular cambium (Fig. 1) that by repeated division produces phloem cells to the outside and secondary xylem (or wood) to the inside. The xylem can be divided into outer *sapwood* containing both living and dead cells and inner *heartwood* comprising entirely of dead cells where formation and deposition of phenolic compounds and other extractives has occurred making heartwood less permeable and more durable.

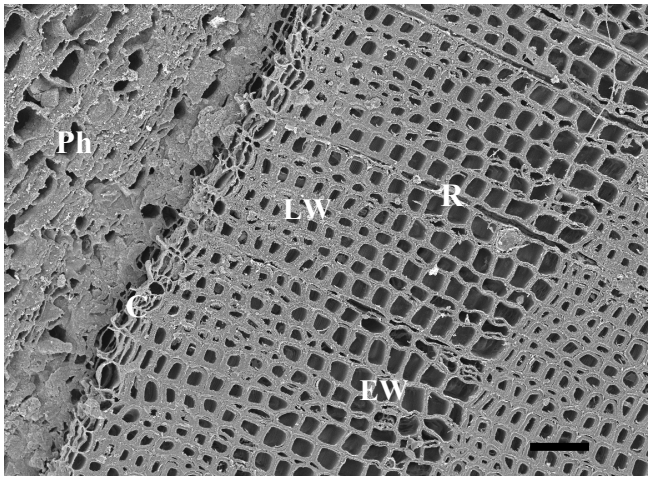


Fig. 1. Transverse section through xylem and phloem of Norway spruce. EW, earlywood; LW, latewood; C, cambium; Ph, phloem; R, ray parenchyma. Bar: 40.0 µm.

In temperate regions, the growth of trees occurs in the spring and summer months of any year giving wood the appearance in cross section of distinct concentrically oriented rings that are referred as annual growth rings. Major cell types formed during spring are called *earlywood* (EW) and are characterized by thin cell walls and large cell lumina while those created during summer, *i.e. latewood* (LW), have thick cell walls and small lumina (Fig. 1).

Softwood consists of a limited number of cells and its structure is more or less uniform and simple compared to hardwood. Softwood xylem is mainly composed of longitudinal tracheids (90-95% of total cell volume) and smaller amounts of ray parenchyma (5-10%; Sjöström, 1993), ray tracheids and epithelial cells surrounding resin canals. Some species like Norway spruce and Scots pine may also possess traces of longitudinal (axial) living parenchyma. Longitudinal tracheids will herein for simplicity be referred as fibres. Softwood fibres are generally long and slender cells that are often 100 times greater in length than in width (*e.g.* in Norway spruce the average length is 2-4 mm and tangential width 20-40 μm; Sjöström, 1993) and have a hollow centre (*i.e.* cell lumen) and closed ends. Fibres formed during summer *i.e.* latewood fibres provide mechanical support and strength to the tree while earlywood fibres are primarily involved in conduction of fluids. Water transport between adjacent wood cells (*i.e.* lateral movement) occurs through natural openings in the fibre cell walls called pits. There are three major pit types namely *simple pits* that connect adjacent ray parenchyma cells, *cross-field pits* (half-bordered pits) that unite ray parenchyma cells with axial fibres and *bordered pits* that unite adjacent axial fibres and axial fibres to ray tracheids.

In contrast, hardwoods like birch are more advanced and complex in their general anatomical organization. The xylem of hardwoods contains fibres (*e.g.* libriform fibres), vessels, tracheids and parenchyma cells. Hardwoods generally contain a greater proportion of parenchyma cells (*i.e.* both ray and longitudinal parenchyma) and often possess large rays and well developed longitudinal parenchyma (*e.g.* birch, 9-12% ray and 2% axial parenchyma; Back & Allen, 2000). Because of cellular encapsulation, wood resin together with resin anatomy and morphological features like small simple pits (*e.g.* birch, Paper V) create major problems (*i.e.* pitch problems) during kraft pulping (Paper V).

1.4 Chemical composition and structure of wood fibres

In this thesis, the predominant raw material came from softwood fibres that represent the main raw material for MP production in Scandinavia. Fibres are complex biocomposites composed of the three main biopolymers, cellulose, hemicellulose and lignin. In addition, there are other minor components in wood such as pectin, inorganic compounds and extractives (< 6%). The approximate chemical compositions of the wood species used in this thesis (*Picea abies* (L.) Karst, *Pinus sylvestris* L. and *Betula pendula* Roth) are given in Table 1.

Table 1. *Chemical composition (mass %) of Norway spruce, Scots pine and birch. Data from Sjöström (1993)*

Species	Cellulose	Glucomannan	Xylan	Other polysacch.	Lignin	Extractives
Norway spruce	42	16	9	3	27	2
Scots pine	40	16	9	4	28	4
Birch	41	2	28	3	22	3

1.4.1 Cellulose

Cellulose is the most abundant biopolymer on earth, and represents the main structural component in wood cell walls accounting for 40-45% of the dry wood. It is an unbranched homopolysaccharide (linear polymer) built up of β-D-glucose units linked together by (1→4) glucosidic bonds. The number of monomeric units making up the cellulose chain (*i.e.* degree of polymerization) varies depending on the source and in wood it is about 10,000 glucose residues (Sjöström, 1993). Since cellulose is a linear polymer, it is capable of forming strong intra- and intermolecular hydrogen bonds that retain the chains of glucose molecules straight and bundles of cellulose chains are aggregated into fibrillar structures (*i.e.* cellulose microfibrils). The cellulose microfibrils are normally ordered into crystalline and amorphous regions (Sjöström, 1993).

The major role of cellulose microfibrils is to impart mechanical strength to fibres and ultimately the tree. They are normally aggregated into larger structures (*e.g.* macrofibrils) in the native cell walls and different terminologies have been used in the literature to describe this fibrillar assembly of cellulose chains as their size varies greatly depending on the methods of preparation and analysis. For example, elementary fibrils of *ca* 3.5 nm diameter in pulp (Heyn, 1977), sub-elementary fibrils of *ca* 1.5-4 nm (Horii, Hirai & Yamamoto, 2000) and cellulose fibrils of 4.0-4.5 nm (Hult, Larsson & Iversen, 2002) have been reported. The term microfibrils will be used in this thesis to denote the smallest fibrillar unit of cellulose and macrofibrils (*e.g.* 30 nm diameter, Paper I) to refer to aggregates of microfibrils that are much larger in size (*e.g.* 10-60 nm range in kraft pulp fibres; Duchesne, 2001). In addition, different terminologies were proposed in Paper I where a structural hierarchy of cellulose fibrils (they are believed to be composed of set number of cellulose aggregates) was noted in mechanical pulp fibre cell walls and names given accordingly (*i.e.* based on their width).

1.4.2 Hemicellulose

Hemicellulose is a diverse group of polysaccharides that are often branched polymers consisting of various sugar units of mannose, xylose, glucose, galactose, arabinose, fucose and rhamnose in addition to some acidic sugars *e.g.* galacturonic-, glucuronic- and 4-*O*-methylglucuronic acid. It is a structural carbohydrate that normally forms 20-30% of the dry weight of wood and plays a supporting role in the fibre cell wall. Structurally, it consists of either a homo- (*i.e.*

only one type of monomer) or heteropolymer (*i.e.* two or several types of monomers) backbone to which short branches of other sugar units are substituted laterally. The backbone substitution and degree of branching can vary considerably between hemicelluloses of the same type (Sjöström, 1993). This structural pattern may be of significance for the supermolecular interactions within the secondary cell wall. Hemicelluloses generally have a lower degree of polymerisation than cellulose, *i.e.* approximately between 100 and 200 (Fengel & Wegener, 1984).

There is a distinct variation in the composition of hemicelluloses between softwoods and hardwoods. In softwood (*i.e.* Scots pine and Norway spruce) the predominant hemicellulose is galactoglucomannan which comprises *ca* 20% while xylan is the most abundant in hardwoods forming up to 30% of the dry wood (Sjöström, 1993).

Softwood galactoglucomannan consists of a linear backbone of (1→ 4) linked β-D-mannose and β-D-glucose residues carrying a side-group consisting of single D-galactose residues attached by α-(1→ 6) linkages. Native galactoglucomanan from softwoods are partially acetylated (*O*-acetyl groups) at C2 or C3 positions of the mannose, on average one group per 3-4 backbone hexose units (Sjöström, 1993).

Hemicelluloses in softwoods are thought to be oriented parallel to the cellulose chains facilitating encrustation of cellulose microfibrils within the hemicellulose/lignin matrix (Page, 1976). Salmén and Olsson (1998) suggest that glucomannans are more intimately associated with cellulose and xylan more with lignin (Fig. 3c). Therefore, hemicelluloses are thought to be located around cellulose microfibrils and also within the matrix between cellulose aggregates (Fig. 3).

1.4.3 Lignin

Lignin is the second most abundant biopolymer on earth and has a very complex three dimensional structure consisting of three aromatic groups, *i.e. p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol depending on the wood type.

Lignin acts as a cementing agent to reinforce cellulose microfibrils in the cell wall by filling the spaces between microfibrils and hemicellulose and thereby making the fibres relatively stiff. The greatest concentration of lignin (g/g) is found in the middle lamellae (ML) and act as glue for keeping cells together in the wood. The secondary walls of the fibres contain the bulk of lignin (Fengel & Wegener, 1984).

1.4.4 The ultrastructure of wood fibre cell walls

Fibres in wood are joined together by the middle lamella (ML; Fig. 2a) that is 0.2-1.0 µm thick (Sjöström, 1993).

The ultrastructure of the wood fibre cell wall is a hierarchical structure ranging from molecular up to fibre cell wall levels. The "true structure" of the fibre cell wall is extremely complex and variable (Bailey, 1938) and controversy still exists and a number of different models have been proposed through the years by scientists (*e.g.* Wardrop, 1954; Dunning, 1969; Fengel & Wegener, 1984; Harada & Côté, 1985; Zimmermann & Sell, 1997). Despite divergence in detail representation, it is well accepted that wood cell walls are composed of a number of different cell wall layers forming the outermost primary (P) and inner secondary cell wall layers namely outer thin S1, thicker middle S2 and innermost thin S3 (Fig. 2a). Brändström (2002) recently suggested a model for Norway spruce fibres (Fig. 2b).

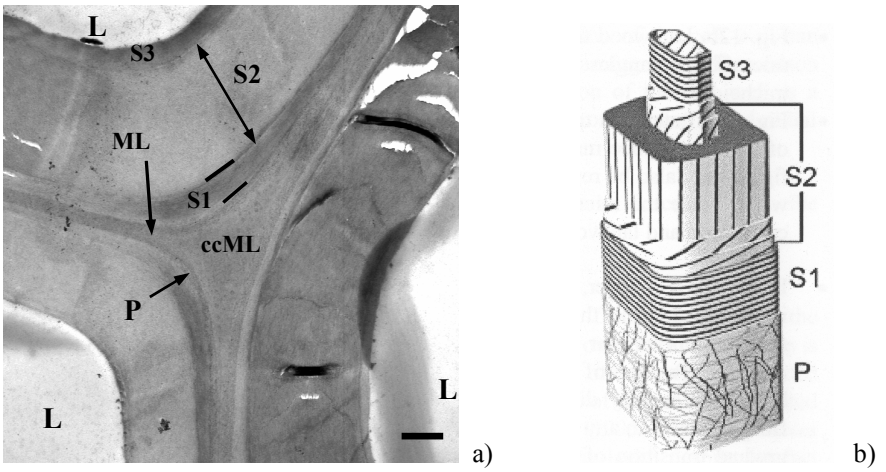
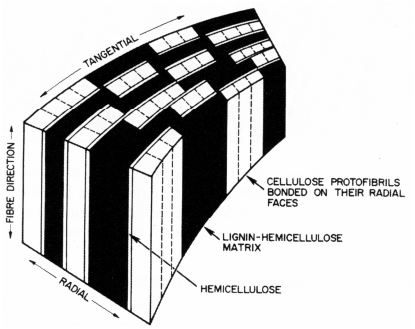


Fig. 2. Major wood fibre cell wall layers. a) Transmission electron micrographs (T.S section) of Norway spruce fibres showing the different cell wall layers; middle lamella (ML), primary wall (P), S1, S2 and S3 secondary cell wall layers. b) Model of cell wall layers in a softwood latewood fibre. Lines in the different layers represent the MFA (Brändström, 2002). ccML= cell corner middle lamella, L= cell lumen. Bar: 0.5 µm.

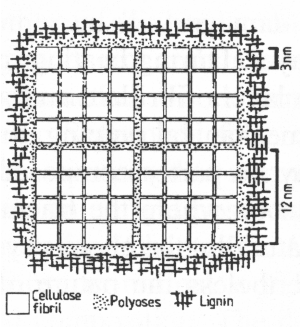
Fibre cell wall layers can be distinguished from one another since the cellulose microfibrils are oriented variably in different layers (orientation of cellulose microfibrils is known as the microfibril angle (MFA)) and wind around the cell axis either to the right (*i.e.* Z-helix) or to the left (*i.e.* S-helix) within the individual layers. The Primary wall (P) layer is comprised of randomly oriented microfibrils (Fig. 2b). S1 is composed of microfibrils oriented approximately perpendicular (*i.e.* horizontal) to the fibre axis (70-90^{o} in Norway spruce; Brändström, 2002) while in S2 they are almost vertical (*e.g.* 2-5^{o}) in Norway spruce LW fibres (Brändström, 2002) and in S3 they are again almost horizontal to the fibre axis (Fig. 2b).

Several models have also been proposed for the molecular arrangement of the three major polymers, cellulose, hemicellulose and lignin in wood cell walls, although no model is fully accepted. A popular model for the ultrastructural architecture of wood cell walls shows the cellulose microfibrils (or sub-elementary

fibrils) to compose the backbone structure and hemicellulose and lignin to exist as matrix materials around the microfibrils (Eaton & Hale, 1993). Furthermore, the microfibrils surrounded by hemicelluloses (*e.g.* glucomannan; Salmén & Olsson, 1998) are aggregated to form "macrofibrils" (cellulose aggregates). Figure 3 shows three models proposed for the ultrastructural organization of the biopolymers in wood fibre cell walls.



a)



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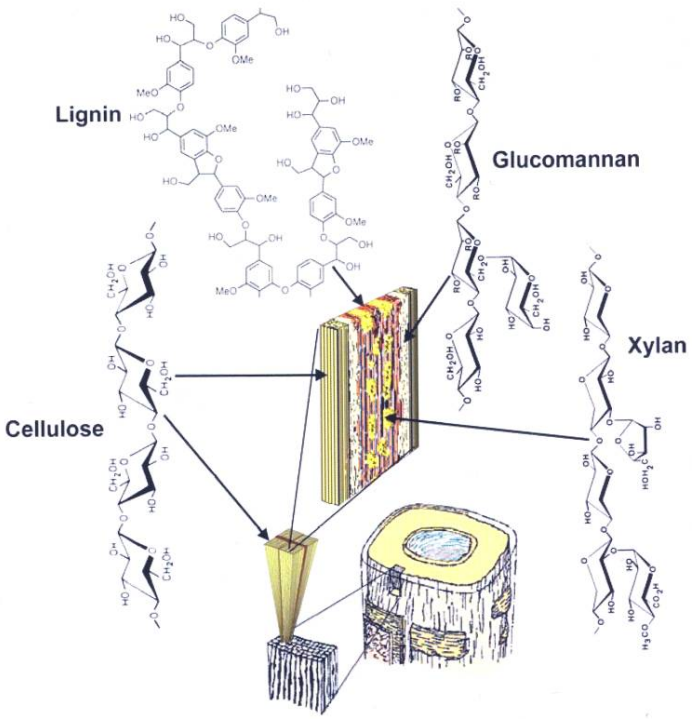

c)

Fig. 3. Proposed molecular arrangement of wood polymers in the fibre cell wall. a) Model illustrating cellulose microfibrils forming interrupted lamellae embedded in a lignin/hemicellulose matrix (from Kerr and Goring, 1975); b) Model proposed by Fengel and Wegener (1984) with elementary fibrils surrounded by monolayer of hemicellulose and larger units (macrofibrils) enclosed with hemicellulose and lignin; c) Salmén and Olsson (1998) where a detailed arrangements of polymers is presented showing glucomannan more closely associated with cellulose and xylan with lignin (from Fahlén, 2005).

1.4.5 Extractives

Extractives are generally low-molecular-weight compounds present as minor components in the wood (Table 1) that are soluble in organic solvents such as acetone. They are non-structural wood constituents comprised of a large number of compounds of both lipophilic (*i.e.* wood resins) and hydrophilic (not concerned in this work) types. Lipophilic extractives (herein for simplicity referred to as *resin*) are those that cause major problems in pulp and paper manufacture to both the machineries and paper products, and can be classified into four major groups; 1) fats and fatty acids (FA), 2) sterols and steryl esters, 3) terpenoids (*e.g.* resin acids) including terpenes, and 4) waxes (*i.e.* fatty alcohols and their esters of FA).

Although there is a general pattern of wood resin chemistry even between softwoods and hardwoods, extractable resin components differ among tree families, genera, and species and even within a tree. For example, most of the resin found in sapwood (*i.e.* fats, steryl esters and waxes) is hydrolysed during heartwood formation resulting in higher amounts of FAs, sterols and fatty alcohols in the heartwood. In addition, specific wood resin components like terpenyl alcohols and phenolics *etc* are usually formed in heartwood (Back, 2002).

Resin is located in certain morphological sites in the wood structure in both softwoods and hardwoods. In native wood they are mainly located in the living parenchyma cells and are frequently called "parenchyma resin". Parenchyma resin in both softwood and hardwoods is mainly composed of fats (*e.g.* predominantly triglycerides) and steryl esters of FAs in addition to minor amounts of hydroxy FAs. Additional resin is located in the resin canals that are more frequent and well developed in pine than in spruce. Resin is synthesized and secreted into the canal by epithelial cells that surround the canal and are alive in sapwood. Thick-walled epithelial cells in spruce (Fig. 4a) compared to thin-walled epithelial cells in pine are a diagnostic feature between the two species and an important factor with respect of deresination during pulping (Back & Allen, 2000).

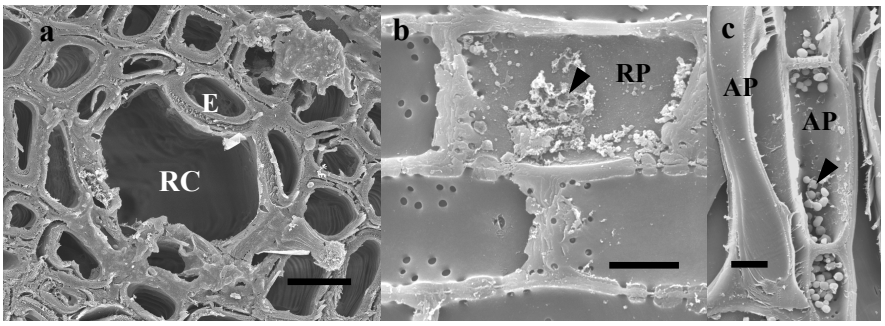

Fig. 4. SEM micrographs showing morphological sites for resin in the native wood structure. a) Resin canal (RC) surrounded by characteristic thick-walled epithelial cells (E) in Norway spruce; b) More abundant ray parenchyma (RP), and c) well developed axial parenchyma (AP) of birch wood showing resin materials (arrowhead) inside. Bars: a, b, c, 10.0 μm.

In contrast, in birch all the resin is located in the parenchyma cells (*i.e.* both ray and axial parenchyma; Fig. 4b, c) that compose a considerable part of the wood tissue (Paper V).

1.5 Mechanical pulping

During mechanical pulping, wood fibres in the integrated wood structure are separated from one another and processed for papermaking by means of purely mechanical treatments. In contrast to chemical pulps, mechanical pulping produces high yield pulps (*i.e.* 97-98% yield for Norway spruce; Sundholm, 1999) in which a broad distribution of particles of various shapes and sizes (*i.e.* bundles of fibres (shives), long intact fibres, broken fibres, fragments of the fibre walls (fines) *etc.*) can be found.

There are two major commercial processes for producing mechanical pulps, the "grinding" and "refining" processes, which produce end-products with significantly different properties. For example, stone groundwood (SGW) process belongs to the former category while the thermomechanical pulp (TMP) process from which the majority of samples were derived in the present work falls into the latter.

During SGW processing, wood logs lying parallel to the grinder shaft are pressed against a water sprayed revolving grindstone. Due to this harsh mechanical action, the wood fibres are severly damaged producing greater amounts of fines and middle fractions from broken fibres and cell walls and reduced amounts of long intact fibres (Kappel, 1999). Groundwood is also produced under pressurized conditions in the grinding chamber (*i.e.* Pressure Groundwood, PGW) where less damaged fibres can be produced compared to SGW (Reme, 2000).

SGW pulps are characterized by superior optical properties like light scattering and better surface smoothness of the final paper products compared to TMP (Honkasalo & Ebeling, 1981; Vaarasalo *et al.,* 1981; Tuovinen & Liimatainen, 1994). However, the strength properties (*i.e.* tensile- and tear strength) of the SGW pulps are lower than that of TMPs.

Today, the TMP process is the dominating process for production of mechanical pulps for wood containing papers. In the TMP process, wood raw materials in the form of wood chips are first preheated with steam and then refined between two metal discs at elevated temperature (*ca* 120 ^{o}C) and pressure. In contrast with SGW, the TMP process involves a thermal softening of the wood structure that is thought to be the key to the superior strength properties of TMPs (Tienvieri *et. al.,* 1999) not only due to an increased longer fibre fraction but also for improved pulp fibre cell wall fibrillation. TMPs therefore have a greater long fibre fraction but are lower in fines and middle fractions compared to SGW pulps.

In addition to the superior strength properties provided by TMPs, the process offers other advantages such as utilization of wood chips and production of tailor-made high quality pulps for specific end-purposes (Mohlin, 1979; Honkasalo & Ebeling, 1981; Reme, 2000). However, the major drawback associated with the TMP process is the high specific energy consumption (SEC) to a given freeness level, in addition to poorer light scattering properties compared to SWG pulps (Reme, 2000).

1.5.1 Fibre development mechanisms during mechanical pulping

Fibre development mechanisms during SGW and TMP processing are well established (Atack, 1971; Mohlin, 1995; Karnis, 1994; Gros, 1997) with respect to process-property relationships. During SGW processing, a sharpen array of protruding grits of the revolving pulpstone indent the wood matrix causing stresses and strains in the wood fibres. As a result, fibre separation (based on fatigue due to mechanical pulses of the grits; Salmén & Fellers, 1982) and subsequent fibre development (*i.e.* fibrillation and flexibilization) occurs depending on for example the wood moisture content and temperature.

Karnis (1994) suggests that there are basically two different mechanisms involved for the development of papermaking potentials of fibres (*i.e.* fibre development) during the TMP refining process. Wood fibres are first disintegrated into either single fibres or small fibre bundles, particularly during the initial stage of the refining process (*i.e.* fibre separation stage). Next comes the fibre development stage, where fibres and fibre bundles are refined to provide suitable fibres with required properties. This process occurs mainly towards the latter part of the refining process. The two stages overlap to some extent within the refiners.

Softening of wood fibres (*i.e.* softening of cell wall polymers) is a very important factor in order to preserve fibre length and also to develop high quality pulps with better bonding ability (Sundholm, 1999).

Although there has been considerable research carried out for decades for understanding fundamentals of fibre development leading to papermaking potentials of wood fibres in mechanical pulping, information is still lacking on ultrastructural aspects of MP fibre cell walls.

Therefore, it is not the intention of this thesis to study in depth the physical aspects of fibre development during processing but rather ultrastructural aspects of fibre development at the cell wall level in relation to morphological and topochemical points of view.

2. Materials and Methods

2.1 Wood and pulps

Samples for the present work came predominantly from industrially produced mechanical pulps with thermomechanical pulps (TMP) used in papers I, III, IV and V and stoneground wood (SGW) pulps as sample material in Paper II. In addition, kraft pulp was used in Paper V.

The industrially produced TMP pulps used in Paper I were sampled for electron microscopy observations, after the primary refining stage which is the most crucial stage for determining pulp properties and where fibre development has started (Kure, Dahlqvist & Helle, 1999; Fuglem *et al.,* 2001). The TMP had a freeness of *ca* 110 ml CSF (Canadian Standard Freenesss). For Papers III and IV, samples came from an extensive WURC investigation where the use of pine and spruce for TMP were compared. Both Scots pine- and Norway spruce sawmill chips were subjected to the same three stage pilot plant TMP process to produce well defined samples for comparative studies on the characteristics (*i.e.* extractives, fibre properties *etc*) of spruce versus pine for TMP pulp and paper production. The pilot plant trials were carried out by Metso, Sundsvall, Sweden. The conditions and parameters of the trials will be published elsewhere. Pulp fibre cell wall ultrastructural studies were carried out on the two wood species with emphasis made on pulps from the primary stage (415 ml CSF of pine pulps vs 464 ml CSF of spruce) as fibre separation was almost completed and fibre development already initiated (Paper IV). Special attention was given to shives of both species during electron microscopy (EM) examination. Shives are bundles of several integrated fibres that are not defibrated during refining and thus represent small pieces of remaining wood material. Studying shives reflects the scenario that occurs in the original wood chips during refining with respect to fibre separation (*i.e.* shives trap the initial effects of the refining process), allowing conclusions on the mechanisms of defibration between the two species to be made.

For ultrastructural characterization of SGW pulp fibres described in Paper II, SGW pulps were produced using both mature and juvenile wood from Norway spruce that was ground using a laboratory scale grinder at Åbo Akademi University, Finland. From five pulps with different CSF values produced from each wood type (*i.e.* wood types 1, 5, 6, 7; Paper II), only the two extreme pulps from the two most distinguishable wood types representing mature (*i.e.* Type 6) and juvenile wood (Type 1; Paper II) were used for EM investigations.

Industrial birch kraft pulps produced at the Smurtfit Kappa Kraftliner mill, Piteå, Sweden, were used in Paper V. Both unbleached and TCF-bleached (OO-Q-PO) pulps were used for histochemical staining of different lipophilic extractives.

2.2 Microscopy for investigation of wood and pulp fibre structure

Microscopy has become an essential tool for analysis of pulp fibres and related materials. The interest has increased considerably over the last decade with dawn of the concept "nano-structure" to pulp fibres. Depending on the objective of the investigation, light-, fluorescence microscopy and electron microscopy (EM) can be applied in order to understand and visualize most fundamental aspects on the behaviour of wood and pulp fibres during pulping processes. For example, both light-, and electron microscopy have made significant contributions to pulp and paper research with respect to assessment of cross-sectional dimensions of wood and pulp fibres (Reme, 2000). To gain insight into the importance of lignocellulosic pulp fibre cell wall ultra-(nano) structure (both morphological and chemical) that govern pulp and paper properties, EM has been used for obtaining novel information on the changes in the cellulose micro- and macrofibrils of kraft pulp fibres (Duchesne, 2001; Fahlén, 2005). However, the use of EM in mechanical pulp fibres has been rather limited possibly because kraft pulps represent the predominant fibre source for paper products. The application of EM techniques for ultrastructural investigations of mechanical pulp fibres was extensively adopted during the present thesis work.

2.2.1 Light- and fluorescence microscopy

History on the use of light microscopy (LM) in wood science research goes back to the seventeenth century when Robert Hooke (1665) was able to differentiate cells (*i.e.* fibres and vessels) in wood cross-sections. Today, LM has become an invaluable tool for analysis of wood raw materials, pulp and paper simply because of its ease of use without the need for sophisticated or complex sample preparation. Light microcopy can be used for pulp and pulp fibre investigations down to the micrometer scale including fibre dimensions and some of the main layers of wood cell walls (Booker & Sell, 1998; Brändström, 2002).

Fluorescence microscopy (FM) has become very popular in recent decades and provides biologists possibilities to visualize subcellular components and processes *in-vivo* (Vonesch *et al.,* 2006). It uses the phenomenon called "fluorescence" which is a property of some atoms and molecules to absorb light of a particular wavelength and then to re-emit light at longer wavelengths. Therefore, the basic principle behind FM is to deliver excitation light to the fluorescing species (*i.e.* the molecule concerned) in the specimen and to separate the weaker emitted fluorescence light from the brighter excitation light. Only fluorescence light emitted from the species reaches the detector and high contrast images are generated (Herman, 1998). High specificity to the components under observation is one major advantage provided by FM.

Correlated LM and FM in combination with histochemistry (see next section) represent an important application for use in pulp and paper research particularly for pitch problems created by wood resin (*i.e.* lipophilic extractives). LM and FM were used in combination with histochemistry during the present work (Papers IV

and V) for investigating different constituents of lipophilic extractives and their spatial distributions in pulp fibres and fines.

2.2.2 Histochemical microscopy for investigating wood resin

Histochemistry, where micro-biochemical methods using specific dyes/probes to study the biochemical state of tissues, and/or individual cells, is a versatile technique that has developed tremendously especially in the fields of medicine and plant biology. However, the application of histochemical methods in pulp and paper research is still at a novel stage, particularly for wood resin research, and further development is needed.

An histochemical approach in conjunction with microscopy was adopted (Papers IV and V) in order to elucidate the nature and behaviour of wood resin (*i.e.* extractives) in their native state and their micro-morphological distribution/redistribution during and after mechanical (Paper IV) and kraft (Paper V) pulping. Different methods were applied depending on the chemistry of the extractives concerned and are briefly outlined below (for further information, see the papers). The histochemical techniques applied not only allowed visualization of lipophilic wood extractives and their micro-morphological distribution, but also provided an opportunity to draw conclusions, coupled with chemical analysis, on possible mechanisms of their behaviour during pulping (Paper V).

Fats are the predominant lipophilic constituent in softwoods (*i.e.* Norway spruce and Scots pine; Back & Allen, 2000) and can be stained using Bromine-Sudan black B. It is a general stain for localizing all lipids including fats and free fatty acids (FA) (High, 1984). Therefore, it is appropriate to apply at the beginning of staining studies in order to determine whether lipids are available within the tissue or pulp under study before analyzing different constituents like triglycerides. Bromine-Sudan black B was used in the papers IV and V.

Further investigations on the chemical nature of specific components like triglycerides and fats containing double bonds were studied using additional specific stains. Nile blue was used for localizing neutral fats *i.e.* triglycerides, in both wood and pulps (Papers IV and V) and stains triglycerides red/pink. The red dye of Nile blue, oxazone, dissolves in neutral fats like triglycerides staining them red/pink (Jensen, 1962; Chayen & Bitensky, 1991).

Osmium tetroxide stains fats containing double bonds (*i.e.* unsaturated fats) black, and was used to localize unsaturated fats/free FAs present in both wood and pulps. In contrast to the above two methods, this staining method is based on a chemical reaction where osmium tetroxide oxidizes the double bonds present in unsaturated fatty acids in the lipid molecule resulting in the formation of a black compound of osmium (Cain, 1950; Pearse, 1953). Due to the chemical reaction taking place during staining, and since osmium is a highly active compound, the duration of staining is very important and therefore was only used for 1 hr at room temperature in the present studies (Jensen, 1962).

Polyene antibiotic filipin has been used widely in animal research as a probe to detect cholesterols (Kruth, 1984; Muller *et al.,* 1984; Severs, 1997) and has recently been used in wood resin research for sterol localization (Speranza *et al.,* 2002). It is a highly specific probe which reacts specifically with 3β-hydroxysterols (Milhaud *et al.,* 1988). Fluorescent sitosterol-filipin complexes formed during staining have absorption and emission bands at 357 and 480 nm respectively and thus can be visualized using epifluorescence microscopy. Filipin was used in Paper V for specific localization of sterols in native birch wood and fibres/parenchyma cells during kraft pulping. Samples were examined with a Leica epifluorescence microscope using 360/40D excitation and 460 nm barrier filters and thereby avoided autofluorescent background from other wood components such as lignin (excitation at ~ 280 nm; Albinsson *et al.,* 1999).

2.2.3 Scanning electron microscopy

Electron microscopy (EM) has a unique ability in providing structural and chemical information over a range of scales down to atomic dimensions and therefore is applicable for all scientists including those working with wood and fibres.

Scanning electron microscopy (SEM) provides information about sample topography (*i.e.* provides three-dimensional views of pulp fibre surface morphology) by scanning the surface of specimens with a finely focussed electron beam.

The principles of conventional SEM are briefly described. Before using SEM, specimens should be stabilized using a suitable method (described below) depending on the objective of the study, and then coated with a thin layer (*ca* 20 nm) of conductive metal such as gold as biological specimens like wood and pulps are non-conductive. Specimen coating not only protects samples from accumulating high voltage charges and specimen damage during electron irradiation, but also acts as an excellent source of secondary electrons.

The electron gun (usually a tungsten filament) at the top of the optical column of the microscope produces an electron beam under the influence of a current. The emitted electron beam is then finely focussed through a vacuum chamber using electromagnetic lenses and scanned in a rectangular raster over the specimen. Apart from other interactions (*e.g.* backscattered electrons, x-rays *etc*) of the primary beam with the specimen, secondary electrons are generated from the specimen surface and these are detected by a secondary electron detector, processed and eventually translated into pixels of the image which can be visualized on a display monitor. Micrographs of pulp fibres in this thesis were acquired digitally using the inbuilt CCD (charge-coupled device) camera.

In addition, Field Emission-SEM (FE-SEM) that provides high resolution and low noise imaging was also used for investigating the surface morphological ultrastructure of mechanical wood pulp fibres with respect to the macro- and

microfibrillar structures of their outer cell walls (Paper I). FE-SEM can have a resolution of *ca* 1nm and magnify over 400,000 times.

Sample preparation is possibly the most crucial stage during any biological EM study and depends on the type of specimen under investigation and the objectives targeted. For SEM, there are several ways to prepare biological samples (water removal and dehydration) before coating with a metal layer, *e.g.* air drying, freeze drying, critical point drying (CPD) and cryofixation (Daniel & Duchesne, 1998).

CPD is a solvent exchange method that preserves original structures with reduced artefacts to the sample (Bozzola & Russell, 1992; Daniel & Duchesne, 1998; Duchesne & Daniel, 1999). In particular, during morphological studies of pulp fibre cell walls, CPD allows the possibility for observing well-defined macrofibrillar and/or microfibrillar structures of the cell wall (Daniel & Duchesne, 1998). Therefore, in the present thesis work CPD was used solely as the sample preparation method (Papers I, II, III and IV) before coating specimens with gold as the conductive layer. During CPD, specimen (*i.e.* wood, pulp fibres) dehydration was performed by gradually replacing water present in the samples using ethanol and then acetone using increasing concentration. Thereafter with samples immersed in acetone, the solvent was completely replaced with transitional fluid (*i.e.* liquid CO_2) under pressurized conditions inside a critical point dryer. At predetermined temperature (31.5 ^{o}C) and pressure (1100 psi), the liquid CO_2 reaches its critical point during which the density of the liquid phase equals the density of the vapour phase and thus the boundary between the two phases (*i.e.* damaging liquid/air interphase with very high surface tension) disappears (Bozzola & Russell, 1992; Hayat, 2000). Since at the critical point only one phase exists and the surface tension is minimum, the original fibrillar structures of the pulp fibre cell walls are preserved and can then be visualized using SEM.

2.2.4 Transmission electron microscopy

In contrast with SEM, the transmission electron microscope (TEM) produces a transmitted two-dimensional image of specimens since the illuminating electrons pass through the sample. In conventional TEM, the primary electrons are emitted from a metal filament of the electron gun after supply of high voltage (*e.g.* 20-200 kV). The beam is then finely focussed onto the specimen by a series of electromagnetic lenses inside the optical column. The electrons that pass through the sample are projected as a magnified image onto a fluorescent screen. Unlike SEM, specimen thickness is crucial and ultrathin sections of *ca* 60-100 nm depending on the objective of the study are normally used. TEM images are acquired on monochrome film and then digitized or nowadays digital images are directly acquired using an inbuilt CCD camera. Depending on electron source, a TEM may have a resolution of *ca* 0.1nm with magnifications over 1 million times.

During this thesis work (Papers IV and V), conventional sample preparation for TEM was adopted where specimens were first dehydrated using an ethanol series of increasing concentration followed by embedding in resin (Daniel, Nilsson & Pettersson, 1989). Ultrathin sections of pre-determined thickness (*ca* 60-90 nm)

were cut using a diamond knife on a ultramicrotome and collected on copper grids for TEM investigations.

Post-staining of sections (*i.e.* Norway spruce and Scots pine TMP fibres) was performed using $KMnO_4$ in order to gain detailed information on lignin distribution across TMP fibre cell walls of spruce and pine (Paper V). $KMnO_4$ has been widely used as a TEM stain in plant and wood cell wall studies to reveal lignin distributions across fibre cell walls at the ultrastructural level (Hepler, Fosket & Newcomb, 1970; Bland, Foster & Logan, 1971; Maurer & Fengel, 1990; Singh & Daniel, 2001; Singh, Daniel & Nilsson, 2002; Schmitt & Melcher, 2004). Lignin-rich regions of the cell wall appear electron-dense while regions poor in lignin have an electron-lucent appearance (Singh & Daniel, 2001). Post-staining was carried out on ultrathin sections of spruce and pine TMP shives using 1% (w/v) $KMnO_4$ (prepared in 0.1% (w/v) sodium citrate) for 3 min. at room temperature (Singh & Daniel, 2001; Schmitt & Melcher, 2004) prior to TEM examination.

2.2.5 Immuno-electron microscopy (Immuno-EM)

In principle, immuno-EM can be used to visualize antigens via the interaction between an antibody and relevant components (*i.e.* antigen) on the surface of biological systems (*i.e.* fibre cell wall) with high specificity (Hayat, 2000). It uses antibodies (*i.e.* IgG) as highly specific markers for localization of the specific macromolecules concerned.

The immunogold EM technique was used in Paper V for the localization of glucomannan. The primary antigen (glucomannan)-antibody complex was visualized using gold labelled secondary antibodies. Labelling of the secondary antibody is generally done using electron-opaque markers such as colloidal gold (*e.g.* 10 nm diameter) and the distribution of the gold particles is used during TEM/SEM to reflect the location of the antigen.

Immuno-EM techniques have been used to study the localization of glucomannan (GM) present in wood fibres (*e.g.* Baba *et al.,* 1994; Maeda *et al.,* 2000) and kraft pulp fibre cell walls (Duchesne, Takabe & Daniel, 2003). The technique has significantly contributed to an increased understanding of the molecular architecture and the developmental dynamics of plant cell walls (Knox, 1997). During the present work, the technique was applied to mechanical pulp fibres to localize and gain information on the glucomannan distribution pattern across the cell walls of spruce and pine TMP pulp fibres.

In Paper V, an antibody raised against softwood glucomannan (GM) i.e. (1-4)-β-mannan and galacto-(1-4)-β-mannan, was used for labelling GM in transverse sections of TMP fibres of Scots pine and Norway spruce. Post-immuno-labelling of TMP fibres of both species was performed during the study. Fibre sections were first incubated with the primary GM antibody followed by incubation with gold (10 nm) labelled anti-mouse IgG secondary antibodies. Gold-labelled GM located over the exposed surfaces of the fibre cell wall was visualized as globular structures using TEM and the number of gold particles counted for comparisons.

3. Results and Discussion

3.1 Surface morphological ultrastructure of mechanical pulp fibres

During recent years, there has been renewed interest on the morphological nano-structure of pulp fibres in order to gain better understanding of fundamental aspects related to pulp fibre cell wall mechanisms and responses to different processes. For example, fibrillation of different fibre cell wall layers (Fig. 5) is considered to play a major role in determining most pulp and paper properties like fibre-fibre bonding that influence paper strength and optical properties. Therefore, improved knowledge of the surface ultrastructure of pulp fibres should not only provide novel understanding of the principles behind property development during processing, but also greater opportunities for the improvement and development of new fibre products.

Several studies have been carried out to characterise the surface morphological ultrastructure of kraft pulp fibres in recent years (Daniel & Duchesne, 1998; Duchesne & Daniel, 2000; Mohlin & Daniel, 2004; Daniel *et al.,* 2004), but less attention has been paid to mechanical pulp fibres regarding these aspects.

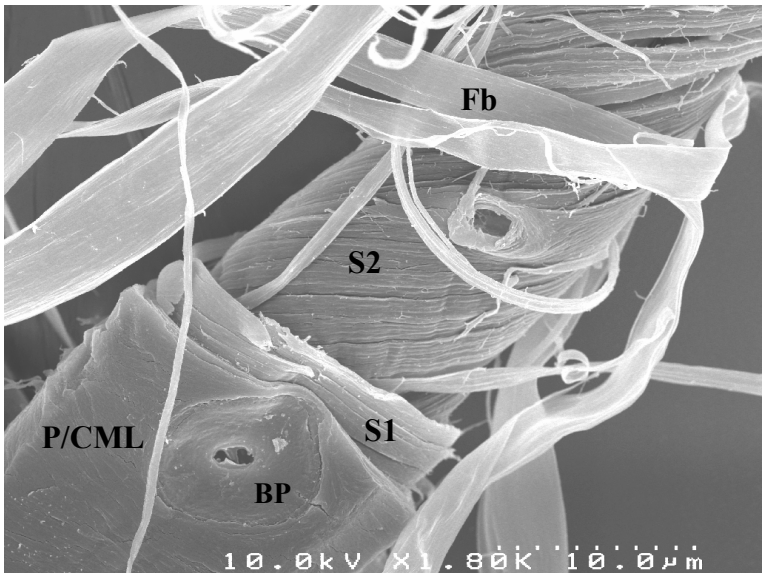

Fig. 5. FE-SEM micrograph of a Norway spruce TMP pulp fibre showing different cell wall layers (*i.e.* primary wall (P)/compound middle lamella (CML), S1 and S2 layers) and external fibrillation (Fb). BP- bordered pit, Fb-fibrils.

During mechanical pulping, wood fibres are subjected to intense mechanical treatment with less effect on the chemical structure of the fibre cell wall compared to kraft pulping. Therefore, unlike kraft pulps most of the wood polymers, especially lignin, are retained during mechanical pulping resulting in high yield

pulps. When lignin and to some extent hemicelluloses are removed during kraft pulping, severe modifications occur in the native fibre cell wall ultrastructure. For example, pores are created in the fibre wall and the fibre surface becomes open with aggregation of fibrillar structures to form larger structural units *e.g.* macrofibrils (Duchesne & Daniel, 2000; Molin, 2002; Fahlén, 2005). In contrast, such severe modifications are minimal in MP fibres and the native fibre cell wall ultrastructure is to a greater extent preserved with respect to macro/microfibrillar structures.

3.1.1 Surface ultrastructure of TMP fibres

Ultrastructural investigations of never-dried TMP fibres (*ca* CSF 100 ml) from Norway spruce using SEM (*i.e.* SEM and FE-SEM) revealed information on the manner in which wood fibre cell walls undergo structural changes that represent the basis for most final pulp and paper properties. Results from the present study (Paper I) suggest that fibre fibrillation (Fig. 6) occurring during primary refining of the TMP process was not random, but rather governed by fibre cell wall architecture and microfibrillar organisation.

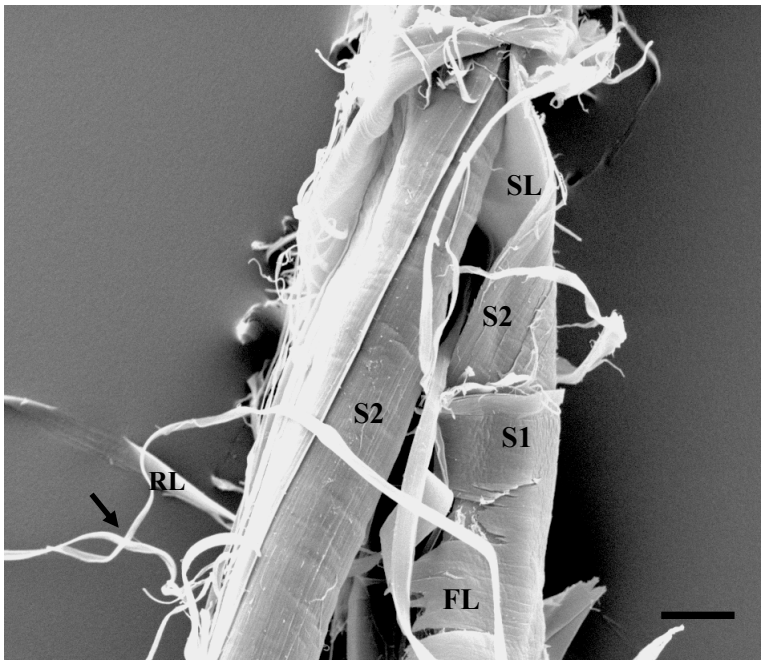

Fig. 6. SEM micrograph of a Norway spruce TMP fibre showing typical cell wall fibrillation, *i.e.* "flake-like" (FL), "sheet-like" (SL) representing concentric lamellation of fibre cell wall and "ribbon-like" (RL and arrow) reflecting the native fibre cell wall ultrastructure. Bar: 10.0 µm.

Fibre cell wall splitting/cracking that led to internal and external fibrillation was initiated at either sites of weakness or abrupt changes in MFA present in the native fibre cell wall structure. In particular, regions in close proximity to pits (*i.e.* cross-

field- and bordered-pits; Paper I, Figs. 1a-c,) where the orientation of cellulose microfibrils (*i.e.* MFA) depart from their normal helicoidal course along the fibre wall and sweep around the pit aperture, were weak sites for initiation of splits. The development of splits then followed the cell wall organization along the fibre wall resulting in different types of external fibrillation.

Based on morphology, two major types of external fibrillation were observed during TMP refining, namely "flake-like" and "sheet/ribbon-like" fibrillation. The type of fibrillation that developed from initial cracking and splitting of the individual cell wall layers was primarily governed by the orientation of cellulose microfibrils (*i.e.* MFA) within the fibre axis. As illustrated in Fig. 3 (Paper I), splits in the S1 layer were oriented perpendicular to the fibre axis (90^o to fibre axis). Thus, "flake-like" fibrillation was derived from the S1 layer due to perpendicular fragmentation and produced shorter and smaller pieces.

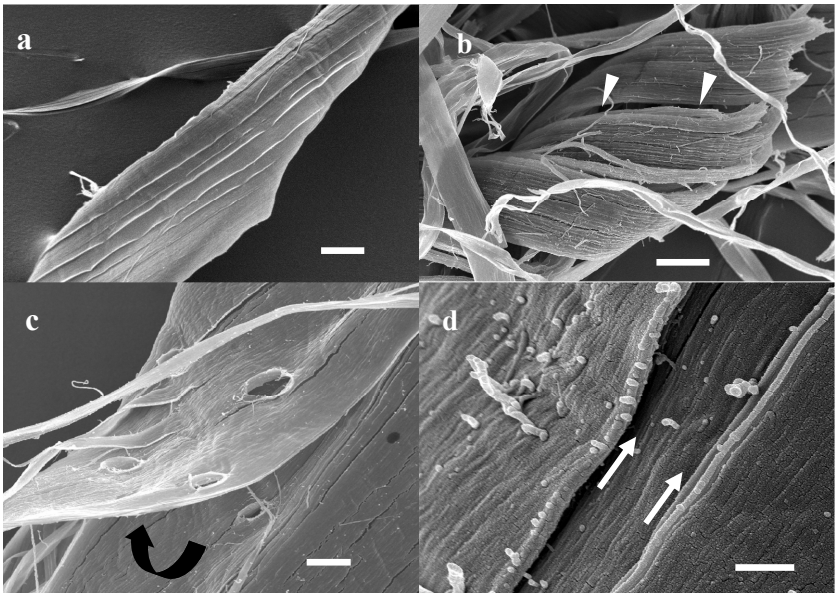

Fig. 7. Spruce fibre fibrillation of S2 layer was governed by the cell wall architecture and cellulose microfibrillar organisation. a) Longitudinal splitting of the S2 layer following the MFA (*i.e.* 0-15^o to the fibre axis); b) Internal helical cracking (arrowheads) of S2 leading to regular-sized ribbons; c) Delamination ("sheet-like") of concentrically layered lamella; d) Ribbon-like fibril peeled off along and between cellulose aggregates (arrows) (macrofibrils). Bars: a, b, c, 5.0 µm; d, 1.0 µm.

The other most important types of fibrillation, *i.e.* sheet/ribbon-like fibrillation was derived from the inner and dominating S2 secondary wall layer (Fig. 7) through longitudinal and concentric splitting that developed in helical fashion (Z-helix) along the fibre axis. Ribbon-like fibrillation is thought to be of prime importance in bonding within the paper web and thereby improve strength and density (Braaten, 1997, 1998; Luukko & Paulapuro, 1999). Splits followed the S2 MFA (0^o – 15^o; Paper I, Fig. 4a-d) that were almost parallel to the fibre axis, and proceeded helicoidally along the fibre (Fig. 7b and Paper I, Fig. 4) reflecting the

native cell wall architecture and microfibrillar organization. As a consequence, S2 fibrillation appeared as lamella-sheets (***sheet-like***) or as thin threads (***ribbon-like***) of cell wall materials that were "peeled-off" due to the refining process. As shown in Fig. 10 (Paper I), sheet-like fibrils (*ca* > 8.0 μm in width) originated by delamination between concentric lamellae (*i.e.* weak sites in S2) of the S2 layer (Scallan, 1974; Kerr & Goring, 1975; Fahlén & Salmén, 2002). Ribbon-like fibrillation occurred as result of splitting and subsequent peeling of the individual lamellae. Detailed studies (Paper I, Fig. 11) showed that during ribbon formation, splitting occurred along and between cellulose aggregates (macrofibrils) (Fig. 7d) where presumably weak sites exist, resulting in thread-like ribbons of equal width along the length (Paper 1, Fig. 12a-d).

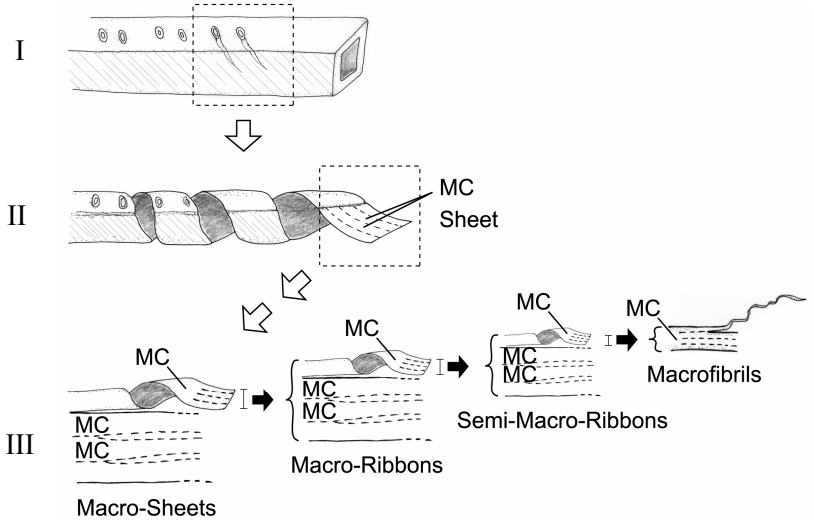

Fig. 8. Schematic diagram illustrating the concept for the development of external fibrillation in the fibre S2 cell wall layer; (I) Initiation of cracks/splits that follow MFA of S2; (II) Development of sheet-like fibrillation by unravelling of helicoidally organized lamellae, and by further propagating of splits along the fibre following its MFA. Internal (micro) cracks may simultaneously occur within the lamella sheet; (III) Internal cracks (micro-cracks) occur between each hierarchical sheet and ribbon within a lamella giving rise to the formation of different types of fibrillation of similar width along its length. MC - micro-cracks.

Four main categories of ribbons were identified (Fig. 8) based on width (width of a particular ribbon was equal along its length) and were named accordingly; macro-sheets, macro-ribbons, semi-macro ribbons and the smallest macrofibrils (Paper 1, Table 4). The ribbons/strings with the smallest width (*ca* 30nm) were probably the smallest macrofibrils, and were composed of an unknown number of cellulose microfibrils. This hierarchy in the ultrastructural breakdown of each lamella of the S2 layer should reflect sites of weakness within the fibre wall and each lamellae (*i.e.* between each referred hierarchical ribbons). These weak areas presumably influence the development of internal micro-cracks and internal fibrillation which in turn could lead to external fibrillation giving rise to the long fibrils of different widths as shown schematically in Fig. 8.

Both EW and LW fibres behaved in similar manner although LW fibres naturally showed greater ribbon development due to their thicker cell wall as described previously by Kure (1997).

In addition to fibrillation, fibre "opening" exposing the cell lumen was observed (Paper I, Fig. 14). This resulted from the initiation of splits on both sides of pits that followed the S2 MFA.

3.1.2 SGW pulp fibre development; Morphological ultrastructural aspects

The purpose of the second paper was to gain insights on fundamental aspects of fibre development during the SGW process at the cell wall level. Effects of the process on different wood types, *i.e.* juvenile wood (JW) and mature wood (MW) were also investigated. JW has shown improved property development although it is commonly known as a poor quality raw material for most pulp and paper products (Brill, 1985; Tyrväinen, 1995).

It is well known that the SGW process generates large amounts of fines with short fibres due to its very harsh mechanical action compared to the TMP process. Ultrastructural examination of pulp fibre surfaces using SEM revealed mechanisms of SGW pulp fibre development at the cell wall level, resulting in the greater percentage of the short fibre fraction and fines generally associated with SGW pulps.

Development of splits in the vicinity of pits as seen with TMP fibres (Paper I) was also similar in SGW fibres indicating weak sites in the fibre wall that cracked as a response to the external forces applied during grinding, no matter the difference between the SGW and TMP processes (Paper II, Fig. 4). Cracking near pits most often led to total fibre breakage due to their advancement across the fibre axis (Fig. 9). This mechanism of transverse fibre breakage occurred more frequently close to the fibre tips resulting in fibre cleavage at both ends (Paper II, Fig. 6) contributing broken fibre tip pieces to the fine fraction and remaining central part to the large middle fraction. Fibre breakage at the tips was attributed to the native Norway spruce fibre morphology close to the ends as described by Sirviö & Kärenlampi (1998). Pit density and pit size increase towards the fibre tips lowering the effective fibre modulus and strength near the fibre ends. In addition, the reduced cell wall thickness in these regions (Okamura, Harada & Saiki, 1974) collectively contributes to the enhanced fibre tip breakage observed during the SGW process.

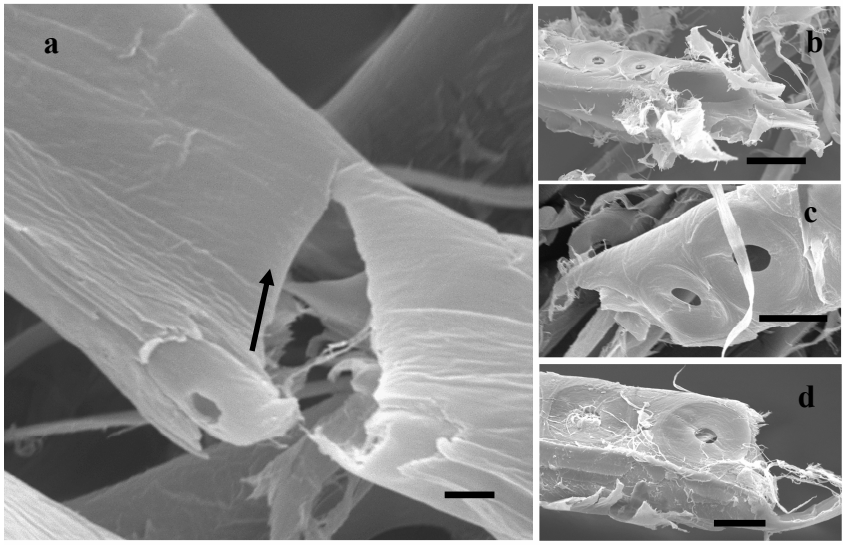

Fig. 9. Transverse fibre breakage mechanism of Norway spruce fibres during the SGW process. Splits initiated in the cell wall near pits (a, arrow) develop across the fibre leading to fibre breakage (b, c, d). Both EW (a, c) and LW (b, d) showed the same mechanism. Bars: a, 2.0 µm; b, 20.0 µm; c, d, 10.0 µm.

In addition, longitudinal fibre breakage resulted in the tearing of fibres along the fibre axis (Paper II, Fig. 7) due to the combing effects generated by the array of grits during the attrition process. The transverse and longitudinal fibre breakage described above are indicative of a glassy/brittle type behaviour of fibres, in contrast to the viscoelastic behaviour shown by TMP fibres (Gros & Lönnberg, 1996; McDonald, Miles & Amiri, 2004) that preserves fibre length and the integrity of fibres during TMP processing. The brittle-like behaviour shown by fibres during the SGW process was most likely attributable to a low degree of softening of the cell wall material (*i.e.* poorly developed viscoelasticity). Possibly, the temperature during stone grinding was insufficient for softening cell wall lignin and also there was no pre-steaming of wood chips as in the TMP process to develop the necessary viscoelastic properties of the fibre cell wall materials.

The most striking difference between TMP and SGW fibres in respect of fibre development was the manner of splitting and subsequent development of fibrillation. As shown in Fig. 8 (Paper II), fibre wall splitting/fracturing and further development occurred randomly over the cell wall of the majority of fibres (both EW and LW) leading to multi-directional fibrillation that did not follow the MFA of the cell wall layers (Paper II). This fibrillation mechanism badly affected the degree of delamination and "peeling" from the thick S2 layer and impeded the generation of ribbons from the main secondary cell wall. In addition, the mechanical action applied perpendicular to the fibre axis from the protruding stone grits of the rapidly revolving grindstone hampered S2 fibrillation, and caused severe fibrillation of the S1 layer. Most of the SGW pulp fibres thus appeared to be severely damaged and highly fibrillated (*i.e.* S1 layer; Paper II, Fig. 9) giving a misleading impression of S2 fibrillation and referred as "*pseudo-fibrillation*". The

materials removed from the S1 layer contributed to the large fine fraction of the SGW pulps as short, but broad "flake-like" fibrils in addition to broken fibre tips and fibre fragments. However, some fibres occasionally also showed ribbon development from the S2 layer due to splitting and subsequent development according to the cell wall architecture; observations consistent with that reported previously (Mohlin, 1977; Jackson & Williams, 1979).

It was suggested that the superior smoothness and the characteristic good opacity provided by the SGW-based furnishes in this study were primarily attributed to the distinct fibre development mechanisms observed. These led to the generation of high amounts of short fibre tips, fragmented fibre parts and short "flake-like" materials from S1. These shorter fines could affectively fill voids within the fibre network of the paper web during papermaking and thereby contribute to improved surface smoothness (de Silveira *et al.,* 1996; 1996; Luukko & Paulapuro, 1999). It has been reported previously that short fines of fibre fragments and flake-like materials from S1 are very effective in light scattering thus contributing to the good light scattering properties of SGW pulps (Luukko, 1999).

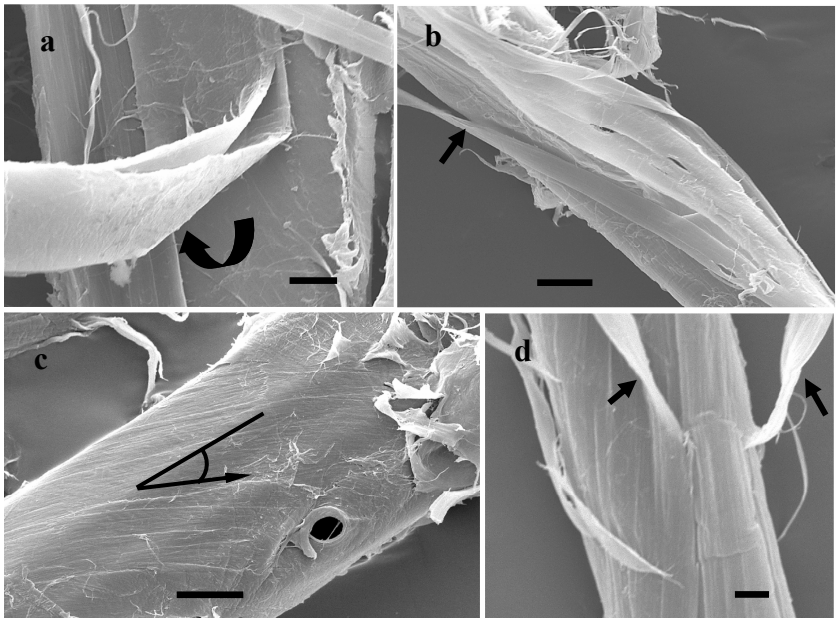

Fig. 10. Norway spruce JW fibre development mechanism during the SGW process. Interestingly, cell wall fibrillation showed features similar to TMP fibre fibrillation (Paper I) where the native fibre micro- and ultrastructure regulates fibre fibrillation. a) Sheet-like fibrillation; b, c, d) Ribbon-like fibrillation giving rise to different types of fibrils of equal width (arrows). Bars: a, 5.0 µm; b, c, 10.0 µm; d, 2.0 µm.

In the SGW trial, pulp and paper properties from JW and MW differed considerably. Pulps from juvenile rich wood materials gave the best electrical energy-tensile strength relation (Paper II, Fig. 2b) in addition to improved sheet properties such as greater light scattering (Paper II, Fig. 10), improved strength

(*i.e.* tensile (Paper II, Fig. 12) and tear (Paper II, Fig. 13)) and higher sheet density (Paper II, Fig. 14) compared to MW. The improvement in pulp and paper properties provided by JW raw materials were explained by the manner of fibre processing and development at the ultrastructural level (Paper II). The JW SGW pulp fibre development mechanism (*i.e.* fibrillation, Fig. 10) observed during the present study (Paper II) showed features similar to that reported in Paper I for TMP fibres where the native morphological fibre ultrastructure (*i.e.* structural hierarchy of the wood fibre cell wall and MFA of S1, S2) plays an important controlling role on how cell wall splitting and fibrillation progresses (Paper II, Fig. 11).

3.2 Cell wall ultrastructure and its effect on TMP fibre development

The fibre cell wall ultrastructure (*i.e.* both morphological and chemical) plays a major role in determining most physical properties of final products. In mechanical pulping, wood fibres are subjected to modification, depending on process, during which changes are made to the cell wall in relation to morphology (*e.g.* cell wall fibrillation) and to a lesser extent the chemistry of cell wall polymers (*e.g.* lignin softening).

Before morphological modifications are made to the cell wall, it is imperative to defibrate fibres (*i.e.* fibre separation from the wood structure) in such a way that subsequent fibre fibrillation and development are achieved most effectively (*i.e.* with a minimal energy used). Defibration is brought about by fracturing between cell walls of integrated wood fibres and this is influenced by the physical conditions during processing such as temperature and moisture content (Höglund, Sohlin & Tistad, 1976; Salmén, 1984; Franzén, 1986). In addition, factors such as wood species, density, cell dimensions and MFA have also been considered to affect fracture properties of the cell wall structure (Wardrop & Addo-Ashong, 1965; Petterson & Bodig, 1983).

However, ultrastructural features of the fibre cell wall (*e.g.* lignin distribution) are proposed to influence the fracturing behaviour between cell walls and thereby considerably affect the properties of final products (Donaldson, 1995, 1997). During the present work (Paper III) attempts were made to study effects of cell wall ultrastructure and their relation to fibre development during processing.

3.2.1 Norway spruce and Scots pine for TMP processing

In Northern Europe, Norway spruce is the major raw material for mechanical pulping and offers superior fibre properties over other softwoods such as Scots pine. Since there is a growing concern for a shortage of Norway spruce in the future due to the current trend of enormous use, it is important to study the use of alternative softwoods for mechanical pulping. A possible alternative for MP is Scots pine which is the second predominant softwood in Northern Europe. Scots

pine is readily available and represents a low cost raw material compared with spruce.

However, the well-known drawbacks associated with Scots pine for TMP pulping, *i.e.* higher SEC and inferior pulp and paper properties (Lindström *et al.,* 1977; Härkönen, Heikkurinen & Nederström, 1989; Reme & Helle 2001; Persson, Sundström & Ku, 2005) have made Scots pine an unfavourable raw material for mechanical pulping. Similar results were obtained during the current study (Paper III) where pine consumed higher electrical energy to reach a certain freeness level compared to spruce (Fig. 11) and produced inferior strength properties (*i.e.* tensile strength; Paper III, Fig. 2). However, according to results shown in Fig. 12, pine appeared to defibrate (*i.e.* fibre separation) more easily than spruce producing significantly lesser amounts of shives during primary refining, although liberated pine fibres were rather difficult to develop further (*i.e.* poor fibre development) to a required freeness (Fig. 11). Understanding reasons for this phenomenon was the major driving force for the study presented in Paper III during which shives from both spruce and pine TMP pulps were subjected to detailed EM studies.

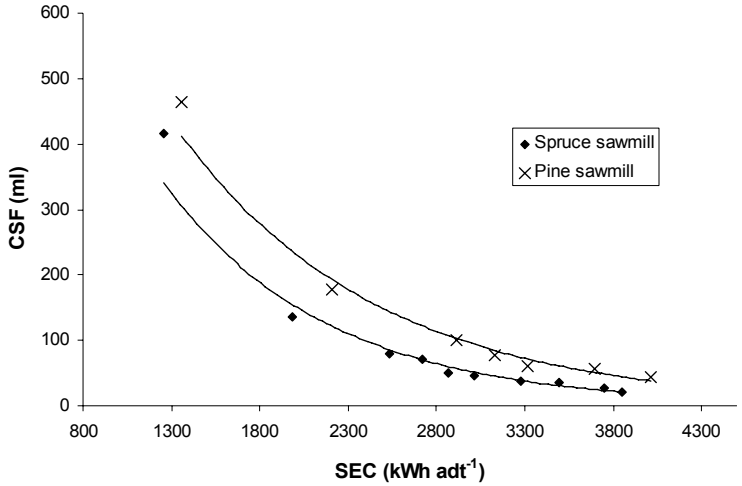

Fig. 11. Freeness (CSF, ml) versus specific energy consumption (SEC, kWh adt^{-1}) for Scots pine and Norway spruce for sawmill chips from mature wood.

Although there are contradictory opinions why pine is difficult to refine to required properties, no clear explanations have yet been forwarded, possibly because the majority of earlier studies are based primarily on physical aspects of fibre processing. Furthermore, there is very little information available on ultrastructural features of Scots pine fibre cell walls, particularly mechanical pulp fibres, compared to Norway spruce (Brändström, 2002). A better understanding of ultrastructural aspects of pine fibre cell walls during processing should therefore provide important clues on the effective utilization of Scots pine for mechanical pulps.

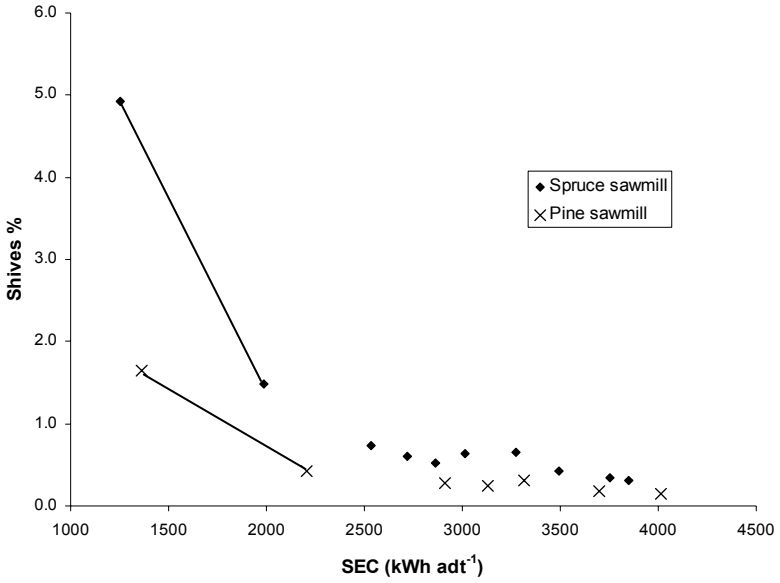

Fig. 12. Percentage of shives produced versus specific energy consumption (kW h adt^{-1}). After primary refining, pine was refined with a significantly lower shive content compared to spruce.

Therefore, the major aim of Paper III was to characterize the ultrastructure of both spruce and pine TMP fibres at early stages of TMP pulping in order to gain insights into the fundamental reasons for the different fibre behaviour shown by the two wood species.

3.2.2 TMP fibre development; morphological cell wall ultrastructure

It is well known that the Norway spruce TMP fibre development mechanism is a two stage process where initial fibre separation (*i.e.* defibration) and subsequent fibre development stages proceed simultaneously within refiners (Karnis, 1994). However, a question arises whether the same principle is applicable for Scots pine since evidence is lacking for the pine TMP process and also because of the negative response of pine to the process as described in Paper III.

As discussed in section 3.2.1, pine consumed higher electrical energy and produced inferior strength properties compared to spruce. However, based on Figures 11 and 12, it appeared there were two different mechanisms involved for the two wood species for fibre development during TMP refining: (1) fibres in pine wood chips tended to defibrate easily during primary refining while liberated fibres showed difficulty to develop the required quality; and (2) in contrast, spruce fibre separation was poor (*i.e.* produced more shives; Fig. 12) but fibre development was easier requiring less energy to reach a certain freeness (Fig. 11).

Interesting information on the different behaviour shown by the two softwood species was revealed by cell wall ultrastructural investigations performed on

shives and TMP pulps using TEM and SEM (Figs 13, 14). Pine fibres (*i.e.* integrated fibres within shives) tended to separate most often through the compound middle lamella (CML)/S1 interphase or through the CML (Paper III, Fig. 3; Fig. 9) compared to spruce fibres where fractures occurred predominantly at the S1/S2 interphase or within the S2 layer (Figures 4, 10; Paper III). Furthermore, spruce fibres showed not only exposed S2 layer (Paper III, arrowheads in Fig. 4a-d) but also S2 fibrillation (Paper III, arrows in Fig. 4a-c) even at the fibre separation stage strongly supporting the proposed pulp fibre development mechanism for spruce where the two stages occur simultaneously. However, based on EM observations, the mechanism of pine fibre development during primary refining of the TMP process contrasted with spruce fibres and the two stages were not concurrent processes within refiners. Thus the fibre separation mechanisms were different for the two species.

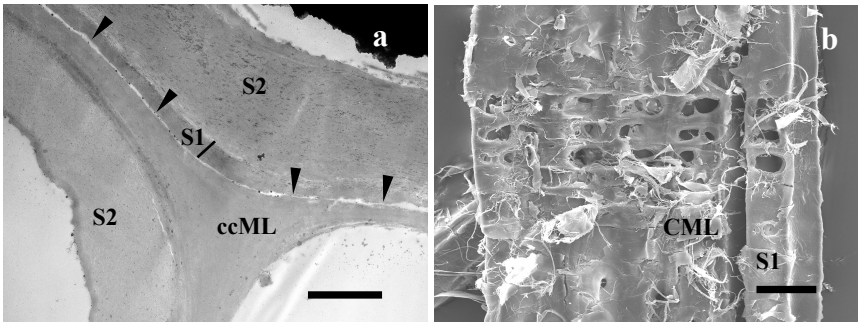

Fig. 13. Fracture behaviour of pine TMP fibres during defibration. a) TEM micrographs of a shive (T.S section) showing predominant fracture that occurred at the CML/S1 interphase (arrowheads); b) SEM of a shive showing S1 and sometimes CML as the outer wall layers indicating that defibration has occurred either at the CML/S1 interphase or through the S1. ccML= cell corner middle lamella. Bars; a, 1.0 µm; b, 50.0 µm.

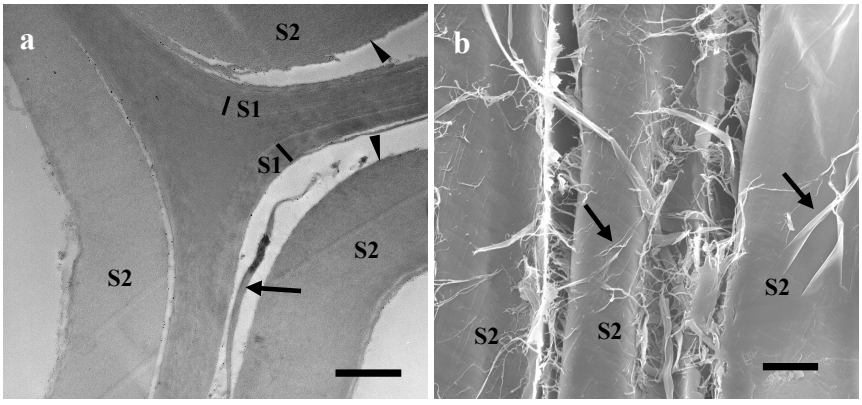

Fig. 14. Fracture pattern of spruce TMP shives after primary stage. a) Fractures occurred primarily at the S1/S2 interphase or through the S2. The defibrating fibres showed not only exposed S2 (arrowheads) but also S2 fibrillation (arrow); b) SEM of spruce TMP shive. Unlike pine, fibres showed smooth surfaces of exposed S2 layer that have already started fibre development via S2 fibrillation (arrows). Bars: a, 1.0 µm; b, 20.0 µm.

Due to the characteristic separation mechanism associated with pine fibres, defibrated TMP pine fibres frequently possessed an S1 layer around the S2 and also less frequently patches of remaining CML (Paper III, Fig. 8a, e; Fig. 9). Furthermore, both SEM and TEM observations showed pine fibres to have an S1 layer with a rather loose lamellar structure at the S1/S2 interphase (Paper III, Fig. 8a-f) enabling the S1 layer to be easily detached from the S2 (Fig. 15) while possessing an elastic character (*i.e.* rubbery-like). Both features of the S1 layer appeared to create a barrier for the required fibrillation of the S2 layer and thus exhibited resistance to pine fibre development. In contrast, spruce fibres had strongly attached lamellae present at the S1/S2 interphase. Strongly bound lamellae at the S1/S2 interphase allowed for enhanced peeling of the cell wall of spruce fibres and materials could be removed from the underlying S2 when the S1 was separated (Paper III).

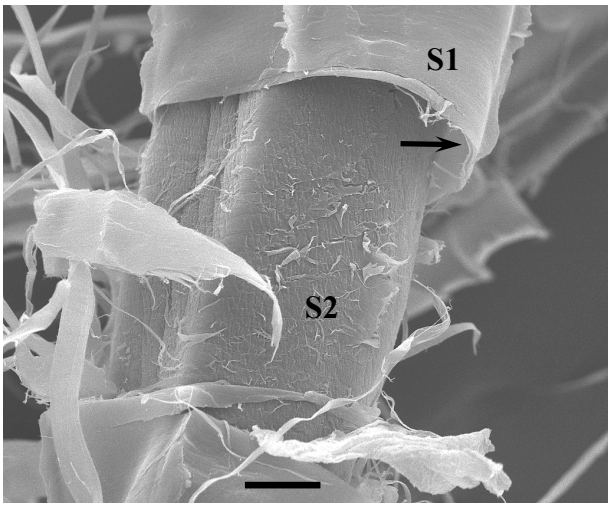

Fig. 15. Due to the loose structure at the S1/S2 interphase, the S1 layer of pine TMP pulp fibres was easily detached from the underlying S2 during refining. Note the large space created between S1 and S2 (arrow) possibly as a result of stretching of the S1 layer. Bar: 10.0 µm.

3.2.3 Cell wall topochemical ultrastructure and its influence on the mechanism of fibre development

3.2.3.1 Glucomannan (GM) distribution in spruce and pine TMP fibres

TEM-immunogold localization of GM across the cell walls of spruce and pine fibres in TMP shives revealed surprising information on its distribution in different cell wall layers in the two wood species. The pattern of labelling (*i.e.* density of gold probes) differed in the secondary cell wall layers where the primary wall was least and the S2 was the highest labelled (Fig. 16). This pattern reflected the general distribution of softwood glucomanan across the cell wall reported in the literature (Meier, 1961; Panshin & de Zeeuw, 1980; Sjöström 1993). Hence, the density of gold probes on different wall layers reflects the surface GM concentration over the transverse cell wall area.

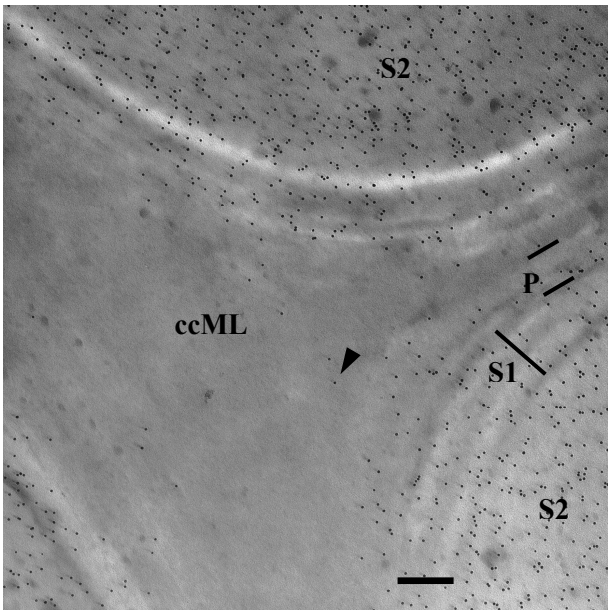

Fig. 16. Anti-glucomannan immunogold labelling of TMP shives from pine (T.S sections). Note the gradual change in labelling intensity across the fibre cell wall where ML was seldom labelled (arrowhead), P has the lowest and then S1 and S2 have the highest intensity labelling. ccML= cell corner middle lamella. Bar: 200 nm.

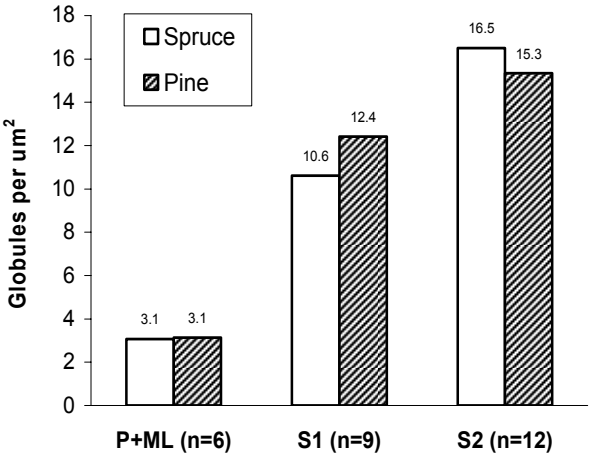

Fig. 17. Number of gold particles per unit cell wall area *(i.e.* per μm^2) calculated from TEM micrographs of spruce and pine. Labelling density gradually increased across the cell wall from P to S2 in increasing order. There was a significant difference ($p = 0.0107$) in the gold density present on the S1 layer between pine and spruce indicating higher amounts of GM in the pine fibre S1 cell wall layer. Labelling of the P wall layer for the two species was similar. Although spruce S2 layer showed slightly higher globular density compared to pine, it was not statistically significant ($p = 0.1183$). Numbers in parenthesis indicate the number of micrographs used in the calculations.

Figure 17 shows the globular density in the different cell wall layers of pine and spruce. There was a significant difference ($p < 0.05$) for the globular density in the S1 layer between pine and spruce where pine fibres showed greater labelling of the S1 (Paper III, Fig. 6). Thus, the pine fibre S1 cell wall layer contained greater amounts of GM across the S1 layer compared to spruce; an observation in agreement with that reported previously by Meier (1961) using chemical analysis in which pine fibres were shown to contain *ca* 2% more GM than spruce within the S1 layer. However, the GM distribution in P and S2 layers appeared to be similar for both pine and spruce (Fig. 17).

Greater amounts of GM per unit area indicates a lesser possibility for other polymers to be present within the cell wall layer, particularly lignin (cellulose is assumed to be similar). This suggests a reduced lignin concentration within the S1 layer of pine fibres compared to that of spruce. Reduced lignification of the S1 layer gave rise to an abrupt change in lignin concentration at the interphase between the lignin-rich ML and the less lignified S1 layer. This abrupt change in lignification at the CML/S1 interphase may have favoured the observed fracturing at the CML/S1 interphase and less frequently within the S1, as earlier suggested by Donaldson (1995, 1996, 1997) for radiata pine fibres. In addition, the branched nature of softwood glucomannan may also add to the above fracture pattern by interfering with lignin within the hemicellulose/lignin network that could also weaken the strength of the entire wall matrix and thus the layer. In contrast, spruce appeared to contain less GM in the S1 layer (Fig. 17) presumably allowing for more lignin to be present and thereby reduced the variation in lignification across this region.

Present microscopy observations did not show any significant influence of primary wall on the fracture behaviour possibly because it is a very thin layer compared to adjoining secondary wall layers. However, the primary wall has been suggested to have high swelling ability primarily due to high proteins associated with lignin within the layer and that fracture in this layer may occur (Salmén & Petterson, 1995).

3.2.3.2 Lignin

In addition, there also appeared a topochemical difference in lignin distribution in the S1 layer between pine and spruce TMP fibres. Staining of lignin with $KMnO_4$ which has been widely used as a stain in plant and wood cell wall ultrastructural studies (Hepler, Fosket & Newcomb, 1970; Bland, Foster & Logan, 1971; Maurer & Fengel 1990; Singh & Daniel, 2001; Singh, Daniel & Nilsson, 2002; Schmitt & Melcher, 2004) showed the S1 layer of spruce fibres to contain two distinct regions in relation to lignin concentration. As shown in Fig. 18a, the outer region of spruce S1 layer adjacent to the CML stained similar to that shown by the ML and ML cell corners (*i.e.* more electron-dense region; Singh & Daniel, 2001), while the inner region adjacent to the S2 layer remained unstained or stained poorly (*i.e.* electron-lucent appearance) with permanganate (arrowheads, Fig. 18a). These observations support the presence of two regions within the S1 layer of

Norway spruce fibre cell walls as proposed earlier by Maurer & Fengel (1991): *viz* a highly lignified outer- (*i.e.* $S1_H$) and less lignified inner regions (*i.e.* $S1_L$). In contrast, pine TMP fibres showed no difference in permanganate staining within the S1 layer (Fig. 18b) suggesting a more homogeneous distribution of lignin.

Based on TEM studies, it appeared that the difference in lignification at the interphase between the CML and S1 of spruce fibres was small (*i.e.* due to high lignin containing $S1_H$ adjacent to CML) compared to that of pine fibres. Therefore the change in lignin concentration over this region of spruce fibre cell walls was most probably insufficient to favour fracture through this region and hence this occurred at other sites in the fibre wall in this study as also observed within the S2 cell wall layer of radiata pine (Donaldson, 1995).

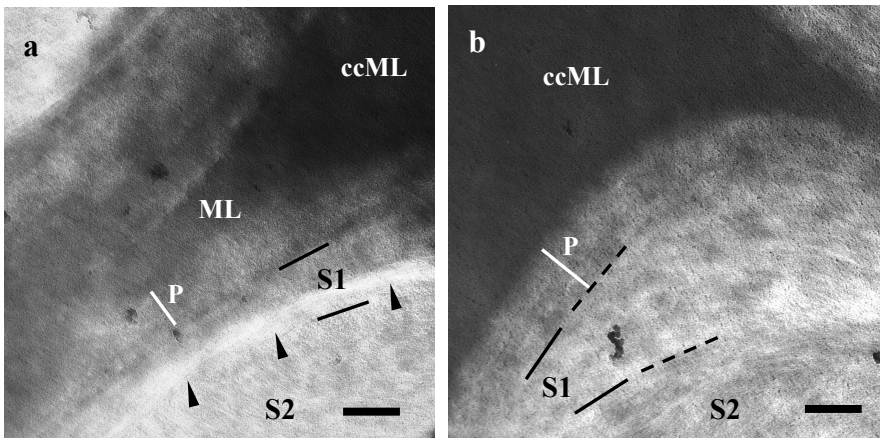

Fig. 18. TEM micrographs (T.S sections) through a cell corner region between adjoining fibres of a TMP shives from spruce (a) and pine (b) after staining with $KMnO_4$. S1 layer of spruce fibres was differentiated into outer electron dense (i.e. close to CML) and inner more electron lucent (i.e. close S2 layer; arrows) regions indicating presence of a more lignified outer (i.e. $S1_H$) and less lignified inner (i.e. $S1_L$) layers within the S1. Pine S1 layer (b) showed no variation in the density across the layer suggesting a more homogeneous lignification across the S1. Bars; a, b, 200.0 nm.

The different topochemical characteristics associated with pine and spruce fibre cell wall layers were considered to govern the different fibre separation mechanisms observed during the present study. As pine fibres showed fracturing in the outer cell wall layers (*i.e.* CML or S1) resulting in defibration through this region, it was obviously much easier in this place rather than deeper in the cell wall. Conversely, fractures deep within the cell wall leading to spruce fibre defibration from within the S2 layer are not as efficient as defibration in the outer layers. These observations explain why pine produced less shives and why spruce refining resulted in higher amounts of shives during the TMP process.

The negative behaviour shown by pine wood during TMP manufacture starts at the very beginning of the process because of its defibration mechanism. Therefore, the majority of pine fibres were surrounded by the S1 layer which acts as a barrier for subsequent fibre fibrillation and fibre flexibility as described in Paper III. The

energy from refining must be used first to remove the S1 and then for S2 peeling. In spruce, S2 peeling is already initiated in the majority of fibres even during the defibration stage. In addition, the characteristic ultrastructural features of the pine fibre S1 layer observed (*i.e.* loose structure of S1/S2 interphase, elastic nature of S1 *etc*) further contributed to the higher energy consumption and poor fibre development leading to inferior pulp properties.

It was therefore concluded that the observed differences in ultrastructural features (*i.e.* morphological and topochemical) associated with pine fibre cell walls may to a great extent explain the negative behaviour shown during TMP refining leading to both higher energy consumption and the inferior pulp properties developed.

3.3 Micromorphology and topochemistry of wood resin

Although the total amount of extractives (*i.e.* wood resin) normally represents only a few percent of wood, they are essential components in the metabolism of living cells like parenchyma cells. However, wood extractives are a major problem in pulp and paper manufacture causing problems in the production process and influence pulp and paper properties negatively (Back & Allen, 2000). Therefore during the present work, a major objective was to further understand the problems associated with lipophilic extractives. In particular, micro-morphological and topochemical aspects and the spatial distribution and redistribution of lipophilic extractives that occur during pulping processes were studied using an histochemical approach in conjunction with chemical analysis.

3.3.1 Pine and spruce wood extractives during TMP processing

As an extension of Paper III, a parallel study (Paper IV) was carried out to investigate the contributions played by spruce and pine wood resins (*i.e.* lipophilic extractives) during TMP processing. In particular, the high amounts of pine wood extractives (*ca* 2.5 times higher than in spruce) have traditionally been claimed as a reason for its limited use in TMP processing (Strand & Brill, 1986; Johansson, 2000; Reme & Helle, 2001). Histochemistry was performed in conjunction with chemical analysis to gain information on the origin of the problem (*i.e.* spatial distribution and micro-morphological features in the native state) using mature sapwood of spruce and pine and TMP pulps of both species after primary refining.

Data from sugar and lignin analysis performed on the native wood of both species (Paper IV, Table 1) showed values of the trees sampled to be representative for average spruce and pine wood. Physical properties such as freeness and strength were as expected where pine consumed higher electrical energy to a certain freeness and produced inferior strength properties (Paper IV, Table 2).

Cytochemical localization of triglycerides, the predominant lipophilic extractive found in the sapwood of both wood species (Ekman & Holmbom, 2000; Dorado *et*

al., 2001; Persson, Sundström & Xu, 2005) showed significant differences in resin anatomy between pine and spruce sapwood (Fig. 19) and in relative abundance that correlated with gross chemical analysis. Differences existed in ray parenchyma, axial parenchyma and epithelial cells surrounding the resin canals between the two species (Paper IV, Fig. 1). Similar results were obtained for fats containing double bonds (*i.e.* unsaturated fats) using osmium tetroxide (Paper IV, Fig. 2). A difference between the two wood species existed with respect to micro-morphological features and the spatial distribution of the native dominating lipophilic extractive fats (*i.e.* triglycerides and FAs) inside parenchyma cells (both radial and axial) and epithelial cells in the sapwood. The problems of Scots pine during TMP processing thus commence at the very beginning with its resin anatomy.

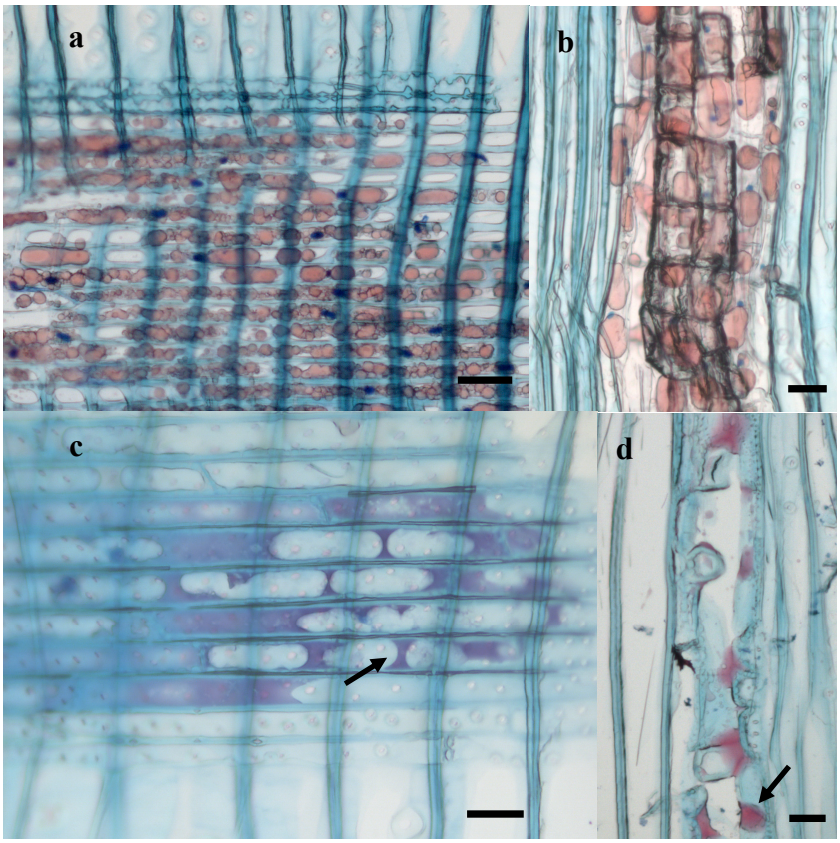

Fig. 19. Localization of native triglycerides in the cell system of Scots pine (top row) and Norway spruce (bottom row) sapwood using Nile blue that stains neutral triglycerides red/pink (all radial longitudinal sections). a) parenchyma cells in rays; b) axial resin canal and its surrounding epithelial cells; c) Spruce ray parenchyma with fats preferentially concentrated in cell corners (arrow); d) axial resin canal of spruce and triglycerides associated with epithelial cells (arrow). Bars: a, b, d, 60.0 µm; c, 30.0 µm.

Clear differences existed between spruce and pine TMP pulps in both the total amount of lipophilic extractives present and the absolute amounts of their different

constituents redistributed during TMP processing (Paper IV, Table 3). The triglyceride content in pine pulps (in both sawmill chips and thinnings) were almost double that present in spruce pulps (Fig. 20).

Neither Nile blue nor osmium tetroxide was sensitive enough to significantly stain triglycerides redistributed on TMP pulp fibres of spruce and pine although it gave strong reaction with fats in native wood. However, as shown in Fig. 21a, some positive staining was obtained with fines (*i.e.* fine fibrils; Paper IV, Fig. 4 a-c) from pine TMP pulps although the fines were seldom stained uniformly red/pink over entire fibrils reflecting complete coverage of triglycerides. The staining reaction of fibrils was very pale pink in colour in comparison to the strong red/pink staining response shown in native wood. This suggested the existence of triglycerides, presumably in very low concentrations over the surfaces of fibrils. In addition, redistributed extractives were observed as small droplets in the pulp slurry (Paper IV, arrow in Fig. 4f) and retained on fibre surfaces (Fig. 21b). Due to the mechanical action in refining, the large globules present in native wood (parenchyma cells) were broken down to much smaller droplets allowing for the removal of extractives from the cells through simple pits (Fernando, Daniel & Lidén, 2005).

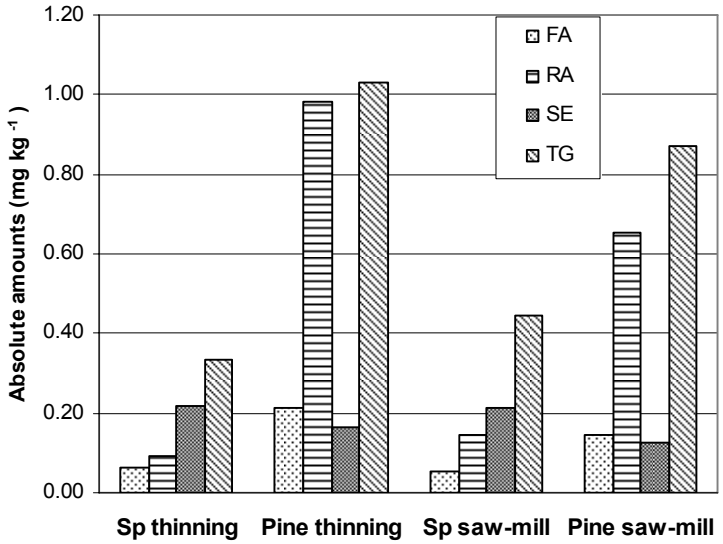

Fig. 20. Weighted absolute composition of the extractive content in spruce and pine TMP from the third stage of refining. Abbreviations, FA, fatty acids; RA, resin acids; SE, steryl esters; TG, triglycerides.

Similar information was given by an indirect cytochemical method for staining the presence of protein on the surfaces of wood fibres. Wood fibres contain proteins as a minor component and as an integrated part of the cell wall structure particularly in primary walls (Westermark, Hardell & Iversen, 1986, Cassab,

1998). Recently, Hafrén (2006) showed Norway spruce TMP to contain protein using cytochemical methods. TMP samples were stained for proteins present on the outer surfaces of pulp fibres using Coomassie blue (Paper IV, Fig. 6). Both pine and spruce showed an effect on staining when extracted TMP pulps (*i.e.* after removing extractives). Pine TMP gave stronger staining after extraction compared with spruce reflecting greater amounts of extractives still present on the outer surfaces of pine fibres.

Recent studies performed on the surface coverage and depth distribution of lipophilic extractives on mechanical pulp fibre surfaces using techniques such as XPS, AFM and ToF-SIMS indicated that liberated extractives could form thin films (*i.e.* most probably as molecular films) over TMP fines and pulp fibres (Koljonen, Österberg & Stenius, 2001; Johansson *et al.,* 2004; Kangas & Kleen, 2004). Based on the evidence provided by cytochemical staining and chemical analyses, present results suggest that lipophilic extractives are effectively covering the majority of pulp fibres and fragments most probably as mono-molecular films on the surfaces; a result consistent with that suggested by Brandal & Lindheim (1966) and more recently by Groff (2002).

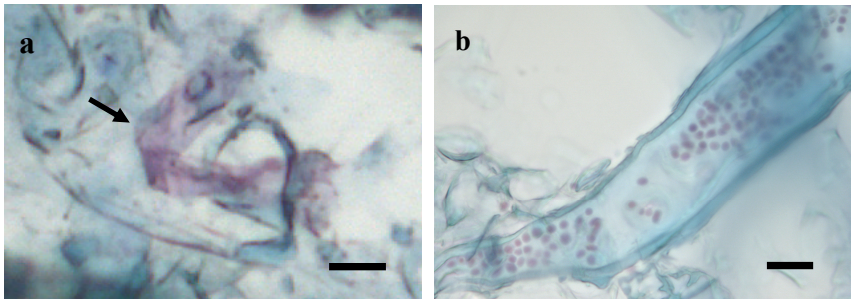

Fig 21. Redistribution of triglycerides among pine TMPs after primary refining as visualized by staining using Nile blue. a) Fines (*i.e.* fibril; arrow) in pine TMP were poorly stained pale pink/purple colour reflecting the presence of extractives possibly as a thin film covering their outer surfaces; b) Many smaller globules of extractives present in localized areas over fibre surfaces (*i.e.* retained outer surfaces of pulp fibres). Bars: a, 100.0 µm; b, 20.0 µm.

According to Garoff (2002), these molecular films of lipophilic extractives covering pulp fibre surfaces effectively create boundary lubrication that reduces paper to paper friction. Based on the results from staining techniques and chemical analysis, it is assumed that the development of thin films by liberated extractives on pulp fibres would have already occurred during the primary refining since the conditions in the refiners are favourable. Hence, the friction between pulp fibres themselves and also between refiners would have been reduced which in turn would have consumed higher energy for required fibre development. Obviously, this process is more strongly associated with pine TMP pulps that contain greater amounts of extractives.

Molecular films of extractives on the fibre surfaces may also impair properties of the pulp preventing bonding between pulp fibres and fines. This may lead to

poor strength properties of handsheets like tensile strength as observed with the pine TMP pulps during this study.

3.3.2 Birch wood extractives during kraft processing

Bleached birch kraft pulp is an important raw material for printing papers like white top liner or white top carton board. However, there have been considerable problems during both pulping and in final products due to birch wood extractives. For example, pitch deposits in refiners, screw transporters *etc* and in final products (*e.g.* dirt specks) cause severe quality problems (Laamanen, 1984; Bergelin & Holmbom, 2003; Lidén & Tollander, 2004). Source of the problems associated with extractives are similar for both mechanical and chemical pulps. Therefore, a study was carried out (Paper V) on birch wood extractives to gain a further understanding of the nature and behaviour of extractives during kraft processing by applying histochemical and microscopy (LM, FM, SEM and TEM) techniques in conjunction with chemical analysis. Localization of extractives involved in pitch problems was carried out in both native birch wood and kraft pulps at different stages (*i.e.* unbleached and TCF-bleached pulps) to obtain information concerning their spatial distribution and redistribution patterns during chemical pulping.

The two major lipophilic extractive constituents found in birch wood xylem are fats (48% triglycerides in petroleum ether extracts) and sterols (free or esterified; 36% petroleum ether extracts; Bergman, Lindgren & Svahn, 1965) that are mainly located in the parenchyma cells (Back & Allen, 2000).

Cytochemical localization of these major birch wood extractives revealed information on their spatial distribution in native wood and also some morphological features affecting the deresination process (Paper V). The three different stains used for fat localization (*i.e.* Bromine-Sudan black B as a general stain for lipids (Paper V, Fig. 3), Nile blue for triglycerides (Paper V, Fig. 4), and osmium tetroxide for unsaturated fats (Fig. 5)) provided similar information confirming their reliability for application in pulp and paper studies. Results showed almost all birch parenchyma cells (axial and ray) to contain fats/free fatty acids (FA) that were unevenly distributed around the cell lumen and concentrated at the ends of cells (Fig. 3-5, Paper V). These fats/free FAs were also located within the chambers of simple pits (Paper V; Figs 1g-h, 3, 5), thus blocking the free passage available during initial deresination. This indicates a further reason why birch pulps are difficult to deresinate.

Sitosterols (*i.e.* the most widely distributed wood sterols; Rowe, 1989) present in birch wood was visualized using filipin stain that specifically binds to sitosterols making fluorescent sterol-filipin complexes that produce fluorescent signals under epi-fluorescent microscopy (Fig. 22). Greatest fluorescent signals reflecting abundance of sterols was observed mainly in parenchyma cells including both ray (Fig. 22, arrow) and axial cells (Fig. 22, inset top left corner). Figure 22 also shows the irregular spatial distribution of sterols inside parenchyma cells and in the bordered pits (arrowhead).

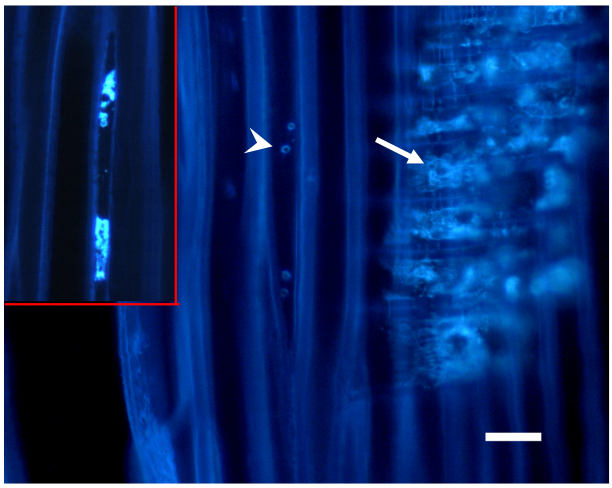

Fig. 22. Epifluorescent micrograph of a radial longitudinal section from birch wood chip showing fluorescent signals (fluorescent sterol-filipin complexes) in both ray (arrow) and axial (inset, top left corner) parenchyma. Distinct fluorescent signals were also visible around the bordered pits (arrowhead) of axial tracheids. Bar: 40.0 µm.

3.3.2.1 Unbleached birch kraft pulps

Staining was performed on unbleached birch kraft pulps using two sample types, one with a low dilution factor (DF *ca* 1.5-2) in brown stock washing and the other with high dilution factor (DF *ca* 4). Staining showed different behaviour exhibited by the extractive constituents, and also a difference between the two samples examined (Paper V). The majority of parenchyma cells in both types of unbleached pulps showed fats (*i.e.* triglycerides, free FAs) located almost exclusively inside cells after cooking (Paper V, Figs 6-9) while filipin gave evidence for the redistribution of sterols mostly on the outside of cells (Paper V, Fig. 6).

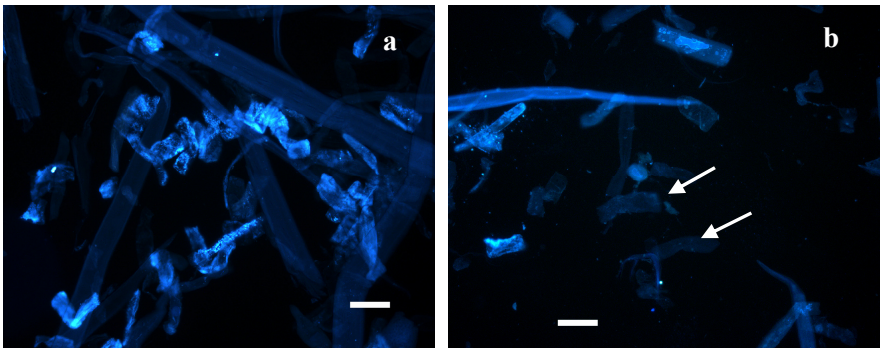

Fig. 23. Filipin staining of unbleached birch kraft pulps. Amounts of cells giving fluorescent signals were considerably higher in the sample of low DF (a) compared to high DF (b) indicating significant removal of sterols due to treatment with high DF (arrows show the unstained parenchyma cells). Bars: a, b, 40.0 µm.

Staining gave clear evidence for effective removal of both fats (Paper V, Figs 7-9) and sterols (Fig. 23a, b) from inside parenchyma cells in samples with high DF compared to that of low DF corroborating results from chemical analysis (Paper V, Table 1).

Staining for fats retained in unbleached pulps indicated that extractives were present as spheres after alkaline cooking and brown stock washing, as reported previously (Paasonen, 1967). Originally fats were distributed irregularly around the cell lumen in native wood. These globular structures were significantly larger than the pore openings (*i.e.* simple pits) of the birch parenchyma cells and thus partly explains why birch pulp can be difficult to deresinate.

From the staining experiments, the amounts as well as the size of the globules were smaller in pulps washed with high DF than in pulps from low DF (Paper IV, *cf* a and b in Figs 7-9), in addition to a lesser number of cells stained. Thus the significant difference in the amount of individual extractive components observed (Paper V, Table 1) was not only due to a lower concentration in the suspension liquor but also due to increased removal of extractives from inside the parenchyma cells.

Correlated information from staining and chemical analysis allowed an understanding of the mechanism by which effective deresination occurred in samples from high DF. Improved deresination in the pulps with high DF resulted from improved diffusion of soap micelles from the suspension liquor through the pits and/or cell walls (*i.e.* both in and out) of parenchyma cells. Because of higher DF, the ionic strength was lowered maintaining adequate micelle concentration for improved diffusion. In contrast, as a result of too low DF, the soap may have precipitated lowering the micelle concentration and thus the driving force for diffusion.

3.3.2.2 Bleached birch kraft pulps

Bleaching is known to be very effective in removing extractives and one major reason is due to chemical attack in the oxidizing bleaching stages (Bergelin & Holmbom, 2003). Satisfactory removal of extractives from bleached- compared to unbleached pulps was clearly shown by the staining methods applied in this study (Paper V).

The number of cells showing fluorescent signals in the bleached pulps was much less compared to unbleached pulps (Paper V, *cf.* Fig. 10a with Fig. 6a, b) indicating significant removal of sterols from parenchyma cells. Furthermore, the number of cells stained with Nile blue (Paper V, *cf.* Fig. 10b with Fig. 8a, b) and osmium tetroxide (Paper V, *cf.* Fig 10c with fig. 9a, b) in the bleached pulps were also significantly lower as the unsaturated fats and triglycerides were easily removed by oxidation. Nevertheless, a few cells were stained by both Nile blue (Paper V, Fig. 10b) and osmium tetroxide (Paper V, Fig. 10c) indicating continual presence of extractives that have not been subjected to oxidation or solubilisation even after TCF-bleaching (totally chlorine free-bleaching).

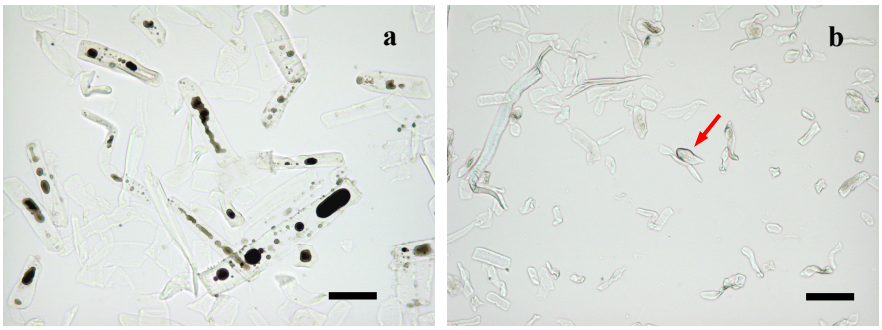

Fig. 24. Localization of unsaturated fats remaining in unbleached (a) and TCF-bleached (b) birch kraft pulps using osmium tetroxide. Note the presence of extractives as spheres inside parenchyma cells after cooking (a) that were significantly larger than pore openings (simple pits) of birch parenchyma cells; a feature negatively affecting deresination. Bleaching significantly removed unsaturated fats from birch kraft pulps allowing only traces of fats to remain that were present primarily on the outside the parenchyma cells (arrow, b). Bars: a, 40.0 µm; b, 80.0 µm.

The presence of dirt specks (*i.e.* pitch deposits) in unsized laboratory made handsheets blemishing the quality of the final product were clearly shown by staining (*i.e.* metal soap of FAs as confirmed using FT-IR spectroscopy) with Nile blue or reaction with osmium tetroxide (Paper V). Similar pitch deposits were not observed in unbleached pulp samples produced after cooking and brown stock washing. Presumably they were formed during the acid bleaching process by agglomerating mixtures of insoluble extractives together as a result of mechanical action in the subsequent stages. Observations of extractive particles stained with osmium tetroxide using SEM showed them to consist of fine cellular materials such as fibre fragments and pits *etc* that together formed larger specks (Paper V, Fig. 11).

4. Concluding remarks

A detailed electron microscope (SEM and FE-SEM) study was performed on Norway spruce thermomechanical pulp (TMP) fibres to observe their surface morphological ultrastructure related to fibre cell wall development and fibrillation. The majority of TMP fibres after primary refining showed the S2 secondary cell wall layer to be exposed and fibrillation of the inner S2 layer to have been initiated. During TMP processing two major types of fibre fibrillation were observed: (1) "flake-like" derived from the S1 layer as a result of splitting along the S1 MFA, and (2) "sheet and thin ribbon" fibrillation from the S2 cell wall layer due to helical cracking of the cell wall layer following the S2 microfibril angle. The outer fibre S2 wall split into regular sized concentrically oriented lamellar-sheets and/or thin ribbons; each ribbon composed of a set number of sub-aggregates. Fibre cell wall splitting developed between the aggregates rather than across them. In addition to providing details on the effects of refining on fibre wall structure, novel information was obtained on fibre cell wall architecture. Results suggest that fibre fibrillation during industrial thermomechanical pulp refining is regulated by the native fibre micro- and nanostructure.

Stoneground wood (SGW) process inflicted severe damage to the Norway spruce fibre structure resulting in transverse and longitudinal fibre breakage. Nevertheless, studies emphasized the importance of the morphological and hierarchical structure of fibre cell walls at the ultrastructural level even during SGW mechanical pulp processing. Differences in pulp and paper properties between juvenile- and mature wood were likely related to the manner of fibre processing and development at the ultrastructural level. Juvenile wood fibre development resulting in improved properties showed features similar to TMP fibres where the native morphological fibre ultrastructure (*i.e.* hierarchy of fibre cell wall and microfibrillar angle of S1, S2) plays an important controlling role on fibre fibrillation. Studies further suggest that raw materials rich in JW (*e.g.* top logs) may be advantageous for the SGW process.

Ultrastructural studies on TMP shives and pulp fibres from Norway spruce and Scots pine revealed differences between the two wood species in relation to both morphological and topochemical features (*i.e.* S1 layer, S1/S2 interphase, lignin and glucomannan distributions across fibre cell walls). The inherent ultrastructural characteristics of Scots pine fibre cell wall governed its fibre development mechanism that was different from Norway spruce during TMP processing. The negative behaviour shown by Scots pine during TMP processing (*i.e.* with respect to both higher electrical energy consumption and inferior pulp properties) was most likely attributable to the observed fibre development mechanism.

Histochemistry was shown to be a versatile technique that can be applied successfully in pulp and paper research for wood resin associated problems. While it may provide a complementary approach to advanced surface analysis techniques like XPS for the analysis of extractives, it also has advantages in that it is relatively easy to use, rapid and inexpensive. In addition, information can be

obtained on the spatial micro-morphological distribution and redistribution of chemical components that can be visualized on single fibres (both internal and external structures) and fractions.

Results obtained using histochemical staining performed on both wood and TMP from Norway spruce and Scots pine on the relative abundance and distribution of extractives (*i.e.* fats) between the two species was found to correlate with chemical analysis done using FT-IR and gel permeation chromatography (GPC). The technique provided further information concerning species-specific distribution patterns of extractives. It further gave evidence for the presence and distribution of extractives possibly as molecular films over pine TMP fibres and fragments that may partly explain the negative response shown by pine fibres.

Birch is known to cause considerable pitch problems during kraft pulping. Localization of lipophilic birch wood extractives involved in pitch problems (*i.e.* sterols and fats in both wood raw materials and kraft pulps) revealed information concerning their spatial mirco-morphological distribution and redistribution during pulping and washing. Sitosterols were specifically localized using polyene antibiotic filipin. Bromine-Sudan black B was used as a general stain for lipids, Nile blue for staining neutral fats *i.e.* triglycerides and osmium tetroxide for unsaturated fats. Based on the correlated information from gas chromatography analysis and staining methods, it was possible to understand not only how much deresination of extractives occurred during kraft pulping but also obtain details about the mechanisms of removal.

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