ENZYMATIC MODIFICATION OF WOODY CELL WALLS FOR IMPROVED STABILITY OF PULP FIBRES

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GRETHE STREY
SIGNED IN PRETORIA ON SEPTEMBER 2009
Al wat staan tussen ’n mens en wat hy uit die lewe wil hê, is dikwils die Wil om te probeer en die Gelooi om te glo dit is moontlik.

Richard M DeVos
TO MY SAVIOUR
WHO GAVE ME THE ABILITY,
FAMILY AND FRIENDS
TO HELP ME REACH A DREAM
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The bonding of fibres in paper is influenced by environmental changes (e.g. moisture) that may cause unstable fibres to move. These movements include cell-wall swelling, fibre lifting and/or puffing that break inter-fibre bonds and lead to reduced strength and surface roughness. Fibre puffing is defined as the expansion of the lumen area as result of changes in the environment.

Puffing was investigated through image analysis of scanning electron micrographs. Detailed images were obtained with samples that were embedded in resin and then etched. Puffing of fibres was then quantified by calculating the ratio of lumen area to fibre area. Stability of softwood and hardwood fibres was studied in this way, and to simulate printing, handsheets were calendered and rewetted. This method was later validated against commercial sheets. Compared to softwood, hardwood fibres were more stable and most of the handsheet properties were retained after rewetting. Mannanase and/or endoglucanase treatments resulted in improved fibre stability by increasing fibre bonding, fibrillation or fibre collapse.
Mannanase improved handsheet smoothness and strength as well as fibre stability, but endoglucanase was less effective. The effect of the enzymes was more difficult to observe on hardwood fibres, because even untreated fibres were more stable under moist conditions. Thin-walled fibres such as earlywood were less stable than latewood fibres, but it responded better to mannanase treatment. Thick-walled fibres (latewood), on the other hand, were more difficult to improve with enzymes.

The potential of enzymes to improve fibre stability of commercial pulp was tested on chemi-thermo-mechanical pulp (CTMP) and bleached CTMP. Enzyme treatment improved fibrillation and reduced beating energy of bleached CTMP. Mannanase again resulted in the most improved fibre stability. On rejects, a lack of response to enzymes was overcome by pre-treating the pulp with alkaline peroxide.

This study provided new insights into the stability of fibres with different morphology. It was also demonstrated that fibre stability can be improved with enzyme treatment and it is expected that this knowledge could have significant commercial value.
CHAPTER 1

LITERATURE REVIEW: FIBRE MOVEMENT IN MECHANICAL PULP AND PROCESSES FOR IMPROVEMENT OF STABILITY

ABSTRACT
Wood is the main source of fibres used industrially to produce paper. These fibres are subjected to different chemical or mechanical processes to produce pulp with specific characteristics. Mechanical fibres are generally stiff and difficult to collapse. When incorporated into paper these fibres often encounter water that may lead to movement within or out of the sheet. These movements include swelling, puffing and lifting, and are responsible for the breaking of inter-fibre bonds that could lead to high surface roughness and reduced paper strength. These potentially unstable fibres can be removed by fractionation or refining processes. Additional treatment of these unstable fibres such as chemical and enzymatic treatments can be combined with these conventional processes. Chemical treatment is often used to remove some of the lignin or hemicellulose, making fibres more flexible and collapsible leading to better paper formation. Enzymes on the other hand, hydrolyse glycosidic bonds in the cell-walls of fibre, to improve fibre collapse.
1.1. INTRODUCTION

Cellulosic fibres find wide application in many pulp and paper operations. These fibres are produced through several wood pulping methods that include chemical treatment or mechanical actions such as grinding.

Pulp fibres encounter water at many different stages during the manufacturing process and conversion to paper; its subsequent behaviour in the presence of moisture will determine its suitability for a given application. Distortion of the fibre network in the presence of water can often be observed. These moving fibres are described as unstable, while stable fibres are characterised by good fibrillation and conformability that allows them to retain their collapsed state even in the presence of moist conditions (Forseth and Lepoutre, 1994). Thick-walled fibres present in mechanical pulp are typically poorly fibrillated. These fibres cause problems during manufacturing of paper, due to their stiffness and low conformability. Different processes used to separate and develop the surface properties of fibres are already applied in the pulp and papermaking industry. However, these methods have limitations and new opportunities are being investigated at present.

Different processes for the treatment of the fibres with either chemicals or enzymes before refining have been investigated. Chemicals such as sulphate are already used in bleaching processes to improve the pulp quality by softening or removing lignin, which acts as a barrier to water during the pulping processes (Salmén, 1984; Lindström et al., 1988). Pre-treating fibres with chemicals can also make the fibre wall more responsive towards refining conditions (Strunk et al., 1990; Meyer-Pinson et al., 2004; Bian et al., 2008). The application of enzymes is also useful to modify components in the cell-wall structure for this purpose (Kibblewhite and Clark, 1996; Mansfield et al., 1996; Clark et al., 1997; Lumme et al., 1999) and can possibility lead to a reduction in chemical use (Hill, 1996). Enzymes can specifically attack compounds in the cell-wall, modifying these compounds to produce higher quality paper with greater strength and surface smoothness (Lumme et al., 1999). The aim of the present chapter is to review previous work on the behaviour of different fibres during papermaking, how environmental conditions influence their stability, and how different methods can be used to improve fibre stability.
1.2. **MECHANICAL PULP**

Pulp consists of wood fibres or other lignocellulosic materials that have been broken down into discrete fibres during pulping. These liberated fibres obtained after pulping can be dispersed in water and reformed into a web to produce a paper sheet. Pulping processes are generally divided into two broad classes, chemical and mechanical, which produce substantially different fibre characteristics. In many papermaking operations, a combination of chemical and mechanical pulps is used to obtain the desired paper characteristics.

Chemical pulping is based on the chemical actions of sodium hydroxide, sulphite or sulphate (Sjöström, 1993; Gullichsen and Fogelholm, 2000). These chemicals degrade and solubilise components of the wood, especially lignin and hemicelluloses, leading to easier separation of fibres (Lindholm and Kurdin, 1999). Very little to no mechanical action is necessary to separate fibres, therefore, making chemical pulping an energy efficient method. The separated fibres show little structural damage, and thereby strong and flexible fibres can be produced from both softwood and hardwood species. Strong papers are produced in this way, since the lignin, which interferes with hydrogen bonding of fibres, is largely removed. However, the high cost of chemicals and low pulp yield obtained (approximately 45% of the dry mass of the wood) are drawbacks of these chemical processes (Biermann, 1996).

Mechanical pulping offers different advantages in the production of pulp and paper. Wood fibres are disintegrated using mechanical actions such as grinding and refining, where heat is generated to soften the lignin for easier fibre separation (Sundholm, 1999). Since the lignin is only softened, it is retained in the pulp and, therefore, high yields of 90 to 95% can be obtained (Biermann, 1996). However, the presence of lignin can restrict fibre swelling during pulping (Ehrnrooth, 1982) and less collapsible fibres are, therefore, produced. It is likely that these lignin-rich fibres form weak paper, because of less inter-fibre bonds that are formed due to the small contact areas and limited hydrogen-bonding. Mechanical actions such as grinding and refining can also damage fibres and contribute further to reduction in strength (Page and El-Hosseiny, 1976; Page and Seth, 1980). The strength properties of mechanical pulp can be improved by either a chemical pre-treatment, mixing chemical and mechanical pulped fibres, or by additional refining steps.
Despite its lower quality, mechanical pulp constitutes 20 to 25% of the world paper production and is increasing due to the high yield of the process and growing competition for fibre resources (Biermann, 1996). Different fibre sources are available for mechanical pulping, and these play an important role in the quality of the pulp produced (Varhimo and Tuovinen, 1999). Softwood species such as spruce, fir, pine and hemlock, are most commonly used (Härkönen et al., 1989; Harris, 1993). It is generally recognised that species from the spruce family are the most suitable raw materials for mechanical pulping, since this species provides pulp with properties ideal for various paper products (Varhimo and Tuovinen, 1999). The superiority of spruce is attributed to the favourable fibre characteristics (fibre length), as well as a lower extractives content and high initial brightness of the pulp.

Poplar is the best suited hardwood resource for mechanical pulping (Varhimo and Tuovinen, 1999), but the morphology of hardwood fibres is more complex than softwood due to the greater variation in fibre types (vessel elements, parenchyma cells etc). Significantly less lignin and correspondingly higher amounts of cellulose and hemicelluloses are present in hardwoods compared to softwoods (Sjöström, 1993). Mechanical pulps from hardwoods also exhibit good light-scattering and sheet properties (such as high surface smoothness), whereas the strength properties are usually poorer due to the short fibre length of 1.2 mm or less (Horn, 1974; 1978; Sjöström, 1993).

Softwood and hardwood species are usually mixed during mechanical pulping to produce higher quality pulp and improve the strength as well as surface properties of paper. However, these pulp blends are usually highly heterogeneous due to the wide variation in fibre morphology, mechanical properties and chemical composition (Biermann, 1996; Koljonen et al., 1997). Pulp blends contain fibres that have good paper-making potential that can be exploited for the production of high quality papers. Mixed pulp also contains a fraction that contributes to poor paper quality unless these fibres are extensively processed. Fibres of low quality are usually present in mechanical pulps and are very stiff and difficult to form into smooth and strong paper. Processing of these stiff fibres fibrillates the cell-wall structure and leads to more flexible fibres. It has been proposed that different mechanical pulping processes can be applied to loosen up the cell-wall layers in different ways (Franzén, 1986; Salmén et al., 1999) and some of these are illustrated in Fig. 1-1.
Refiner mechanical pulping (RMP) is used to separate fibres only by mechanical actions, causing cracks in the S2-layer of the secondary cell-wall (Lindholm and Kurdin, 1999). These cracks in the cell-walls create fibre bundles that are not ideal for papermaking. However, the amount and size of these bundles can be reduced through extensive refining (Huusari, 1999). Stone groundwood (SGW) and pressure groundwood (PGW) are more common mechanical pulping methods, and separate fibres by pressurising the grinder with steam that heats and softens wood prior to the grinding action (Biermann, 1996). These two methods (SGW and PGW) split fibres between the S1- and S2-layers of the secondary cell-wall, leading to better separation, but some fibre damage may still occur (Lindholm and Kurdin, 1999; Fig. 1-1).

The thermo-mechanical pulping (TMP) method was developed 10 years after RMP, and has become the most important mechanical pulping method due to the higher strength of fibres that are produced. In TMP processes, wood chips are subjected to high temperatures (140°C to 160°C) in combination with mechanical refining (Lindholm and Kurdin, 1999). The effect on the fibre cell-wall structure is the same as observed with SGW and PGW.
A pulp yield of 91 to 95% can be attained with all these mechanical methods (Biermann, 1996), however, fibre damage that can degrade the pulp quality can occur. Less fibre damage is observed when chemi-thermo-mechanical pulp (CTMP) is produced. During this process heat is combined with small amounts of chemicals that partly dissolve or soften lignin. The chemical treatment before refining causes fibres to separate preferentially along the primary cell-wall (P) and the middle lamella (ML) (Lindholm and Kurdin, 1999; Fig. 1-1). These different mechanical pulping processes were investigated by Lindholm (1980), who demonstrated the effect of the typical action of each process on fibre properties.

1.3. FIBRE BEHAVIOUR

Mechanical pulp fibres can be significantly modified through refining processes, thus developing pulp properties to suit specific end-products, such as newsprint, uncoated papers and light weight coated (LWC) papers (Biermann, 1996). Two important requirements that need to be met for most end-products are high paper strength and smooth surfaces. Paper strength is necessary for good runability, or the ability of the web to withstand failure or breakages on the paper machine. In many cases when paper strength requirements are not met, the speed and productivity of the system may be limited by these web breaks. Surface smoothness, on the other hand, plays an important role in the quality of the printed images (Bristow and Ekman, 1981). These requirements for strength and smoothness are influenced by the ability of the fibres to collapse and conform during paper making.

1.3.1. Fibre collapse

The ability of fibres to collapse can be improved by removing some of the lignin in the cell-wall, modification of the cell-wall, or applying pressure on the fibre after drying. Lignin present in the cell-wall seems to act as a non-binding spacer between fibres and also limit fibre swelling during pulping (Lindström et al., 1988; Ehrnrooth, 1982) and it is likely that these fibres will have poor collapsibility and less bonding. Some of the lignin can be modified or removed with chemical treatments (sodium sulphate, for example) followed by refining. Refining processes are used to modify the cell-walls of fibres (fibrillation), by removing the middle lamella, primary wall and S1-layer and parts of the S2-layer. Material that is only partly removed (fibrils) will give the fibre a larger surface area favourable for
fibre bonding. Refining induces fibrillation, weakens the cell-walls and makes fibres more flexible and easier to collapse (Mohlin, 1975; Paavilainen, 1993). After chemical treatment and fibre development, these fibres are distributed unto a forming fabric, drained and some pressure is applied to induce fibre collapse. Wet pressing collapses fibres and brings them closer together (He et al., 2003). Additional methods such as calendering also apply pressure to achieve a smoother surface with higher gloss (Rounsley, 1991; Holmstad et al., 2004; Nesbakk and Helle (2002). While Skowronski (1990) showed that smoothness of a sheet made from TMP is achieved principally by fibre lumen collapse during calendering, little is known about the behaviour of fibres during calendering.

The degree of fibre collapsibility can vary, because of differences in fibre morphology among wood species and the effect of different pulping methods on the structure of the cell-walls. The lumen area is a good indication of the degree of collapsibility of fibres and is reduced or even eliminated when a fibre collapses (Fig. 1-2).

Figure 1 - 2: Illustration of a fibre in cross-section showing changes in the lumen area for an A: uncollapsed fibre; B: a partially collapsed fibre and C: a totally collapsed fibre (adapted from Jang and Seth, 1998).

Fibre collapsing is important in paper formation, because it can result in denser sheets with higher strength. The density of sheets is increased due to fibres that easily collapse and conform, creating fewer voids in the paper network. These highly collapsed fibres create large contact areas with more inter-fibre bonds, which are reflected in the stronger paper
produced. Having recognised the importance of fibre collapse for papermaking, different methods for its measurement have been investigated (Robertson, 1964; Page, 1967; Kibblewhite and Bailey, 1988; Kibblewhite and Bawden, 1991; Jang et al., 1996, Jang and Seth, 1998 and Williams and Drummond, 2000).

Water retention measurements were used by Robertson (1964) to determine an index for collapse fibres and the reduction in the water content was associated with the loss in lumen volume. However, that method was only applicable to never-dried fibres, because the difference in the water content was associated not only with loss of lumen volume, but also with the fibre shrinkage and the collapse of fibrils. Page (1967) developed a sample preparation technique in which fibres were embedded in a medium that had the same refractive index as the fibre cell-wall. When this sample was viewed using a light microscope the totally collapsed fibres became invisible, while partially or uncollapsed fibres remained visible. While it was possible to discern the amount of collapsed fibres contained in the pulp, this method could not quantify the degree of collapse. Kibblewhite and Bailey (1988) investigated the shape of the fibres using microscopy and image analysis to measure fibre cross-sectional dimensions.

Examination of the cross-sectional shapes of fibres using microscopy appears to be the most accurate reflection of fibre collapse. The preparation procedure for this technique is tedious and requires aligning, resin embedding and microtome sectioning of the fibres (Kibblewhite and Bailey, 1988). The sample preparation technique is, however, very important for accurate results, and should not influence the state of the fibre present in the network. When representative cross-sections were obtained, image analysis could be applied to determine the dimensional parameters of fibers in micrographs (Jang et al., 1991; 1992; 1995; Fjerdingen et al., 1997; Reme et al., 1998; 1999). The most relevant of these dimensions are illustrated in Fig. 1-3.
The definition derived for fibre collapse is historically based upon the measuring techniques and image analysis software available. A collapse index (CI) was defined by Jang et al. (1995; 1996) using different area parameters such as fibre area (FA), lumen area (LA) and cell-wall area (CWA). The CI represents the change in the lumen compared to an estimated uncollapsed lumen. Kibblewhite and Bawden (1991) measured the fibre shape by using the aspect ratio (AR) as an index of fibre collapse. Fibre height is divided by fibre width, where a small AR implies a high degree of fibre collapse. Page (1967) expressed the collapse index as a function of the fibre-wall thickness only, since, wood fibres are tubular in shape and it is expected that thin-walled fibres collapse with greater ease than thick-walled fibres (Hallamaa et al., 1999). Xu et al. (1997) discussed a method that can determine the effect of the CWT at any stage of collapse, by calculating the ratio between fibre height and fibre width of a fibre cross-section. Image analysis software was also used by Reme et al. (2002) to measure the effect of CWT using the ratio of the CWA and the centreline perimeter, but this method has only limited application to fibres that are almost uncollapsed.
Those studies concluded that thin-walled fibres collapse to a higher degree than thick-walled fibres during paper production. Fibre morphology, therefore, can influence the paper structure, as well as its physical properties, but the impact of fibre collapse on paper properties is mainly due to its influence on conformation of fibres in the paper web.

1.3.2. Fibre conformation

Fibres that have the ability to bend and match the shape of adjacent fibres form large contact areas for fibre-to-fibre bonding, resulting in good sheet formation and stronger and smoother paper (Kure et al., 1999; Retulainen et al., 1998). Such fibres are described as conformable and the degree of conformability depends on the degree of fibre collapse. However, the conformability and collapsibility of a fibre is influenced by pulping conditions as well as the composition of the furnish.

Mechanical fibres are generally stiffer and less conformable when compared to chemical pulp fibres (Skowronska, 1990). Since mechanically-pulped fibres seldom collapse totally in the paper network, smaller contact areas are formed between fibres that lead to less bonding. It is, therefore, generally difficult to obtain well-formed sheets with the required strength and smoothness with mechanical pulps. However, different mechanical pulping methods are employed to produce specific end products, since CTMP fibres are, for example, more conformable with a higher degree of lumen collapsibility than TMP (Biermann, 1996). Fibres are tubular, and thus bend and conform more easily when collapsed or flattened. The degree of fibre collapse is, therefore, considered to be a good indicator of the fibre conformability (Jang and Seth, 1998).

It is clear that a high degree of fibre collapsibility and conformability are required to produce paper with good strength and printing surface. However, paper is subjected to processes such as coating and printing, where high moisture can reverse the collapsing of fibres (Retulainen et al., 1995; Forseth and Helle, 1996). Fibres with the ability to remain collapsed and resist movement can be described as stable fibres. High quality papers with good strength and surface properties are not only composed of collapsed fibres, but are also characterised by the stability of these fibres.
1.4. **Fibre stability**

The instability of fibres usually becomes evident when the relative humidity increases or when the fibres in the sheet come into contact with moisture. Paper has hygroscopic characteristics that attract moisture (Stone and Scallan, 1968). When moisture is present, water molecules penetrate between the hydrogen-bonded fibres in paper and into the cell-walls of the fibres, leading to changes in the paper and cell-wall structure (Skowronski and Lepoutre, 1985). The mechanism for individual fibre response under these conditions is still unclear.

The amount of water absorbed by the paper network depends on the fibre morphology and the state of collapse in the lumen (Aslund et al., 2005). Since pulps are characteristically heterogeneous, it is likely that paper sheets could contain stable fibres as well as unstable fibres that react differently with water molecules, and can cause undesirable surface roughness and a loss in paper strength. Unstable fibres can, therefore, move and cause negative changes in the paper structure. This is due to three types of fibre movement. Firstly, fibres that lift from the sheet surface (fibre rising); secondly, swelling of the fibre cell-wall and; thirdly, fibres that lose their collapsed shape (puffing). These movements become evident during operations such as coating and printing and are especially predominant in grades that contain mechanical pulp (Skowronski and Lepoutre, 1985; Aspler and Béland, 1994).

1.4.1. **Fibre rising**

Fibre rising can be observed in the fibre network after sizing, coating, printing or flexography (Hoc, 1989; Aspler and Béland, 1994). During these operations fibres are subjected to high moisture conditions, where one or both ends of the unstable fibres rise out of the paper surface (Forseth and Helle, 1997). In some cases when both ends are anchored, segments in the middle may rise. These movements are triggered by diffusion of water molecules into the fibre cell-wall (Hoc, 1989). Béland et al. (1993) showed that this movement can cause distortion in the fibre network and changes in the fibre dimensions,
but the causal mechanism is still unclear. It was also observed by Skowronski and Lepoutre (1985) and Forseth and Helle (1997), that the distortion is irreversible and causes the surface to roughen and reduce the gloss in printed areas.

The extent to which paper roughness develops is influenced by the type of pulp used and when only mechanical pulp fibres were used, fibre rising was more evident, resulting in rougher sheets (Skowronski and Lepoutre, 1985; Hoc, 1989; Aspler and Béland, 1994). Fibre rising was also more noticeable in poorly-bonded sheets and, according to Hoc (1989), an increase in fibre length leads to a greater extent of fibre rising. Fibre rising is, therefore, expected to be more evident in softwood than in hardwood species, due to the relatively longer fibres. However, Mohlin (1989) emphasised that this problem was also due to the presence of thick-walled fibres typical in mechanical pulp.

1.4.2. Cell-wall swelling

Cell-wall swelling occurs when water molecules diffuse into the fibre wall, causing an increase in some the fibre dimensions (Page et al., 1985). Cell-wall swelling in the present study refers to the reaction of fibres in a dried paper sheet when exposed to water during coating or printing. These swelling forces inside the paper can be extremely powerful, and it is likely to cause bond breakage, leading to unbalanced stress that may cause fibre segments to lift out of the sheet surface. According to Hoc (1989), these segments that lift out of the surface because of cell-wall swelling can contribute to fibre rising. The cell-wall swelling effect was primarily observed in chemical pulp by Forseth and Lepoutre (1994).

Cell-wall swelling can also contribute to increased bulk and surface roughness during coating (Skowronski et al., 1988). Stone and Scallan (1968) used microscopy to observe the elementary fibrils present in the fibre wall (Fig. 1-4). In the model subsequently proposed, a dry fibre wall was described as being nonporous. The fibre wall in its dry state consists of fibrils bonded together in a close-packed array (Fig. 1-4 A). When these fibres start to adsorb water, hydrogen bonds are broken and the structure swells (Figs. 1-4 B to C). The fibrils are then separated by lamellar spaces, and water molecules can penetrate between the hydrogen bonded fibrils into the fibre cell-wall. The more water penetrates, the more internal bonds between the cell-walls are lost, to cause distortion or deformation of the fibres in the paper network (Figs. 1-4 C to D).
1.4.3. Puffing

The recovery of the fibre shape to a tubular form has been referred to as decollapse (Reme et al., 1998; 1999), but is better described as fibre puffing. Puffing can be defined as the expansion of the lumen of a fibre, under increasing moisture conditions. A study by Forseth and Helle (1997) showed that the puffing of fibres was not related to changes in the thickness of the cell-wall or the cell-wall area. Fibre puffing has not been widely investigated but a number of studies have been done to examine changes in the dimensions of the fibre lumen. In these studies, puffing of fibres was usually brought about by wetting of fibres (Skowronski, 1990; Forseth and Lepoutre, 1994; Forseth and Helle, 1996; 1997). Skowronski (1990) also investigated the wetting of fibres under different stress conditions that included uncalendered and calendered paper. It was evident that puffing of unstable fibres was more pronounced in calendered handsheets that were subjected to high moisture (Forseth et al., 1997).

1.5. IMPROVEMENT OF FIBRE STABILITY

Current technology such as refining and fractionation makes it possible to isolate and treat unstable fibres and thus control the fibre movements described above. Fibres are modified during refining, to increase cell-wall fibrillation for better bonding and conformability (Corson and Ekstam, 1994; Karnis, 1994; Mohlin, 1997). Since fibres react differently towards refining conditions, and since all fibres are not subjected to the same
amount of refining action, a fraction of unfibrillated fibres often remains after refining. These unfibrillated fibres could add to instability, they are separated from the main stream using fractionation processes (Hautala et al., 1999; Kappel, 1999). Further improvement of such fibres can be achieved using these mechanical processes combined with either chemical treatments or enzymatic treatments aimed at making the cell-wall more susceptible to fibrillation and collapse.

1.5.1. Refining

The post-refining of mechanical pulp is one of the critical operations in the production of a desired end-product. One of the objectives of fibre modification during refining is to increase the bonding ability of fibres (Mohlin, 1975; 1997; Hiltunen, 2003). The increased bonding leads to stronger paper (Karnis, 1994; Kure, 1997) and reduced fibre movement. Fibre stability can, therefore, be improved by increasing the flexibility and fibrillation of the cell-walls. The flexibility of the cell-wall can be improved by internal fibrillation of fibres, while external fibrillation increases inter-fibre bonding. As mentioned earlier, internal fibrillation refers to loosening of the internal fibre-wall structure by rupture of bonds in the cell-wall (Mohlin, 1991), making it possible for the fibre to swell more during the refining process. As a result, the collapsibility and flexibility of fibres are increased and these fibres then conform effectively during sheet formation.

The high number of impacts associated with efficient refining causes cell-walls to also fibrillate externally. Scanning electron microscopy (SEM) showed that fine cellulosic bundles are loosened and in some cases parts of the cell-wall are peeled off, but not completely removed from the fibre (Kibblewhite, 1972; Kerekes, 2005; Molin and Lennholm, 2000). Since these external fibrils remain attached to the fibres, more fibre-to-fibre bonds are formed (Page et al., 1985, Page, 1989). Strength properties such as tensile and Scott bond of Kraft pulp have been shown to increase by 20 and 46%, respectively, through efficient refining (Kang and Paulapuro, 2006).

The response of fibres to refining depends to a large extent on fibre morphology, such as cell-wall thickness (Huang et al., 2007). It was observed that fibres with thin walls absorbed more energy and were easier to rupture under standard refining conditions when compared to thicker-walled fibres. After refining, most of the thin-walled fibres were split
and collapsed and short elements and fines were also generated (Reme et al., 1999). When thick-walled fibres were refined, changes were not obvious, but higher refining levels caused reduction in the cross-sectional dimensions to produce more collapsed fibres (Corson and Ekstam, 1994; Karnis, 1994; Mohlin, 1997). Therefore, even thick-walled fibres can be modified through refining so that they can collapse to increase fibre and pulp stability (Norgren et al., 2004), and it is likely that these stable fibres can result in stronger paper with better surface properties. However, refining is an energy intensive process and mills include other processes to reduce these thick-walled poorly fibrillated fibre portions.

1.5.2. Fractionation

Mechanical pulp typically contains a wide range of fibres with different morphologies, including highly and poorly fibrillated fibres (Paavilainen, 1988). Fractionation refers to the selection processes that can be used to separate fibres which are suitable from those unsuitable for paper making (Vomhoff and Grundström, 2003). The result of using only desirable fibre is that pulp with a higher quality and more uniformity can be produced (Abubakr et al., 1994).

Fractionation processes are generally applied after refining, and consists of hydrocyclone cleaners and screens. Hydrocyclone cleaners use the principle of centrifugal separation, and remove fibres on the basis of specific gravity (Paavilainen, 1992). The result of cleaning is, therefore, to reject thick-walled fibres that are often responsible for printing defects (Hoc, 1989; Paavilainen, 1992; Kure et al., 1999). Handsheets made from the cleaned (thin-walled) fibres showed significantly higher surface smoothness and higher strength properties when compared to the rejected fibre fraction (Paavilainen, 1992; Vomhoff and Grundström, 2003).

Fractionation also includes screens that are usually constructed with foils to create pressure across the slots and are generally referred to as pressure screens (Biermann, 1996). Pressure screens primarily aim to separate long fibres as rejects (Kure et al., 1999). However, it was also observed that it was possible to separate out some of the coarse, thick-walled fibres using hydrocyclones (Kappel, 1999). By removing these long undeveloped fibres, paper properties such as smoothness can be improved (Mohlin, 1989).
Removal of unsuitable fibres (rejects) results in a reduction of pulp yield. In fractionation processes with low reject rates, fibres can be re-circulated by returning rejected fibres to the pulp stream before refining. Alternatively, with higher reject rates, rejected fibres can be improved through additional reject-refining stages. Reject refining specifically concentrates on promoting flexibility and collapsibility of these rejected fibres (Kure et al., 1999). Fibre development through only refining and fractionation are limited to the degree of fibre modification that can be achieved. Integrating these processes with pre-treatments such as chemicals or enzymes may offer new opportunities to improve pulp properties even further.

1.5.3. Chemical treatment

The effects of chemical pre-treatments before refining have been described in terms of pulp and paper properties (Franzén, 1983; Gummerus, 1986; Sferrazza et al., 1988; Strunk et al., 1988, 1990; Meyer-Pinson et al., 2004; Bian et al., 2008). The effect of different chemicals such as sodium sulphate, alkaline peroxide and oxalate were investigated on reject fibres. Sulphate treatments improved paper formation as well as surface properties (Franzén, 1983; Gummerus, 1986), while alkaline peroxide modified fibre characteristics and caused an increase in the paper strength and reduced refining energy requirements (Strunk et al., 1988, 1990; Bian et al., 2008). Paper strength can also be increased by using a biomimetic treatment, consisting of sodium oxalate (Meyer-Pinson et al., 2004). The benefit of chemical pre-treatments is that it can promote the quality of pulp, by softening or removing some of the lignin in the cell-walls before refining, (Heitner and Atack, 1983; Bian et al., 2008). Therefore, in most of the chemical treatments better fibrillation, increased fibre flexibility and swelling, followed by a high degree of fibre collapse and conformability are obtained. Oxalate treatment specifically modifies the hemicellulose-pectin complex, making the cell-wall more susceptible towards refining (Meyer-Pinson et al., 2004). Although chemical treatments could improve fibre characteristics, these have generally not been applied to improve the quality of mechanical pulp rejects. Therefore, other ways to pre-treatment of mechanical fibres before refining were investigated.
1.5.4. **Enzymatic treatment**

The natural polymeric components of paper (cellulose, hemicelluloses and lignin), has led to investigations into the potential use of enzymes to improve certain fibre characteristics by modifying these polymers (Pere *et al*., 1995; Bhardwaj *et al*., 1996; Clark *et al*., 1997; Mansfield and Saddler, 1999). Commercial enzymes that include cellulases and hemicellulases act on these polymers to promote specific fibre properties such as strength (Mansfield *et al*., 1996; Mansfield and Saddler, 1999; Kibblewhite and Wong, 1999). These enzyme applications were studied on a laboratory scale (Kibblewhite and Clark, 1996; Mansfield *et al*., 1996; Kibblewhite and Wong, 1999; Lumme *et al*., 1999; Mansfield *et al*., 1999a; 1999b; Mansfield and Saddler, 1999; Wong and Mansfield, 1999; Wong *et al*., 1999; 2000; Mansfield and Dickson, 2001), but until recently only a few processes have reached mill-scale application (Viikari *et al*., 1994).

**Cellulases**

Cellulases are used to modify the main component (cellulose) of the cell-wall and constitute the most widely studied of the fibre-treating enzymes. Cellulose forms approximately 80% of the dry material in all fibres and serves as an essential stress bearing constituent of all plant fibres (Sjöström, 1993). Cellulases have the ability to adsorb onto the cellulose molecule through a cellulosic binding region before attacking the glycosidic bonds (Béguin and Aubert, 1994; Lynd *et al*., 2002). Since cellulases occur in multiple forms with different specific activities, total degradation of the cellulose is possible when multi-component formulations are applied. When mono-component enzymes are used, the cellulose will be modified rather than totally degraded (Bledzki and Gassan, 1999; Lynd *et al*., 2002).

A multi-component cellulase system consists of three classes of enzymes with specific actions including endoglucanase (EG), cellobiohydrolases (CBH) and glucosidase (Béguin and Aubert, 1994). Endoglucanases attack the amorphous region of native cellulose, at multiple internal sites causing a rapid decrease in the length of the polymer chain (Pere *et al*., 1995). By opening the substrate, the surface area is increased for subsequent attack by CBH (Howard *et al*., 2003). Cellobiohydrolases form the major component of cellulase systems accounting for 40 to 71% of the total cellulase protein that hydrolyses highly crystalline cellulose structures (Béguin and Aubert, 1994). The CBH cleave mono and dimeric groups from different sites of the glucose chain. A-type CBH cleave from the reducing ends of the cellulose chain, while those enzymes that cleave from the non-reducing
ends are known as the CBH II (B-type). Glucosidase is a considerably smaller enzyme that cannot bind or degrade crystalline cellulose. This enzyme can only hydrolyse cellobiose to D-glucose.

The potential applications of cellulases to the pulp and paper industry are numerous. As early as 1959, cellulases were applied to improve fibre fibrillation (Bolaski and Gallatin, 1959). Cellulases are also known to promote the drainage ability of pulp by decreasing finer fibre elements (Mansfield and Saddler, 1999). The ability to deink recycled fibres (Tausche, 2005) can also be improved by using these enzymes. Cellulases also enhance the potential for improvement of fibre stability through increased fibrillation. Treatments with different cellulases were evaluated on chemical pulp (Kibblewhite and Clark, 1996; Mansfield et al., 1996; Mansfield and Saddler, 1999) and strength properties such as tensile, tear and burst were increased. However, it was observed that these enzyme treatments reacted differently to unbleached and bleached chemical pulps. Unbleached fibres treated with a multi-component cellulase did not show any significant changes in length and coarseness and fibres remained essentially uncollapsed when compared with bleached fibres (Kibblewhite and Clark, 1996). They found that treatments with EG were more effective, resulting in more collapsible fibres. Although it was possible to increase fibre flexibility through EG treatment, fibre strength was negatively influenced and reflected in lower tear and tensile properties (Kibblewhite and Clark, 1996; Kibblewhite and Wong, 1999; Lumme et al., 1999). The effect of the multi-component cellulase and EG were also evaluated on fully bleached fibres, and the effect of the enzymes was more evident. The fibres were more collapsed in both treatments, but strength loss was still a problem (Mansfield et al., 1996).

Strength properties of both bleached and unbleached chemical pulp were reduced when treated with enzymes (Clark et al., 1997). The highest strength loss was observed with EG treatment that mainly caused weakening of the individual fibres and not of inter-fibre bonds or overall fibre length (Clark et al., 1997). Strength reduction of the fibres due to EG treatment correlated strongly with loss of the degree of polymerisation in cellulose, suggesting that enzymatic degradation was focused on accessible areas of the fibre and created weak points within these areas.
To minimise the loss in fibre strength, only selected fibres fractions were treated with enzymes (Mansfield et al., 1996; Mansfield and Saddler, 1999). These selected fractions contained fibres that were more resistant to chemical treatments and refining processes and mostly consisted of thick-walled long fibres. Fractions that contained long and coarse fibres were collected on a laboratory scale, with the use of a Bauer-McNett classifier, and on a mill scale rejected fibres were collected after screening and cleaning (Mansfield et al., 1996; Mansfield and Saddler, 1999). Enzymatic treatments were applied to these pulp fractions and reduced fibre strength was observed. However, 10% less refining energy was required to reach the target freeness when enzyme treatments were applied compared with untreated samples. Furthermore, the integration of an enzymatic treatment stage with refining could provide a means of incorporating coarse fibres into the manufacture of fine paper (Mansfield et al., 1999b).

The influence of cellulases on long thick-walled fibres was also investigated on mechanical pulp (Pere et al., 1996). When these fibres were pre-treated with EG no significant changes were observed. However the application of CBH showed improvement in tensile strength of handsheets. The difference in the effect of the enzyme may be due to the selectivity of the enzyme action as well as the different fibre properties (Pere et al., 1996).

Fibre properties of thick-walled fibres are difficult to improve during refining, and can influence paper formation negatively (Hallamaa et al., 1999; Kure et al., 1999). Compared to thin-walled fibres, the thick-walled fibres are not as susceptible to refining, unless they were previously treated with cellulase. Strength properties of handsheets made with coarse fibres were also improved with cellulase treatment, although the effect of the enzyme was more evident on less-coarse fibres (Lumme et al., 1999). When coarse and less-coarse fractions were treated with endoglucanase (mono-component of cellulase), the tensile properties improved irrespective of the fibre coarseness.

Increased strength properties of paper can be related to weakening of the cell-wall to improve fibre collapse (Mansfield et al., 1996, 1998). In that study two commercial enzymes (a multi-component cellulase and EG) caused the densities of the handsheets to increase, suggesting that collapsibility had been improved. These results implied that the limited hydrolysis caused by the mono-component enzyme treatment was enough to enhance fibre fibrillation.
**Hemicellulases**

The hemicellulose content of dry wood is approximately 20%. The structure of hemicelluloses is very complex and consists of monomeric compounds that include D-glucose, D-mannose, D-galactose, D-xylose and L-arabinose (Sjöström, 1993). The composition of the heteropolysaccharides differs in hardwood and softwood species and xylan is the main hemicellulose in hardwood fibres comprising 25 to 30% of dry weight (Sjöström, 1993). Xylan has been shown to contribute to fibre-wall swelling and flexibility (Eriksson *et al.*, 1991).

The heterogeneous nature of hemicelluloses makes hydrolysis very complex and the hemicellulytic system contains the following enzymes: (i) endo-1,4-β-D-xylanases (EC 3.2.1.8), (ii) exo-1,2-β-D-xylosidases (EC 3.2.1.37), (iii) endo-1,4-D-mannanases (EC 3.2.1.78), (iv) β-mannosidases, (v) α-D-glucuronidases, (vi) acetyl xylan esterases, (vii) α-L arabino-furanosidase and (viii) α-galactosidase (Viikari *et al.*, 1994). Hemicellulases in the form of xylanases are commercially applied to enhance delignification during bleaching (Kantelinen *et al.*, 1993; Viikari *et al.*, 1994; Wong *et al.*, 1999). The effect of xylanase treatment on improving individual fibre properties has also been investigated (Noé *et al.*, 1986; Pham *et al.*, 1995; Kibblewhite and Wong, 1999; Moss and Pere, 2006). Partial removal of xylan by enzymes improved the potential of fibres to collapse (Kibblewhite and Wong, 1999; Moss and Pere, 2006). Highly collapsible fibres resulted in increased tear strength of handsheets (Kibblewhite and Wong, 1999). Most of these studies with xylanase included chemical pulp and showed that strength properties were retained, contrasting with some of the results obtained with endoglucanase treatments.

The effect of xylanases was more evident on hardwood species because of the high xylan content. Xylanase-treatment reportedly did not cause any significant differences in any of the fibre or handsheet properties (Lumme *et al.*, 1999). The main hemicellulosic component of softwood is mannanase and application of mannanase could potentially have the same benefits on softwood that xylanase had on hardwood.
1.6. CONCLUSIONS

Chemical or mechanically pulped fibres are used to produce paper with specific strength and surface properties, which depend on the collapsibility and conformation of fibres. Paper produced from mechanical pulp contains stiffer and less collapsible fibres, especially due to the presence of latewood (thick-walled) fractions (Skowronsiki, 1990). These less-collapsible fibres cause lower strength and rougher paper surfaces, because of the smaller bonding areas. These thick-walled fibres are unstable when subjected to moisture, and was reflected in fibre movement (Forseth and Helle, 1996). These movements (fibre rising; cell-wall swelling and puffing) caused higher surface roughness and lower paper strength (Page et al., 1985; Hoc, 1989; Reme et al., 1999).

There are two ways of dealing with unstable fibres, the fibres can either be removed through fractionation (Vomhoff and Grundström, 2003), or by improving their stability through cell-wall modification. Refining is the conventional way to modify the cell-wall to achieve improved collapsibility (Paavilainen, 1993). These highly collapsible fibres lead to stronger bonding (Kure et al., 1999) which is believed to improve fibre stability. However, in moist conditions, fibre movement still remains a problem. Alternative treatments that could lead to more stable fibres include chemical (Strunk et al., 1990; Meyer-Pinson et al., 2004) and/or enzymatic treatments (Mansfield and Saddler, 1999; Kibblewhite and Clark, 1999); but these treatments should be combined with refining for the best results.

Chemical treatments are often used to remove some of the lignin or hemicelluloses, making fibres more flexible and collapsible for better paper formation. Enzymatic treatments weaken the cell-wall through breaking glycosidic bonds that result in better collapsibility or fibrillation. When combined with refining, these treatments can have the potential to extend the efficiency of refiners to obtain more stable fibres that will produce stronger and smoother paper.
1.7. **Problem Statement and Objectives**

The stability of fibres in a paper network is clearly important to obtain high quality printed images. When environmental conditions change (e.g. moisture), unstable fibres react and move to cause changes in sheet properties such as surface roughness as well as reduced strength. Improvement of fibre stability under moist conditions was, therefore, investigated in the present study. It was noticed that refining could improve the quality of the pulp by fibrillating the cell-wall and by fractionation to remove some of the long- and thick-walled fibres. The effect of these processes is to increase surface smoothness and sheet strength. Additional treatments with enzymes have the potential to improve the stability of fibres and were, therefore, also a focus of the research presented in this thesis.

It is important to quantify the changes in the fibre shape after treatments and a method to observe and quantify these changes was developed (Chapter 2). It was also important to understand the influence of different treatments on a variety of wood species and fibre types (Chapters 3 to 5). The relevance of these methods was evaluated on commercial pulps and sheets (Chapters 6 to 8). The potential of the enzyme treatment for improvement of stability in different fibre types and the proposed mechanism for such development are presented in conclusion (Chapter 9).
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SAMPLE PREPARATION AND QUANTIFYING OF FIBRE MOVEMENT

ABSTRACT
When mechanical pulp fibres come in contact with water, they can move and cause poor surface properties such as high roughness. Microscopy can be applied to observe these movements and understand the basis for roughness. However, obtaining reliable images of the fibres in the paper formation is essential. In the present study, details of the surface and internal structure of the fibre network was examined. Different methods that included cutting (blade, freezing and laser) and embedding (wax and resin) of handsheet cross-sections were evaluated. Resin embedding was selected as the method that gave the best detailed and reliable fibre images, and was subjected to image analysis that allowed measuring of fibre dimensions. Fibre morphology, the cutting angle and degree of collapse can influence these fibre parameters. Fibres displaying different features were modelled to illustrate the impact of each of these features. Different parameters were evaluated and it was determined that puffing of fibres in cross-sections can be quantified with a ratio of the lumen area to fibre area. Resin-embedded samples imaged with a conventional scanning electron microscope allowed puffing to be quantified by using image analysis.
2.1. **INTRODUCTION**

When mechanical paper comes into contact with water; for example during coating and printing, the structure of the fibres in the paper network can change. These changes are caused by fibre movements leading to a rough paper surface (Mao, 2001), which lowers the quality of printed images (Ginman and Visti, 1973; Oittinen and Saarelma, 1998). Movement such as fibre lifting was observed by Hoc (1989) when fibres lifted from the surface of paper. Lifting was more evident in paper that contained large proportions of long fibres and especially after supercalendering. Skowronski and Lepoutre (1985) observed cell-wall swelling when fibres were exposed to coating suspensions, suggesting that the swelling movement was irreversible because of debonding and stress relaxation occurring in the surface structure. A fibre movement called fibre puffing and defined here as the expansion or “decollapsing” of the lumen area, was also observed. While all the fibre movements (fibre rising, cell-wall swelling and puffing) have been observed and described in mechanical pulp, quantifying these movements still remains a challenge.

Different methods were published to observe changes caused by fibre rising and cell-wall swelling under high-moisture conditions (Hoc, 1989; Jang *et al.*, 1991; 1992; 1995; 1996; Forseth and Helle, 1997; Jenkins and Donald, 1997; Jang and Seth, 1998; Williams and Drummond, 2000; Reme *et al.*, 2002). However, a standard method to quantify puffing is not available yet. Most of these published methods include microscopy that makes it possible to visualise changes that occur in the structure of the fibre in a paper network in the presence of water. The fibre structure in paper can be observed using non-invasive as well as invasive microscopy methods. Confocal laser scanning microscopy (CLSM) provides a non-invasive method of visualising a paper network in two or three dimensions (Jang and Seth, 1998; Singh *et al.*, 2008). With this method, fibre morphology can be examined by means of optical sectioning of the paper network. Alternatively, fibres in the paper network can be exposed mechanically by sectioning.

Irrespective of the microscopic techniques used, it is important to follow sample preparation protocols that will not distort fibres and allow free response to environmental changes. Therefore, in order to obtain sections of fibres by means other than CLSM, the fibre network needs to be supported to withstand the mechanical cutting process. Supporting
media such as wax or resin can be used, and previous research has shown that fibre and paper structure can be preserved well and artefacts minimised after embedding (Page, 1965; Nanko and Ohsawa, 1990; Szikla and Paulapuro, 1989; Forseth and Helle, 1997). In contrast, other studies reported that areas within these thin sections fail to fully represent significant structural features, and that reliable structure assessment is made difficult by the frequent artefacts that are created during sectioning (e.g. Reid and Beesley, 1991). These artefacts include scratches, compressed fibres and distorted paper structure which can lead to inaccurate measurements. Gibbon et al. (1989) explored an alternative method to thin sectioning by grinding and polishing methods to evaluate the resin-embedded samples. However, this sample preparation method was less suitable for detailed examination in the scanning electron microscopes (SEM) due to the lack of contrast between the fibres and the surrounding embedding material after polishing. This lack of detail was overcome by Williams and Drummond (2000) who, in addition to grinding and polishing to produce the section plane, also removed a thin layer of the supporting resin by chemical etching to reveal the internal structure of paper. The surface of an embedded sample could, therefore, be examined for puffing by etching the surface.

Conventional scanning electron microscopy (cSEM) can be used to view dry samples (Reimer, 1985) and puffing could, therefore, only be imaged before and after rewetting, because samples have to be dried before viewing. In contrast, environmental scanning electron microscopy (ESEM) makes it possible to control the environmental conditions in the specimen chamber, and thus to observe fibre structural changes in real time as the paper is gradually wetted (Danilatos, 1993; Jenkins and Donald, 1997).

These changes observed by cSEM and ESEM can then be quantified by measuring fibre dimensions, therefore, contributing towards increased understanding of the nature of puffing. Changes in fibre structure, due to movement (such as puffing) can be converted into reliable measurements using image-analysis programmes. These programmes can be used to record fibre dimensions such as cell-wall thickness (CWT), fibre area (FA), lumen area (LA) and the outer circumference of the lumen and fibre (Reme et al., 1998; 1999). These parameters can be statistically analysed to determine the effects of treatments on fibre stability after rewetting. Forseth and Helle (1997) successfully applied image analysis to cSEM images to quantify the changes in the LA during rewetting of mechanical pulped fibres. An image analysis programme was also used by Reme et al. (2002), to determine the
variation in CWT in pulp fibres, using cSEM micrographs. Jang et al. (1996) used CLSM combined with image analysis to examine fibre collapse and changes in CWT in mechanical pulp at different refining levels. In further research the state of the lumen was defined by a collapse index (Jang and Seth, 1998).

The present study was motivated by the difficulties previously experienced in obtaining compression-free sections of handsheets, as well as in observing and quantifying puffing. Therefore, the objectives of the present study were to a) establish a reliable method to obtain artefact-free and detailed cross-sections of handsheets of chemi-thermo-mechanical pulp (CTMP); and b) to find a reliable method to visualise - and wherever possible quantify - fibre movement, following rewetting. Fibre movement was observed with cSEM and ESEM on both sheet surfaces and in cross-sections, and in keeping with previous work (Hoc, 1989; Jang et al., 1991; 1992; 1995; 1996; Forseth and Helle, 1997; Jenkins and Donald, 1997; Jang and Seth, 1998; Williams and Drummond, 2000; Reme et al., 2002), the present study also used image analysis as a tool to quantify puffing.

2.2. MATERIALS AND METHODS

2.2.1. Pulp

Pulp containing 75% spruce (softwood) and 25% poplar (hardwood) was received from a CTMP mill. The samples were made up to a 3% consistency and disintegrated at 3000 revolutions using a MK IIIC disintegrator (Messmer Instruments Limited, UK) to separate fibres. Handsheets with a basis weight of 60 g m\(^{-2}\) were subsequently made with a Rapid Köthen handsheet-former according to the 5269/2 standard ISO method. Samples from the handsheets were then processed as detailed below.

2.2.2. Sample preparation and examination

**Surface areas of handsheets**

Four random samples were cut from the handsheets and mounted with double-sided tape onto a stub, with the sheet surface area uppermost. The surface of handsheets was imaged with ESEM (Philips XL 30) to examine the movement of the fibres when exposed to
water (15.0 kV, GSE 10.0 and a 350x magnification). The fibres on the surface were initially viewed in the dry state after which the humidity was raised to the point where water was visible on the sample. Images were recorded at various stages during the wetting and subsequent drying process.

**Cross-sections of handsheets**

The following preparation methods were tested to identify the most reliable method to obtain clean cross-sections that did not distort fibre or handsheets structure. In each instance, three to five samples were taken randomly from handsheets and processed as described below.

**Cutting:**

The following cutting methods were investigated and include: (a) **razor-blade**: cut handsheet-samples by using a new razor blade, (b) **freeze drying**: razor-blade cutting after samples of dry handsheets were frozen in an attempt to immobilise fibres in the network and thus prevent distortion during cutting. Samples were put into liquid nitrogen for 30 s and quickly cut with a sharp blade before thawing and (c) **laser cutting** where handsheets were cut with a Coherent G-100 laser (LaserCAMM™) with a laser power of 100 W.

**Microtome Cutting:**

Before paper samples can be cut with a microtome a supporting medium is needed. Two mediums were investigated during the present study: (a) **wax embedding**: samples of handsheets were embedded in histology-grade paraffin wax, as this method is relatively quicker than resin embedding. Samples were placed in plastic moulds, intrafiltrated with molten wax at 50°C and left for 30 min to cool. The embedded wax samples were then cut by hand using a microtome to expose the paper web and (b) **resin embedding**: samples were embedded in Quetol 651 resin, according to the method described by Van der Merwe and Coetzee (1992). Microtome sectioning took place from the middle of each sample to avoid the mechanical distortion at the edges of the sample after trimming with a razor blade.

The sectioned face was etched with a solution of saturated sodium methoxide for approximately 40 min to expose the fibre network (Iwadare *et al.*, 1990).
After etching, the blocks were rinsed thoroughly in ethanol (3 x 30 s) with the aid of an ultrasonic water bath, and allowed to dry. Chemical etching was used to expose fibre detail and increase the contrast of the sample. However, as chemicals used for etching could cause changes in the fibre structure, an experiment was set-up initially to investigate this possibility. In this experiment, fibres from resin-embedded samples were measured before and after etching to determine if any differences in handsheets structure could be detected.

The sample cross-sections obtained using razor-blades, laser ablation or microtomes were mounted with double-sided carbon tape onto stubs, with the cut area uppermost. Samples were viewed with an ESEM and images were recorded under the same conditions as for the surface samples. All the handsheet cross-sections were also photographed using a cSEM (LEO 1450, Carl Zeiss, Germany; and JSM 840, JEOL, Tokyo, Japan). The samples were rendered conductive in the vapour of a 0.5% RuO$_4$ solution (Van der Merwe and Peacock, 1999) before viewing to avoid charging artefacts. The influence of the etching chemicals was also examined with cSEM. Samples embedded in resin and cut with a microtome were photographed in backscatter-electron imaging mode (BEI) before etching. After etching, samples were viewed in normal secondary-electron imaging mode (SEI). Before and after images were compared to establish if movement due to etching had occurred.

### 2.2.3. Quantifying fibre dimensions

The “ImageTool” software (www.http://ddsdx.uthscs.edu/, University of Texas, San Antonio) was used to measure the area and circumference of fibres and lumina (Fig. 2-1). Only fibres with an intact cell-wall and with a visible lumen (i.e. not fully collapsed) were measured. The diameter of the fibres was also measured, and the CWT was calculated as follows:

\[
CWT = \frac{(\text{fibre diameter} - \text{lumen diameter})}{2} \tag{1}
\]

Many fibres are not cut perpendicularly during sectioning, because of the random orientation of fibres in a handsheet network. These fibres were thus excluded from the measurements as they can lead to over- or underestimated fibre values. The influence of cutting angle and fibre dimensions is explored in the next section.
2.2.4. **Modelling of fibre morphology and orientation**

A graphics package (AutoCAD, 2004, Autodesk Inc., U.S) was used to investigate the extent to which fibre orientation (cutting angle) or fibre morphology influenced parameters that could indicate fibre collapsing or puffing. Arbitrary values (units) were assigned to the fibre outer circumference and fibre area (defined as the whole area within the outer circumference) for this modelling (Fig. 2-1). Parameters were defined to create thin-walled and thick-walled fibres with the same fibre circumference, but with different circumferences for their lumina.

The profiles of the thin-walled and thick-walled fibres were then manipulated to produce collapsed and uncollapsed fibres sectioned at 90° or 45° angles. During this manipulation, the circumference of the fibre and lumen were kept constant. The following parameters from the models were selected: fibre area (FA); lumen area (LA) and cell-wall thickness (CWT) in both the x- and y-directions (Fig. 2-1).

![Figure 2 - 1: Schematic representation of an uncollapsed and collapsed fibre in cross-section to illustrate parameters defined for modelling.](image_url)

2.3. **RESULTS AND DISCUSSION**

2.3.1. **Surface examination using ESEM**

Examination with ESEM proved to be an ideal technique to observe hydration of fibres on a paper surface in dynamic experiments. It was possible to examine the movement of fibres on the paper surface without any sample preparation method (Figs. 2-2 and 2-3).
After rewetting, some of the fibres appear to be twisted, possibly due to irregular cell-wall swelling as the cell-wall was gradually impregnated with water (Fig. 2-2).

In this study, other fibres appeared to remain rigid and lift out of the plane of the web when rewetted (Fig. 2-3). It was also observed by Skowronski and Lepoutre (1985) that fibre ends rise out of the paper surface, due to weak inter-fibre bonds that brake between fibres in the presence of water (Skowronski and Lepoutre, 1985). Fibre lifting was also observed during rewetting in previous studies done by Hoc (1989; 2005) and could cause irreversible changes such as increased paper roughness.

**Figure 2 - 2:** Micrographs obtained with ESEM showed, A: a collapsed fibre in a dry handsheet and B: twisting behaviour of the same fibre in a rewetted handsheet.

**Figure 2 - 3:** Micrographs obtained with ESEM showed, A: a collapsed fibre in a dry handsheet and B: moving behaviour of the same fibre in a rewetted handsheet.
2.3.2. Examination of cross-sections

The cutting of cross-sections in paper with a blade led to mechanical distortion and most of the fibres were compressed, obscuring lumina and making measurement of individual fibre dimensions impossible (Fig. 2-4A). When samples were frozen to make handsheets more rigid and amenable to sectioning, fibres were brittle and appeared to fracture rather than cut evenly (Fig. 2-4B). The image of the freeze cross-section also showed some debris present, which made the cross-section detail of fibres unclear. Laser cutting appeared to “melt” the cell-walls, making these samples unsuitable for measurements (Fig. 2-4C).

Figure 2 - 4: Scanning electron micrographs of different sample preparation techniques including, A: blade hand-cutting, B: frozen and hand-cut, C: laser cut, and D: resin embedding and etch method. (working distance = 12 and 5 KV)

While wax embedding was faster than resin embedding, samples lacked contrast during SEM viewing, which meant that fibre details were difficult to discern. Attempts to etch blocks with xylene to expose fibres before viewing with cSEM were also unsuccessful (not shown). Sectioning of resin embedded samples followed by etching appeared to be the
best method, producing clear cross-sections (Fig. 2-4D). This method was, therefore, adopted for the rest of the study. One disadvantage of this method was that the method was lengthier than the others. It was also important to ensure that the etching solution was rinsed thoroughly before drying to prevent details being obscured by partially dissolved resin (Fig. 2-5).

![Figure 2-5: Scanning electron micrograph of a paper cross-section that was not rinsed properly before drying. (working distance = 11 and 5 KV).](image)

**Effect of etching solution on fibre structure**

A comparison of the dimensions of fibres and handsheets before and after etching was done to establish that the etching solution did not distort the fibre or handsheet structure (Fig. 2-6). Backscattered-imaging showed sufficient differences in contrast between the fibres and the surrounding resin in the unetched sample to make it possible to measure fibre dimensions (Fig. 2-6A). The etched sample could be viewed better in normal secondary-electron imaging mode (SEI) (Fig. 2-6B). Measurements of the fibre dimensions using image analysis before and after etching showed no significant differences ($p \leq 0.05$) between any of the fibre dimensions measured (Table 2-1).

**Table 2-1: Mean fibre dimensions before and after etching. A Student’s t-test showed no significant differences at 95% confidence interval.**

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Before etching</th>
<th>After etching</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibre area ($\mu m^2$)</td>
<td>454.4</td>
<td>457.0</td>
</tr>
<tr>
<td>Fibre circumference ($\mu m$)</td>
<td>98.1</td>
<td>96.4</td>
</tr>
<tr>
<td>Lumen area ($\mu m^2$)</td>
<td>105.4</td>
<td>98.3</td>
</tr>
<tr>
<td>Lumen circumference ($\mu m$)</td>
<td>55.0</td>
<td>52.8</td>
</tr>
<tr>
<td>Cell-wall thickness ($\mu m$)</td>
<td>5.3</td>
<td>5.4</td>
</tr>
</tbody>
</table>
The cross-section of the fibre structure in resin-embedded samples during rewetting was observed using ESEM. It was possible to examine dynamic changes in the LA and CWT (Fig. 2-7). In this study it was observed that fibre cell-walls can absorb water leading to an increase in thickness by up to 23% (Fig. 2-7B) and fibres also puffed during rewetting. However, puffing in these cross-sections during rewetting was not as marked as had been anticipated and occurred in relatively few fibres. A possible explanation for this is that swelling and puffing of fibres may have been restricted by resin remaining within the cell-walls of the fibres and thus limiting the degree of movement observed. Further etching of samples must be tempered against the possibility of interfering with the fibre network as more resin is removed deeper into the handsheet web.
Figure 2 - 7: ESEM micrographs of fibres, A: under dry conditions and B: during the rewetting stage. The fibre shapes that were observed can be described as, a: partially collapsed fibre, b: totally collapsed fibre, c: uncollapsed fibre, d: fibre puffing observed after rewetting and e: fibre swelling after rewetting.
2.3.3. Image-analysis parameters and cutting angle

Image analysis was used to quantify fibre dimensions, but it was not clear initially which parameter was the most suitable to quantify puffing (Table 2-2). The models drawn with AutoCAD clearly showed that the cutting angle and the CWT influenced some of the parameters (Table 2-2). Ideally, fibres should be cut at a 90° angle, but fibres in sheets (especially handsheets) are randomly orientated and, therefore, it is not always possible to achieve this or to determine accurately at what angle fibres were cut. The models produced in this study showed that the CWT of fibres cut at 45° is overestimated in the x-direction, but not in the y-direction. These results were confirmed in models for both thin-walled and thick-walled fibres (Table 2-2).

Table 2 - 2: Relative dimensions using arbitrary units of computer-generated model fibres for collapsed and uncollapsed thin-walled and thick-walled fibres cut at a 90° or a 45°.

<table>
<thead>
<tr>
<th>Appearance of fibre in cross-section</th>
<th>Thin-walled fibres</th>
<th>Thick-walled fibres</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uncollapsed 90°</td>
<td>Uncollapsed 45°</td>
</tr>
<tr>
<td>x-CWT (units)</td>
<td>12.50</td>
<td>12.68</td>
</tr>
<tr>
<td>y-CWT (units)</td>
<td>12.50</td>
<td>12.50</td>
</tr>
<tr>
<td>FA (units²)</td>
<td>7854</td>
<td>11271</td>
</tr>
<tr>
<td>LA (units²)</td>
<td>4418</td>
<td>6370</td>
</tr>
<tr>
<td>LA:FA ratio</td>
<td>0.560</td>
<td>0.565</td>
</tr>
</tbody>
</table>

* x-CWT: cell-wall thickness measured in x-direction, y-CWT: cell-wall thickness measured in y-direction, FA: fibre area, LA: lumen area, LA:FA: lumen area to fibre area ratio, units: arbitrary units defined in the AutoCAD software.

When modelling was applied to fibres cut at different angles, it was possible to obtain the same FA (7854 units²) for both thin- and thick-walled uncollapsed fibres cut at 90° (Table 2-2). At a 45° cutting angle, the FA of both these uncollapsed fibre types increased to 11271 units² and when the fibres cut at 90° were collapsed, the FA decreased for the thin-walled (4779 units²) as well as for the thick-walled fibres (7186 units²). When the collapsed thick-walled fibre was cut at a 45° angle, the FA increased to 10748 units² which is similar to the 11272 units² of the uncollapsed fibres. The FA increased when the same fibre was cut at an angle. The impression was, therefore, created that a collapsed fibre was uncollapsed.
A similar increase in the FA and LA was also observed for the thin-walled fibres. To overcome these discrepancies caused by the cutting angle, calculation of the LA:FA ratio proved useful. These ratios showed only small variation due to cutting angle. When a fibre collapses, the area of both the fibre and lumen becomes smaller and this is reflected in a smaller LA:FA ratio. This effect was illustrated with modelling when an uncollapsed thick-walled fibre cut 90° had an LA:FA ratio of 0.141 units but after collapsing a LA:FA of 0.076 units was calculated (Table 2-2).

A drawback of using LA:FA-ratios is that the CWT could potentially obscure differences between collapsed and uncollapsed fibres when pulp mixtures are examined. The models showed a small LA:FA ratio (0.144 units) for an uncollapsed thick-walled fibre compared to 0.280 units for a collapsed thin-walled fibre. According to these results, the thick-walled fibres appeared to be more collapsed than the thin-walled fibre. Separating the data according to CWT should, therefore, be considered to correctly interpret the state of the fibre collapse in heterogeneous pulp mixtures.

2.4. **CONCLUSIONS**

Environmental scanning electron microscopy was invaluable to capture fibre movement during rewetting in real time. Quantifying the movements (lifting and twisting) of fibres and other structural changes occurring in the surface of paper during rewetting was not possible, but quantification of cell-wall swelling as well as puffing in cross-sectional images was not a problem. However, using ESEM to evaluate puffing in cross-sectional images was time consuming, and embedded fibres were restricted in the amount of movement allowed by the resin even after etching. In contrast, even if cSEM allows no real-time observation, detailed images of samples before and after rewetting were deemed equally informative, and were used for the rest of the study. Image analysis was invaluable in quantifying the effect of rewetting on the fibre dimensions in the cross-sectional area.
Different sample preparation techniques were investigated and required representative cross-sectional images of the paper structure. Resin embedded samples that were sectioned and etched, optimally preserved the structure of the network and fibres being examined. The absence of changes in handsheet structure before and after etching (Fig. 2-6) added confidence that this step did not introduce any spurious puffing, swelling or any other form of fibre movement.

The influence of the cutting angle and degree of fibre collapsibility on the various parameters measured was investigated. Computer modelling of fibres indicated that the LA:FA ratio was the most suitable parameter to quantify puffing and the modelling data was supported by analysis of cSEM and ESEM micrographs. Based on this approach, the ratio of LA:FA was found to be a reliable measure to quantify puffing. Despite the fact that cutting angles can influence fibre dimensions, it was shown that the effect of the cutting angle on the LA:FA ratio was very small. Cell-wall thickness of fibres can influence the LA:FA ratio in such a way that this may obscure findings regarding puffed and/or collapsed fibres. This confusion can be overcome by dividing the fibre data into thin-walled and thick-walled fibre classes, an approach which is described in the following chapters.
2.5. References


CHAPTER 3

THE INFLUENCE OF ENZYMATIC TREATMENT ON THE FIBRE STABILITY OF SPRUCE CTMP

This chapter was published as:

ABSTRACT
An increase in moisture during printing can lead to movement of fibres such as puffing (return of a fibre to its uncollapsed state), twisting and lifting of fibres out of the paper web. Spruce fibres are especially subject to these movements contributing to weak bonding and increased surface roughness. The aim of the present study was to treat spruce pulp with enzymes to improve fibre bonding and stability. Spruce chemi-thermo-mechanical pulp was prepared in the laboratory and treated with mannanase, endoglucanase and with a combination of these enzymes. Strength properties were evaluated before and after wetting of handsheets and their transverse sections were examined by scanning electron microscopy. Handsheet properties before and after rewetting as well as measurement of lumen area of fibres proved to be effective in evaluating fibre movement and puffing. The treatment of pulp with mannanase or endoglucanase improved fibre stability; however, endoglucanase treatment resulted in loss of strength. Mannanase appeared to modify the cell-walls, making them more susceptible to beating and resulting in improved fibre development and inter-fibre bonding. Endoglucanase possibly damaged fibrils, and fibre degradation instead of fibre development occurred during beating. Stability of thin-walled fibres improved more than thick-walled fibres after enzyme treatment.
3.1. INTRODUCTION

The strength of paper is derived from both the strength of individual fibres in the network and the strength of the bonding between fibres (Page, 1969). Strength properties, such as tensile and burst indices, are especially dependant upon fibre-to-fibre bonding. Wetting of paper, such as during coating and printing, weakens hydrogen bonds and allows fibres to relax (Skowronski and Lepoutre, 1985; Hoc, 1989). During this process, fibres can return to their round, less collapsed state, undergo twisting movements and lift out of the paper web. The end result is that surface roughness is increased and strength properties are reduced.

Fibres with thick-walls, large diameters, and low degree of collapsibility are reportedly more subject to such movement under high moisture conditions (Forseth, 1997; Reme et al., 1998) and are designated as unstable. Thick fibre cell-walls are also considered to be a dominant morphological factor that reduce the flexibility of wet fibres and weaken the bonding properties of the fibre network (Hattula and Niemi, 1988). Softwood fibres are included in paper for their positive contribution to strength, though they are coarse (16 to 67 mg 100 m\(^{-1}\)) and thick-walled with large diameters (Varhimo and Tuovinen, 1999). Softwood pulp can, therefore, be expected to contain a large number of unstable fibres that could contribute to weak bonding and increased sheet roughness after exposure to moisture.

Many attempts have been made to improve the paper-making quality of spruce pulp and these efforts have included methods, such as high-temperature refining, chemical treatments, and even fractionation (Kappel, 1999; Meyer-Pinson et al., 2004; Norgren et al., 2004). The aim of those studies has essentially been to modify cell-walls or to select fibres with suitable cell-wall properties. The modification of the cell-wall can potentially be achieved by removal of specific components from the cell-wall and thereby improving characteristics such as flexibility, swelling and collapsibility.

Improved fibre-wall properties have been achieved by increased fibrillation during refining (Mohlin and Pettersson, 2002) and also through selective enzymatic modifications with multi-component cellulase and endoglucanase (Mansfield and Saddler, 1999; Mansfield et al., 1999). These enzyme treatments resulted in reduced coarseness as well as
denser and smoother handsheets (Mansfield et al., 1996). However, these fibre modifications were usually achieved at the expense of fibre and sheet strength (Edgar et al., 1998; Mohlin and Pettersson, 2002). Similarly, mannanase has been applied to reduce refining energy and improve tensile properties of mechanical pulp (Taylor et al., 2005). Although enzymes have not been widely applied for fibre improvement on an industrial scale, the enzymatic modification of fibre cell-walls holds promise, especially when combined with mechanical action.

The aim of the present study was, therefore, to treat spruce pulp with enzymes to improve fibre stability in order to retain surface smoothness and sheet strength by maintaining strong inter-fibre bonding in handsheets. Spruce, chemi-thermo-mechanical pulp (CTMP) was prepared in the laboratory and treated with mannanase (MAN), endoglucanase (EG), or a combination of these two enzymes (MAN+EG). The influence of the enzymes was determined by characterising fibre morphology, evaluating strength properties of handsheets and by examining the ultrastructure of fibres in transverse sections before and after rewetting. The return of fibres to an uncollapsed state was regarded as the critical response of unstable fibres to wetting and is referred to as puffing in this paper.

### 3.2. Materials and Methods

#### 3.2.1. Pulp preparation

Simulated CTMP was done by cooking spruce chips (10.8 kg bone dry) for 60 min to 165°C and 60 min at 165°C with a sodium-bisulphite charge of 5.6% and liquor to wood ratio 2.5:1. The resulting pulp, at a chlorine number of 28, was subjected to two-stage refining for separation and fibrillation of the fibres. The first stage refining was performed with a Sprout Bauer refiner by the CSIR (Forestry and Forest Products Research Centre, Durban) and the second stage with a low-consistency pilot refiner by the Sappi Technology Centre (Pretoria).
3.2.2. Enzyme treatment

The pulp (35 g per sample) was made up to a consistency of 3.5% in 1 ℓ of tap water and the pH adjusted to 7.0 with H₂SO₄ before treatment with mannanase NZ 510230, endoglucanase Novozyme 476 (both from Novozymes, Denmark) or a combination of the two. The enzymes are considered to be mono-component formulations of endo 1,4-βD-mannan mannanohydrolase (EC 3.2.1.78) and 1,4-(1,3;1,4)-β-D-glucan 4-glucanohydrolase (EC 3.2.1.4), respectively. The activity of MAN was 10.7 KU ℓ⁻¹, assayed with locust-bean gum (Stålbrand et al., 1993) and that of EG was 38.1 IU ℓ⁻¹, assayed with carboxymethyl cellulose (CMC) (Ghose, 1987). Each enzyme was applied at a relatively high dosage of 28.6 µℓ g⁻¹ pulp to compensate for sub-optimal physical conditions. The combined treatment consisted of 28.6 µℓ g⁻¹ of each enzyme and no enzyme was added to the control. All treatments were incubated at 60°C for 30 min with constant stirring and the enzyme activity was not terminated after treatment. Following incubation, the pulp was disintegrated (1500 revolutions) and beaten for 3000 revolutions using a PFI mill. The samples were disintegrated again and diluted with tap water to approximately 6 ℓ and left over-night to reduce latency of fibres.

3.2.3. Fibre characterisation and pulp properties

Fibre characterisation of each pulp sample was done with a MorFi LB-01 Fibre Quality Analyser (TECHPAP, France) and fibre length (weighted in length), fibre width, coarseness, curl, kinked fibres, fines (% length and % area) were measured according to the user’s manual. Handsheets with a base weight of approximately 60 g m⁻² were prepared from each sample according to the ISO 5269/2 Rapid-Köthen method. The handsheets from each treatment were divided into two groups; the first was kept dry in a conditioning room (23°C, 50% relative humidity), while the second group was rewetted by dipping into water and then placed out to condition. Roughness, burst and tensile indices were determined for all the treatments (in dry and rewetted state) by the International Standard Methods (ISO 8791-2 1990; ISO 2758 2001; ISO 1924-3 2005). The tear strength of the pulps could not be determined due to the small amounts of treated pulp samples available. The response of the pulp to rewetting was calculated by comparing the properties of rewetted handsheets to the dry controls.
3.2.4. SEM examination

Samples (3 x 10 mm$^2$) were cut randomly out of each of three handsheets with a razor blade and embedded in Quetol 651 (Van der Merwe and Coetzee, 1992). Transverse sections were cut with a microtome from the middle of each sample, and the sectioned face was immersed in a saturated solution of sodium methoxide to etch the resin away from the fibres (Iwadare et al., 1990). The etching was done for approximately 40 min to expose a clean transverse section of the handsheet. Samples were then air dried, mounted and rendered conductive in the vapour of a 0.5% RuO$_4$ solution (Van der Merwe and Peacock, 1999). A JSM 840 instrument (JEOL, Japan) at 5 kV and a working distance of 10 mm was used to record ten SEM micrographs of each sample. Image Tools software (University of Texas, San Antonio) was used to measure the surface area of fibre walls in transverse section and the area of the lumina (lumen area or LA) in each image. The fibre area (FA) was calculated by adding the area of the fibre cell-wall and LA and these dimensions were then used to calculate the ratio of lumen area to fibre area (LA:FA) as the measure to quantify fibre collapse as a result of different treatments. The median of the measured cell-wall thicknesses was calculated, with the help of which the data for thin- and thick-walled fibres were separated for numerical analysis. The influence of different enzyme treatments on the movement of fibres in handsheets was also determined by measuring the mean height of each cross-sectional image of the handsheets. At least six measurements were done for each sample.

3.2.5. Experimental design and statistical analysis

A completely randomised experimental design was used to test the influence of the enzyme treatments on fibre characteristics as well as the response of the treated pulp in handsheets to rewetting. Each treatment was replicated three times and the data were subjected to one-way analysis of variance and means were tested for significant differences (Q-value) with Tukey’s multiple-range test at a 95% confidence level.

Due to the small number of measurable fibres in some of the electron micrographs, data were grouped across replications. For comparison of LA:FA ratio and sheet thickness, all rewetted treatments (control, MAN, EG, and the combination of enzymes) were compared with the dry control sample by Student’s t-test at a 95% confidence level.
3.3. RESULT AND DISCUSSION

3.3.1. Pulp characteristics

Neither of the fibre treatments with MAN, EG or the combination of these enzymes caused any significant changes in the morphological characteristics of fibres (Table 3-1). A previous report suggested that improvement of fibre characteristics should be reflected by a decrease in coarseness (Mansfield et al. 1996) and, therefore, a reduction in the cell-wall thickness (CWT). However, in the present study, the enzymes did not significantly change any of the fibre characteristics related to coarseness (Table 3-1).

### Table 3 - 1: Fibre characteristics after beating of untreated (control) pulp and pulp treated with mannanase (MAN) and endoglucanase (EG) or a combination of these enzymes (MAN+EG).

<table>
<thead>
<tr>
<th>Fibre characteristics</th>
<th>Control</th>
<th>MAN</th>
<th>EG</th>
<th>MAN+EG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibre length: weighted in length (µm)</td>
<td>739</td>
<td>760</td>
<td>745</td>
<td>741</td>
</tr>
<tr>
<td>Fibre width (µm)</td>
<td>32.83</td>
<td>32.97</td>
<td>32.80</td>
<td>32.70</td>
</tr>
<tr>
<td>Coarseness (mg g⁻¹)</td>
<td>0.29</td>
<td>0.26</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Curl (%)</td>
<td>9.67</td>
<td>9.53</td>
<td>9.60</td>
<td>9.53</td>
</tr>
<tr>
<td>Kinked fibres (%)</td>
<td>21.53</td>
<td>21.60</td>
<td>21.70</td>
<td>21.53</td>
</tr>
<tr>
<td>Fines (% length)</td>
<td>76.57</td>
<td>75.87</td>
<td>76.57</td>
<td>77.00</td>
</tr>
<tr>
<td>Fines (% area)</td>
<td>16.30</td>
<td>15.73</td>
<td>17.71</td>
<td>16.46</td>
</tr>
</tbody>
</table>

*ab: Values in the row followed by the same letter do not differ significantly where applicable (p ≤ 0.05, Tukey’s multiple-range test).

3.3.2. Influence of enzymes on handsheet properties before rewetting

In comparison to the control, treatment with MAN, EG and the combination of the two enzymes significantly decreased roughness (p ≤ 0.05) (Table 3-2). The in-plane strength properties (tensile and burst indices) were influenced differently by each enzyme treatment. Tensile strength increased significantly after MAN treatment, but the EG and the combination of the two enzymes resulted in a significant decrease in the tensile values. Burst index was not significantly increased by MAN treatment, but treatment with EG and the combined enzymes reduced the burst index significantly in comparison to the control (Table 3-2).

### Table 3 - 2: The influence of different enzyme treatments on properties of dry handsheets.

<table>
<thead>
<tr>
<th>Handsheet properties (dry)</th>
<th>Control</th>
<th>MAN</th>
<th>EG</th>
<th>MAN+EG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roughness (ml min⁻¹)</td>
<td>3334</td>
<td>2592</td>
<td>2504</td>
<td>2342</td>
</tr>
<tr>
<td>Tensile index (mN m⁻² g⁻¹)</td>
<td>50.30</td>
<td>53.70</td>
<td>44.19</td>
<td>37.70</td>
</tr>
<tr>
<td>Burst index (kPa m⁻² g⁻¹)</td>
<td>2.40</td>
<td>2.49</td>
<td>1.31</td>
<td>1.58</td>
</tr>
</tbody>
</table>

*a b c d: Values in each row followed by the same letter do not differ significantly (p ≤ 0.05, Tukey’s multiple-range test).
Fibre modification with enzymes is often achieved at the expense of strength (Edgar et al., 1998), but in the present study, treatment with MAN not only maintained burst strength, it also significantly increased tensile strength in the sheets. The increased strength properties introduced by the MAN treatment, suggest that the enzyme degraded the mannans bound to the cellulose fibrils to some extent. This modification may have made the cell-wall structure more amenable to beating for better fibrillation or conformation. After treatment with EG, collapsibility of fibres increased (as evidenced by reduced roughness). However, the enzyme degraded the cellulose in cell-walls to the extent where fibrillation decreased and inter-fibre bonding weakened as a result. The strength properties were consequently reduced as described by Edgar et al. (1998). Furthermore, the combined action of the two enzymes (MAN+EG) caused even greater strength loss than the action of the EG alone. It is likely that the combined enzymes degraded the cell-walls to the extent where external fibrils were detached from the fibre during beating and could not contribute to bonding characteristics.

3.3.3. Response of handsheet properties to rewetting

When untreated handsheets were exposed to water, roughness increased while strength properties decreased, possibly as a result fibre movement. When MAN- or EG-treated handsheets were rewetted, roughness was significantly less when compared to the rewetted control sample (Fig. 3-1). These results can be interpreted that fibres were more stable or better conformed as a result of better fibre collapse or increased fibrillation. The increased stability caused less movement on rewetting. However, a combination of MAN and EG resulted in increased roughness, similar to that of the rewetted control, possibly indicating that the degradation caused a loss of fibrillation. With the loss of fibrillation, inter-fibre bonding decreased and it, therefore, reflects the important role that the fibrils play in anchoring fibres within the network to control movement.
Figure 3.1: The influence of rewetting on the roughness of untreated pulp and pulp treated with mannanase (MAN), endoglucanase (EG) or a combination of these enzymes (MAN+EG). Bars with the same letter (a, b) indicate that treatments did not differ significantly (p ≤ 0.05, Tukey’s multiple-range test).

The tensile strength of the control decreased significantly (p ≤ 0.05) after rewetting (Fig. 3-2), possibly due to breaking of hydrogen bonds between fibres and decreased contact between fibres. However, the tensile index from MAN-treated pulp after rewetting was significantly higher than the rewetted control. The high tensile strength observed despite rewetting supports the earlier hypothesis that the MAN treatment led to greater external fibrillation and stronger inter-fibre bonding. Rewetting of EG-treated pulp resulted in a significant (p ≤ 0.05) decrease in the tensile index of handsheets and this effect was even more marked in the treatment with MAN+EG (Fig. 3-2).

The trends observed for burst after rewetting (Fig. 3-3) mirrored those observed for tensile strength (Fig. 3-3) and rewetting decreased the burst index of the control significantly (p ≤ 0.05). However, when MAN treated handsheets were rewetted, the burst index remained significantly above that of both dry and rewetted controls. An even greater strength loss was observed in pulp treated with EG and the MAN+EG than in the rewetted control. These observations again reflected improved fibre development as result of MAN treatment and reduced inter-fibre bonding consequence of EG and MAN+EG treatment.
Figure 3 - 2: The influence of rewetting on the tensile index of untreated pulp and pulp treated with mannanase (MAN), endoglucanase (EG) or a combination of these enzymes (MAN+EG). Bars with the same letter (a, b, c, d) indicate that treatments did not differ significantly (p ≤ 0.05, Tukey’s multiple-range test).

Figure 3 - 3: The influence of rewetting on the burst index of untreated pulp and pulp treated with mannanase (MAN), endoglucanase (EG) or a combination of these enzymes (MAN+EG). Bars with the same letter (a, b, c, d, e) indicate that treatments did not differ significantly (p ≤ 0.05, Tukey’s multiple-range test).
3.3.4. **SEM examination**

No differences in the collective response of pulp fibres between enzyme treatments could be measured by SEM and this was possibly due to large variation in the fibre morphology. However, trends became apparent when measured data were separated into thin- and thick-walled groups by means of the calculated median of the CWT (2.65 µm) (Fig. 3-4). Separate analysis of these groups highlighted morphological changes that were not previously evident and the ratio of lumen area to fibre area (LA:FA) appeared to be the best parameter to quantify puffing, especially after rewetting.

The LA:FA ratio of thin-walled fibres was significantly larger in handsheets that were rewetted than in those that were kept dry, indicating that lumen volume had increased when fibres came in contact with water (Fig. 3-4). The high LA:FA ratio, therefore, reflects the return of a fibre to its uncollapsed state (puffing). Pulp treated with MAN, EG or MAN+EG showed no significant change in the LA:FA ratio after rewetting when compared to the dry control. These enzyme treatments, therefore, appeared to modify the wall-structure of fibres sufficiently to ensure that fibres remained in the collapsed state after wetting.

![Figure 3 - 4](image)

*Figure 3 - 4:* The ratio of lumen area (LA) to fibre area (FA) reflects the degree of puffing for thin-walled and thick-walled fibres. Bars with the same letter (a, b) indicate that treatments of thin-walled fibres did not differ significantly. No significant differences were found between treatments of thick-walled fibres ($p \leq 0.05$, Tukey’s multiple-range test).
The changes in LA:FA ratio (or the puffing effect) were observed in cross-sections of handsheets (Fig. 3-5) and measurements of sheet thickness (Fig. 3-6). The untreated handsheets showed a dense cross-section with only a few lumina and inter-fibre spaces visible (Fig. 3-5A). Rewetting increased the thickness of the handsheet, with more lumina visible and larger inter-fibre spaces (Fig. 3-5B), likely reflecting fibre movement as result of disrupted inter-fibre bonding. Mannanase-treated fibres were more stable after rewetting and only a few puffed lumina were visible (Fig. 3-5C), appearing similar to the control. These measurements of individual fibres were confirmed by measurement of the thickness of whole handsheets (Fig. 3-6) and wetting of the control handsheets resulted in an increase in sheet thickness while no change was recorded after treatment with either MAN, EG or MAN+EG.

Results from thick-walled fibres were highly variable, and although small differences between treatments were observed, no significant effects of the enzyme treatments could be detected (Fig. 3-7). It is well-established that thick-walled fibres do not collapse readily, even after beating. However, the present study also demonstrated that these fibres are less responsive to rewetting than previously reported (Forseth, 1997; Reme et al., 1998), especially when compared to thin-walled fibres.
3.3.5. Proposed mechanisms

The enzymes tested here apparently modified the cell-walls and predisposed fibres in different ways to the beating that followed. These differences became apparent in surface and strength properties of handsheets before and after wetting as well as in fibre puffing. However, interpretation is limited by the fact that surface morphology of fibres and sheets was not imaged and samples were too small to allow the testing of fibre or tear strength. Within the constraints of the present dataset, the following scenarios for enzymatic action, beating and rewetting on fibres are proposed (Fig. 3-7):

Modification of fibres possibly manifests itself as a weakening of the fibre wall through degradation of the cell-wall structure (Fig. 3-7B-D). The reduction of strength properties in dry handsheets (Table 3-2) appeared to indicate that this weakening was detrimental in the EG-treated fibres (Fig. 3-7C-D). Beating caused fibrillation of fibres in all treatments, but the slightly increased tensile strength of the MAN-treated pulp (Table 3-2) could reflect a possible increase in fibril length or number (Fig. 3-7F). However, the reduction in strength properties of the EG-treated pulps probably indicates a manifestation of fibrillation loss (Fig. 3-7G-H) when compared to the control. The amount of fines in beaten pulps did not differ significantly between treatments (Table 3-1), possibly indicating that only very small fibrils detached from the EG-treated pulp or that the fines were fully degraded.
The improved conformation of the three enzyme-treated pulps was reflected in reduced surface roughness of handsheets. Improved conformation is possibly due to increased collapsibility of the fibres (Table 3-2), where the enzyme-treated fibres (Fig. 3-7J-L) collapsed to a greater extent than the control fibres (Fig. 3-7I). However, when these handsheets were rewetted, puffing of the untreated fibres (Figs. 3-4 and 3-7M) took place and, in combination with relatively little fibrillation, led to increased roughness and reduced tensile and burst indices (Figs. 3-1 to 3-3). Puffing did not occur in the enzyme-treated pulps (Figs. 3-4 and 3-7N-P), but due to less fibrillation of the EG-treated pulps, inter-fibre bonds were disrupted. The breaking of these bonds is evidenced by reduced burst and tensile strength, but no change in surface roughness (Figs. 3-1 to 3-3) is observed. A higher level of fibrillation in the MAN-treated fibres and the resulting fibre stability retained the handsheet structure in terms of strength (Figs. 3-2 and 3-3) and surface smoothness (Fig. 3-1).

This proposed mechanism does not seem to describe the effect of enzymes on thick-walled fibres, since thicker fibre walls were less responsive to enzyme treatment. Differences in fibrillation levels, collapsibility and stability were, therefore, more difficult to observe. It is possible that the response of fibres to the enzymes could increase with longer incubation, but penetration of the enzyme into the fibre wall will play an important role in improving fibre collapsibility.
Figure 3 - 7: Schematic representation of the proposed mechanism for enzymatic modification of thin-walled fibres when combined with beating and rewetting. (A to D: pulp subjected to enzyme treatments, E to H: pulp after beating, I to L: pulp formed into handsheets and, M to P: pulp after handsheets were rewetted).
3.4. **Conclusions**

Moisture causes disruption of inter-fibre bonding and fibre movement, in the course of which the surface roughness deteriorates (Skowronski and Lepoutre 1985). Enzymes can be applied to improve fibrillation (Mohlin and Petterson, 2002) that can result in denser and smoother handsheets (Mansfield et al., 1996). Enzymatic modification can also improve fibre stability as shown in the present study. Handsheet properties and SEM studies, both demonstrated that enzymes such as EG or MAN improved fibre stability. The maintenance of strength and smoothness after MAN treatments were the result of better fibrillation and collapsibility for higher fibre stability in handsheets. However, the EG treatment was associated with strength loss. Mannanase increased the strength of dry en rewetted handsheets despite inclusion of thin-walled and thick-walled fibres in these sheets. The reduction in puffing was only observed in thin-walled fibres.

Fibre stability depends on two factors: firstly, collapsibility of fibres and their ability to remain collapsed results in a larger area for inter-fibre bonding that persists after rewetting of handsheets. This property was reflected in surface smoothness. Secondly, it is proposed that the degree of fibrillation determined the strength of fibre bonding as it was reflected in strength properties of handsheets. A high degree of fibrillation resulted in increased tensile and burst strengths that were retained after rewetting. The model for enzymatic modification to effect fibrillation, collapse, and stability of fibres supported the empirical data, but a similar model could not be developed for thick-walled fibres. The experiments and model also placed an emphasis on fibrillation and fibre bonding and future work should investigate the impact of the enzymes on fibre strength and tear strength of pulps.
3.5. References


CHARACTERISATION OF EARLYWOOD AND LATEWOOD CTMP FIBRES AND THE EFFECT OF ENZYMATIC TREATMENT ON THEIR STABILITY

ABSTRACT
Softwood pulps are morphologically more variable than those of hardwoods, mostly in relation to thickness of cell-walls due to the presence of earlywood and latewood. In pulp, latewood fibres form weak inter-fibre bonds with bad conformation, while earlywood fibres form stronger bonds due to higher conformability achieved through refining. Sheet formation places stress on fibres and when inter-fibre bonds are broken, stress is relieved and fibres are allowed to return to their original shape. Thin-walled earlywood fibres conform better, but are less stable and move more when inter-fibre bonds are broken. This lack of stability can potentially be improved by treating the pulp with enzymes to improve conformation. The aim of the present study was to compare the stability of earlywood and latewood fibres and to study the impact of mannanase on fibre morphology, handsheet properties and stability. This study on pinewood chemi-thermo mechanical pulp showed that earlywood produced smoother handsheets with more strength, but less stability that resulted in an increase in roughness and puffing after wetting. Due to the bad conformation of the latewood fibres, less fibre movement was observed and the change in surface roughness was less obvious. Enzymatic treatment improved burst and tensile indices of latewood, possibly through improved fibrillation of the thick-walled fibres. Wetting of handsheets showed that fibre stability improved with mannanase, especially when earlywood fibres were treated. Handsheet smoothness and some of the strength properties were consequently retained after rewetting.
4.1. INTRODUCTION

Mechanical softwood pulps are characteristically more variable in terms of fibre morphology than hardwood pulps, with most of this variation occurring in the thickness of cell-walls (Reme et al., 1999; Reme and Helle, 2001). This variation is mainly due to morphological differences between the earlywood (EW) and latewood (LW) fractions (Sjöström, 1993). Earlywood fibres have thin walls and large lumina (Sjöström, 1993), and their main function is to transport nutrients and water. Earlywood fibres absorb more energy during refining leading to better fibrillation, but are also easier to damage resulting in weakening of the cell-wall (Rudie et al., 1994; Huang et al., 2007). After refining, strong inter-fibre bonds form between the large contact areas provided through fibrillation and fibre collapse. The higher conformation of EW fibres results in smoother sheet surfaces with higher tensile index, but lower tearing resistance in comparison to sheets with more LW fibres (Paavilainen, 1992). In contrast, LW fibres are longer that EW fibres and have thicker cell-walls and smaller lumen volumes and serve more as mechanical support in trees (Biermann, 1996; Kure, 1999). However, LW fibres are mechanically more resistant, and fibrillate to a lower extent during refining (Huang et al., 2007). These long, thick-walled fibres present in mechanical pulp collapse less, leading to smaller surface contact areas and thus weaker inter-fibre bonding (Forseth et al., 1997). The poor inter-fibre bonding between the fibres can lead to rougher paper (Mohlin, 1989; Honksalo, 2004).

Calendering can improve surface smoothness possibly by forcing fibres closer together, encouraging greater fibre collapse and increasing inter-fibre contact and bonding. The greater conformation forced into fibres by calendering can, therefore, create higher strain among fibres. When moisture is increased, the bonds that maintain the position of these fibres in the paper web are broken, stress is relieved and fibres can return to their original shape (Forseth et al., 1997). It was observed by Forseth and Helle (1996) that thin-walled fibres conform better during sheet formation, while thick-walled fibres mostly retain their original shape. Therefore, EW fibres revert to its tubular shape when rewetted such as during printing processes. However, in an earlier study, the effect of fibre morphology was investigated and it was shown that thin-walled fibres of spruce were less stable and moved more during rewetting handsheets (Strey et al., 2009). That study also showed that fibre stability could be improved by treating mechanical pulp with enzymes such as
mannanase (MAN) and endoglucanase (EG) before refining. The EG-treated pulp retained surface properties after rewetting, but not strength properties, whereas MAN led to retaining of both surface and strength properties.

Therefore, the aim of the present study was to compare the stability of EW (thin-walled) and LW (thick-walled) fibres and to study the impact of enzyme treatment on these fractions. Mannanase was selected for the treatment as it was the enzyme that gave the most promising results (Strey et al., 2009). The impact of the enzyme was evaluated on fibre characteristics and handsheet properties, before and after rewetting. This study was conducted on pinewood, since pine contains a high ratio of LW to EW when compared to either spruce or fir (Lindström et al., 1977; Varhimo and Tuovinen, 1999). Pine pulp thus produced an ideal furnish to test the potential of MAN to improve pulp stability.

4.2. MATERIALS AND METHODS

4.2.1. Wood sampling and pulping

A Pinus patula log (approximately 12 years old) was sawn into disks of about 2 cm thick. Each disk was then sawn into strips (3 cm wide) and segmented with a chisel to produce EW and LW chips. The EW and LW fractions were each separated into six 50 g samples to be pulped and treated separately. Each sample was pulped in cooking liquor in its own container, but pulping was done simultaneously in one digester. The pulping conditions and fibre separation in a refiner simulated a chemi-thermo-mechanical pulping (CTMP) process. A charge of 6.6% sodium bisulphite with liquid to wood ratio of 2.5 : 1, a ramp-up time of 90 min and a reaction time of 60 min at 165°C were used. After cooking, fibres were separated using a three-stage process on a Sprout-Waldron refiner. On the first pass through the refiner a 1.1 mm gap was used followed by two passes with the gap set at 0.4 mm. Fibre characteristics and strength properties were determined to confirm effective separation of EW and LW.
4.2.2. Enzyme treatment

A sample of 1ℓ of pulp at a 3.5% consistency in tap water was prepared from each replicated fraction, in order to assess the impact of MAN on each of the replications of the EW and LW fractions. The pH was adjusted to 7.0 using H₂SO₄ (0.1M). Mannanase NZ 51023 (MAN) from Novozymes, Denmark was applied at a dosage of 360 U g⁻¹ pulp and water was used as a control. The treatment was incubated at 60°C for 2 h with manual shaking every five to 10 min and was based on the conditions existing in the mill. After treatment, each sample was beaten at 3000 revolutions using a PFI mill. The samples were then disintegrated at 1500 revolutions using a MK IIIC disintegrator (Messmer Instruments Limited, UK), where after it was diluted to approximately 6 ℓ with tap water and left overnight to reduce latency.

4.2.3. Fibre characterisation and handsheet properties

Fibre characteristics for both EW and LW samples after fibre separation and after beating were determined using a MorFi LB-01 Fibre Quality Analyser (TECHPAP, France). These fibre characteristics included cell-wall thickness (CWT), fibre length, fibre width, coarseness, density, curl, kinked fibres, broken ends and the proportion of fines.

Ten handsheets with a basis weight of 60 g m⁻² were prepared for each sample according to the Rapid-Köthen method (ISO 5269/2). Each set of handsheets was divided into two groups. One group was kept dry and conditioned overnight at a relative humidity of 50% and 23°C. The second group was rewetted by briefly dipping handsheets into water before conditioning to a constant weight under the conditions described above. The surface roughness, tensile and burst indices of handsheets were determined using ISO 8791-2 (1990), ISO 1924 (2005) and ISO 2758 (2001), respectively.

4.2.4. SEM examination

Three samples (3 x 10 mm) were randomly cut to be representative of the surface of the handsheets and embedded in Quetol 651 (Van der Merwe and Coetzee, 1992). A microtome was used to obtain transverse sections through the middle of the sample, and the sectioned face immersed in a saturated solution of sodium methoxide for
approximately 40 min to etch the resin away from the fibres and thus expose a clean transverse section of the handsheet (Iwadare et al., 1990; Chapter 2). Samples were briefly rinsed in methanol, air dried, mounted on stubs with double-sided carbon tape with etched surfaces uppermost, and rendered conductive in the vapour of a 0.5% (w/v) RuO₂ solution (Van der Merwe and Peacock, 1999).

A SEM (JSM 840, JEOL, Tokyo, Japan) at 5 kV and a working distance of 10 mm was used to record five electron micrographs per replicated treatment. Transverse dimensions of the fibre and lumen were measured by using ImageTool software (University of Texas, www.http://ddsdx.uthscs.edu/) and calculations of the ratio of lumen area to fibre area (LA:FA) were done as described previously to quantity puffing (Chapter 2).

4.2.5. Experimental design and statistical analysis

A completely randomised experimental design with three factors (wood fraction, enzyme treatment and handsheet wetting) was used to compare fibre morphology, handsheet properties and fibre stability for each treatment. The whole data set was initially subjected to analysis of variance, but because all interactions were significant, the impact of each factor was evaluated separately by doing one-way ANOVA and means tested for significant differences (Q-value) with Tukey’s multiple-range test at a 95% confidence level.

4.3. RESULTS AND DISCUSSION

4.3.1. Pulp characteristics

Earlywood consists of thin-walled fibres, while LW fibres are longer with thicker walls (Sjöström, 1993). In the present study, the significant difference in CWT between fractions confirmed that EW and LW had been successfully separated before pulping (Fig. 4-1, Table 4-1). Fibres in the EW fraction had a CWT of approximately 3 µm, whereas that of the LW fibres was 6 µm (Fig. 4-1). The fibres in the LW fraction were also significantly longer than the EW fibres, however, fibre characteristics such as width, density and curl did not differ significantly between the two fibre fractions (Table 4-1).
Coarseness was higher in LW fractions (Table 4-1) reflecting the longer and thicker fibres as described by Sjöström (1993). More EW fibres were kinked in comparison to LW fibres after refining (Table 4-1), possibly because EW fibres absorbed more energy, resulting in structural changes during refining (Huang et al., 2007). In LW fibres, broken ends increased (Table 4-1) suggesting that fibres broke rather than kinked due to their stiff, unyielding structure as described by Forseth et al. (1997). The amount of fines was significantly higher in the LW fraction and accounted for the reduction in the fibre length that was observed (Table 4-1). Corson and Ekstam (1994) also observed that LW fibres were more often broken down into fines than EW fibres.

**Figure 4 - 1: Distribution of cell-wall thickness in earlywood and latewood fibres in laboratory CTMP.**

**Table 4 - 1: Fibre characteristics of pine earlywood and latewood after beating with a PFI mill as determined with the MorFi fibre analyser.**

<table>
<thead>
<tr>
<th>Fibre characteristics</th>
<th>Earlywood</th>
<th>Latewood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average cell-wall thickness (µm)</td>
<td>4.180ₐ</td>
<td>5.518ₘ</td>
</tr>
<tr>
<td>Fibre length: weighted in length (µm)</td>
<td>1313ₐ</td>
<td>1431ₘ</td>
</tr>
<tr>
<td>Fibre width (µm)</td>
<td>40.13ₐ</td>
<td>38.4ₘ</td>
</tr>
<tr>
<td>Fibre coarseness (mg g⁻¹)</td>
<td>0.65ₐ</td>
<td>0.94ₘ</td>
</tr>
<tr>
<td>Fibre density (million g⁻¹)</td>
<td>1.80ₐ</td>
<td>1.35ₘ</td>
</tr>
<tr>
<td>Curl (%)</td>
<td>9.00ₐ</td>
<td>8.90ₘ</td>
</tr>
<tr>
<td>Kinked fibres (%)</td>
<td>26.2ₐ</td>
<td>24.9ₘ</td>
</tr>
<tr>
<td>Fines (% length)</td>
<td>72.1ₐ</td>
<td>78.8ₘ</td>
</tr>
<tr>
<td>Broken ends (%)</td>
<td>51.6ₐ</td>
<td>56.1ₘ</td>
</tr>
</tbody>
</table>

ₐ b: Values in each row followed by the same letter do not differ significantly (p ≤ 0.05, Tukey’s Multiple-Range test).
4.3.2. **Influence of earlywood and latewood fractions on handsheet properties**

The thick-walled LW fibres yielded handsheets with higher surface roughness when compared to EW fibres (Table 4-2), possibly reflecting the presence of coarse fibres that did not collapse or conform well. Fibres that do not have a high degree of conformation result in smaller contact areas between fibres and weaker inter-fibre bonding. This is supported by the fact that burst and tensile indices were lower for LW handsheets than for EW sheets (Table 4-2).

<table>
<thead>
<tr>
<th>Handsheet properties</th>
<th>Earlywood</th>
<th>Latewood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roughness (ml min⁻¹)</td>
<td>1423ᵃ</td>
<td>3648ᵇ</td>
</tr>
<tr>
<td>Tensile index (mN m² g⁻¹)</td>
<td>18.59ᵃ</td>
<td>16.06ᵇ</td>
</tr>
<tr>
<td>Burst index (kPa m² g⁻¹)</td>
<td>0.675ᵃ</td>
<td>0.421ᵇ</td>
</tr>
</tbody>
</table>

ᵃᵇ: Values in each row followed by the same letter do not differ significantly (p ≤ 0.05, Tukey’s Multiple-Range test).

4.3.3. **Influence of mannanase on fibre characteristics**

Treatment with MAN did not yield significant changes in most of the fibre characteristics of EW, but the coarseness of EW fibres was reduced significantly, possibly reflecting improved flexibility (Table 4-3). On the LW fraction, MAN treatment did not result in any significant changes in the fibre characteristics (Table 4-4). These results confirm previous results where enzymes did not induce any significant changes in the morphological characteristics of spruce fibres (Strey *et al.*, 2009).

<table>
<thead>
<tr>
<th>Fibre characteristics</th>
<th>Control</th>
<th>MAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibre length: weighted in length (µm)</td>
<td>1316ᵃ</td>
<td>1322ᵃ</td>
</tr>
<tr>
<td>Fibre width (µm)</td>
<td>40.13ᵃ</td>
<td>39.90ᵃ</td>
</tr>
<tr>
<td>Fibre density (million g⁻¹)</td>
<td>2.13ᵃ</td>
<td>2.48ᵃ</td>
</tr>
<tr>
<td>Curled fibres (%)</td>
<td>9.00ᵃ</td>
<td>8.83ᵃ</td>
</tr>
<tr>
<td>Kinked fibres (%)</td>
<td>23.23ᵃ</td>
<td>23.17ᵃ</td>
</tr>
<tr>
<td>Fibre coarseness (mg g⁻¹)</td>
<td>0.71ᵃ</td>
<td>0.49ᵇ</td>
</tr>
<tr>
<td>Fines: length (%)</td>
<td>72.13ᵃ</td>
<td>72.63ᵃ</td>
</tr>
</tbody>
</table>

ᵃᵇ: Values in each row followed by the same letter do not differ significantly (p ≤ 0.05), Tukey’s multiple-range test.
Table 4-4: Fibre characteristics of latewood fibres before and after mannanase (MAN) treatment and beating with a PFI mill.

<table>
<thead>
<tr>
<th>Fibre characteristics</th>
<th>Control</th>
<th>MAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibre length: weighted in length (µm)</td>
<td>1414</td>
<td>1445</td>
</tr>
<tr>
<td>Fibre width (µm)</td>
<td>38.43</td>
<td>37.93</td>
</tr>
<tr>
<td>Fibre density (million g⁻¹)</td>
<td>1.34</td>
<td>1.79</td>
</tr>
<tr>
<td>Curled fibres (%)</td>
<td>8.30</td>
<td>8.87</td>
</tr>
<tr>
<td>Kinked fibres (%)</td>
<td>19.97</td>
<td>20.97</td>
</tr>
<tr>
<td>Fibre coarseness (mg g⁻¹)</td>
<td>0.94</td>
<td>0.70</td>
</tr>
<tr>
<td>Fines: length (%)</td>
<td>78.80</td>
<td>77.83</td>
</tr>
</tbody>
</table>

No significant difference at p ≤ 0.05

4.3.4. Influence of mannanase on handsheet properties

In EW pulp, the enzyme treatment resulted in the formation of smoother handsheet surfaces and also increased burst strength. However, no significant changes in the tensile strength were observed (Table 4-5). Mannanase improved properties of EW pulp (Table 4-5), confirming the findings from Strey et al. (2009), where MAN reduced roughness and increased tensile and burst indices. Since both smoothness and burst were improved, it is possible that enzyme treatment led to higher fibre collapsibility and cell-wall fibrillation.

Table 4-5: The influence of mannanase (MAN) treatment on dry handsheet properties from earlywood and latewood.

<table>
<thead>
<tr>
<th>Wood fraction</th>
<th>Handsheet properties (dry)</th>
<th>Control</th>
<th>MAN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Roughness (ml min⁻¹)</td>
<td>1423</td>
<td>1277</td>
</tr>
<tr>
<td></td>
<td>Tensile index (mN m⁻² g⁻¹)</td>
<td>19.52</td>
<td>18.37</td>
</tr>
<tr>
<td></td>
<td>Burst index (kPa m⁻² g⁻¹)</td>
<td>0.675</td>
<td>0.717</td>
</tr>
<tr>
<td>Earlywood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Roughness (ml min⁻¹)</td>
<td>3648</td>
<td>3746</td>
</tr>
<tr>
<td></td>
<td>Tensile index (mN m⁻² g⁻¹)</td>
<td>13.21</td>
<td>13.78</td>
</tr>
<tr>
<td></td>
<td>Burst index (kPa m⁻² g⁻¹)</td>
<td>0.419</td>
<td>0.470</td>
</tr>
<tr>
<td>Latewood</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a b: Values in each row followed by the same letter do not differ significantly (p ≤ 0.05), Tukey’s multiple-range test.

It is difficult to modify fibres with thicker cell-walls (such as LW fibres) through refining (Huang et al., 2007) and, therefore, only a small effect can be obtained on the fibre morphology and handsheet properties (Kure, 1999). However, in the present study MAN treatment followed by refining increased the tensile and burst indices of LW-containing handsheets significantly (Table 4-5), probably due to the better fibrillation of enzyme-modified cell-walls. Beating of these thick-walled LW fibres after enzyme treatment did not have a significant impact on the surface properties of handsheets (Table 4-5), suggesting that the enzymes did not improve collapsibility and conformability to a larger extent.
4.3.5. Stability of earlywood fibres

When handsheets from untreated softwood pulp were rewetted, surface roughness increased and strength properties were reduced, possibly as result of movement of unstable fibres (Skowronska and Lepoutre, 1985; Strey et al., 2009). In the present study, handsheets from untreated EW pulp also showed a significant increase in roughness when rewetted (Table 4-6), supporting the earlier suggestion that fibres reverted to their original shape and this disrupted the paper web. As before with spruce, treatment of pine fibres with MAN controlled this fibre movement and surface roughness could be maintained within 99% of the dry control (Table 4-6).

Table 4 - 6: The influence of treatment with mannanase (MAN) and rewetting on pulp properties of handsheets from earlywood pulp.

<table>
<thead>
<tr>
<th>Handsheet properties</th>
<th>Control (dry)</th>
<th>Control (rewetted)</th>
<th>MAN (rewetted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roughness (ml min⁻¹)</td>
<td>1423⁹</td>
<td>1562⁶</td>
<td>1405⁸</td>
</tr>
<tr>
<td>Tensile index (mN m⁻² g⁻¹)</td>
<td>19.52⁹</td>
<td>16.80⁶</td>
<td>17.03⁸</td>
</tr>
<tr>
<td>Burst index (kPa m² g⁻¹)</td>
<td>0.675⁹</td>
<td>0.607⁶</td>
<td>0.706⁸</td>
</tr>
</tbody>
</table>

a b: Values in each row followed by the same letter do not differ significantly (p ≤ 0.05), Tukey’s multiple-range test.

In previous investigations on spruce pulp (Strey et al., 2009), the tensile as well as the burst indices of untreated pulp decreased after rewetting, but in the present study, tensile and burst indices were not significantly different before and after rewetting (Table 4-6). This lack of puffing due to rewetting may be typical of the thicker-walled pine fibres used in the present study. The different levels of reaction to water may also be explained by the structure and chemical differences between these species (Sjöstrom, 1993). When these EW fibres were treated with MAN, a significant increase in burst index was observed before rewetting (Table 4-5) and this improvement was retained even after rewetting (Table 4-6). Therefore, MAN has also the potential to modify cell-walls of EW fibres, increase collapsibility and strengthen inter-fibre bonds. However, this effect was not evident for tensile strength (Table 4-6).
4.3.6. Stability of latewood fibres

After rewetting handsheets made from LW, no significant changes were observed in surface roughness (Table 4-7). This is not surprising, as it was previously demonstrated that thick-walled fibres (such as those found in LW) did not collapse in the sheet, and thus retained their rigid structure (Strey et al., 2009). The fact that the roughness of handsheets from LW (Table 4-7) fibres in this study was more than double the roughness of that measured in EW handsheets (Table 4-6) confirms the poor collapsibility before and after rewetting.

The tensile index of the untreated handsheets was significantly reduced after rewetting (Table 4-7). Bonding was improved with MAN treatment before rewetting (Table 4-5) but, in contrast to EW, the stability of these bonds was not maintained after rewetting (Table 4-7). The reduction in tensile strength of LW may reflect less fibre fibrillation during refining, causing weaker inter-fibre bonding that was easily broken in the presence of water. The burst index, on the other hand, was not significantly influenced for the control or enzyme-treated handsheets when subjected to rewetting.

<table>
<thead>
<tr>
<th>Table 4 - 7: The influence of mannanase (MAN) treatment rewetting on pulp properties of handsheets, from latewood pulp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Handsheet properties</td>
</tr>
<tr>
<td>Roughness (ml min(^{-1}))</td>
</tr>
<tr>
<td>Tensile index (mN m(^2) g(^{-1}))</td>
</tr>
<tr>
<td>Burst index (kPa m(^2) g(^{-1}))</td>
</tr>
<tr>
<td>a b: Values in each row followed by the same letter do not differ significantly (p ≤ 0.05), Tukey’s multiple-range test.</td>
</tr>
</tbody>
</table>

4.3.7. SEM examination

Calculating the ratio of lumen area to fibre area (LA:FA) appeared to be a suitable parameter to evaluate the effect of rewetting on fibre stability, as demonstrated previously (Strey et al., 2009). The stability of EW and LW was, therefore, quantified by calculating the LA:FA ratio of dry and rewetted handsheets. The LA:FA ratio in untreated EW fibres increased significantly (by 35%) after rewetting, indicating that the lumen volume increased as fibres puffed (Table 4-8). However, in LW handsheets the LA:FA increased by 10%,
but this change was not significant when comparing dry and rewetted controls. Enzymatic treatment allowed fibre shape to be maintained for both EW and LW fibres when compared to the dry control samples. Fibre puffing occurred in untreated EW fibres, and it was possible to reduce this effect with MAN treatment (Table 4-8), leading to a smoother paper with higher strength.

<table>
<thead>
<tr>
<th>Wood fraction</th>
<th>Control (dry)</th>
<th>Control (rewetted)</th>
<th>MAN (rewetted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Earlywood (LA:FA)</td>
<td>0.212&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.326&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.140&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Latewood (LA:FA)</td>
<td>0.141&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.157&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.133&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a b:</sup> Values in each row followed by the same letter do not differ significantly (<i>p</i> ≤ 0.05), Tukey’s multiple-range test.

### 4.4. CONCLUSIONS

Softwood is a heterogeneous material containing EW and LW fibres, with different fibre characteristics that influence the handsheet properties (Vomhoff and Grundström, 2003). In the present study the EW fraction of pine CTMP contained shorter fibres with thinner cell-walls and less coarse structure and produced handsheets with a much smoother surface and higher strength when compared to the LW fibres. The surface smoothness obtained with EW fibres reflected higher collapsibility and better conformation, while improved strength revealed better fibrillation. However, EW fibres were unstable, which was reflected in an increase in roughness and puffing (LA:FA ratio) when subjected to moisture. However, the strength properties were retained possibly due to good conformation.

Instability of LW fibres was seen only in the reduction of tensile strength and possibly reflected more water penetration into the open structure of badly-conformed sheets. Due to the bad conformation of these fibres, less fibre movement was observed and the change in surface roughness was less obvious. In agreement with work reported by Hattula and Niemi (1988), CWT appeared to be the dominant factor in determination of fibre instability.
Enzymatic treatment improved burst and tensile strengths of LW, possibly through improved fibrillation of the thick-walled fibres during beating with a PFI mill. Fibre stability was improved with MAN treatment especially when EW fibres were treated. Handsheet smoothness and some of the strength properties were consequently retained after rewetting. Increased fibre stability after MAN treatment was confirmed through SEM and image analysis of fibres in cross-sections.
4.5. REFERENCES


TECHPAP, MorFi LABO (LB-01) user’s manual (1997).


CHAPTER 5

THE IMPACT OF ENZYME TREATMENT ON THE FIBRE STABILITY OF POPLAR CTMP

ABSTRACT
Blends of softwood and hardwood fibres are generally used in mechanical paper grades to obtain the required smoothness and strength properties. Hardwood fibres are in general thinner and collapse easier, and are included in the mixture to contribute to surface smoothness. Moisture can lead to deterioration of both smoothness and strength, due to the instability of fibres. In the present study, when handsheets from hardwood pulp were exposed to water, surface roughness was not increased. However, strength properties (tensile and burst) were reduced. It was possible to retain these strength properties by treating fibres with mannanase while most of the strength properties deteriorated after endoglucanase treatment. Since wetting did not influence pulp properties to the same extent as observed previously in softwood, it was concluded that hardwood fibres were more stable. This can possibly be explained by the difference in fibre length and coarseness and chemical composition of the cell-wall. The present study confirmed that it is possible to use a mannanase treatment on a mixed furnish containing softwood and hardwood without any detrimental effects.
5.1. INTRODUCTION

Printing performance is becoming increasingly important to all paper grades containing mechanical pulp. These quality aspects include adequate strength and smooth surface properties after coating and printing. However, due to differences in the fibre morphology (e.g. fibre length, width and cell-wall thickness) the pulp quality can vary (Dinwoodie, 1965; Horn, 1974, 1978). Softwood pulps typically contain long fibres (2 to 4 mm), with a width of 0.02 to 0.04 mm and cell-wall thicknesses (CWT) that vary between 2 and 4 µm in earlywood and 4 to 8 µm in latewood. In contrast, hardwood pulps contain shorter fibres that are only 1.1 to 1.2 mm in length, 0.014 to 0.04 mm wide with an average CWT of 3 to 4.4 µm (Sjöström, 1993).

It is generally accepted that paper with desirable strength properties must include long fibres such as those of softwood pulps (Dadswell and Wardrop, 1959; Barefoot et al., 1964; Horn, 1978). However, the increased fibre length, width and CWT associated with softwood are accompanied by a corresponding decrease in fibre collapsibility (Forseth and Helle, 1996; Forseth et al., 1997), which can lead to an increase in surface roughness. To overcome this, hardwoods with typically thinner cell-walls and higher flexibility are used in blends with softwood to obtain the required surface smoothness (Sjöström, 1993). Additionally, the modification of softwood fibres through enzyme treatment can also be used successfully to produce more stable, smoother and stronger paper (Strey et al., 2009; Chapter 4). Enzymes such as endoglucanase (EG) and mannanase (MAN) used on their own or in combination reduced surface roughness, while MAN also contributed to better strength properties (Strey et al., 2009; Chapter 4). However, since most of the mechanical pulps are blends of softwood and hardwood fibres, it was necessary to investigate the effect of these two enzymes on the hardwood fibres, and this forms the subject of this chapter.

Although hardwoods have more flexible fibres with thinner cell-walls, they contain approximately the same amount of cellulose as softwood (Sjöström, 1993). However, the composition and structure of the hemicelluloses in hardwoods differ from that of softwoods. The main component of hardwoods is xylan (15 to 30%), with only 2 to 5% glucomannan, compared to the 25 to 30% glucomannan, which is the main component in softwood.
Accordingly, the enzymes tested on softwood (Strey et al., 2009; Chapter 4) may not have the desired impact on hardwood fibres or may even have negative effects when mixed furnishes are treated. The aim of the present study was, therefore, to determine the effect of EG and MAN on fibre characteristics and pulp properties of hardwood fibres. Conducting this study on a single hardwood species reduces the potential complexity of the response from a mixed furnish. The influence of the enzymes was assessed by monitoring fibre characteristics, handsheet strength and stability of poplar fibres during rewetting.

5.2. MATERIALS AND METHODS

5.2.1. Pulp preparation

Poplar chemi-thermo-mechanical pulp (CTMP) was produced in the laboratory at the Sappi Technology Centre (STC) at Pretoria under milder conditions than those used for the pulping of spruce (Strey et al., 2009). These conditions were chosen to compensate for the thinner cell-walls of hardwood fibres, which make these more reactive towards pulping conditions and, therefore, requiring milder processing. The wood was pulped by cooking chips using a ramp-up time to 165°C of 60 min, followed by 60 min at 165°C and a 1% sodium-bisulphite charge and a liquor to wood ratio of 2.5 : 1. After pulping, two-stage refining was done to separate and fibrillate fibres. The first-stage refining was performed with a Sprout Bauer refiner at the CSIR (Forestry and Forest Products Research Centre, Durban). On the first pass through this refiner a 1.1 mm gap was used followed by two passes with the gap set at 0.4 mm. The second refining stage was performed with a low-consistency pilot refiner at the STC. During secondary refining, milder conditions that involved low intensity plates and a programme for hardwoods were used.

5.2.2. Enzyme treatment

Two enzymes were used: mannanase NZ 51023 (MAN) and endoglucanase Novozyme 476 (EG), both from Novozymes, Denmark. Combinations of these two enzymes were found to be detrimental to the strength properties of handsheets made from spruce fibres possibly due to a reduction in the fibre strength (Strey et al., 2009). Therefore, the enzyme combination of MAN and EG was not used in the present study. Pulp samples were prepared
at a consistency of 3% and treated separately with MAN and EG for 2 h by following the same method as previously (Strey et al., 2009).

5.2.3.  **Freeness and fibre characterisation**

The freeness (Canadian Standard Freeness) of each pulp sample was determined with a DFR 04 analyser (Mütek, Germany), followed by evaluation of the fibre characteristics with a MorFi LB-01 Fibre Quality Analyser (TECHPAP, France).

5.2.4.  **Handsheet properties**

Handsheets with a basis weight of 60 g m\(^{-2}\) were prepared from each sample according to the ISO 5269/2 Rapid-Köthen method. The handsheets were divided into two groups, where the first group was kept dry and the second group was rewetted by dipping into water and placing them out to dry and condition overnight. Roughness, porosity, burst and tensile indices were determined for all the samples using the appropriate ISO methods (Strey et al., 2009). The stability of pulp was determined by comparing the properties of the rewetted handsheets with that of the dry control as described previously (Strey et al., 2009).

5.2.5.  **SEM examination**

Three random samples were cut from each handsheet and embedded in Quetol 651 (Van der Merwe and Coetzee, 1992). A microtome was used to make sections in the middle of the sample and the sectioned face was then immersed in a saturated solution of sodium metoxide for approximately 40 min to etch the resin away from the fibres and expose a clean transverse section of the handsheet (Iwadare et al., 1990). Samples were air dried, mounted with double-sided carbon tape on stubs after which they were rendered conductive in the vapour of a 0.5% RuO\(_4\) solution (Van der Merwe and Peacock, 1999). Scanning electron microscopy (SEM) (JSM 840, JEOL, Tokyo, Japan) was used to examine the samples at 5 kV and a working distance of 10 mm. Ten representative electron micrographs were recorded for each sample and analysed with ImageTool software (University of Texas, San Antonio, www.http://ddsdx.uthscs.edu). Transverse dimensions of the fibre-walls and lumina were measured and the ratio of the lumen area (LA) to fibre area (FA) was calculated as described previously (Strey et al., 2009; Chapter 2). This ratio quantifies the collapsibility of fibres and a change in this ratio reflects unstable fibres that undergo a movement referred to as puffing.
The median of the observed CWT was used to separate the data from thin- and thick-walled fibres for separate statistical analysis of fibre populations that behave differently in paper sheets (Strey et al., 2009).

5.2.6. Experimental design and statistical analysis

A completely randomised experimental design was used to test changes in characteristics of enzyme treated pulp before and after rewetting. Each treatment was replicated three times. The data were subjected to a one-way analysis of variance and means were tested for significant differences (Q-value) with Tukey’s multiple-range test at a 95% confidence level.

5.3. RESULTS AND DISCUSSION

5.3.1. Freeness and fibre characterisation

Treating of poplar CTMP with MAN did not cause a significant difference in fibre length in comparison to the control after beating, but a significant reduction in fibre length was observed after treatment with EG (Table 5-1). This reduction in length could indicate that the cell-walls of poplar fibres are more responsive towards EG-treatment than MAN-treatment, reflecting the presence of amorphous cellulose. Endoglucanase treatment caused weak areas in the cell-wall structure making fibres more fragile and prone to breaking during beating. None of the enzymatic treatments resulted in a change in the fibre width. The coarseness and fibre density were reduced by approximately 50% following MAN treatment, but EG did not change any of these characteristics. The reduction in coarseness and density possibly reflect greater swelling and fibrillation of the MAN-treated fibres, but also some loss of cell-wall material as fines that reflect the increase in the percentage fines (Table 5-1). Both enzymes (MAN and EG) reduced curl, possibly as result of relaxation of the fibre structure. Although the percentage kink did not increase after enzymatic treatment, an increase was observed in the percentage broken ends after MAN treatment, but EG did not have any significant effect (Table 5-1).

<table>
<thead>
<tr>
<th>Fibre characteristics</th>
<th>Control</th>
<th>MAN</th>
<th>EG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibre length weighted in length (µm)</td>
<td>661&lt;sup&gt;a&lt;/sup&gt;</td>
<td>547&lt;sup&gt;b&lt;/sup&gt;</td>
<td>534&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fibre width (µm)</td>
<td>31.94&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>32.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.43&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fibre coarseness (mg g⁻¹)</td>
<td>0.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.315&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.62&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fibre density (g million⁻¹)</td>
<td>0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.27&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Fibre stability of poplar CTMP

5.3.2. Influence of enzymes on handsheet properties before rewetting

Roughness was not changed with either MAN or EG treatments and tensile and burst indices of the handsheets were not affected by MAN treatment (Table 5-2). However, EG reduced the tensile and burst indices significantly, possibly as a result of the degradation of cellulose in the cell-wall which led to loss of fibrillation and lower bonding strength after beating. The reduction in fibre length after treatment with EG (Table 5-1) also likely contributed to the poor strength properties (Table 5-2).

Table 5 - 2: The influence of different enzyme treatments on dry handsheet properties.

<table>
<thead>
<tr>
<th>Handsheet properties (dry)</th>
<th>Control</th>
<th>MAN</th>
<th>EG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roughness (ml min⁻¹)</td>
<td>2798ᵃ</td>
<td>2597ᵇ</td>
<td>2570ᵇ</td>
</tr>
<tr>
<td>Tensile index (mN m² g⁻¹)</td>
<td>37.71ᵃ</td>
<td>32.53ᵇ</td>
<td>25.72ᵇ</td>
</tr>
<tr>
<td>Burst index (kPa m² g⁻¹)</td>
<td>1.706ᵃ</td>
<td>1.679ᵇ</td>
<td>1.549ᵇ</td>
</tr>
</tbody>
</table>

ᵃᵇᶜ: Values in each row followed by the same letter do not differ significantly (p ≤ 0.05, Tukey’s test).

5.3.3. Response of handsheet properties to rewetting

A reduction in surface smoothness and strength properties in handsheets after rewetting can be used as an indication of unstable fibres (Strey et al., 2009; Chapter 4). In contrast to spruce fibres, roughness was not influenced by rewetting either before or after enzyme treatment (Fig. 5-1). The unaltered surface properties may be due to hardwood fibres being less coarse and, therefore, more stable. However, in softwood, roughness increased after rewetting, but enzymatic treatment preserved the surface smoothness after rewetting (Strey et al., 2009; Chapter 4).
A significant decrease in tensile and burst indices was found after rewetting the control handsheets, but strength was retained when MAN-treated handsheets were rewetted (Fig. 5-2 and Fig. 5-3). This retention of tensile strength was not observed after treatment with EG, and lower strength was measured compared to the control after handsheets were rewetted. Similar trends were observed on softwood pulp (Strey et al., 2009), indicating that EG damages the fibres, causing a reduction in strength similar to strength loss described by Mohlin and Petersson (2002) when using a multi-component cellulase.

Figure 5 - 1: The influence of rewetting on the roughness of handsheets from untreated and enzyme treated pulp ($p \leq 0.05$, Tukey’s multiple-range test).

Figure 5 - 2: The Influence of rewetting on the tensile index of handsheets from untreated and enzyme treated pulp ($p \leq 0.05$, Tukey’s multiple-range test).
5.3.4. SEM examination

Previously (Strey et al., 2009) separated data for thin and thick-walled spruce fibres revealed the positive effects of enzymes on puffing. A similar approach was followed when large variations in the data for poplar fibres were observed. The data for thick-walled fibres (cell-walls thicker than a median of 2.04 µm) and the thin-walled (< 2.04 µm) fibres were then analysed separately. Rewetting did not have any significant effect on the LA:FA ratio of fibres with either thin- or thick-walls (Figs. 5-4 and 5-5). When these fractions were subjected to enzyme treatment, a slight decrease in LA:FA was observed, but it was not significant for either of the enzymes. Hardwood fibres, therefore, appeared to be more stable than softwood, possibly due to their lower coarseness (Sjöström, 1993).
Figure 5 - 4: The influence of rewetting, mannanase (MAN) and endoglucanase (EG) on puffing of thin-walled fibres ($p \leq 0.05$, Tukey’s multiple-range test).

Figure 5 - 5: The influence of rewetting, mannanase (MAN) and endoglucanase (EG) on puffing of thick-walled fibres ($p \leq 0.05$, Tukey’s multiple-range test).
5.4. **CONCLUSIONS**

The effect of enzymatic treatment on a mixed furnish (containing softwood and hardwood) has not been described previously. However, a study of single-species pulp can reduce the complexity when the response of fibres to rewetting as well as enzymatic modification is investigated. The response of fibres can be influenced by the differences in fibre characteristics and biochemical composition of the cell-wall between different species (Sjöström, 1993).

Hardwood fibres are often included in a mixed furnish to increase the paper smoothness (Varhimo and Tuovinen, 1999), due to their thin cell-walls and high degree of collapsibility. In the present study it was observed that rewetting and enzyme treatment did not influence the surface roughness of handsheets from hardwood. This study confirmed the role of hardwood to improve smoothness. Paper strength, on the other hand, was reduced after rewetting but when treated with mannanase these strength properties were maintained, but not after EG treatment, possibly due to fibre damage by EG (Mohlin and Petersson, 2002).

When MAN is applied to mixed pulps, the effect on hardwood fibres is not expected to be detrimental, and most of the paper properties (smoothness and strength) can be retained after rewetting. However, the presence of EG in a mixed furnish might degrade the strength properties of the hardwood fraction of the pulp.
5.5. REFERENCES


CHAPTER 6

FIBRE STABILITY IN COMMERCIAL PRODUCED PAPER SAMPLES FROM DIFFERENT PRODUCTION PROCESSES

ABSTRACT
Stability of fibres becomes more important in mechanically-produced paper when exposed to changing environmental conditions such as moisture associated with production processes such as coating, supercalendering and printing. Mechanically-produced paper that is subjected to this calendering pressure, experience more movement during moisture as a result of pressed fibres that recover to their original shape more than uncalendered paper. Previous research investigated the impact of wood type, fibre morphology and enzyme treatments have on fibre stability during exposure to moisture. The investigation was based on fibre characterisation, handsheet properties and cross-sectional behaviour of mechanically-produced fibres in handsheets. The findings from previous studies reflected on laboratory produced calendered and rewetted handsheets and did not necessary reflect the behaviour of fibres in commercially produced paper sheets. The aim of the present study was to determine the stability of commercially produced paper from chemi-thermo-mechanical pulp across different production processes and also to compare the results obtained from laboratory-produced pulp and handsheets with commercial pulp and sheets. Surface and strength properties of commercial paper samples (base-sheet, coated, and supercalendered-paper) were tested. A significant increase in roughness was observed in all three samples after exposure to moisture and strength loss was most evident in supercalendered-paper after rewetting. These three samples (base-sheet, coated and supercalendered-paper) including a commercially printed paper sample was examined with a scanning electron microscope combined with image analysis. The puffing effect observed in the rewetted supercalendered paper was similar to that of a printed sample. This study on commercially produced paper across different production processes showed similar results to those obtained previously with handsheets after calendering and rewetting. Therefore, the reliability of test methods (calendering and rewetting) applied to handsheets on laboratory scale could be validated as a measure to quantify puffing and fibre stability.
6.1. INTRODUCTION

A key factor for high-quality printing is the stability of the fibres when exposed to changing environmental conditions such as moisture and heating. These changes in moisture occur during processes such as coating and printing. The moisture originates from water-based inks, ink-water emulsions or the aqueous fountain solution in offset printing (Biermann, 1996), whereas coating suspensions are also made up with water.

The response of fibres towards these changes in moisture, and the characteristics of the paper produced, are influenced by the fibre morphology and processing conditions. Previous research has shown that the fibre behaviour is influenced by the morphology of different wood species used in the furnish (Horn, 1974; Laine, 1997). Paper properties can also be controlled through processes such as blending, fractionation and refining of fibres, and coating and calendering of sheets (Biermann, 1996; Kure et al., 1999; Holmstad et al., 2004; El-Sharkawy et al., 2008). Coating is applied to improve the surface properties of the base-sheet in terms of smoothness, brightness, gloss and overall uniformity (Biermann, 1996). As a result, coatings also improve aspects important to printing process such as surface smoothness (Skowronski and Lepoutre, 1985). However, in some papers containing mechanical pulp, the interaction with water may lead to movement of unstable fibres as result of reduced inter-fibre bonding or fibre puffing (Aspler and Bélard, 1994). Calendering or supercalendering is commonly applied after the paper surface is coated to remedy flaws such as high surface roughness. However, Skowronski (1990) reported that application of calendering pressure may introduce strain in the paper that can cause an even greater increase in roughness following exposure to moisture as unstable fibres tend to recover their original shape.

In order to understand the response of rewetted fibres, the behaviour of single-specie pulps (Strey et al., 2009; Chapter 5), as well as that of different fibre types (earlywood and latewood; Chapter 4) were investigated under controlled laboratory conditions. Fibre puffing was common in softwood pulp, contributing to surface roughness following rewetting (Strey et al., 2009).
In contrast, hardwood fibres such as poplar puffed relatively less after rewetting, maintaining both surface smoothness and strength properties (Chapter 5). The differences observed between the response of pulp from spruce and poplar were ascribed to corresponding differences in coarseness, collapsibility, as well as upon the amount of fibrillation, all of which contribute to fibre stability.

While the trends observed have increased present understanding of fibre stability, these results have been obtained using selected raw material and processes under laboratory conditions, and thus still need to be validated under industrial conditions where a heterogeneous furnish is the norm. The aim of the present study was, therefore, to investigate the stability of fibres from a mixed furnish contained in commercial paper.

6.2. MATERIALS AND METHODS

6.2.1. Sampling

Three commercially produced paper samples containing a mixture of poplar (30%) and spruce (70%) were received from a bleached-chemi-thermo mechanical pulp (BCTMP) and paper mill. Samples were collected during a single sampling session from a mill that produces a base-sheet that subsequently undergoes on-machine calendering, coating and off-machine supercalendering, depending in the end product required. The base-sheet sample (40 g m\(^{-2}\)) was collected after on-machine calendering. The second sample (80 g m\(^{-2}\)) was collected after coating, and a third was a supercalendered sample. These three samples were tested by comparing surface smoothness, tensile and burst strength before and after rewetting, as well as fibre puffing after rewetting. A fourth sample of the same product was a coated-supercalendered paper which had been printed on both sides by a commercial printer. This sample allowed the extent of puffing occurring under commercial printing conditions to be determined.

6.2.2. Properties of commercial paper samples

Surface roughness, tensile and burst strengths of the three commercial paper samples (base-sheet, coated-sheet and supercalendered-sheet) were measured using appropriate ISO methods as described in Strey et al. (2009). Corrected strength indices, based on a 40 g m\(^{-2}\)
base-sheet were calculated for each sample. Scanning electron microscopy (SEM) was used to examine the surface structure of these samples. As before, image analysis was used to determine the lumen area to fibre area ratio (LA:FA) of dry sheets and the impact of rewetting on the cross-sectional behaviour of fibres in the base-sheet, coated-paper, supercalendered-paper and a commercially-printed sample.

6.2.3. Experimental design and statistical analysis

Given the limited size of the samples provided, replicated evaluation of the material was not possible. Pairs of treatments were compared using the Student’s t-test at a 95% confidence level. The properties compared were roughness, burst and tensile indices as well as puffing (LA:FA ratio) for dry and rewetted samples.

6.3. RESULTS AND DISCUSSION

6.3.1. Properties of commercial paper samples

Coating, followed by supercalendering increased the smoothness of base-sheets as observed in SEM micrographs (Fig. 6-1). However, the relative increase in smoothness was most evident in supercalendered-paper (Figs. 6-1 and 6-2). This effect was probably due to the smoothening effect of the calendering rollers on the coated surface (Fig. 6-1c) and the corresponding increase in compression of the fibres.

![Figure 6-1: Representative scanning electron micrographs showing the surface structure of the A: base-sheet, B: coated-paper and C: the supercalendered-paper.](image-url)
After rewetting, the surface roughness of all three paper samples increased significantly when compared to the dry samples (Fig. 6-2). The base-sheet showed only a two-fold increase in roughness after rewetting, compared to the five-fold in the coated-paper and a 42-fold increase in the supercalendered-paper. Notably, the roughness of the base-sheet from this mixed furnish increased more after rewetting than the corresponding changes observed in handsheets from spruce in a previous study (Strey et al., 2009). This large increase was possibly due to more effective calendering achieved in the mill than achieved during calendering of handsheets in the laboratory. Commercial calendering possibly led to greater compaction of fibre in the network and supports the conclusions of Skowronski (1990).

Coating contributed to an increase in strength properties when compared to the base-sheet (Figs 6-3 and 6-4), but tensile and burst strengths of the coated and supercalendered-papers were similar (Figs 6-3 and 6-4, respectively), suggesting that the additional calendering pressure had no additional effect on paper strength. Rewetting did not influence the tensile or burst strength of the base-sheet or coated-paper when compared to the dry samples (Figs 6-3 and 6-4), given that the calendering pressure of the base-sheet and coated-paper sample was the same. In contrast, both tensile and burst indices of
supercalendered-paper samples were lowered by rewetting. It is possible that the higher pressure exerted on paper during supercalendering induced greater strain into the fibre network upon drying, and that during rewetting this strain was relieved, leading to movement of unstable fibres, lower inter-fibre bonding and loss of paper strength.

**Figure 6 - 3:** The influence of rewetting on the corrected tensile index of production paper from a commercial paper mill. Columns with the same letter do not differ significantly, $p \leq 0.05$ (pairs of treatment means were compared using a Student’s t-test).

**Figure 6 - 4:** The influence of rewetting on the corrected burst index of production paper from a commercial paper mill. Columns with the same letter do not differ significantly, $p \leq 0.05$ (pairs of treatment means were compared using a Student’s t-test).
6.3.2. Stability of fibres in commercial paper

Fibre instability is reflected in the degree of puffing defined earlier as the increase in the ratio of lumen area (LA) to fibre area (FA) (Strey et al., 2009). The LA:FA ratio of the dry samples showed that the base-sheet contained a significantly larger amount of uncollapsed fibres compared to the more collapse fibres in the coated and supercalendered papers (Table 6-1; Fig. 6-5). Comparing the coated and supercalendered paper, no significant changes were observed in the LA:FA (p=0.44), although it was expected that the pressure applied to the fibres during supercalendering would lead to greater fibre collapse (Skowronski, 1990) and thus a significant reduction in LA:FA ratio.

<table>
<thead>
<tr>
<th>Commercial paper samples</th>
<th>Dry</th>
<th>Rewetted</th>
<th>Significance a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base-sheet (LA:FA)</td>
<td>0.214</td>
<td>0.272</td>
<td>p = 0.38</td>
</tr>
<tr>
<td>Coated-paper (LA:FA)</td>
<td>0.188</td>
<td>0.316</td>
<td>p = 0.01</td>
</tr>
<tr>
<td>Supercalendered-paper (LA:FA)</td>
<td>0.167</td>
<td>0.252</td>
<td>p = 0.04</td>
</tr>
<tr>
<td>Printed-paper (LA:FA)</td>
<td>0.256</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

a: Values of p determined in a t-test comparing dry and rewetted treatments of the same sample.

Figure 6 - 5: Transverse sections of dry and rewetted samples from different treatments (A: dry base-sheet, B: rewetted base-sheet, C: dry coated-paper, D: rewetted coated-paper, E: dry supercalendered-paper, F: rewetted supercalendered-paper and G: printed-paper).
Significant increases in the LA:FA ratio after rewetting were observed only in the coated and supercalendered samples, but not in the base-sheet (Table 6-1; Fig. 6-5). After rewetting an increase in puffing was observed in coated-paper but not in the base-sheet. It was unexpected because, as mentioned earlier, both the base-sheet and coated-paper were subjected to the same pressure. Therefore, it is possible that supercalendering pressure has an effect on fibre puffing (as was initially thought), but an interaction between fibres and the water-based coating suspension could also lead to puffing (Table 6-1). However, there is incomplete information in this study to support or reject this suggestion.

One of the critical questions addressed in this study was whether or not rewetting conducted under laboratory conditions replicated reliably the extent of rewetting that occurs during commercial printing. Results indicate that the LA:FA ratio of supercalendered-paper after printing (Fig. 6-5g) was significantly greater (p=0.04) than that of the dry supercalendered-paper (Fig. 6-5e; Table 6-1), indicating that puffing occurred. Furthermore, the LA:FA ratio of the supercalendered-paper after rewetting (Fig. 6-5f) was similar compared to the printed sample (0.252 and 0.256, respectively), indicating that the extent of puffing did not differ significantly (p=0.94). The rewetting of supercalendered-paper in the laboratory had a similar effect to that of the printing process.

6.4. CONCLUSIONS

Laboratory studies indicate that the response of paper to rewetting is influenced by the type of wood fibres present (Strey et al., 2009; Chapters 2; 4 to 5) and that the relative stability of fibres is influenced by processes such as calendering. However, it was important to test the validity of these laboratory findings against paper from mixed furnish produced under commercial processing conditions.

The paper properties of the commercial samples were measured before rewetting and surface smoothness was increased by supercalendering. Strength (corrected tensile and burst indices) was not influenced in the base-sheet or coated-paper after rewetting. However, after supercalendering, a reduction in both these strength properties was observed when fibres were exposed to water. Therefore, in the present study, when supercalendered-paper was rewetted the presence of unstable fibres was confirmed. Since the majority of the furnish consisted of
spruce (70%) it was assumed that the long fibres were the source of instability. Previous work on spruce showed that rewetting of handsheets caused increased roughness while tensile and burst strength were reduced due to unstable fibres (Strey et al., 2009). In contrast, poplar fibres were more stable; therefore, surface and strength properties could be maintained after rewetting (Chapter 5).

Calendered fibres have a greater tendency to react with moisture and move, because of the strain introduced to the paper (Skowronski and Lepoutre, 1985). The present study showed that unstable fibres are more evident in paper with fibres under strain caused by supercalendering, compared to the base-sheet and coated-paper. The lack of change between the base-sheet and coated-paper after rewetting reflects more stable fibres. However, these samples (base-sheet, coated-sheet and supercalendered-paper) contained the same furnish, therefore, highlighting the important role of the converting processes in controlling fibre puffing.

The dry base-sheet contained the largest amount of partially-collapsed fibres, as shown by the largest LA:FA ratio observed in SEM images. After supercalendering was applied, the smallest LA:FA ratio was measured. Calendering increased fibre collapse in the present study, but during rewetting the instability of these fibres became evident. The puffing effect in the base-sheet was insignificant, but in the coated and supercalendered samples, significant puffing was observed reflecting the presence of unstable fibres.

For validation of laboratory calendering and rewetting processes, a printed sample was evaluated and compared to the dry and rewetted commercial samples. The magnitude of puffing observed in rewetting of supercalendered-paper samples was similar to the amount of puffing observed in the printed-paper sample of the same product. It was concluded that the technique of rewetting successfully imitated the effects of the printing process and the test method (calendering and rewetting) applied to handsheets was validated as a technique to measure puffing and quantify stability. However, care should be taken in interpreting these results, since the sampling was not replicated.
6.5. REFERENCES


Chapter 7

The potential of cell wall-modifying enzymes on the development of BCTMP fibres

Abstract
It has been reported that refining can be combined with enzymatic treatment to improve cell-wall development, paper properties and even save energy. In the present study, the impact of mill refining on bleached-chemi-thermo-mechanical pulp was quantified through fibre characterisation as well as handsheet testing. Pulp was subsequently treated with enzymes (mannanase, endoglucanase and a combination of the two) and fibrillated under simulated mill conditions where after the pulp properties and fibre stability were assessed. Mill refining led to more developed fibres that resulted in stronger bonding between fibres and, therefore, greater strength and smoothness of the handsheets. Enzymatic treatments combined with beating did not show a significant effect on the fibre characteristics such as coarseness, fibre length or fibre width. However, the freeness of pulp was lower than the control, reflecting better fibre fibrillation and a potential for reduction in refining energy. Treatment of bleached-chemi-thermo-mechanical pulp with mannanase improved pulp properties such as burst and roughness. All of the enzymes reduced the impact of wetting on handsheets and this effect was considered to reflect an increase in fibre stability. These results indicate that treatment with mannanase can be used to improve pulp strength. The enzymes were also effective in reducing refining energy.
7.1. INTRODUCTION

Fibre stability is an important factor to maintain smooth paper surfaces after paper is subjected to moisture during coating and printing. One way of increasing fibre stability is through refining, which on a mill scale is achieved using disc refiners, while in the laboratory this is commonly done with a PFI mill. The aim of refining is to produce fibres that are more flexible and collapsible and with lower coarseness to form paper with stronger inter-fibre bonds and higher surface smoothness (Page, 1989; Paavilainen, 1993; Braaten, 1998). Light- and scanning electron microscopy (SEM) studies have shown that promoting fibrillation through refining plays an essential role in the quality of the end-products (Molin and Lennholm, 2000; Laine et al., 2004). Fibrillation is achieved through weakening of cell-walls and partially releasing fibrils which can increase inter-fibre bonding and paper strength. These fibrils sometimes peel off completely to form fines, which can increase surface smoothness (Kibblewhite, 1972). However, the degree of fibrillation achieved through refining can be influenced by the morphology of the fibres.

Previous studies have highlighted the differences that exist in the response of different species and fibre types to beating or refining (Strey et al., 2009; Chapters 4 to 5; Huang et al., 2007). Pulps that contain a high proportion of thick-walled fibres are sometimes not optimally developed through refining. These fibres typically show low collapsibility, which can cause disruption in the paper structure during rewetting. Increasing refining energy to overcome this is not always advisable, as extensive refining energy can lead to undesirable decreases in freeness and fibre shortening (Kure et al., 1999) especially to thin-walled fibres. Increase in refining energy could result in lower tear and burst indices (Chapman and Allen, 1970) and the situation is worsened in pulps containing mixtures of softwood and hardwood fibres. Therefore, alternative means of developing fibre surfaces and stability without undesirable fibre shortening or decrease in freeness are required.

The efficiency of refining has been increased through application of cell wall-modifying enzymes such as cellulases and hemicellulases mixtures (Bhardwaj et al., 1996; Dienes et al., 2004; Dickson et al., 2000). The impact of these enzymes can be observed in a higher portion of fibrillated fibres and the result is increased inter-fibre bonding. Stronger paper has the potential to reduce the likelihood of breaks in the paper machine, thus
increasing production rates (Noé et al., 1986; Pommier et al., 1990). Additionally, highly fibrillated fibres can be obtained using less refining energy to achieve fibre development when pre-treated with enzymes (Pere et al., 1996; Dickson et al., 2000). However, the influence of the enzyme on stability of commercial pulp has not been investigated.

The aim of the present study was firstly to characterise bleached chemi-thermo-mechanical pulp (BCTMP) fibres before and after mill refining. Secondly, to develop beating curves, using a PFI mill to simulate the refining on a laboratory scale, and finally, to evaluate the effect of beating on the stability of BCTMP fibres when combined with an enzyme pre-treatment. Scanning electron microscopy was not required in the present study, since the degree of puffing was reflected in pulp properties (Strey, et al., 2009; Chapters 4 to 6).

7.2. MATERIALS AND METHODS

7.2.1. Impact of refining on pulp

Never-dried, peroxide-BCTMP consisting of approximately 75% spruce and 25% poplar fibres and were collected from a mill before and after refining. Fibre characteristics such as fibre length (weighted in length), fibre width, coarseness, curl, kink and fines of BCTMP were measured before and after refining using a Fibre Quality Analyzer (MorFi LB-01). The freeness (Canadian Standard Freeness) of each sample was determined according to the Tappi method (T227). Individual fibre strength and bonding strength between these fibres was measured with a Pulmac (Z-span TM 300) fibre tester. Laboratory handsheets (60 g m\(^{-2}\)) were prepared and roughness, tensile and burst indices were tested using the ISO methods described previously (Strey et al., 2009).

7.2.2. Impact of PFI beating on pulp

The pulp sample collected from a mill before refining was beaten with a PFI mill at either 250, 500, 750, or 1000 revolutions. A beating curve was drawn to relate the freeness of beaten pulps to the commercially refined pulp. Additionally, roughness, tensile and burst strength of handsheets were determined at each level of beating and compared with handsheet properties of the commercially-refined pulp. The appropriate beating level that reproduce similar fibre properties to refining was used in further experiments.
7.2.3. **Influence of enzymatic pre-treatments**

Mannanase NZ 51023 (MAN) from Novozymes, Denmark and endoglucanase Buzyme2535 (EG) from Buckman Laboratories, USA were used for all treatments. The 30 g bone dry (b.d.) samples of BCTMP (unrefined) were made up to a 3% consistency. Each sample was preheated to 70°C and treated with 1 000 µl g⁻¹ b.d. pulp of MAN or EG for 60 min at pH 5.3. The activity of the MAN and EG was determined as described by Strey *et al.* (2009). Combinations of these two enzymes (MAN+EG) were found to be detrimental to the strength properties of handsheets made of spruce fibres (Strey *et al.*, 2009) and were, therefore, not tested here.

Freeness (Canadian Standard Freeness) values were obtained according to the Tappi method (T227) after beating untreated and enzyme-treated samples at different revolutions (0, 500, 1000, 2000, 3000 and 6000) with a PFI mill. Further evaluation was only done at the level of beating that showed the most similar freeness and handsheet properties to commercially refined pulp. Fibre Quality Analyzer (MorFi LB-01) was used to evaluate the fibre characteristics before and after enzyme treatment. Handsheet properties (roughness, tensile and burst strength) of each treatment were determined on dry handsheets as described previously (Strey *et al.*, 2009). Fibre stability was also determined by dipping the handsheets in water before conditioning overnight at 23°C and 50% relative humidity, followed by evaluating the same handsheet properties as for the dry samples (Strey *et al.*, 2009).

7.2.4. **Experimental design and statistical analysis**

The quantity of BCTMP obtained from the commercial mill after a single collection did not allow for replicated evaluation of the impact of commercial refining on fibres. Enzymatic-treatment of pulp was replicated three times in a completely randomised design to test the influence of the enzyme treatments on fibre characteristics as well as the response of the treated pulp to rewetting. The data were subjected to one-way analysis of variance and means were tested for significant differences (Q-value) with Tukey’s multiple-range test at a 95% confidence level.
7.3. **RESULTS AND DISCUSSION**

7.3.1. **Impact of refining**

After refining at a pulp mill, the freeness of the BCTMP was lowered by 11.5% suggesting improved fibre development (Table 7-1). The commercial refining in the present study led to a reduction in fibre length that is often associated with refining, and which can lead to reduced paper strength (Miles and Karnis, 1991; Kure *et al*., 1999). Fibre width was not influenced by refining, but changes that could contribute to higher paper quality, e.g decrease in coarseness, curl, kinked fibres and a small increase in the fines were observed (Table 7-1). Further improvements included increased cell-wall thickness (CWT). This increase in CWT could possibly indicate internal fibrillation that reflected loosening the cell-wall structure to make fibres more flexible and collapsible for papermaking (Mohlin., 1991). An increase in individual fibre strength and bonding between fibres after refining was also evident (Table 7-1).

Evaluation of handsheets before and after commercial refining showed that surface roughness decreased after refining (Table 7-2), possibly due to more flexible fibres with lower coarseness (Table 7-1). Tensile and burst indices of handsheets also increased after refining, possibly reflecting more fibrillated fibres at lower freeness that led to stronger inter-fibre bonds between the fibres. The negative effect of fibre shortening after refining (Table 7-1) was not reflected in either of these handsheet properties.
Table 7 - 1: Fibre characteristics of pulp collected before and after commercially refining (Each value represents the mean observation of three measurements for the two surveys).

<table>
<thead>
<tr>
<th>Fibre characteristics</th>
<th>Before refining</th>
<th>After refining</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeness (ml)</td>
<td>113</td>
<td>100</td>
<td>11.5</td>
</tr>
<tr>
<td>Fibre length: weighted in length (µm)</td>
<td>776</td>
<td>749</td>
<td>-3.5</td>
</tr>
<tr>
<td>Fibre width (µm)</td>
<td>26</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>Fibre coarseness (mg g⁻¹)</td>
<td>0.283</td>
<td>0.279</td>
<td>-1.4</td>
</tr>
<tr>
<td>Curl (%)</td>
<td>8.70</td>
<td>8.37</td>
<td>-3.8</td>
</tr>
<tr>
<td>Kinked fibres (%)</td>
<td>26.15</td>
<td>23.96</td>
<td>-8.4</td>
</tr>
<tr>
<td>Fines (% length)</td>
<td>76.09</td>
<td>76.91</td>
<td>+1.1</td>
</tr>
<tr>
<td>Fines (% area)</td>
<td>23.09</td>
<td>23.91</td>
<td>+3.4</td>
</tr>
<tr>
<td>Fines (numbers)</td>
<td>246636</td>
<td>248177</td>
<td>+0.6</td>
</tr>
<tr>
<td>Cell-wall thickness (µm)</td>
<td>4.01</td>
<td>4.12</td>
<td>+2.7</td>
</tr>
<tr>
<td>Fibre strength (N cm)</td>
<td>54.4</td>
<td>55.0</td>
<td>+1</td>
</tr>
<tr>
<td>Bonding strength (%)</td>
<td>2.12</td>
<td>2.31</td>
<td>+8</td>
</tr>
</tbody>
</table>

Table 7 - 2: Handsheet properties of BCTMP commercial pulp before and after commercial refining.

<table>
<thead>
<tr>
<th>Handsheet properties</th>
<th>Before refining</th>
<th>After refining</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roughness (ml min⁻¹)</td>
<td>484</td>
<td>446</td>
<td>-8</td>
</tr>
<tr>
<td>Tensile index (mN m² g⁻¹)</td>
<td>34.974</td>
<td>38.298</td>
<td>+10</td>
</tr>
<tr>
<td>Burst index (kPa m² g⁻¹)</td>
<td>1.374</td>
<td>1.817</td>
<td>+32</td>
</tr>
</tbody>
</table>

7.3.2. Beating response

Handsheet properties after mill refining were used as a reference to evaluate the impact of PFI beatings. Due to low drainage of pulp, handsheets could be made only with pulp that was beaten for up to a 1000 revolutions. Beating levels at 250, 500, 750 and 1000 revolutions produced handsheets with similar properties to commercially-refined pulp (not shown), with 500 revolutions being the closest (Table 7-3). Pulp beating of 500 revolutions was, therefore, applied in the present study. At this level, freeness (CSF) was reduced by 12.5%, which closely represents the 11.5% reduction seen on the commercial pulp sample (Table 7-1).

Table 7 - 3: Handsheet properties of BCTMP commercial pulp before and after commercial refining or after beating at 500 revolutions with a PFI mill.

<table>
<thead>
<tr>
<th>Handsheet properties</th>
<th>Before refining</th>
<th>After refining</th>
<th>Change (%)</th>
<th>500 rev beatings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roughness (ml min⁻¹)</td>
<td>484</td>
<td>446</td>
<td>-8</td>
<td>433</td>
</tr>
<tr>
<td>Tensile index (mN m² g⁻¹)</td>
<td>34.974</td>
<td>38.298</td>
<td>+10</td>
<td>45.778</td>
</tr>
<tr>
<td>Burst index (kPa m² g⁻¹)</td>
<td>1.374</td>
<td>1.817</td>
<td>+32</td>
<td>1.866</td>
</tr>
</tbody>
</table>
7.3.3. Influence of enzymatic pre-treatment

*Energy savings*

The impact of the enzyme treatments on fibre fibrillation was reflected in freeness values; therefore, estimated saving of refining energy was calculated by fitting a beating curve to freeness data before (control) and after enzyme treatment (MAN and EG; Fig. 7-1). The following equations 1 to 3 were used to determine the potential of energy that can be saved after enzyme treatments.

\[
\begin{align*}
F &= 1.3 \times 10^{-6} (R^2) - 0.01327 R + 77.814 \quad \text{Equation 1} \\
F &= 6.1 \times 10^{-7} (R^2) - 0.00938 R + 71.569 \quad \text{Equation 2} \\
F &= 1.7 \times 10^{-6} (R^2) - 0.01351 R + 76.053 \quad \text{Equation 3}
\end{align*}
\]

where \( F \) = Canadian Standard Freeness (CSF) and \( R \) = beating revolutions

The beating curves showed that a 11.5% reduction in freeness on the control sample can be achieved through beating for 725 revolutions (Fig. 7-1). However, with MAN a 11.5% reduction could be reached after only 295 revolutions and with EG after 555 revolutions. Alternatively expressed, the freeness of MAN treated pulp could be reduced by 16.4% and by 14.1% in EG treated pulp at the same energy expenditure (725 revolutions). Therefore, enzymes treatments can be used to save energy or to lead to higher cell-wall fibrillation.

*Figure 7 - 1: Beating curves for untreated (control) BCTMP and pulp treated with mannanase (MAN) and endoglucanase (EG).*
**Handsheet properties**

Fibre characteristics did not change significantly between control and enzyme treated samples (Table 7-4). Previous studies also indicated that the enzyme treatment had no effect on the fibre characteristics (Strey *et al.*, 2009; Chapter 4 to 5). However, the effect of the enzymes was reflected in the surface roughness of handsheets properties (Table 7-5). Mannanase-treated handsheets were smoother than the control, as previously described (Strey *et al.*, 2009) and an increase in roughness was caused by EG treatment (Table 7-5). This increase in roughness could be ascribed to weaker inter-fibre bonds that allowed unstable fibres to move in the presence of water and disrupt the sheet surface. The negative effect of the EG was also observed previously (Strey *et al.*, 2009). Although the reduction in surface roughness with MAN or EG was not reflected in the strength properties, the impact of the enzyme may become evident only when water is present.

**Table 7 - 4:** Fibre characteristics of untreated (control) BCTMP and pulp treated with mannanase (MAN) and endoglucanase (EG) after beating at 500 revolutions.

<table>
<thead>
<tr>
<th>Fibre characteristics</th>
<th>Control</th>
<th>MAN</th>
<th>EG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibre length: weighted in length (µm)</td>
<td>805</td>
<td>813</td>
<td>793</td>
</tr>
<tr>
<td>Fibre width (µm)</td>
<td>25.5</td>
<td>25.3</td>
<td>25.7</td>
</tr>
<tr>
<td>Fibre coarseness (mg g⁻¹)</td>
<td>0.343</td>
<td>0.331</td>
<td>0.282</td>
</tr>
<tr>
<td>Curl (%)</td>
<td>6.55</td>
<td>6.64</td>
<td>6.56</td>
</tr>
<tr>
<td>Kinked fibres (%)</td>
<td>19.67</td>
<td>19.75</td>
<td>19.85</td>
</tr>
<tr>
<td>Fines (% area)</td>
<td>24.74</td>
<td>25.52</td>
<td>26.47</td>
</tr>
<tr>
<td>Cell-wall thickness (µm)</td>
<td>3.80</td>
<td>3.81</td>
<td>3.90</td>
</tr>
</tbody>
</table>

No significant difference at p ≤ 0.05

**Table 7 - 5:** The influence of different enzyme treatments on dry handsheet properties. All pulps were beaten at 500 revolutions.

<table>
<thead>
<tr>
<th>Handsheet properties (dry)</th>
<th>Control</th>
<th>MAN</th>
<th>EG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roughness (ml min⁻¹)</td>
<td>564a</td>
<td>498a</td>
<td>601a</td>
</tr>
<tr>
<td>Tensile index (mN m⁻² g⁻¹)</td>
<td>42.307a</td>
<td>40.396a</td>
<td>41.728a</td>
</tr>
<tr>
<td>Burst index (kPa m⁻² g⁻¹)</td>
<td>1.747a</td>
<td>1.844a</td>
<td>1.640a</td>
</tr>
</tbody>
</table>

a b c d: Values in each row followed by the same letter do not differ significantly (p ≤ 0.05, Tukey’s test)
7.3.4. Stability of BCTMP fibres

Rewetting of handsheets can cause paper roughness to increase in hardwood and softwood pulp (Strey et al., 2009; Chapter 4), and this effect was repeated in the mixed pulp used in the present study (Fig. 7-2). While the impact of the enzyme in increasing fibre stability was not as marked as observed in previous studies, it was still possible to restrict roughness development with both MAN and EG relative to that observed in the control sample after rewetting. The best result was obtained with MAN.

![Figure 7 - 2: Roughness of dry and rewetted handsheets from untreated and enzyme-treated pulps (p ≤ 0.05, Tukey's multiple-range test).](image)

In the present study, the tensile index of the untreated sample was reduced after rewetting when compared to the dry control (Fig. 7-3), similar to the decrease in strength properties observed in previous studies on softwood (Strey et al., 2009) and described by Skowronske and Lepoutre (1985). After enzymatic treatments with MAN and EG, the loss in tensile strength was smaller than for the rewetted control, indicating that fibre stability was increased. In this experiment the impact of MAN or EG on the tensile properties of the handsheet showed a similar decrease, but contrary to previous studies, which showed that treatment with EG could not retain strength properties (Strey et al., 2009; Chapter 4). However, the difference in response may be due to the difference between spruce pulp (Strey et al., 2009) and pine pulp used in the present study.
The burst index was also reduced when rewetting occurred (Fig. 7-4). After pulp was treated with MAN it was possible to maintain burst strength after rewetting when compared to the dry control, which suggests improved fibre stability. In contrast, EG treatment resulted in a significant loss of burst strength (Fig. 7-4). Therefore, the greatest benefit was obtained with MAN treatment to improve strength.
7.4. **Conclusions**

Refining (a standard processes in papermaking) can lead to an increase in fibrillation and collapsibility of fibres in a paper network (Karnis, 1994). The impact of a PFI mill on the fibres differs from the impact of a commercial refiner (Karnis, 1994). However, since this study could not be performed on large scale, it was simulated by using a PFI mill to develop fibres. The freeness and handsheet properties obtained with commercial refining were used as a reference to obtain beaten pulp with similar properties, which will add more confidence to the study of the impact of enzymes on the stability of BCTMP pulp.

According to the present study, commercial refining reduced the pulp freeness, but also improved fibre characteristics including lower fibre coarseness, kinked and curled fibres and caused a slight increase in the fines of the pulp. Inter-fibre bonding also increased. The changes in fibre characteristics and bonding strength (Pulmac) were reflected in the handsheet properties. Surface smoothens as well as strength properties (tensile and burst) were increased. Refining thus improved the quality of paper before it came in contact with water. However, even when the properties of mechanical pulp can be improved, the stability of these fibres still has to be investigated. In the presence of water, surface roughness increased and significant loss in strength properties occurred. Refining was, therefore, able to achieve a limited degree of fibre improvement and enzymatic pre-treatment of fibres has potential to increase refining efficiency and thus reduce refining energy.
7.5. REFERENCES


ABSTRACT
Fibre fractionation generally follows pulping to separate fine elements, shives and underdeveloped fibres from the main stream and thus improve pulp quality. These reject fibres are separated on the basis of their length using screens and surface area or density by using cleaners. The rejected fibres can be described as thick-walled, undeveloped fibres with a low degree of collapsibility. The chemi-thermo-mechanical pulp (CTMP) in the present study was produced in a mill using a low reject rate (4%) and these rejects were re-circulated to secondary-refiners. Enzyme, biomimetic and chemical treatments were evaluated for more efficient fibrillation of rejects in the secondary refiner. It was observed that the rejects did not respond to the mannanase or the oxalate treatment, and the lack of response may have been due to the high lignin content of CTMP. However, when CTMP rejects were pre-treated with alkaline peroxide, fibre stability was improved, possibly because fibre swelling was increased, leading to fibres with higher conformability and collapsibility. The pulp pre-treated with alkaline peroxide was then subjected to mannanase and oxalate treatments to effect additional improvements in fibre stability. These improvements may be due to increased response of the cellulose and hemicellulose to the enzyme when some of the lignin barrier was removed. The alkaline peroxide treatment represents a technique to improve the quality of CTMP pulp rejects, especially in systems where a low reject rate is applied.
8.1. INTRODUCTION

Wood pulps, especially mechanically-defibrillated softwood pulps, are heterogeneous due to the presence of earlywood (EW) and latewood (LW) fibres (Sjöström, 1993). Latewood fibres are longer, stiffer and have thicker walls than EW fibres (Kure et al., 1999). This makes them mechanically more resistant to structural modification through refining (Huang et al., 2007), which can result in mechanical pulp with unacceptably-large amounts of coarse, underdeveloped fibres. Latewood fibres can also have negative effects on paper properties such as low inter-fibre bonding and rough surfaces (Reme, 1997), and their instability during rewetting can cause further reductions in strength and surface smoothness (Forseth and Helle, 1997; Chapter 5).

Commercial fractionation processes such as pressure screens and centrifugal cleaning (Duffy, 1999; Wakelin et al., 1999) can improve pulp quality by removing these under-developed, thick-walled fibres after refining (Fig. 8-1). It has been suggested that pressure screens fractionate pulp primarily on the basis of fibre length (Karnis, 1997). On the other hand, it is possible to separate fibres according to their specific surface area (Høydahl and Dahlqvist, 1997) or degree of cell-wall fibrillation (Wood and Karnis, 1979). Due to the potential loss of pulp yield after fractionation of the reject fibres, opportunities exist to modify this pulp with additional treatments, whereafter it can be re-circulated back into the refining line (Kappel, 1999).

There are various means of improving the quality of these rejected fibres. Work presented in Strey et al. (2009) and Chapters 4; 5 and 7 has shown that mannanase treatment can be used to improve pulp quality and reduce energy consumption during refining. These improvements may be due to greater fibrillation achieved during refining, which resulted in stronger paper with smoother surfaces and higher stability. An alternative biomimetic approach is to treat fibres with oxalate as described by Meyer-Pinson et al. (2004). In that study the treatment of thermo-mechanical pulp (TMP) rejects with oxalate resulted in modification of the hemicellulose-pectin complex to increase fibrillation of the S2-layer as well as internal fibrillation and delamination. A third approach uses a chemical pre-treatment consisting of alkaline peroxide (H₂O₂) to treat rejects (Strunk et al., 1988; 1989; 1990). It was found that H₂O₂ treatment can lead to an increase in strength properties (tensile and
burst) as well as a reduction in the refining energy required. The improved pulp quality was most likely a result of increased fibre flexibility and conformability. However, in some cases pulp yield decreased. These results were also confirmed when TMP rejects were pre-treated with alkaline H₂O₂ (Bian et al., 2007; 2008).

![Diagram](image)

**Figure 8 - 1:** Diagrammatic presentation on the impact of screening and cleaning on pulp fibres after refining (adapted from Theunissen, 1998). The screening system separates fibres on the basis of length and the cleaning process on the basis of fibrillation.

The objectives of the present study were, therefore, (a) to determine the efficiency of fibre fractionation in a chemi-thermo-mechanical pulp (CTMP) mill by characterising the properties of fibres in the reject streams and (b) to evaluate the influence of different fibre modifying processes (enzymatic, biomimetic and chemical pre-treatment) upon the characteristics of fibres and the properties of handsheets made from the rejected pulp. The impact of the H₂O₂ pre-treatment on the enzyme and oxalate treatments was evaluated, since the lignin modification caused by H₂O₂ could facilitate greater efficiency of the secondary treatments.

### 8.2. MATERIALS AND METHODS

#### 8.2.1. Fibre audit of screens and cleaners

Samples of CTMP that contained a blend of softwood (70%) and hardwood fibres (30%) were collected from a screening and cleaning system in a pulp mill as shown in
Fig. 8-2. The screening operation consisted of a two-stage process of pressurised basket screens (4-% reject rate), while the cleaning operation consisted of four stages of hydro-cyclones (3 to 4-% reject rate). Each sample was analysed using a MorFi LB-01 Fibre Quality Analyser (TECHPAP, France) to determine its fibre characteristics, whereafter handsheets (6.5 cm diameter) were made with a Pulmac (Z-Span 3000, USA) handsheet maker. Cross-sections of these handsheets were obtained, embedded and analysed using scanning electron microscopy (SEM) as described previously (Chapter 2).

![Diagrammatic representation of the fibre fractionation process at the CTMP mill (1 to 6 indicate sampling points).](image)

The efficiency of the fibre fractionation processes (screens and cleaners) was evaluated using fibre characteristics as well as the collapsibility (lumen area to fibre area ratio) of the separated fibres as described in Chapter 2. The screening and cleaning process were each evaluated by comparing the rejected and accepted fibres during each stage to the inlet stream.

8.2.2. Reject treatments

A CTMP sample was collected from the reject tank (Fig. 8-2), for use in three treatments that included, mannanase (MAN), oxalate and $\text{H}_2\text{O}_2$. After each of the treatments described below, the pulp samples were disintegrated at 1500 revolutions and beaten for 3000 revolutions using a PFI mill as done previously (Strey et al., 2009). The beaten samples were then disintegrated again and diluted to approximately 5 ℓ with tap water and left soaking overnight to reduce latency of the pulp. Each treatment was replicated three times.
Enzyme treatment

Mannanase NZ51023 (MAN) was obtained from Novozymes (Denmark) and used as the first treatment. This treatment was done on 1 ℓ of reject pulp at a 3.5% consistency and a pH of 7.3. The replicates were incubated for 60 min each with shaking every five to 10 min. No enzyme was added to the control treatment. After treatment, the samples were thickened to a consistency of 10% and washed twice with 1 ℓ of water.

Biomimetic treatment

In the second treatment, a sodium oxalate buffer with a final concentration of 200 mM was used to make up a 1 ℓ reject sample with a 3.5% consistency as described by Meyer-Pinson et al. (2004). The pH of the pulp was only adjusted to pH 3.5, with the sodium oxalate buffer, because further adjustment to reach the target of pH 2.5 required excessive amounts of buffer. The treated pulp was incubated at 25°C for 6 h. After incubation, the pulp was recovered by vacuum filtration and two washing steps with 1 ℓ of hot water (50°C).

Chemical pre-treatment

The third treatment evaluated the impact of H₂O₂ as a pre-treatment to MAN and oxalate. Chemi-thermo-mechanical pulp rejects were made to a 3.5% consistency in 5 ℓ of hot water (50°C) which contained Na₂SiO₂ (2% w/w), NaOH (1.5%) and H₂O₂ (0.75%). In the first stage of the H₂O₂ treatment, the pulp was incubated at 70°C for 1 h and then centrifuged (Eppendorf 5810 R, Germany) for 4 min at 313 x g. A washing step with approximate 5 ℓ of tap water followed by centrifugation (313 x g) was repeated three times. The second stage of the H₂O₂ treatment consisted of pulp suspended in 5 ℓ of hot (± 50°C) water that contained Na₂SiO₂ (2%), NaOH (2.5%) and H₂O₂ (1.25%). The pulp was incubated at 70°C for 1 h, followed by centrifugation and three washing steps as before. The pulp was then mechanically ‘fluffed’ and stored at 4°C until treatment with MAN and oxalate followed as described above.

Pulp evaluation after treatments

The freeness (Canadian Standard Freeness) of each treated sample was determined after beating and latency reduction. The fibre characteristics were then determined using a MorFi LB-01 Fibre Quality Analyser (TECHPAP, France). Six handsheets (60 g m⁻²) were prepared from each sample according to ISO 5269/2 Rapid-Köthen method. All the handsheets were calendered and divided into two groups. One group was immediately
conditioned for 24 h (25ºC and 50% relative humidity), the other group was rewetted, and placed out to condition overnight at the same conditions as before (Strey et al., 2009). Roughness, tensile and burst indices were measured as before on all handsheets using the appropriate ISO methods.

8.2.3. Experimental design and statistical analysis

The collapsibility of fibres in screened and cleaned samples was compared to their corresponding inlet samples using a Student’s t-test at 95% confidence level. A completely randomised experimental design was used to compare the effect of different treatments (MAN, oxalate, peroxide and rewetting) on fibre characteristics and handsheet properties. The data were subjected to one-way analysis of variance and means were tested for significant differences (Q-value) with Tukey’s multiple-range test at a 95% confidence level.

8.3. Result and Discussion

8.3.1. Fibre audit of screens and cleaners

Evaluation of screens

The changes in the fibre characteristics across the screens showed that the shorter fibres were accepted (Fig. 8-2; sample 4) and longer fibres were rejected (sample 5), when compared to fibres at the inlet (Table 8-1; sample 1). These results confirm the findings of Kure et al. (1999), who concluded that screens separated fibres primarily on the basis of their length. Average fibre width before screening (sample 1) was not different from that of samples after screening (sample 4), although the rejected stream (sample 5) contained fibres with slightly smaller width. The longer rejected fibres had thicker cell-walls than those fibres present in the accepted fraction. Previous studies (Chapter 4), which showed that LW fibres of softwood are typically long with thicker cell-walls much like the rejected samples in this study. It was also observed that the characteristics of the accepted fibres (sample 4) were similar to those fibres observed in hardwood species (Chapter 5). Coarseness measurements supported these results, showing that rejects were 20% coarser compared to pulp at the inlet.
Table 8-1: The fibre characteristics of samples collected before and after screening. The change relative to the inlet sample is indicated in brackets.

<table>
<thead>
<tr>
<th>Fibre characteristics</th>
<th>Inlet (sample 1)</th>
<th>Accepts (sample 4)</th>
<th>Rejects (sample 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibre length: weighted in length (µm)</td>
<td>913</td>
<td>891 (-2%)</td>
<td>1486 (+39%)</td>
</tr>
<tr>
<td>Cell-wall thickness (µm)</td>
<td>3.031</td>
<td>2.813 (-7%)</td>
<td>3.832 (+21%)</td>
</tr>
<tr>
<td>Fibre width (µm)</td>
<td>37.2</td>
<td>37.2 (0%)</td>
<td>35.4 (-5%)</td>
</tr>
<tr>
<td>Coarseness (mg g⁻¹)</td>
<td>0.326</td>
<td>0.323 (-1%)</td>
<td>0.408 (+20%)</td>
</tr>
</tbody>
</table>

When these screened samples were studied with SEM, the collapsibility of the fibres from the inlet (sample 1) did not show any significant differences from the screened fibres (sample 4) (Fig. 8-3). However, fibres that were rejected by the screens (sample 5) had significantly lower collapsibility (Fig. 8-3) than those from the inlet fibres (sample 1), and were similar to LW fibres (Strey et al., 2009; Chapter 4). Therefore, the results suggest that the reject pulp was dominated largely by the LW fraction of pulp.

![Figure 8-3: Ratio of lumen area (LA) to fibre area (FA) as a reflection of the degree of collapsibility of fibres from different screening stages (bars with the same letters do not differ significantly, Student’s t-test, p ≤ 0.05).](image)

Evaluation of cleaners

The cleaning stage did not lead to any noticeable difference between the fibre length of the inlet (sample 6) and accepted pulp (sample 3). However, the accepted pulp contained slightly wider fibres with 20% thicker cell-walls (Table 8-2). The rejected fraction after cleaning (sample 2) consisted of fibres that were 11% longer and 2% wider when compared to the inlet sample (sample 6). The rejects also contained fibres with thicker cell-walls (16%) compare to the inlet (sample 6). Efficient separations of long and thick-walled fibres were
also observed after cleaning, but the ‘cleaning’ step was not as effective as the screening process. While the increase in cell-wall thickness (CWT) and fibre width in both the rejected (sample 2) and accepted (sample 3) fibre streams appears to be inconsistent, this is very likely due to the removal of small fibre elements (also known as fines) into the waste stream (Fig. 8-2). The cleaners, in contrast to the screens, led to no significant difference in the degree of collapsibility of fibres between any of the three different streams (Fig. 8-4).

**Table 8 - 2:** The fibre characteristics of samples collected before and after cleaning. The change relative to the inlet sample is indicated in brackets.

<table>
<thead>
<tr>
<th>Fibre characteristics</th>
<th>Inlet (sample 6)</th>
<th>Accepts (sample 3)</th>
<th>Rejects (sample 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibre length: weighted in length (µm)</td>
<td>891</td>
<td>879 (-1%)</td>
<td>1005 (+11%)</td>
</tr>
<tr>
<td>Cell-wall thickness (µm)</td>
<td>2.813</td>
<td>3.505 (-20%)</td>
<td>3.331 (+16%)</td>
</tr>
<tr>
<td>Fibre width (µm)</td>
<td>37.2</td>
<td>38.3 (+3%)</td>
<td>38.0 (-2%)</td>
</tr>
<tr>
<td>Coarseness (mg g⁻¹)</td>
<td>0.323</td>
<td>0.301 (-7%)</td>
<td>0.316 (+2%)</td>
</tr>
</tbody>
</table>

**Figure 8 - 4:** Ratio of lumen area (LA) to fibre area (FA) as a reflection of the degree of collapsibility of different samples collected at different cleaning stages (bars with the same letters do not differ significantly, Student’s t-test, p ≤ 0.05).

8.3.2. Influence on mannanase on freeness and fibre characteristics

Treatment of rejects with MAN did not cause any significant (p ≤ 0.05) change in the freeness of the pulp or fibre characteristics (Table 8-3) as previously observed, especially for thick-wall fibres (Strey *et al.*, 2009; Chapter 4). This study confirms that thick-walled fibres are not as easily modified by enzymes as thinner walled fibres (Chapter 7).
Table 8 - 3: The impact of mannanase (MAN) on the freeness and fibre characteristics of reject fibres.

<table>
<thead>
<tr>
<th>Fibre characteristics</th>
<th>Control</th>
<th>MAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeness (ml)</td>
<td>530</td>
<td>533</td>
</tr>
<tr>
<td>Fibre length: weighted in length (µm)</td>
<td>953</td>
<td>892</td>
</tr>
<tr>
<td>Cell-wall thickness (µm)</td>
<td>3.900</td>
<td>3.795</td>
</tr>
<tr>
<td>Fibre width (µm)</td>
<td>32.8</td>
<td>32.8</td>
</tr>
<tr>
<td>Kinked fibres (%)</td>
<td>15.15</td>
<td>16.28</td>
</tr>
<tr>
<td>Curl (%)</td>
<td>6.23</td>
<td>6.40</td>
</tr>
<tr>
<td>Broken ends (%)</td>
<td>44.10</td>
<td>44.63</td>
</tr>
<tr>
<td>Fines (% length)</td>
<td>48.833</td>
<td>48.059</td>
</tr>
<tr>
<td>Fines (% area)</td>
<td>7.843</td>
<td>7.509</td>
</tr>
</tbody>
</table>

No significant differences were observed between treatments (p ≤ 0.05; ANOVA)

8.3.3. Influence of mannanase on the stability of reject fibres

Fibre stability was tested through rewetting of handsheets. The roughness of handsheets in the control increased significantly (p ≤ 0.05) after rewetting (Table 8-4). The MAN treated pulp also showed a significant increase in roughness after rewetting, which indicates that the enzyme was not effective. A similar effect of Man on roughness was also observed in previous studies done on spruce and LW pine fibres (Strey et al., 2009 and Chapter 4, respectively). Both untreated (control) and MAN-treated samples showed a significant decrease in the tensile and burst indices after rewetting, indicating that the enzyme treatment did not lead to cell-wall modification or improved fibrillation of rejected fibres. These results reflect the difficulty of improving the quality of thick-walled fibres noted previously (Strey et al., 2009; Chapter 4).

Table 8 - 4: The influence of MAN treatment and rewetting on the properties of handsheets from reject pulp.

<table>
<thead>
<tr>
<th>Handsheet properties</th>
<th>Control (dry)</th>
<th>Control (rewetted)</th>
<th>MAN (rewetted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roughness (ml min⁻¹)</td>
<td>230 a</td>
<td>2058 b</td>
<td>2278 b</td>
</tr>
<tr>
<td>Tensile index (m Nm² g⁻¹)</td>
<td>1.991 a</td>
<td>1.027 b</td>
<td>0.772 b</td>
</tr>
<tr>
<td>Burst index (kPa m² g⁻¹)</td>
<td>0.642 a</td>
<td>0.311 b</td>
<td>0.301 b</td>
</tr>
</tbody>
</table>

a b: Values in the same line followed by the same letter do not differ significantly (p ≤ 0.05; Tukey’s test)

8.3.4. Influence of biomimetic treatment on the stability of reject fibres

Meyer-Pinson et al. (2004) reported that oxalate treatment had a significant impact upon the fibre width and amount of curl of poplar fibres. However, in the present study, oxalate treatment did not lead to significant (p ≤ 0.05) differences in the pulp freeness or fibre morphology when compared to the control, at least under the conditions tested here (Table 8-5).
Table 8-5: The impact of oxalate treatment on the freeness and fibre characteristics of reject fibres.

<table>
<thead>
<tr>
<th>Fibre characteristics</th>
<th>Control</th>
<th>Oxalate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeness (ml)</td>
<td>490</td>
<td>493</td>
</tr>
<tr>
<td>Fibre length: weighted in length (µm)</td>
<td>855</td>
<td>896</td>
</tr>
<tr>
<td>Cell-wall thickness (µm)</td>
<td>3.830</td>
<td>3.998</td>
</tr>
<tr>
<td>Fibre width (µm)</td>
<td>28.77</td>
<td>28.63</td>
</tr>
<tr>
<td>Kinked fibres (%)</td>
<td>10.83</td>
<td>10.97</td>
</tr>
<tr>
<td>Curl (%)</td>
<td>5.02</td>
<td>5.06</td>
</tr>
<tr>
<td>Broken ends (%)</td>
<td>39.48</td>
<td>39.67</td>
</tr>
<tr>
<td>Fines (% length)</td>
<td>38.92</td>
<td>38.98</td>
</tr>
<tr>
<td>Fines (% area)</td>
<td>4.40</td>
<td>4.22</td>
</tr>
</tbody>
</table>

No significant differences were observed between treatments (p ≤ 0.05; ANOVA).

Surface roughness of the control and oxalate-treated handsheets increased significantly while the strength properties of handsheets were significantly reduced (p ≤ 0.05; Table 8-6) leading to similar conclusions as drawn above that oxalate treatment did not contribute positively towards inter-fibre bonding, surface roughness or strength. The fact that none of the fibre improvements described by Meyer-Pinson et al. (2004) were observed could be as result of the slightly higher pH used in the present experiment. Recent discussion revealed that pH may be critical for success (B. Kurek, personal communication, kurek@reims.inra.fr). Nevertheless, it does not appear to be commercially feasible to reduce the pH to 2.5 as described (Meyer-Pinson et al., 2004).

Table 8-6: The influence of oxalate treatment and rewetting on the properties of handsheets from reject pulp.

<table>
<thead>
<tr>
<th>Handsheet properties</th>
<th>Control (dry)</th>
<th>Control (rewetted)</th>
<th>Oxalate (rewetted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roughness (ml min⁻¹)</td>
<td>836 a</td>
<td>2839 b</td>
<td>2504 b</td>
</tr>
<tr>
<td>Tensile index (k Nm⁻¹ g⁻¹)</td>
<td>1.903 a</td>
<td>0.916 b</td>
<td>0.931 b</td>
</tr>
<tr>
<td>Burst index (kPa m² g⁻¹)</td>
<td>0.603 a</td>
<td>0.226 b</td>
<td>0.208 b</td>
</tr>
</tbody>
</table>

a b: Values in the same line followed by the same letter do not differ significantly (p ≤ 0.05; Tukey’s test)

8.3.5. Influence of chemical pre-treatment

Hydrogen peroxide (H₂O₂) did not lead to any significant (p ≤ 0.05) change in the freeness or the fibre characteristics in comparison to the control (Table 8-7). Pre-treating pulp with H₂O₂, followed by MAN and oxalate treatments was investigated, but these treatments also did not change any of the fibre characteristics measured.
**Table 8 - 7: The impact of peroxide (H\textsubscript{2}O\textsubscript{2}) pre-treatment followed by enzyme and oxalate treatment on the freeness and fibre characteristics of pulps.**

<table>
<thead>
<tr>
<th>Freeness and fibre characteristics</th>
<th>Control</th>
<th>H\textsubscript{2}O\textsubscript{2}</th>
<th>H\textsubscript{2}O\textsubscript{2} + MAN</th>
<th>H\textsubscript{2}O\textsubscript{2} + Oxalate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeness (ml)</td>
<td>488</td>
<td>483</td>
<td>502</td>
<td>550</td>
</tr>
<tr>
<td>Fibre length: weighted in length (µm)</td>
<td>825</td>
<td>907</td>
<td>906</td>
<td>854</td>
</tr>
<tr>
<td>Fibre width (µm)</td>
<td>28.7</td>
<td>29.0</td>
<td>28.6</td>
<td>28.4</td>
</tr>
<tr>
<td>Curl (%)</td>
<td>4.6</td>
<td>4.8</td>
<td>4.9</td>
<td>5.5</td>
</tr>
<tr>
<td>Kinked fibres (%)</td>
<td>8.4</td>
<td>9.7</td>
<td>10.5</td>
<td>12.8</td>
</tr>
<tr>
<td>Broken ends (%)</td>
<td>41.4</td>
<td>40.5</td>
<td>40.0</td>
<td>40.4</td>
</tr>
<tr>
<td>Fines (% length)</td>
<td>46.3</td>
<td>37.9</td>
<td>38.4</td>
<td>46.3</td>
</tr>
<tr>
<td>Fines (% area)</td>
<td>5.1</td>
<td>3.7</td>
<td>3.8</td>
<td>5.2</td>
</tr>
<tr>
<td>Cell-wall thickness (µm)</td>
<td>3.741</td>
<td>3.614</td>
<td>3.781</td>
<td>3.578</td>
</tr>
</tbody>
</table>

Table 8 - 8: The influence of peroxide pre-treatment on the properties of handsheets from reject pulp, followed by MAN and oxalate treatment.

<table>
<thead>
<tr>
<th>Handsheet properties</th>
<th>Control (dry)</th>
<th>Control (rewetted)</th>
<th>H\textsubscript{2}O\textsubscript{2} (rewetted)</th>
<th>H\textsubscript{2}O\textsubscript{2} + MAN (rewetted)</th>
<th>H\textsubscript{2}O\textsubscript{2} + Oxalate (rewetted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roughness (ml min\textsuperscript{-1})</td>
<td>1257\textsuperscript{a}</td>
<td>1874\textsuperscript{d}</td>
<td>1610\textsuperscript{c}</td>
<td>1574\textsuperscript{bc}</td>
<td>1393\textsuperscript{b}</td>
</tr>
<tr>
<td>Tensile index (m Nm\textsuperscript{2} g\textsuperscript{-1})</td>
<td>1.740\textsuperscript{a}</td>
<td>0.974\textsuperscript{b}</td>
<td>1.585\textsuperscript{b}</td>
<td>1.295\textsuperscript{b}</td>
<td>1.000\textsuperscript{b}</td>
</tr>
<tr>
<td>Burst index (kPa m\textsuperscript{2} g\textsuperscript{-1})</td>
<td>0.497\textsuperscript{a}</td>
<td>0.220\textsuperscript{b}</td>
<td>0.362\textsuperscript{ab}</td>
<td>0.326\textsuperscript{b}</td>
<td>0.250\textsuperscript{b}</td>
</tr>
</tbody>
</table>

a b c d: Values in the same line followed by the same letter do not differ significantly (p ≤ 0.05; Tukey’s test).
8.4. CONCLUSIONS

Fibre stability of mechanical pulp can be increased by removing large amounts of long- and thick-walled fibres through fractionation using screens and cleaners (Kappel, 1999). In the present study, efficient separation of these unstable fibres in chemi-thermo-mechanical pulp (CTMP) was possible. It was shown that both screens and cleaners can reject long- and thick-walled fibres from the pulp stream. Screens primarily rejected long fibres with thick cell-walls, whereas cleaners separated fibres on the basis of density, which could reflect the degree of fibrillation (Wood and Karnis, 1979). In the present study the reject fraction from the cleaners generally contained poorly fibrillated fibres. These rejected fibres from each process (screens and cleaners) were similar in characteristics to the LW fraction of pine as studied previously (Chapter 4). This large amount of rejects that are removed using fractionation justifies application of additional treatment methods for improved development of reject fibres and utilisation with other suitable fibres.

While enzyme treatments successfully modified the cell-wall structure and increased stability in previous studies (Strey et al., 2009; Chapters 4 to 7), these enzymes did not show any positive changes in the fibre characteristics or handsheet properties before and after rewetting. This lack of response by fibres may also be due to the high lignin content present in CTMP (Sundholm, 1999), which could restrict these modifying treatments (especially enzymes). Lignin could possibly form a barrier that restricts binding of the enzyme to the substrate (Palonen, 2004). Chemical treatment with oxalate was also investigated but without success. According to previous research by Meyer-Pinson et al. (2004) and Sferrazza et al. (1988), strength improvement was observed, but these studies were done on poplar fibres, which contain thinner cell-walls and were possibly more responsive to oxalate than the rejects treated in this study. However, when an \( \text{H}_2\text{O}_2 \)-treatment was used at the same concentrations as on poplar, a significant reduction in surface roughness was observed, possibly because this treatment made fibres more flexible by removing some of the lignin that reduced the activity of MAN and oxalate. Fibres pre-treated with both \( \text{H}_2\text{O}_2 \) and MAN or oxalate increased surface smoothness. An increase in surface smoothness was also obtained with \( \text{H}_2\text{O}_2 \) alone. Even further improvements were made when \( \text{H}_2\text{O}_2 \)-pre-treatment was combined with treatments with oxalate. These improvements were possibly due to the fact that fibres tend to swell more under alkaline conditions (Engstrand et al., 1991). Treatment with alkaline \( \text{H}_2\text{O}_2 \),
therefore, presents an opportunity to improve CTMP rejects, especially in systems where low reject rates are used during fibre fractionation and separate refining of rejects is not feasible.
8.5. REFERENCES


Improvement of fibre stability of CTMP reject fibres


In the papermaking process, blends of softwood and hardwood species are often used to meet strength and smoothness objectives for specific paper grades (Varhimo and Tuovinen, 1999). Strength is obtained with longer fibres from softwood species, while hardwood fibres are used to improve the surface smoothness (Horn, 1978; Koljonen et al., 1997). Although the specific paper properties are relatively easy to meet, exposure to coating and printing processes can lead to deterioration of these properties. The challenge is, therefore, to maintain these properties after paper is subjected to the environmental changes associated with coating and printing processes. Changing environmental conditions such as moisture, can weaken hydrogen bonding and cause unstable fibres to move, leading to a reduction in the sheet smoothness and strength properties (Skowronski and Lepoutre, 1985). Methods of addressing the instability of fibres have one objective in common: modifying the cell-wall to promote fibrillation and fibre collapse. Fibrillation and collapsing of fibres increases the number of hydrogen bonds that can form between fibres and could improve fibre stability. Refining is used to modify the cell-wall structure by increasing fibrillation and making fibres more collapsible (Mohlin, 1997; Hiltunen, 2003), although maintaining fibre stability can still remain a problem. In the present study, the stability of different fibre types during rewetting was studied, as well as the application of enzymes to increase fibre stability.
The first challenge faced in this study was to develop a suitable approach to visualise fibres in a paper network and their response to moisture. This problem was addressed by using conventional microscopic methods including scanning electron microscopy (cSEM) and environmental scanning electron microscopy (ESEM). Reliable images were obtained using both methods and while fibre movement was observed on the paper surface after rewetting in cSEM, in ESEM this process could be observed in real time. However, fibre movements such as fibre lifting, cell-wall swelling and puffing were difficult to quantify and, therefore, fibre cross-sections were also examined. Cross-sectional imaging of paper samples required an appropriate sample-preparation technique and the best results were observed after fibres were embedded in Quetol resin. Resin embedding required an etching step to produce specimens with detailed images, and in the present study it was observed that it did not have an effect on the shape of fibres. Therefore, it was possible to get reliable images of fibre cross-sections that could be analysed and used to observe the effect of moisture.

A very important aspect of this study to improve fibre stability was to quantify the changes observed in the fibre shape in the SEM images after exposure to moisture. In previous studies, movements such as fibre rising, cell-wall swelling and puffing were described for unstable fibres (Skowronski and Lepoutre, 1985; Aspler and Béland, 1994). The first two movements, fibre rising (Page et al., 1985) and cell-wall swelling (Hoc, 1989) were defined and quantified, but puffing has not been defined previously. The term de-collapse (referred to here as puffing) has been used previously and methods for quantifying it were investigated (Reme et al., 1998; Forseth and Helle, 1997; Skowronski, 1990). Forseth and Helle (1997) used cSEM combined with image analysis and the average lumen opening was plotted as a function of the cell-wall thickness (CWT). Reliable results were obtained, but only fibres that were cut at a right angle were measured. Therefore, the amount of fibres that could be measured was limited in each sample. In the present study, the change in the cross-sectional shape of fibres was described as fibre puffing and defined as the degree to which the lumen returns to its original shape. In order to accurately quantify puffing the cross-sectional dimensions including lumen area (LA), fibre area (FA) and CWT were measured using image analysis software. The reliability of these measurements was tested by comparing models of fibres (both thin- and thick-walled) at different cutting angles. Puffing was calculated by determining the lumen area to the fibre area ratio (LA:FA), which was not influenced by the cutting angle.
In the present study, the ratio LA:FA that defined puffing was first applied to softwood CTMP fibres (Strey et al, 2009; Chapters 4 to 5). Measurements obtained for these fibres showed great variation, but it was realised that different CWT classes may be the cause of the problem. The effect of the CWT was also observed in a previous study by Forseth and Helle (1997) and confirmed observations on models of thin- and thick-walled fibres (Chapter 2). Therefore, the data were separated for thin-walled and thick-walled fibre fractions. It was then possible to quantify fibre stability and the relevance of this technique was confirmed when it was later applied to commercial CTMP and bleached-CTMP pulps and sheets.

The complex response of mixed (heterogeneous) pulps to treatments obscured the effect of treatments and, therefore, pulps were separated into more homogeneous fractions to make the response of the fibres apparent. In the present study, it was necessary to produce softwood, hardwood as well as earlywood and latewood fractions from softwood separately in the laboratory. These laboratory produced pulp were then subjected to industrial processes that were simulated in the laboratory. Industrial processes such as calendering and printing also have an impact on the sheet properties and a second challenge was to validate the results obtained with laboratory-scale processes against commercial samples.

Once confidence in the methods to quantify fibre stability in the laboratory had been established, these were then applied to different wood species. Softwood fibres were generally unstable (especially in the thin-walled fibre fraction), causing a reduction in the surface smoothness as well as strength properties of paper when rewetted. The thick-walled fibres in softwood were not collapsible, and puffing was not as evident as in the thin-walled fraction after rewetting. The earlywood (thin-walled) and latewood (thick-walled) pulps behaved in a similar manner. Thin-walled and thick-walled fibres in the hardwood fraction showed high collapsibility and, after rewetting, both these fractions underwent little or no puffing. The difference in stability between hardwood and softwood is probably related to the difference in fibre morphology and chemical composition (Sjöström, 1993).

Regarding the differences between hardwood and softwood, it was observed that some fibre fractions (especially fractions that contained thick-walled fibres) still did not conform after refining. The fibres rejected from the main stream presented a particular problem and alternative treatments were, therefore, investigated. One option for improved quality of
rejected fibres was to increase the fibre collapse by using enzymes such as cellulases or hemicellulases (Mansfield et al., 1996; Kibblewhite and Wong, 1999). Previous studies have shown that endoglucanase (EG) increase collapsibility, but that its influence on fibre strength was negative (Kibblewhite and Wong, 1999; Lumme et al., 1999). In the present study, EG was applied to softwood fibres, reducing puffing especially to the thin-walled fraction. Furthermore, the reduction in strength properties caused by EG was confirmed in the present study (Strey et al., 2009; Chapters 4 to 5). Application of the mannnase (MAN) to the softwood pulps showed that it was possible to retain both strength and smoothness properties of paper from the thin-walled fibres. The thicker cell-walls were less responsive to both these enzymes and the response of the hardwood fibres was difficult to evaluate, due to the lack of response to rewetting. These results suggested that hardwood fibres were more stable, and that MAN treatment maintained strength properties even after paper was rewetted.

The present study proposes a model of fibre development and enzyme action on the stability of spruce fibres during rewetting. The enzymes tested here apparently modified the cell-walls and predisposed fibres in different ways to the beating that followed. Modification of fibres possibly manifested itself as a weakening of the fibre wall through alteration of the cell-wall structure. Beating caused fibrillation of fibres in all treatments, but the slightly increased strength properties of MAN-treated pulp could reflect a possible increase in fibril length and/or in number. However, the reduction in strength properties of the EG-treated pulps probably reflected fibrillation loss. This model was valid for the earlywood fraction (thin-walled fibres) in softwood pulp. The latewood fibres as well as the hardwood fibres respond less to moisture than softwood fibres. Although, the lack in response made it difficult to correlate it with the results from the model, no contradiction is suggested for HW pulp.

Tests on laboratory pulps and sheets showed that enzymes can increase fibre stability. When these enzymes were applied to commercial pulps a positive effect on stability of bleached-CTMP was observed, possibly due to large amount of thin-walled fibres present in the pulp. Another factor that may have caused better response to the enzyme could be the reduced amount of lignin present on the surface of the bleached fibres. However, when these enzymes were tested on CTMP rejects, the effect of the enzyme was difficult to observe, possibly due to the presence of more thick-walled fibres and higher amount of lignin. This lack in response was overcome by pre-treating the CTMP fibres with alkaline hydrogen
peroxide, thereby, making the cell-wall more responsive towards the enzyme by removing some of the lignin. The chemical treatment alone can improve fibre stability, but when combined with enzymes, or oxalate the effect of the chemical treatment was enhanced. The application of the enzyme under mill conditions is feasible and the greatest potential exists in treatment of the rejected CTMP fibres, since a relatively small stream of pulp will require treatment. However, enzyme and chemical application will require a treatment vessel with capacity to allow adequate retention time for the chemical and/or enzyme to react with the fibres.

Future studies of fibre stability should focus on the depth of penetration by the enzyme and the impact of enzyme action on a molecular level. The size of enzymes may prevent them from penetrating between microfibrils. However, the methods used in the present study did not warrant speculation on this aspect of enzyme action and the model for enzyme action was proposed with this in mind. The impact of enzymes on a molecular level is probably one of the major issues to be resolved in terms of understanding the action of increasing fibre stability. Further investigations using atomic force microscopy (AFM) and immuno-labelling of enzymes may resolve some of these questions. Since the AFM scanning is limited to very small areas of the fibre, sample selection during studies will be very important.


9.1. REFERENCES


