UNIVERSITÉ DE REIMS CHAMPAGNE - ARDENNE

THÈSE DE DOCTORAT

Spécialités biochimie et science des matériaux

Solvants pariétaux et aptitude à la transformation des fibres de chanvre

par

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à Vezzan et Paşa

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SOMMAIRE

Contexte général de l'étude

Les lignocelluloses issues des co-produits de l'agriculture représentent des ressources renouvelables et disponibles en quantité abondante. Pour répondre à des besoins d'ordres économiques et environnementaux, d'importants efforts au niveau de la Recherche ont été réalisés afin d'exploiter au mieux cette matière première. La transformation des lignocelluloses constitue un enjeu majeur dans l'utilisation de ces dernières comme par exemple, l'utilisation de fibres agricoles dans les matériaux composites. Différents traitements existent afin de préserver la production de fibres cellulosiques intactes, permettant ainsi d'obtenir des substrats spécifiques pour la production optimale de matériaux à base de fibres agricoles. Cependant, la difficulté est de mettre en place des procédés technologiques économiquement intéressants pour la valorisation des lignocelluloses. La connaissance des propriétés mécaniques à l'échelle micro et macro s'avère être indispensable pour l'optimisation des procédés afin de réduire les coûts. C'est dans ce cadre de valorisation de produits agricoles que s'inscrivent nos travaux.

Le déroulement de cette étude a été réalisé au sein de l'Unité Mixte de Recherche – Fractionnement des Agroressources et Environnement (INRA - Reims). La thématique générale du laboratoire est de comprendre les mécanismes de construction et de déconstruction de la paroi végétale.

Les recherches ont été réalisées sur un co-produit issu de la tige de chanvre dépourvue de ses fibres longues afin de générer des applications nouvelles. Ce co-produit nommé chènevotte, provenant d'un procédé industriel de défibrage mécanique propre à la Chanvrière de l'Aube, présente un aspect proche de celui de copeaux de bois. La tige

de chanvre possède la particularité de renfermer des fibres longues très peu lignifiées et des fibres plus courtes, fortement lignifiées, proche du celle du bois des plantes pérennes. Généralement utilisée dans la fabrication de pâte à papier ou litière de chevaux, la chènevotte connait des applications limitées due au manque de connaissances sur ses propriétés de transformation lorsqu'elle est soumise à des stress mécaniques et environnementaux (torsions, cisaillement ; élongation ; température, pression ; hygrométrie).

L'objectif de cette étude est de maitriser l'aptitude à la transformation des fibres de chanvre via la caractérisation de ses propriétés micro et macromécaniques suite à des extractions avec différents solvants. Ce travail représente un aspect fondamental du rôle des extractibles dans la cohésion pariétale. Les extractions ont pour effet de retirer facilement des entités « extractibles » de la paroi végétale sans la déstructurer. Ce choix de matériel d'étude est d'autant plus pertinent qu'il s'agit de plantes d'applications sur lesquelles des projets ont été réalisés (deux projets Agrice 2003-2005 et 2005-2007; un projet du MAP 2005-2007; un projet labellisé Pôle de Compétitivité Agro-Industrie ; 2006-2008)

L'étude comporte deux volets distincts : la caractérisation biochimique des extractibles au sein de l'équipe parois végétales et la détermination de leurs propriétés physico-chimiques fonctionnelles (interactions vis-à-vis des autres constituants pariétaux) au sein de l'équipe matériaux de l'INRA de Reims.

Ces travaux ont été co-financé par la Région Champagne-Ardenne et l'INRA, et ils ont été présentés lors de différentes manifestations scientifiques au niveau national et européen. Le bilan de ces activités est recensé ci-dessous :

- Un poster présenté à « 6th Plant Biomechanics Conference » à Cayenne (Guyanne) le 19 Novembre 2009 + communication orale de 5 min : **« Impact of the selective extractives removal on the micro and macromechanical properties of *hemp core* »**
- Une communication orale à Wageningen à l'occasion du COST E50 Cell Wall Macromolecules and Reaction Wood (CEMARE) le 10 Juillet 2009 : **« Selective removing of cell wall extractive molecules influencing lignin and hemicelluloses viscoelastic properties in woody hemp core »**
- Un poster présenté à une conférence européenne à Vienne (Autriche) COST FP0802 Experimental and Computational Micro-Characterisation Techniques in Wood Mechanics le 11 Mai 2009 + communication orale de 5min : **« Viscoelastic properties of in situ cell wall polymers from woody hemp core (chenevotte) »**
- Une communication orale à Boussens (Toulouse) à l'occasion du Réseau Français de la Paroi (RFP) le 27 Mars 2008 : **"New methods for woody hemp chenevotte characterization"**

CHAPITRE I

1 Introduction sur la paroi végétale.

La paroi végétale a longtemps été considérée comme une structure statique, mais suite aux premiers modèles décrivant son architecture, celle-ci est définie comme une structure extracellulaire organisée, dynamique délimitant le contour d'une cellule. En effet, la cellule régule les dépôts de substances synthétisées de nature essentiellement polysaccharidiques (cellulose, hémicelluloses), protéiques et phénoliques (lignine et acides phénoliques) et généralement de type polymérique. La cellule gouverne également l'organisation supramoléculaire des composés pariétaux, dont l'architecture qui en résulte joue un rôle prépondérant dans la physiologie de la plante. Grâce à sa composition et son architecture, la paroi assure de nombreuses fonctions vitales, mécaniques et morphologiques à la plante entière. (Goodwin et Mercer 1990)

Dans la suite de ce chapitre, nous nous intéresserons principalement aux parois végétales du bois.

2 Composition chimique de la paroi

Les principaux constituants chimiques des parois végétales sont en général : la cellulose, les hémicelluloses et la lignine. D'autres composés polymériques sont présents en plus faibles quantité comme les pectines et les protéines. En plus des ces composés macromoléculaires, de nombreux constituants de faible masse moléculaire peuvent être présents en faible quantité. Ces constituants sont des matières extractibles d'origine organiques ou des composés inorganiques (minéraux) solubles dans l'eau par exemple.

Dans le bois, la proportion relative des différents constituants varie en fonction des essences. La répartition moyenne des polymères est représentée dans la figure 1.

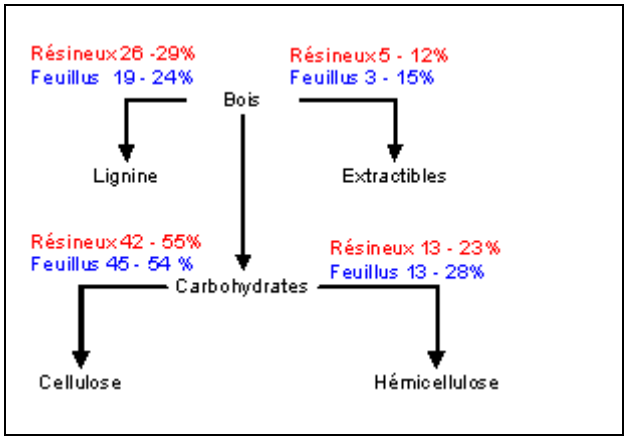

Figure 1. *Composition chimique générique des bois résineux et feuillus*

Tous ces éléments présents en différentes proportions coexistent dans la matière et présentent des structures, des proportions et des interactions diverses et complexes.

2.1 Les polysaccharides

2.1.1 La cellulose

La cellulose est le polymère le plus abondant du monde végétal. Dans les parois végétales, elle représente de 30 à 60% de la matière sèche. C'est un polymère linéaire composé de molécules de glucose reliés entre elles par des liaisons β-1,4 (figure 2).

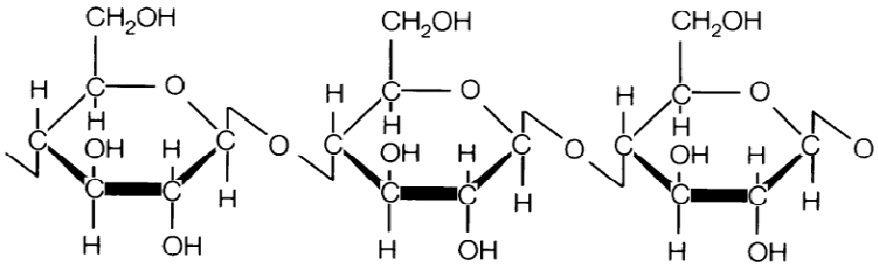

Figure 2. *Structure chimique de la cellulose*

Le degré de polymérisation de la cellulose est compris entre 2500 et 4500 dans les parois primaires et peut atteindre jusqu'à 15000 dans les parois secondaires. Grâce aux

liaisons hydrogènes intra et intermoléculaires, les molécules de cellulose adoptent des structures en microfibrilles très rigides et résistantes. Ces microfibrilles de celluloses sont constituées par l'alternance de régions hautement ordonnées (régions cristallines) et de régions plus relâchées (région amorphes) (figure 3).

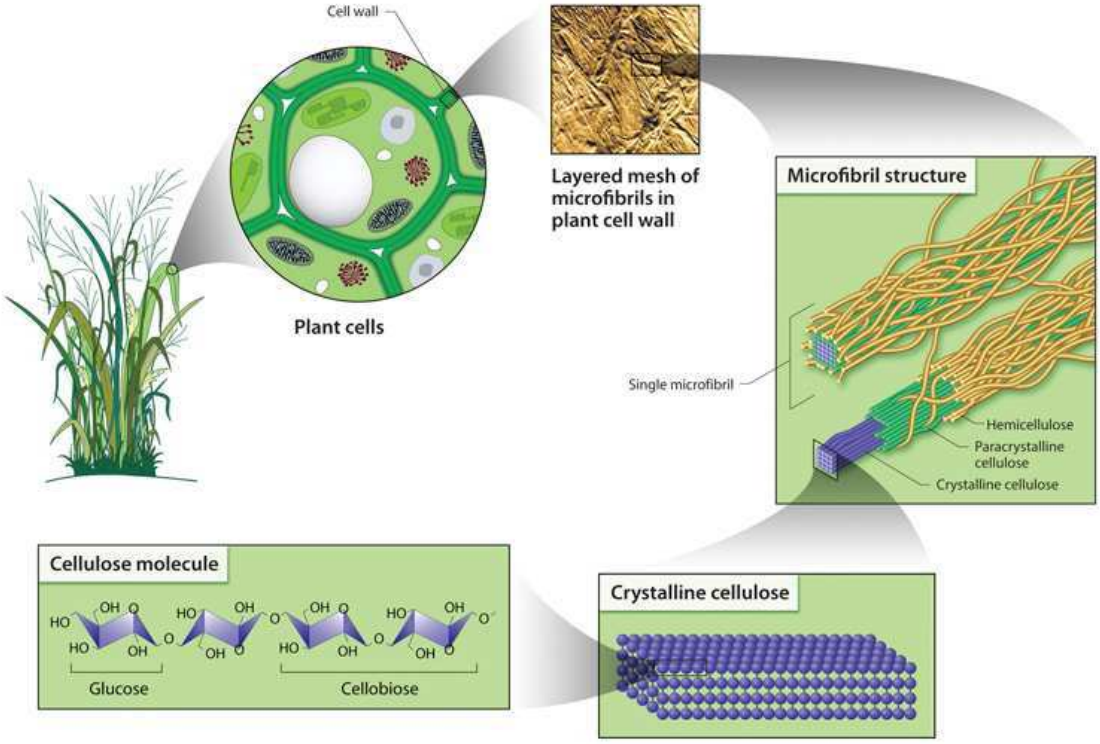

Figure 3. *Microstructure de la fibre cellulosique d'après (Rose et al. 1999)*

Les microfibrilles sont ensuite agglomérées en fibrilles puis en fibres de celluloses. La structure de la cellulose apporte à la paroi élasticité et résistance à la traction.

2.1.2 Hémicelluloses

Les hémicelluloses sont une classe de polymère très variées et très hydrophiles. Ceux sont des polymères linéaires ou ramifiés formés à partir de pentoses ou d'hexoses autres que le glucose. Quelle que soit l'espèce on retrouve la même structure pour la cellulose alors que les hémicelluloses ont des compositions et des structures qui varient considérablement selon qu'elles proviennent de feuillus ou de résineux. Les

hémicelluloses de feuillus sont généralement plus riches en pentoses, que celles des résineux qui habituellement contiennent davantage d'hexoses.

Leurs unités constitutives sont les suivantes :

- hexoses (D-glucose, D-mannose et D-galactose)

- pentoses (D-xylose, L-arabinose et D-arabinose)

- désoxy-hexoses (L-rhamnnose et L-fucose)

- faibles quantités d'acides hexuroniques (acides D-4-O-methylglucuronique, D-glucuronique et D-galacturonique) (figure 4).

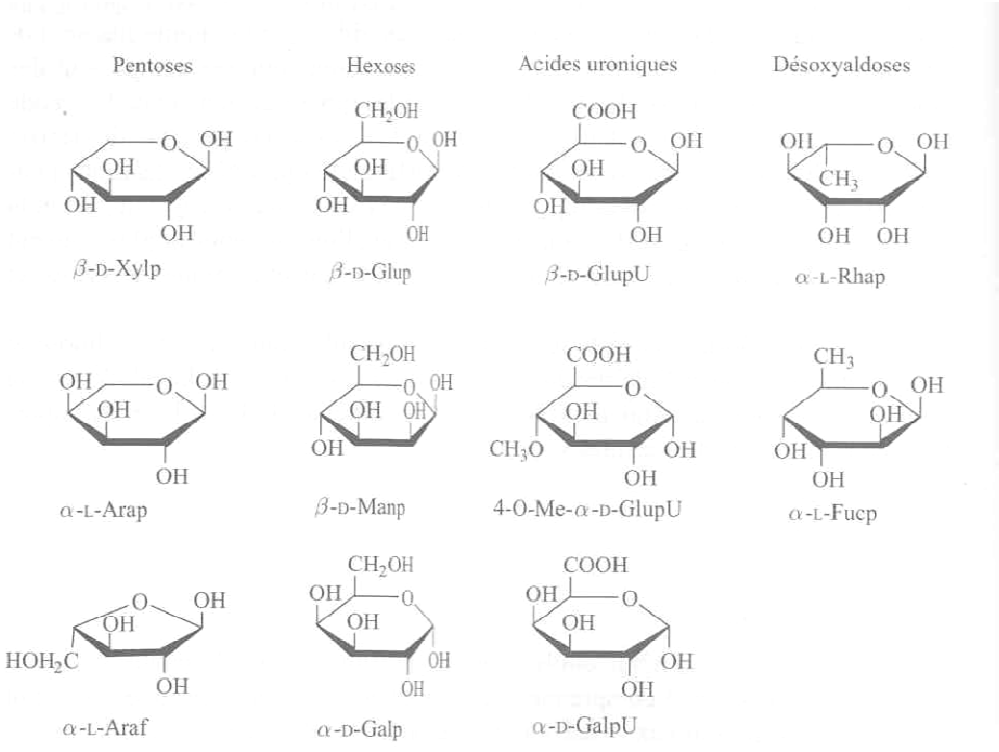

Figure 4. *Pentoses, hexoses, acides uroniques et désoxyaldoses composant les hémicelluloses du bois.*

Dans les bois feuillus, les hémicelluloses les plus importants sont :

- Les glucuronoxylanes (xylanes) (20 à 30% de la matière sèche du bois) sont constitués d'une structure linéaire d'unités β-D-xylopyranose liées en (1→4) contenant des branchements en (1→2) d'acide 4-O-methyl-α-D glucuronique (figure 5).
- les glucomannanes (< 5% de la matière du bois) sont formés par une chaîne linéaire principale de β-D-glucopyranose et β-D-mannopyranose liées par des liaisons (1→4) ni substitués, ni acétylés (Ebringerová 2005).

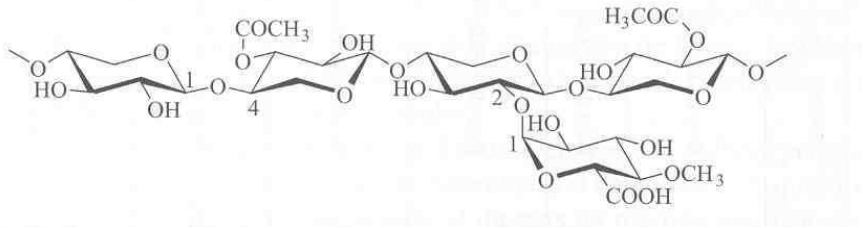

Figure 5. *Structure partielle d'un glucuronoxylane du bois feuillus (O-acétyl-4-O-méthylglucuronxylane)*

Dans les bois résineux, les hémicelluloses sont regroupées sous trois types :

- les glucommannes, similaires à ceux des feuillus, possèdent des ramifications supplémentaires α-D-galactopyrannose (1→6) et acétyles.
- Les galactoglucomannanes se différencient des glucomannanes par une proportion plus importante d' α-D-galactopyrannose (figure 6).
- Les arabinoglucuronoxylanes, composés d'un squelette de xylane, sont substituées par des enchainements arabinofurannoses et d'acides glucuroniques.

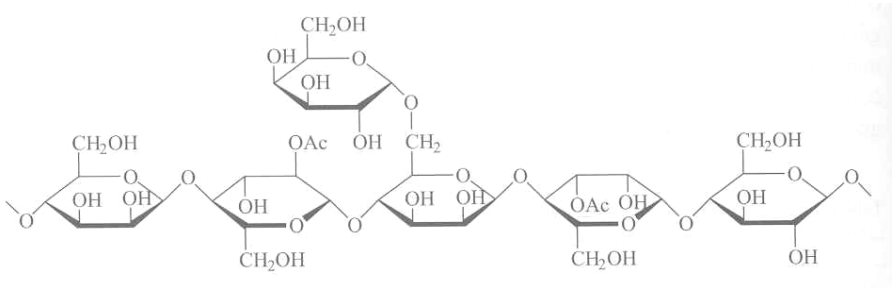

Figure 6. *Structure partielle d'un glucomannane d'un bois résineux*

Les structures d'hémicelluloses sont différentes selon l'origine de la plante ainsi leur identification sert de marquage et permet généralement de remonter à la nature de la plante considérée. La présence de chaînes latérales empêche celles-ci de s'organiser en microfibrilles mais elles s'associent grâce à des liaisons hydrogènes à la cellulose. Les hémicelluloses sont également susceptibles de former des liaisons covalentes avec la lignine et ainsi jouent un rôle fondamental dans le maintien d'une structure pariétale (Stevanovic T. et al. 2009).

2.1.3 Pectines

Les pectines sont caractéristiques des lamelles moyennes, et des jonctions tricellulaires et des parois primaires. Leur présence dans la paroi secondaire est moins connue. Ce sont des polysaccharides acides ou neutres extractibles par l'eau, les agents chélateurs et les acides dilués.

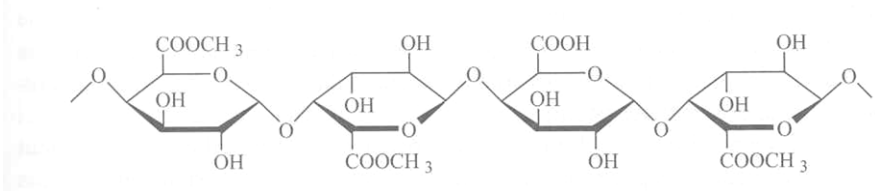

Figure 7. *Structure du principal polysaccharide pectique, le polygalacturonane*

La structure caractéristique de la pectine est une chaîne linéaire d'acides galacturoniques interconnectés par une liaison α-D-(1→4), qui forme la chaine principale, nommé polygalacturonane (figure 7) (Stevanovic T. et al. 2009).

Sur cette chaine principale, il ya des régions où l'acide galacturonique est remplacé par une molécule L-rhamnose(1→2). À partir de ces résidus rhamnose, des chaînes latérales de différents sucres neutres peuvent être ramifés. Ce type de pectine est appelé Rhamnogalacturonane I. Les sucres neutres ramifiés sont principalement le D-galactose, le L-arabinose et le D-xylose ; les types et les proportions de ces sucres varient avec l'origine de la pectine (de différentes plantes). Les Rhamnogalacturonanes sont un groupe de polymères de la paroi qui contiennent une chaine principale de motif répétitif disaccharidique: 4)-α-D-acide galacturonique-(1,2)-α-L-rhamnose-(1,2).

Un troisième type de structure de la pectine est Rhamnogalacturonane II (RG-II), qui est un complexe moins fréquent et très ramifié. Il est composé de 7 unités acide D-galacturonique liées ensemble par la liaison osidique α(1→4), chacune portant des ramifications constitutées par quatre différents oligosaccharides composés de sucres inhabituels. Les RG-II ont été identifiés dans les substances pectiques de toutes les plantes étudiées à ce jour. Chez les angiospermes, les RG-II existent sous forme mono et dimérique, et dans ce cas, leur association se fait par des diesters établies par un ion bore (Ridley et al. 2001).

Les pectines peuvent également former une structure stable via un arrangement particulier : elles peuvent piéger des ions de calcium. Le calcium se lie avec l'environnement électronégatif des atomes d'oxygène situés sur les deux chaînes de polymères adjacentes (Sedan et al. 2007). Lorsque cette fixation est répété entre deux chaînes, une structure très stable appelée ''boîte à oeufs'' est alors formée. (figure8)

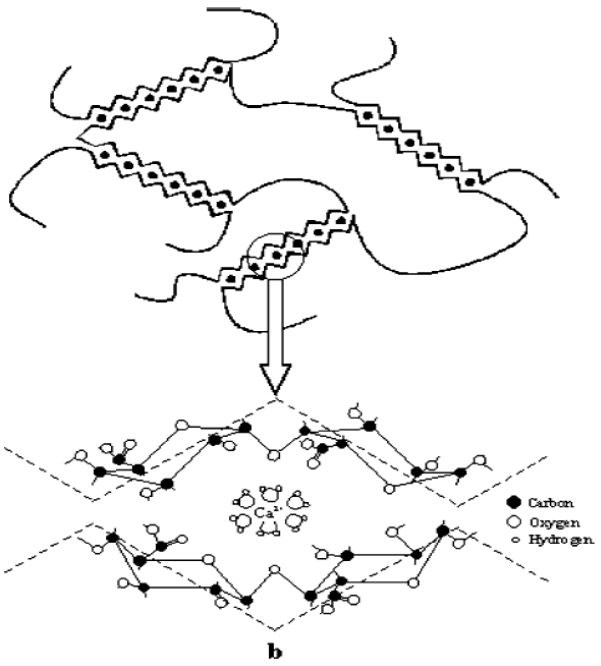

Figure 8. *Structure en « egg-box », d'après Sedan, 2007*

2.2 Lignine

La lignine représente 20 à 25% de la matière sèche du bois chez les feuillus. Elle appartient aux polymères phénoliques tridimensionnels. Elle peut être définie comme une macromolécule résultant de la polymérisation oxydative d'une ou plusieurs des trois unités phénylpropanoïques précurseurs (figure 9):

- l'alcool *p*-coumarique (qui donne par polymérisation oxydative l'unité H non méthoxylée de lignine)

- l'alcool coniférylique (donne l'unité G monométhoxylée)

- l'alcool sinapylique (donne l'unité S diméthoxylée)

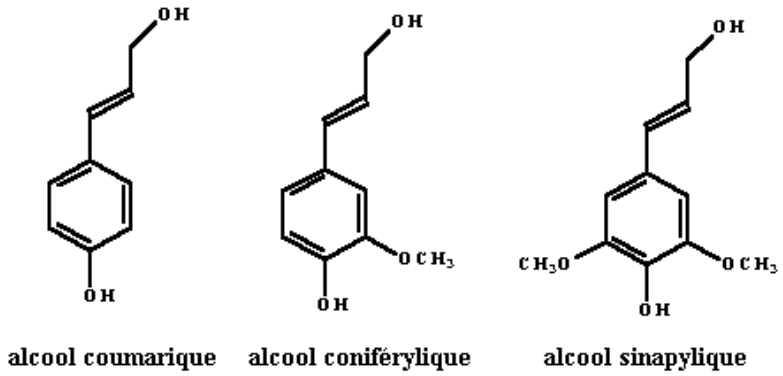

Figure 9. *Alcools précurseurs du polymère de lignine.*

La proportion dans le polymère de chacun des monomères dépend de l'origine et de l'espèce du végétal. Ces alcools précurseurs copolymérisent et forment la structure complexe du polymère de lignine. La réticulation des monomères phénylpropanes est assurée par un grand nombre de liaisons classées en deux grandes catégories :

- Les liaisons éther appelées liaisons non condensées, sont largement prépondérantes (plus de 2/3 de liaisons et surtout la liaison β-O-4 (40 à 60% du total des liaisons)
- Les liaisons carbone-carbone de type condensé

La figure 10 illustre les différents types de liaisons en fonction de leur fréquence qui peut varier selon l'emplacement morphologique de la lignine. Les principales connues sont les suivantes:

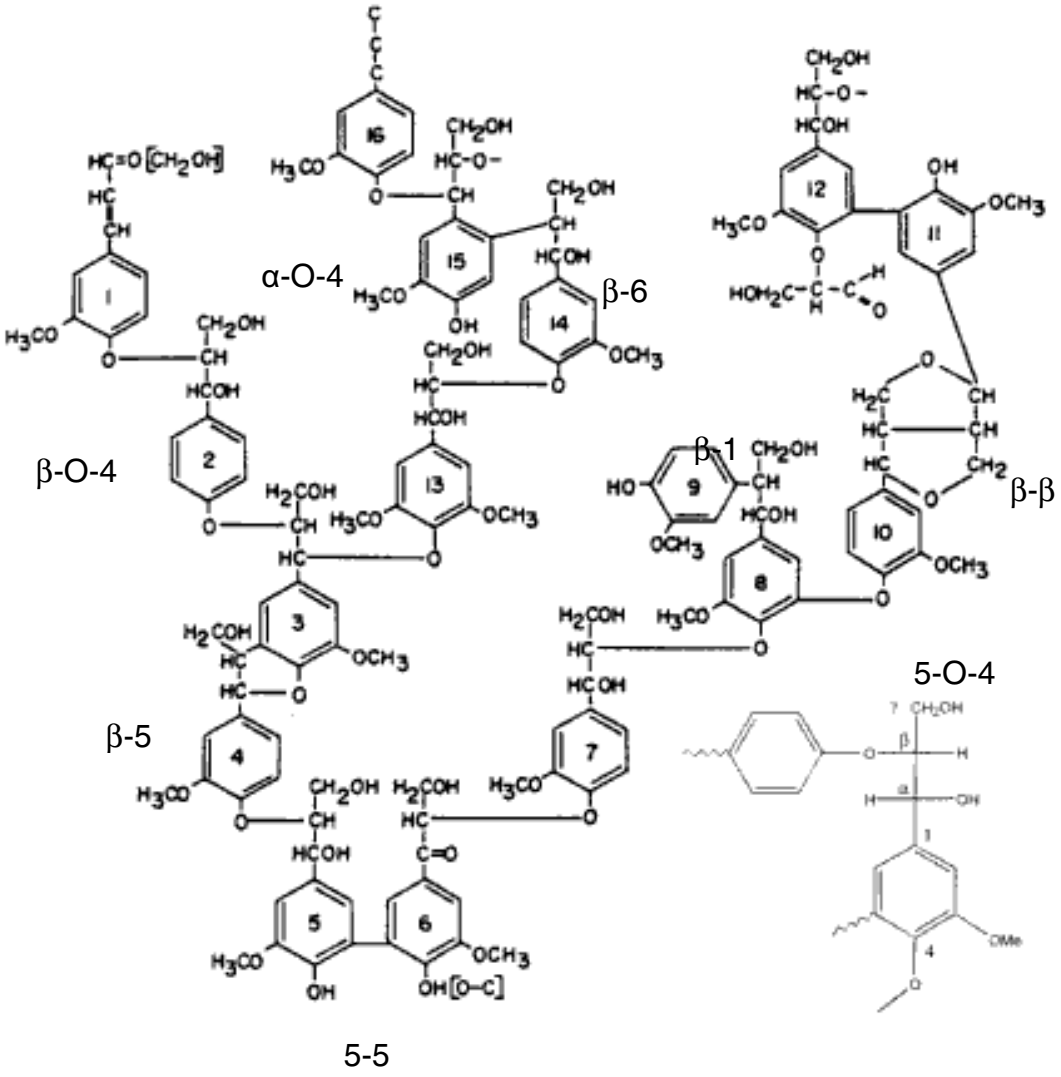

Figure 10. *Structure des lignines et les différents types de liaisons existantes : Molécule 1-molécule 2 : liaison β-O-4, Molécule 3-molécule 4 : liaison β-5, Molécule 5-molécule 6 : liaison 5-5, Molécule 8-molécule 9 : liaison β-1, Molécule 8-molécule 10 : liaison 5-O-4, Molécule 10-molécule 11 : liaison β-β, Molécule 14-molécule 15 : liaison β-6, Molécule 15-molécule 16 : liaison α-O-4.*

Au cours de la lignification, les lignines les plus condensées sont synthétisées en premier dans les points de jonctions tricellulaires et leur formation se propagent sur les zones mitoyennes. Les formes moins condensées se situeraient dans les couches internes de la paroi secondaire enrobant les microfibrilles de celluloses et assurant la rigidité des parois.

2.3 Protéines

De nombreuses études existent sur les protéines de la paroi, compte tenue de leur forme complexe et de leur rôle encore inconnu dans la paroi. Cependant, ils sont classifiés en quatre classes selon leur composition en acides aminés et de leur schéma de glycosylation:

- protéines riches en hydroxyproline (HRGP),
- protéines riches en glycine (GRP),
- protéines riches en proline (PRP),
- et les arabinogalactan- protéines (AGP) (Girault et al. 2000)

Les extensines, protéines structurales majeures des parois des cellules primaires de la plupart des dicotylédones, sont des glycoprotéines riches en hydroxyproline glycosylé avec de l'arabinose, arabinobiose, arabinotriose et rabinotretraose et avec du glucose.

Les AGPs représentent une famille de proteoglycans généralement composées d'une chaîne principale HRGP à laquelle sont ramifiée des 3,6-β-D-galactanes attachés par des liaisons O-glycosidiques. Certains AGPs ont été détectés dans les parois cellulaires lors de l'utilisation d'anticorps monoclonaux tel que JIM8 (Abreu et al. 2004) et anticorps polyclonaux anti 1,6-β-galactotetraosyl (Willats et al. 2000) . Bien que la plupart des AGPs sont solubles, certains pourraient être immobilisées dans les parois cellulaires, et ont un certain rôle dans, ou une certaine influence sur, l'extensibilité de la paroi cellulaire (Girault et al. 2000).

2.4 Minéraux

Les composés minéraux se retrouvent dans les cendres (obtenu par incinération à 550±50℃ dans un four à moufle pendant 4h). Bien que le ur quantité soit faible dans le bois (généralement moins de 1%), les minéraux peuvent avoir un rôle structural (comme le bore et le calcium vus dans les paragraphes précédents). Certains minéraux bien que minoritaires et non structuraux peuvent être détectables dans le xylème suite à leur rôle lors de la croissance de la plante:

- K, ion principal des cellules cytoplasmique qui possède un rôle fondamental dans le processus d'échange transmembranaires
- Mn, Fe, Cu, Zn minéraux qui font parties des constituants de nombreux enzymes.

Les minéraux peuvent donc provenir de la sève mais aussi directement de la paroi lorsque les polymères qu'ils stabilisent sont solubilisés. Le tableau qui suit représente la quantité de divers éléments présents dans les cendres de bois de feuillus

Conc., ppm	Eléments									
400-1000	K	Ca								
100-400	Mg	P								
10-100	F	Na	Si	S	Mn	Fe	Zn	Ba		
1-10	B Ru	Al Pd	Ti Cd	Cu Te	Ge Pt	Se	Rb	Sr	Y	Nb
0.1-1	Cr	Ni	Br	Rh	Ag	Sn	Cs	Ta	Os	
<0.1	Li	Sc	V	Co	Ga	As	Zr	Mo	In	Sb
	I	Hf	W	Re	Ir	Au	Hg	Pb	Bi	

Tableau 1. Classification des divers éléments présents dans les cendres de bois

2.5 Extractibles

Ce dernier terme générique désigne les molécules solubles dans des solvants de différentes polarités (eau, éthanol, toluène, etc…) qui peuvent provenir des différentes parois du bois, des vestiges du métabolisme mais peuvent également être issus des vaisseaux conducteurs. Par conséquent, elles peuvent être d'origines structurales et non-structurales, et même organiques ou non. Cependant ces molécules constituent en majorité des mono ou di ou trimères des polymères principaux de la paroi énumérés précédemment. La quantité et la composition des matières extractibles varient avec l'espèce du bois considérée et la maturité du bois (Mészáros et al. 2007). Ces derniers sont des molécules associés généralement aux constituants structuraux que par des interactions intermoléculaires de faible énergie. Elles sont généralement présentes en faibles concentrations ce qui rend difficile la détermination de leur distribution dans la paroi.

Celles d'origine organique et connues dans le bois se divisent en trois catégories :

- Les composés aromatiques phénoliques ou tanins : ils sont divisés en deux groupes, les tanins hydrolysables et les tanins condensés ; Les tanins ont la propriété de s'associer et de se combiner avec les protéines.
- Les terpènes et terpenoïdes : les terpènes sont des hydrocarbures volatils, polymères de l'isoprène. Les terpenoïdes sont des diterpènes contenant des fonctions acides carboxyliques, ils sont appelés acides résiniques et peuvent représenter 5% des bois résineux (Uçar et al. 2003).
- Les acides gras : dans le bois ils se retrouvent sous forme d'ester de glycérol principalement dans une proportion de 1 à 5%.

3 Formation, structure et organisation de la paroi végétale

3.1 Formation de la paroi

La croissance du bois dans les arbres se manifeste par son allongement (croissance primaire) mais également par l'augmentation du diamètre du tronc (croissance secondaire). Cette croissance primaire est due à l'activité d'un tissu du méristème. Ce méristème situé sur la partie apical des tiges commence à former les tissus de la tige, produit des excroissances qui deviennent des feuilles et commence à former des branches latérales juste au-dessus de l'aisselle de la feuille (jonction de la feuille et de la tige). Pour la croissance secondaire, d'autres méristèmes, ou méristèmes secondaires, contribuent considérablement à la formation de la plante, plus particulièrement chez les arbres et les arbustes. Le cambium vasculaire et le cambium du liège forment des tissus supplémentaires dans le système vasculaire (conducteur) et le système dermique (protecteur) respectivement et sont un autre exemple du remplacement des cellules par ajout (figure 11). Le cambium vasculaire est une couche de cellules méristématiques située entre le xylème et le phloème. Par division longitudinale de ses cellules parallèlement à la surface de la tige, il forme le xylème secondaire ou bois vers l'intérieur et le phloème secondaire vers l'extérieur. Chez les arbres et les arbustes, cette activité peut durer de nombreuses années. Le cambium du liège, situé près de la surface, produit cependant un périderme (écorce) composé en grande partie de cellules de liège subérisées (contenant de la subérine) qui réduisent la perte d'eau ((Mauseth 2003)

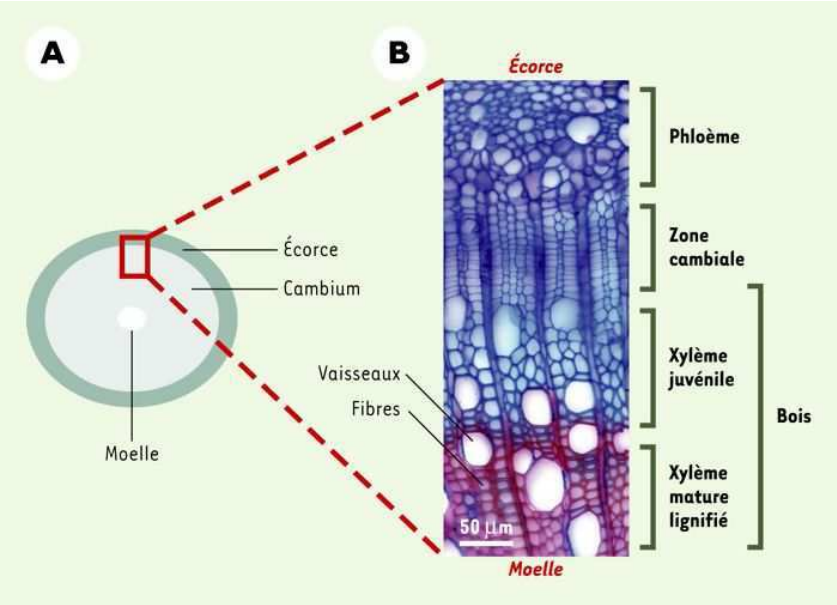

Figure 11. *A. Schéma anatomique d'un tronc d'arbre. Le rectangle correspond à la place de la coupe de la partie B. B. Coupe transversale d'une tige de peuplier après coloration au safranine/bleu astra (photo F. Laurans).*

Dans le bois feuillu, le xylème est formé de cellules spécialisées dans la conduction de la sève brute, ce sont les vaisseaux, tandis que d'autres participent au soutien mécanique de l'arbre, ce sont les fibres. Le phloème quant à lui transporte la sève élaborée (produits de photosynthèse, substances de réserve) du feuillage jusqu'aux racines. Les rayons primaires du cambium produisent des cellules des rayons secondaires (principalement les parenchymes qui servent à emmagasiner les réserves de nutriments).

D'une manière générale, au cours de la phase de croissance, après division cellulaire, une paroi primaire très mince et plastique recouvre le protoplasme de chaque cellule (croissance primaire). Ensuite, la paroi cellulaire s'épaissit et une paroi secondaire se forme (croissance secondaire) alors que la dernière phase du développement cellulaire (lignification) débute avec l'incrustation par la lignine de la lamelle moyenne puis de la paroi primaire. Elle gagne ensuite le reste de la paroi.

3.2 Structure morphologique de la paroi

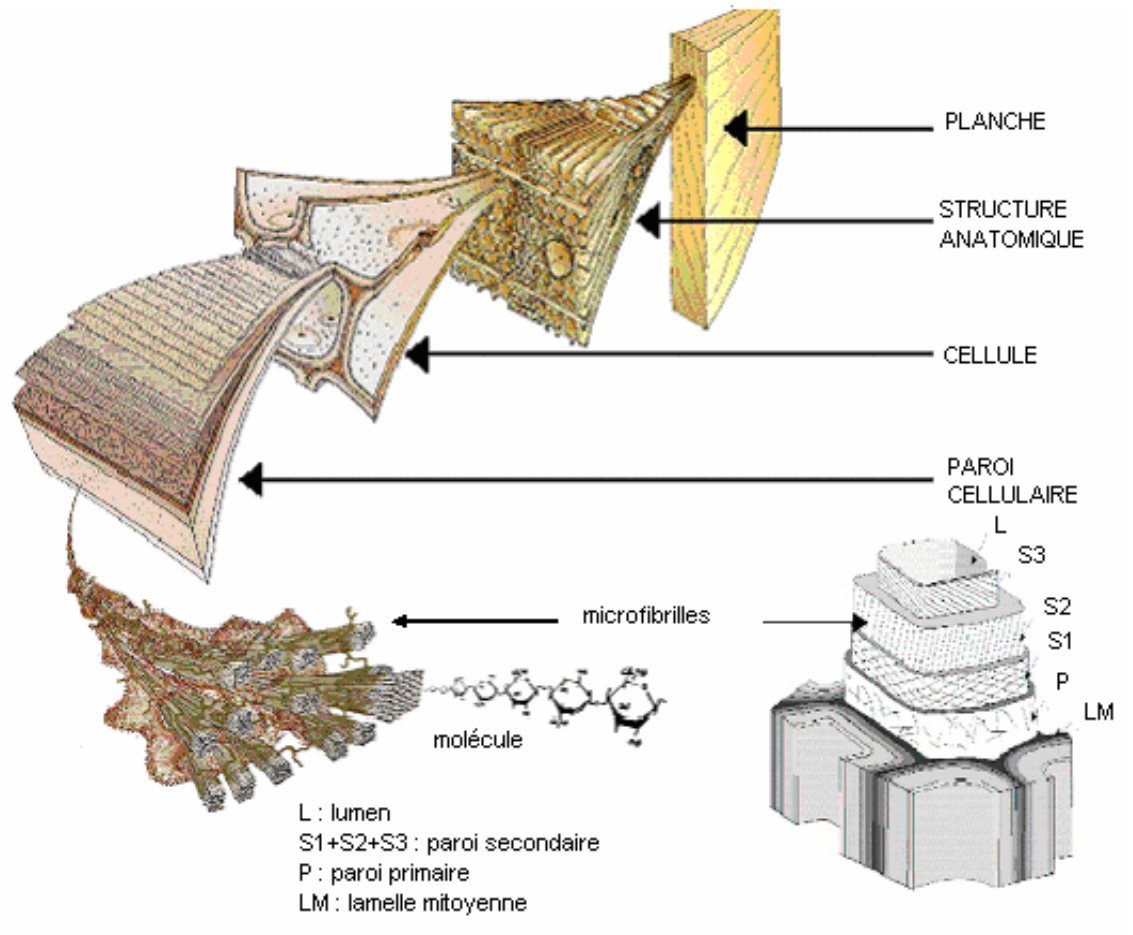

Figure 12. *Changements d'échelle dans le bois, d'après Harington*

On distingue la paroi primaire et secondaire constituée de deux à trois sous-couches S1, S2 et S3 (figure 12), les fibres étant assemblés les une par rapport aux autres par la lamelle moyenne.

- La lamelle moyenne (LM sur la figure 12) est une zone intercellulaire d'épaisseur variant entre 0,2 et 1 µm qui enveloppe entièrement chaque cellule et assure la cohérence de la structure. Aux points de rencontre des fibres adjacentes, il y a accumulation de lignine qui assure le remplissage des angles de jonctions intercellulaires.

- La paroi primaire, très mince, est constituée de microfibrilles orientées de manière aléatoire (figures 12 et 13). L'appréciation de la limite entre paroi primaire et lamelle moyenne est rendue difficile par la très faible épaisseur de la paroi primaire, on l'appelle alors lamelle moyenne composite (LMC).
- La paroi secondaire, d'une épaisseur comprise entre 1 et 9 µm, représente environ 60% du volume de la paroi cellulaire (figure 12). Elle est constituée de trois couches dénommées S1, S2 et S3.
  - La couche S1, d'épaisseur 0,1 à 0,3 µm est composée de deux lamelles microfibrillaires d'orientation hélicoïdales alternées, disposées systématiquement le long de l'axe longitudinal de la cellule. Cette couche renferme le pourcentage de lignine le plus élevé de la paroi secondaire.
  - La couche S2, d'épaisseur 1 à 8 µm est constituée de nombreuses sous-couches dans lesquelles les microfibrilles suivant un angle de quelques degrés par rapport à l'axe longitudinal de la cellule. Cette couche assure la rigidité de la fibre grâce à la présence d'anneaux concentriques de lignine localisés entre les lamelles de S2. La couche S2 représente environ 80% de l'épaisseur de la paroi des fibres. La lignine et les polysaccharides sont liés chimiquement entre eux et constituent dans la matrice de S2 des complexes lignine-polysaccharides ou LCC (Lignine Carbohydrate Complexes).
  - La couche S3 est la couche la plus interne de la paroi, ouverte sur le lumen (figure 13). Elle est plus ou moins épaisse (environ 0,1 µm) selon l'espèce végétale et elle peut quelquefois être absente. Côté lumen, elle est recouverte d'une membrane plus ou moins verruqueuse provenant de la disparition du cytoplasme.

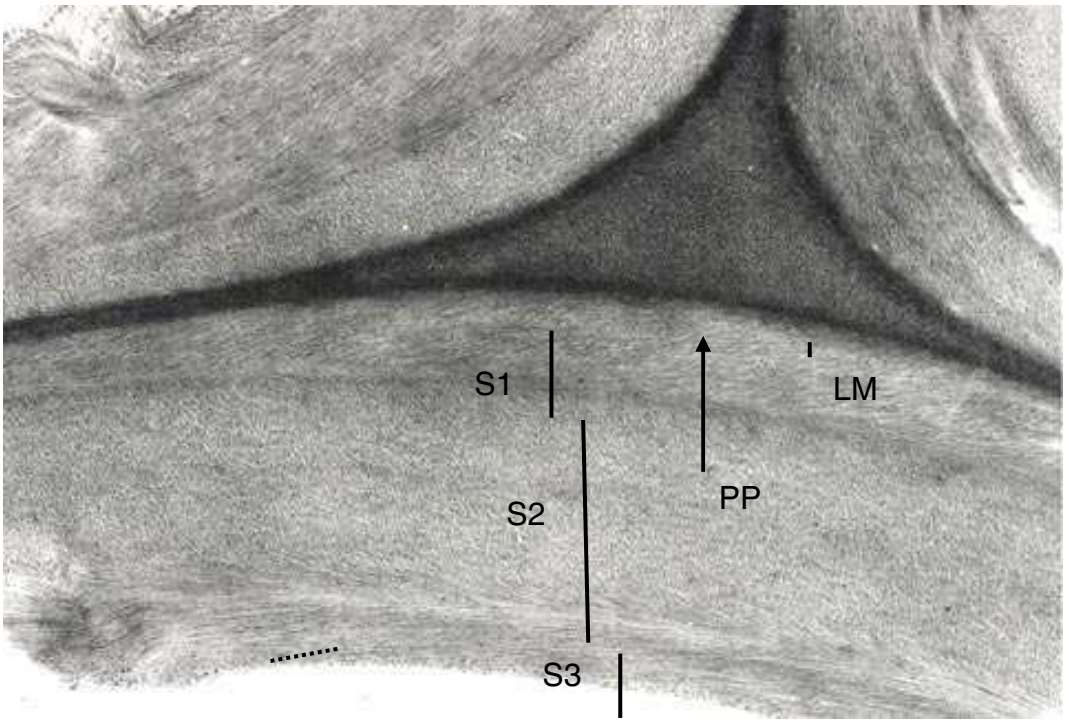

Figure 13. *Microscopie électronique à transmission. Coupe transversale d'une tige de lin: lamelle moyenne (plus sombre et mince), paroi primaire mince (PP, flèche) et paroi secondaire (S1, S2, S3) très épaissie, on peut distinguer par endroit les orientations de microfibrilles (····). D'après Driouich*

Toutefois, cette organisation de la paroi est générale et il existe une variabilité structurale importante des constituants et de leur concentration dans la paroi selon la localisation dans le bois, selon la maturité (âge) de l'espèce et selon l'espèce considérée (bois de tension, bois de compression, bois juvénile, etc…).

3.3 Organisation de la paroi

Les parois cellulaires végétales sont donc constituées de microfibrilles de cellulose incluses dans une matrice de pectines, d'hémicelluloses et de lignine. La figure 14 illustre un exemple de la complexité des structures et des interractions qu'il peut exister au sein d'une paroi primaire.

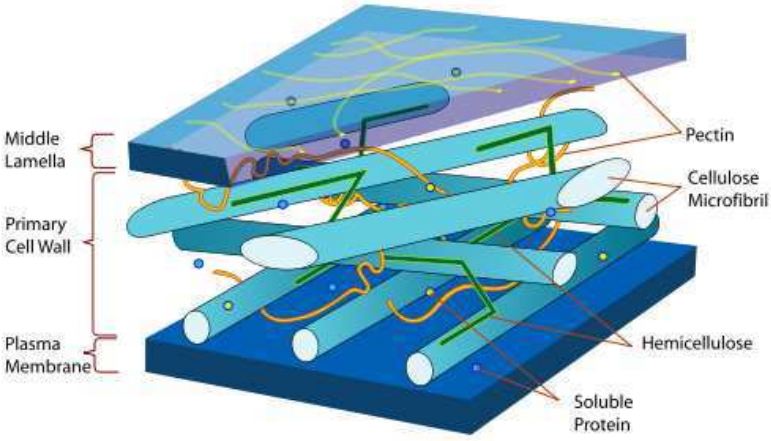

Figure 14. Organisation supramoléculaire de la paroi primaire montrant les différents polymères constitutifs (cellulose, hémicellulose, pectin).

Les différentes macromolécules, détaillées dans les paragraphes précédents, sont étroitement liées en réseau quelque soit leur endroit dans la paroi. Leur cohésion est assurée aussi bien par des liaisons faibles comme des liaisons de Van der Waals et les liaisons hydrogènes que par des liaisons ioniques et covalentes.

De ces interactions dépendent les propriétés des parois.

- *Liaisons covalentes*

  Ce sont des liaisons covalentes lignine-hémicellulose, LCC. La stabilité chimique de telles liaisons et leur résistance vis-à-vis de traitements acides ou alcalins ne dépend pas seulement du type de liaison impliquée mais également de la structure chimique de la lignine et du sucre associé par cette liaison. Les types de liaisons les plus fréquentes sont les liaisons suivantes :

  - Liaisons éthers : elles sont formées chez les bois résineux entre le Cα de la lignine et le C3 (ou C2) des unités L-arabinose des xylanes ou le C3 des Unités D-galactose des glucomannanes. La lignine serait également associée aux polysaccharides pectiques (galactanes et arabinanes) par des liaisons éthers.

- Liaisons esters : elles existent entre le Cα de la lignine et l'acide glucuronique des xylanes de feuillu et résineux.
- Liaisons glycosidiques : elles sont possibles par réaction des extrémités réductrices des hémicelluloses avec le groupement hydroxyles phénoliques des lignines.

- *Liaisons non covalentes*
  - Les chaines d'acide polygalacturonique des polymères pectiques peuvent s'associer par l'intermédiaire d'ions Ca^{2+} (cf paragraphe pectines).
  - Des liaisons hydrophobes sont impliquées dans la cohésion de la lignine mais également entre la lignine et d'autres molécules hydrophobes comme les protéines.
  - Les liaisons hydrogènes permettent la stabilisation des microfibrilles de cellulose (liaisons intrachaines) mais aussi l'association des microfibrilles entre elles (liaisons interchaines).

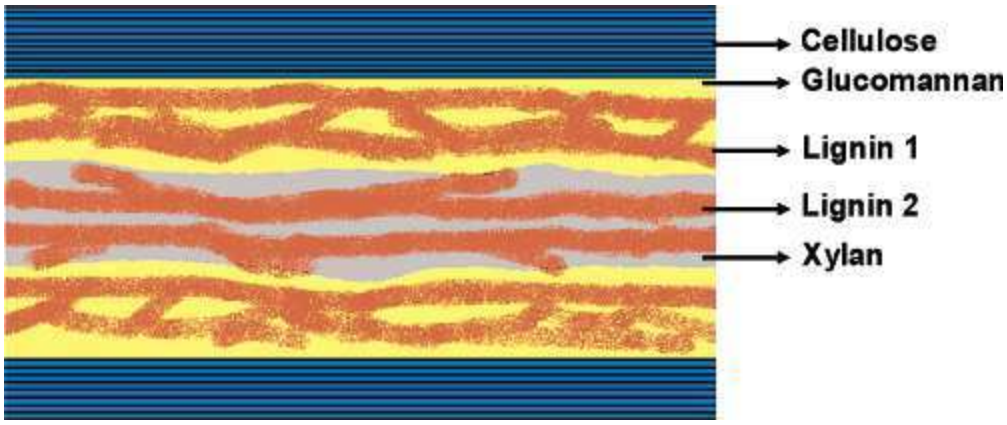

Figure 15. *Vue schématique de l'organisation pariétale et des interactions qui peuvent exister au sein d'une paroi de bois d'épicea d'après (Lawoko et al. 2005)*

4 Répartition des différents composés de la paroi

Les trois constituants structuraux du bois, cellulose, hémicelluloses et lignine ne sont pas distribués uniformément dans les différentes sous-couches du bois, et leurs proportions relatives peuvent varier considérablement selon la région morphologique et l'âge du bois (figure 16). La connaissance de la répartition exacte des principaux constituants à l'intérieur des couches de la paroi cellulaire permettrait de mieux comprendre les propriétés chimiques et physiques du bois.

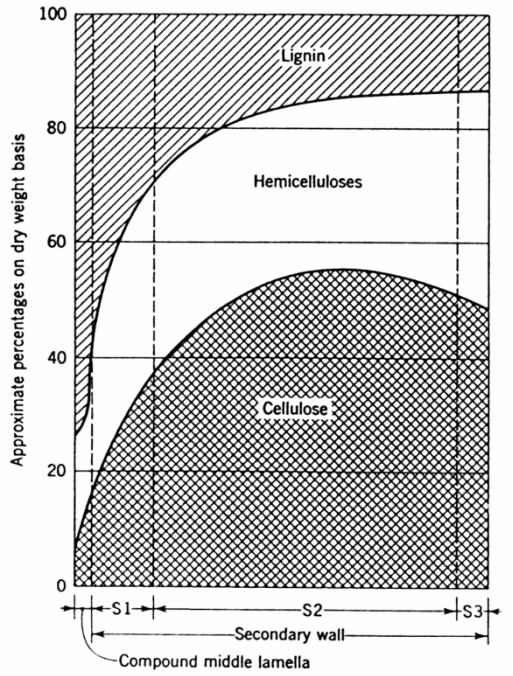

Figure 16. *Répartition des trois composés principaux du bois dans les différentes couches de la paroi végétale*

La lignine présente une distribution hétérogène tant par sa nature que par sa concentration à l'intérieur des couches de la paroi cellulaire. Les lignines de bois feuillus ne sont pas formées uniquement d'un copolymère de monomères G et S mais des fractions de lignines G et S prédominent selon les types cellulaires. Sur le bouleau

(tableau 2), la paroi secondaire des fibres et des rayons ligneux renfermeraient essentiellement des lignines de types S alors que celle des vaisseaux serait constituée principalement de lignine de type G, de même que la lamelle moyenne (Goring 1985). L'utilisation de marqueurs immunocytochimiques en microscopie UV a également révélé l'hétérogénéité de la distribution quantitative des lignines dans les différents tissus des fibres longues de chanvre (Cronier et al. 2005).

Région morpho.	LM+P	S1	S2	S3	LM+P	S1	S2	S3	LM+P	S
Vol. de tissu dans le bois (%)	7,6	11,4	58,5	3,5	0,8	1,6	4,3	2,3	2,0	8,0
Concentration en lignine (%)	0,36	0,14	0,14	0,12	0,40	0,26	0,26	0,27	0,38	0,12
Elément	Fibre				Vaisseau				Rayon ligneux	

Tableau 2. Distribution de la lignine dans le bouleau, d'après Goring, 1985

La variabilité de la distribution des polysaccharides à l'intérieur de la paroi cellulaire a également été mise en évidence par Imai (Imai et al. 1999) par une méthode de marquage topographique par microauto-radiographie des polysaccharides. Ce marquage a été réalisé dans les cellules matures grâce à la radioactivité incorporée dans les polysaccharides.

Certains polysaccharides ont été recensés dans les principales couches constituant la paroi des fibres de bouleau et d'épicéa (tableau 3, (Meier 1961)). D'après ces données, les galactanes et arabinanes se trouvent principalement dans la paroi primaire alors que les glucuronoxylanes et la cellulose sont plus présents dans la paroi secondaire pour ces deux essences.

Polysaccharides	Paroi primaire	Paroi secondaire (%)		
	%	S1	S2 (paroi externe)	S2+S3 (paroi interne)
Bouleau				
Galactane	16,9	1,2	0,7	0,0
Cellulose	41,4	49,8	48,0	60,0
Glucomannane	3,1	2,8	2,1	5,1
Arabinane	13,4	1,9	1,5	0,0
Glucuronoxylane	25,2	44,1	47,7	35,1
Epicea				
Galactane	20,1	5,2	1,6	3,2
Cellulose	35,2	61,5	66,5	47,5
Glucomannane	7,7	16,9	24,6	27,2
Arabinane	29,4	0,6	0,0	2,4
Arabino-glucuronoxylane	7,3	15,7	15,7	19,4

Tableau 3. Distribution des polysaccharides dans les différentes couches de la paroi de fibres du bois, d'après Meier, 1961.

5 Propriétés mécaniques du bois

Le bois est un matériau qui présente un comportement complexe lié à sa nature composite. Il peut être considéré comme une superposition d'une matrice amorphe composée de lignine et hémicelluloses et un renforcement de semi-cristallinité par des fibres de cellulose.

Pour de faibles sollicitations, le bois a un comportement linéaire élastique. Pour des sollicitations plus importantes, la limite élastique est dépassée et le matériau présente une déformation permanente. La zone plastique est atteinte. Lorsque la déformation permanente est mesurable, des fractures microscopiques apparaissent dans le bois. Si la contrainte devient trop importante, la déformation permanente peut être visible à l'échelle macroscopique(Stanzl-Tschegg 2006).

Les propriétés à rupture du bois sont encore mal comprises puisqu'elles dépendent de facteur naturel lié à la nature de l'essence et à l'origine géographique du bois, de présence de défaut et des procédés technologiques de transformation. Cependant, quelques travaux sur la modélisation de la propagation de fracture selon le pourcentage de cellulose et sur l'effet de l'orientation des microfibrilles ont été réalisés (Åkerholm et al. 2001; Färber et al. 2001; Hinterstoisser et al. 2001; Keckes et al. 2003; Abe et al. 2005). Il a été démontré que la rigidité de la cellulose est primordiale dans les propriétés mécaniques de la paroi ainsi que l'élasticité longitudinale est déterminée par l'orientation des microfibrilles dans la couche S2. D'autres paramètres influencent la mécanique du bois, comme la densité du matériau ainsi que son contenu en lignine (Bardet et al. 2003).

La description du déplacement de la fracture à l'échelle de la paroi manque de références mais reste décrite de manière schématique.

Schématiquement les directions du matériau sont la direction longitudinale (L) des trachéides ou des fibres, la direction radiale (R) du rayon et la direction tangentielle (T) aux anneaux de croissance. (Fig. 17 (a)). En conséquence, il ya six principaux systèmes de propagation de la fissure, nommé par une paire de lettres: TL, RL, LR, TR, RT et LT (Fig. 16 (b)). La première lettre donne la direction de la normale au plan de fissure et la seconde lettre indique la direction de propagation des fissures. La propagation des fissures dans le plan LR ou LT (à savoir, à travers les fibres de bois) est rarement observée. Parmi les autres systèmes de propagation de la fissure (par exemple, le long des fibres de bois), les systèmes RL et TL systèmes constituent les plus fréquents en raison de la conception particulière des structures de bois (Silva et al. 2006).

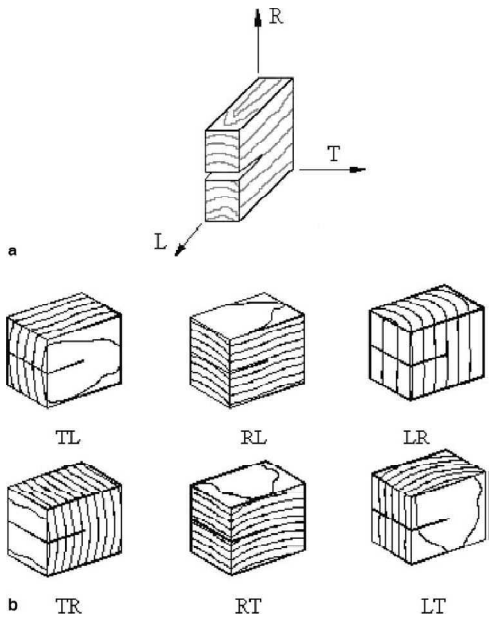

Figure 17. *(a) Image référence LRT, (b) principaux systèmes de propagation de fissure dans le bois.*

La fracture peut être longitudinale trans-pariétale, intra-pariétale, intercelullaire et transverse trans-pariétale (figure 18). Celle trans-pariétale est caractéristique des cellules à paroi minces comme le parenchyme ou vaisseaux des bois durs. Dans ce cas la fracture casse la couche mince des fribrilles et laisse apparaitre une surface lisse (a). La fracture intra-pariétale est caractéristique des cellules à paroi épaisse et petit diamètre. Dans ces cellules, qui ont une couche S2 résistante, la propagation des fissures se fait préférentiellement sur un plan entre S1 et S2 plutôt qu'à travers les fibrilles (b). Lorsque le bois est sec et la force appliquée est lente, la fissure passe à travers la région lamelle moyenne/ paroi primaire fortement concentré en lignine, pour donner une rupture intercellulaire (c). Selon le bois considéré et la sollicitation appliquée, la fracture conduit à des surfaces différentes en termes de nature et morphologies. Selon sa propagation, il apparait une surface plus ou moins rugueuse (River et al. 1991).

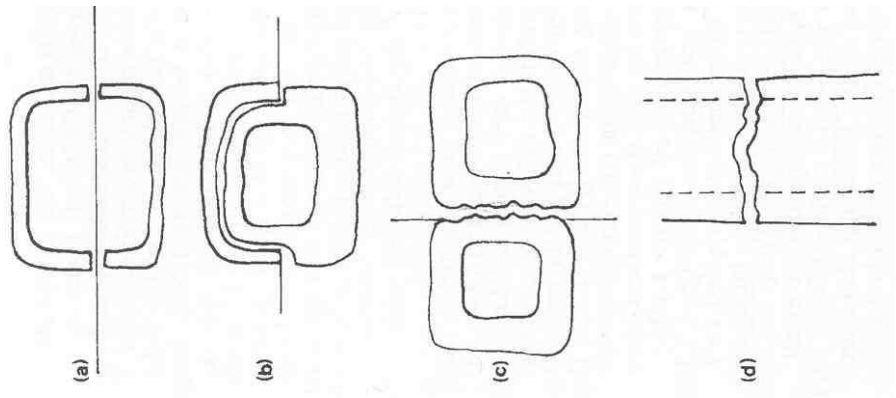

Figure 18. *Schéma de la localisation de la fracture dans le bois (a) longitudinal trans-pariétale, (b) intra-pariétale, (c) intercelullaire et (d) transverse trans-pariétale d'après River et Vick (1991).*

La propagation des fissures est décrite dans la littérature, soit comme issue d'une modification chimique (présence de défaut ou dégradation des matériaux) soit comme une modification de la mobilité d'une famille de macromolécules. La première explication appelle à une chute de la cohésion au niveau des zones de défauts bien déterminées dans la paroi. La seconde peut conduire à un déplacement de la zone de contraintes (Green 1999). De plus, la diminution de mobilité peut également modifier le mécanisme de rupture de la famille de macromolécules en question. Le passage d'une rupture ductile (dans le domaine plastique) à une rupture fragile (dans le domaine élastique) peut perturber la nature de la zone d'amorçage de la rupture mais également sa zone de propagation(Green 1999).

Pour la fabrication de pâtes à papier par exemple, la préparation consiste à isoler les fibres et préserver intacts leurs propriétés mécaniques et morphologiques, en cherchant à éliminer la lignine qui sert de 'matière collante'. Pour cela, il existe deux procédés principaux:

- La voie mécanique : dans ce cas, les composés organiques du bois, la cellulose, les hémicelluloses et la lignine, sont préservés. Néanmoins, certains produits, en bonne partie des matières extractibles (résines, tannins, colorants, cires, alcaloïdes, etc.), s'évaporent. En effet, que ce soit par l'utilisation d'un défibreur (meule) ou d'un raffineur (disques), les températures dépassent aisément les 100℃ à cause du frottement.
- La voie chimique : cuisson du bois par des traitements acides (bisulfite) ou alcalins (sulfate) et ajout de produits chimiques pour dissoudre la lignine et récupérer les fibres de cellulose.

Dans l'industrie pour la fabrication de pâte à papier, le procédé continu d'obtention de pâte mécanique de raffineur ou défibreur à disques sous pression de vapeur est utilisé. Ce procédé est à l'origine des pâtes TMP [Pâte thermomécanique] et CTMP [pâte chimico-thermomécanique].

Les connaissances sur la fracture des parois végétales restent primordiales pour la maitrise des processus de transformation de cette ressource tels le défibrage ou le raffinage des copeaux de bois et permettre leur utilisation dans de nouvelles applications.

Ainsi, l'étape de séparation des fibres est essentielle car la nature de la surface extérieure des fibres va déterminer les caractéristiques mécaniques et physico-chimiques des fibres obtenues et ainsi trouver les meilleures conditions pour leur application dans les matériaux composites. La localisation de la fracture entre les fibres dépend des différentes caractéristiques de chaque couche de la paroi et des stress qui leur sont appliqués.

A basse température, la lignine est rigide et les fractures se développent au hasard, le long de la fibre et au travers de la paroi ce qui conduit à une grande quantité de fibres 'cassées'.

A plus haute température, la zone de fracture est déplacée vers l'extérieur de la fibre près de la paroi primaire et de la couche S1 de la paroi secondaire ce qui conduit à l'obtention d'une plus grande proportion de fibres longues.

Enfin, avec un traitement chimique avant de défibrer, les propriétés des lignines sont altérées et sa température de transition vitreuse est plus faible, ce qui améliore la séparation des fibres. Certains traitements (à l'oxalate par exemple) facilitant la

séparation des fibres sont connus pour changer les propriétés des polymères *in situ* (Sugiyama et al. 2003; Xie et al. 2004; Kenealy et al. 2007; Kenealy W. et al. 2007; Jiang et al. 2008).

La température est donc un paramètre physique très important, qui conditionne le comportement mécanique du bois car le comportement rhéologique du bois en fonction de la température est lié au comportement viscoélastique des constituants de base. Pour la fabrication de pâtes à papier, les fibres du bois sont individualisées par le passage de copeaux entre les disques du défibreur où ils sont soumis à des forces de compression et de cisaillement.

Le ramollissement des polymères amorphes du bois dépend également du pourcentage d'eau qu'il existe au sein du matériau. La revue bibliographique de Green et Kretschmann (Green et al. 1994), sur la plupart des propriétés du bois à différents taux d'humidité, informe que dans le domaine de service des structures poreuses (entre le point de saturation et 7%-8% de la teneur en eau), toutes les propriétés du bois (la résistance en traction, compression, flexion, cisaillement, le module d'élasticité et la résistance en rupture) progressent quand le bois sèche. Mais lorsque l'humidité oscille entre 12 et 20 % dans le bois, sa structure voit certaine de ces propriétés varier de presque 50 %.

L'eau, la température ainsi que les traitements chimiques (extractions) vont donc modifiés le comportement viscoélastique du bois au cours du défibrage (Åkerholm et al. 2001; Olsson et al. 2004).

6 Propriétés viscoélastiques du bois

Les propriétés viscoélastiques sont décrites par les mobilités moléculaires de chaque constituant amorphe de la paroi. La connaissance du comportement rhéologique de ces constituants est un point essentiel pour la maitrise des procédés de transformation du bois.

La mobilité moléculaire est déterminée par le degré de l'ordre (il est moins important dans un état plus rigide), elle dépend des associations qui peuvent être développées entre les molécules. Par exemple, la mobilité diminue avec la masse moléculaire et le degré de réticulation, elle augmente avec la teneur en eau ou en fonction de la teneur d'autres espèces plastifiante. C'est la mobilité moléculaire de chaque constituant qui détermine les différentes propriétés macroscopiques accessibles de la plante permettant de mieux comprendre la structure et le rôle des différents composants du 'ciment' pariétal.

Le bois possède une structure hétérogène. Les propriétés de ses composés amorphes de la paroi cellulaire sont intéressantes pour l'étude de modification chimique ou physique légère qui pourrait faciliter ou améliorer le défibrage ou le raffinage des copeaux de bois. En augmentant la température, le ramollissement de ces composantes est observé, soit le passage d'un état vitreux, rigide et cassant à un état caoutchouteux, où les chaines sont plus mobiles. Ainsi, l'assouplissement du matériau se caractérise par une température spécifique : la température de transition vitreuse, Tg. Les propriétés mécaniques du bois sont affectées par la transition vitreuse de chaque composant amorphe, qui elle-même, est influencé par la température, l'humidité et l'échelle de temps de l'expérimentation (cf paragraphe précédent). En d'autres

termes, les conditions expérimentales sont d'une importance primordiale lors d'analyse de transitions observées.

Pour mette en évidence cette transition dans un matériau, il est parfois nécessaire de monter très haut en température, ce qui peut conduire à la dégradation des matériaux. Pour rendre plus accessible la mesure d'une transition, l'ajout de plastifiants qui sont en général des molécules de petite taille qui ont pour rôle de gonfler la matrice, permet le glissement des chaînes moléculaires. La température de transition vitreuse pour des molécules plastifiées apparait à des plus faibles températures. Des travaux menés précédemment (Salmén 1982) ont permis de tracer l'évolution de ces transitions vitreuses pour les polymères amorphes de la paroi en fonction du pourcentage d'eau (plastifiant) dans les échantillons (figure 19).

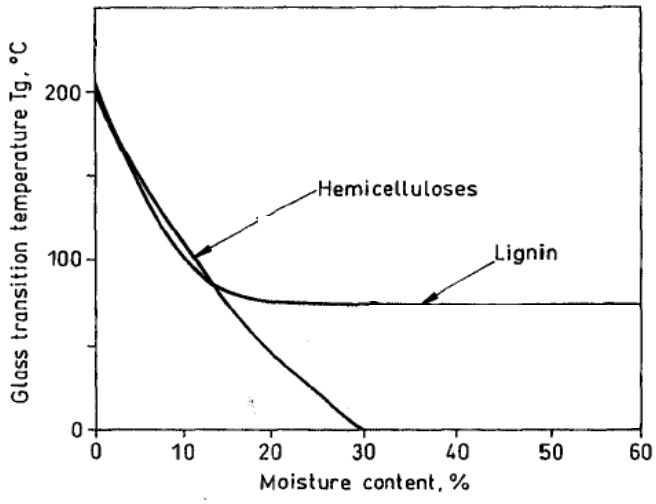

Figure 19. *Evolution des températures de transitions vitreuses pour les lignines et hémicelluloses en fonction du pourcentage d'eau dans le matériau*

Salmen observe une forte diminution de la Tg des hémicelluloses avec le taux d'humidité dans le matériau ainsi qu'une forte diminution de la Tg des lignines jusqu'à un certain seuil d'humidité où les valeurs atteignent un palier autour de 80°C.

Les facteurs qui affectent la mobilité des polymères sont :

- La maturité et les espèces : l'étude de Lenth sur le pin et le peuplier a mis en évidence les effets d'espèces et de maturation sur la position des transitions vitreuses des hémicelluloses, leur sensibilité à l'eau, leur activation thermique et même le sens de la variation de la mobilité selon l'espèce considérée (Lenth et al. 2001).
- La plastification *in situ* : l'eau reste le plastifiant naturel le pus approprié pour la plastification des polysaccharides due à leur caractère hydrophile. Cependant les lignines semi polaire ont fait l'objet de plus vastes études quant à la nature de leurs plastifiants (Bouajila et al. 2006). Le système le plus utilisé après l'eau est l'éthylène glycol qui présente un paramètre de solubilité plus proche des lignines que l'eau et une fonctionnalité adaptée à l'établissement de liaisons hydrogène. Salmén l'utilise en tant qu'agent gonflant pour caractériser les lignines à basse température en immersion (Salmén et al. 1996).
- Les extractibles : Matsunaga a étudié l'effet de leur introduction dans la paroi, contrairement à une extraction qui peut conduire également à une modification et une extraction de polymères. (Matsunaga et al. 1999). Il laisse suggérer que les extractibles auraient un effet plastifiant des polymères de la paroi. De plus dans la fabrication de pâtes à papier leur élimination par voie chimique améliore l'obtention des fibres cellulosiques.

Les derniers paragraphes décrient l'état des connaissances générales et actuelles sur le bois. Dans la suite du manuscrit, nous nous intéresserons particulièrement à l'objet de cette étude : la tige de chanvre dépourvue de fibres longues et assimilée au bois.

7 Le chanvre

Angiosperme dicotylédone de la famille des Cannabinaceae, le chanvre est divisé en 4 sous-espèces : Sativa, Indica, Afghanica, Ruderalis. Parmi ces dernière se trouve le chanvre cultivé Canabis Sativa, Les variétés cultivées aujourd'hui en France ont des teneurs en delta-9-tétrahydrocannabinol (THC) à caractère psychotrope, extrêmement faibles. En 2008, en France, le taux de THC devrait être inférieur à 0,2 % conformément à la règlementation européeenne. En Europe du Nord, les plantes de chanvre peuvent atteindre jusqu'à 2,5 m de hauteur selon le cultivar, la lumière du soleil et les conditions météorologiques.

7.1 Morphologie de la plante

Le chanvre (*Cannabis sativa L.*), est une plante annuelle herbacée à feuilles palmées, contenant des vaisseaux dans le cœur ligneux (xylème) comme le bois dur.

Il est cultivé pour sa tige qui contient les fibres longues (la filasse) sur les parties externes et qui est constituée d'une forte proportion de fibres intérieures plus courtes fortement lignifiées (son bois). Cette tige, une fois dépourvu de ces fibres longues récupérées par traitement mécanique donne la chènevotte (bois de chanvre).

La filasse qui représente environ 30% de la masse sèche de la tige est utilisée dans le textile, les textiles techniques, le papier et les matériaux de construction et d'isolation.

Le bois de chanvre quant à lui, représente environ 70% de la masse de la tige. Il est également utilisé dans les matériaux de construction (béton de chanvre), les litières animales ainsi que pour le paillage des sols.

Ses graines sont également valorisées pour leur caractère nutritif ; la graine entière est riche en protéines (25%) en glucides (30%) ainsi qu'en fibres insolubles, carotène, potassium, magnésium, soufre, calcium, fer et zinc, vitamines E, C, B1, B2, B3 et B6 (~15%). Ainsi, de ses graines est extraite l'huile de chanvre utilisée dans l'alimentation, les cosmétiques et même les produits techniques tels que les peintures à l'huile ou vernis.

Ses fleurs et feuilles sont généralement utilisées dans le domaine médical. Par exemple, une étude, réalisée en Suisse, a mis en évidence l'efficacité des extraits de cannabis pour la relaxation musculaire et contre la douleur de malades atteints de la sclérose en plaque.

7.2 Anatomie de la tige

La tige (figure 20) se compose d'un cylindre creux comportant le xylème de 1-5 mm d'épaisseur couvert par le cambium de 10-50 µm, le cortex de 100-300 µm, l'épiderme de 20-100 µm et la cuticule de 2-5 µm (Garcia et al. 1998).

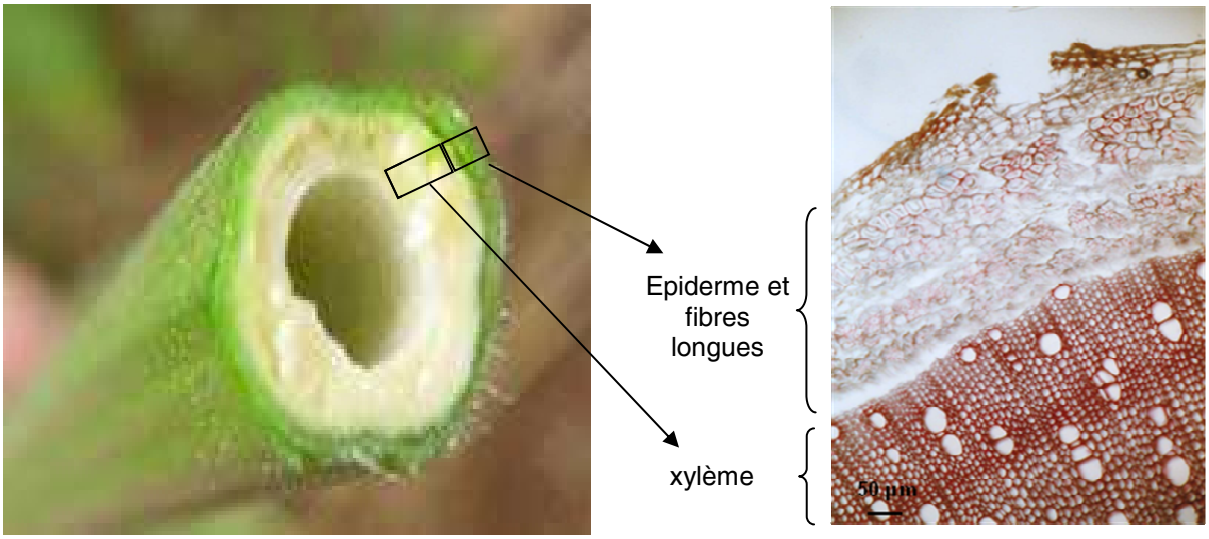

Figure 20. *A gauche, tige de chanvre la chenevotte étant issue de la partie centrale. A droite, coupe transversale d'une tige de chanvre d'après Chabbert*

Les fibres longues et souples sont situées au niveau de l'écorce (cortex) sur la surface de la tige, en périphérie des tissus conducteurs. Elles ont un rôle de maintien de la tige. Elles peuvent facilement être décollées de la surface du xylème à la main ou à la machine (figure 20, gauche).

L'examen au microscope d'une tige de chanvre permet de distinguer quelques zones concentriques (figure 20, droite). La tige présente une couronne de faisceaux de fibres localisée entre les tissus périphériques de l'écorce et les tissus du cylindre central formés, de l'extérieur vers l'intérieur, par le phloème secondaire, le cambium vascualire et le xylème secondaire (càd le bois).

7.3 La chenevotte

Obtenue par défibrage mécanique, la chènevotte est la partie centrale et moelleuse de la tige de chanvre (figure 20). Elle représente des morceaux de bois hétérogène de la tige de chanvre. On l'assimile ses propriétés à celles des bois durs.

La chènevotte représente environ 45% du poids de la paille. Cette dernière contient typiquement 37 à 40% de cellulose, 16 à 20% d'hémicelluloses, 17 à 22% de lignines et un faible pourcentage d'extractibles. Ces extractibles sont composés d'oligomères d'hémicellulose et lignine ainsi que d'autre composés tel protéines, lipides, cires, cendres.

La chènevotte correspond à la partie qui a véhiculé la sève pendant la période de croissance du chanvre, elle est donc très hydrophile et peut contenir jusqu'à 4 fois son poids en eau. Ces utilisations sont principalement dans les matériaux de construction.

Bien être animal	Matériaux de construction	Autres usages
Litières pour animaux: chevaux, bovins, etc.	<u>Voie sèche</u> Remplissage d'espaces vides pour isolation (plancher-plafond), Panneaux d'agglomérés, Mélange avec du bitume pour construction des chapes isolantes. <u>Voie humide</u> Briques de chanvre, Blocs à maçonner, Remplissage d'espaces vides, Cloisons, Crépissage, etc.	Paillage, Combustible.

Tableau 4. les principales utilisations actuelles de la chènevotte

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9 Présentation du sujet

Dans le contexte précédent, les travaux réalisés au laboratoire en 2005 sur le bois de peuplier ont permis de mettre en évidence l'impact de traitements acides sur le défibrage des copeaux de bois et de démontrer l'existence d'un gain d'énergie lors du processus de raffinage dans la fabrication de pâte à papier. Parmi les divers mécanismes expliquant ce phénomène, la participation des extractibles comme plastifiants des polymères pariétaux a été proposée.

Résumé des travaux de Valérie Meyer :

Valérie Meyer démontre que l'imprégnation des copeaux de peuplier par de l'oxalate de sodium à pH 2.5, entraîne un gain d'énergie de raffinage de 20%. Parallèlement, les résultats en biochimie montrent une diminution du pourcentage de pentosanes. Cette dépolymérisation partielle des hémicelluloses est supposée être la cause de la diminution de la consommation d'énergie lors du processus de raffinage. La composition en lignines des copeaux raffinés, montre un pourcentage élevé de monomères syringyl liés en β-O-4 supposant un meilleur accès des agents de thioacidolyse aux lignines. L'analyse des fibres au SEM montre que les fibres issues des copeaux prétraités à l'oxalate présentent une augmentation de la fibrillation des parois secondaire et/ou primaire.

Relation entre la mobilité des polymères *in situ* et énergie de raffinage :

Il a été démontré que lors du 'pulping' mécanique, la température de relaxation (ramollissement) des lignines est d'une importance primordiale pour la qualité du 'pulping' et pour la consommation d'énergie de raffinage. En augmentant la température et en diminuant la rigidité du bois, la zone de fracture est progressivement déplacée de

la paroi secondaire à la primaire pour finalement être dans la lamelle moyenne. Paradoxalement, l'augmentation des liaisons hydrogène entre les fibres (qui est un important paramètre pour la qualité de pulping) est possible, si les fibres ont un pourcentage maximum en polysaccharides à leur surface. Le processus de raffinage a un impact considérable sur la disponibilité des interactions fibre/fibre du papier. D'après leur structure morphologique, les fibres doivent être séparées dans la paroi secondaire (s1 et s2). C'est pourquoi, le raffinage des copeaux de bois, doit être aussi sélectif que possible. Un grand challenge est de contrôler le processus de raffinage.

Nous supposons donc que ces modifications d'interactions entre polymères ont un impact sur la cohésion pariétale, sur les propriétés mécaniques d'un matériau et par conséquent sur son mode de fracture.

Il est donc intéressant de réaliser le même type de recherche sur la chènevotte afin de revaloriser son utilisation. Par ce type d'approche, on pourra déterminer une corrélation entre structure et propriétés mécaniques des polymères de la chènevotte.

Cette étude se focalise sur l'impact de traitements d'extraction sur les propriétés mécaniques à rupture de la chènevotte. Dans le cadre de ce projet, nous testons l'hypothèse d'une relation entre ces traitements et les variations de température de transition vitreuse de la lignine : ces variations sont susceptibles d'influencer les propriétés à rupture du bois dans des conditions de température et d'humidité proches de la zone de transition vitreuse de la lignine et proche des conditions d'usage dans le défibreur pour la séparation des fibres. La qualité des fibres d'usage dans la chènevotte étant optimale si la rupture a lieu préférentiellement au niveau de la lamelle moyenne.

Ce travail est constitué dans un premier temps de l'analyse des composés extractibles et de l'impact de leur retrait sur la mobilité moléculaire des polymères amorphes constitutifs de la paroi. Dans la logique du raisonnement précédent, les conséquences sur le mode de rupture des morceaux de chènevotte seront étudiées dans une seconde partie. La troisième partie traitera de corrélations possibles entre les différentes variables (solvants) et leurs réponses associées (composition, comportements viscoélastique et mécanique). Enfin, une dernière partie sera consacrée à un traitement type de référence.

Chapitre II :

Viscoelastic properties of cell wall polymers *in situ* of woody hemp core,
Article accepté pour publication dans Holzforschung, Juin 2010

Le travail présenté dans ce chapitre porte sur les effets d'extractions appliqués à la chènevotte et sur les interactions entre polymères au sein de la paroi. Pour déterminer les variations de propriétés viscoélastiques, une méthodologie originale et fiable a été développée, utilisant un échantillonnage spécifique couplé à des analyses en DMA et DEA. Ces deux techniques renseignent sur la mobilité moléculaire et les relaxations structurales des polymères par l'application d'une déformation pour le DMA ou d'une tension pour le DEA pour des matériaux qui présentent un moment dipolaire permanent. Les polymères modifiés par les traitements étant les polymères amorphes de la paroi cellulaire, on s'intéresse ici plus particulièrement aux hémicelluloses et aux lignines.

Chapitre III

Fracture behaviour changes after selective removing of cell wall extractives from woody hemp core,
Article en révision pour l'anglais

La première partie de ce chapitre est consacrée à la recherche de la zone de température de transition (pour le test de caractérisation concerné) de l'état fragile à ductile d'échantillons de chènevotte que l'on attribue essentiellement à la transition vitreuse de la lignine qui constitue le ciment dans la matrice.

Par la suite, des tests mécaniques sur des échantillons traités avec différents solvants seront réalisés dans cette zone de température en adaptant la méthodologie décrite précédemment à ce type de test à rupture. Le but de ces tests est d'observer les modifications apportées par les solvants sur les modes de fracture dans les conditions sélectionnées.

Chapitre IV

Relationships between biochemistry, modulation of cell wall viscoelastic properties and fracture behaviour in woody hemp core by correlation study,
Article en révision pour l'anglais

Ce chapitre reprend les résultats des deux chapitres précédents et traite d'une manière plus approfondie les corrélations entre les différentes réponses mesurées (composition chimique, propriétés viscoélastiques, paramètres de mécanique à rupture) selon les solvants d'extractions considérés.

Cette étude a été réalisée à l'aide d'un outil mathématique développé pour les analyses en composantes principales (the Unscrumbler) et aussi à l'aide de calculs sur les indices de corrélation que propose Excell.

Chapitre V

Polymer mobility in lignified cell walls impregnated by strong mineral acids,
Article en voie de soumission dans Wood Science and Technology

Les traitements décrits dans les parties précédentes sont des traitements limités à de faibles taux d'extraction, qui retirent principalement des entités de faibles masses de la paroi végétale et qui impliquent très peu de modifications chimiques au sein des structures. Cette partie sera consacrée par conséquent à un cas plus complexe et plus courant d'extraction à l'acide chlorhydrique qui aurait à priori des impacts majeurs sur les structures et sur la quantité de 'ciment' dans le matériau. L'objectif de ce chapitre est de mettre en évidence l'effet de changements des variables prépondérantes (par la création de défauts chimiques) sur les propriétés mécaniques de la chènevotte.

Ce manuscrit se terminera sur un résumé des principales conclusions qui ressortent de cette recherche. La dernière partie dressera une liste de perspectives à envisager pour la suite des travaux sur les extractibles et leur rôle dans la paroi.

CHAPITRE II

Viscoelastic properties of *in situ* cell wall polymers of woody hemp core

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Key words: dynamic mechanical analysis (DMA), dielectric analysis (DEA), extractives, glass transition temperature (Tg), hemicelluloses, hemp core (chènevotte) lignin, viscoelastic properties, wood

ABSTRACT

The aim of this work was to determine the effect of removing extractives from the woody core of hemp (chènevotte) on the chain mobility of hemicelluloses and lignin, which may react during technological transformation like defibering and/or composite materials elaboration. Extractives are low molecular weight molecules derived from the cell wall matrix and readily removed by solvents. The nature and amounts of extractives, removed under different conditions and with solvents of different polarities, were determined. The mobility and structural relaxations of lignin and hemicelluloses were studied *in situ* by dynamic mechanical analysis (DMA) and dielectric analysis (DEA) under controlled moisture content. Low temperature extractions led to rigidification of lignin and plasticizing of the hemicelluloses, probably due to local changes by the selective removal of molecules interacting with the polymers, and/or better accessibility to plasticizing water, at controlled humidity. In contrast, hot extractions including water induced rigidification of the hemicelluloses and plasticizing of lignin. This could be related to a combination of molecule extractions and chemical modifications of both polymers, supported by the variation of activation energy for hemicelluloses relaxations. Each type of extraction had a clear specific effect on the relaxation properties of the amorphous cell wall polymers.

Introduction

Hemp (*Cannabis sativa* L.) is a non-wood material suited for paper making and preparation of composites (Rowell et al. 1997). The woody core (chènevotte) accounts for 70% of the hemp stem. Industrial hemp core is obtained by mechanical separation of the long fibres from the hemp xylem. During transformation, the hemp core is subjected to mechanical stress, high temperatures and/or controlled humidity conditions, which lead to partial or total defiberization. It is thus important to understand and control the mechanical phenomena which are dependent on the properties and organization of the cell wall polymers.

Typically, the woody hemp core contains 33–37% cellulose, 16–20% hemicelluloses, and 17–22% lignin. It also harbours small amounts (1–5%) of extractives, ash and silica (Cappelletto et al. 2001).

It was previously shown that poplar extractives and amorphous polymers play an important role in cell wall cohesion during defiberization and affect energy consumption during wood refining (Meyer-Pinson et al. 2004). Pre-treatment with oxalate, as in paper making for example, also facilitates fiber removal and changes the properties of *in situ* polymers (Sugiyama et al. 2003; Xie et al. 2004; Kenealy et al. 2007; Kenealy W. et al. 2007; Jiang et al. 2008). Based on these data, cell wall polymers and their organization may affect the ability of wood to be transformed at the macroscopic level.

The properties of amorphous polymers are generally determined by dynamic characterization methods, sensitive to the behaviour of constituents with high viscoelastic properties. Salmén et al. (1998) and Lenth et al. (2001) initiated research on several substrates to develop methods of characterizing in situ polymers and the relationship between structure and mechanical properties. These authors proposed a

method adapted to wood substrates which allowed characterization of the glass transition (Tg) of *in situ* hemicelluloses. Tg is an intrinsic characteristic of a given polymer chain, dependent on the presence of water and plasticizing molecules (which decrease Tg). The environment of such associated macromolecules may modify the intrinsic mobility, solicitation frequency, and level of organization of the neighbouring crystalline phase.

Among the various factors affecting mobility via the direct environment of the macromolecules, the effect of extractives has rarely been examined. Matsunaga et al. (2000) studied the effect of introducing cell-wall extractives into specimens and demonstrated the formation of direct hydrogen bonds between impregnated extractives and wood components under wet conditions. In contrast, when the specimen was at a higher relative humidity, the formation of direct hydrogen bonds was disturbed by clusters of water molecules, and some extractives were acting as a plasticizer. Obataya et al. (1999) also demonstrated a change in the mechanical relaxation of reed used for construction of musical instruments *(Aroundo donax L.*) on removal of water soluble extractives. Although several authors claimed that wood mechanical properties depend on extractives, no in-detail studies have been devoted to this topic.

This study highlights the effect of extractives on the mobility of amorphous polymers taking into account the variability of the substrate depending on its origin within the stem. To study the effects of extractives on polymer cohesion, the approach is promising in the course of which the mobility changes of polymers are observed in the presence and absence of extractives. This can be done by measurement of the glass transition temperature (Tg) of each polymer *in situ*. The hemp core model (hemp core) in the present study contains ~ 5% extractives. The experiments were focussed on the

variability in the softening points of *in situ* lignin and hemicelluloses (i.e. their Tg values) in the presence and absence of extractives.

Materials and methods

Samples were obtained from the stem of hemp, cultivated and refined according to the procedures of La chanvrière de l'aube (LCDA) in Bar sur Aube (France). To limit to some extend natural variability, only one cultivar, Fedora 17, was investigated. Sticks of the woody part of the stem (hemp core), devoid of detached fibres and visible cracks, were selected for the experiments, on the basis of their regular parallelepipedic form (i.e. a geometric solid whose six faces are parallelograms), ~ 2 mm large and ~ 1 mm thick, 10 to 40 mm in length). Figure 1 illustrates (a) the microscopic view of woody core of hemp coming from the xylem of the plant and (b) the schematic view of the sticks and their use in DMA and DEA. For DMA investigations, the samples were cut into two pieces for paired-comparisons. One half of the stick was extracted with solvents and the other half remained untreated (defined hereafter as the reference sample). For dielectric analysis (DEA) investigations, one stick of hemp core was cut into five small cubes (around 1 x 1 x 1 mm^3) in transversal directions; four of them were treated and one was left untreated as a reference. This sample traceability reduced the number of specimens to be studied, as well as the effect of natural variability on the measured characteristics.

Extractions

The sticks (or cubes, see above) were extracted with three solvents either independently or in combination and at two temperatures. Extraction time: 6 h; liquor to

material ratio: 26.6 mL g^{-1}. One extraction was done with a mixture of toluene/ethanol-95% (2/1 by v/v) (Tol/EtOH), another with ethanol-95% (EtOH), and a final one with ultra pure water (water). The combined extractions were performed using these last solvents (successive). The “hot” extractions were done in a Soxhlet apparatus and the “cold” ones at 20℃ in a batch vessel by simple mixing wit h the solvent. The extracts were concentrated and freeze-dried. The dry matter obtained was weighed and subjected to analyses.

Analysis of hemp core and its extracted products

Before chemical characterization, the unextracted and extracted sticks were milled to powder with a rotary mill and freeze dried. Carbohydrate analysis: (1) acid hydrolysis of 10 mg samples consisting of a pre-hydrolysis with 12 M H_2SO_4 followed by (2) a hydrolysis with 1 M H_2SO_4 for 2 h at 100℃. (3) The released monosaccharides were separated by high performance anion-exchange chromatography (HPAEC); triplicate analysis, as described by Beaugrand et al.(2004).

The acid-insoluble lignin content, corrected for the ash content, was estimated gravimetrically according to the procedure described by M.J. Effland (1977) and modified by Monties (1984). The composition of the extracted products was determined as described above for the polysaccharides but without the pre-hydrolysis step (1).

Due to the low amount of sample to be analyzed, the lignin content was measured spectrophotometrically from the absorbance at 278 nm according to the acetylbromide method: (1) 10 mg of samples are hydrolyzed at 70℃ for half an hour with a 5 mL mix of acetyl bromide (98%), acetic acid (99.8 %) solution and 0.2 mL

perchloric acid. (2) 5 mL of 2M NaOH was added to 2 mL of this mixture (1) and adjusted to 20 mL with acetic acid. (3) The mixture (2) was left half an hour in a dark place. (4) The absorbance was measured at 280 nm; all analyses were performed in triplicate (Day et al. 2005).

Elemental analyzer (NA 1500, Carlo Erba) coupled to a mass spectrometer (Fisons Isochrom) was applied for C, N analysis of 3 mg (dry matter) samples (duplicate determination). He gas flow: 100 mL/min; Lucine was the standard for calibration.

The amount of soluble protein was determined colorimetrically in duplicate with the microscale Bradford reagent from Sigma-Aldrich. Standard: Bovine albumin serum. Protein concentrations based on UV absorbance at 595 nm (Beaugrand et al. 2004).

Ash content of the extracts and woody hemp core: by incinerating 10 mg of samples in a muffle furnace at 525 ±25℃ for 3.5 h according to (Tappi T 211. om-07). Mineral determination: atomic emission spectrometer ICP (Varian Liberty series II); 10 mg of dried extracts were dissolved in 25 mL of ultrapure water, filtered (0.45 µm) and the contents of Ca, Cu, Fe, K, Mg, Mn, Na, and Zn were determined.

Selection of methods dedicated to the characterization of lignin and hemicelluloses mobility

The interpretation of wood behavior through the relaxation mechanisms of its components requires a description of the specific behavior of each component. Thus the issue is to define *specific* methods dedicated to the characterization of the different families of wood macromolecular components.

As the relaxation temperatures of lignocellulosic polymers vary as a function of water content, the solution is to look for environmental conditions which contribute to the separation of a given relaxation of a given polymer. This is easy with the main relaxation of wood associated to lignin glass transition, which is obtained around 70-100°C, in water saturated wood, with no superposition with other relaxation mechanism.

At the contrary, in these same plasticization conditions, hemicelluloses glass transition is located below 0°C. An overlapping is then obtained with a lot of relaxation phenomena associated to water and local side chain movements of all polymers (cellulose, hemicelluloses and lignin).

Due to this practical issue, water saturated wood allows the characterization of lignin only. Salmén proposed methods based on DMA studies of water saturated wood and ethylene glycol saturated wood, ethylene glycol showing the advantage of a higher boiling point (Salmén et al. 1996).

The reduction of water content from saturation conditions lead to an increase of the glass transition temperatures of amorphous polymers. Lignin relaxation becomes hardly accessible, as its relaxation temperature increases above 100°C. It is also practically very difficult to obtain good DMA data from hydrophilic polymers like hemicelluloses in the temperature range 100-200°C, as high pressure cells are needed, and such equipment is not commercially available and/or under development only. Placet proposed a first DMA prototype which allows the characterization of wood under pressure but for the moment the system is limited to temperatures above 130°C (Placet et al. 2007). Thus very few data are found in literature, and the main significant work has been done by Kelley et al (1987) in non equilibrium conditions with a classical DMA cell.

The glass transition of hemicelluloses varies from negative temperatures to 200℃ as a function of water content (Salmen et al. 1986). At intermediate water content, hemicelluloses Tg falls in the good window (room temperature to 100℃), i.e. with no interference with low temperature relaxations and in a temperature range which allows the use of classical equipments (Salmén et al. 2004).

In their work on wood characterization by DMA characterization, Kelley et al. (1987) showed a small damping peak that he associates to hemicelluloses relaxation. Considering that the more recent works done by Salmen and Placet which use humidity controlled DMA machines do not show evident tan delta damping peak, it could be concluded that the phenomena evidenced by Kelley are more complex than a simple hemicelluloses relaxation as proposed initially. Nevertheless, our own attempts to obtain measurable and reproducible data for the characterization of hemicelluloses from RH regulated DMA system were unsuccessfull which lead us to test alternative method.

An original work dedicated to wood DEA characterization at intermediate water contents was proposed by Lenth et al (2001). Thanks to an adapted sensor system which allowed the characterization of equilibrated samples, Lenth showed the variations of a high and broad relaxation of wood as a function of water content, centered at 50℃ for 5Hz and 12% moisture content. He proposes an attribution of this relaxation to hemicelluloses and disordered cellulose. But considering (i) the high sensitivity of this relaxation towards water (data shown going from 200℃ down to negative temperatures), which is more the characteristic of a free amorphous component (Vartzeli-Nikaki et al. 2003), and (ii) the intensity of this dielectric relaxation, which suggests that the polymers associated to this relaxation are partially charged like rhamnogalacturonans, constituting hemicelluloses of hemp (Vignon et al. 1996) among

other oligomers (glucomanans) (Cronier et al. 2005), the relaxation shown by Lenth could be attributed to hemicelluloses.

In conclusion, according to the advantages/limitations of the methods available for the determination of polymer mobility in wood, we choose DMA and DEA to characterize *in situ* lignin and hemicelluloses respectively.

Dynamic mechanical analysis (DMA) in tension and immersion mode

DMA is the method of choice to characterize *in situ* lignin relaxation because the peaks observed in the 80-110℃ range are related to the g lass transition (Tg) of *in situ* lignin (Salmén et al. 1996).

The test specimen was clamped between the movable and stationary fixtures, and then enclosed in a sealed container (thermal chamber). The storage modulus (***E***'), loss modulus (***E***''), and **tan *δ*** responses for woody hemp core were plotted as a function of temperature at a given frequency. As with synthetic polymers, the values of E' decrease from a glassy plate to a rubbery state. E'' and tan δ show the same evolution with a maximum peak when the material softens.

The clamped sample was placed in a container filled with ethylene glycol and heated by two heating films glued on two of the exterior walls. The sample was about 1-2 mm large, 0.5-1 mm thick and 15 mm long. The instrument was in single frequency mode (1 Hz), the amplitude of the oscillation constant at 12 μm in "autostrain method" with a static force of 112%. Ethylene glycol elevates the precision above 100℃ with a heating rate of 2℃ min^{-1}. Wood impregnated with ethylene glycol is expected to have a

similar softening behaviour as water-saturated wood (Salmén et al. 1996; Bouajila et al. 2006).

Dielectric analysis (*DEA) in controlled humidity environment*

Some materials, including wood, possess a dielectric constant (ε') that characterizes the extent of electrical polarization which can be induced by an electrical field. In case of an alternating electrical field (as in this paper), polarization lags behind the field by a phase angle, due to partial dissipation of the stored energy. The dissipated energy is proportional to the dielectric loss (ε") and the stored energy to the dielectric constant (ε'). The values of ε" and ε' are measured by DEA.

The samples were equilibrated and stored in a constant relative humidity (RH) box (65% RH). For the analysis, the samples were placed in a modified sample holder to prevent water evaporation during the measurements. This consisted of a silicone flat joint glued between the electrodes with silicone grease. The water content, in samples with less than 20% water content, did not vary up to 150℃. A constant force of 350 N was applied during the experiment. Frequencies of 1, 5, 10, 50, and 100 Hz were studied (the best for observing softening effects) with a heating ramp of 2℃ min^{-1}.

This DEA technique is well suited to characterize the chain mobility of *in situ* hemicelluloses. The observed peaks are generally associated with the different relaxations of hemicelluloses (William L. James 1975) depending on the frequency and RH (Lenth et al. 2001). At each frequency range, the sample softens at a given temperature, and increasing the frequency leads to an increase the softening temperature. Frequency and temperature supposed to follow an Arrhenius plot. Thus

the apparent activation energy of relaxation (Ea) can be calculated from Eq. 1 based on the time/temperature diagram log(freq) = f(1000/T), (Lenth et al. 2001).

$$f_T = A^{-Ea/RT} \qquad \text{(Eq. 1)}$$

Results

Chemical analyses

Raw hemp core consists of 40% carbon and 0.2% nitrogen (by wt), distributed between polysaccharides (in total 67%; 42% cellulose + 25% hemicelluloses and pectin), lignin (20.7%), and a small amount of ash (2.7%). After extraction with various solvents, the composition of treated chènevotte samples slightly differs from the one of reference samples (Table 1 and 2). The solvent-extracted products originate mainly from the carbohydrates and lignin (Table 3). According to the elution profile obtained from the GPC (Mw < 800 g/mol) and TC (data not shown); the extracts have low molecular weights. Moreover, ultrafiltration with membranes with a cut-off of 3 kDa confirmed that the molecular mass of 80% of these entities was less than 3000 Da. They also consist of proteins and ash (Table 3). Water extracts mainly mineral elements and polysaccharides, whereas toluene removes a higher amount of phenolic compounds. These results are independent of temperature. Separate or successive extractions show the same pattern but, as expected, the yields are higher under hot conditions (e.g., 0.6% and 2.4% for cold and hot Tol/EtOH extractions, respectively).

Table 4 gives the percentage of monosaccharides present in extracts from the solvents sequence for the two temperature conditions. A high amount of glucose was extracted with every solvent. Under cold conditions, the highest monosaccharide

content was obtained with water. Water disolves the most galactose, xylose, mannose, rhamnose and uronic acids. Under hot conditions, the amounts of extracted arabinose, galactose, xylose and mannose are higher.

The analysis of aromatic compounds (lignin) by the acetylbromide method (Table 5) shows that the toluene and ethanol extracts contained three times more lignin than the raw material. After water extraction, the lignin content was the same as in the raw material. The yield of lignin increased with temperature (e.g., 0.2% cold Tol/EtOH and 1.5% hot Tol/EtOH) independently of solvent type, except in the case of hot water where the yield was constant (0.7%).

The water soluble extracts contain a high amount of minerals, with approximately 15% of potassium (Table 6).

Viscoelastic behaviour

Figure 2 presents the plots E and tan δ determined on untreated specimens at 1 Hz vs. temperature. On the tan δ curve, a maximum peak is noticed (see arrow) at around 115℃ at ethylene glycol saturation representing a relaxation. This softening temperature range is very wide (more than 60℃).

Figure 3 shows that the Tg data obtained on parallel stick samples (as a stick was separated into two equal parts) have almost the same Tg values. Accordingly, an accurate relative comparison of extracted and non-extracted samples is possible.

Figure 4a illustrates the effects of Soxhlet (hot) extractions on the Tg of lignin when the extraction was performed independently or in succession. It is obvious that the

extraction mode and the solvents have an effect on Tg. Treatments with Tol/EtOH mixtures increases the Tg of lignin whereas extraction with hot water leads to a decrease of the softening temperature. The same situation is described under cold conditions in Figure 4b. Temperature has a clear effect. Both cold Tol/EtOH and water treatments had an increasing effect on Tg values while EtOH exhibited intermediate behaviour.

Figure 5 describes the dependency of tan δ (=ε"/ε') from temperature and frequency. As in the DMA plot in Figure 2, the softening temperature is given by the maximum of the tan δ peak. The value for hemicelluloses relaxation in hemp core was around 10℃ at 1 Hz and around 35℃ at 5 Hz (both a t 65% RH). The softening temperature increased with the frequency. Each frequency decrease leads to a shift of nearly 10℃ to a higher Tg value.

Figure 6a is the Arrhenius plot of the relaxation temperature of hemicelluloses determined for samples obtained from the same piece extracted separately in a Soxhlet apparatus. The relaxation temperatures decrease as the amplitude function of solvent polarity increases. After successive extractions, hemicelluloses have a lower Tg (Figure 6b). Moreover, with this relaxation mechanism, an increase in the apparent activation energy (Ea) from 176 kJ mol^{-1} to 237 kJ mol^{-1} was observed.

Figures 7a and 7b show the Arrhenius plots of samples subjected to independent and successive cold extractions, respectively. The former (Figure 7a) leads to an intermediate behaviour, with an irregular effect of ethanol. After successive extractions, Tg is higher than in the raw material and Ea has almost the same value as the reference sample (145 kJ /mol compared to 130 kJ/mol (Figure 7b)).

Discussion

Biochemistry of hemp chènevotte

The aim of this study was to understand the impact of removing low molecular weight compounds from the cell walls, on the chain mobility of hemicelluloses and lignin. The amounts of extracts obtained under the described conditions were generally low (below 5% see Table 3). As a consequence, this had only slight effect on the overall neutral sugars and lignin compositions of the hemp core pieces, within the accuracy of the methods used for the determinations (Tables 1 and 2).

Only a very small amount of sugar was removed from the cell wall (Tables 3 and 4). Nevertheless, higher amounts of fucose, rhamnose, galactose, and uronic acids were extracted with water than with toluene and ethanol. These sugars probably originated from pectins such as type I rhamnogalacturonan (Vignon et al. 1996; Ridley et al. 2001). Higher amounts of arabinose, xylose, and mannose were extracted by hot water treatment, probably due to the auto-hydrolysis of lateral chains of various hemicelluloses such as D-galacto-D-mannans, D-gluco-D-mannans, L-arabino-D-xylans or D-xylo-L-arabinanes (Lavarack et al. 2002; Cronier et al. 2005). Aromatic compounds were readily extracted with apolar solvents, as expected from their known hydrophobic properties (Table 5). One characteristic of the samples was the high amount of minerals (Table 6). This was probably due to the covering of soil dust on the hemp core which was not removed during the defiberization process because the samples were not washed before extraction to avoid the elimination of some organic extracts from the cell wall.

Polymer mobility characterization

The cell wall consists of the amorphous polymers (lignin, hemicelluloses and pectins) and the crystalline cellulose arranged to a complex supramolecular architecture (Monties 1980). The viscoelastic properties of the *in situ* polymers have been interpreted in the present paper based on the model of Salmén and Olsson (1998): the dispersion of the hemicelluloses in a continuous lignin phase. The quoted authors demonstrated by successive removal of the polymers from wood that in the fibre wall xylan is associated with lignin and glucomannan with cellulose.

In spruce, lignin relaxation occurs at around 90°C at the frequency of 1 Hz (Salmén 1984) and at around 80°C in beech at fibre saturation (Becker et al. 1968), which is in accordance with our results for hemp core (figure 2). Figure 3 illustrates the dispersion of the Tg value observed in our raw material. This can be explained by the natural variability of the samples. The structure and organization change according to the position of the sample in the stem (Salmén 1984; Cronier et al. 2005). As the original location of each piece of hemp core within the stem was unknown, each stick was cut into two similar pieces to avoid this natural variability and always keep a reliable untreated sample that was directly related to the treated one.

Plasticization and deplasticization

Cold extractions of hemp core led to an increase in lignin Tg with every solvent, except ethanol which showed irregular behaviour. Several subsequent scenarios are possible:

1. The first hypothesis is that deplasticizing of the lignin phase occurs by a simple physicochemical modification as a result of the removal of low molecular weight entities. These consist of 80% of molecules below 3 kDa, and are mainly composed of phenolic compounds. The lignin-like structures extracted by Tol/EtOH may act as plasticizers in the native wood network (Li et al. 2005). In

contrast, hemicelluloses become less mobile. As hemicelluloses are embedded in a continuous lignin phase, a modification of the lignin environment may also affect the viscoelastic properties of the hemicelluloses (Karlsson et al. 2004). When lignin becomes less mobile, hemicelluloses have a greater free volume around the chains, enabling them to be more flexible, i.e. their Tg is lowered. The accessibility to water is also expected to be higher (Pejic et al. 2008). Tests performed at different humidities (data not shown) revealed and enhanced plasticizing effect. Cold water solubilised uronic acids from the cell wall, and large quantities of calcium and iron were found in the ash of the extracts (Tables 4 and 6). As these compounds may form cross-linkages by complex organometallic structures (Cosgrove 2005), their removal could also explain the increment in hemicelluloses mobility. The Tg of hemicelluloses are highly dependent on frequency (Figure 5), as also shown by Lenth et al. (2001). However, in the presents paper the absence of an apparent activation energy change (225 ±32 kJ/mol was demonstrated for the unextracted (raw) material. The energy change for the water-extracted sample was 204 ±1 kJ/mol). It can be concluded that water extraction, which solubilises ions plus pectic material, has no impact on the main relaxation mechanism of hemicelluloses.

2. The second hypothesis is that the lignin polymer chains are reorganized resulting in a Tg increase. This could occur either by a larger number of lignin interactions within the lignocellulosic network, at the level of the interfaces between polymers, or by modification of the complexity of the lignin phase, due to polymerization of the rearrangements. However, no clear correlation can be found between the nature of the extracts and the impact of the increased Tg of lignin. This supports the hypothesis that some extractable molecules are embedded within the lignin

phase. Their removal induces its reorganization with a subsequent effect on the reorganisation of hemicelluloses, as discussed above. With the removal of impurities, it could also become easier for plasticizers such as water to access the larger free volume around the hemicelluloses chains.

3. Hemicelluloses are known to be plasticized by water molecules (Matsunaga, Obataya et al. 2000; Lenth et al. 2001; Obataya, et al. 2001; Salmén et al. 2004). In our experiments, a RH 65% was applied which is well suited for observing hemicelluloses relaxation. The amount of local water concentration can change as a function of the accessibility of hemicelluloses to the H_2O molecule, and as a function of the extraction yield of 'impurities' surrounding the hemicelluloses as assumed in the literature (Pejic et al. 2008)**.**

These last two mechanisms may act simultaneously because, for each type of solvent, the same entities were extracted but in different amounts. The structural modification and organization of the polymers *in situ*, (water distribution, complexity, etc.) would need to be studied directly to elucidate the relative significance of the two mechanisms.

Chemical modification of the polymers

In the case of separate or successive hot extractions, the results revealed a decreased mobility of the chains of hemicelluloses. As for lignin, this can be explained by a deplasticizing effect due to the higher relative amount of sugar monomers or dimers extracted. The two solvents at two temperatures affected mainly the percentage of extractives recovered, and only slightly their composition. These small sugar molecules seem to act like plasticizers in the hemicelluloses phase as suggested for the aromatic compounds extracted from the lignin phase (see above).

After successive hot extractions, the apparent activation energy increased from 176kJ/mol to 237kJ/mol, indicating that the mechanism of relaxation had changed, and suggesting that a complex chemical modification had occurred. The nature of the extracted products recovered, as well as the amount of polysaccharides remaining within the hemp core shows that hemicelluloses were degraded (Lavarack et al. 2002). These modifications could be directly related to the changes in Tg values of hemicelluloses which were higher after a Soxhlet extraction. However, in all cases, the extraction step involving hot water led mainly to a decrease in mobility (see above). This suggests a complex changes within the amorphous polysaccharide phase which could induce both scission and non-covalent cross-linking mechanisms (Crescenzi et al. 1997; Fry 1998).

Water has a greater effect on both hemicelluloses and lignin relaxation compared to other separate solvents. The Tg increase or decrease of amorphous polymers observed after successive extractions can mainly be attributed to the effect of water extraction alone. Whereas no differences between cold and hot conditions were observed with toluene and ethanol, hot water extraction led to a decrease of lignin Tg. Complex chemical mechanisms might occur, which involve the lignin phase and also other macromolecular components. Moreover, in experiments carried out in ethylene glycol, lignin may have submitted to chemical modification at high temperatures (above 100℃) like to that of organosolv delignification p rocesses (Thring et al. 1990)**.** When the ethylene glycol liquid was analysed by UV spectrometer, a band at 280 nm indicated the presence of aromatic compounds. Moreover after 4 h of hot water extraction, the pH decreased from 7.1 to 6.1 and a strong yellow colour appeared as a sign of the simultaneous occurrence of autohydrolysis and delignification (Murphy et al. 1981).

Certainly, a combination of different physical and chemical reactions, undefined as yet, affects the mobility of *in situ* cell wall polymers.

Conclusions

This study highlighted some viscoelastic properties of the woody core of industrial hemp core, in relation to the effect of extractives on the mobility of lignin and hemicelluloses. The removal of some material soluble in toluene and ethanol led to an increase in the softening temperatures of both lignin and hemicelluloses. The impact of water extraction is of particular interest. The removal of molecules at low temperatures resulted in an increase of the lignin softening temperature and a concomitant decrease of that of hemicelluloses. However the opposite phenomena were observed when hot water extraction was performed.

All extracts obtained consisted of the same oligomers derived from lignin and polysaccharides, but in different ratios. Accordingly, the different behaviour of the non extractable structural polymers within the cell wall are not related to a particular compound and/or oligomer removed by extraction, but more probably to the reformation of new associations within the supramolecular complexes of hemicelluloses and lignin.

All together, our data confirm that low molecular weight compounds influences the viscoelastic properties of the cell wall to a high extent.

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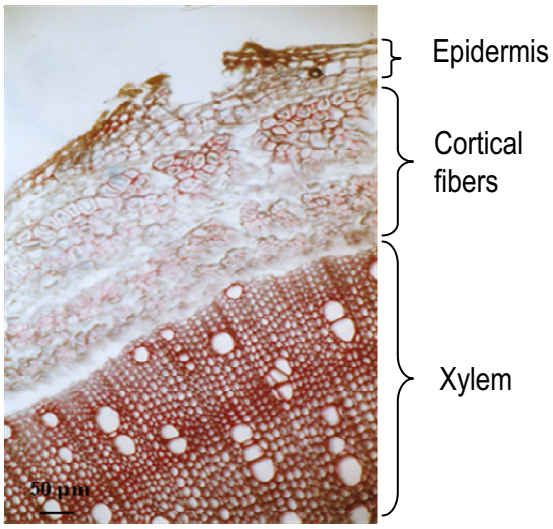
Figures

Figure 1

a) Anatomic picture of the hemp stem



b) Schematic picture of an analyzed stick:
(♦) untreated (raw), (A) Tol/EtOH, (B) EtOH, (C) water, (D) successively extracted samples.

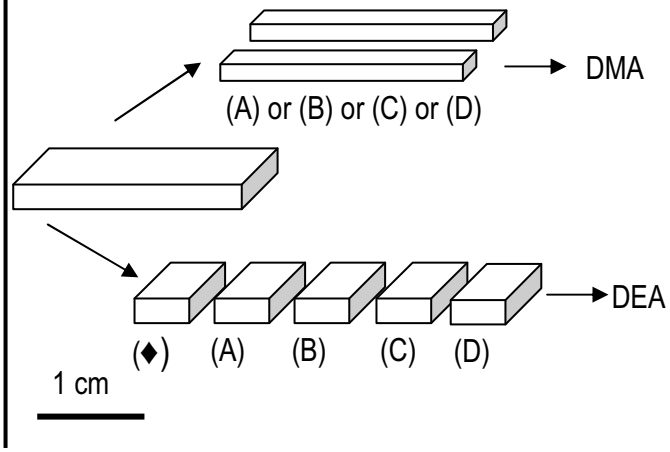


Figure 2

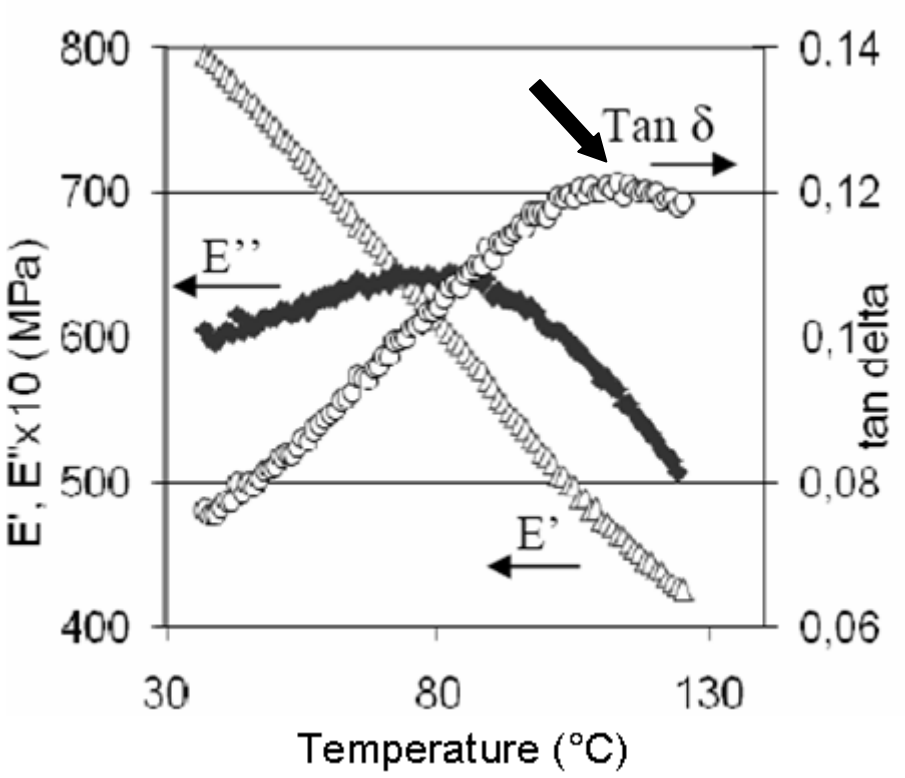


Figure 3

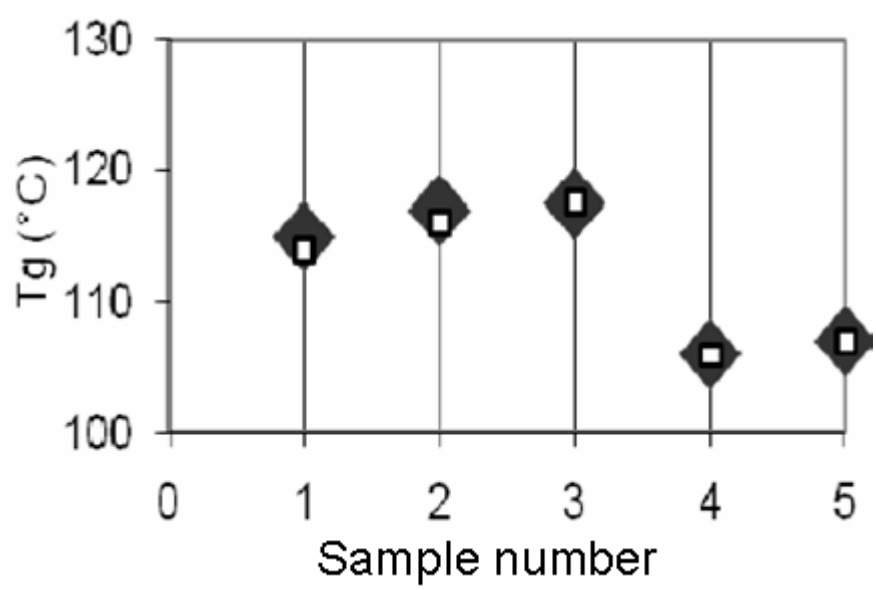


Figure 4

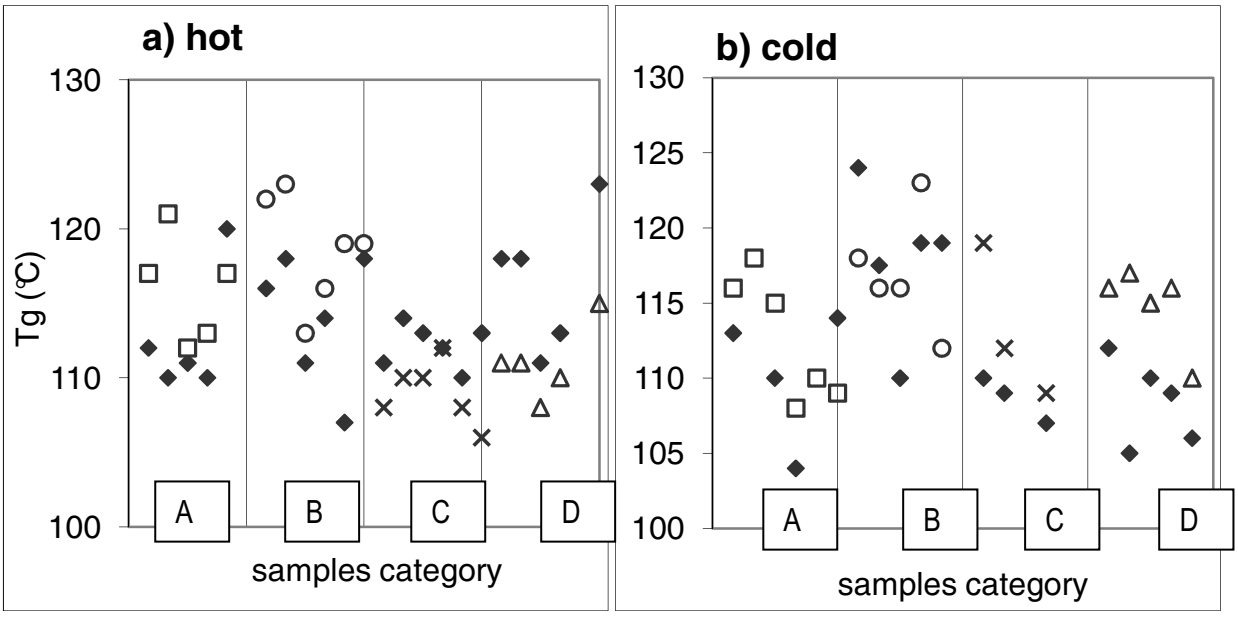


Figure 5

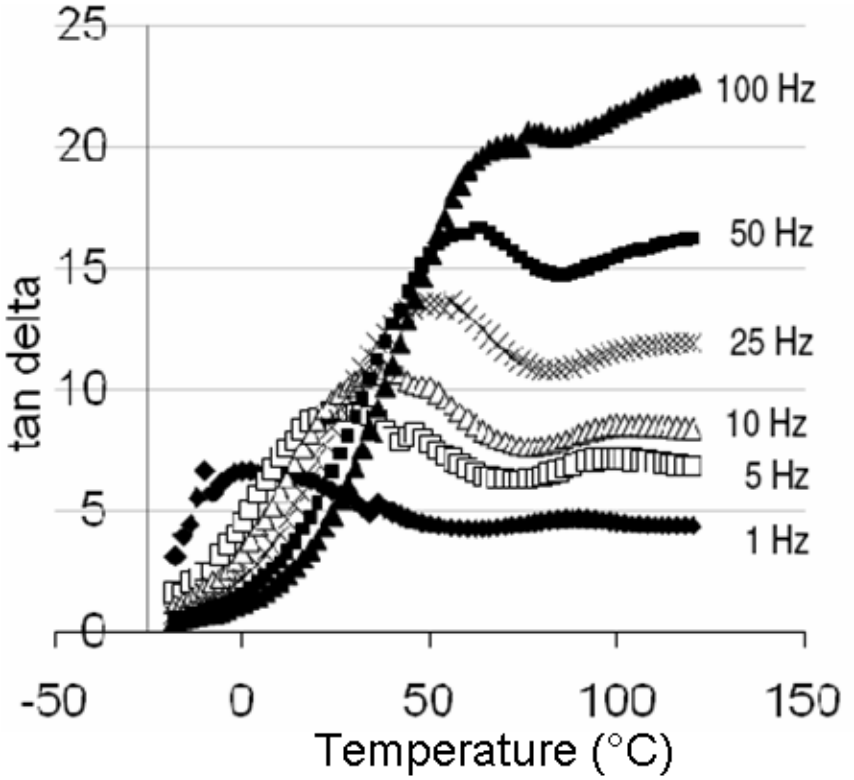


Figure 6

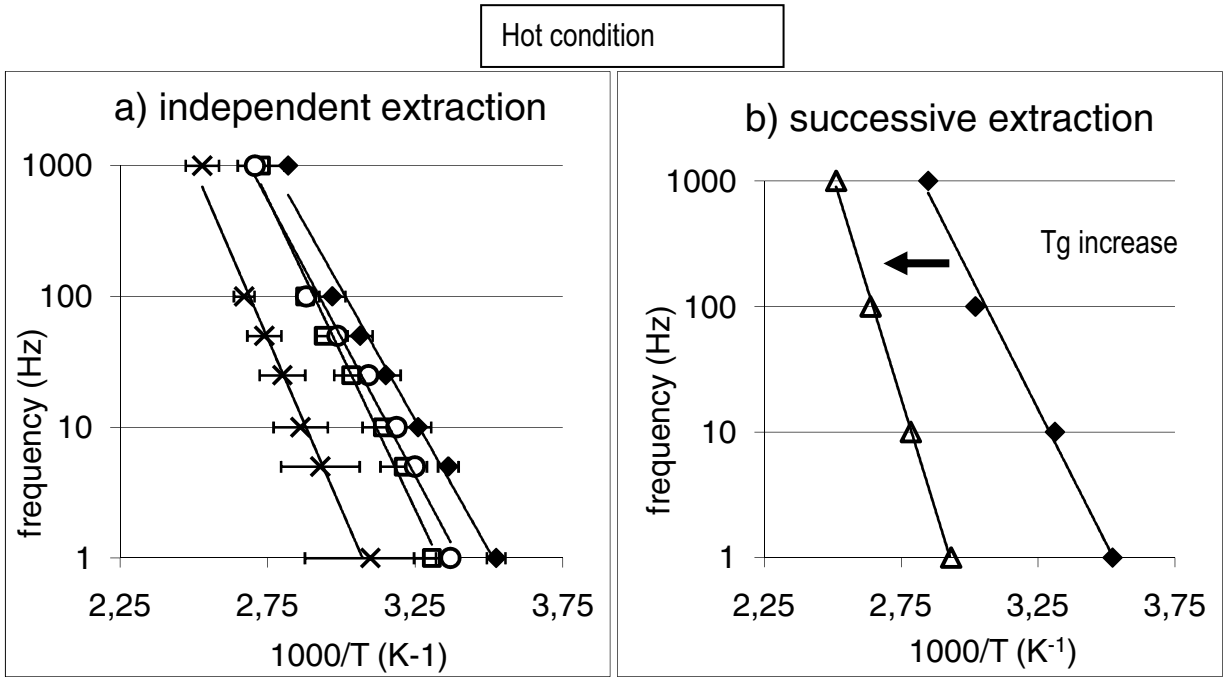


Figure 7

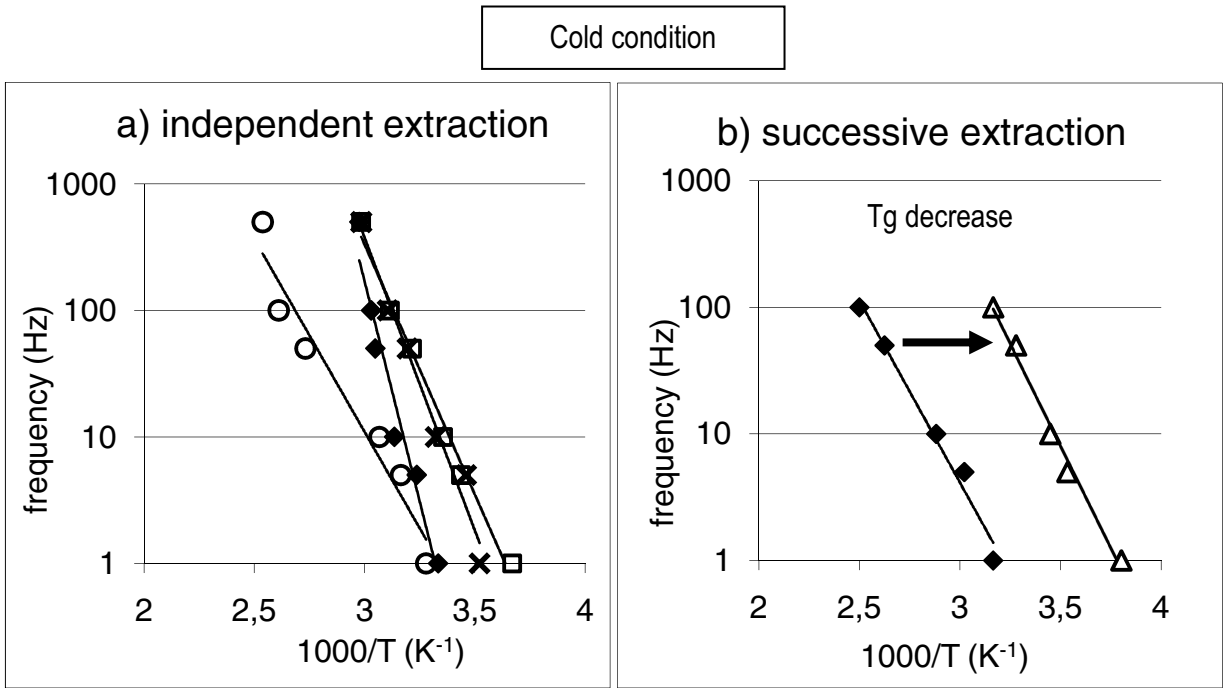

Cold condition
a) independent extraction
1000
100
10
1
frequency (Hz)
2
2,5
3
3,5
4
1000/T (K-1)
b) successive extraction
Tg decrease
1000
100
10
1
frequency (Hz)
2
2,5
3
3,5
4
1000/T (K-1)

Figure captions

Figure 1: Picture of hemp stem and description of the sticks use: (a) Microscopic view of hemp stem, woody hemp core corresponds to the xylem part of the stem after mechanical separation of fibres (from Chabbert, INRA) and (b) schematic view of the stick and their use with DMA and DEA

Figure 2: Plots of storage E' (∆), loss modulus E''x10 (♦), and tan delta (o) date vs. temperature. Lignin softening (thick black arrow)

Figure 3: Lignin softening temperatures (Tg) for a raw piece sample cut into two equal parts: raw1 (♦), raw2 (□).

Figure 4: Lignin softening temperatures (Tg) determined from a temperature scan in tension mode in ethylene glycol submersion. a) Soxhlet (hot) extraction. b) cold extraction. Captions: raw material (♦), A: part extracted with toluene/ethanol (□), B: part extracted with ethanol (o), C: part extracted with water (x), D: part extracted with all solvents in succession (∆).

Figure 5: Dielectric curves vs. temperature and frequency: 1 Hz (♦), 5 Hz (□), 10 Hz (∆), 25 Hz (x), 50 Hz (■) and 100 Hz (▲) on raw chènevotte at 65% RH.

Figure 6: Activation energy of hemp core. a) independent extraction. Captions: raw material (♦), extracted with toluene/ethanol (□), extracted with ethanol (o), and extracted with water (x). b) successive extraction. Captions: raw material (♦), extracted with all solvents (∆) under hot conditions.

Figure 7: Activation energy of hemp core. a) independent extraction. Captions: raw material (♦), extracted with toluene/ethanol (□), extracted with ethanol (o), and extracted with water (x). b) successive extraction. Captions: raw material (♦), extracted with all solvents (∆) under cold conditions

Tables

Table 1: Results of neutral sugars analysis of hemp core after extractions

Sugar	Without extraction[a]	After extraction with the solvents[b]		
		Tol/EtOH	EtOH	Water
		(%)	(%)	(%)
Fucose	n.d.	n.d.	n.d.	n.d.
Rhamnose	0.25 ± 0.01	0.29 ± 0.01	0.29 ± 0.01	0.25 ± 0.01
Arabinose	0.44 ± 0.01	0.47 ± 0.01	0.46 ± 0.01	0.41 ± 0.01
Galactose	0.83 ± 0.02	0.82 ± 0.02	0.83 ± 0.03	0.87 ± 0.07
Glucose	42.6 ± 0.6	43.4 ± 0.5	40.4 ± 0.9	40.9 ± 1.5
Xylose	18.9 ± 0.1	19.5 ± 0.7	19.8 ± 0.5	19.2 ± 0.7
Mannose	1.4 ± 0.08	1.44 ± 0.05	1.47 ± 0.05	1.50 ± 0.06
Gal. acid	2.04 ± 0.08	2.07 ± 0.02	2.8 ± 1.05	2.1 ± 0.09
Gluc. acid	0.50 ± 0.02	0.53 ± 0.04	0.7 ± 0.25	0.52 ± 0.03
Total	66.9	68.4	66.9	65.7

nd: not detectable, [a] % based on raw material; [b] % based on dry material extracted

Table 2: Klason lignin percentages of woody hemp core obtained after extractions

Extraction temp.	Without Extraction[a] (%)	Lignin % obtained after extraction with[b] Tol/EtOH (%)	EtOH (%)	Water (%)	Successive extr. (%)
Cold	20.7±0.6	21.0±0.4	21.7±0.1	21.9±0.1	20.6±0.3
Hot	20.7±0.6	20.5±0.5	21.0±0.2	21.3±0.3	19.9±0.3

[a] % based on raw material; [b] % based on dry material extracted

Table 3: Extraction yields and composition of extractives (in % based on the raw material extracted)

		Composition of the extract[b]					
	Extract yield[a] (%)	N (%)	C (%)	Protein (%)	Sugar (%)	Lignin (%)	Ash (%)
Cold Tol/EtOH (1)	0.6	0.5	69.9	0.1	4.6	61.1	1.0
Cold EtOH (2)	0.6	0.8	61.7	0.2	9.2	65.8	10.1
Cold water (3)	2.5	2.3	45.1	1.8	7.4	26.0	57.7
Succ. extraction (1)+(2)+(3)	3.0	1.3	41.4	1.3	7.7	30.1	37.2
Hot Tol/EtOH (4)	2.4	0.5	62.2	n.d.	5.1	64.0	2.2
Hot EtOH (5)	2.2	1.3	51.6	0.2	11.1	69.0	7.8
Hot water (6)	2.6	1.5	30.1	1.3	15.3	27.4	36.2
Succ. extraction (4)+(5)+(6)	5.0	0.9	49.3	0.9	15.4	52.8	27.6

[a] % based on raw material; [b] % based on extractives; n.d. not detectable

Table 4: Percent yield of neutral sugars (based on extract) analysis on extracts obtained from different solvents under hot and cold extraction conditions

Sugar	Tol/EtOH		EtOH		Water		Succ. extraction	
	Cold (%)	Hot (%)	Cold (%)	Hot (%)	Cold (%)	Hot (%)	Cold (%)	Hot (%)
Fucose	nd	nd	nd	nd	0.10 0.01	0,05 0.01	nd	nd
Rhamnose	0.27 (0.05)	0.21 (0.06)	0.44 (0.01)	0.35 (0.02)	1.12 (0.05)	1.13 (0.03	0.94 (0.11)	1.60 (0.08)
Arabinose	n.d.	n.d.	n.d.	n.d.	n.d.	0.48	n.d.	0.62 (0.02)
Galactose	0.30 (0.04)	0.26 (0.06)	0.52 (0.04)	0.72 (0.10)	1.29 (0.18)	1.94 (0.02)	1.17 (0.14)	2.38 (0.12)
Glucose	3.73 (0.55)	4.28 (1.17)	7.76 (0.15)	9.58 (1.12)	1.62 (0.17)	9.11 (0.23)	4.16 (0.79)	7.70 (0.50)
Xylose	0.07 (0.04)	0.05 (0.01)	0.10 (0.01)	0.09 (0.01)	0.55 (0.09)	0.75 (0.03)	0.39 (0.04)	0.92 (0.09)
Mannose	0.05 (0.03)	0.08 (0.03)	0.14 (0.02)	0.24 (0.05)	0.28 (0.03)	0.95 (0.06)	0.44 (0.09)	0.93 (0.07)
Gal. acid	0.02 (0.00)	0.11 (0.12)	0.02 (0.00)	0.01 (0.00)	1.35 (0.10)	0.52 (0.06)	0.31 (0.04)	0.70 (0.02)
Glu. acid	0.15 (0.03)	0.09 (0.07)	0.12 (0.07)	0.06 (0.00)	1.14 (0.15)	0.38 (0.01)	0.22 (0.02)	0.48 (0.05)
Total	4.6	5.10	9.2	11.07	7.4	15.3	7.7	15.4

n.d. not detectable. In parenthesis: standard deviation

Table 5: Lignin determination (“aromatic compounds”) by the acetyl bromide method in the extracts indicated

	Yield of aromatic compounds in extracts with the solvents			
Extr. temp.	Tol/EtOH (%)	EtOH (%)	Water (%)	Succ. extraction (%)
Cold	61.1±1.6 (0.2)	65.8±6.4 (0.4)	26.4±6.7 (0.7)	30.1±2.1 (0.9)
Hot	64.0±2.7 (1.5)	68.9±4.4 (1.5)	27.4±1.9 (0.7)	52.8±1.9 (2.6)

% based on extract ± standard deviation. In parenthesis: based on raw material

Table 6: Mineral determination by atomic emission spectrometry (% based on extract)

Extraction with	Extr. temp.	Total minerals (%) consisting of Ca, Cu, Fe, K, Mg, Mn, Na, and Zn
Tol/EtOH (1)	Cold	1.05
	Hot	1.26
EtOH (2)	Cold	4.61
	Hot	15.72
Water (3)	Cold	22.87
	Hot	17.43
Successive extr. (1)+(2)+(3)	Cold	10.12
	Hot	7.79

Le travail précédent contribue à comprendre les mécanismes par lesquels les « petites» molécules extractibles des parois cellulaires du bois de la tige de chanvre sont impliquées dans la cohésion, par le biais de la modulation des propriétés viscoélastiques des macromolécules *in situ*. Pour se faire, des solvants de différentes polarités ont été sélectionnés en combinaison avec la température de traitement pour réaliser des extractions seules ou successives sur un lot de chènevotte.

Les extractibles retirés des cellules pariétales ont ensuite été caractérisés quantitativement et qualitativement ainsi que la mobilité et les transitions des chaines de lignine et hémicelluloses en utilisant l'analyse mécanique dynamique et l'analyse diélectrique respectivement. Parmi les résultats obtenus, les traitements au toluène et à l'éthanol pourraient induire une 'rigidification' de la lignine et une 'plastification' des hémicelluloses tandis que l'eau agit différemment lors d'une extraction à température élevée, puisque les hémicelluloses sont plus rigides et les lignines plastifiées.

Cependant quelques points supplémentaires doivent être discutés quant à la méthodologie employée :

- *Variabilité naturelle du substrat*

Lors des analyses précédentes, nous ignorons l'endroit d'où provient la chènevotte analysée sur la tige. Cependant, la sélection selon la géométrie de l'échantillon peut être un critère sélectif dans certains cas. Généralement les morceaux issus de la partie basale sont plus épais que les parties provenant de la partie apicale. Sachant que les parties basales et apicales n'ont pas la même composition, les résultats sur les valeurs dispersées de la température de transition vitreuse sont alors prédictibles. Par conséquent, il serait plus rigoureux d'envisager une campagne de manipulations avec

des morceaux prédécoupés et identifiés sur la tige de chanvre. Les résultats obtenus devraient illustrer une moins grande dispersion et des valeurs de Tg proches pour des morceaux voisins dans une tige puisque c'est le cas pour un échantillon brut coupé en deux.

- *Mesures dans le bain d'éthylène glycol*
  - Mesures comparatives sur des échantillons bruts avec et sans bain d'éthylène glycol

Le diagramme suivant (figure 1) illustre l'évolution de Tg avec immersion dans un bain d'éthylène glycol et sans immersion mais imprégné d'éthylène glycol sous vide pendant 3h. Le relevé des Tg se fait sur le maximum du pic du module de perte (E'') puisque les expériences sans immersion impliquent le séchage des échantillons (changement des dimensions du bois analysé) et par conséquent leur décrochage des mors au dessus de 100°C et rendent l'exploitation sur tan δ impossible. Pour les six échantillons testés, la paire immergée a une valeur de Tg inférieure à sa paire sans immersion. L'immersion a donc pour effet de diminuer les valeurs de Tg (gonflement des chaines et donc plastification).

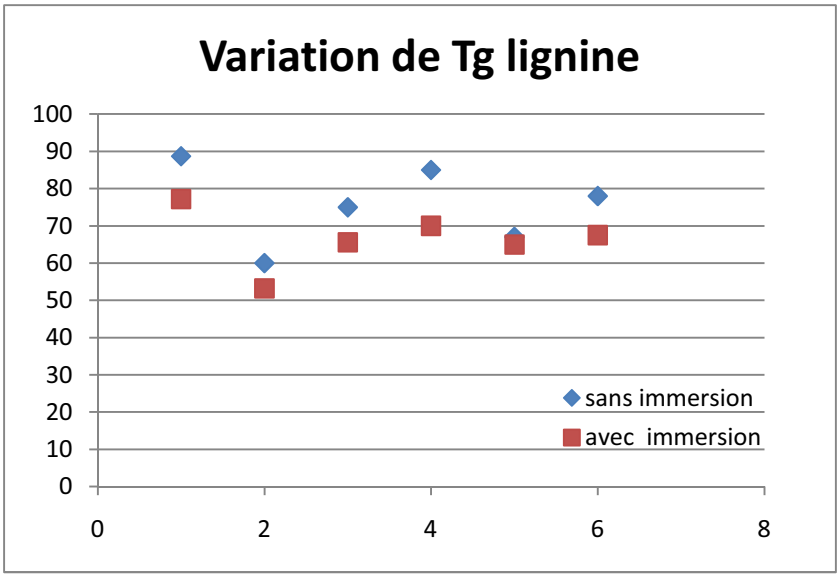


Figure 1 :Variation de Tg des lignines avec et sans immersion

- Variation de Tg avec et sans immersion après extraction

Les deux diagrammes suivants (figure 20) illustrent l'effet de l'immersion après extraction. L'étude se fait par comparaison sur des paires.

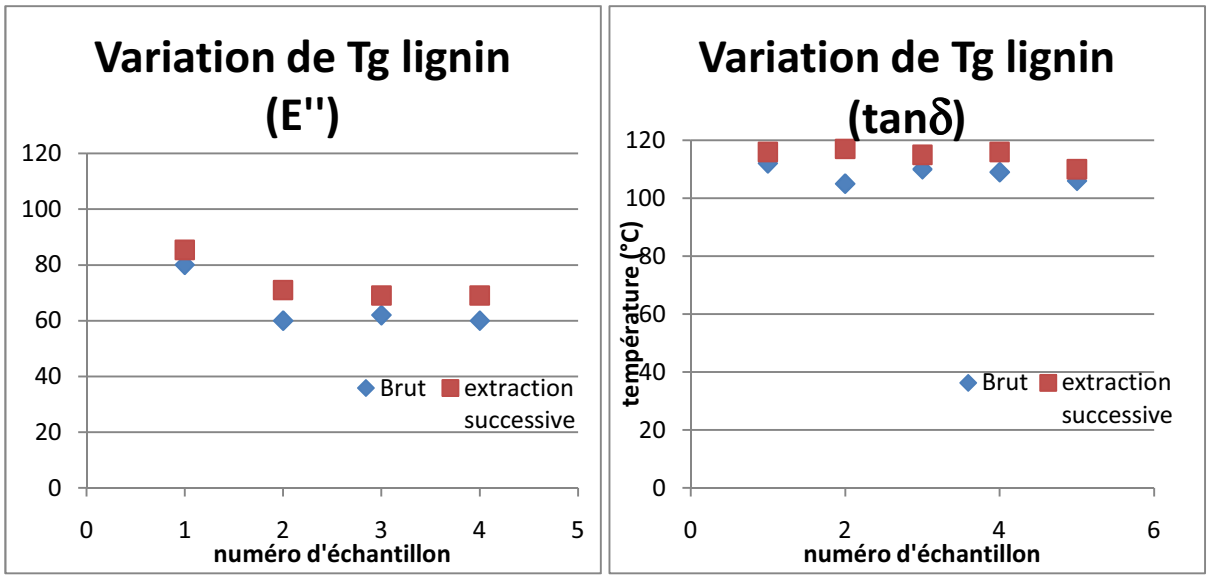


Figure 2 : Variation des Tg lignine après une extraction sans (à gauche) et avec (à droite) immersion

L'extraction, comme il a été démontré précédemment, a pour effet d'augmenter les Tg des lignines. L'exploitation des courbes sans immersion ne peut se faire qu'en dessous de 100°C et sur E'', l'intérêt de travailler en immersion est d'augmenter la plage de température d'analyse qui peut atteindre 120°C dans l'éthylène glycol car aucune perturbation ne vient troubler l'analyse aux alentours de 100°C (perturbation qui peut être due à l'évaporation de l'eau) et ainsi d'observer un pic net et distinct sur tanδ. L'immersion n'efface pas l'effet de l'extraction sur les Tg : la différence de Tg est toujours aussi marquée qu'il s'agisse d'une mesure en immersion ou non. Bien que les échanges de composés entre solvant et matière existent (la couleur de la solution d'EG après la rampe en température pour l'analyse passe de transparent à jaune : l'analyse en UV de cette solution d'immersion par comparaison à la solution pure révèle un épaulement aux alentours de 280nm caractéristique de composés aromatiques), les échantillons sont préalablement saturés en EG pendant deux jours à 20 °C pour éviter les extractions supplémentaires. La différence entre les shifts de Tg étant identique (et hiérarchie des Tg conservée), ceci ne suppose pas d'extraction supplémentaire qui viendrait influencer considérablement les valeurs de Tg pendant la mesure.

- *Mesure dans l'eau*

Les expériences ont généralement été conduites dans l'éthylène glycol car ce dernier est un aussi bon plastifiant des lignines que l'eau et qu'il permet d'élargir le champ de température de mesure. Cependant quelques expériences ont été réalisées dans l'eau à différentes fréquences (figure 21). Ces manipulations supplémentaires ont ainsi permis de calculer l'énergie apparente d'activation pour le mécanisme de relaxation des lignines. A partir de la pente du diagramme d'Arrhenius, une valeur d'énergie

d'activation de Ea=390 kJ/mol pour un matériau brut. Cette valeur est comparable à celle de 395 kJ/mol révélée par Salmen sur le bois mouillé d'Epicea mesurée en torsion dans la direction radiale **(Salmén, 1982)** pour des variations de fréquence comprise entre 1 et 10Hz.

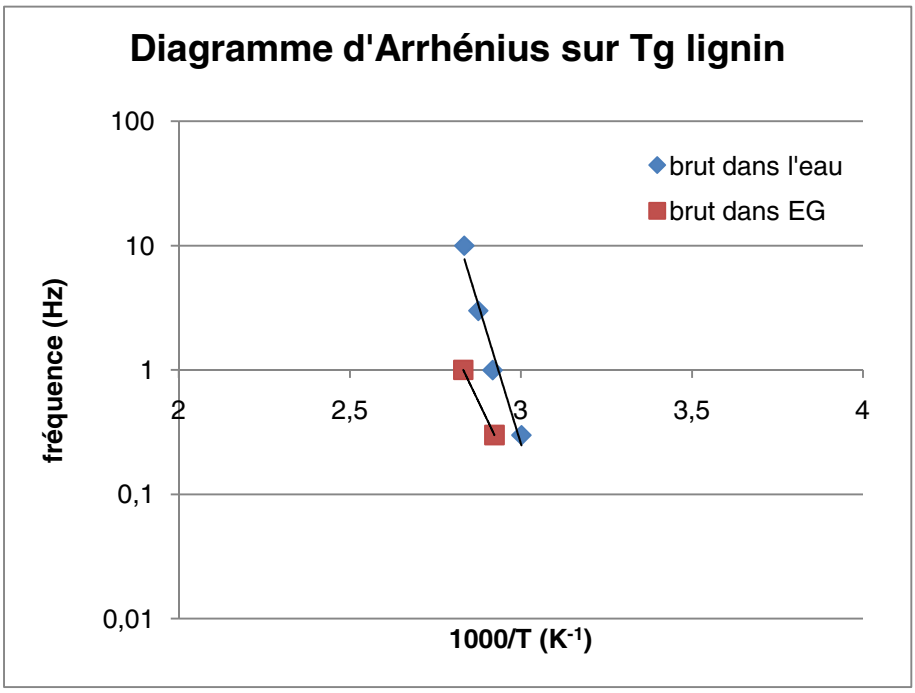


Figure 3 : Diagramme d'Arrhenius des variations de Tg lignine dans l'eau

Comme il apparait sur ce diagramme, l'éthylène glycol est un plastifiant moins efficace que l'eau mais il reste assez comparable.

Ea eau (kJ/mol)	396
Ea EG (kJ/mol)	253

Ainsi, ce diagramme nous permet de localiser la température de transition vitreuse de la lignine pour le même type de substrat lorsque celui-ci est sollicité à des vitesses variables ou sur des tests mécaniques comparables.

- *Mesure à différentes RH pour les hémicelluloses*

La figure 4 représente les diagrammes d'Arrhenius pour les variations de Tg des hémicelluloses pour des échantillons bruts stockés à différentes humidités relatives

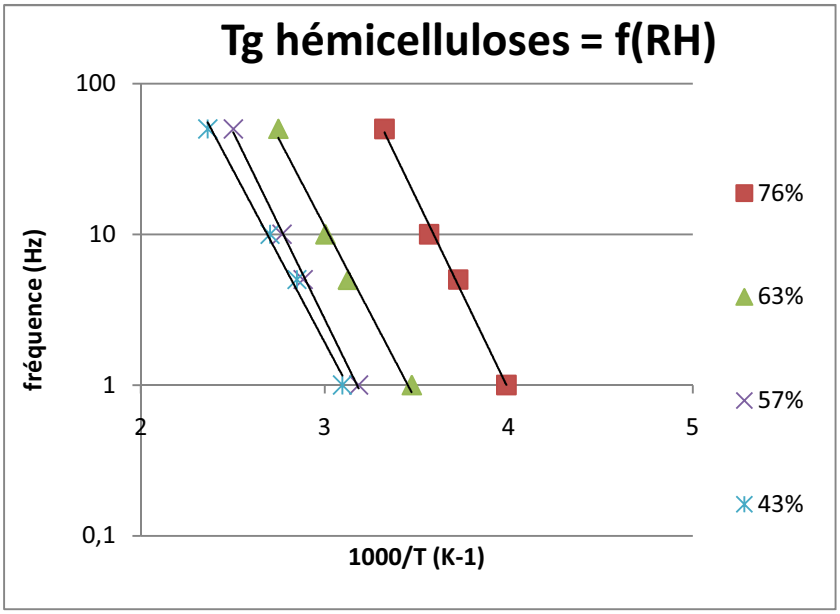


Figure4 : Diagramme d'Arrhenius des variations de Tg hémicelluloses pour des échantillons stockés à différentes humidités relatives

Les pentes de chaque courbe sont presque identiques et pour des valeurs croissantes de RH, la Tg des hémicelluloses diminue. Ceci souligne l'effet plastifiant de l'eau, plus la quantité d'eau dans l'échantillon est élevée plus, il y aura des chances que ces molécules d'eau soient placées entre les chaînes par conséquent plus les chaînes pourront être mobiles.

De plus, les valeurs calculées des énergies apparentes d'activation comprises entre 100 et 110 kJ/mol (tableau 1) illustrent la conservation du mécanisme de relaxation.

RH (%)	Ea (kJ/mol)
76	111
63	103
57	109
43	101

Tableau 1 : Valeurs de Ea pour différents RH sur un même échantillon brut

Pour conclure sur ce chapitre, nous avons mis en évidence une relation entre extractibles et mobilité. L'objectif étant d'optimiser le processus de défibrage, il est donc primordial d'étudier la conséquence de ces extractions sur les modes de rupture des morceaux de chènevotte avant et après extractions. Pour ce faire, nous devons soumettre les échantillons à des conditions idéales de mesure sachant, via le chapitre précédent, que de petits changements de la mobilité de la lignine (principal facteur influant sur la propagation de la rupture) apparaissent suite à des extractions (maximum 10℃ de hausse sur la température de transition vit reuse de la lignine).

Le chapitre suivant décrira le cheminement pour le choix des conditions opératoires et dans un second temps, les résultats des conséquences des extractions sur les mécanismes de rupture seront discutés.

Référence

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CHAPITRE III

Fracture behaviour changes after selective removing of cell wall extractives from woody hemp core

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Key words: extractive molecules, fracture, lignin, mechanical properties, wood.

ABSTRACT

The mechanical properties of woody hemp core were studied after the extraction with four combinations of solvent with two thermal conditions. Four point bending tests were investigated on samples which present a natural and technological variability. The responses measured on breaking properties did not have the same values in the same untreated stock. Comparison by pairs, starting from a raw material divided into two parts is necessary (one part untreated and the other part extracted). The difference in breaking energy indicates that for toluene ethanol and ethanol extractions, the failure mode have changed. Generally after these non polar solvent treatments, the samples become more rigid and break easily compared to the raw material. This change in behaviour is attributed to the lignin content of the cell wall and not directly to the Tg variation of lignin shown in previous work. Natural variability of samples is also a parameter which disturbs the failure mode.

Introduction

Wood is a complex natural composite material, with great mechanical properties in spite of its low density. It is not only composed of cellulose (~ 40%); it contains between 40 and 50% of hemicelluloses, pectin, lignin and extractives. The characterization of the properties of each constituent will contribute to the knowledge of the global behaviour of the material. One of the interesting parameters is to understand the fracture behaviour of wood, to better valorize direct applications of their transformation process, defibrization and material mechanics.

Frühmann (2003) described the crack propagation in beech; he highlighted the cell wall breaking or splitting function to a critic value of relative density of wood. He observed by ESEM investigations the crack propagation happening mainly by separating the cell along their middle lamella (in lignin-rich fraction) without cell wall breaking (Frühmann et al., 2003). Different models exist highlighting different aspects of cell wall structure and proportion: Yamamoto considered the Young modulus highly depending on the moisture content (Yamamoto et al., 2001). Moreover, a good knowledge on cellulose microfibrils and their angle which confer wood rigidity have been investigated by mechanical and structural characterizations (Åkerholm et al., 2004; Färber et al., 2001; Stevanic and Salmen, 2009; Tze et al., 2007). Additionally to this field, Keckes and Burgert proved, by making experiments on single fibers and foils, a molecular stick-slip mechanism (like Velcro connection) and they highlighted the importance of the cell wall matrix by explaining this 'velcro behaviour' mediated by the hemicelluloses attached to the cellulose fibrils, which are flexible enough to entangle and disentangle with the rest of the lignin-rich matrix (Keckes et al., 2003).

As wood is widely used in materials like in composite making which induce transformation steps, the mechanical properties of each component must be well described to enhance optimizing process. Meyer-Pinson's previous studies support the dependency of the fiber mechanical separation on the matrix composition and lignin and hemicelluloses *in situ* proportion. It was supposed that extractives have a role in energy saving for mechanical pulping of poplar (Meyer-Pinson et al., 2004) and that they highly contribute to the viscoelastic behaviour of *in situ* amorphous polymers. After selective removing with toluene, ethanol or water, hemicelluloses glass transition temperature (Tg) decreases in cold conditions of extraction and it increases in hot conditions. For lignin softening, every solvent leads to an increase of Tg except when water hot extraction occurs (Bag et al., 2009). Indeed, our data confirmed that low molecular weight compounds are determining to a significant extend the viscoelastic properties of the cell wall. Considering their possible impact on the mechanisms by which fibres are separated during the high yields thermo mechanical processes, this opens the possibility to control the energy levels required by simple extractions. This study is made on woody hemp core. In order to revalorize, the woody residues from the separation of hemp stem long fibres, some research have been launched on woody hemp core for the optimization of the defribrization process of little fibres and explore them in composite application. This paper aims to study if those extractives can also contribute to the breaking mode of woody hemp core.

Materials and methods

The samples studied were issued from the stem of hemp cultivated and refined according to the procedures of La chanvrière de l'aube (LCDA) in Bar sur Aube (France). Only one cultivar Fedora 17 was used throughout this work. For the

experiments, sticks of the woody part of the stem, devoid of visible cracks, were selected on the basis of regular parallelepipedic form (~ 2mm large and ~ 1mm thick, 10 to 40mm length size). For breaking investigations, samples were cut into two pieces in longitudinal direction to make paired-comparisons. One half part of the stick was extracted with the solvents and the other part remained untreated (defined as the reference sample).

Preparation of samples:

Samples, never dried, are fully impregnated with water under controlled environment during four days in boxes with saturated salt solution using $CuSO_4$ powder in order to place them at 95% RH. This precaution of saturating woody core before each experiment aims to avoid new extraction phenomena by water.

Four point bending test:

The mechanical tests were performed on a Test Well machine model 108.2Kn.H. Strain measurements were obtained using sensors placed between the fixed platen and the moving one. The displacement rate was 15 mm mn^{-1} until the sample cracks and reaches 50% of the max stress then the experiment is stopped. The obtained curve is analysed with the Test Winner 922 software.

The home made four point bending test device is described in figure 1. The samples are placed on the device with the inner part of the stem down. Specimens and testing system were placed into a water bath controlled at constant temperature. The thermal regulation is assured using an electrical heating. The rupture occurs in the transverse plane. This test was chosen due the small geometry of the samples.

Optimizing method

Because the breaking properties is associated to the better propagation of cracks in abundant lignin phase, this step is to find the glass transition of *in situ* lignin for this mechanical testing**:** samples were tested at different temperatures by making the breaking tests in water container equilibrated at different temperature from 40 to 70℃ at intervals of 5℃. For each temperature condition 8 samples was tested until fracture.

Referring to the curve of force (N) against displacement (mm), Figure 2(a), four parameters can be calculated: E, the stiffness criterion is calculated from the slope of the initial linear part (Eq 1), σ_{max}, the strength criterion, derivate from the maximum of the stress before cracking (Eq 2), where d_{max} is the associated displacement and finally $W_{20\%}$, the energy criterion which is calculated from the area below the curve at 20% decrease of the maximum stress.

Eq 1: $E = \frac{L^3 * slope}{4 * l * e^3}$

Eq 2: $\sigma_{max} = \frac{3 * Fmax * (L' - l')}{2 * l * e^2}$ (see figures 1 and 2 for symbols)

Extractions

The removal of extractives was made by using three solvents separately or in combination and two temperature conditions. One extraction was done with a mixture of toluene/ethanol-95% (2/1 by v/v) (Tol/EtOH), another with ethanol-95% (EtOH), and a final one with ultra pure water (water). Successive extraction was done the above order. They were performed in two temperature conditions: (1) The “hot” extraction was done

in a Soxhlet apparatus and (2) the “cold” one was done at 20℃ in a batch vessel by simple mixing with the solvent. The extracts were concentrated and freeze-dried. The dry matter obtained was weighed and subjected to analyses.

Breaking mechanisms on extracted samples

After being extracted as described above, the sticks were tested with four-point-bending test at the temperature condition found in the optimizing method to better see the difference in failure behaviour near Tg lignin. From each curve obtained the four parameters E, σ_{max}, d_{max} and $W_{20\%}$ were calculated on the extracted parts and reference parts assimilated.

Results

Glass transition temperature of lignin

It’s known in literature that the breaking of fibres propagates preferentially in the middle lamella where lignin continuous phase is abundant (Frühmann et al., 2003). The tested hypothesis in this work is to affect the failure mode to the change in lignin phase transition; that’s why in this step the ideal temperature is searched to see the impact on fracture close to its Tg changes.

The typical curves obtained were separate into two classes regarding their forms: ductile breaks noted D and brittle breaks noted B. Figure 2 illustrates respectively the shape D (a) and B (b). The first linear part of the curve corresponds to the elastic behaviour of the material; the second curved part reveals the plastic behaviour of the sample. When a sample break in the linear part, the breaking is characterized as brittle

(figure 2b), as the visible failure happens in the plastic domain, the sample is defined as ductile (figure 2a). After classification and inventory of rupture mode differentiated with shape D and B described above, the histogram in Figure 3 is obtained. With increasing temperature the forms of the curves vary, demonstrating that the breaking mode is influenced by the temperature and the condition of experiment (in saturated water). Above 55℃, mainly shape B curves (brittle behaviou r) appear on the graph and up to this temperature shape D curves (ductile behaviour) are predominant. This change in samples failure mode proves a shift in behavior and an existing phenomenon at this temperature. If failure mode has changed, the propagation has also changed; it's harder for the materiel to break.

Figure 4 shows the evolution of elastic modulus from 40 to 70 ℃ for a raw woody core. In general, the elastic modulus versus temperature curves of polymers exhibit qualitatively four regions of visco-elastic behavior. The first region is called the glassy region. In this state the polymer has a relatively high modulus and is rigid. The next phase is the glass transition region, which is characterized by a sharp decrease in the elastic modulus of the material. In the rubbery plateau region, the elastic modulus versus temperature curve reaches a lower plateau. Upon further heating, the polymer reaches the rubbery flow region, where its modulus decays further out of the measurable range. In the evolution of elastic modulus versus temperature (Figure 4), the values of E present a decrease from 600 MPa to nearly 200 MPa with an inflection of the curve at around 55 ℃ confirming a change in mechanical properties. This change is also in coherence with the shape change of the curves in figure 3. This change is possibly associated to the transition mode assimilate to the glass transition of lignin amorphous phase according to the literature (Bardet et al., 2003). The decrease in elastic modulus is the relevance of a change in elasticity of the material: polymer chain

environment and interactions modified the flexibility of the material and is probably the consequence on the *in situ* amorphous polymers mobility. However, a small increase can be recognized after 70℃ that can be attributed to the effect of heating inducing new extractions during experiments.

Figure 5 represents the mean strength values recorded for samples at various temperatures. It decreases from 12 to 6 MPa. The inflection point of the curve seems to be also around 55 ℃. This supports again the local isation of brittle/ductile transition that would be associated to that of lignin Tg.

For the next part of the work, the experiments were done at 55℃, found in the last paragraph, to better characterize the impact of extractions on the fracture of sample.

Effect of extractions

Figure 6 shows the average values of the *in situ* lignin Tg difference between a treated and untreated part of a stick of woody hemp core from with the same extractions solvents (Bag et al., 2009). Each extraction has an impact on the Tg values of lignin. Toluene and ethanol extractions have an increasing impact on Tg (except for the case of ethanol at room temperature). In cold condition, water and successive extractions have the same behaviour as toluene and ethanol whereas in hot condition they have a decreasing impact on lignin softening temperatures. Indeed, clearly when water extraction occurs (separately or successively), it has the higher positive or negative impact on *in situ* lignin Tg.

From the mechanical testing, the elastic part of the curves is not exploited because woody hemp core sticks are small and thin, regarding to classical wood

characterization. So, additionally to natural variation of samples, the breaking happens too early to have a stabilized and reliable portion on elastic response of the samples. So, the results on elastic modulus variations are not considered as reliable as the standard deviation is very high, and no conclusion about the extraction impact on the elastic behaviour of the samples can be released. However, we must take in mind that if variation of Tg exists, variation on modulus must also exist regarding the basic definition of Tg.

Figure 7 represents the average difference (between treated sample and the reference untreated pair) in the strength value at failure defined as the maximum. Tol/EtOH mix and water lead to a decrease in strength criterion from maximum 2 MPa (~18%), whereas ethanol alone and successive extractions are scarcely decreasing the stress values. The differences between samples suppose a systematic decrease tendency in hot condition whatever the solvent used. In those cases, the samples need less force to start breaking.

Figure 8 represents the displacement value difference when the maximum force is applied (just at failure starting). The samples extracted by Tol/EtOH and EtOH present an increase in the cross-head displacement from maximum 0.4 mm (~16%) for both extraction temperature conditions whereas water presents an opposite behavior (a decrease of maximum 0.1 mm). Successive extraction has a divergent behavior between hot and cold extraction conditions for not much than 0.1mm also. This figure shows a little but clearer difference on maximum displacement at breaking than the strain parameter. When the displacement increases, this reflects an increase in irreversible changes in the material, expressed by a wider plastic part in the curve compared to the reference.

From Figure 9, even if the accuracy of the method is high, the evolution of breaking energy looks the same as maximum displacement. Toluene and ethanol extractions increase the energy values for 0.5 $J.m^{-2}$ to 1.3 $J.m^{-2}$ for ethanol cold condition. The impact of water and successive extractions is not as clear; their breaking energy shifts are close to zero and the accuracy of the method is high. As a result, these two combinations of solvent don't seem to affect the breaking energy of woody hemp core.

To better understand and compare the different parameters of mechanical testing, student test on the average values of E, σ_{max}, d_{max} and $W_{20\%}$ with the four combinations of solvents at the two temperature conditions were investigated (Figure 10). These tests were calculated using a unilateral argue and working with series of pairs between treated and untreated sticks. The maximum value to consider the value acceptable, in other terms to affirm that the two series are different, is fixed at 0.1. As it was discussed above, the elastic modulus has upper values than 0., this parameter is hardly related to the extraction solvents, however a better correlation in hot condition appears from this test values. The other parameter's analyze highlights the evidence of correlations with Tol/EtOH treatment in both cold and hot conditions as they are nearly all around the fixed limit 0.1. From a general point of view, the strain has two cold extraction conditions were the student test value are not pertinent for the separation of the two populations (extracted and reference), whereas for breaking energy, there are three hot extraction conditions that presents the same conclusions.

Discussion

Standing out from all these measurements on σ_{max}, d_{max} and $W_{20\%}$, Tol/EtOH and EtOH extractions have a dissociable impact on the breaking mode, even if water extraction presents the widest difference in Tg values of lignin (figure 7). Therefore, the fracture energy of samples presents a clear difference for polar solvents and not when water extraction occurs. As a consequence, breaking mode is not directly dependent on lignin mobility but on the combination of extraction rate. This extraction rate includes different percentage of small entities as lignin-like or sugar components function to the nature of solvent. Indeed, cell wall is not only constituted of lignin: polysaccharides are also attached to lignin phase that can also influence the lignin mobility. Hofstetter demonstrated the importance of interactions between lignin hemicelluloses and water clustered with extractives conferring to wood its specific elastic behavior (Hofstetter and Gamstedt, 2009). The removal of a low quantity of polysaccharides (not up to 16% of the total extractives whatever solvent is used, data shown in Bag et al. (2009)), don't seem to affect considerably the mechanical properties of wood at the macroscopic level. However, the elastic modulus measurements are not precise enough to highlight the hemicelluloses removal impact on samples elasticity.

In this work, all investigations done on fracture function to Tg variations are done with a macromolecular point of view while the mechanism of breaking is more likely to be a local phenomenon, generally starting at middle lamellae (Frühmann et al., 2003).

These results were taken from experiments considering all kind of *in situ* lignin wherever they are localized in the cell wall but lignin must be considered as follow:

- Lignin is a minor polymer in the plant composite material compared to cellulose, which is essentially crystalline. From a more general aspect, it is known that

wood rigidity is mainly dependent on the percentage of cellulose cristallinity and also of cellulose micro fibril angles (Färber et al., 2001). These simple extractions have no impact on cellulose degradation in cold condition but are more likely to hydrolyze this phase in higher temperatures. This may explain the lower values of student test in hot condition that can be reliable to the clearer effect of the extractions on the sample elasticity.

- But as lignin is not homogeneously distributed in the cell wall, and as the localization of extraction could not be controlled, this study does not take into account the origin of entities removal. As it was demonstrated, that mechanical properties are highly dependent on global relative density of wood and lignin content (Bardet et al., 2003), local composition differences in the cell wall may also have an impact on the mechanical properties from this point of view. There could be a gradient of properties inside the different level of the cell wall, which are differently composed from a structure and chemical view (Österberg et al., 2006).

As the exact place from where such extractives are removed is ignored yet, such a study should be investigated by developing a characterization method at a smaller level. This new research should take into account the extractives presence or not and also the water local concentration in the different level of the cell wall.

Finally the other conclusion is that this work is an illustration of the complexity of the various parameters governing the fracture behavior of wood (defaults or *in situ* polymers mobility or chemical composition). Indeed, other factors as the composition of each stick of woody hemp core or the not visible and systematically existing cracks, due

to the transformation process, should be taken into account to highlight these solvent extractions effect on the fracture propagation in woody hemp core. From all these study the extraction with Tol/EtOH has the clearer impact on the mechanical properties. Biochemical results on extractives with this kind of solvents revealed a higher amount of aromatic compounds (essentially lignin) is better removed from the cell wall compared to polar solvents: aromatic compounds compose up to 60% of the total extractives with Tol/EtOH or EtOH in cold or hot cases, data shown in (Bag et al., 2009). As lignin phase is known to govern the failure mode in the middle lamellae, the existing natural default presence should have occurred where lignin amount is high (propably in the middle lamellae). In the defaults sites, lignin phase is easily accessible for lignin solubilizing solvents (as Tol/EtOH). The lignin removal, the sample breaking and natural default presence are highly correlated. For all these reasons, Tet/EtOH treated samples have a clearer consequence on their mechanical properties.

Conclusions

This study has shown that when viscoelastic property changes occur after toluene and ethanol extractions, a modification in the macromechanical properties with this condition of characterization can be noticed. Extraction of high amount of aromatic compounds influences viscoelastic and mechanical properties. Samples'breaking energy decreases after such extraction, they become more brittle. Therefore, the chemical composition of the extractives removed seems to play an important role in the macromechanical properties of woody hemp core.

However, for water and successive extraction, the effects on breaking type seem to be smoothed by other parameters as the experimental condition (in water at 55°C) or the natural defaults function to samples. These experiments are not sensitive enough to highlight the change in the global behaviour of woody hemp core.

Noteworthy, the measure of the main mechanical parameters has given the values calculated above. A study on correlation with the biochemical composition of the extracts removed, the viscoelastic properties of each amorphous polymer and these last investigations is necessary to better explore these results and go deeper in these conclusions.

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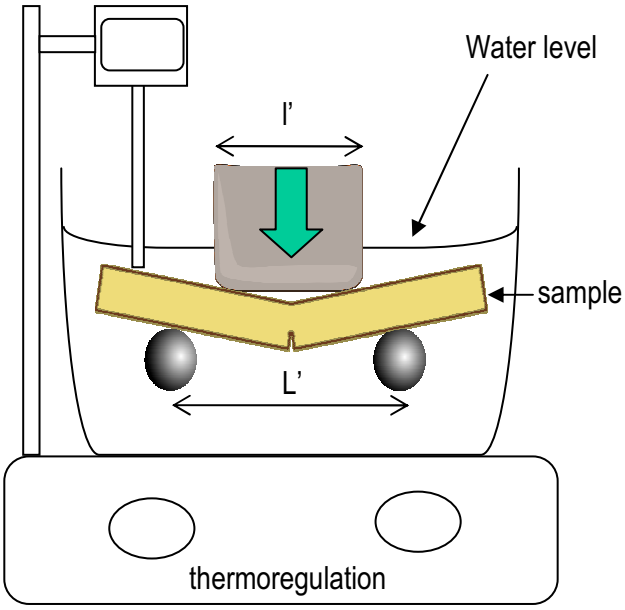

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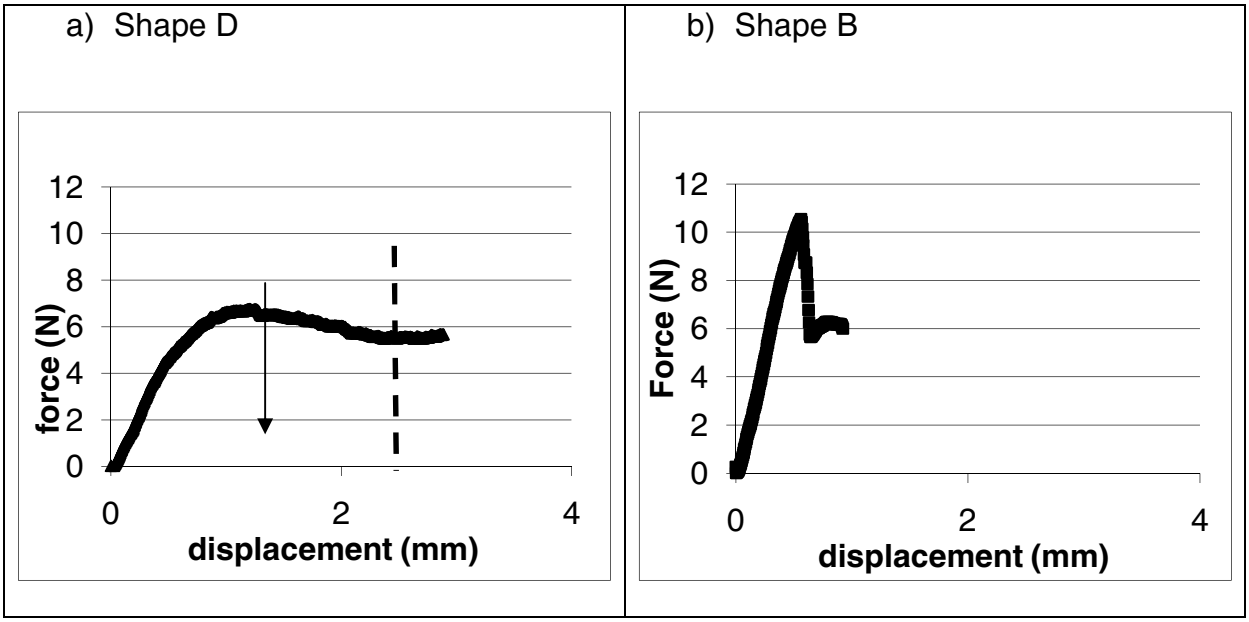

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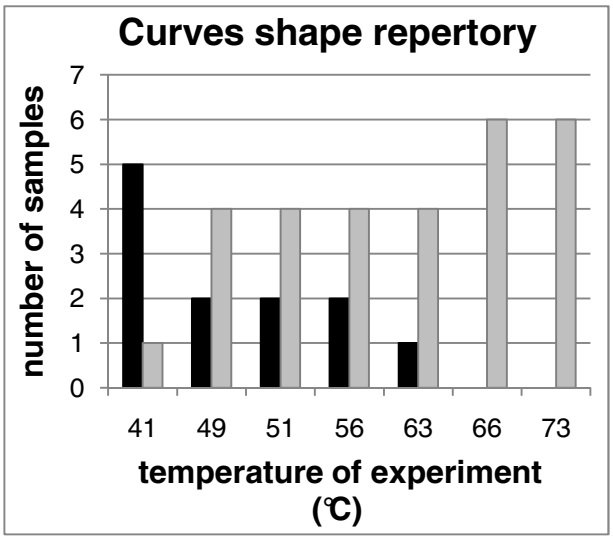

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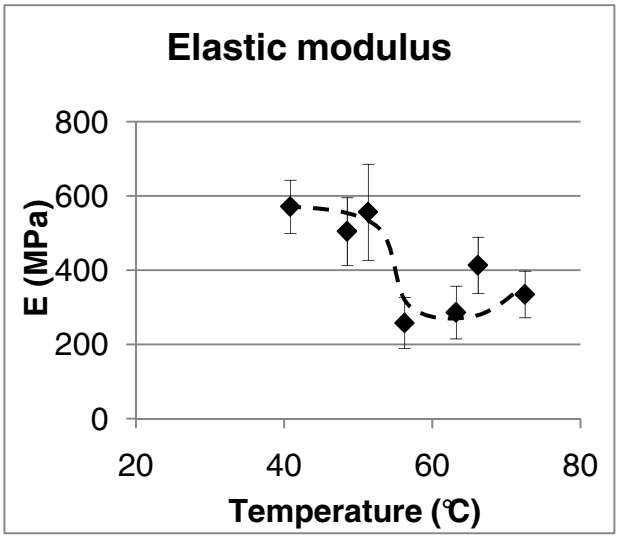

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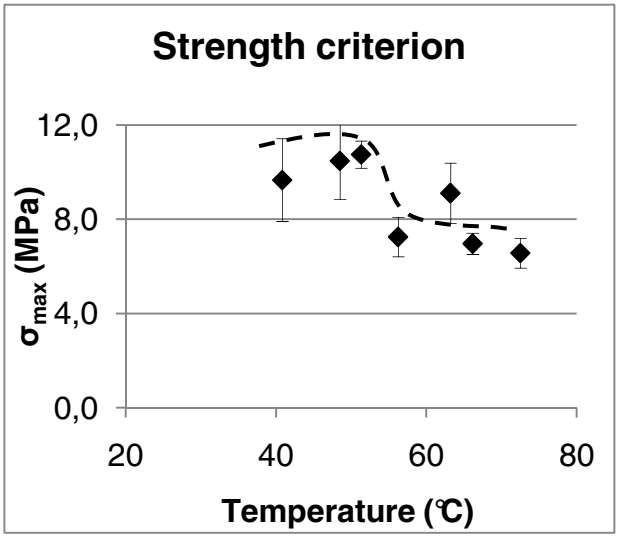

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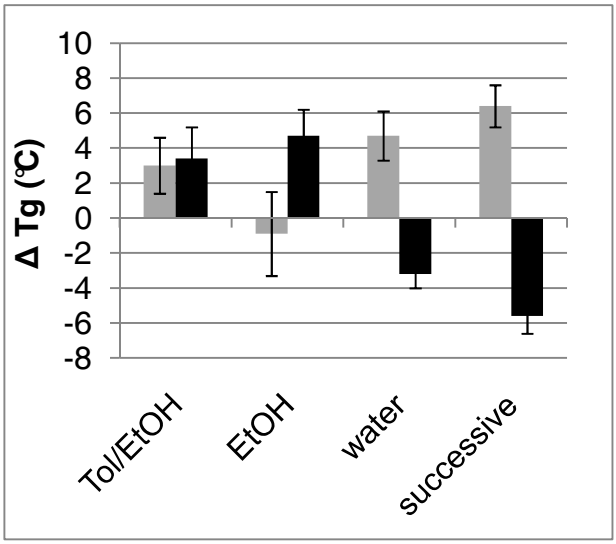


Figure 6

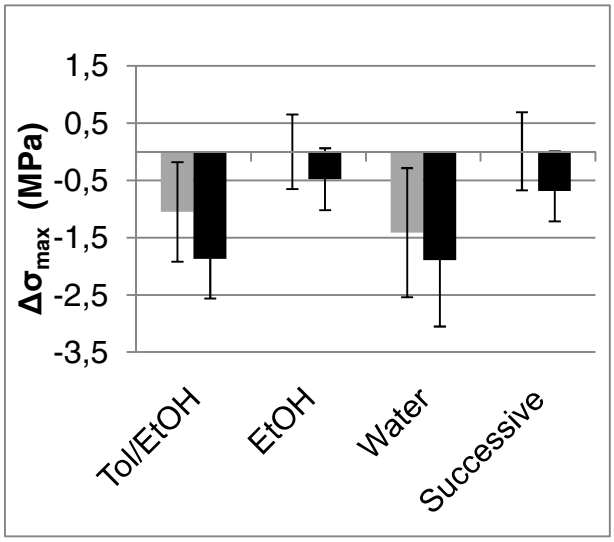


Figure 7

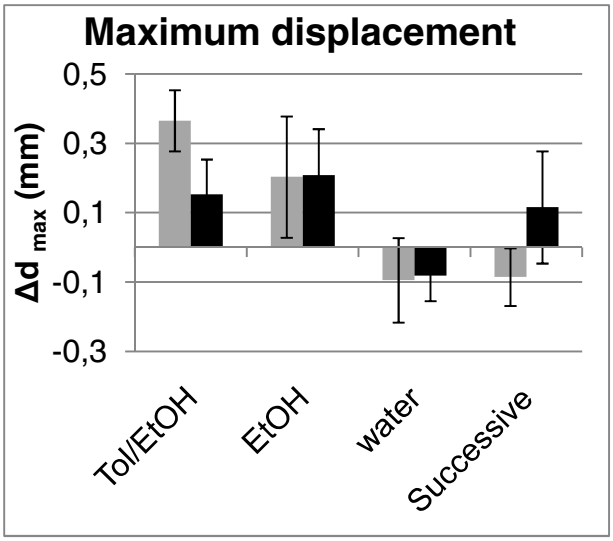


Figure 8

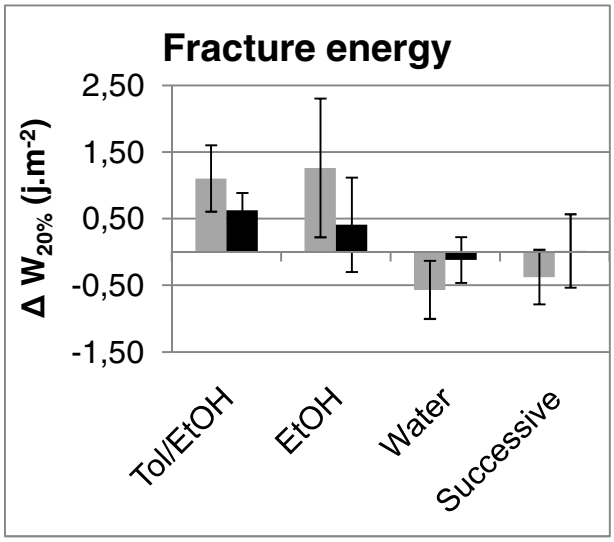
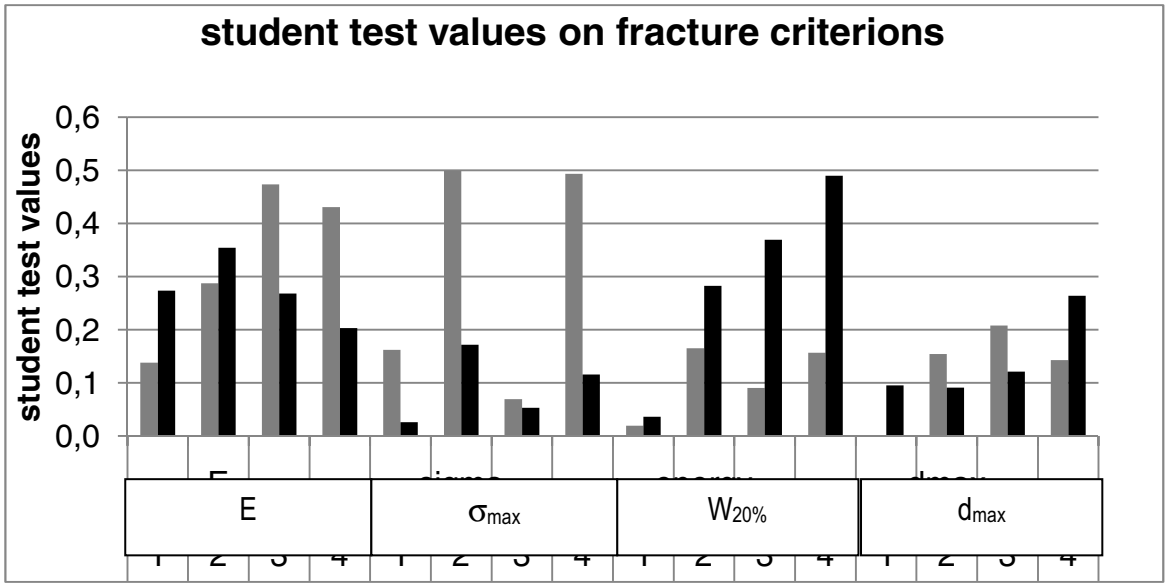


Figure 9


student test values on fracture criterions
student test values
0,6
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E
σmax
W20%
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1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4

Figure 10

Figure captions

Figure 1: Four point bending device: l'=3.96mm, L'=6.82mm, l is the sample width and e is the sample thickness.

Figure 2: Illustration of shapes a) Shape D and curve's exploitation, b) Shape B

Figure 3: Shape distribution of fracture curves function to temperature. Captions: (grey): shape D; (black): shape B

Figure 4: Average value of Elastic modulus of raw material function to temperature

Figure 5: Average value of strength criterion of raw material function to temperature

Figure 6: Average value of the shifts of *in situ* lignin glass transition function to extraction solvents and temperature condition according to Bag (2009). Captions: (grey): cold, (black): hot conditions

Figure 7: Average value of the shifts on strain function to extraction solvents and temperature condition. Captions: (grey): cold, (black): hot conditions

Figure 8: Average value of the shifts on maximum displacement function to extraction solvents and temperature condition. Captions: (grey): cold, (black): hot conditions

Figure 9: Average value of the shifts on fracture energy function to the extraction solvents and the temperature condition. Captions: (grey): cold, (black): hot conditions

Figure 10: Unilateral student test with pairs for E, σ_{max}, $W_{20\%}$ and d_{max}. Captions: (1)=Tol/EtOH; (2)= EtOH; (3)= water; (4)= successive extractions, (grey): cold, (black): hot conditions

L'étude du mode de fracture de la chènevotte ne semble pas démontrer de manière évidente un changement brusque des propriétés à rupture suite aux extractions sélectives des extractibles de la paroi. Le choix des expériences en flexions quatre points en mode immergé dans l'eau à 55 °C découle d'une suite d'expérimentations qui confirme la valeur de la température de transition moyenne des lignines pour ce mode de caractérisation autour de 55 °C. Cette valeur semble plus faible par rapport à la valeur de Tg lignine trouvée au dessus de 100 °C en DMA. Cependant, il ne faut pas oublier que les fréquences de sollicitations et la force appliquée ne sont pas les mêmes durant ces deux expérimentations. La manifestation de la transition vitreuse est fortement dépendante des conditions expérimentales qui d'ailleurs introduit probablement de nouveaux artéfacts dans la méthodologie de mesure. En effet, il convient de noter que les expériences se font à 55 °C en immersion dans l'eau. Bien que la précaution de saturer l'échantillon en humidité soit prise, certains échanges de molécules avec l'eau (solubilisation supplémentaire d'extractibles avec le chauffage) ne sont pas impossibles. De ce fait, la variation des propriétés à rupture avant et après extraction peut ne pas être mesurable par cette adsorption supplémentaire. Cette technique de mesure ne semble pas être suffisamment sensible pour permettre de déceler une différence prononcée dans le comportement global du bois.

Les valeurs du module élastique ne sont pas présentées dans cette partie car elles sont discutables. Les valeurs des différences du module élastique avant et après extraction montrent une grande incertitude numérique. En effet, le calcul de la pente sur le début de chaque représentation graphique dépend de la forme de la courbe. Celle-ci est plus ou moins lissée en fonction de la stabilité de l'échantillon dans l'eau (perturbations existantes dues à l'agitation de l'eau). Les conclusions aboutissent, dans certains cas, à aucun changement sur le module après extraction alors que des modifications de Tg

existent dans le chapitre précédent. Si Tg est décalé vers d'autres valeurs de température, le module élastique doit également être modifié, par définition de la mesure de Tg sur les courbes de relaxation. Si telles sont nos conclusions, la précision de ces valeurs est mise en doute par la technique de mesure. Pour toutes ces raisons et par soucis d'interprétations, il a été préférable de ne pas représenter ces valeurs de module en fonction des solvants d'extraction.

Le but du chapitre suivant est de croiser les données expérimentales pour permettre de ressortir des relations non visibles entre les différents paramètres des échantillons. Ainsi, nous pourrons éventuellement attribuer le changement de propriétés mécaniques ou viscoélastiques à l'extraction majoritaire ou sélective d'un type de composés. En somme, les valeurs numériques, bien que peu marquées en terme de différences, sont celles mesurées. Elles nécessitent malgré tout une étude approfondie : l'étude d'analyse de corrélations entre la composition biochimique des extraits, les propriétés viscoélastiques des polymères amorphes de la paroi et les propriétés mécaniques du matériau s'avère nécessaire. Par conséquent, le chapitre suivant traitera uniquement des liens entre mobilité et rupture que nous tenterons d'attribuer à l'extraction d'un ou plusieurs types de composés par des analyses de composantes principales et des calculs d'indice de corrélation.

CHAPITRE IV

Relationships between biochemistry, modulation of cell wall viscoelastic properties and fracture behaviour in woody hemp core by correlation study

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Key words: extractives, fracture, glass transition temperature, hemicelluloses, lignins, principal component analysis, wood.

ABSTRACT

Extractions impacts of woody hemp core cell wall through various solvents polarity at two thermal conditions were investigated. Biochemical analyses of extractives, viscoelastic behaviour of lignins and hemicelluloses plus mechanical properties of woody hemp core were carried out. The large results data set obtained was refined by principal component analysis (PCA) and correlation index providing evidence of relationships between extractives removal linked to polymers mobility and/or breaking properties. The major novelty are the support of moderately extracted low molecular mass carbohydrates and aromatic compounds influence the cell wall *in situ* amorphous polymers mobility and are also able to affects the fracture behaviour at the macroscale. With the help of correlation index in part, it was found that the polymers mobility changes can impact the breaking property if at minima a sufficient threshold of extractives is removed from cell wall.

Introduction

The growing interest on using the biomass to reduce the impact on environment encourages manufacturers to choose their potential raw material from lignocelluloses. In order to revalorize the woody residues from the separation of hemp stem long fibres, some research have been launched on woody hemp core for the optimization of the defibring process of short fibres and explore them in composite application. Although the woody hemp core represents approximately 70% of the hemp plant, it is still incompletely valorized (like in animal litters). New applications start to emerge, but a better description of different properties, taking into account the natural variability, is required. Woody hemp is composed of 33–37% cellulose, 16–20% hemicelluloses, 17–22% lignins, 1-5% proteins and ashes and a small amount of extractives, around 5% (Cappelletto et al., 2001). Extractives are composed in variable proportions of a mix of mainly low molecular weight molecules arising from the main molecule family. As illustration, extractives present di- and oligo-mers of carbohydrates (thereafter called sugars for simplicity reasons), lignins (almost oligomers), some lipids and polyesters, peptides plus proteins and ash compounds which is rich in minerals.

Woody hemp core has a wood like structural arrangement with fibre cell units themselves assembled as a complex composite material (Rose, 2003). The universal fibre cell wall is organised with superposed layers differing in thickness and biochemical composition (Buchanan et al., 2000). In woody hemp core the cell wall present a typical secondary type structure, the middle lamellae enriched in pectins compounds, the thin primary cell wall enriched in lignins, the secondary cell wall divided into three sublayers. They are composed with cellulose microfibrils oriented with different angles function to their location in the secondary cell wall. Several parameters affect the strength of a

plant material: the order and the amount of cellulose microfibrils, generally along the fibre axis in the secondary cell wall, drive fibres strength mechanical properties (Åkerholm et al., 2004; Stanzl-Tschegg, 2006; Wang et al., 2007; Yamamoto et al., 2001)

The viscoelastic behaviour of matrix amorphous polymers in the cell wall give a trend of the state of the material from fragile to viscous (Assor et al., 2009; Daniels, 1989; Placet, 2009). The cell wall content in extractive compound-family was very recently shown to affect the mobility properties of the amorphous phase (Bag et al., 2009) and it is furthermore suspected to take part in failure modes during refining process (Meyer-Pinson et al., 2004). However, these questions still suffer a lack of knowledge, leading to imprecise conclusion regarding the intrinsic effects of extractives natures and levels on the fracture process. Because it is of fundamental and applicative importance in one part and that has not been extensively investigated in previous works. In a second part, this work aspires to give new insights concerning roles and mechanisms relied on extractives when both polymers mobility and failure behaviour modification occurs. To do that, woody hemp core were used as woody substrate and analytical results already published were further process by coupled PCA and correlation index values-calculation. PCA involves a mathematical procedure that transforms a number of possibly correlated variables into a smaller number of uncorrelated variables called principal components. The first principal component accounts for as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible. Using this method, the data are expressed to highlight the similarities and differences between different samples. When samples are similar, they are close to another in principal components' space. At the opposite, when samples are different, there is a

considerable distance between them. Therefore, samples are arranged into groups. So, PCA is a powerful tool to acquire quick information about the similarities in a group of samples (Shlens, 2005). Correlation index is a method that can provide systematic analysis of correlation only between two selected variables, independently from the others (Guadalix et al., 1996).

The extractives have been selectively removed from raw cell wall material by combination of solvents differing in polarity and their components were biochemically described in (Bag et al., 2009). The consequences of the removal of extractives on the mobility of the *in situ* amorphous polymers were studied with thermal analysis. In parallel, the fracture behaviour was described in the resulting samples (Bag et al., 2009). This pluridisciplinary approach coupled to a statistic analysis would provide us somewhat pertinent correlations, if any, between viscoelastic, mechanical and biochemical data permitting some attribution of changes to certain parameters or class of extractives.

Experiments

Material, extractions and analyses

The woody hemp core plant material is from the cultivar Fedora 17 supplied by La chanvrière de l'aube (LCDA) in Bar sur Aube (France). This woody lot is issued from the stem of hemps, cultivated and refined according to the procedures in LCDA. For the experiments, regular parallelepipedic sticks (~ 2mm large and ~ 1mm thick, 10 to 40mm length size) of woody core, devoid of both phloem fibres and visible cracks, were selected.

For breaking and thermal properties investigations, samples were cut into two pieces to permit a comparison by pair. One half part of the stick was extracted with solvents as described hereafter, whereas the other halfpart remained untreated and defined here after as the reference sample (Bag et al., 2009).

The removal of extractives was done at two temperatures: hot (H) and cold (C) and by using three solvents: the first was a mixture of toluene/ethanol-95° (2v/v) named TEt; the second was ethanol-95° named Et and finally the third was ultra pure water named Water. In addition, extractions conditions were conduct with one of the three solvents independently or in a successive combination.

The extractives extracted from the woody hemp core were collected, lyophilized and submitted to biochemical analyses as described in (Bag et al., 2009). Briefly, carbohydrates, ashs, aromatic compounds, mineral compounds and proteins were examinated.

The mobility behaviour of *in situ* amorphous polymers was measured in woody core by T_α relaxations of hemicelluloses and lignins using thermal analyses: DEA and DMA respectively for each polymers according to (Bag et al., 2009). Those T_α records on both the extracted halfparts and the reference one were assimilated to Tg, also called softening temperatures in wood technology (Salmén and Olsson, 1998). The differences between the reference and treated halfpart were calculated.

The fracture behaviour was investigated by four point bending tests. From each curve obtained, the four parameters E, σ_{max}, d_{max} and $W_{20\%}$ were calculated on the extracted parts and reference parts assimilated. The differences between the both parts were calculated on six samples (Bag et al., 2009).

Principal component analysis (PCA):

Collections of data sets from (Bag et al., 2009; Bag et al., 2009) were selected to study the specific influence of each type of extractions in two temperature conditions on the following variables: mean value of the difference in mobility; biochemical composition or mechanical properties between a raw and an extracted sample.

The Unscrambler 9.8 software was used as the programming tool for PCA calculations. Sets of data were organized as a matrix of 8 samples, representing different conditions of extractions which are Tet, Et, Water and Successive extractions in cold (C) or hot (H) temperature conditions, and 28 variables listed below:

- extractive percentage
- total carbohydrate content of extractives called sugar (plus the details of nine monomers of constitutive sugars)
- total aromatic compound content
- ash content
- protein content
- mineral analysis (Ca, Cu, Fe, K, Zn, Mg, Mn, Na)
- glass transition temperature difference for lignins calculated as value between raw reference and extracted material
- glass transition temperature difference for hemicelluloses between a raw and extracted material
- elastic modulus difference between a raw and extracted material, ΔE
- strength difference between a raw and extracted material, $\Delta\sigma_{max}$
- maximum elongation difference between a raw and extracted material, Δd_{max}

- fracture energy difference between a raw and extracted material, $\Delta W_{20\%}$

Prior to the analysis, variable's columns were first mean centred: the average across each column was subtracted allowing an equal importance to each variable.

Correlation index:

The correlation index is calculated from the following formula: $\rho_{x,y} = \frac{Cov(X,Y)}{\sigma_x * \sigma_y}$ where Cov(X,Y) is the covariance of two matrix X and Y, given for one of the variables cited above at a given condition (solvent extraction in a temperature condition) and σ_x, σ_y are the mean value of the values in the matrix X or Y.

Results and discussion

The Tables 1-3 (biochemical), Table 4 (thermal) and Table 5 (mechanical) results obtained from the overall experiments are summarized as function of the extraction solvents used. All these variables herein given for different type of extraction came from a set of studies where specific questions were addressed (Bag et al., 2009; Bag et al., 2009): i) the composition of extractives, ii) the variation of Tg lignins and hemicelluloses and iii) the variation of mechanical properties. Only the mean values of these last data set are given.

PCA:

Solvents and biochemical analysis

From the PCA analyses between extractions and biochemical contents of extractives (figure 1 A), three different groups are clearly distinguishable: one is composed of

organic solvents and the two other includes Water and successive extractions discernible by the temperature of extraction (H and C). These groups are far from each other proving their respective independence regarding both their nature and temperature extraction condition. When analysing the loading plots (figure 1 B), their differences can be explained by the various content of their extractives in lignins, in sugars and in ashs, respectively. The extraction rate and the protein content (close to the N content as expected: proteins have amino acid groups justifying the proportionality between protein concentration and N contents) seem to be independent variables from the extractions. These results confirm that the entities previously removed are in accordance with the polarity nature of the solvent.

Solvents, biochemical analysis and ∆Tg in situ polymers

Similar correlation was done in a second round with taking into account the Tg variations of amorphous polymers in the woody core. The resulting score is given in figure 2. For this analysis, two components are taken into account since more than 90% of the total variance is explained. The figure in the left (C) represents both the scores and loading to better see the dependence of each variable to each extraction solvent. The lignins Tg variation is not affected by one solvent nature specifically, whereas the variation of hemicelluloses Tg is closed to water extractions under H condition. This behaviour can be explained by the correlation loadings (D): ∆Tg lignins is isolated from the rest of the variables, which means that it does not depend on a specific biochemical component. Therefore the removal of extractives does not disturb directly the lignins phase. Whereas ∆Tg hemicelluloses is close to sugars extracted and with hot water extraction. For this reason, in the bi-plot (C), sugars content in extractives appears close to Tg hemicelluloses variations. This supports the hypothesis that mono/di- or oligo-

saccharides possibly coming from cell wall (extracted with hot water) disturb the organization of the hemicelluloses phase.

To verify this last conclusion, it can be proposed to run the PCA analysis with every type of sugar, to attribute potential reliance on ΔTg.

Solvents, ΔTg in situ polymers and monosaccharides

Each monosaccharide is here correlated to mobility and solvents (figure 3). These variables are described essentially by one principal component, 99% of the total variance. First, in the scores and loading bi-plot, all sugars are centred and near ΔTg hemicelluloses, but since they are far from ΔTg lignins one can conclude that sugars removed have solely an impact on hemicelluloses but no against lignins. Second, not any variables are found to be close to the extraction condition, so they are not specific to one extraction nature. From the correlation loadings (figure 3 F), the mobility changes (lignins or hemicelluloses) appear far from one another and from the sugars; they seem to be not correlated to one specific monosaccharide. Interestingly, this deeper analysis reveals that this conclusion is in some extent not completely true regarding (figure 3 E) conclusions, since we can observe two groups well separated from the Tg hemicelluloses. Uronic acids and fucose together in one of the groups are commonly assigned to pectin substances. Therefore, this highlights the extraction of entities from the same origin from the cell wall. And the second group composed of neutral sugars, illustrates the extraction of some chemical features from the hemicelluloses main chain like xyloglucans or arabinogalactans, known to compose woody hemp core (Cappelletto et al., 2001).

Solvents, ΔTg in situ polymers and mechanical properties

The ACP correlation between those parameters did not show an obvious correlation with ΔTg lignins and the fracture parameters. This kind of ACP calculation did not help to highlight correlations, if any, between breaking and lignins mobility. Perhaps the method is not powerfull enough due to weak ΔTg variations. Moreover, the dispersion of extraction rate in hot and cold conditions is wide which generates wide Tg variations (compare table 1 column 1 and table 4) and impacts in a negative way the ACP analysis strength. In that case, for estimating if hidden correlations exist between mobility and fracture, the decoupling of experimental conditions may reveal it using for instance correlation index.

Solvents, mechanical properties and biochemical analysis

Even if failure mode appears not directly correlated to lignins softening, there might be a chemical compound dependency as we shown that polymers mobility and extraction rate were correlated. With the idea that the small difference in mechanical behaviour may be related to one specific nature of extract as above, the mechanical properties measurements were correlated to each extractives sub-group (figure 4). The variance is described for 99% by two principal components. All the variables are centred unless three of them: the lignins content along positive value of PC2, the ashs content along negative value of PC2 and thirdly the variation of elastic modulus ΔE along positive value of PC1 (G). The first principal component describes the flexibility of the samples. The second principal component seems to describe, for its positive part, the lignins content in samples, and for its negative part the ashs content in samples in accordance with the solvent nature. Nevertheless, the correlations loadings (H) informs on the dependence of the lignins content in extractives with the breaking energy and the maximum elongation of samples. This result is in accordance with the works done by

Bardet, who illustrates the breaking parameters related on lignins percentage (Bardet et al., 2003). If it is the case for different trees containing different amount of lignins, one can arguably hypothesize that it can be the same in woody hemp core, where treatments lead to extraction of more or less lignins and so residual amount in cell walls.

Solvents, mobility, mechanical properties and minerals

The correlation between each mineral and the different properties of samples (mobility and mechanical variables) was also studied but no conclusive results which were worth to be developed. Minerals were not entities which seems able to disturb the samples fracture mode, but in other hand they could be structural *in muro* thus if extracted in higher amount than in this study, their lack may possibly disturb the polymer environment or arrangement. Once again, this arguably assumption still to be confirmed since it was not demonstrated in our work.

Finally, the different kind of entities the solvents removed preferentially is illustrated here by the PCA calculations. Broad-spectrum correlations appeared; it was shown that toluene and ethanol solvents extracts lignins in turn impact on breaking energy and maximum elongation. Hot water extractions remove more sugars than during cold condition, which are presumably from cell wall matrix and can modulate both the hemicelluloses mobility and sample elasticity. If PCA was powerful to unmasked the above-mentioned statement, it failed to highlight direct link between mobility of amorphous polymers and the failure mode. The correlations were made with all data together without temperature conditions separation, and consequently the natural imprecision linked to each parameter assessments were globalized perhaps leading to a weakening of the PCA resolution capacity. Temperature effect leads to a greater

amount of extractives removed and disturbs the way of softening (plasticizing / deplasticizing). And so can transform the mechanism of relaxation as it was demonstrated on hemicelluloses softening in previous works (Bag et al., 2009). As intermediate conclusion, the analysis should be done with separation of these two differing extraction thermal energy levels. Beside, this parameter has to be considered in the correlation index paragraph.

Correlation index:

In attempt to get deeper in the analysis, a correlation test with distinguishing the extraction temperature condition was necessary due to the reasons explained above. Only correlation index values above 0.5 from a scale ranging from 0 to 1 were accepted as a valid correlation.

Mobility and extractives' biochemical compounds:

Figure 5 represents the correlation between polymers mobility and biochemical composition of extractives in H and C condition one by one. In cold case (A and B), first point, the main correlation observed was on lignin softening (figure 5 A) which seems attributably to a global effect of extraction rates, proteins and ashes. Looking the specific correlation with sugars (figure 5 B), relations with rhamnose, galactose, xylose and mannose exist. The nature of this latest saccharide panel (Buchanan et al., 2000) sustains the fact that concomitant extraction of pectins related occured in addition to hemicelluloses originated monomers (Morvan et al., 2003). Second point, hemicelluloses' softening is scarcely affected by lignin extraction (figure A), and unsurprisingly our study shown that no correlation exists with one particular

monosaccharide (figure 5 B). Hemicelluloses mobility variation seems to be a consequence of aromatics molecules generally associated to lignin. They are well known to be able to covalently link and sometimes crosslink both polysaccharides backbone and aromatics polymers: this could plausibly explain the sugar extraction dependency on lignin softening as their relationship has been proved in hemicelluloses partial recalcitrance to enzyme hydrolysis (Beaugrand et al., 2004). Focusing on polymers mobility, it may be of interest to recall that since these two amorphous matrix polymers are arguably interconnected to each other by plenty of low and high energy interactions, they have an *in muro* complex organization still imperfectly know and it makes the interpretation complex.

In hot case (figure 5 C and D), lignin softening was only correlated to lignin extraction rate when the hemicelluloses extraction was correlated to the full component panel monitored (figure 5 C). In cold extraction case, lignin softening behaviour showed an interestingly opposite trend than the one reported in hot condition. Analyzing in details (figure 5 D), all the sugars were correlated to hemicelluloses softening. In, hot condition extraction only, every sugar plays a similar and strong influence on hemicelluloses viscoelastic behaviour certainly implying an undifferentiated mechanism.

Mobility and fracture behaviour

The correlation between mobility and fracture behaviour in cold (figure 6 E) and hot (figure 6 F) conditions tested individually is represented figure 6. No high correlation exists in cold condition (figure 6 E). Mobility does not seem to affect the way of breaking in these conditions. But following this temperature splitting scheme, we were then able to see a better correlation occurring in hot condition: Lignin' softening was correlated to both the breaking energy and displacement variations in one hand, and hemicelluloses'

softening is correlated to elastic modulus variations (figure 6 F) in a second hand. The preponderant difference between the cold and hot conditions was the global amount of extraction, higher under a warm environment, with minor differences in the distribution of the nature of entities extracted more sprayed whilst temperature increased. To explain ΔTg and breaking energy correlation, we propose that when a sufficient amount of aromatic compounds was extracted from the total lignins phase, it induces a consequent lignins chains rearrangement that can be perceptible even at a macro scale (fracture behaviour) (Gindl and Teischinger, 2002). In the case of ΔTg and elastic modulus correlation, we further suggest that again, if a sufficient amount of sugar is extracted from the hemicelluloses phase, it can lead to elasticity changes. These last correlations did not appear with the first ACP global analysis approach. To better illustrate these last two hypothesis, the following correlations between the fracture parameters and the biochemical compounds was done in cold (figure 7 G and H) and hot (figure 7 I and J) conditions.

Facture behaviour and biochemical compounds

Breaking energy and maximum elongation were clearly related on lignins extraction in the two temperature conditions (figure 7 G and I). In cold condition, the strength criterion and the elasticity of the samples were correlated to the sugar extraction (figure 7 G) and specifically to the arabinose level (figure 7 H). In hot condition, the elastic modulus varied essentially due to the global effect of extraction rate (figure 7 I), however correlations can be attributed to all sugars at the noticeable exception of fucose and mannose (J), two carbohydrates often pectin related units.

Lignin extractions seem to be directly correlated to breaking properties (energy and elongation) either in cold or hot extractions which is confirmatory to what was previously

demonstrated in another cell wall origin (Bardet et al., 2003). This result corroborates the hypothesis we have formulated in paragraph *Mobility and fracture behaviour* where we proposed the fracture dependency on the amount of lignins removed even with extractions in mild conditions.

In cell wall, the hemicelluloses content influence the fibril aggregates and therefore the flexibility of the material as it was demonstrated for Norway spruce pulps: in fibres with a low hemicelluloses content, the fibril aggregates (macro fibrils) formed a much more compact surface structure (Duchesne et al., 2001). Therefore, the numerous correlations between sugars and elastic modulus highlighted (compare figure 7 H (cold) and J (hot)) can be explained by a higher amount of sugar extracted in hot condition. It surely leads to fewer sugars in the cell wall.

Conclusions

Exhaustive correlation studies between the biochemical analysis on extractives, the viscoelastic behaviour of *in situ* amorphous polymers and the breaking properties of woody hemp core were investigated by two complementary statistical methods.

The first one, PCA calculations revealed the high reliance of natures of entities extracted function to the solvent polarity used. In addition, it pointed out particularly the temperature condition significance with water as solvent. Organic solvents extract better lignins related components, cold water condition is an efficient solvent for ashes extraction and finally hot water extraction removes easier sugar families.

The mobility of the remaining cell wall polymers is not attributed to a specific removing of extractive compounds but more to a global ‘amount’ effect which must lead

to consequences as the rearrangement of the two main amorphous phases in the cell wall matrix. Globally with the PCA analysis, the extractions practised herein were supposed to have a negligible effect on the macromechanical properties due to their minus amount. Nonetheless analysing the data with correlation index calculations, by separating the extraction temperature condition, permit to lift a veil from existing correlations. The lignins content in the extracted samples affects their fracture energy (as less as 0.5% extraction of the woody dry matter). But correlation between lignins softening and breaking mechanism appears only if a sufficient but low (≥ 1.5% of the woody dry matter) threshold amount of lignins is cell wall extracted. The conclusion for polysaccharides extraction followed the same rule: only when a sufficient amount of sugar is extracted that a correlation between elasticity and hemicelluloses softening revealed. In parallel to what is observed in others wood substrate, in the woody hemp core the global amount of cell wall hemicelluloses present one of the factors governing its elasticity. The more hemicelluloses are present in the cell wall, the more flexible is woody hemp core.

Finally, we demonstrated that mild extractions on woody hemp core can modulate the viscoelastic properties which in turn have measurable impacts on macromechanical properties.

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Figure caption

Figure 1: Principal Component analysis of the biochemical composition analysis from the treated samples: scores plots (A) and loadings (B). The first principal component describes 94% of the total variance; the second is responsible for 4% and the third for 2%

Figure 2: Principal Component analysis of the biochemical composition analysis from the treated samples: scores and loadings bi-plots (C) and correlation loadings (D). The first principal component describes 62% of the total variance; the second is responsible for 36%.

Figure 3: Principal Component analysis of the monosaccharide analysis from the treated samples: scores and loadings bi-plots (E) and correlation loadings (F). The first principal component describes 99% of the total variance; the second is responsible for 1%.

Figure 4: Principal Component analysis of the monosaccharide analysis from the treated samples: scores and loadings bi-plots (G) and correlation loadings (H). The first principal component describes 77% of the total variance; the second is responsible for 22%.

Figure 5: Representation of correlation index between different biochemical composition of extracts function to temperature: cold (A and B), hot (C and D) and the mobility of lignins and hemicelluloses

Figure 6: Representation of correlation index between mobility of lignins and hemicelluloses and the fracture properties in cold (E) and hot condition (F). DE=ΔE; Dsigma=$\Delta\sigma$; DW 20%= $\Delta W_{20\%}$ and Ddmax=Δd_{max}.

Figure 7: Representation of correlation index between different biochemical composition of extracts function to temperature: cold (G and H), hot (I and J) and the fracture properties. DE=ΔE; Dsigma=$\Delta\sigma$; DW 20%= $\Delta W_{20\%}$ and Ddmax=Δd_{max}.

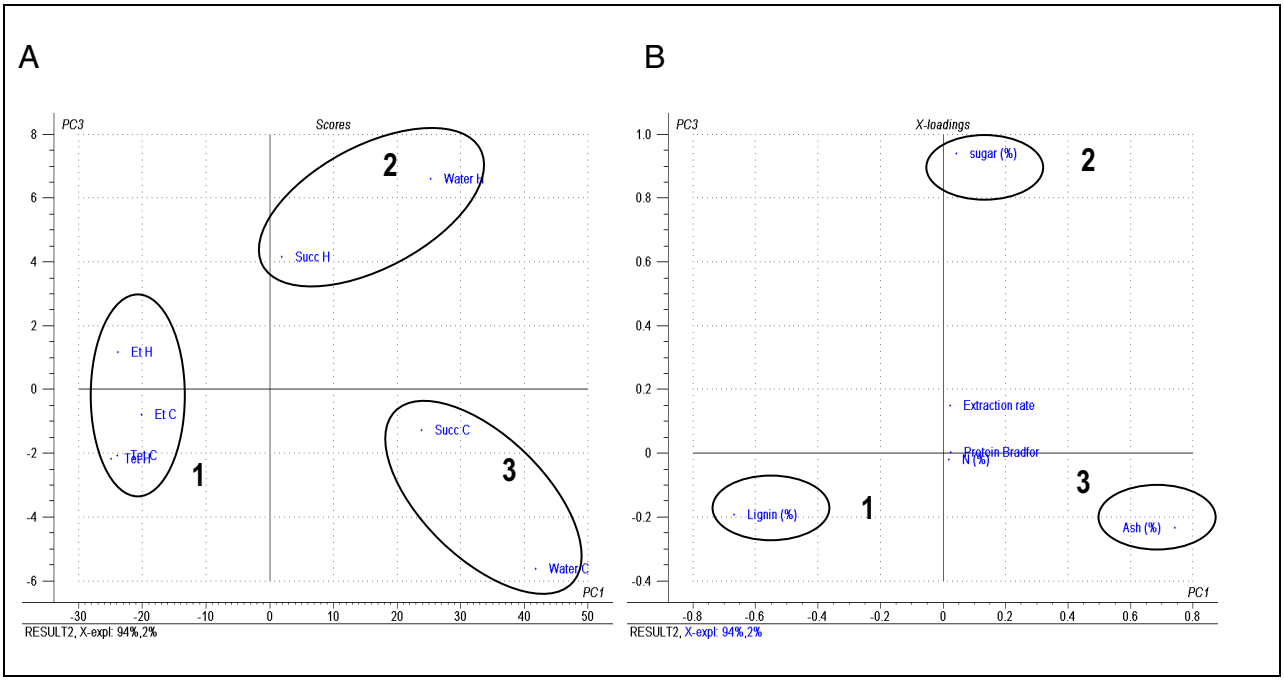
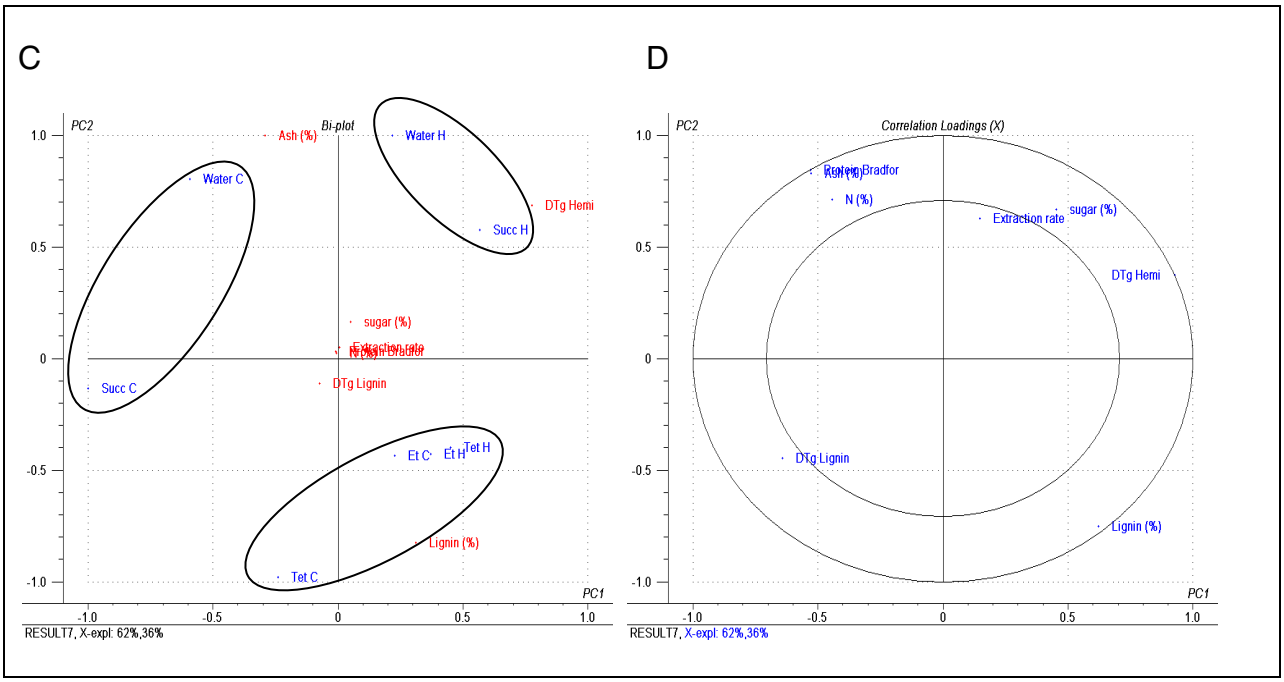


Figure 1



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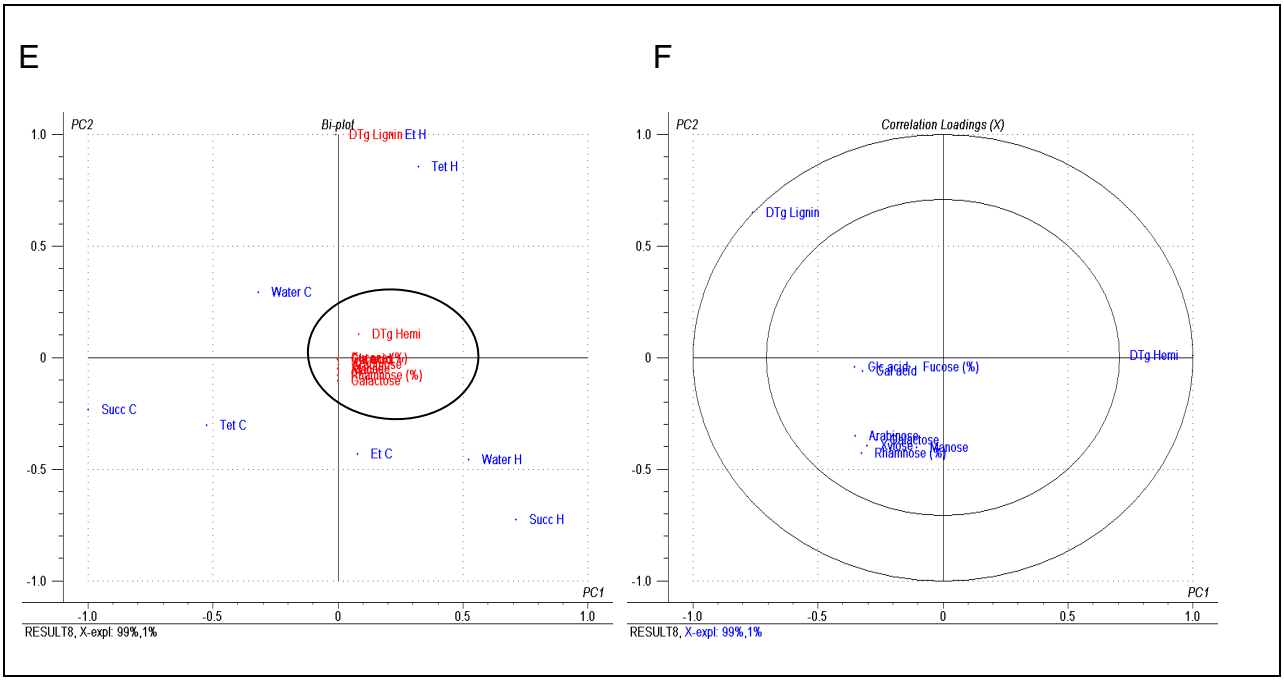


Figure 3

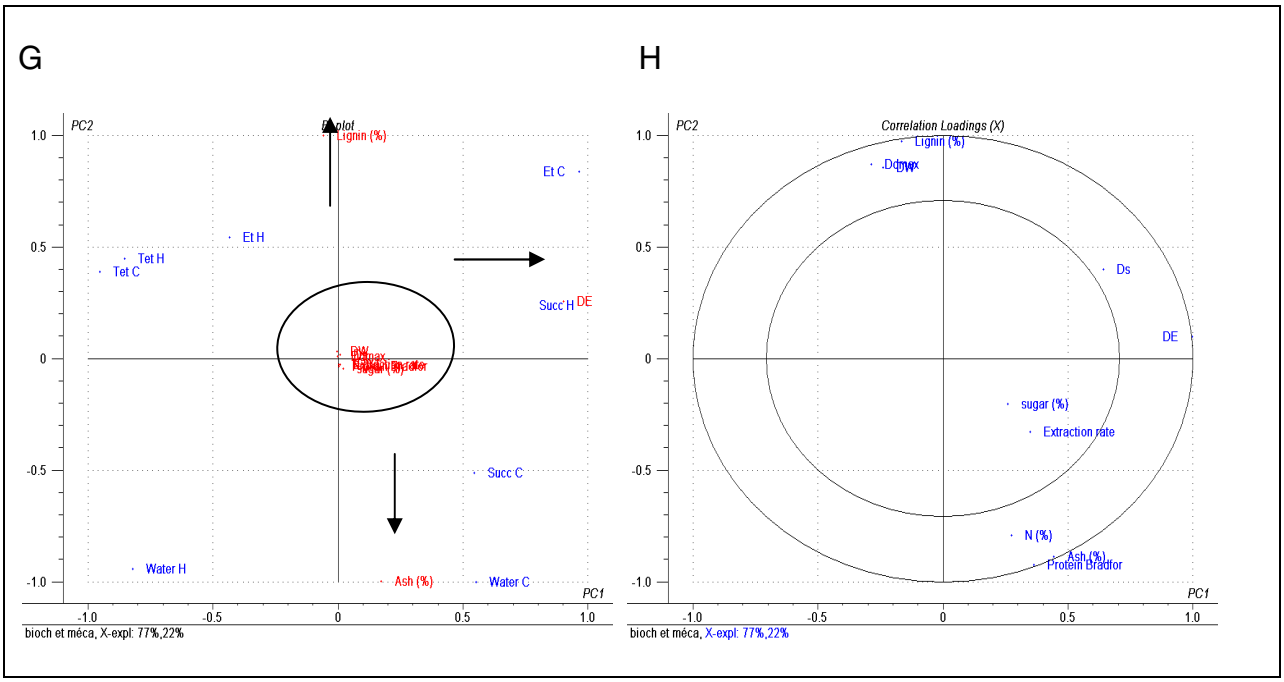
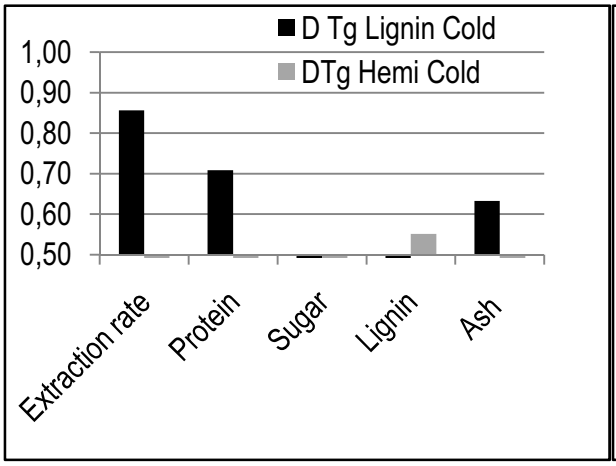


Figure 4

A



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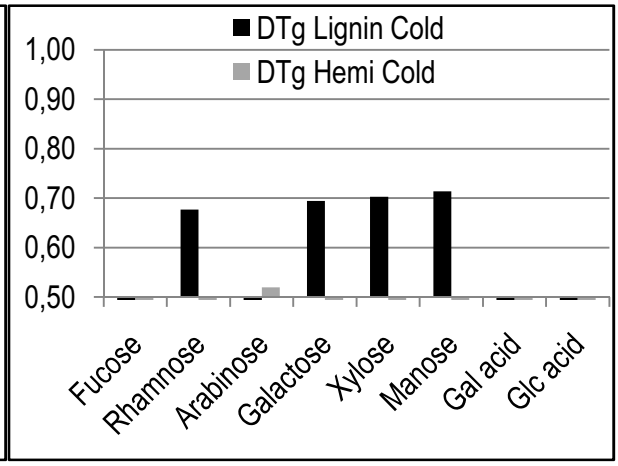

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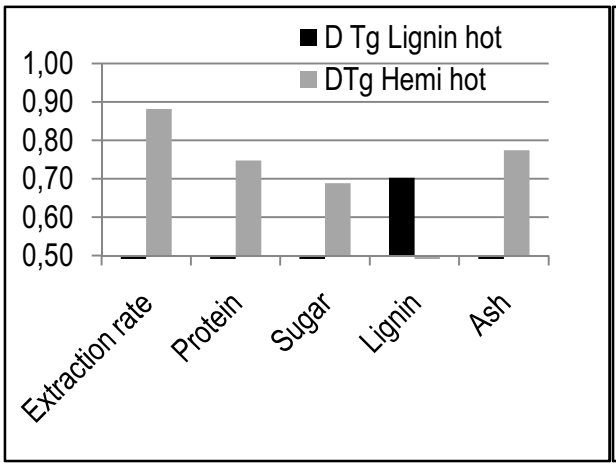


D

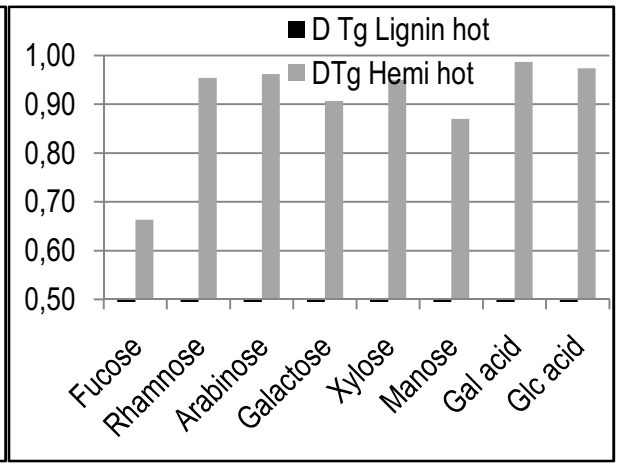
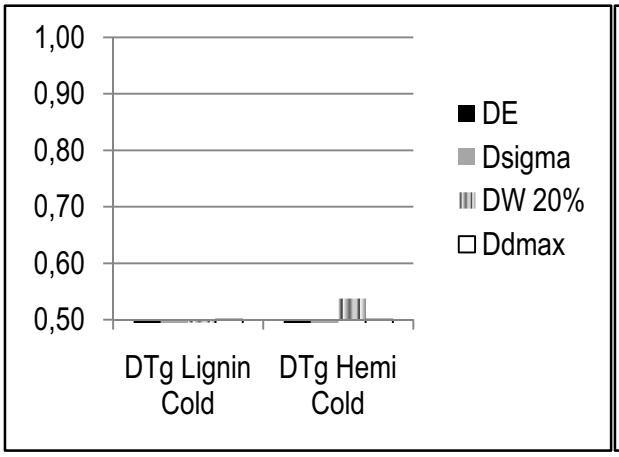
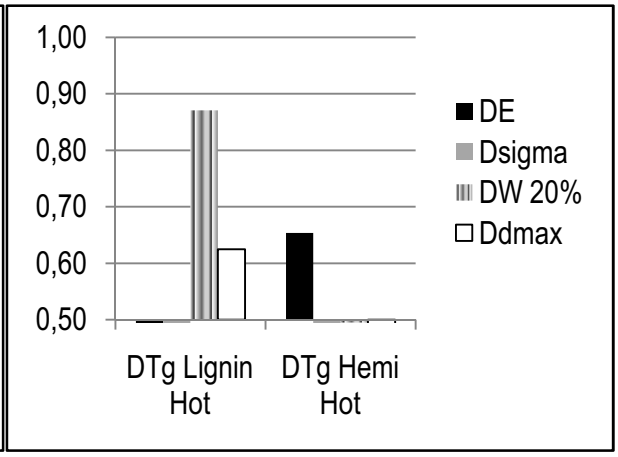


Figure 5

E


1,00
0,90
0,80
0,70
0,60
0,50
DE
Dsigma
DW 20%
Ddmax
DTg Lignin Cold
DTg Hemi Cold

F


1,00
0,90
0,80
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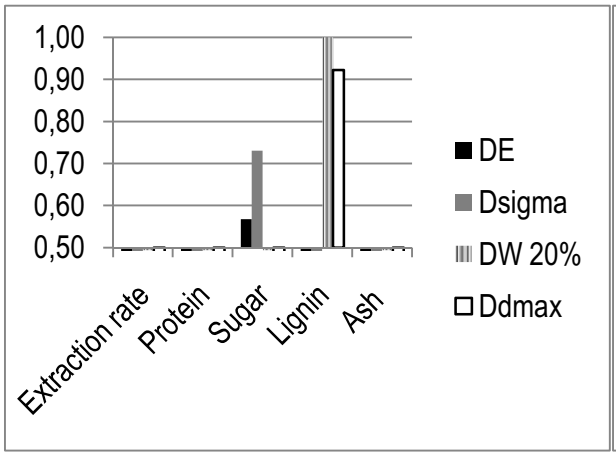
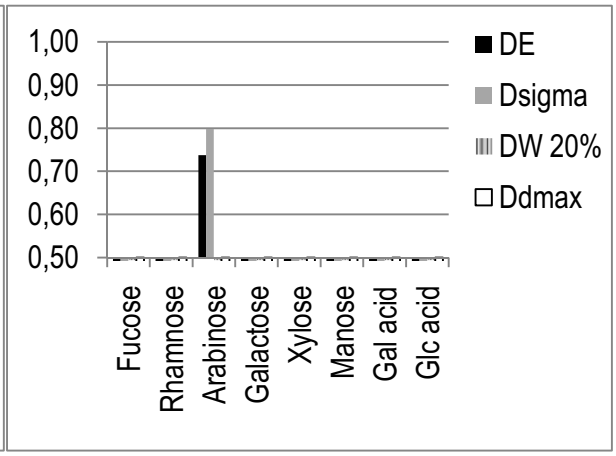
DE
Dsigma
DW 20%
Ddmax
DTg Lignin Hot
DTg Hemi Hot

Figure 6

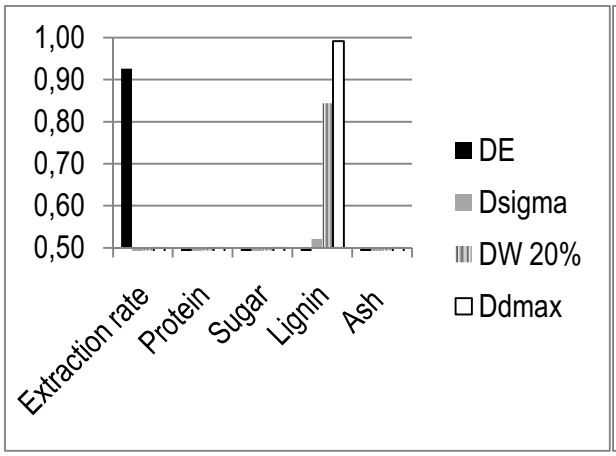
G



H



I



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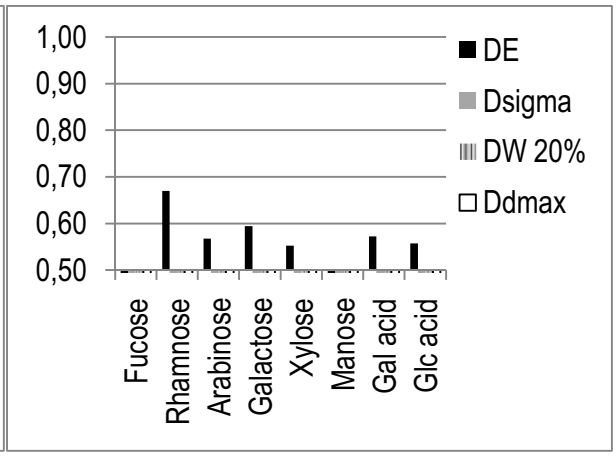

Figure 7

Table caption

Table 1: Extraction rate and chemical compounds analysis according to (Bag et al. 2009)

Table 2: Sugar composition by monosaccharide content based on % of extractives according to (Bag et al. 2009)

Table 3: Mineral composition based on % of extractives according to (Bag et al. 2009)

Table 4: Variation of Tg between an extracted and a treated part for lignins and hemicelluloses according to (Bag et al. 2009)

Table 5: Variation of mechanical properties between an extracted and a treated part according to (Bag et al. 2009)

Table1:

	Extraction yield[a] (%)	Composition of the extracts[b] Proteins (%)	Sugars (%)	Lignins (%)	Ashs (%)
Cold Tol/EtOH	0.6	0.1	4.6	61.1	1.0
Cold EtOH	0.6	0.2	9.2	65.8	10.1
Cold Water	2.5	1.8	7.4	26.0	57.7
Cold Succ.	3.0	1.2	7.7	30.1	37.2
Hot Tol/ EtOH	2.4	0.05	5.1	64.0	2.2
Hot EtOH	2.2	0.2	11.1	69.0	7.8
Hot Water	2.6	1.3	15.3	27.4	36.2
Hot Succ.	5.0	0.9	15.4	52.8	27.6

[a] % based on raw material; [b] % based on extractives

Table 2:

	Fuc	Rha	Ara	Gal	Glc	Xyl	Man	Gal acid	Gluc acid
Cold Tol/EtOH	0.01	0.27	n.d.	0.30	3.73	0.07	0.05	0.02	0.15
Cold EtOH	0.03	0.44	0.05	0.52	7.76	0.10	0.14	0.02	0.12
Cold Water	0.10	1.12	n.d.	1.29	1.62	0.55	0.28	1.35	1.14
Cold Succ.	0.04	0.94	0.02	1.17	4.16	0.39	0.44	0.31	0.22
Hot Tol/ EtOH	0.02	0.21	n.d.	0.26	4.28	0.05	0.08	0.11	0.09
Hot EtOH	0.03	0.35	n.d.	0.72	9.58	0.09	0.24	0.01	0.06
Hot Water	0.05	1.13	0.48	1.94	9.11	0.75	0.95	0.52	0.38
Hot Succ.	0.03	1.60	0.62	2.38	7.70	0.92	0.93	0.70	0.48

n. d.: not detectable

Table 3:

	Ca	Cu	Fe	K	Mg	Mn	Na	Zn
Cold Tol/EtOH	0.10	n.d.	0.31	0.43	0.01	n.d.	0.18	0.01
Cold EtOH	0.16	n.d.	0.01	4.22	0.02	n.d.	0.18	0.01
Cold Water	1.19	n.d.	0.04	21.19	0.24	n.d.	0.18	0.01
Cold Succ.	0.58	n.d.	0.08	9.21	0.11	n.d.	0.12	0.01
Hot Tol/ EtOH	0.09	n.d.	0.02	0.96	0.01	n.d.	0.17	n.d.
Hot EtOH	0.07	0.01	n.d.	14.34	0.03	n.d.	1.26	0.01
Hot Water	0.78	0.02	0.01	15.55	0.22	n.d.	0.84	0.01
Hot Succ.	0.38	0.02	0.02	6.70	0.17	n.d.	0.49	0.01

n. d.: not detectable

Table 4:

	ΔTg Lignins (℃)	ΔTg Hemicelluloses (℃)
Cold Tol/EtOH	3.0	-27.5
Cold EtOH	-0.9	5.0
Cold Water	4.7	-16.0
Cold Succ.	6.4	-53.0
Hot Tol/ EtOH	3.4	18.7
Hot EtOH	4.7	13.0
Hot Water	-3.2	29.0
Hot Succ.	-5.6	39.0

Table 5:

	ΔE (MPa)	$\Delta\sigma$ (MPa)	$\Delta W_{20\%}$ (J. m^{-2})	Δd_{max} (mm)
Cold Tol/EtOH	-67.78	-1.05	1.10	0.37
Cold EtOH	42.34	0.00	1.26	0.20
Cold Water	6.70	-1.41	-0.57	-0.09
Cold Succ.	10.32	0.01	-0.38	-0.09
Hot Tol/ EtOH	-62.11	-1.87	0.63	0.15
Hot EtOH	-38.57	-0.48	0.41	0.21
Hot Water	-69.25	-1.89	-0.12	-0.08
Hot Succ.	40.04	-0.68	0.01	0.12

Ce chapitre nous a permis de mettre en évidence par des outils élaborés, des corrélations entre les résultats expérimentaux des deux premiers chapitres de ce manuscrit. Cette étude illustre principalement la dépendance du mode de rupture au pourcentage de lignine extraite de la paroi qui aurait une influence, lorsque celle-ci est extraite en grande quantité, sur la relation entre mobilité des chaines de lignine et rupture du morceau de chènevotte. Aussi, l'extraction en forte quantité de polysaccharides semble perturber l'élasticité du matériau.

La chènevotte issue d'un procédé industriel est un coproduit résultant du défibrage des fibres longues de la tige de chanvre. Sa formation est le résultat d'une rupture naturelle de la tige de chanvre suite aux sollicitations mécaniques à plusieurs endroits. Via ce procédé, chaque morceau de chènevotte est susceptible de posséder d'emblée des défauts avant extractions et caractérisations mécaniques. Ainsi, cette présence de défauts masque l'effet des faibles variations de mobilité de la lignine car l'amorce de la rupture peut principalement avoir lieu au niveau de ces artéfacts et n'est pas obligatoirement liée aux propriétés intrinsèques macromoléculaires. Par conséquent, il est logique d'étudier l'impact d'une présence de défauts en grand nombre sur ce même type de substrat. De ce fait, un traitement référence est appliquée à la chènevotte: le traitement par une solution d'acide chlorhydrique à pH 2 est choisi pour cette étude créant des changement ultra structuraux sur les chaînes de polymère au sein de la paroi par hydrolyse et extraction de la matière dans la matrice de ce matériau composite naturel.

CHAPITRE V

Polymer mobility in lignified cell walls impregnated by strong mineral acids

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Key words: hydrolysis, cellulose, extractives, fracture, glass transition temperature, hemicelluloses, lignin, micromechanics, wood.

ABSTRACT

The effect of acid impregnation on the viscoelastic and breaking properties of woody hemp core was studied. Lignin and hemicellulose mobilities, expressed by the variation of glass transition temperatures (Tg), were analyzed by dynamic mechanical analysis (DMA) and dielectric analysis (DEA), respectively. The fracture mode was investigated by three point bending test and the broken surfaces were examined by Scanning Electron Microscope. The biochemical compositions of the products extracted by dilute mineral acid and water were determined. The Tg values of lignin and hemicelluloses were not affected by mild acid treatment, whereas the mechanism of relaxation of hemicelluloses, expressed by the apparent activation energy, was modified. The opposite phenomenon was observed with the reference water extraction. The changes in sample strength were highlighted by the study of fracture mode: the samples treated with HCl become more brittle whereas no modification of fracture behavior was observed in water-treated samples. HCl treatment resulted in depolymerization and the extraction of entities whereas water had a simple extracting effect on the samples.

Introduction

Wood is readily exposed, under natural conditions, to damage caused by fungal decay. Brown rot fungi attack the cell wall carbohydrates, leading to a rapid decrease in the strength properties of wood. Brown rot fungi produce oxalic acid which results in local acidification and favours the action of Fenton-like reactions involving transition metals and hydrogen peroxide (Green and Highley, 1997). The system developed by such fungi considerably modifies the structures involved in wood cohesion, particularly at the level of amorphous polymers, lignin and hemicellulose.

Recently, the use of oxalic acid to pretreat wood chips for paper making, in order to save energy during defiberization and mechanical pulping and to enhance fiber properties during refining, has been proposed.

The principle is based on modification of the cohesion between the wood cell walls, and of the composite and lamellar organization within the cell wall. It has also been recently suggested that a treatment with oxalic acid, prior to removal of the hemicellulosic sugars from the chips, will also extract small amounts of other cell wall components. This could play a determinant role in the mechanism of defiberization where mechanical stresses are applied at high temperature and humidity in the refiner (Kenealy et al., 2007; Limare and Kurek, 2007; Meyer-Pinson et al., 2004).

The entities removed by simple extractions do not result in chemical modification of the wood, but have a recognized impact on the mobility of amorphous polymers *in situ* (Bag et al., 2009). However, the impact on strength properties, particularly on the fracture mechanism leading to individualization of the wood fibers in a mechanical pulping process remains unclear.

Our aim in this paper is to describe the modifications induced by a strong mineral acid, applied under mild conditions, on cell wall properties at different scales, ranging from the polymer to the wood particle.

Studies were carried out on woody hemp core, a coproduct of the hemp stem defiberization process. This material is particularly suitable, due to its high amorphous polymer content and its potential new applications in composites. During transformation, mechanical stresses are applied to the woody core under given temperature and humidity conditions, which are known to affect the final properties of the material. Better knowledge of the intrinsic properties and mechanisms is thus required to improve control of this process.

Material and methods

The chenevotte in our study came from the industrial defiberizing process at La Chanvrière de l'Aube (LCDA) in Bar-sur-Aube (France). A single hemp cultivar, Fedora 17, was used throughout the study. Only sticks of chenevotte with no visible cracks were used in the experiments. They were then selected on the basis of geometrical shape (~ 2mm large and ~ 1mm thick, 10 to 40mm length).

For the DMA and DEA investigations, the samples were cut into two pieces to allow paired comparisons. One half of the stick was treated and the other half (hereafter defined as the reference sample) was left untreated.

Treatments

The samples were impregnated for 5 hours in either a solution of hydrochloric acid (pH=2) at room temperature or in ultra pure water (reference treatment) at room temperature.

Biochemical analysis of the extracted products:

The extracts were concentrated and freeze-dried. The dry matter obtained was weighed and analyzed.

Total monosaccharide content was determined by acid hydrolysis of 10 mg of the extractives using a solution of 1M H_2SO_4 for 2h at 100°C. After hydrolysis, the released monosaccharides were separated by high performance anion-exchange chromatography (HPAEC) as described previously (Beaugrand et al., 2004). The fractions were analysed in triplicate.

The glucose released from the cell wall was determined by thin-layer chromatography (TLC), as described previously (Zilliox and Debeire, 1998): 70 mg of cellulose (from Whatman filter) was impregnated with 1 mL of ultrapure water or 1mL of HCl solution. 15 µL of the solutions were then spotted onto silica gel plates. The chromatography chamber was saturated with vapor from the solvent system, which consisted of: butan-1-ol/acetic acid/water (2:1:1 by volume), before plate development. The solvent was allowed to migrate until it was about 2cm from the top of the plate. The plate was then removed and air-dried for half a day. The separated sugars were revealed using an orcinol-sulphuric acid spray reagent (200 mg orcinol/100 ml 20% sulfuric acid) heated for 10 min at 130°C.

The lignin content of the extractives was determined by measuring the UV absorbance at 278 nm with a method using acetyl bromide: (1) 10 mg of samples were

hydrolyzed at 70℃ for half an hour with a 5 mL mix of acetyl bromide (98%), acetic acid (99.8 %) solution and 0.2 mL perchloric acid. (2) 5 mL of 2M NaOH was added to 2 mL of this mixture (1) and adjusted to 20 mL with acetic acid. (3) The mixture (2) was left for 30 minutes in a dark place. (4) The absorbance was measured at 280 nm by analysis of triplicates (Day et al., 2005).

The protein content of the extractives was calculated by measuring the UV absorbance at 595 nm using a Bradford protein assay procedure (Beaugrand et al., 2004).

The ash content was determined by thermo gravimetric analysis (TGA) by incinerating 10 mg of the samples at 550℃ until a plateau was reached on the curve of mass vs temperature.

Mineral contents, notably Ca, Cu, Fe, K, Mg, Mn, Na and Zn, were measured with an ICP atomic emission spectrometer (Varian Liberty series II), using 10mg of dried extracts dissolved in 25mL of ultrapure water and filtered (0.45 µm).

Determination of the viscoelastic properties of hydrolyzed chenevotte:

The polymers constituting the cell wall exhibit viscoelastic responses during deformation that are frequency, temperature and water content dependent. The techniques used to determine the Tg values of lignin and hemicelluloses were DMA and DEA, respectively.

Dynamic mechanical analysis in tension mode in immersion (DMA)

DMA is specifically used to characterize *in situ* lignin relaxation because the peaks observed in the range of 80-110℃ represent the glassy transition of *in situ* lignin (Salmén et al., 1996; Sun and Frazier, 2007) under the same conditions.

The sample geometry was as described above. The instrument was in single frequency mode (1Hz), the amplitude of the oscillation constant was set at 12µm in "autostrain method" with a static force of 112%. Experiments were done in ethylene glycol, to better determine the softening temperature, and measurements were obtained above 100℃ with a heating rate of 2℃/min. Wood impregna ted with ethylene glycol has been claimed to exhibit similar softening behavior to water-saturated wood (Bouajila et al., 2006; Salmén et al., 1996). The storage modulus (E′), loss modulus (E″), and tan δ responses for woody hemp core were plotted as a function of temperature at a given frequency solicitation. As with synthetic polymers, the values of E' decreased from a glassy plate to a rubbery state, and E' and tan δ showed the same evolution with a maximum peak when the material softened.

Dielectric Analysis in controlled humidity (DEA)

DEA is specifically used to characterize the *in situ* chain mobility of hemicelluloses. The observed peaks are generally associated with different relaxations of hemicelluloses (William L. James, 1975) depending on the frequency and relative humidity (Lenth and Kamke, 2001; Sugimoto and Norimoto, 2005). The samples were equilibrated and stored in a constant relative humidity box (65%RH) but were placed in a modified sample holder for the analysis, so as to prevent water evaporation during the measurements. No variation in water content was observed in samples that did not exceed 20% water content, up to 150℃. A constant f orce of 350N was applied during the experiment. The frequencies examined were 1-5-10-50-100 Hz (the best for observing softening effects) with a heating ramp of 2℃/min. The sample softened at a given temperature for each frequency solicitation, and increasing the solicitation frequency led to an increase in the softening temperature. By considering paired values of frequency and temperature, and acknowledging softening to be an Arrhenian-type

mechanism, an Arrhenius plot could be traced. The apparent activation energy of relaxation (Ea) was calculated from the time/temperature diagram log(freq)=f(1000/T) using eq 1 (Lenth and Kamke, 2001; Sun and Frazier, 2007).

$$f_T = A^{-Ea/RT} \qquad \text{eq 1}$$

where f_T is the frequency, A is a constant, E_a is the activation energy, R is the universal *gas constant* = 8.3145 J/mol K, and T is the temperature in K.

Determination of the fracture mode and properties of hydrolyzed woody hemp core:

Mechanical tests were performed on a Test Well machine model 108.2Kn.H. Strain measurements were obtained by placing sensors between the fixed plate and the moving one. The displacement rate was 15 mm mn^{-1}. The experiment was stopped when the force applied to samples attained 50% of the maximum force in the part just after crack initiation. The curve obtained by plotting the load (force in N) against displacement was analysed with the Test Winner 922 software and various criteria, including E, σ_{max}, d_{max} and $W_{20\%}$, were calculated. The experiments were done under a controlled atmosphere with 50% relative humidity and a temperature of 20℃, using a homemade three point bending test.

Investigation of SEM pictures

Scanning Electron Microscopy micrographs of fracture sections from untreated and treated woody hemp core were obtained with a scanning electron microscope, model MEB XL 30 from PHILIPS. The samples were fixed on carbon adhesive bearings and coated with gold-palladium using a plasma sputtering apparatus, model SCD 040 from BALZERS, prior to SEM investigation.

Results and discussion

Biochemical analysis

Table I gives the composition of raw chenevotte. The sugars consisted of 36.7% glucose, probably a constituent of crystalline and amorphous cellulose but also of the main chains of hemicellulosic glucans. They also contained 15.6% xylose, probably coming from xyloglucans, the principal hemicellulose present in hemp (Cappelletto et al., 2001). The very small quantities of other hexoses and pentoses suggest the presence of other types of hemicellulose such as galactoglucomannans (1.33% mannose and 0.86% galactose). The other monomers should come from peptic substances, such as rhamnogalacturonans as suggested by the presence of rhamnose and galacturonic acid (Ridley et al., 2001). Other types of hemicellulose (arabinoxylans) mentioned in the literature probably coexist but in very minor amounts (Ebringerová, 2005). In any case, the exact attribution of each sugar to a particular component would require specific fractionation methods and was not attempted here.

After acid impregnation, 2.9% of the dry matter was removed from the samples. This was similar to that obtained after water impregnation (2.5% of the raw material). In both cases, the extractives contained various cell wall constituents (Table II). The HCl extracts contained nearly two times more sugars and lignin than the water extracts, which contained more ash and protein (1.5 and 8 times more, respectively). The higher amount of sugars and lignin extracted with HCl, as compared to water, corroborates the hypothesis that acid treatment concomitantly extracts lignin and carbohydrates as either separate or covalently linked structures, such as LCC (Choi et al., 2007).

Table III shows the amount of total monosaccharide analyzed in the HCl and water extracts. As water should not split linkages within the cell wall at ambient temperature, the glucose in the extracts may not only come from the chains of low molecular weight hemicelluloses but also from monomers of residual glucose produced by plant growth metabolism (Cosgrove, 2005). In contrast, the higher amount of glucose in the HCl extracts suggests that some is derived from degraded cellulose, as was demonstrated by the formation of glucose from amorphous or crystalline cellulose when hydrolysis was performed under the same experimental conditions (Figure 1). Indeed, Thin Layer Chromatography (TLC) revealed a significantly higher amount of glucose when cellulose was treated with HCl than with water (spots 1 and 2). A higher amount of glucose was also apparent in extracts from acid-treated chenevotte (spots 3 and 4). Moreover, the products extracted with water contained other sugars than could be dimers or trimers of glucose compounds, whereas the HCl extracts presented a single spot at the level of reference glucose. This may indicate that the other sugars extracted with water were degraded by mild hydrolysis.

Glucuronic and galacturonic acids accounted for nearly 30% of the total amount of water extractives and were probably derived from pectins. They included rhamnogalacturonans ramified with chains composed of arabinose (0.56%) and galactose (1.14%). These pectins are known to be abundant in the primary cell wall and middle lamellae (Ridley et al., 2001). Galactose was the main non-cellulose monomer removed from woody hemp core by both extractions. A small percentage of xylose was removed, supposedly derived from xyloglucans which are known to be quite abundant in the primary cell wall (Buchanan et al., 2000). The amount of mannose (representing ~ 5% of the total extractives for each solvent) suggested the presence of glucomannans which can be easily isolated from water-soluble extractives (Jacobs et al., 2003). The

larger proportion of arabinose extracted with water, compared to HCl solution, indicated the occurrence of arabinogalactans, possibly linked to protein. Indeed, as highlighted in table II, water concomitantly extracts nitrogen products and oligosaccharides, so that arabinogalactan protein would be expected to be present in the recovered products (Girault et al., 2000; Parsons and Hogg, 1974).

Significant amounts of inorganic compounds were also found in the extracts. The minerals essentially consisted of potassium (Table IV). The chenevotte in our experiments was used as received from the factory, without a washing step, in order to avoid any extraction by water. The high proportion of potassium in the extracts might be derived from fertilizers in the soil dust on the chenevotte (Sedan et al., 2007).

When the mineral ratio was expressed in relation to the mass of raw material and without taking into account the presence of the exogenous potassium contaminant, only calcium and magnesium were extracted in significant amounts by water and dilute HCl (table IV). Calcium is known to be a structural mineral, which contributes to the arrangement of pectins. Even though the amount of pectin in the secondary cell wall is low, some removal of pectic calcium may occur with the acid treatment, leading to greater modification of the cell wall structure than with water.

The main difference between the HCl and reference water extractions was due essentially to the simultaneous removal of low amounts of glucose and lignin. This was probably a consequence of i/ cellulose degradation ii/ the solubilization of some LCC complexes and iii/ the extraction of hemicelluloses and pectin-related substances. All of these modifications could destabilize the global environment of polysaccharides and lignins, as indicated below by the viscoelastic properties of the cell wall.

Variation of Tg after treatments

Figures 2 and 3 show the changes occurring in lignin Tg and the Arrhenius plot of the Tg of hemicelluloses, respectively, after HCl (A and C) or water (B and D) impregnation. The data show a positive or negative variation of Tg depending on the stick considered, suggesting that the impact of hydrochloric acid treatment on the softening behavior of the polymer was complex. In contrast, water extraction led to a net increase of Tg. The average Tg values for the HCl treatment were not significantly different, (109±10°C for the treated and 112±12°C for the untreated portions) whereas they were more clearly distinguished for water (106±5°C and 110±7°C respectively).

Hemicelluloses seemed to be affected by both impregnations (figure 3), but not in the same way. With HCl, the Tg values were slightly modified, but the apparent activation energy required to enhance polymer mobility was lower after the extraction. This decrease of Ea (from 389 to 254 kJ/mol), revealed a significant change in the softening mechanism. With water, the mechanism of relaxation remained the same as the slope of the curve after impregnation remained unchanged, whereas the Tg values were clearly increased.

The removal of low molecular mass hemicelluloses and lignin-like components from the cell wall during water impregnation seemed to have a 'deplasticizing' effect. In contrast, the relaxation mechanism for the amorphous matrix seemed to be more complex after acid hydrolysis. Indeed, some hemicellulose fragments or domains might be hydrolyzed and removed from the network. Furthermore, acid washing of Ca2+ ions might alter the pectins structure, and lead to changes in the viscoelastic properties of the material (Lootens et al., 2003). Clearly, the main impact of the mild hydrochloric acid treatment was on polysaccharides, although the lignin environment was also affected.

Indeed, a greater amount of lignin was removed from the cell wall by acid treatment, (45 % of the extracts) but these conditions were not strong enough to cleave the ether bonds of the lignin and induce their degradation, thus indicating their probable linkage to the removed oligosaccharides. However some structural modification was still possible, as repolymerization can occur at pH 2 at room temperature (Meyer-Pinson et al., 2004; Pouteau et al., 2005), which might partly explain the observed increase of lignin Tg. All these data strongly suggest the simultaneous removal of linked or interacting cell wall components rather than of isolated products, any disturbance of the physico-chemical environment of one component affecting the behavior of the others.

The acidic treatment seemed to be equivalent to a cold water extraction combined with degradation related to acid hydrolysis. However, a concomitant change in the softening properties of the matrix chains was also noted.

Breaking properties

Figure 4 represents the shifts in elastic modulus (E and F) and stress properties (G and H) after impregnation. As the geometries of the analyzed samples were all different the curves are represented as a function of the chenevotte stick thickness. The elasticity modulus decreased with sample thickness while it must be a parameter that should be independent of sample geometry but dependent on the intrinsic properties of the material. This thickness dependency can be explained by the natural variability of samples function to their provenance from the base to the top of the stem. Thicker samples were more likely to come from the basal region and thinner ones from the apical region of the stem. The observed dependency on thickness probably reflected the difference in chemical composition from one sample to another, supposing that the

different sticks came from different place in the stem. (Cronier et al., 2005). A gradient in chemical composition can also exist within the thickest samples. However, the acid treatment did not produce a change in the modulus. On the contrary, the strength criterion $\Delta\sigma$ decreased after acid treatment but was unchanged after water treatment. The student test value for the HCl treatment was small (0.002 – on 12 samples) and the average difference was negative (Table V). In other words, the strength of the HCl treated samples had clearly decreased and the samples were less resistant to force (12 repetitions).

Figure 5 (I and J) show the displacement value (in mm) as a function of sample thickness before and after treatment. The drop was dependent on sample shape. The thicker is the sample, the harder it was to break it in both cases. Figure 5 (I) shows that the drop decreased after acid treatment, whatever the sample thickness, which meant that the first crack occurred earlier in the test and that the sample plasticity had changed. No real change was observed in the water-treated samples, as confirmed by the student test values (0.22) and average values of Δdmax (0.1 ± 0.1). Moreover, in figure 5 (K), the values dependent on sample thickness are also clearly lower proving that the samples were more brittle after acid treatment. The fracture energy after water impregnation (figure 5 L) stayed about the same, as confirmed by the average value of $\Delta W_{20\%}$ (-1.4 ± 1.1). These results indicate that the HCl treatment had a similar decreasing effect on both fracture energy and drop value whereas water extraction had no real impact on energy evolution.

The mechanical properties of the woody hemp core were clearly affected by acid treatment but not by water extraction. Wood rigidity is mainly dependent on the amount of crystalline cellulose and orientation of the fibril angle: (Åkerholm et al., 2004; Stanzl-

Tschegg, 2006; Wang et al., 2007). In our case the decline in mechanical properties would be due to the slight cellulose degradation evidenced, and probably to i) the associated degradation of hemicelluloses, ii) the extraction of higher amounts of lignin and/or LCC complexes, and iii) modification of the pectic substances remaining in the cell wall as a result of Ca++ extraction.

Fracture mode

Structural modification of the woody hemp core was also confirmed by SEM imaging (Figure 6) of the untreated (6A) and HCl-treated (6B) parts of a sample. The surface of the HCl-treated sample seemed to be smoother than that of the water-impregnated chenevotte, as if it had been cleaned.

Analysis of the fracture curves in figure 6 (C and D) revealed ductile breaking of the raw sample, with two elastic and plastic domains distinguishable. The fracture was different in the HCl treated sample (D), occurring sooner and indicative of the brittle nature of the material. These results confirmed the structural modification of woody hemp observed by SEM (figure 6B).

Deeper analysis of the breaking surface (*500 zoom) showed that the failure was sharper and transverse to the fiber direction (F), which could explain the smaller drop values. Propagation of the fracture did not seem to be as straightforward, in the untreated sample (E), which might explain the higher drop values of the raw material.

The fracture was more likely to be stepped fracture and straight in the HCl-treated sample and more random in the untreated sample. The impact of a mild acid

treatment on the breaking properties and associated decrease in polymer strength was confirmed.

Under our experimental conditions, water had a simple extraction effect and only affected polymer chain mobility. This modification could be due to the plasticizing effect of the water-soluble entities, as previously suggested (Obataya and Norimoto, 1999; Obataya et al., 1999). However, no measurable or observable consequences on the breaking mechanism were apparent.

In contrast, after mild acid hydrolysis, the fracture mode and appearance of the two parts of the same stick were different. This showed that the changes in properties brought about by this simple extraction of woody hemp core were not directly due to a modification of polymer chain mobility, as was the case with water extraction. The changes in strength properties were of course due to the removal and depolymerization of structural entities from the cementing matrix which induced chemical alterations and thus affected sample brittleness. A study of fracture type provides a good way of monitoring the propagation of such defaults. The combined effects of depolymerization and entities extraction on modification of the fracture and matrix were demonstrated in this study.

One hypothetical reason for these results is the use of a non ideal raw material derived from the mechanical separation of fibers from hemp stem. Indeed, several defaults are already present inside sticks of woody hemp core due not only to natural growth but also to the separation process. The presence of such defaults is likely to be a predominant factor determining the site of acid solution attack, making the samples

more fragile at these sites. Thus the macromechanical properties of chevenotte may be influenced by viscoelastic properties or by the presence of defaults.

Conclusion

The impact of hydrochloric acid treatment on the viscoelastic and breaking properties of woody hemp core was demonstrated. Mild acidic treatment, in addition to extracting lignin and cations, resulted in hydrolysis of cellulose and therefore of the hemicellulose chains, thereby creating defaults inside the polymer network. Hydrolysis modified the viscoelastic behaviour of *in situ* amorphous polymers, decreased the activation energy and altered the fracture behaviour of the samples. The samples became more brittle after treatment and were easier to break. The presence of an increased number of defaults, resulting from chemical modification by mild HCl, had a clear impact on sample strength.

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Table I: Biochemical composition of raw material in % of raw material

Chemical compound	Mean value	SD
Lignin	20.7	0.6
Ash	2.7	0.2
Sugar	57.8	
Fucose	0.03	0.01
Rhamnose	0.33	0.01
Arabinose	0.39	0.05
Galactose	0.86	0.02
Glucose	36.78	0.37
Xylose	15.57	0.26
Mannose	1.33	0.01
Gal Acid	1.96	0.07
Glu Acid	0.53	0.04

Expressed in g per 100g of raw material

Table II: Biochemical composition of extractives treated with HCl and water as % of extracted components

	Protein (%)	Sugar (%)	Lignin (%)	Ash (%)
HCl	0.3	16.6	43.9	36.0
Reference water	2.5	7.2	23.5	45.5

Based on % of extracts

Table III: Monosaccharide composition of extractives treated with HCl and water in % of extracted components

	HCl extracts	SD	Reference water extracts	SD
Fucose	0.03 (0.01)	0.00	Nd (nd)	
Rhamnose	1.29 (0.04)	0.14	0.60 (0.02)	0.05
Arabinose	0.42 (0.01)	0.02	0.56 (0.01)	0.00
Galactose	2.38 (0.07)	0.29	1.14 (0.03)	0.09
Glucose	10.14 (0.29)	1.22	1.45 (0.04)	0.08
Xylose	0.55 (0.02)	0.07	1.02 (0.03)	0.23
Mannose	0.93 (0.03)	0.11	0.35 (0.01)	0.03
Gal Acid	0.53 (0.02)	0.13	0.68 (0.02)	0.05
Glu Acid	0.31 (0.01)	0.07	1.40 (0.04)	0.08
Total	16.58 (0.48)		7.19 (0.18)	

Expressed in g per 100g of extractives; (in brackets) : g in 100g of raw material, SD: standard deviation

Table IV: Mineral composition of extractives treated with HCl and water in % of extracted components

	Ca	Cu	Fe	K	Mg	Mn	Na	Zn
HCl	3.97 (0.12)	Nd	0.05 (nd)	22.14 (0.64)	0.88 (0.03)	0.01 (nd)	0.52 (0.02)	0.01 (nd)
Ref. Water	1.19 (0.03)	Nd	0.04 (nd)	21.19 (0.53)	0.24 (0.01)	nd	0.18 (nd)	0.01 (nd)

Expressed in g per 100 g of extractives; (in brackets): g in 100g of raw material

Table V: Student test values for E, σ_{max}, d_{max} and $W_{20\%}$ and average values of ΔE, $\Delta\sigma_{max}$, Δd_{max} and $\Delta W_{20\%}$ for samples treated with HCl and water

	HCl			
Student test	E 0.248	σmax 0.002	dmax 1.6E-06	W20% 6.9E-05
	ΔE (MPa)	Δσ (MPa)	Δdmax (mm)	$\Delta W_{20\%}$ (J/m²)
Mean value SD	85 261	-7.4 4.7	-0.3 0.1	-2.3 1.2
	Reference water			
	E	σmax	dmax	$W_{20\%}$
Student test	0.329	0.269	0.223	0.066
	ΔE (MPa)	Δσ (MPa)	Δdmax (mm)	$\Delta W_{20\%}$ (J/m²)
Mean value SD	-33.5 78.9	1.7 2.8	0.1 0.1	-1.4 1.1

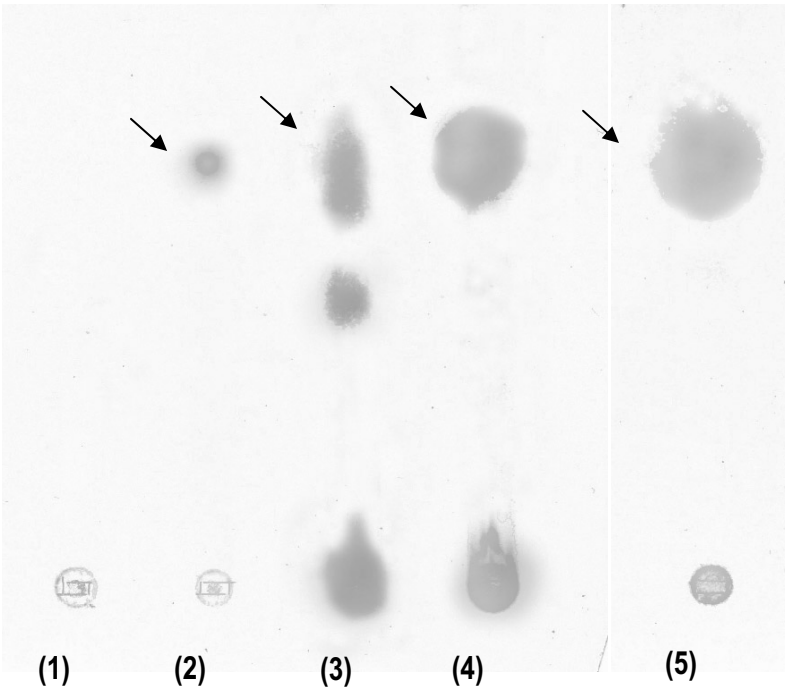

Figure 1

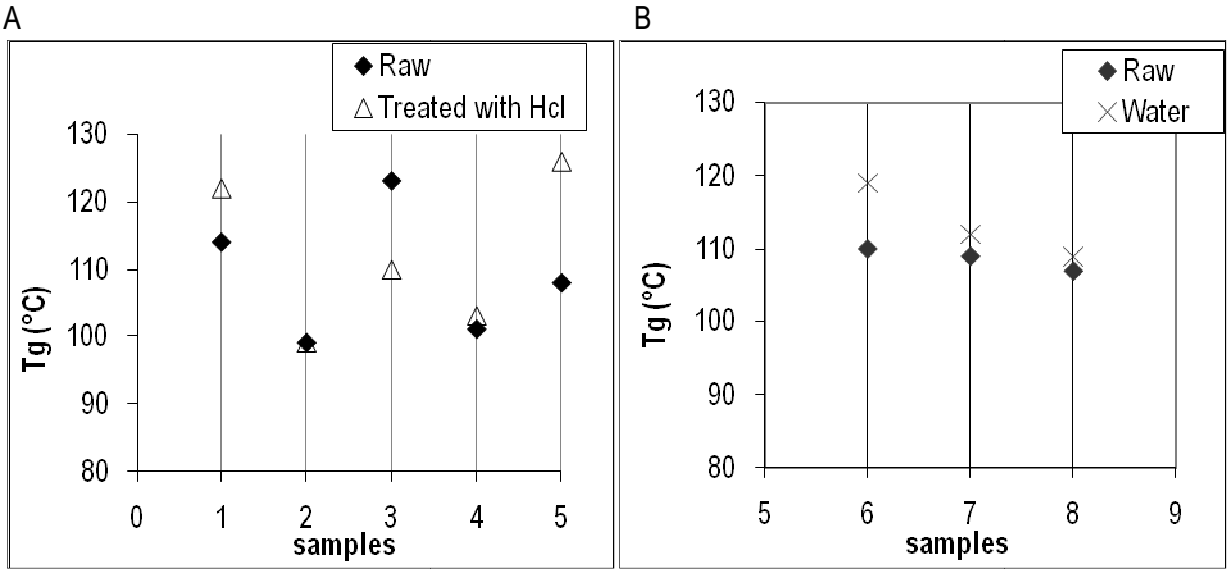

Figure 2

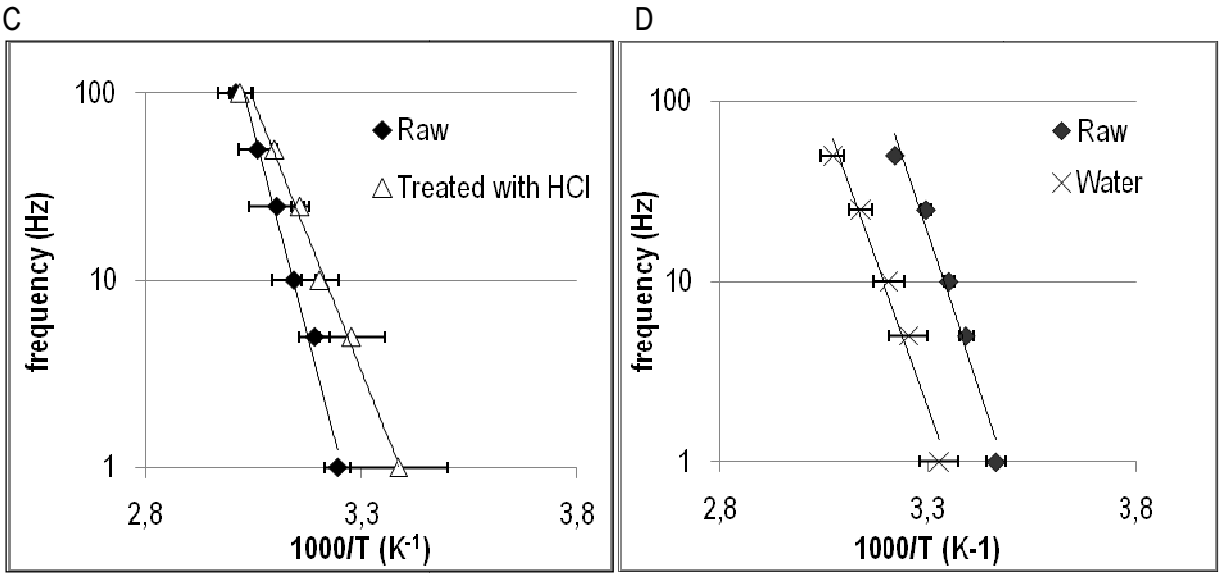
C
100
10
1
frequency (Hz)
Raw
Treated with HCl
2,8
3,3
3,8
1000/T (K-1)
D
100
10
1
frequency (Hz)
Raw
Water
2,8
3,3
3,8
1000/T (K-1)

Figure 3

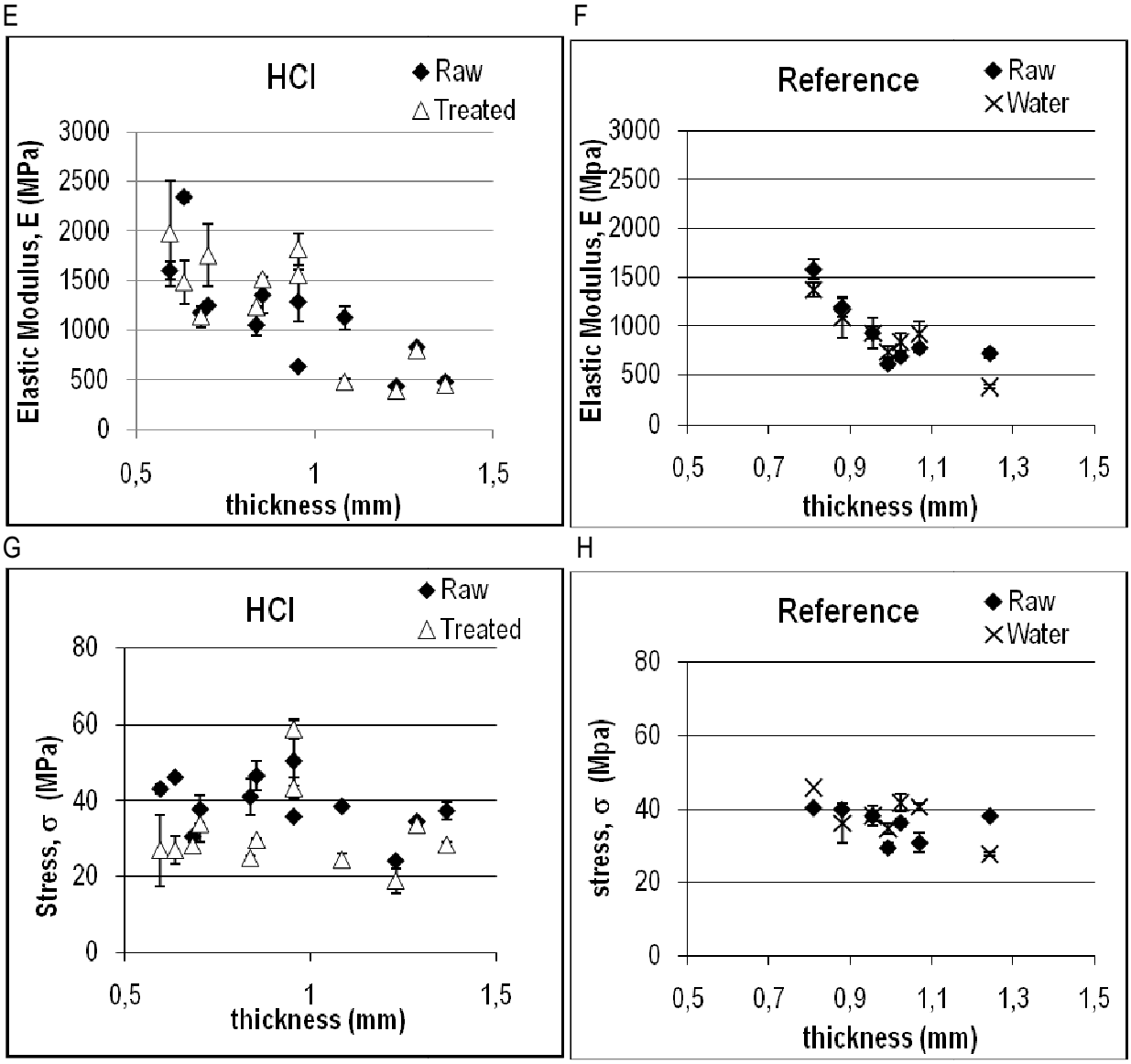
E
HCl
Raw
Treated
Elastic Modulus, E (MPa)
3000
2500
2000
1500
1000
500
0
0,5
1
1,5
thickness (mm)
F
Reference
Raw
Water
Elastic Modulus, E (Mpa)
3000
2500
2000
1500
1000
500
0
0,5
0,7
0,9
1,1
1,3
1,5
thickness (mm)
G
HCl
Raw
Treated
Stress, σ (MPa)
80
60
40
20
0
0,5
1
1,5
thickness (mm)
H
Reference
Raw
Water
stress, σ (Mpa)
80
60
40
20
0
0,5
0,7
0,9
1,1
1,3
1,5
thickness (mm)

Figure 4

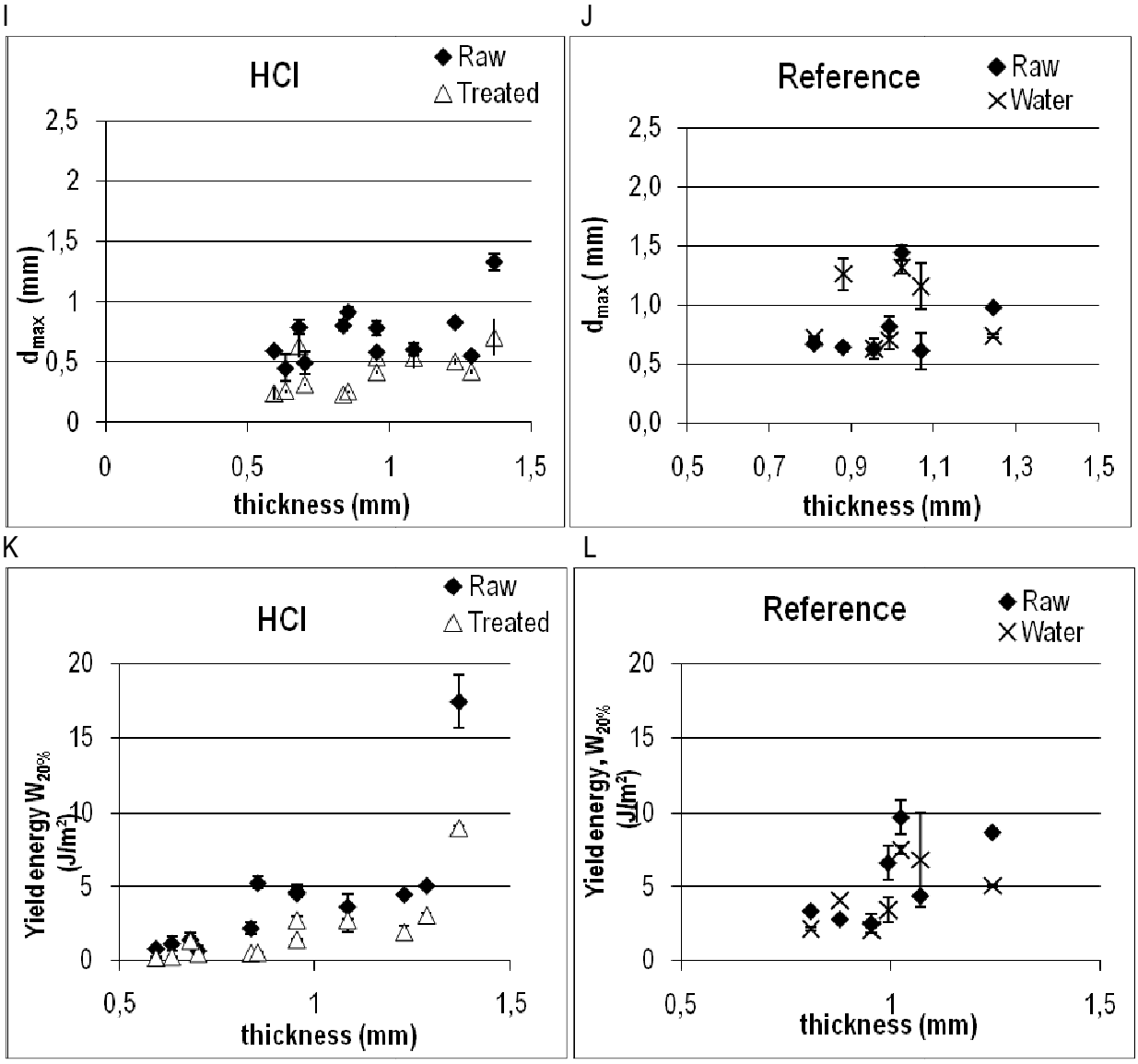
I
HCl
Raw
Treated
2,5
2
1,5
1
0,5
0
d_{max} (mm)
0
0,5
1
1,5
thickness (mm)
J
Reference
Raw
Water
2,5
2,0
1,5
1,0
0,5
0,0
d_{max} (mm)
0,5
0,7
0,9
1,1
1,3
1,5
thickness (mm)
K
HCl
Raw
Treated
20
15
10
5
0
Yield energy $W_{20\%}$ (J/m²)
0,5
1
1,5
thickness (mm)
L
Reference
Raw
Water
20
15
10
5
0
Yield energy, $W_{20\%}$ (J/m²)
0,5
1
1,5
thickness (mm)

Figure 5

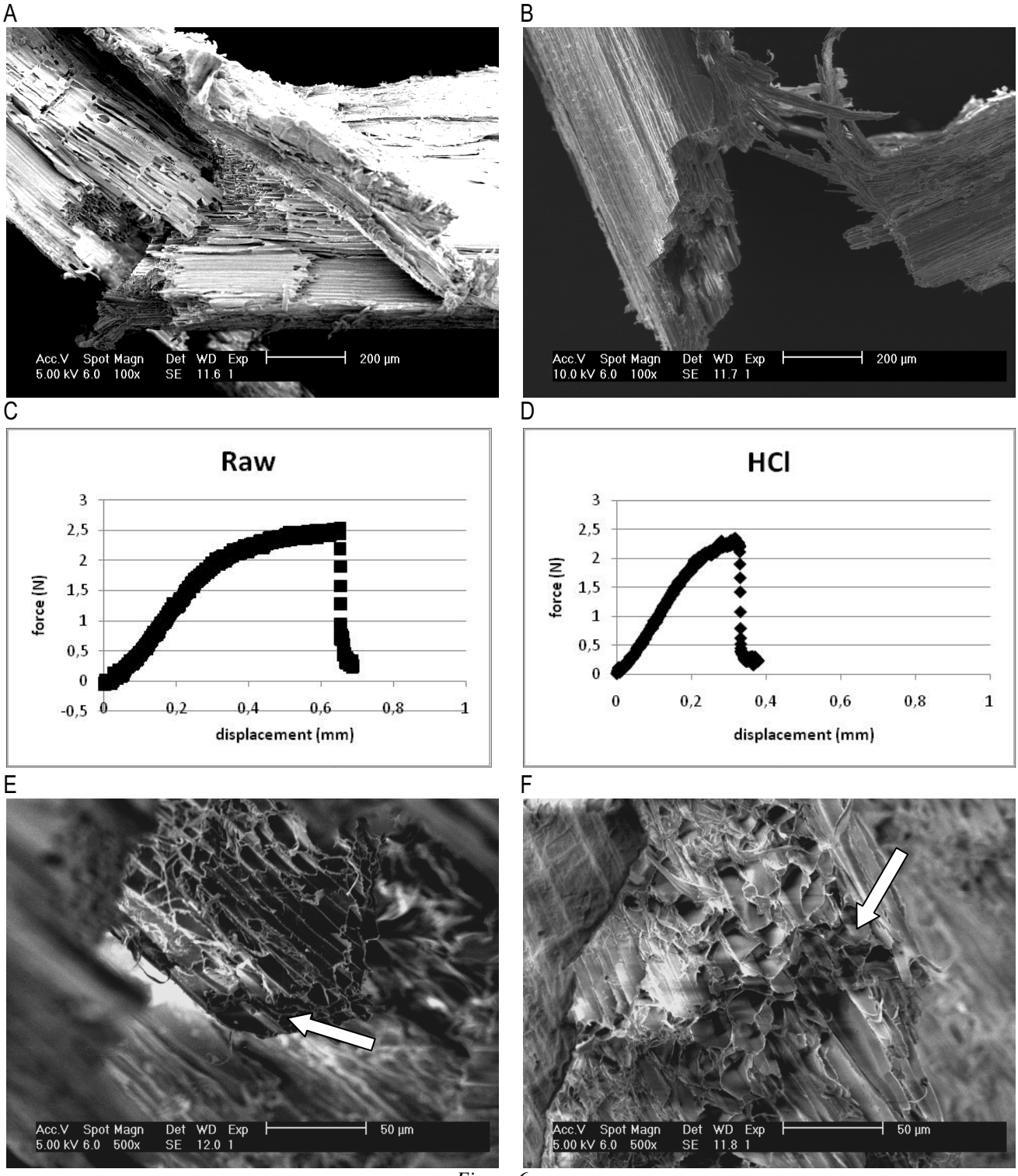
A
Acc.V Spot Magn Det WD Exp
5.00 kV 6.0 100x SE 11.6 1
200 µm
B
Acc.V Spot Magn Det WD Exp
10.0 kV 6.0 100x SE 11.7 1
200 µm
C
Raw
force (N)
displacement (mm)
D
HCl
force (N)
displacement (mm)
E
Acc.V Spot Magn Det WD Exp
5.00 kV 6.0 500x SE 12.0 1
50 µm
F
Acc.V Spot Magn Det WD Exp
5.00 kV 6.0 500x SE 11.8 1
50 µm

Figure 6

Figure Captions

Figure 1: Thin Layer Chromatography of (1): water-impregnated and (2):HCl-impregnated cellulose, (3):water and (4): HCl extracts from woody hemp core (the arrows represent glucose)

Figure 2: Softening temperatures of lignin (Tg) Captions: (♦) raw, (Δ) treated with HCl (x) treated with water (diagram B is according to (Bag et al. 2009))

Figure 3: Arrhenius plot of hemicelluloses. Captions: (♦) raw, (Δ) treated with HCl and (x) treated with water (diagram D is according to (Bag et al. 2009))

Figure 4: Mechanical properties: E in MPa (diagram E and G) and σ in MPa (diagram F and H) as a function of sample thickness (in mm). Captions: (♦) raw, (Δ) treated with HCl and (x) treated with water

Figure 5: Mechanical properties: dmax in mm (diagram I and K) and $W_{20\%}$ in J/m² (diagram J and L) as a function of sample thickness (in mm). Captions: (♦) raw, (Δ) treated with HCl and (x) treated with water

Figure 6: SEM images of raw [(A) and (E)], HCl treated [(B) and (F)] and fracture curves for raw (C) and treated (D) samples.

Conclusions Générales et perspectives

L'objectif de ce travail était d'étudier les mécanismes par lesquels les molécules extractibles de la paroi végétale affectent les propriétés des polymères *in situ* et par voie de conséquence, les propriétés macroscopiques de rupture du matériau.

Quels sont les résultats fondamentaux qui ressortent de cette étude ?

Cette étude, réalisée sur la chènevotte, représente un premier volet d'une recherche sur le rôle des extractibles dans la cohésion pariétale. Ces derniers ont été obtenus par quatre combinaisons de solvant de polarités différentes, utilisés dans deux conditions thermiques (température ambiante et point d'ébullition des solvants). Les molécules extractibles sont assimilées à de petites entités de faible poids moléculaire constitutives de la matrice de la paroi végétale. La nature des entités extraites a été systématiquement identique mais leurs quantités diffèrent selon le solvant et la température d'extraction utilisés.

Dans un premier temps, l'effet de l'extraction de la chènevotte par les solvants sur la mobilité des chaînes d'hémicelluloses et lignine démontre une rigidification de la lignine et une plastification des hémicelluloses lorsque le toluène et l'éthanol sont utilisés. Des changements dans les concentrations locales de plastifiants pourraient être à l'origine de ces effets. En revanche, l'extraction à l'eau conduit à la rigidification des hémicelluloses et la plastification de la lignine, mais seulement après une extraction à chaud. En plus des changements physiques locaux, l'eau à haute température induit des modifications chimiques de la chènevotte, ayant un impact sur la structure des polymères notamment la cellulose et les hémicelluloses. Chaque type d'extraction a un

effet spécifique clair sur les propriétés de relaxation des polymères amorphes de la paroi.

Dans un second temps, l'étude des propriétés mécaniques de ces mêmes échantillons met en évidence une différence dans l'énergie de rupture pour des extractions à l'éthanol et au toluène : les modes de fracture ont changé, les échantillons deviennent plus rigides et se brisent plus facilement par rapport aux échantillons témoins non traités.

Une analyse en composante principale et des calculs d'indice de corrélation indiquent que les changements des propriétés viscoélastiques et mécaniques sont attribués à l'extraction globale quand celle-ci présente un rendement supérieur à 2,5% de matière initiale. Le cas échéant les défauts macroscopiques préexistants semblent l'emporter sur l'amorce de la rupture. En effet, le changement de comportement mécanique à rupture est attribué principalement à la teneur en lignine de la paroi et non pas directement à la variation de Tg de la lignine. D'autre part, l'élasticité du matériau est étroitement liée à la teneur en hémicelluloses de la paroi.

Pour finir et démontrer la prépondérance des défauts dus à la transformation de la matière première (défibrage de la tige de chanvre), l'imprégnation à l'acide chlorhydrique dans des conditions douces a mis en évidence l'augmentation nette de la fragilité de l'échantillon face à de faibles variations de mobilité. Cette modification chimique (changement dans l'ultra-structure des chaînes) est soutenue par l'effet opposé avec une extraction simple à l'eau, pris comme référence: La valeur de la transition vitreuse de la lignine est plus élevée, celle des hémicelluloses est plus faible et leur mécanisme de relaxation est presque inchangé. L'étude du mode de rupture met en évidence le changement dans la faiblesse de l'échantillon: les échantillons traités

avec HCl deviennent plus fragiles et se cassent facilement tandis que ceux traités à l'eau n'ont pas de changement drastique dans leur comportement mécanique. HCl, en condition douce, a ainsi dépolymérisé une fraction de la cellulose *in situ* et créé par conséquent des macro-défauts qui l'emportent sur les changements de mobilité des polymères.

Nos conclusions soulèvent quelques questions concernant la nature chimique de chaque extrait, les mécanismes de relaxation des lignines et des hémicelluloses et le mécanisme de propagation de la rupture. Les mécanismes de relaxation encore inconnus ont probablement changé selon les informations régies par le calcul des énergies apparentes d'activation de la relaxation des hémicelluloses. L'origine des extractibles et leur localisation dans les sous-couches pariétales reste une information à déterminer. La nature de chaque composé extrait est classifiée selon des grandes familles sans qu'on puisse leur attribuer un rôle spécifique sur les effets observés (variations de Tg). Toutefois, la maitrise de la nature et du taux de plastifiants naturels dans la paroi est une piste intéressante à poursuivre lorsque l'on veut moduler les caractéristiques mécaniques d'un biomatériau.

Quels sont les perspectives à envisager ?

Analyse de la structure de chaque composé extrait :

Via ce travail original sur la chènevotte, nous connaissons les grandes familles d'extractibles (lignines, hémicelluloses, pectines, minéraux et protéines) retirés de la paroi et nous savons qu'ils sont généralement de faibles masses. Dans nos expériences, nous n'avons pas cherché à attribuer des structures chimiques à chaque

famille d'extractibles. L'étape suivante serait donc de donner une carte d'identité à chaque type de composés extraits en mélange puis de purifier chacun de ces composés isolés.

Imprégnation sélective dans une paroi modèle :

Les travaux de recherche qui ont inspiré ce travail sont ceux de Matsunaga qui avait procédé à une imprégnation de son substrat par des molécules extractibles. Les changements de propriétés de relaxation lui ont permis de soumettre l'hypothèse de plastification par les extractibles. La détermination de l'aspect structural de chaque type de composé pourra nous permettre d'identifier et de classer les molécules susceptibles de plastifier les polymères *in situ*. La connaissance et la séparation de chaque entité, nous permettra d'envisager l'imprégnation de ces petites molécules extraites et connus sur des parois modèles et de vérifier la réciproque sur l'évolution de la Tg. Ainsi, cette démarche permettra de confirmer l'hypothèse de plastification par les extractibles.

Analyse des propriétés élastiques et plastiques locales :

Durant toutes ces expériences, nous avons extraits des entités dont nous ignorons la localisation. S'il existe un gradient d'extraction (fortement probable selon l'organisation complexe et le type de liaisons et interactions et composition inhomogène dans la paroi végétale), il existe certainement des gradients de propriétés au sein des différentes sous-couches de la paroi. Le changement des propriétés macromécaniques n'est pas prépondérant après nos extractions, mais nous savons que la mobilité des polymères est affectée par ces extractions. Logiquement, il serait intéressant de pousser l'étude à une échelle plus fine et étudier les transitions ainsi que les modules et contraintes locales selon les couches pariétales. Toutefois, les techniques de caractérisation ne sont pas encore au point. On peut supposer que des études de

nanoindentation, et d'analyses microscopiques à partir de mesure locales de propriétés thermiques donneraient quelques informations intéressantes sur ce point.

Analyse de la composition locale en eau :

Dans la même logique de la composition locale, l'extraction de certains composés dans les différentes couches de la paroi, doit affecter l'accessibilité des molécules d'eau, qui sera plus ou moins facilitée selon l'encombrement de chaque phase. Ainsi, la plastification des polymères *in situ* serait plus ou moins efficace changeant ainsi la mobilité des polymères. Comme l'illustre l'ampleur des changements de Tg pour les hémicelluloses (hydrophiles), il est probable que certains sites occupés par des molécules extraites, puissent donner une meilleure accessibilité aux molécules d'eau qui assureraient ensuite une meilleure plastification de la phase considérée.

Le travail réalisé dans cette thèse apporte un éclairage nouveau sur les interactions entre polymères amorphes et molécules mobiles dans la paroi, et ouvre ainsi des pistes intéressantes sur les mécanismes aux niveaux moléculaires et supramoléculaires qui impactent sur le défibrage réalisé à forte température en milieu hydraté.

Résumé

La transformation des polymères et leur caractérisation dépendent fortement des conditions dans lesquels ils sont manipulés. Dans les plantes, qui sont des matériaux composites naturels, la caractérisation de chaque nature de composé s'avère nécessaire et délicate pour la compréhension de leurs propriétés physiques et mécaniques. Celles ci sont généralement décrites par l'état de chaque polymère dans le composite. Ce travail de recherche a pour modèle d'étude un matériau naturel, le bois de chanvre ou chènevotte, qui est issu d'un défibrage de la tige de chanvre et qui représente 70% de la plante. A l'heure actuelle, sa production en France représente 18 000 t/an et est encore insuffisamment valorisée malgré des caractéristiques intéressantes. Ce bois est composée majoritairement de cellulose (42%), de lignine (20%) et d'hémicelluloses (25%), mais également d'environ 5 % de composés dits extractibles. Dans le domaine du bâtiment émerge avec le béton de chanvre un débouché prometteur, qui rencontre néanmoins des limitations ayant pour origines un manque de maitrise de la chènevotte. L'apport de connaissances fondamentales sur ce substrat permettra d'en lever certaines et d'envisager de nouvelles applications comme dans les matériaux composites à base de polymères synthétiques. Des études sur le peuplier ont souligné l'importance de pré-traitements de la plante qui supposait un rôle de plastification tenue par les extractibles dans les étapes de transformation aval du bois. Cette thèse a ambitionné de vérifier cette hypothèse sur la chènevotte. Pour clarifier le rôle des extractibles dans la paroi cellulaire, nous les avons quantifié et partiellement qualifié après extraction sélective par affinité de solvant et en fonction d'énergie thermique. L'impact de ces traitements sur les propriétés de relaxations des polymères amorphes pariétaux a ensuite été réalisé par des analyses thermiques (DMA-lignine et DEA-hémicelluloses). Les conséquences sur le mode de rupture des substrats de chènevotte ont ensuite été étudiées. Et à partir des caractéristiques en micro- et macromécaniques, nous avons identifié des corrélations entre traitement (solvants) et réponses associées (biochimie des extractibles, comportements viscoélastique et mécanique). Un dernier axe de la thèse est dédié à l'imprégnation douce à l'acide chlorhydrique à l'ambiante et pH 2. Le retrait des extractibles avec des extractions simples influe sur la mobilité de la phase amorphe de la paroi végétale mais ce changement n'altère pas considérablement le mode de rupture des buchettes de bois. Cependant lorsque l'ultra-structure de la phase organisée (cellulose) est modifiée par l'acide (hydrolyse), le matériau est fragilisé et devient plus cassant. L'existence de néo-défaut chimique au sein du matériau semble masquer l'impact des extractibles sur le mode de rupture. De plus, un lien statistiquement établit entre la lignine extraite, l'énergie de rupture et la mobilité de ses chaines apparait quand celle-ci est extraite en quantité suffisante.

Mots clés : chènevotte, extractibles, fracture, hémicelluloses, hydrolyse, lignine, mobilité

Polymer processing and characterization depend heavily on conditions in which they are handled. In plants, which are natural composite materials, the characterization of each type of compound is necessary and critical for the understanding of their physical and mechanical properties. These are usually described by the state of each polymer in the composite. This research is to study a model of natural material, woody hemp core or chenevotte that comes from a transformation process of the hemp stem and represents 70% of the plant. Currently, production in France is 18 000 t/year and is still insufficiently valued in spite of interesting features. This wood is mainly composed of cellulose (42%), lignin (20%) and hemicelluloses (25%), but also about 5% of compounds known as extractives. In the emerging field of building, promising and concrete composite application with cement matrix exist but there are still limitations arising out of a lack of mastery on woody hemp core. The contribution of fundamental knowledge on the substrate enables to remove some and to consider new applications such as in composite materials based on synthetic polymers. Studies on poplar stressed the importance of pre-treatment plant that involved a plasticizing role held by extracting steps in downstream processing of wood. This thesis has aspired to verify this hypothesis on woody hemp core. To clarify the role of extractives in the cell wall, we have partially characterized and quantified after selective solvent and affinity extraction based on thermal energy. The impact of these treatments on the relaxation properties of amorphous cell wall polymers was then performed by thermal analysis (DMA-lignin and DEA-hemicelluloses). The consequences of the failure mode of substrates were then studied. And based on the characteristics of micro-and macromechanical, we have found correlations between treatment (solvent) and associated responses (extractives biochemistry, viscoelastic and mechanical behaviors). A final focus of the thesis is dedicated to the mild impregnation with hydrochloric acid at room temperature and pH 2. The removal of entities with simple extractions affects the mobility of the amorphous phase of the plant cell wall but this change does not alter significantly the failure mode of sticks of wood. However, when the ultra-structure of the organized phase (cellulose) is amended by acid (hydrolysis), the material becomes more fragile and brittle. The existence of neo-chemical defect in the material seems to mask the impact of extractives on the failure mode. In addition, a statistically established link appears between the extracted lignin, the fracture energy and the mobility of their chains when lignin is removed in sufficient quantity.

Keywords: woody hemp core, extractives, fracture, hemicelluloses, hydrolysis, lignin, mobility