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THÈSE

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ADHESIFS NATURELS A BASE DE TANIN, TANIN/LIGNINE ET TANIN/GLUTEN POUR LA FABRICATION DE PANNEAUX DE BOIS

Sous la responsabilité d'Antonio PIZZI et Caroline ROGAUME

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SOMMAIRE

I INTRODUCTION 15

II ETUDE BIBLIOGRAPHIQUE ET METHODES DE CARACTERISATION 19

II.1. GENERALITES 19
II.1.1. **Urée formaldéhyde** 20
II.1.2. **Phénol formaldéhyde (PF)** 20
II.1.3. **Mélamine urée formaldéhyde (MUF)** 21
II.1.4. **4,4'-méthyl diphényl diisocyanate (pMDI)** 21
II.1.5. **Colles naturelles** 21

II.2. LES TANINS 23
II.2.1. **Classification** 25
II.2.1.a. Tanins hydrolysables *25*
II.2.1.b. Tanins condensés *25*
II.2.2. **Réactivité** 28
II.2.3. **Réactions avec les aldéhydes** 29
II.2.3.a. Formaldéhyde *29*
II.2.3.b. Trishydroxylméthyl nitrométhane (TN) *32*
II.2.3.c. Hexamine *33*
II.2.3.d. Glyoxal *35*
II.2.3.e. Autres aldéhydes *36*
II.2.4. **L'autocondensation de tanins** 37
II.2.5. **Méthodes de caractérisation** 41
II.2.5.a. Analyse thermomécanique (TMA) *41*
II.2.5.b. Spectroscopie de Résonance Magnétique Nucléaire (RMN ^{13}C) *43*
II.2.5.c. Spectrométrie de Masse MALDI ToF *44*
II.2.5.d. Autres techniques de caractérisation *45*
II.2.6. **Autres applications des tanins** 45
II.2.6.a. Teintures de cuir *45*
II.2.6.b. Préservation du bois *47*
II.2.6.c. Mousses *48*
II.2.6.d. Anti corrosifs *48*
II.2.6.e. Antioxydants *50*
II.2.6.f. Utilisations pharmacologiques *51*

II.3. LA LIGNINE 52
II.3.1. **Types de lignine selon le procédé d'obtention** 58
II.3.1.a. Lignine kraft *58*
II.3.1.b. Lignosulfonate *58*
II.3.1.c. Lignine Organosolv *59*
II.3.2. **Caractérisation** 60
II.3.2.a. Hydroxyles phénoliques *60*
II.3.2.b. Hydroxyles aliphatiques *60*
II.3.2.c. Metoxiles *61*
II.3.2.d. Carbonyles *61*

II.3.2.e. Carboxyliques *61*
II.3.2.f. Sulfonates *61*
II.3.3. **Réactivité** 62
II.3.3.a. Hydroxymetilation *62*
II.3.3.b. Phenolation *64*
II.3.3.c. Démethylation *65*
II.3.3.d. Fractionnement *65*
II.3.3.e. Dépolymérisation *67*
II.3.4. **Applications** 68

II.4. GLUTEN 71

II.5. EMISSIONS DE FORMALDEHYDE ET DE COV 73
II.5.1. **Antécédents** 73
II.5.2. **Méthodes pour la détermination d'émissions de formaldéhyde et autres COVs** 79
II.5.2.a. Méthode dessiccateur (A 5905) *79*
II.5.2.b. Méthode WKI ou bocal (EN 717-3) *80*
II.5.2.c. Méthode perforateur (EN 120) *80*
II.5.2.d. Méthode de gaz (EN 712-2) *80*
II.5.2.e. La méthode de la chambre (EN 717-1) *81*
II.5.2.f. Méthode de la chambre d'essai (NF ISO 16000-9) *81*

II.6. REFERENCES BIBLIOGRAPHIQUES 85

III RESULTATS 97

III.1. COLLES POUR PANNEAUX DE BOIS A BASE DE TANIN 98
III.1.1. **Caractérisation de tannin industriel de pin maritime par 13C RMN et Maldi-tof** 98
III.1.2. **Caracterisation de deux tanins de pin maritime pour leur utilisation comme colle de bois** 106
III.1.3. **Effet de la teneur en humidité sur la cohésion interne au sein de panneaux de particules fabriqués avec des colles à base de tannin de pin radiata** 134
III.1.4. **Inhibition de la réactivité de tannins condensés à travers des réarrangements interneS : Détection CP-Mas ^{13}C RMN** 138
III.1.5. **Extraction de tannins condensés à partir de pulpe de raisin pour utilisation comme adhésifs** 150

III.2. COLLES POUR PANNEAUX A BASE DE TANNIN ET LIGNINE 157
III.2.1. **Colles pour panneaux bois à base de tannin et lignine de bas poids moléculaire sans colle synthétique** 157
III.2.2. **Faibles emissions de formaldehyde a partir de bio materiaux de bois fabrique avec de colles a base de tanins/lignine glyoxalée** 182
III.2.3. **Etude de la réaction entre la lignine el le glyoxal àu moyen de la spectrometrie de masse MALDI-ToF et de la ^{13}C-NMR** 202
III.2.4. **Étude par spectrométrie de masse MALDI-ToF de la distribution d'oligomères dans la lignine glyoxalée à stabilité durable** 216
III.2.5. **Extraction, caractérisation et utilisation de lignine organosolv de miscanthus pour l'elaboration de colles pour bois erespectueuses de l'environnement** 230

III.3. COLLES A BASE DE GLUTEN 243
III.3.1. **Colles A base de protéine de glutens pour panneaux de bois** 243

III.4. EMISSION DE FORMALDEHYDE ET COVS 259
III.4.1. **Impact sanitaire et dégagement de formaldéhyde** 259
III.4.2. **Formalddehyde and COVs emissions from bio-particleboards** 291

IV CONCLUSION 315

V PERSPECTIVES 319

LISTES DES FIGURES

FIGURE 1: HISTOIRE DES COLLES..... 19
FIGURE 2: LES EXTRACTIBLES SE TROUVENT PRINCIPALEMENT DANS L'ECORCE INTERNE (PHLOEME). 23
FIGURE 3: PROCEDE TRADITIONNEL D'EXTRACTION DE TANINS. 24
FIGURE 4: DIFFERENTS TYPES DE TANINS HYDROLYSABLES. 25
FIGURE 5: SCHEMA D'UN FLAVONOÏDE. (PIZZI, 1983). 26
FIGURE 6: QUATRE REPRESENTATIONS POSSIBLES DES STRUCTURES DE TYPE MONOFLAVONOÏDE (PIZZI 1983). [A]NOYAU: A DE TYPE RESORCINOL ET NOYAU: B DE TYPE PYROGALLOL. [B] NOYAU: A DE TYPE RESORCINOL ET NOYAU: B DE TYPE CATECHOL. [C] NOYAU: A DE TYPE PHLOROGLUCINOL ET NOYAU: B DE TYPE PYROGALLOL. [D]NOYAU: A DE TYPE PHLOROGLUCINOL ET NOYAU: B DE TYPE CATECHOL. 27
FIGURE 7: REPRESENTATION DES SITES REACTIFS DES FLAVONOÏDES PAR BROMATION. 29
FIGURE 8 : MECANISME DE REACTION ENTRE UN TANIN ET LE FORMALDEHYDE. 31
FIGURE 9: TEMPS DE GEL TANIN-PARAFORMALDEHYDE EN FONCTION DU PH. 32
FIGURE 10: TEMPS DE GEL EN FONCTION DU PH DES COLLES DE TANIN DE PIN RADIATA, MIMOSA MODIFIE ET QUEBRACHO MODIFIE AVEC 12% DE TRIS[HYDROXYMETHYL]NITROMETANE (TROSA ET PIZZI, 2001). 32
FIGURE 11: TEMPS DE GEL EN FONCTION DU PH POUR UNE COLLE DE TANIN DE MIMOSA AVEC 5% D'HEXAMINE. 34
FIGURE 12: EXEMPLE DE RETICULATION DE TANIN A TRAVERS DE PONTS BENZYLAMINE PAR REACTION AVEC UNE BASE IMINE-AMINE METHYLENE OBTENUE PAR LA DECOMPOSITION D'HEXAMINE EN PRESENCE DE TANINS. 35
FIGURE 13: TEMPS DE GEL EN FONCTION DU PH DE COLLES A BASE DE TANIN DE PIN RADIATA AVEC 8% DE PARAFORMALDEHYDE ET 9% DE GLYOXAL. (BALLERINI ET AL. 2005). 36
FIGURE 14: DEGRADATION DES TANINS A CATECHINE ET ANTHOCYANIDINE 37
FIGURE 15: AUTOCONDENSATION ACIDE PAR HYDROLYSE DES HETEROCYCLES 38
FIGURE 16: AUTOCONDENSATION ALCALINE: RUPTURE DE LA LIAISON INTERFLAVANOÏDE ET AUTOCONDENSATION FINALE. EXEMPLES: TANINS DE PIN ET DE NOIX DE PECAN. (PIZZI ET AL.1993). 39
FIGURE 17: REARRANGEMENT CATECHINIQUE. 40
FIGURE 18: APPAREIL D'ANALYSES THERMOMECANIQUES (TMA). 41
FIGURE 19: ANALYSE THERMOMECANIQUE: MISE EN PLACE DE L'ECHANTILLON. 43
FIGURE 20: APPAREIL (A) ET SCHEMA DE RMN (B). 44
FIGURE 21: PRINCIPE DE FONCTIONNEMENT D'UN APPAREIL MALDI-TOF. 45
FIGURE 22: QUELQUES APPLICATIONS DE TANINS AVEC LE CUIR. 47
FIGURE 23: STRUCTURE DE LA PAROI CELLULAIRE (LA LIGNINE SE TROUVE DANS LA LAMELLE MOYENNE) 52
FIGURE 24: LES UNITES A LA BASE DE LA LIGNINE: (A) 3-[4-HYDROXYPHENYL]-2-PROPEN-1-OL OU ALCOOL P-COUMARYLIQUE, (B) 3-[3-METHOXY-4-HYDROXYPHENIL]-2-PROPEN-1-OL OU ALCOOL CONIFERYLIQUE ET (C) 3-[3,5-DIMETHOXY-4-HYDROXYPHENYL]-2-PROPEN-1-OL OU ALCOOL SINAPYLIQUE. [FENGEL ET WEGENER, 1989]. 53
FIGURE 25: STRUCTURE DE LA LIGNINE DANS LES CONIFERES. 54
FIGURE 26 : LIAISONS ETHER RENCONTREES DANS LES MOLECULES DE LIGNINES [B-O-4, α-O-4 ET 4-O-5]. 55
FIGURE 27 : LIAISONS CARBONE-CARBONE PRESENTES DANS LES MOLECULES DE LIGNINES [5-5, B-5, B-1, B-B]. 55
FIGURE 28: DESHYDROGENATION DES MONOLIGNOLS [ICI L'ALCOOL CONIFERYLIQUE] PAR DES PEROXYDASES ET DES OXYDASES LACCASES A L'ORIGINE DE LA POLYMERISATION DE LA LIGNINE. 56
FIGURE 29: HYDROXYMETYLATION D'NOYAUX PHENOLIQUES D'UNITES DE LIGNINE TYPE GUAYACYL. 63
FIGURE 30: REACTION DE CANIZARRO 63
FIGURE 31: REACTION DE TOLLENS. 64
FIGURE 32: ACCROISSEMENT DES CENTRES ACTIFS DANS LES FRAGMENTS DE LA LIGNINE PAR PHENOLATION. 65
FIGURE 33: DE PRINCIPALES STRUCTURES PRESENTES DANS DES FRACTIONS DE LA LIGNINE KRAFT OBTENUES A TRAVERS ULTRAFILTRATION. STRUCTURES B-5; STRUCTURES B-O-4 ; F: FERULATES ; H: ACIDE P-COUMARIC ; G: ALCOOL DU GUAIACYL ; S: ALCOOL DU SYRINGYL. (TOLEDANO ET AL. 2010) 67
FIGURE 34: STRUCTURE D'UN GRAIN DE BLE. 71
FIGURE 35: SOURCES EXTERIEURES ET INTERIEURES POSSIBLES DE LIBERATION DE FORMALDEHYDE. 73
FIGURE 36: POSSIBLE FORMATION DE FORMALDEHYDE A PARTIR DE LES POLYSACCARIDES DE BOIS. 77
FIGURE 37: VOIES POSSIBLES DE FORMATION DE FORMALDEHYDE A PARTIR DE LA LIGNINE 78
FIGURE 38: METHODES STANDARD POUR LA DETERMINATION DE FORMALDEHYDE LIBERE A PARTIR DE MATERIAUX A BASE DE BOIS. (SALTHAMMER, 2010). 79
FIGURE 39: SYSTEME D'ANALYSE CHROMATOGRAPHIQUE GC – MS 82
FIGURE 40: SYSTEME D'ANALYSE HPLC. 84

I INTRODUCTION

L'unanimité quant aux ressources fossiles et en particulier au pétrole s'exprime aujourd'hui plus volontiers par un inéluctable épuisement que sur des échéances précises concernant celui-ci. Le pétrole a trouvé sa place dans la vie des hommes, que ce soit dans le domaine de l'énergie (chauffage, transport,…), des matériaux avec notamment les matières plastiques, des cosmétiques et autres engrais ou insecticides. Parmi les éléments de cette liste non exhaustive, on trouve également l'utilisation de cette matière première dans la formulation d'un composant essentiel à la fabrication de panneaux à base de bois : la colle. Le phénomène d'épuisement de cette ressource conjugué à une demande de plus en plus importante ne reste pas sans conséquence sur le plan économique avec une instabilité notable des coûts qui augmentent de jour en jour. La majorité des systèmes économiques mondiaux reposant sur ce que l'on appelle communément le «prix du baril», les effets se font ressentir dans tous les domaines: économique biensûr mais aussi politique et social.

Parallèlement à ces constats, ces dernières décennies ont vu naître autour du globe une volonté croissante de développements écologiques avec la protection et la préservation de l'environnement en leitmotiv. Le bilan carbone de la filière pétrolière divergeant quelque peu avec ces attentes, on a assisté à un réel regain d'intérêt pour toutes les actions pouvant être entreprises dans les sens de ces démarches éco-responsables. Le développement des énergies renouvelables s'est alors accru et de nombreuses recherches quant à l'intégration de produits naturels dans les procédés industriels ont vu le jour. Ce fût le cas notamment dans l'industrie du panneau de bois avec un objectif précis de diminuer considérablement voire de remplacer intégralement les résines à bases de dérivés pétroliers utilisées actuellement.

Un développement durable ne peut par ailleurs être mené sans l'intégration dans la conception d'un produit d'une dimension écologique de sa naissance à sa fin de vie et voire même au-delà avec les notions de recyclage qui ne peuvent désormais plus être ignorées.
La construction et l'industrie du meuble restent les principales applications des quelques 170 millions de mètres cube de panneaux à base de bois produits annuellement (2004). Concernant les panneaux de particules, les colles représentent environ 10% en masse de la

formulation. Cette proportion est d'environ 5% pour les panneaux d'OSB (oriented strand boards) et peut atteindre 20% pour les panneaux de MDF (Medium Density Fibreboards). D'autre part, des études ont d'ores et déjà montré un caractère nocif pour l'homme et pour l'environnement de composés émis par ce type de matériaux et en particulier de la colle qu'ils contiennent. Le fondement des recherches menées dans ce travail reposent sur les faits et observations développés précédemment.

L'idée de substituer toutes ou partie des résines figurant actuellement dans la composition des panneaux de particules semblent être une alternative intéressante et prometteuse pour plusieurs raisons. La première relève des domaines économique et écologique concernant les stocks disponibles de matériaux de substitution. Parmi ceux-ci, les tanins peuvent être récupérés des écorces que l'on trouve en grande quantité dans les industries de premières transformations du bois (scieries en particulier) à l'issue de la phase d'écorçage. Les liqueurs noires issues de l'industrie papetière renferment une quantité très importante de lignine qui doit être ôtée pour l'obtention d'un papier de qualité. Outre l'utilisation énergétique qui reste l'application actuelle principale, on peut imaginer récupérer ce matériau pour les substitutions recherchées. Enfin, une application tout aussi intéressante et dans les mêmes finalités (formulation de colles pour panneaux) peut voir le jour pour le gluten qui doit être retiré de la farine de blé destinée aux personnes allergiques à cette substance. La disponibilité de ces matériaux, leur répartition géographique mais aussi leur faibles coûts de revient sont autant d'aspects non négligeables pour envisager la mise au point de ces «colles du futur».

Malgré des recherches qui se développent de jour en jour autour de cette thématique de «colles vertes», les applications industrielles restent peu développées pour deux raisons principales: les coûts qui doivent défier la rude concurrence de ceux engendrés par l'utilisation de colles synthétiques et l'aspect technique pour obtenir des performances au moins égales à celles utilisées actuellement.

Par ailleurs, un autre phénomène vient influencer de manière non négligeable le développement de ces produits sur le plan de la législation. Les progrès en termes de mesure et de prévention concernant la pollution de l'environnement (intérieur et extérieur) et notamment d'impacts sur la santé et l'environnement sont depuis quelques années considérables. De nouvelles exigences concernant les émissions de certains composés sont

apparues et rendent les alternatives écologiques pour le développement des colles d'autant plus intéressantes.

Avant de dévoiler les objectifs de mes travaux et les différentes parties autour desquelles s'articulent ces derniers, je voulais mentionner ici un exemple concret qui témoigne du véritable engouement suscité par l'intérêt de la mise au point des ces colles environnementales. L'entreprise chilienne Arauco travaille en effet actuellement à la mise au point d'un système d'extraction industrielle de tanin à partir de pin radiata en vue de la fabrication de colles naturelles pour l'intégrer aux panneaux de bois qu'elle produit.

Dans ce contexte, les objectifs de ce travail sont les suivants:
(i) Evaluer le potentiel de différents tannins en provenance de déchets d'écorces et développer des colles à base de tannins de pin maritime obtenus à travers deux procès et de tannins d'alepe, de douglas, etc. Différents durcisseurs ont été utilisés.
(ii) Evaluer le potentiel de lignines de différentes origines et développer des colles à base de tannin de mimosa et de lignine.
(iii) Développer des colles à base de tannin et de protéine de gluten.
(iv) Evaluer les émissions de formaldéhydes et d'autres composés organiques volatiles (COV) à partir de panneaux de particules fabriqués avec les colles naturelles mises au point précédemment.

La présentation des résultats de mes travaux effectués lors de cette étude adopte le schéma suivant:

- Une partie «Etude bibliographique et méthodes de caractérisation» dresse un état de l'art concernant les colles utilisées actuellement dans l'industrie du panneau. Celle-ci est également l'occasion de faire le point sur les propriétés des matériaux d'origine naturelle susceptibles de participer à la formulation des colles vertes.

- Une partie «Résultats» constituée par les différentes publications rédigées lors de la thèse présente les expériences mises au point avec les formulations de colles et leurs conclusions. Le caractère écologique de chacune des colles ainsi obtenues a été approuvé par la réalisation de tests répondant aux exigences de normes concernant l'émission de différents composés.

- Une partie «Conclusion» reprend l'ensemble des résultats trouvés au cours des ces travaux.

II ETUDE BIBLIOGRAPHIQUE ET METHODES DE CARACTERISATION

II.1. GENERALITES

Depuis ses origines, l'homme a puisé dans la nature, que ce soit dans le monde animal ou végétal les ressources pour la fabrication de colles pour assembler le bois. Par exemple à travers l'histoire, il a utilisé des protéines comme l'albumine, l'amidon, la gélatine ou de la colle issue du poisson. Plus tard, on a utilisé la caséine, une autre protéine dans la fabrication des avions par exemple, et, après 1920, des colles à base de soja ont vu le jour et ont été employées dans les panneaux contreplaqués.

La figure 1 donne un aperçu des colles utilisées dans le domaine du bois au fil du temps.

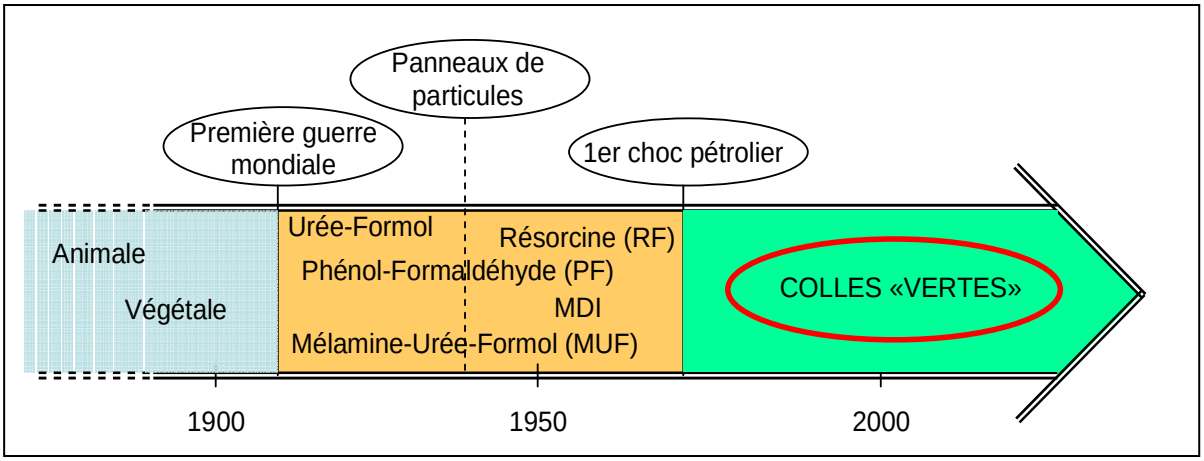


Figure 1: Histoire des colles.

La colle peut représenter jusqu'à 40 à 60 % du coût total d'un produit fini. Pour cette raison, il est nécessaire de chercher de nouvelles alternatives pour diminuer ou du moins maintenir le prix en conservant les propriétés du matériau composite.

Les colles thermodurcissables les plus utilisées aujourd'hui dans l'industrie du bois sont les résines dites urée-formaldéhyde (UF), phénol-formaldéhyde (PF), mélamine-urée-formaldéhyde (MUF) et isocyanates. La volonté de protection de l'environnement qui a émergé ces dernières années s'est traduite par un intérêt particulier pour les colles à base de matériaux naturels. Cependant, on dénote que seule une infime quantité de colles vertes à base de tanins est utilisée dans le monde industriel actuel.

Les colles qui sont utilisées aujourd'hui dans l'industrie du panneau sont brièvement décrites ci-après.

II.1.1. **Urée formaldéhyde**

Les résines UF qui sont des systèmes de collage parmi les plus anciens avec les PF restent prépondérantes. L'urée a été découverte en 1824 par Wöhler. La première résine UF a été fabriquée aux USA en 1920, mais son utilisation comme colle commerciale débute quelques années plus tard. Son développement s'est s'accéléré en raison de la pénurie des colles d'origine animale et végétale.

De nos jours, les résines UF, améliorées ou non, plus ou moins chargées, sont les plus utilisées dans l'industrie du bois. En terme de mise en application, les résines UF présentent des caractéristiques intéressantes: la gamme étendue de températures lors de la fabrication (généralement de 90 à 200°C), leur vitesse de prise (de quelques secondes à quelques minutes), leur couleur blanche, leur prix relativement peu élevé, et enfin leur possibilité d'utilisation pour chaque essence de bois.

Les problèmes des résines UF sont liés à leur absence de résistance à l'eau et à l'émission de formaldéhyde, bien que dans le cas de certaines résines, désignées E1, le problème a été considérablement réduit avec des modifications entraînant des baisses d'émissions de formaldéhyde.

II.1.2. **Phénol formaldéhyde (PF)**

Les résines phénoliques sont les produits issus de la polycondensation du phénol avec le formaldéhyde. Ce sont les vrais premiers polymères synthétiques qui ont été développés commercialement. Les premières résines PF datent de 1872 [BAYER], mais il faut attendre la fabrication industrielle du formaldéhyde à la fin du siècle pour voir leur fabrication et leur commercialisation s'accroître en raison de leur prix relativement avantageux par rapport à celui des autres résines extérieures. C'est seulement durant la deuxième guerre mondiale et pendant les années suivantes que l'emploi des résines PF s'est élargi à tous les types de panneaux à usage extérieur. Les phénols polyfonctionnels peuvent réagir avec le formaldéhyde aussi bien en position ortho qu'en position para du groupe hydroxyle. Cela signifie qu'il existe un très grand nombre d'isomères pour une longueur de chaîne donnée. Cela a pour conséquence de rendre très complexe la chimie de ces réactions et donc particulièrement difficile une étude cinétique. Elles ont été commercialisées dès 1908 et ont été les premières résines totalement synthétiques à avoir été développées.

Elles sont les plus utilisées en Amérique du Nord, en Europe du Nord et en Allemagne. En France, en Italie et en Espagne, leur utilisation est très limitée en raison de la loi régissant les dégagements de phénol particulièrement toxique.

II.1.3. **Mélamine urée formaldéhyde (MUF)**

Elles sont de meilleure qualité que les résines UF et ont une meilleure résistance à l'eau. Pour cette raison, elles sont souvent employées dans la fabrication des panneaux de contreplaqué et de particules pour remédier à la mauvaise tenue en milieu humide des résines UF et pour diminuer le coût des résines MF pures. La mélamine est ainsi co-condensée avec l'urée et le formaldéhyde pour obtenir des résines MUF à usage semi-extérieur. Elles résistent mieux aux intempéries que les UF et elles sont moins onéreuses que les résines MF. Elles sont aussi incolores, ce qui représente un avantage par rapport aux résines PF.

Les MUF sont les plus utilisées en Europe pour un usage extérieur, en particulier en France, en Italie et en Espagne. Leur prix de revient est assez élevé. En Europe, la capacité de production de mélamine est inférieure à la demande en raison de son utilisation par l'industrie du papier et l'industrie chimique.

II.1.4. **4,4'-méthyl diphényl diisocyanate (pMDI)**

Il s'agit du produit synthétique le plus récent employé dans le domaine des résines thermodurcissables [Deppe et Ernst, 1971; Deppe 1977]. Il trouve d'importantes applications pour l'isolation [mousses thermo-isolantes] ainsi que pour le collage bois- métal et bois-plastique en combinaison avec des résines polyacétates de vinyle PVA. Il garantit de bonnes performances extérieures et des temps de pressage courts. Il ne libère pas de formaldéhyde mais la présence de groupes – N=C=O libres peut être dangereuse pour la santé publique.

Les colles pMDI sont une alternative aux résines à base de formaldéhyde pour la production des panneaux bois. L'intérêt de ces colles réside dans leur faible adhésion aux plaques de la presse à panneaux, la réduction de la quantité de colle nécessaire et l'amélioration de la réactivité (température de presse basse, applications froides).

La consommation de pMDI pour la fabrication de panneaux de particules, MDF et OSB est en constante augmentation dans les pays comme la France, l'Allemagne et l'Italie malgré le coût du produit qui peut être considéré comme un inconvénient.

II.1.5. **Colles naturelles**

Le terme «colles naturelles» couvre n'importe quel monomère/oligomère/polymère ou pré-polymère dérivé de la nature qui durcit [normalement irréversiblement] pour former une

liaison durable. Ce terme inclut les colles d'origine animale (sang, colle de poissons) et végétale (dérivés de plantes).

Les dérivés de plantes sont divisés en cinq catégories:

Tanins:

- Hydrolysables
- Condensables

Lignine:

- Sulfite
- Organosolve

Carbohydrates:

- Amidon
- Cellulose
- Hémicellulose

Huiles insaturées:

- Triglycérides d'oléagineux
- Coquilles de noix de cajou

Protéines:

- Soja
- Gluten

De nos jours, seuls les tanins et la lignine sont utilisés par l'industrie du bois et cela, en très faibles quantités.

Pratiquement tous les tanins sont utilisés dans l'hémisphère sud (Afrique du Sud, Australie, et Amérique du Sud) avec comme domaine principal d'utilisation l'industrie du panneau. Actuellement, la lignine (Organosolv et liqueur noire dérivée du procédé au sulfite en provenance des usines du papier) est employée comme co-réactif avec la colle PF commerciale pour ajouter une propriété spécifique à la résine. Le tanin et la lignine sont employés comme substituts phénoliques naturels dans les colles synthétiques à base de phénol/résorcinol.

Quant aux colles à base de polysaccharides et de triglycérides, peu d'utilisations sont connues pour l'assemblage du bois. La suite de ce paragraphe présente les colles à base de tanin, de lignine et de gluten.

II.2. LES TANINS

Les tanins sont des produits qui se trouvent dans toutes les plantes en différentes proportions. Les écorces des arbres, plus particulièrement la partie interne, contiennent en général la quantité la plus significative, mais les tanins sont présents dans l'ensemble du cytoplasme de toutes les cellules végétales [Haslam, 1989] (figure 2).

Les tanins sont considérés comme étant le quatrième groupe de composés d'origine végétale le plus abondant après la cellulose, les hémicelluloses et les lignines, représentant ainsi une part importante du carbone de la biomasse terrestre [Hernes et al., 2000; Kraus et al., 2003].

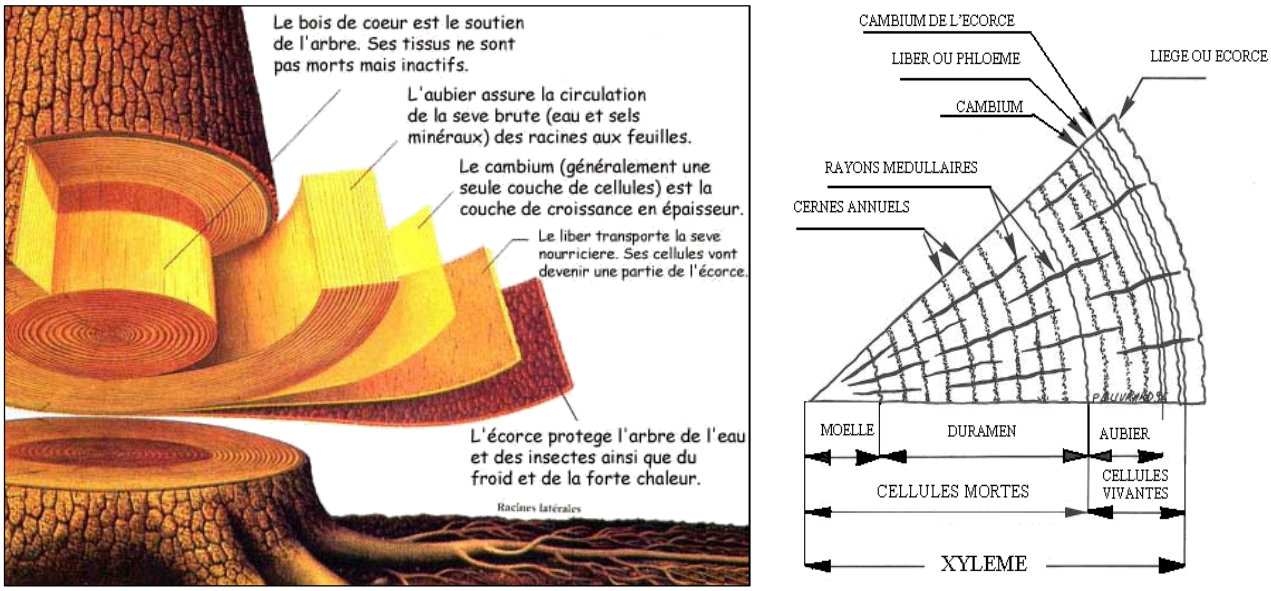


Figure 2: Les extractibles se trouvent principalement dans l'écorce interne (phloème).

Différents types de tanins ont été utilisés très tôt pour la production de colles. Quelques recherches datant des années 50 relatent déjà les travaux de Dalton, Plomley, Narayanamurty etc, effectué avec des tanins [Manas, 1979]. Acacia molisima, Callitiris calcarata, C. glauca, Eucalyptus renduca, E. grebra et E. consideniana, etc. sont seulement quelques tanins étudiés pour la fabrication de colles.

L'extrait tannique est le résultat de la concentration par évaporation de l'eau de la solution obtenue par lessivage méthodique de la matière tannique naturelle [Calleri, 1989].

L'extrait obtenu peut être liquide, c'est alors une solution concentrée contenant 50 % de matières sèches. Il est aujourd'hui le plus souvent solide, en poudre et renferme 90 à 96 % de matières sèches. Les opérations nécessaires pour préparer un extrait tannique sont

sensiblement les mêmes quelle que soit la matière tannique concernée. Dans la figure 3, un exemple de procédé d'extraction de tanin à partir d'écorces est présenté.

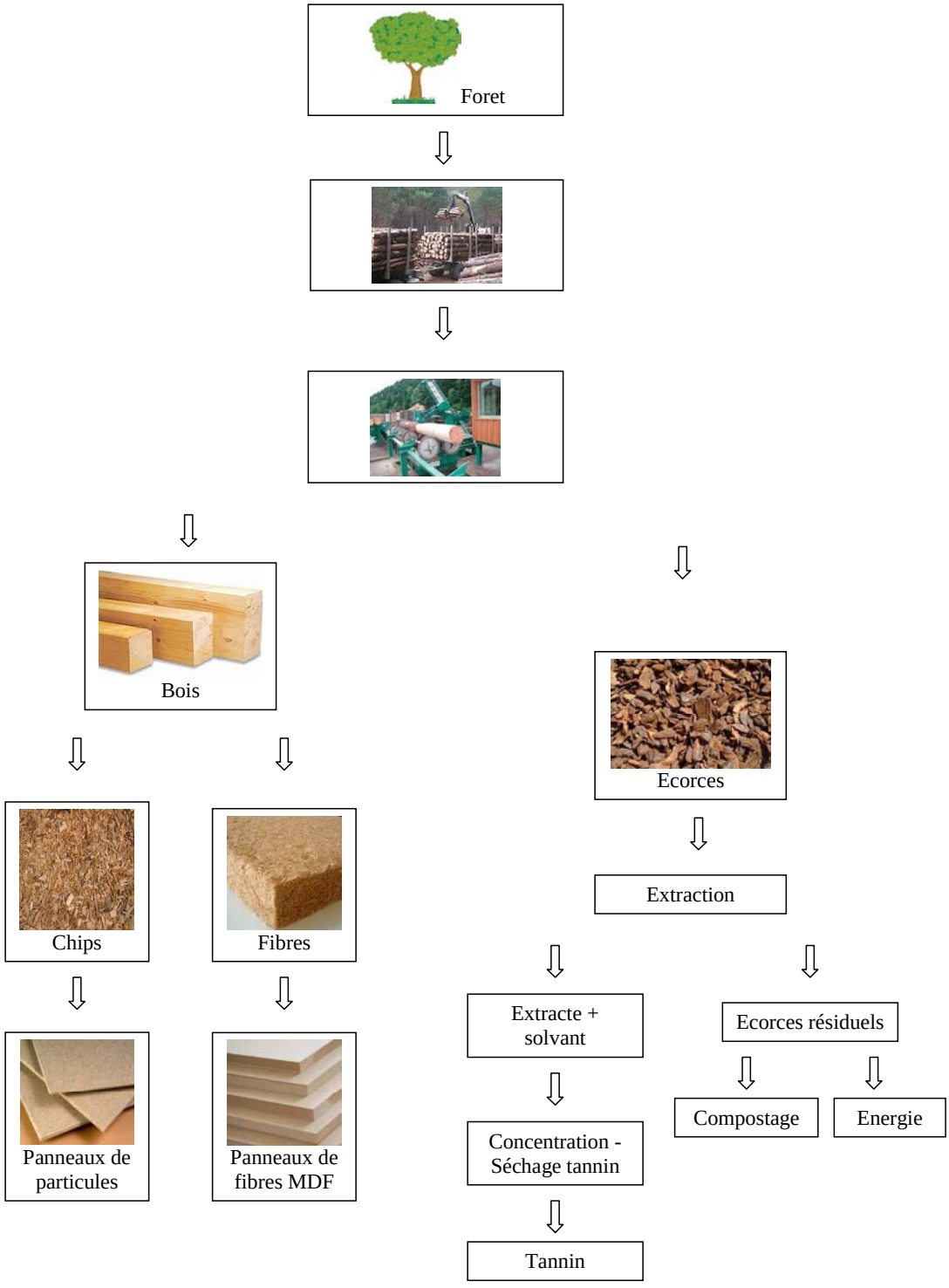


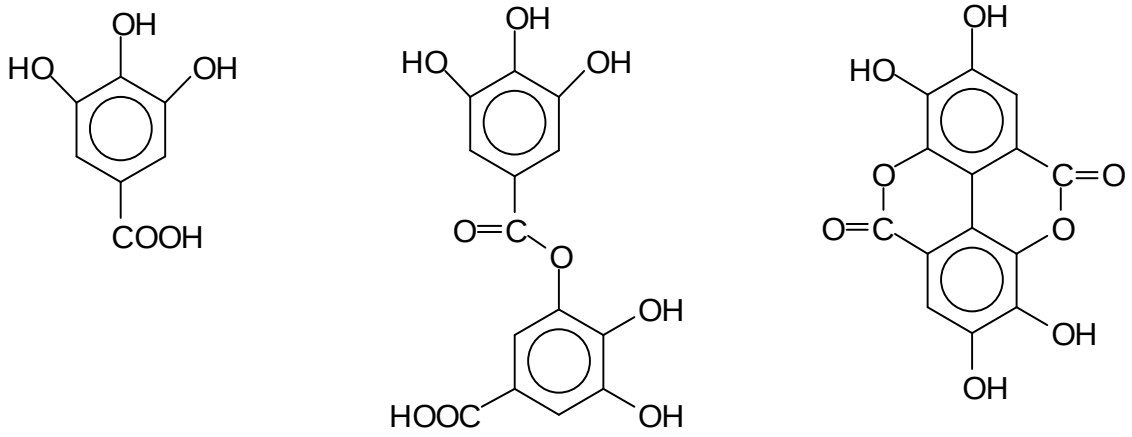
Figure 3: Procédé traditionnel d'extraction de tanins.

II.2.1. **Classification**

II.2.1.a. Tanins hydrolysables

Ces tanins, comme la dénomination l'indique, s'hydrolysent aisément par des acides et des bases, par l'intermédiaire d'enzymes et ils sont généralement de formation pathologique [Carretero, 2000].

Ce sont des mélanges de phénols simples comme le pyrogallol, l'acide ellagique et de sucres, principalement le glucose et les acides gallique et digallique (figure 4) [Pizzi, 1994; Roffael et al. 2000]. Leur utilisation comme colles est inappropriée en raison d'une part, de leur faible réactivité avec le formaldéhyde et d'autre part pour son approvisionnement limité. Toutefois, quelques tanins hydrolysables ont déjà été utilisés comme substituts partiels du phénol dans la fabrication de résines phénol formaldéhyde [Kulvik, 1976; 1977].



Acide gallique Acide digallique Acide ellagique ellagique

Figure 4: Différents types de tanins hydrolysables.

II.2.1.b. Tanins condensés

Ce type de tanins est produit dans le métabolisme normal des végétaux ce qui explique qu'ils sont considérés comme physiologiques et sont largement distribués dans le monde végétal [Carretero, 2000]. Ils peuvent être présents dans les écorces, les tiges, le phloème, les graines, les feuilles et aiguilles des plantes dicotylenes [Zucker, 1983].

Les tanins condensés sont constitués d'unités flavonoïdes. Présentant différents degrés de polymérisation. Deux types de noyaux phénoliques de réactivité différente avec le formaldéhyde sont présents dans chaque flavonoïde: noyaux A et B (figure 5). Ils sont

toujours constitués d'un mélange de quatre types de polyflavonoïdes (figure 6) en proportions diverses.

Ainsi, pour les noyaux A, la fréquence de noyau de type phloroglucinol par rapport au type résorcinol permet de prévoir la réactivité du tanin avec un aldéhyde et de présager de la qualité du réseau d'enchevêtrement. Pour le noyau B, la fréquence des noyaux de type pyrogallol par rapport au type catéchol influe sur l'ouverture de l'hétérocycle pyranique et détermine le type de réarrangement à envisager pour la structure sous conditions alcalines ou basiques [Pizzi et Stephanou, 1994 (1), (2)].

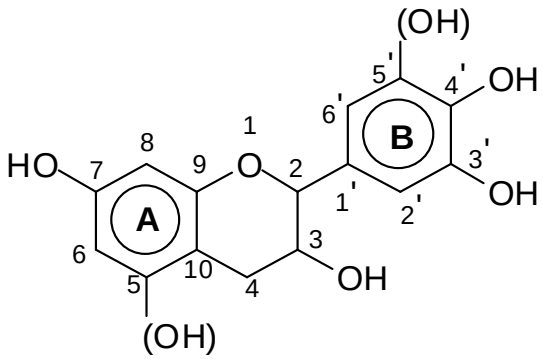


Figure 5: Schéma d'un flavonoïde. (Pizzi, 1983).

Une technique d'analyse ^{13}C RMN des tanins condensés a permis de mettre en évidence les types de polyflavonoïdes rencontrés pour un tanin déterminé. Néanmoins, cette méthode ne permet pas de différencier les prodelphinidines des procyanidines, ni les prorobinetinidines des profisetinidines [Thompson et Pizzi, 1995]. C'est la raison pour laquelle on compare [prodelphinidines + procyanidines] à [prorobinetinidines + profisetinidines].

Ces tanins sont des polyflavonoïdes de nature polyphénolique qui peuvent durcir par réaction avec le formaldéhyde [Pizzi, 1983] au par autocondensation induite [Meikleham et al. 1994].

De nombreuses études ont été réalisées sur l'utilisation de ces tanins condensés [Roux, 1972; Roux et al, 1975; Pizzi, 1983; 2006]. Ils sont utilisés dans de nombreuses applications industrielles: colles pour produits bois ou teintures pour cuir [Pizzi, 1983]. Ils sont composés majoritairement d'unités répétées de flavan-3-ols, comme profisétidine, prorobinétidine, procyanidine, prodelphinidine, et de petites fractions de sucres simples et autres polysaccharides, des carbohydrates, ainsi que des traces d'amino et imino acides [Hemingway et al. 1976]. D'après Roux et al. [1975], seuls les flavonoïdes de type flavan-3-ol

et flavan-3,4-diol participent à la formation des tanins condensés. En effet, le groupe carboxyle en position 4 des autres types de flavonoïde réduit le caractère nucléophile et occupe une des positions de condensation. La substitution méta du noyau A de type résorcinol des flavan-3,4-diols par les groupes hydroxyles et l'oxygène de l'hétérocycle crée une très forte nucléophilie pour les positions C6 et C8. Ainsi, les unités des tanins condensés sont principalement liées entre elles par des liasons C4-C6 ou C4-C8. La première liaison prédomine dans les tanins composés principalement de profisétidines [résorcinol noyau-A: catéchol noyau-B] et prorobinétidines [résorcinol noyau A: pyrogallol noyau-B]. Par ailleurs, les unités répétées dans les procyanidines [phloroglucinol noyau-A; catéchol noyau-B] et prodelphinines [phloroglucinol noyaux A; pyrogallol noyau-B] sont liées aux liaisons C4-C8 [Pizzi, 1994; Meiklehand et Pizzi, 1994].

On parle réellement de tanins condensés pour une répétition de 3 à 8 unités de flavonoïdes.

Les centres nucléophiles dans le noyau A de l'unité flavonoïde tendent à être plus réactifs que ceux présents dans le noyau B. Ceci est dû aux substituts hydroxyles voisins, qui entrainent une activation dans le noyau B sans effets spécialement localisés contrairement à ceux observés dans le noyau A [Pizzi, 2003].

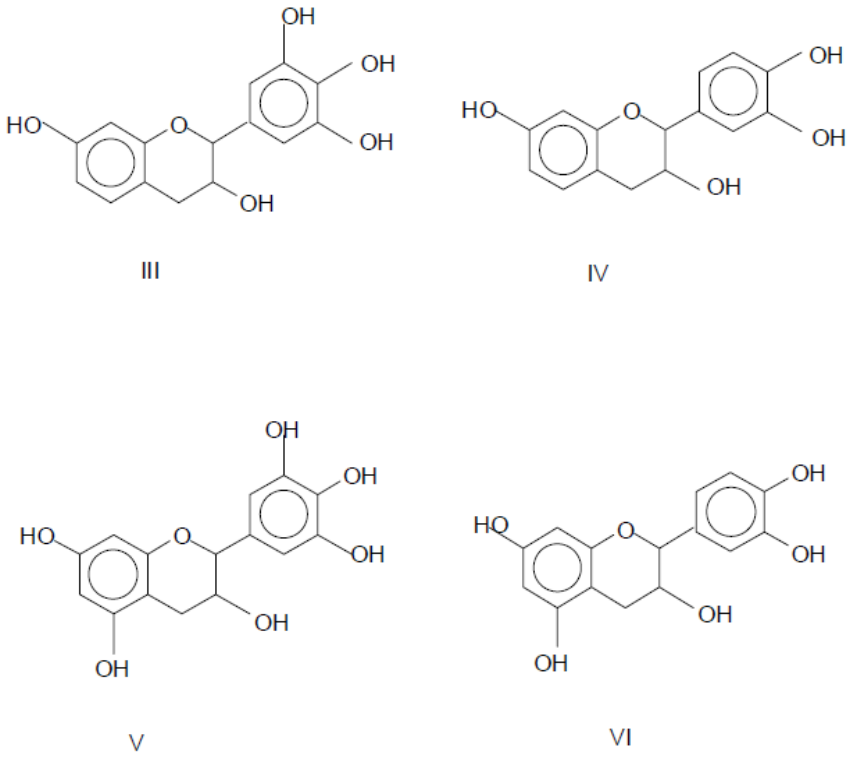


Figure 6: Quatre représentations possibles des structures de type monoflavonoïde (Pizzi 1983). [a]Noyau: A de type résorcinol et noyau: B de type pyrogallol. [b] Noyau: A de type résorcinol et noyau: B de type catéchol. [c] Noyau: A de type phloroglucinol et noyau: B de type pyrogallol. [d]Noyau: A de type phloroglucinol et noyau: B de type catéchol.

Les C6 et/ou C8 libres dans le noyau A sont les sites qui réagissent avec le formaldéhyde, du fait de leur forte nucléophilie pour former des colles.

Les carbohydrates simples (hexoses, pentoses et disaccharides) ainsi que des glucuronates complexes (gommes hydrocolloïdales) sont souvent présents en quantités suffisamment élevées pour influencer les propriétés physiques et chimiques (viscosité, réactivité).

Les tanins condensés constituent plus de 90% de la production mondiale des tanins (actuellement de 220 000 tonnes par an); ils se sont révélés, par ailleurs, très intéressants dans la préparation d'adhésifs et de résines car les réseaux polymères formés sont denses et présentent de bonnes propriétés.

Les tanins condensés suivants sont commercialement exploités [Pizzi 1994]:

- Acacia [Mimosa, extraction à partir de l'écorce]. L'extrait utilisé pour les colles est modifié avec de l'anhydride acétique et de la soude caustique;
- Schinopsis [Québracho, extraction à partir du bois]. L'extrait utilisé pour les colles est modifié avec de l'anhydride maléique et de la soude caustique;
- Tsuga [Hemlock, extraction à partir de l'écorce];
- Rhus [extrait de Sumac];
- Pinus [Pin radiata, extraction à partir de l'écorce].

Il convient de citer également les tanins extraits de diverses variétés de pin et de pécan (Carya Illinoensis).

II.2.2. **Réactivité**

La relative accessibilité et/ou réactivité des flavonoïdes a été étudiée grâce à la bromation sélective dans la pyridine de modèles des familles du phloroglucinol et du résorcinol. La bromation se produit préférentiellement en C8 sur la [+]-tétra-O-méthylcatéchine [Roux et al. 1975] et c'est uniquement lorsque ce site est occupé que la substitution commence en C6.

Le noyau B n'est pas réactif à moins qu'il n'y ait un excès de réactif de bromation : on observe alors un faible degré de substitution en C6'. La séquence de bromation de la [+]-tétra-O-méthylcatéchine 8>6>>6' (figure 7A).

Cependant, pour le résorcinol équivalent, cas de la [-]-tri-O-méthylfustine, la séquence de substitution devient 6>8>>6' (figure 7B).

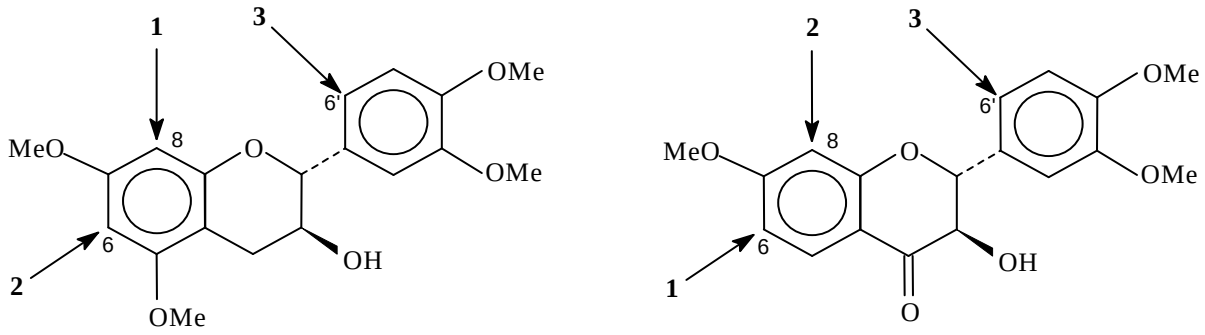


Figure 7: Représentation des sites réactifs des flavonoïdes par bromation.

La substitution préférentielle des flavonoïdes de type phloroglucinol en C8 et de type résorcinol en C6 doit vraisemblablement être liée à la plus grande accessibilité de ces sites. En utilisant le résorcinol comme modèle simplifié, il apparaît que les di-tri- et tétramères formés lors de la réaction [en milieu acide ou alcalin] de celui-ci avec le formaldéhyde, ne privilégient pas la position en ortho des deux groupements hydroxydes. Cette substitution préférentielle qui semble être respectée dans la structure des biflavonoïdes, et confirmée par les réactions de modèles phénoliques, n'est cependant pas respectée à un degré de réticulation supérieur.

II.2.3. **Réactions avec les aldéhydes**

II.2.3.a. Formaldéhyde

Le formaldéhyde réagit avec des tanins pour amorcer la polymérisation à travers des ponts méthylène dans les sites actifs des molécules de flavonoïdes, principalement les noyaux A (figure 8) [Pizzi, 2003]. Les noyaux B, de type pyrogallol ou catéchol, ne sont donc pas réactifs, sauf lorsqu'il y a formation d'anions à des pH relativement élevés [≈ 10] [Roux et al, 1975]. Ceci est dû aux groupements hydroxyles qui provoquent une activation de la globalité du noyau B sans localisation de la réactivité comme cela se produit sur le noyau A. Parfois, les noyaux B de type catéchol sont réactifs à températures plus élevées [Osman et Pizzi, 2002]. Malheureusement la réactivité des noyaux A vis-à-vis du formaldéhyde est alors tellement élevée que la trop faible durée de conservation des résols tanins- formaldéhyde les rend industriellement inutilisables [Pizzi, 1978].

Il a été démontré que les tanins polyphénoliques réagissent avec le formaldéhyde, même à température ambiante. Toutefois, la réactivité de différents tanins varie considérablement [Roffael et al. 2000]. Pendant la réaction des tanins polyflavonoïdes avec le formaldéhyde, deux réactions compétitives peuvent se produire. [i] La réaction de l'aldéhyde avec les tanins

condensés à proprement parler [ii] La formation d'un nombre important de ponts de type méthylène éther [-CH_2OCH_2-] instables à partir du formaldéhyde non réagi. Ces ponts sont aisément réarrangés pour former des ponts méthylène [-CH_2-] avec libération de formaldéhyde [Pizzi, 2003].

En pratique, seuls les noyaux A interviennent dans la formation du réseau tridimensionnel. Cependant, il suffit d'un faible degré de condensation avec le formaldéhyde pour que la taille et la configuration des molécules de tanins leur imposent l'immobilité, ceci à un point tel que les sites réactifs sont alors trop éloignés pour que puisse s'établir un pont de type méthylène [Roux et al, 1975]. Le résultat en est une polymérisation incomplète, d'où des faiblesses et fragilisations souvent caractéristiques des adhésifs tanins-formaldéhyde. Cependant, certaines indications suggèrent que dans des milieux légèrement acides ou basiques avec un excès de résorcinol, les noyaux B de type pyrogallol sont capables de réagir avec le formaldéhyde [Pizzi, 1977]. Ainsi, lors de l'étude de molécules modèles à température ambiante, Pizzi a observé la formation de dimères pyrogallol-formaldéhyde, ainsi que de dimères et de trimères résorcinol-formaldéhyde. Ceci indique qu'en dépit d'une participation limitée des noyaux B dans la formation d'un réseau tridimensionnel tanin-formaldéhyde, un tel réseau reste toujours faible.

Afin de résoudre ce problème, des agents de réticulation comme des résines phénoliques ou aminoplastes à fort taux de polymérisation ont été utilisées, elles permettent l'établissement de liaisons entre des sites trop distants pour être reliés par un pont de type méthylène [Pizzi et Scharfetter, 1978; Pizzi et Roux, 1978].

Bien que le catéchol et les noyaux B de type catéchol ne réagissent pas avec le formaldéhyde à des pH inférieurs à 10, l'addition d'acétate de zinc permet aux noyaux B de type catéchol de réagir avec le formaldéhyde à ces pH, la gamme optimale de pH étant comprise entre 4.5 et 5.5, comme le montre la plus grande quantité de formaldéhyde consommée [Hillis et Urbach, 1959]. L'acétate de zinc permet donc une réticulation du système sans employer de résines synthétiques phénoliques ou aminoplastes. Ainsi l'utilisation d'une petite quantité d'acétate de zinc (5 à 10% par rapport à la résine solide) permet un haut degré de réticulation ne conférant cependant pas au réseau une force comparable à celle obtenue grâce à l'addition de résines synthétiques [Osman et Pizzi, 2002].

Si l'on considère la dépendance de la réaction tanins-formaldéhyde vis-à-vis du pH, il est généralement reconnu que la réaction des tanins de Mimosa avec le formaldéhyde est plus lente entre 4.0 et 4.5 et, pour les tanins de pin, entre 3.3 et 3.9.

A pH neutre, le formaldéhyde réagit rapidement avec les unités monomères en C6 et C8, mais également en C2’ et C6’ des noyaux B de type catéchol ou pyrogallol bien que plus lentement. La Figure 8 [Pizzi, 1994], montre la dépendance du temps de gel du tanin de Mimosa avec le paraformaldéhyde en fonction du pH à 100°C (figure 9).

Le formaldéhyde est l’aldéhyde généralement utilisé lors de la préparation et du durcissement d’adhésifs à base de tanins. Il est normalement additionné aux tanins en solution, au pH désiré, sous la forme de formaline liquide ou sous la forme du paraformaldéhyde polymère, capable de se dépolymériser rapidement en milieu alcalin.

La réaction du formaldéhyde avec les tanins peut être contrôlée par l’addition d’alcools dans le système. Par exemple, l’utilisation de méthanol conduit à la formation d’hémiacétals quand la colle polymérise à températures élevées en diminuant la libération de formaldéhyde [Pizzi et Scharfetter, 1978].

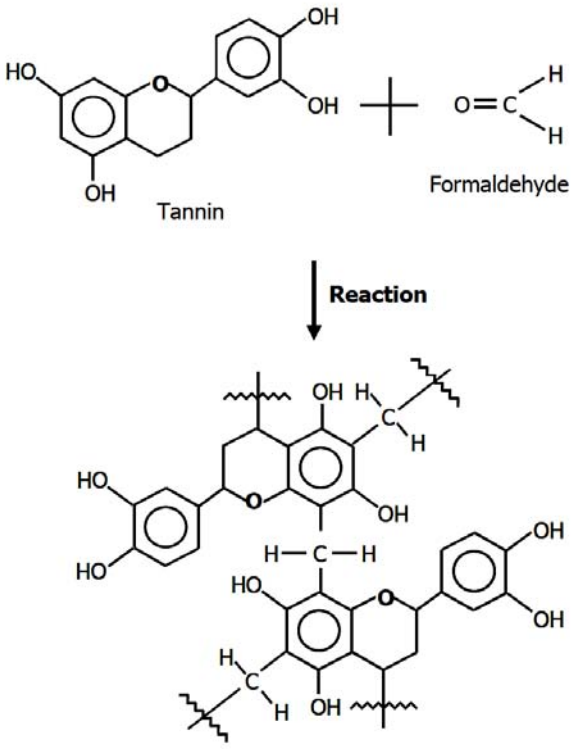


Figure 8 : Mécanisme de réaction entre un tanin et le formaldéhyde.

La recherche pour diminuer ou éliminer complètement les émissions de formaldéhyde provenant de produits collés à base de bois, a été articulée autour de deux axes principaux : (a) L’utilisation de durcisseurs qui n’émettent pas de formaldéhyde, et (b) l’autocondensation des tanins.

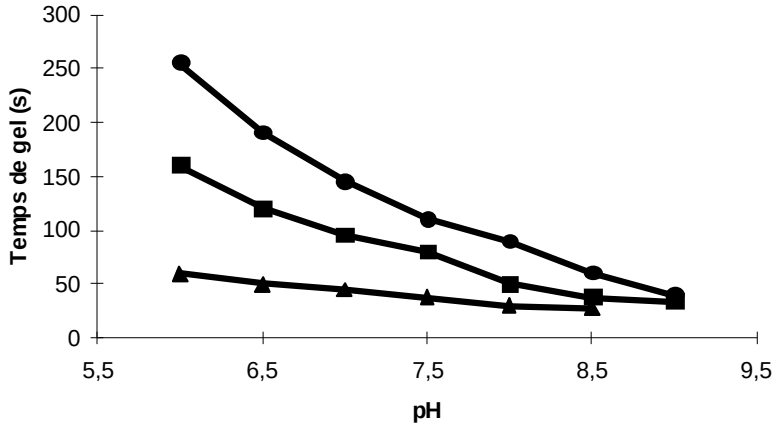


Figure 9: Temps de gel tanin-paraformaldéhyde en fonction du pH.

(Pizzi, 1994).

II.2.3.b. Trishydroxylméthyl nitrométhane (TN)

Il s'agit du plus simple et du moins cher des nitroparaffines méthylolates. Il fonctionne bien comme durcisseur pour les colles à base de tanins et les produits type panneaux de particules, MDF, et contreplaqués. De plus, la durée de vie de pot est largement augmentée ce qui constitue un réel avantage [Trosa et Pizzi, 2001]. Sa réactivité dépende de son pH (figure 10).

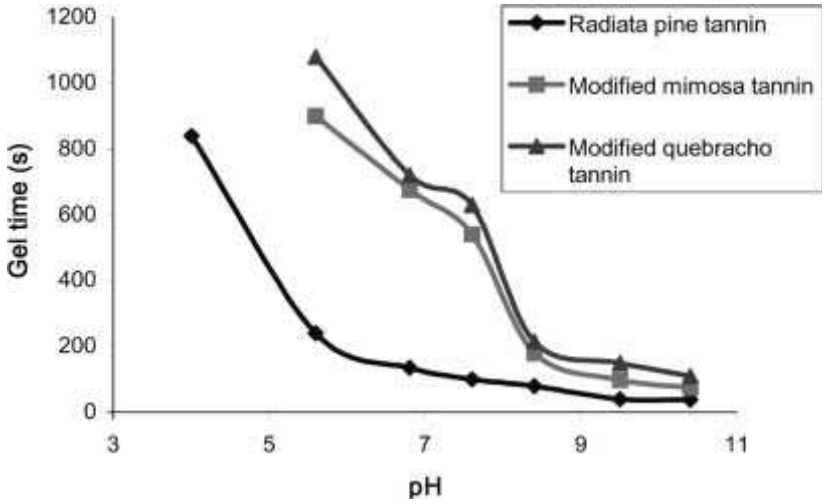


Figure 10: Temps de gel en fonction du pH des colles de tanin de pin radiata, mimosa modifié et quebracho modifié avec 12% de tris[hydroxymethyl]nitrometane (Trosa et Pizzi, 2001).

En outre, les niveaux d'émissions de formaldéhyde sont réduits avec l'utilisation de TN comme durcisseur. Ces émissions sont de l'ordre de celles de formaldéhyde émis naturellement par chauffage du bois. Cette observation montre que les colles préparées

avec TN peuvent être classées dans la catégorie «émission type zéro» et confirme que TN est complètement stable sous toutes les conditions de température, malgré une réactivité plus faible que celle du formaldéhyde (le durcisseur traditionnel) [Trosa et Pizzi, 2001].

Les mêmes auteurs ont signalé que TN peut être utilisé en combinaison avec des durcisseurs à base de formaldéhyde. Cette option semble intéressante pour réduire les émissions de formaldéhyde des panneaux (de particules ou autres tels MDF, OSB,…). La production de panneaux contre-plaqués est également concernée mais le coût serait plus important en raison de la quantité de TN plus importante à utiliser. Dans le cas des assemblages aboutés, TN peut être utilisé de façon avantageuse dans des colles polymérisant sous radiofréquence et/ou par activation thermique.

II.2.3.c. Hexamine

La préparation de colles phénoliques et d'hexaméthylènetétramine (plus communément hexamine) a déjà été étudiée de manière assez détaillée. Ces colles sont utilisées industriellement pour la production de panneaux de type intérieur à base de tanins avec des émissions de formaldéhyde faibles voire inexistantes. [Pizzi, 1977; 1999; Pizzi et Stephanou, 1994; Pichelin et al. 1999].

Selon Saayman [1971], l'hexamine se décompose en formaldéhyde dès 65°C en milieu acide. Elle est instable sous ces conditions mais devient plus stable lorsque le pH augmente. Cependant, en milieu alcalin, la libération de formaldéhyde peut ne pas être aussi rapide et efficace. En effet, en milieu acide, l'hexamine (soluble dans l'eau jusqu'à 50% en masse) se décompose en 6 molécules de formaldéhyde et 4 molécules d'ammoniaque alors qu'en milieu alcalin, seules 3 molécules de formaldéhyde sont libérées, cette libération s'accompagnant de la formation de triméthylamine [Walker, 1964].

Par ailleurs, les solutions d'hexamine et de tanins de Mimosa en milieu alcalin ont l'avantage d'avoir une durée de vie en pot très importante à température ambiante [Pizzi, 1977]. Néanmoins, les matériaux fabriqués à partir de ces solutions peuvent manquer de résistance et ne résistent pas à l'eau bouillante [Saayman, 1971], contrairement à ceux formés par utilisation de formaline ou paraformaldéhyde comme durcisseurs Pizzi, [1977]; McLean et Gardner, [1952] ont montré que la présence d'hexamine permettait l'existence de liaisons aussi solides, si ce n'est plus, que le paraformaldéhyde.

Lorsque l'hexamine est incorporée à des colles naturelles ou synthétiques à prise rapide, (renfermant toutes peu de formaldéhyde), principalement à base de tanins [Pizzi et Tekely, 1995], résorcinol-formaldéhyde [Pizzi et Tekely, 1996], et mélamine formaldéhyde [Pizzi et

Tekely, 1996], son comportement est différent [Pichelin et al. 2006]. Elle ne se décompose ni en formaldehyde et en ammoniac en milieu acide ni en formaldéhyde et triméthylamine en milieu basique [Pizzi et Tekely 1995, 1996; Pichelin et al. 1999; Kamoun et al. 2003]. Les intermédiaires amino-immines fortement réactifs issus de cette décomposition sont à l'origine de réactions avec les espèces phénoliques ou aminoplastiques présentes, sans passage par la formation de formaldéhyde [Pizzi and Tekely 1995, 1996; Pichelin et al. 1999; Kamoun et al. 2003]. Cependant, ces réactions conduisent à la production de bases amino-imino métilène du type CH_2=N-CH_2^+ [Pichelin et al. 1999; Pizzi, 1999; Kamoun et Pizzi 2000 a, b; Kamoun et al. 2003]. Ce mécanisme de réaction est fondé sur la capacité de l'espèce réactive présente à réagir avec les bases amino-imino méthylène avant la décomposition.

Selon Pichelin et al. [2006], une colle de tanin avec un pH de 10 est adaptée pour la fabrication de panneaux. Cependant, un pH basique augmenterait l'absorption d'eau et donc le gonflement des panneaux fabriqués avec un système adhésif tanin-hexamine. A pH plus faibles, la décomposition plus rapide de l'hexamine et la réactivité moins élevée du tanin conduisent à la formation de formaldéhyde sous forme de traces et à sa volatilisation sous températures élevées lors du pressage du panneau, conduisant parfois à une diminution de cohésion du matériau.

La figure 11 montre que le temps de durcissement d'une colle à base de tanin de mimosa avec de l'hexamine augmente en fonction du pH [Pizzi, 1979; Pichelin et al. 1999; Pichelin et al. 2006]. Ce comportement est lié au taux de décomposition de l'hexamine, qui dépend fortement de pH [Pichelin et al. 2006].

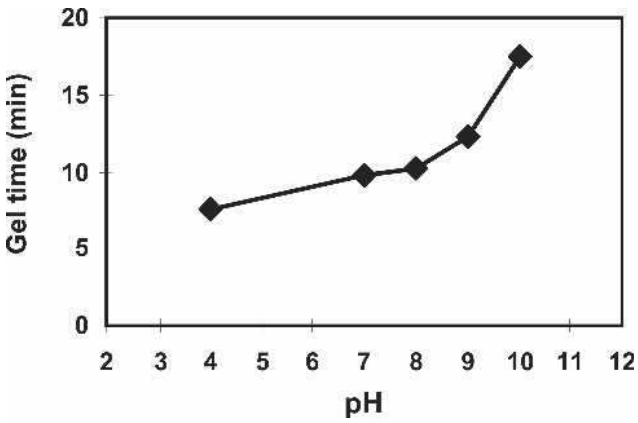


Figure 11: Temps de gel en fonction du pH pour une colle de tanin de mimosa avec 5% d'hexamine. (Pichelin et al. 2006).

Le mécanisme de réaction de l'hexamine montre la formation de ponts benzylamine entre les molécules des tanins (figure 12).

Lors des réactions entre les tanins et l'hexamine, il est nécessaire de bien définir et prendre en compte les conditions de pH dans les processus chimiques [Peña et al. 2009].

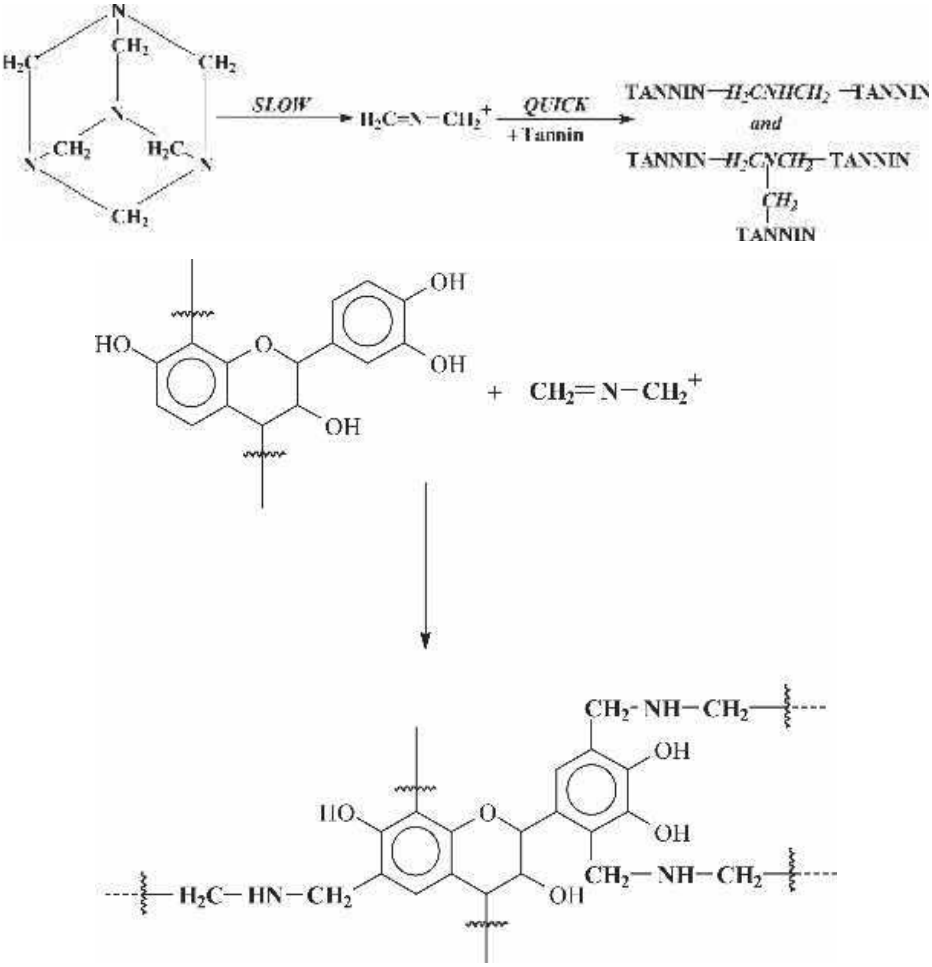


Figure 12: Exemple de réticulation de tanin à travers de ponts benzylamine par réaction avec une base imine-amine méthylène obtenue par la décomposition d'hexamine en présence de tanins.
(Pichelin et al. 2006).

II.2.3.d. Glyoxal

Le glyoxal présente plusieurs avantages importants sur le formaldéhyde. Sa toxicité est si basse qu'il est classé comme composé non-toxique par tous les organismes de santé. Ainsi, alors que le LD50 du formaldéhyde est d'environ 70-140 mg/kg, les valeurs de glyoxal s'échelonnent de 3000 à 9000 mg/kg (Organisation Mondiale de la Santé 2004). Son caractère biodégradable fait qu'il peut être rapidement transformé par les bactéries ou les champignons. En outre, le glyoxal n'est pas volatil, contrairement au formaldéhyde, éliminant ainsi la possibilité d'émission nocive du gaz pendant la fabrication des panneaux ou leur utilisation. Il est produit en grandes quantités et reste par conséquent aussi bon marché que le formaldéhyde. Le principal inconvénient du glyoxal est sa réactivité avec les

phénols, l'urée et la mélamine qui est plus basse que celle observée avec le formaldéhyde [Ballerini et al. 2005].

On a comparé le temps de gel d'une solution de tanin de pin en fonction du pH avec comme durcisseur le formaldéhyde ou le glyoxal (figure 13). A taux de réticulation comparable, on obtient le même temps de gel pour la formulation tanin-glyoxal et tanin-formaldéhyde mais pour des plages de ph respectivement de 8-9.5 et 6-7. Cela signifie que pour des pH de l'ordre de 8-9.5, une colle à base de tanin-glyoxal devrait donner des résultats similaires à une colle à base de tanin-formaldéhyde à pH 6-7.

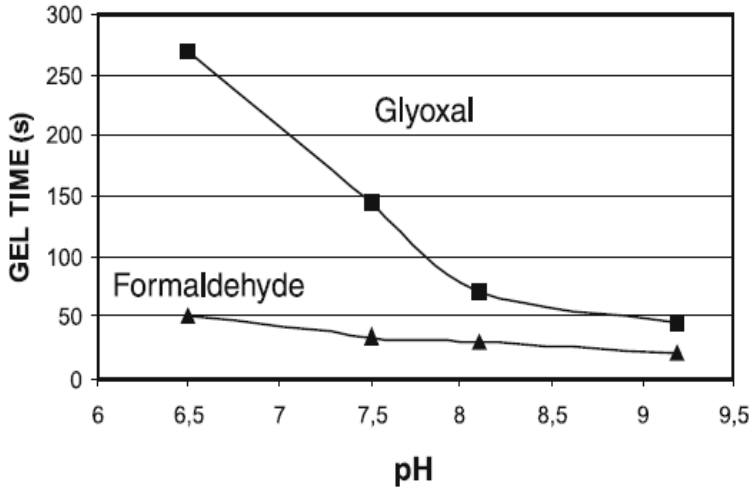


Figure 13: Temps de gel en fonction du pH de colles à base de tanin de pin radiata avec 8% de paraformaldéhyde et 9% de glyoxal. (Ballerini et al. 2005).

Le travail réalisé par Ballerini et al. [2005] a montré que dans les panneaux de particules fabriqués avec du tanin de pin radiata et du glyoxal, la cohésion interne est plus faible que celle des panneaux à base de colles tanin/ paraformaldéhyde. Les émissions de formaldéhyde sont, en revanche, considérablement plus basses pour le premier système. L'utilisation de ce durcisseur peut être envisagée dans des applications pour lesquelles le formaldéhyde est interdit [Tondi, 2009].

II.2.3.e. Autres aldéhydes

La cinétique de réaction du formaldéhyde avec les tanins condensés de type résorcinol et phloroglucinol a été comparée à celle d'autres aldéhydes (acétaldéhyde, propionaldéhyde, iso-butyraldéhyde et furaldéhyde) [Rossouw, 1979]. Il ressort que la réaction sur le formaldéhyde est systématiquement plus rapide que celle observée avec les autres aldéhydes testés.

D’autres études ont été menées avec le benzaldéhyde et le gluteraldéhyde qui sont capables de réagir eux-aussi sur les tanins mais avec là aussi une cinétique beaucoup plus lente.

II.2.4. **L’autocondensation de tanins**

A chaud, et en présence d’acides minéraux forts, les tanins sont susceptibles de réagir de deux manières différentes. Une des réactions dégrade les polymères et mène à la formation de catéchine et d’anthocyanidines comme l’illustre le biflavanoïde typique de la figure 14.

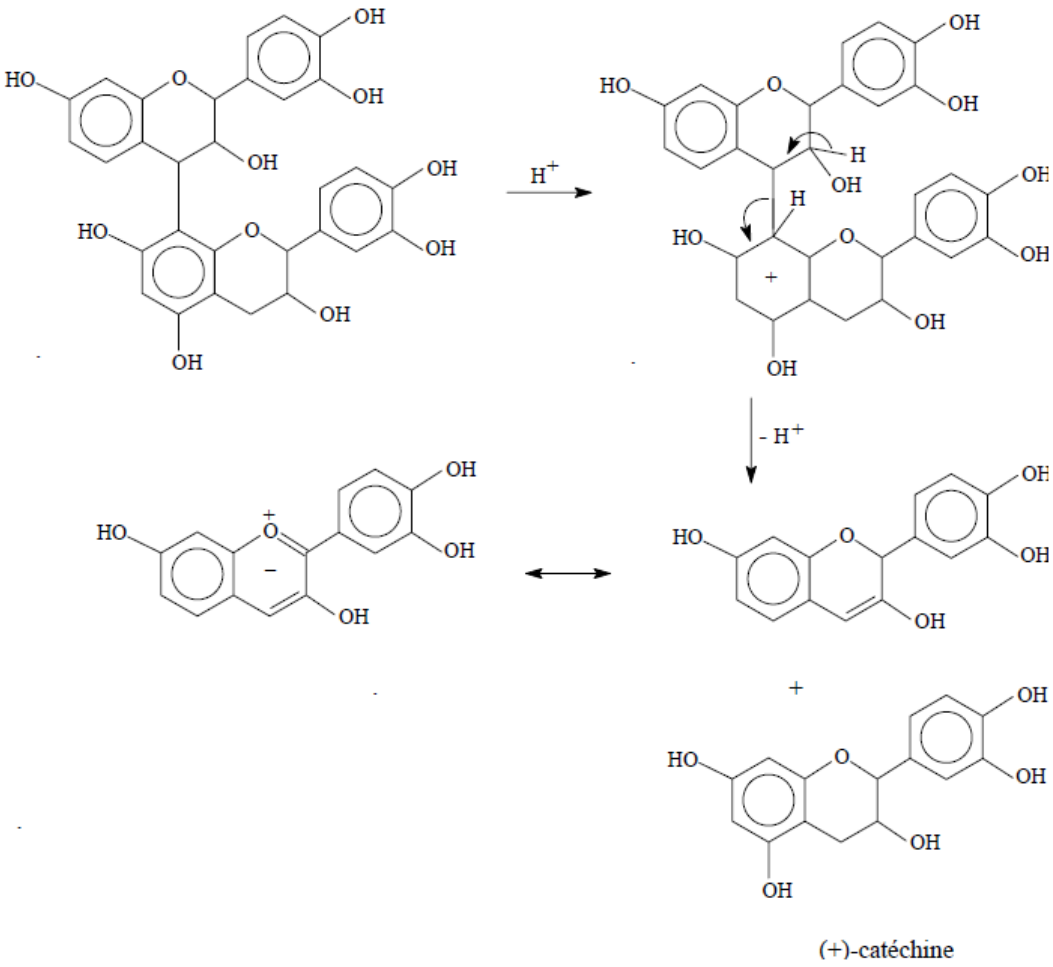


Figure 14: Dégradation des tanins à catéchine et anthocyanidine

(Pizzi 1983).

La deuxième réaction est une condensation résultant de l’hydrolyse des hétérocycles (liaisons p-hydroxybenzyléther) (Roux et al. 1975). Les ions p-hydroxybenzylcarbonium (figure 15) créés se condensent alors avec les sites nucléophiles d’autres unités de tanin pour former les phlobaphènes.

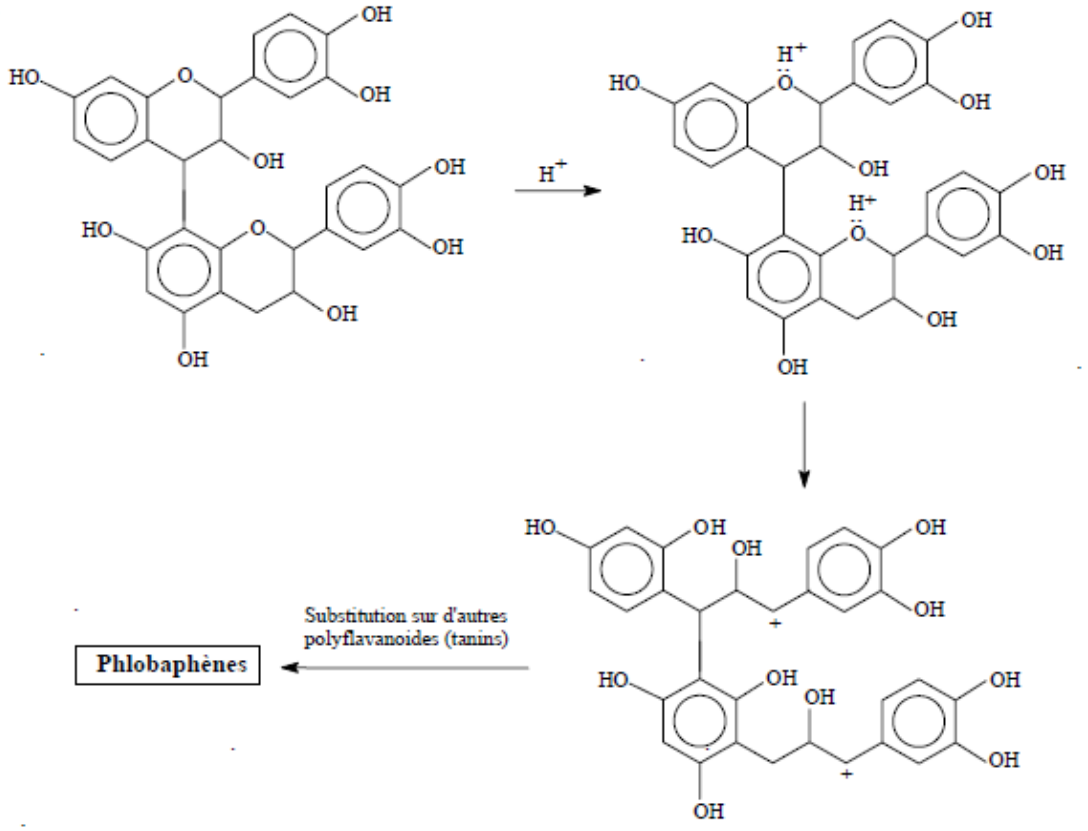


Figure 15: Autocondensation acide par hydrolyse des hétérocycles

(Pizzi 1983).

En milieu alcalin, les tanins sont également susceptibles de réagir de deux manières différentes. La première réaction (figure 16) est la rupture de la liaison interflavanoïde 4-8. Cette réaction intervient dans les tanins de pin et de noix de Pécan (Pizzi et al.1993), mais pas (ou très peu) dans le tanin de Mimosa (Pizzi et al.1993). Le carbocation formé peut mener à une autocondensation « alcaline ».

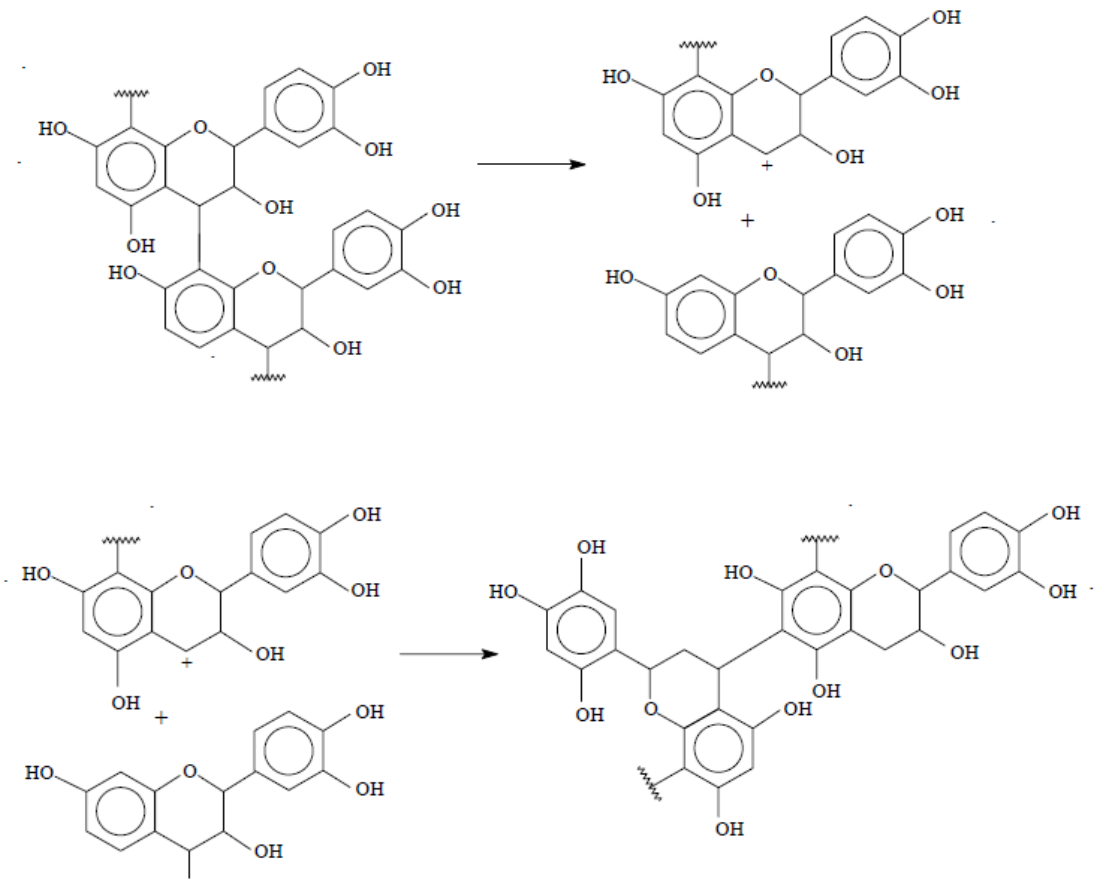


Figure 16: Autocondensation alcaline: rupture de la liaison interflavanoïde et autocondensation finale. Exemples: tanins de pin et de noix de pécan.
(Pizzi et al.1993).

La seconde réaction (figure 17) est une autocondensation partielle due à l'augmentation de réactivité provoquée par l'ouverture de l'hétérocycle. Récemment, il a été montré que les tanins de noix de pécan, majoritairement prodelphinidique, s'autocondensent rapidement, sans pour autant former un réseau tridimensionnel comme une résine durcie [Pizzi et Stephanou 1993; Pizzi 1994]. Ceci est clairement visible lorsqu'en milieu alcalin, la catéchine, monomère, en solution s'autocondense lors du réarrangement catéchinique bien connu [Ohara et al. 1991].

Les réactions d'autocondensation caractéristiques des tanins polyflavonoïdes ont été utilisées à partir de 1994 pour la préparation de colles polycondensées durcies en absence d'aldéhydes [Meikleham et al. 1994].

La réaction d'autocondensation se base sur l'ouverture, dans des conditions alcalines et/ou acides de la liaison O1-C2 du flavonoïde et la condensation ultérieure de le centre réactive forme à C2 avec les sites liberes d'une unité flavonoïde sur une autre chaine de tanin [Meikleham et al. 1994, Pizzi et Stephanou, 1993; Pizzi et Maikleham, 1995; Garcia et Pizzi, 1998 a,b].

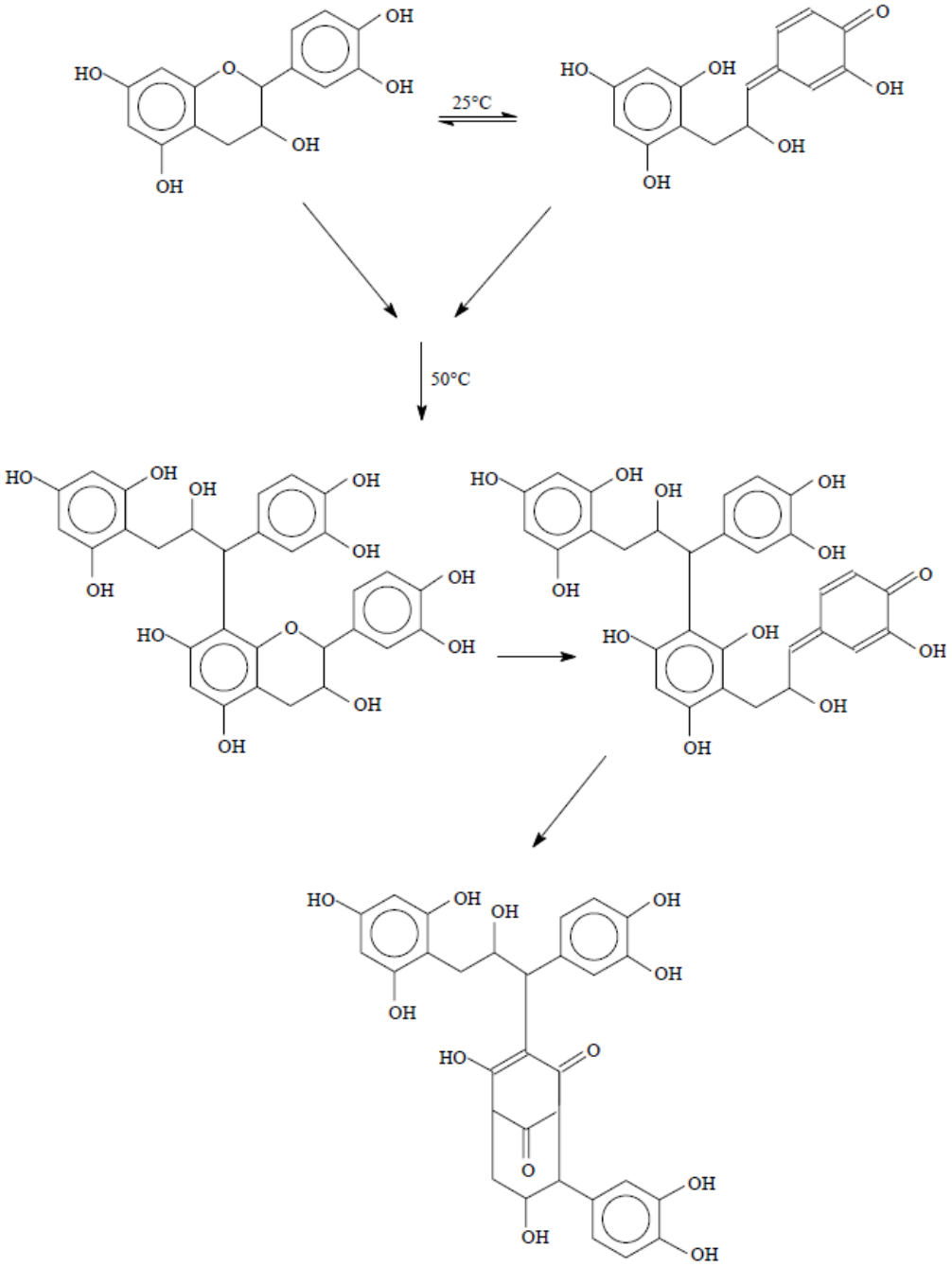


Figure 17: Réarrangement catéchinique.
(Ohara et al. 1991).

L'autocondensation induite aussi est menée à bien en employant des acides de Lewis faibles, silice et acide silicique [Merlin et Pizzi 1996, Masson et al. 1996]. Un mécanisme ionique et radical existe pour cette réaction pour tous les tanins polyflavonoïdes [Meikleham et al. 1994; Merlin et Pizzi 1996; Masson et al. 1996; Pizzi, 1994]. La différence dans la cinétique de réaction dépend de la structure du tanin [Masson et al. 1997].

La réaction d'autocondensation peut permettre un accroissement considérable de la viscosité, mais il n'y a pas de gélification [Pizzi et Stephanou 1993] sauf en présence d'un catalyseur comme la silice ou une surface lignocellulosique [Pizzi, 1993].

Selon Garcia et Pizzi [1998a], quelques coréactants, tels que le paraformaldéhyde, semblent renforcer l'autocondensation de tanin tout en laissant toujours une petite contribution de cette réaction à la formation de la réticulation finale. D'autres coréactants, comme l'hexametitetramine, semblent amplifier la formation de réseau final par synergie entre les deux mécanismes de condensation pour les tanins qui réagissent plus lentement, tandis que pour les tanins avec réaction plus élevé (procyanidin), ce qui est hexametilentetremine, ne montrent aucune interférence.

II.2.5. **Méthodes de caractérisation**

Différentes techniques peuvent être utilisées pour la caractérisation de polymères. Dans le cas de colles à bois d'origine naturelle (tanins, lignines, etc.), certaines des techniques les plus utilisées pour cette caractérisation sont décrites ci-après.

II.2.5.a. Analyse thermomécanique (TMA)

Un analyseur thermomécanique permet de déterminer les variations dimensionnelles d'un échantillon placé dans un environnement thermiquement contrôlé (Figure 18). Selon la méthode adoptée (compression, flexion, traction ou pénétration) et la charge appliquée, statique ou dynamique, il est possible de suivre et de déduire les comportements relatifs à la variation dimensionnelle. Celle-ci peut, en effet, traduire le coefficient d'expansion thermique, la température de transition vitreuse ou le ramollissement et indirectement le module de Young.



Figure 18: Appareil d'analyses thermomécaniques (TMA).

L'analyse thermomécanique en flexion trois points (figure 19) a permis de quantifier l'enchevêtrement du réseau par détermination du nombre moyen de degrés de liberté des segments de polymères au cours de l'autocondensation des tanins [Garcia et Pizzi, 1998a]. Dans l'analyse des thermogrammes obtenus, l'augmentation du module d'Young est liée à deux phénomènes: la progression de la réaction d'autocondensation du tanin par paliers successifs correspondant aux différences de réactivités des sites disponibles sur le noyau A et le noyau B mais aussi l'autocondensation à basse température initiale. Cette autocondensation conduit à la formation de polymères linéaires dont la longueur augmente jusqu'à une valeur limite qui dépend de la température et de la concentration, longueur critique à partir de laquelle se forme un réseau d'enchevêtrement de ces polymères linéaires, et qui correspond à un autre palier sur la courbe du Module d'Young [Garcia et Pizzi, 1998b]. Dans le cadre de résines de type phénol - formaldéhyde, l'analyse TMA a permis de mettre en évidence le point de gel, correspondant au début de l'augmentation du Module d'Young, et le début de la vitrification de la résine, repéré par le point d'inflexion sur la courbe du module [Yin et al, 1995; Riesen et Sommeraurer, 1983].

Dans le cas des tanins condensés qui sont des composés phénoliques complexes, il n'est pas possible de définir ces deux points, puisque la courbe du Module d'Young comporte plusieurs pics et plusieurs paliers. Ainsi, on identifiera les différents points d'inflexion obtenus, à savoir les pics de la dérivée première du module, suivant leur ordre d'apparition: le premier point d'inflexion est identifié comme le début de l'enchevêtrement des polymères linéaires, sorte de pseudo vitrification, suivie par le vrai début de la vitrification due à la formation du réseau d'enchevêtrement tridimensionnel induit par la réactivité des sites du noyau A, et enfin le renforcement du réseau précédemment formé par réactions sur les sites du noyau B [Garcia et Pizzi, 1998b].

A travers cette technique, il est possible d'obtenir une indication concernant le comportement final d'une colle et il est possible évaluer la réaction de durcissement d'une colle en fonction de la température.

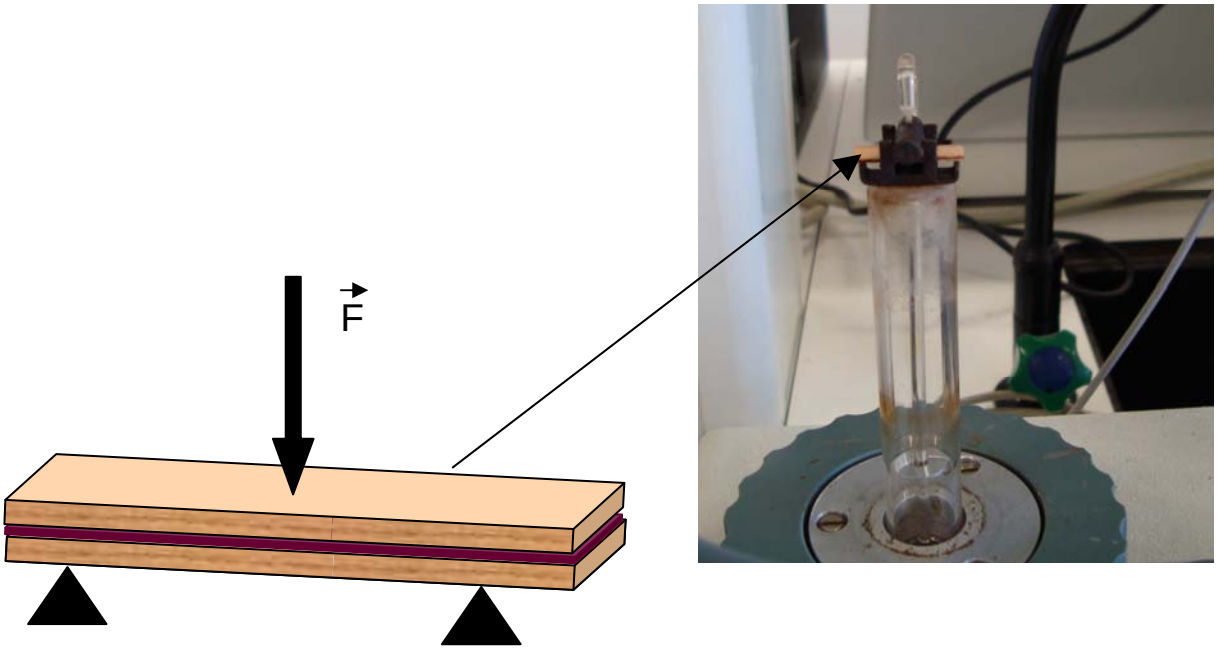


Figure 19: Analyse thermomécanique: mise en place de l'échantillon.

II.2.5.b. Spectroscopie de Résonance Magnétique Nucléaire (RMN ^{13}C)

Méthode utile pour déterminer la structure des composés, la spectroscopie de Résonance Magnétique Nucléaire (figure 20) permet de préciser la formule développée, la stéréochimie et dans certains cas la conformation du composé étudié [Breitmaier et Voelter, 1987; Mansouri et Pizzi 2006, 2007, Mansouri et al., 2007].

La RMN tire des informations de l'interaction qui naît entre les noyaux des atomes et certains éléments présents dans l'échantillon étudié et le champ magnétique intense et constant, produit par un aimant, auquel on le soumet. Le document de base fourni par tous les appareils est le spectre RMN. Il s'agit d'un diagramme représentant des signaux de résonance.

Le spectre RMN correspond à l'absorption par certains atomes de l'échantillon, de certaines des fréquences présentes dans la source électromagnétique. L'interprétation de ces signaux (position, aspect, intensité) conduit à un ensemble d'informations d'où l'on déduit des détails de structure concernant l'échantillon, ce qui est d'autant plus facile qu'il s'agit d'un composé pur.

Ces essais peuvent être effectués en phase liquide ou solide.

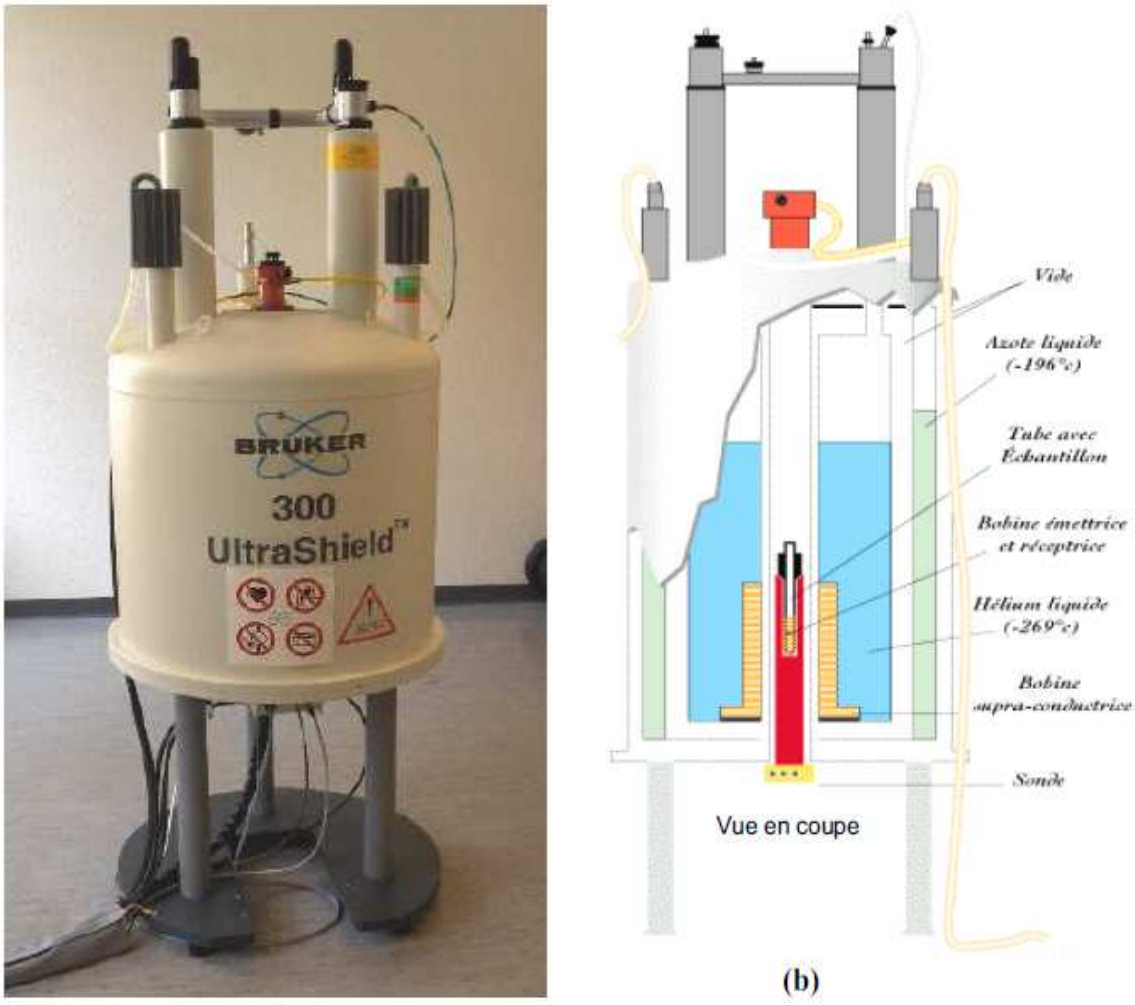


Figure 20: Appareil (a) et Schéma de RMN (b).

II.2.5.c. Spectrométrie de Masse MALDI ToF

Ces dernières années, une technique innovante a été développée. Il s'agit de la spectrométrie de masse «Matrix Assisted-Laser Desorption/ionization-Time of Flight» (MALDI-ToF) qui permet d'évaluer le poids moléculaire des fragments polymères (figure 21). Depuis son introduction par Karas et Hillenkamp en 1987, son utilisation n'a cessé de se développer.

Cette technique douce d'ionisation est en grande partie combinée avec les analyseurs de masse à temps de vol (ToF). Elle présente l'avantage de fournir un spectre de masses complet, pour une gamme de masses pratiquement illimitée, à partir de peu d'échantillon et pour un coût d'équipement relativement faible.

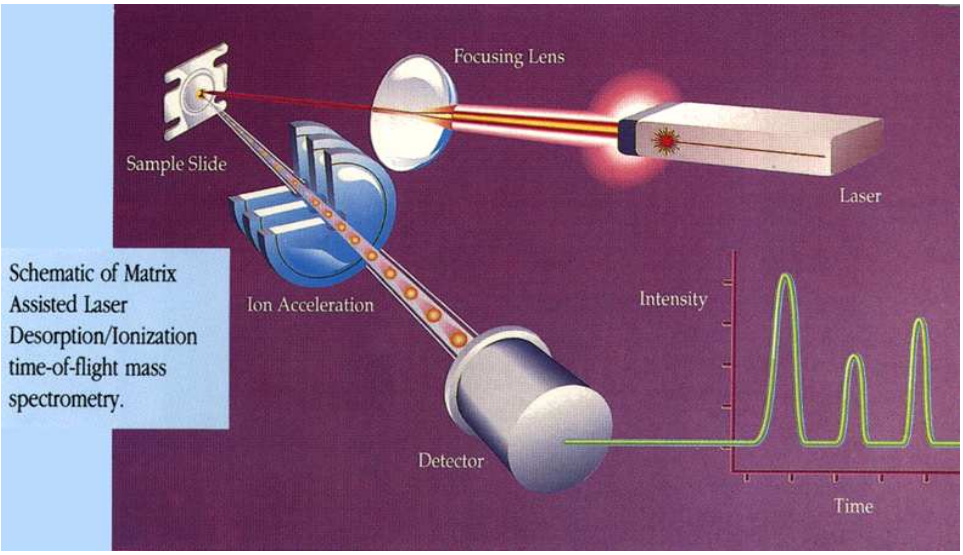


Figure 21: Principe de fonctionnement d'un appareil MALDI-ToF.

L'introduction de cette technique dans l'investigation des tanins [Pasch et al., 2001] a permis une meilleure connaissance des enchaînements des flavonoïdes dans les polymères tanniques.

II.2.5.d. Autres techniques de caractérisation

Diverses techniques d'analyse ont permis d'isoler et d'identifier les molécules constituant les tanins ainsi que leur degré de polymérisation comme par exemple la chromatographie liquide haute performance [Cheynier et al., 1999], et la chromatographie à perméation de gel [Cadahía et al., 1996]. La spectroscopie d'absorption dans l'ultraviolet [Kirby et al., 1953; Muralidharan, 1997] permet d'identifier globalement la famille d'un tanin. Par ailleurs, la spectroscopie infrarouge à transformée de Fourier permet de qualifier un tanin donné [Nakagawa et Sugita, 1999] et la spectroscopie dans le proche infrarouge permet également ce type d'analyse [Donkin et Pearce, 1995].

II.2.6. **Autres applications des tanins**

II.2.6.a. Teintures de cuir

Les propriétés les plus intéressantes des tanins sont dues à leur capacité à se combiner avec diverses substances en formant des complexes. Ils ont ainsi la propriété de transformer la peau en cuir, c'est-a-dire, de rendre la peau imputrescible par leur réaction sur des macromolécules telles les hydrates de carbone et les protéines [Carretero, 2000].

Pendant plusieurs milliers d'années, les tanins végétaux ont été utilisés seuls ou en mélange avec d'autres agents de tannage pour changer la couleur des cuirs [Giovando et al. 2009].

Déjà les Egyptiens et les Babyloniens connaissaient l'art de traiter les peaux. Les tanins végétaux sont très employés encore dans l'industrie du cuir, mais on rencontre également des tanins chimiques synthétiques (comme pour le tannage au chrome), qui présentent l'inconvénient majeur d'être plus polluant.

L'industrie du cuir est l'une des plus anciennes qui existe. Bien que la technologie ait évolué ces derniers temps, les principes de la production sont restés les mêmes. Les protéines de la peau, principalement le collagène, sont rendues insolubles et dimensionnellement plus stables par traitement avec des produits chimiques capables de se fixer sur elles et de les rendre plus résistants et moins exposées aux attaques biologiques et autres [Pizzi et al. 2004].

On établit des liaisons entre les fibres de collagène de la peau et l'agent de tannage; les tanins et les macromolécules réagissent grâce aux groupes phénoliques des premiers en formant des liaisons hydrogène et des liaisons covalentes qui elles sont durables dans le temps. Ceci requiert que le tanin possède une masse moléculaire comprise entre des limites bien définies, pas trop importante pour qu'il puisse être intercalé entre les espaces inter fibrillaires, ni trop petite, afin qu'il forme un nombre suffisant de liaisons [Carretero, 2000].

Les tanins polyphénoliques naturels comme le châtaignier sont toujours largement utilisés, principalement pour la manufacture de cuirs rigides pour les semelles de chaussures, les selles, les ceintures, et autres produits d'usage fréquent (figure N° 22) [Pizzi et al., 2004]. L'effet astringent des polyphénols naturels les fixe efficacement sur la structure du collagène [Pizzi et al., 2004].

Les tanins diffèrent d'un arbre à un autre. La durabilité et la couleur du cuir sont déterminées en partie par le type de tanin utilisé. Quebracho, mimosa, châtaigner et autres espèces sont utilisées dans l'industrie du cuir. Le tanin de pin radiata a été utilisé jusque dans les années 1988. Plus récemment, ont été étudiés les tanins des déchets viticoles [AIICA, 2004-2007].



Figure 22: Quelques applications de tanins avec le cuir.

II.2.6.b. Préservation du bois

Aujourd'hui, la préservation du bois est généralement effectuée par l'utilisation de produits chimiques qui empêchent l'attaque des organismes xylophages. Ce sont des composants chimiques relativement simples ou des mélanges de différentes formulations [Tondi, 2009]. Actuellement sur le marché, trois catégories existent : les produits à l'huile, les substances synthétiques dispersées dans un milieu organique et les sels minéraux solubles dans l'eau.

Les tanins condensés sont des agents de conservation et des fongicides naturels [Zucker, 1983]. Ils aident à protéger les plantes contre l'attaque de nombre d'agents pathogènes comprenant les bactéries et les champignons.

La majorité de ces agents pathogènes sécrètent une enzyme extracellulaire comme la cellulase et la lignase pour rompre le tissu cellulaire des plantes. Les tanins condensés agissent comme des inhibiteurs de ces enzymes en complexant ces protéines pour bloquer leur action [Laks et McKaig, 1988].

La résistance à l'attaque des champignons pour un bois traité par des flavonoïdes est au minimum doublée par rapport à un bois sans protection [Pizzi et Conradie, 1986].
Les extractibles en provenance de duramen peut protéger le bois parce qu'ils ont des propriétés fongicides et antioxydantes [Schultz et al. 1995, Schultz et Nicholas 2000, 2002].

II.2.6.c. Mousses

Les mousses sont des matériaux complexes constitués par la dispersion d'un gaz dans un milieu condensé. La formation des mousses est un processus que l'on observe régulièrement dans la nature quand en particulier des gaz sont mélangés mécaniquement dans un liquide. Selon le type de milieu condensé, les mousses peuvent être liquides ou solides. Les premières sont des matériaux que l'on utilise quotidiennement: les mousses tensioactives, la mousse à raser, la mousse du lait, du cappuccino ou de la bière sont des exemples très pertinents pour s'apercevoir que les mousses se trouvent partout. Les deuxièmes, en général sont produites à partir du dégagement d'un gaz dans un milieu liquide de haute viscosité qui durcit pendant que le gaz s'échappe [Tondi, 2009].
Les mousses solides peuvent être classées selon (i) le type des cellules: ouvertes et fermées; et (ii) sur les propriétés physiques: élastiques ou rigides. Les premières sont utilisées dans des secteurs spécifiques comme les matelas et les accessoires sportifs. Elles sont majoritairement constituées de polyuréthanes mais il existe des alternatives naturelles. Les secondes sont utilisées pour l'isolation thermique et acoustique des bâtiments et parfois comme anti-chocs dans les automobiles. Dans cette catégorie, pratiquement aucun produit naturel n'est proposé en substitution des produits synthétiques [Tondi, 2009]. Cependant, dans le travail de Tondi (2009), une innovation technologique a été proposée à savoir une mousse rigide à base de tanin de mimosa.

II.2.6.d. Anti corrosifs

Les tanins hydrosolubles et les tanins condensés en phase solvant ou aqueuse ont été utilisés comme prétraitement. Ces formulations peuvent être appliquées sur des substrats partiellement oxydés, pour faciliter le nettoyage de la surface par abrasion avec du sable ou par une autre méthode. Ces tanins sont appelés «convertisseurs d'oxydes» et sont plus stables et résistants à la corrosion [Rahim et al. 2007].
Généralement, les tanins naturels produisent des tannates ou des chélates de fer à différents degrés d'oxydation, avec des valences -1, +1 et +2, en facilitant une oxydation ultérieure. Les tanins de faible poids moléculaire sont les moins réactifs vis-à-vis du fer mais ont une plus grande capacité à absorber de l'oxygène [Pizzi, 1980; Matamala et al. 1994].

Jusqu'à aujourd'hui, des recherches ont principalement été effectuées sur les tanins provenant d'écorce de pin et d'acacia [Matamala et al. 2000], ainsi que sur des tanins provenant d'espèces de mangroves [Rahim et al. 2007].

Le processus d'oxydation est amorcé avec la perte d'ions négatifs par le passage de Fe à Fe^{2+}. La réaction continue avec l'oxydation de Fe^{2+} en Fe^{3+} par l'oxygène en formant FeOOH [Rahim et al. 2007]. Les tanins agissent sur les ions du fer disponibles de trois manières différentes. Premièrement, les tanins peuvent former des complexes d'ions Fe^{2+} pour former des tannates-ferroses qui peuvent facilement s'oxyder au contact de l'oxygène en tannates-ferriques, composés bleu foncé. Deuxièmement, les tanins peuvent directement agir sur les ions Fe^{3+} disponibles pour former des tannates-ferriques. Troisièmement, les tanins peuvent réduire les oxydes de Fe(III) en ions Fe^{2+} étant donné leur pouvoir de réduction plus important. Ces ions Fe^{2+} peuvent alors être facilement complexés par les tanins, en tannates-ferriques en présence d'oxygène. La conversion de Fe(III) en tannates de fer est expliquée par le mécanisme suivant [Rahim et al. 2007]:

$$nFe^{3+} + ne^{-} \rightarrow nFe^{2+}$$

$$\tan inos \rightarrow \tan ato + ne \; + nH^{+}$$

$$nFe^{3+} + \tan inos \rightarrow (Fe^{2+})_n \tan ato + nH^{+}$$

puis,

$$nFe^{3+} + \tan inos \rightarrow (Fe^{2+})_n \tan ato + ne^{-}$$

$$(1/2O_2 + 2H^{+} + 2e^{-} \rightarrow H_2O) \times \frac{n}{2}$$

$$(Fe^{2+})_n \tan ato + 1/4nO_2 + nH^{+} \rightarrow (Fe^{3+})_n \tan ato + 1/2nH_2O$$

Toutefois, le traitement chimique des aciers avec des tanins de pin augmente la durée de protection anticorrosive de minimum 50%, avec une économie significative du coût anticorrosif protecteur.

Cette application commerciale a été présentée sur le marché chilien aux débuts des années 1993 [Matamala et al. 2000]. Cependant, sa production a été arrêtée et le tanin de pin radiata n'est plus disponible pour ce marché.

II.2.6.e. Antioxydants

Les réactions chimiques des radicaux libres se déroulent dans les cellules de notre corps et sont nécessaires pour la santé, mais le processus doit être contrôlé avec une protection antioxydante adéquate. L'antioxydant est une substance capable de libérer des électrons qui neutralisent l'action des radicaux libres. Des problèmes de sante surviennent quand notre organisme doit supporter un excès de radicaux libres pendant des années. Ces radicaux sont produits plus intensément en présence de polluants externes (pollution atmosphérique, fumée de cigarettes – qui contient des hydrocarbures aromatiques polycycliques –, aldéhydes…) [Avello et Suwalski, UDEC].

Il existe un groupe de composés naturels, les polyphénols qui sont des antioxydants puissants présents dans les fruits et les légumes. Dans cette famille, on trouve les flavonoïdes, les acides phénoliques et les tanins.

Les tanins se comportent comme des capteurs de radicaux libres [Carretero, 2000]. Ce sont les composés antioxydants les plus abondants dans notre régime alimentaire et leur ingestion a un rôle actif dans la prévention de nombreuses maladies dégénératives [Manach et al. 2005; Scalbert et al. 2005]. En particulier, les flavonoïdes ont été bien étudiés du point de vue de leur purification, caractérisation propriétés et leur influence positive sur la santé. [Cork et Krockenberger, 1991].

Les polyphénols possèdent une structure chimique adéquate pour exercer une action antioxydante. Ils contiennent des noyaux aromatiques avec des groupements hydroxyles, ce qui leur permet d'agir comme donneurs d'hydrogène ou d'électrons, ou de recueillir des radicaux libres.

Dans de nombreux cas, la capacité antioxydante de ces produit est associée à une famille de composés: les catéchines et les proanthocyanidines qui constituent une proportion majoritaire de la famille des flavonoïdes [Gonzalez-Paramas et al. 2004; Yilmaz et Toledo, 2004].

La bioactivité des procyanidines est reconnue pour dépendre de leur structure et particulièrement de leur degré de polymérisation [Svedström et al. 2002]. Ces molécules ont attiré considérablement l'attention dans les domaines tels que la nutrition, la santé et la médecine du fait de leurs activités physiologique et anti oxydante [Bors et al., 2000].

Elles possèdent la faculté de capter des radicaux libres et ont des propriétés antivirales [De Bruyne et al. 1999].

Bien que les extraits aqueux de quelques espèces étudiées aient une activité plus faible que les extraits éthanoliques, ils peuvent aussi être considérés comme une importante source de composés anti-radicaux libres. Une quantité plus importante est alors requise pour obtenir une activité antioxydante équivalente, mais ils présentent l'avantage d'être obtenus par un processus plus simple et économique [Rosales et al. 2006].

Il existe des espèces de bois, sources de composés phénoliques comme les écorces de bouleau [Kahkonen et al. 1999], et de pin maritime, qui présentent une excellente capacité de capture de radicaux libres [Packer et al. 1999].

II.2.6.f. Utilisations pharmacologiques

Les tanins présentent des propriétés astringentes, tant par voie interne qu'externe. Par voie interne, on les emploie comme anti-diarrhéiques, favorisant cette activité par un certain effet antiseptique, puisqu'ils précipitent les enzymes extracellulaires secrétées par les micro-organismes responsables des infections. Ils possèdent aussi des propriétés vasoconstrictrices, ce qui explique pourquoi on les utilise en interne pour traiter des infections vasculaires comme les varices ou les hémorroïdes et dans le cas de blessures légères. En utilisation externe, ils sont préconisés pour traiter divers problèmes dermatologiques et se retrouvent dans les cosmétiques comme toniques astringents [Carretero, 2000]. Ils présentent aussi des effets anti-microbiens [Cowg an 1999], anti-inflammatoires [Santos-Buelga et Scalbert, 2000], et anti-allergiques [Bagchi et al. 2000], etc.

II.3. LA LIGNINE

La lignine s'apparente à un groupe de polymères phénoliques qui confèrent force et rigidité à la paroi cellulaire des plantes (figure 23). Après la cellulose, la lignine est la substance organique polymère la plus abondante dans le monde [Fengel et Wegener, 1989]. La proportion de lignine, entre 20-40%, varie selon les espèces de plantes [Fengel et Wegener, 1989].

Il existe trois groupes principaux de lignines: (i) les lignines de feuillus (gymnospermes), (ii) les lignines de résineux (angiospermes), et (iii) les lignines en provenance d'herbes [récoltes non-arborées ou herbacées] [Buranov et al. 2008]. Ces dernières sont particulièrement intéressantes pour deux raisons essentielles: leur importance en termes de quantités (1549 millions de tonnes/année dans le monde) et leur caractère renouvelable [Kim et Dale, 2004].

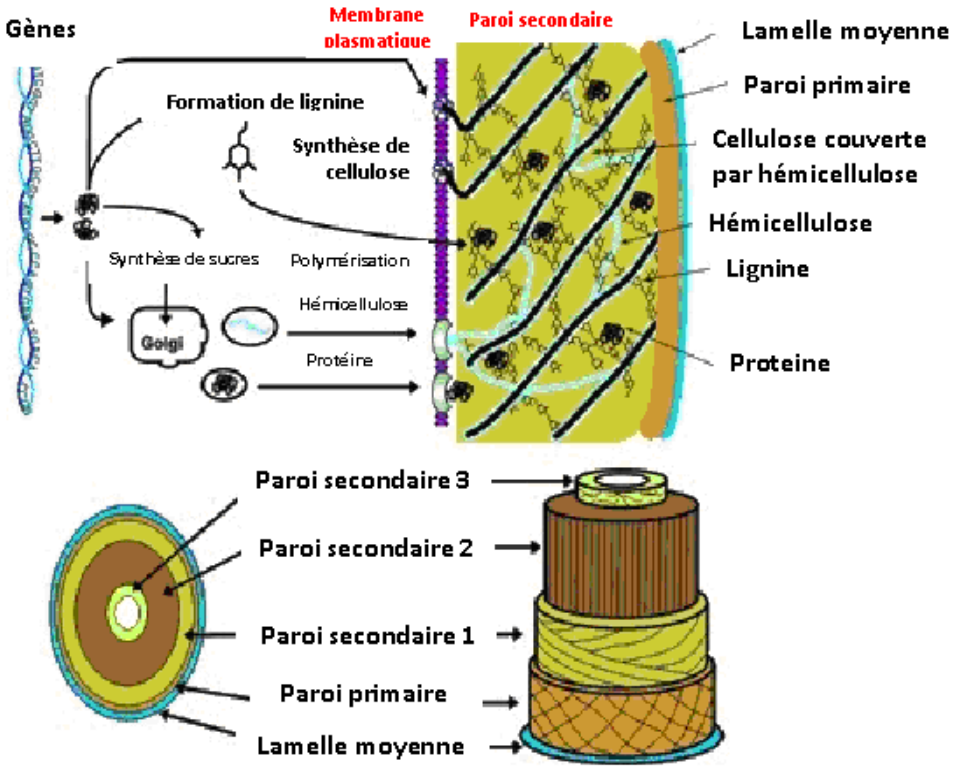


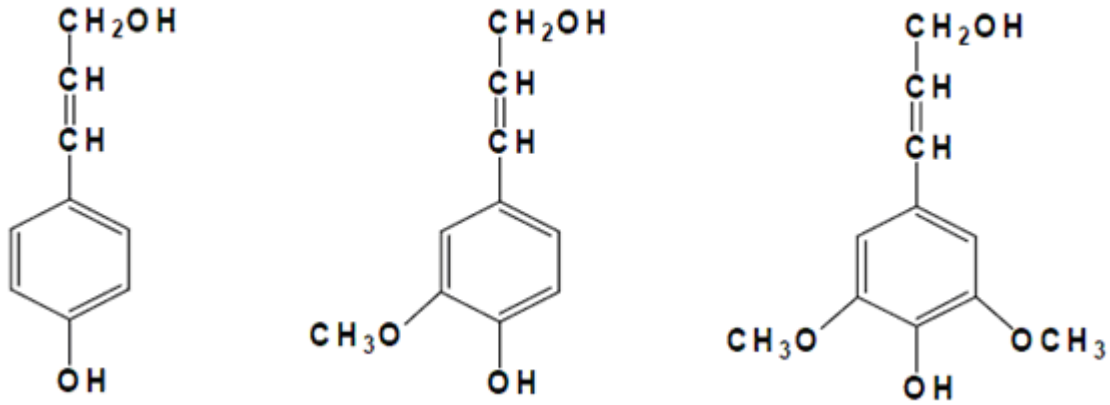
Figure 23: Structure de la paroi cellulaire.

(Neal Stephenson 2010).

La composition de la lignine a été étudiée à de multiples reprises. La conclusion reste la même: celle-ci relève de phénomènes biologiques, biochimiques et chimiques complexes. La composition de la lignine dépend de surcroît de facteurs, tels son âge et sa localisation dans

la paroi cellulaire [Alonso, 2002]. La formation de cette lignine, sa structure et ses liaisons avec le reste de la paroi cellulaire demeurent difficilement explicables du fait de la grande hétérogénéité de polymères au niveau subcellulaire et le manque de méthode pour l'isolement non destructif de ce polymère [Radotic, 1998].

La lignine est un polymère de type phénolique avec la présence de phénylpropane et des associations moléculaires bien définies [Jolivet et al. 2001] : alcool conifférylique, alcool sinapylique et alcool p-coumarylique (figure 24). La recherche structurelle de la lignine a été un grand défi, étant donné sa structure moléculaire hétérogène et complexe, son manque de régularité et son poids moléculaire variable selon l'espèce de la plante et l'origine de l'échantillon [Ysambertt, 2009]. La lignine est un polymère naturel tridimensionnel comportant de nombreuses ramifications avec une quantité très importante de groupes fonctionnels qui renferment des centres actifs chimiques et biologiques [El Mansouri, 2006].



(A) (B) (C)

Figure 24: Les unités à la base de la lignine: (A) 3-[4-hydroxyphényl]-2-propén-1-ol ou alcool p-coumarylique, (B) 3-[3-méthoxy-4-hydroxyphénil]-2-propén-1-ol ou alcool conifférylique et (C) 3-[3,5-diméthoxy-4-hydroxyphényl]-2-propén-1-ol ou alcool sinapylique. [Fengel et Wegener, 1989].

Suivant la composition structurelle de ses unités (figure N° 25), la lignine est divisée en trois classes [Alder, 1977].

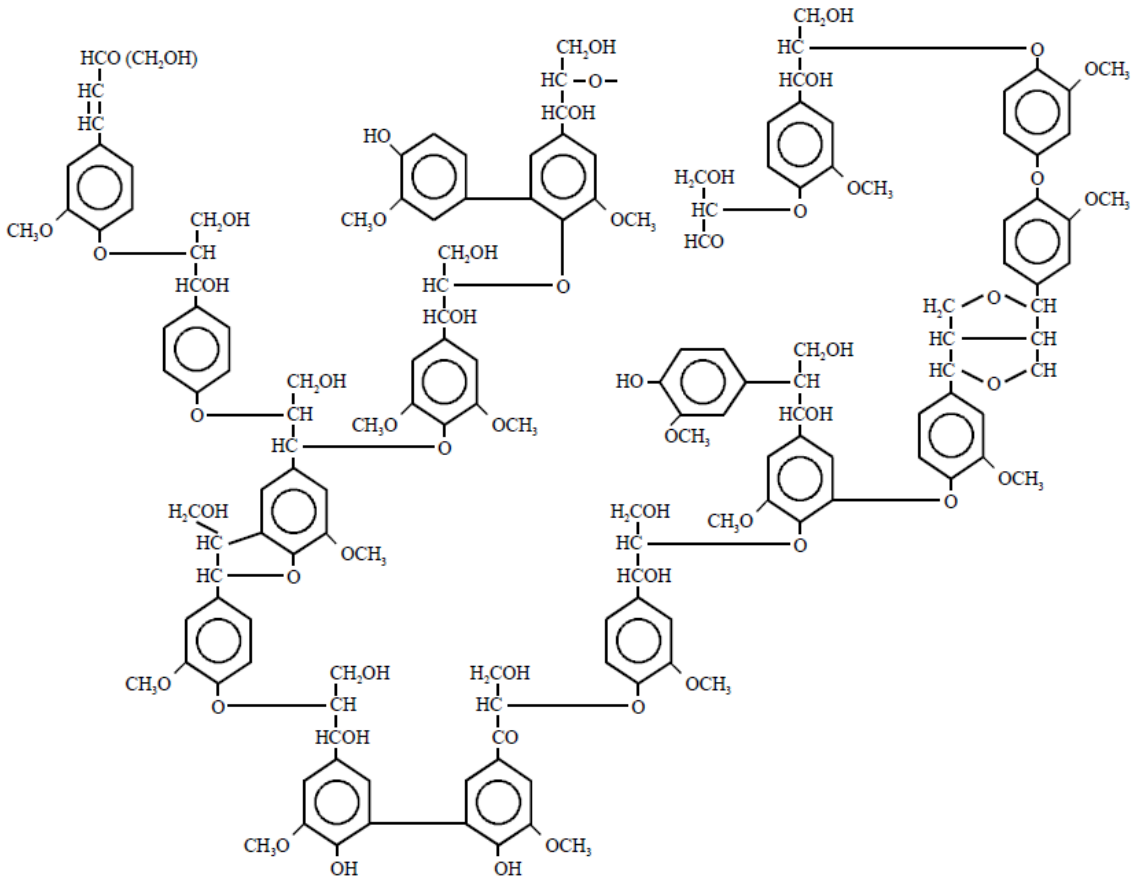


Figure 25: Structure de la lignine dans les conifères.
Alder [1977].

Les lignines de conifères, isolées par différentes méthodes et à partir de différentes espèces ont une structure très similaire. Elles contiennent des unités guaïacylpropane G avec de faibles quantités d'unités d'alcool conifférylique H. Ces structures sont liées par une multitude de liaisons de types éther et carbone-carbone (figures 26 et 27). Les liaisons éther s'établissent entre la chaine de propane d'un monomère de la lignine et un atome de carbone du noyau benzénique d'une autre unité phénylpropane. La liaison la plus courante est de type β-O-4. La liaison α-O-4 est moins fréquente et se rompt plus facilement. L'autre type de liaison est constitué par association entre deux atomes venant de deux noyaux benzéniques différents, comme par exemple la liaison 4-O-5. Les liaisons de type carbone-carbone sont plus difficiles à briser que les liaisons éther. Quant aux liaisons de type β-β, elles proviennent de l'union d'un carbone d'un noyau benzénique avec celui d'une chaine aliphatique d'une autre unité (liaisons β-5 ou β-1), et les liaisons entre carbones de noyaux benzéniques.

Les structures sont de ce fait complexes et hétérogènes. Cette hétérogénéité dépend de l'espèce de plante, du processus d'obtention et des moyens par lesquels la lignine est récupérée de la liqueur [Vazquez et al. 1997].

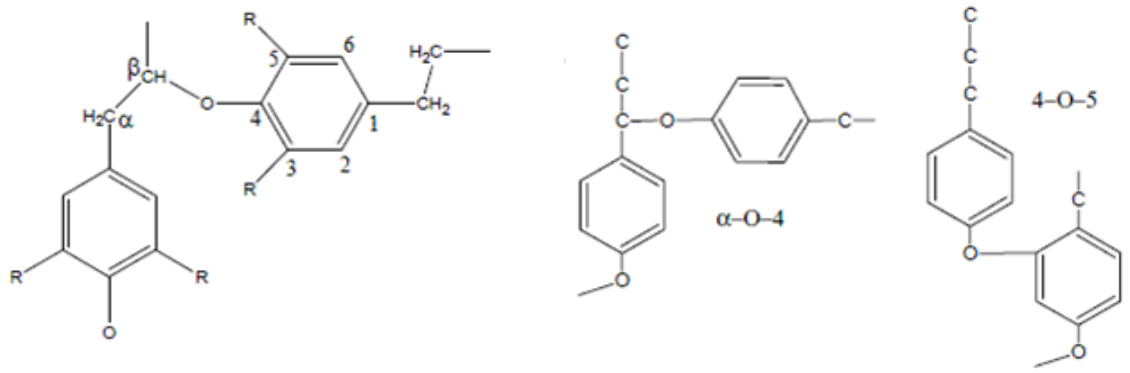


Figure 26 : Liaisons éther rencontrées dans les molécules de lignines [β-O-4, α-O-4 et 4-O-5].

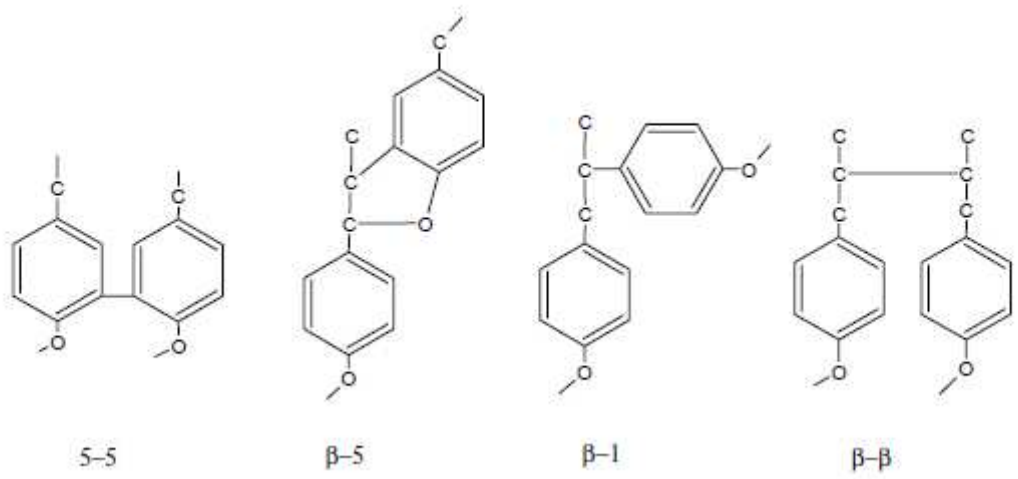


Figure 27 : Liaisons carbone-carbone présentes dans les molécules de lignines [5-5, β-5, β-1, β-β].

Contrairement aux lignines de confères, les lignines de feuillus contiennent des unités de guaïacylpropane G et de syringylpropane S [Carrott et Carrott, 2007], avec de faibles quantités d'unités H dans des proportions que varient depuis 4:1 jusqu'à 2:1, étant les alcools coniferylique et sinapylique ce qui sont les monomères de base [El Mansouri 2006].

Pour les angiospermes monocotylédones (herbes), les lignines contiennent les trois unités H, G et S [Carrot and Carrott, 2007; Wertz, 2010].

Les trois structures H, G et S définissent le modèle de base des différents types de lignines que l'on appelle la formule C9 qui prend en compte les six carbones du noyau aromatique et les trois carbones de la chaîne aliphatique. La variation de la composition de la lignine est

beaucoup plus grande chez les feuillus que chez les conifères. Le contenu des groupes methoxyle dans les lignines varie entre 1.20 et 1.52 mol par unité de phényl-propane (C9). Les lignines d'espèces herbacées présentent des syringylpropanes en faibles quantités, ce qui indique que le pourcentage de groupes methoxyle par unité C9 est aussi moins important [Alonso, 2002].

La lignification est un processus qui débute au niveau de la lamelle moyenne au moment où la paroi secondaire s'épaissit, puis s'étend progressivement à l'ensemble des parois primaire et secondaire. La teneur en lignine est plus élevée dans la zone de la lamelle moyenne-paroi primaire dont l'épaisseur ne dépasse pas 1 µm [Brett et Waldron, 1996], et la paroi secondaire, généralement de 1-5 µm, contient la plupart des lignines de la plante [Wardrop, 1981].

La lignification de la paroi cellulaire provoque la diffusion d'unités de phénylpropane, précurseurs de la lignine. Leur polymérisation commence par une déshydrogénation enzymatique (figure 28) conduisant à la formation de radicaux phénoxy. A cause de la nature aléatoire de la réaction de polymérisation, il n'existe pas une structure définie pour la lignine.

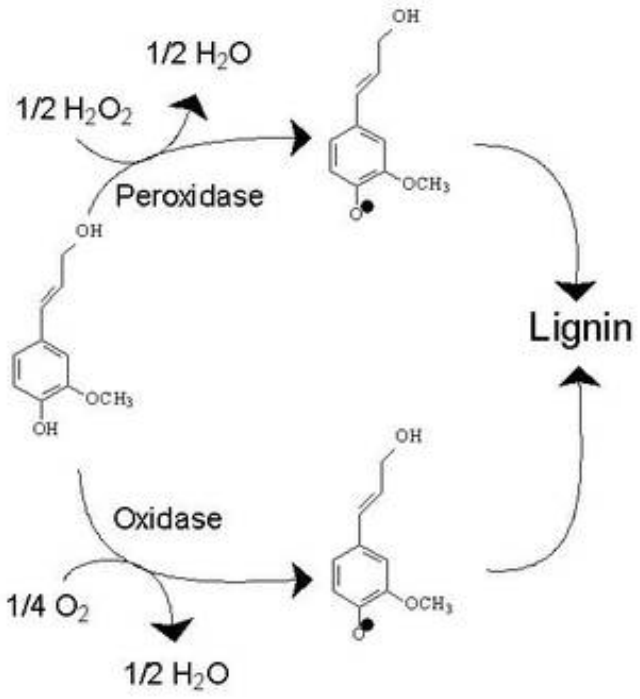


Figure 28: Déshydrogénation des monolignols [ici l'alcool coniférylique] par des peroxydases et des oxydases laccases à l'origine de la polymérisation de la lignine.

La polymérisation de la lignine s'effectue par radicalisation oxydative des phénols, suivie d'un couplage radicalaire combinatoire [Lapierre et al. 1995]. La déshydrogénation des monolignols fait intervenir des peroxydases et/ou des laccases pour fournir la capacité oxydante dans la paroi cellulaire. Tous les composés phénoliques qui entrent dans la paroi cellulaire peuvent devenir des radicaux et ainsi s'incorporer dans le polymère de lignine en fonction de leur oxydation chimique et de leur propension au couplage [Werzt, 2010].

Il existe une grande diversité des interactions impliquant les acides phénoliques dans la lignification [Scalbert et al. 1985]. L'acide férulique peut être estérifié sur la position O-5 des unités d'arabinose des chaînes d'arabinoxylanes et éthérifiés à la lignine. Cet acide phénolique augmente la réticulation des polymères pariétaux en permettant la formation de ponts entre les polysaccharides pariétaux et/ou entre la lignine et les polysaccharides pariétaux [Grabber et al., 2004].

La lignine n'a pas de point de fusion, elle se ramollit entre 100 et 200°C. Elle se décompose à partir de 270°C. Comme pour tous les polymères, la température de transition vitreuse de la lignine est affectée par la présence de petites molécules d'eau et par sa structure. C'est un polymère thermoplastique qui peut être mis en œuvre avec la plupart des thermoplastiques pour faire des composites [Liu et Wang, 2010].

Les propriétés physiques et chimiques de la lignine sont étroitement liées aux méthodes d'obtention, car elle est facilement modifiée par des composés chimiques d'où une variation très rapide de ses propriétés [El Hadji B. LY, 2008].

En dépit de nombreuses recherches sur la lignine en provenance de paille de blé, la littérature concernant ce matériau reste très limitée [Buranov et Mazza, 2008]. Cette lignine contient les trois unités monolignols (p-hydroxyphényl, guaïacyl et syringyl) en quantités significatives : respectivement 5, 49 et 46 % [Lapierre et al. 1995].

Un aspect particulier de sa paroi cellulaire est l'existence d'une lignine qui est facilement soluble en milieu basique et représente jusqu'à 20% de la lignine totale. La récupération de cette lignine augmente la digestibilité du matériel et également la bioconversion microbienne ou enzymatique. Ceci est particulièrement significatif pour tous les processus de fractionnement et représente le premier pas de la délignification pour la production de fibres dans plusieurs processus d'obtention de pulpe [Zilliox et Debeire, 1998].

II.3.1. **Types de lignine selon le procédé d'obtention**

Les lignines techniques sont divisées en deux catégories [Gosselink et al. 2004]. (i) La première contenant du soufre comprend les lignosulfonates et les lignines kraft, qui sont produites en quantités très importantes. Les lignines conventionnelles qui sont employées en industrie sont principalement obtenues à partir de bois de résineux. (ii) La deuxième catégorie correspond aux lignines sans soufre, obtenues à partir de différents processus (traitement à la soude, procédé Organosolv, par explosion à la vapeur, issues de bioéthanol ou de procédés de délignification par l'oxygène). Elles ne sont pas encore produites commercialement [Mancera et al. 2011].

La description de certaines de ces lignines est donnée ci-après.

II.3.1.a. Lignine kraft

La délignification se produit à travers l'action de NaOH et de Na_2S sur les liaisons éther de la molécule de lignine. La rupture de ces liaisons libère des groupes hydroxyles phénoliques en favorisant la dissolution de la lignine en milieu alcalin. La pression et le temps de cuisson peuvent varier de 7-10 bar et de 0.5-2 h, respectivement, à une température autour de 180 °C. La liqueur noire obtenue à l'issue de ce processus est concentrée dans des évaporateurs et le résidu est brulé dans des chaudières pour produire l'énergie nécessaire à la transformation et les réactifs sont récupérés.

Pour isoler la lignine kraft, on peut utiliser une méthode de précipitation acide en deux étapes en employant CO_2 et H_2SO_4 jusqu'à un pH final de 2-3 [Garcia et al. 1984; Norhey, 1992].

II.3.1.b. Lignosulfonate

La lignine issue du processus au sulfite est considérée comme la lignine technique la plus importante du point de vue des quantités commercialisées. Ce procédé permet de produire des pâtes facilement «blanchissables» avec des caractéristiques spécifiques dans la production de papiers spéciaux, comme celui pour la photographie. Il est subdivisé en différents types de cuissons (acide au bisulfite, bisulfite, neutre au sulfite, basique au sulfite, multiétapes au sulfite, sulfite catalysé avec anthraquinone).

Dans le processus au soufre, on utilise un mélange acide sulfureux et ion bisulfite pour dégrader et rendre la lignine soluble. En outre, le mécanisme d'attaque chimique élimine la lignine sous forme de sels d'acide lignosulfonique, en laissant la structure moléculaire quasiment intacte. La cuisson est effectuée dans une solution de dioxyde de soufre avec des

quantités variables de solutions basiques (comme celles mentionnées précédemment). Les températures employées sont généralement de l'ordre de 120-135°C, et le temps de cuisson supérieur à 4 h. Ce processus peut être effectué dans un vaste intervalle de pH. L'utilisation de sels de calcium, magnésium, sodium ou ammonium, plus solubles, permet d'opérer dans des conditions moins acides. Ces procédures de cuisson permettent l'obtention de pâtes de haut rendement et avec de meilleures propriétés.

La délignification dans les processus au soufre se produit dans une certaine mesure par la rupture de quelques liaisons, mais fondamentalement il y a des réactions de sulfonation qui solubilisent la molécule de lignine. Les lignosulfonates sont récupérés pour la plupart lors de la dernière étape de ce procédé. Ils sont cependant difficiles à séparer et à purifier. La séparation est possible seulement quand on a recours à l'utilisation de réactifs chimiques, comme des solvants organiques totalement solubles dans l'eau quel que soit le pH.

II.3.1.c. Lignine Organosolv

Ces lignines sont obtenues par utilisation d'un solvant organique comme milieu de réaction. La lignine qu'on souhaite dissoudre étant lipophile, un solvant organique hydrophobe peut faciliter l'extraction de la matrice cellulosique par rapport aux procédés traditionnels. Alcools, acides organiques, aminés, esters, etc. ont été utilisés comme solvants [Rodriguez et al. 1998].

Ce processus présente plusieurs avantages, parmi lesquels: (i) obtention de la lignine de manière naturelle d'où un processus économiquement possible; (ii) lignines extraites présentant une structure aromatique hautement réticulée, semblable à celle des résines PF; (iii) utilisation possible comme matériel macromoléculaire sans traitement préalable [Nimz, 1983]; (iv plus faible impact environnemental puisque les composés organiques se récupèrent plus facilement des liqueurs noires par distillation; (v) rendements en pulpe importants; (vi) peu de dégradations subies par la lignine; et (iv) blanchiment de la pâte plus aisé [El Mansouri, 2006]**.**

Quelques procédés organosolvs développés : éthanol, acide acétique [Gottlieb et al. 1992; Berg et al. 1995], acide acétique et formique [Nimz et Schoene, 2000], acide formique et peroxyde d'hydrogène, méthanol.

Le processus Alcell est une méthode auto-catalysée (sans aditif) acide qui utilise du méthanol en solution aqueuse à 50% en poids. La température de cuisson varie entre 190-200 °C [Lora et Aziz, 1985]. Dans ces conditions, on peut obtenir des pâtes avec des rendements supérieurs à ceux des processus conventionnels et avec des viscosités

acceptables. Le solvant est récupéré par rectification des liqueurs noires. Ensuite, la lignine est obtenue par précipitation comme sous produit. D'autres sous-produits comme le furfural et une solution riche en sucres sont également obtenus.

Pour isoler la lignine acetosolv, on peut utiliser une méthode de précipitation acide à un pH 2 et éliminer simultanément le solvant [Gilarranz et al. 1998]. Dans les processus organosolv acides, la précipitation de la lignine est obtenue par dilution de liqueur noire avec de l'eau [Lora et Aziz, 1985].

II.3.2. **Caractérisation**

Les quantités de lignine sont déterminées par la méthode de Klason qui permet de quantifier les fractions de lignine soluble et insoluble.

La lignine peut être caractérisée par le biais de ses groupes fonctionnels, puisqu'elle en possède une grande variété qui influence ses propriétés et il existe différentes techniques pour leur détermination. Ainsi, dans les lignines se trouvent les groupes fonctionnels suivants:

II.3.2.a. Hydroxyles phénoliques

La détermination des groupes hydroxyles phénoliques donne des informations sur la structure et la réactivité de la lignine. Ainsi, la présence de ces groupes augmente sa réactivité avec le formaldéhyde. Cependant, la détermination des groupes hydroxyles phénoliques n'est pas facile et peut se faire de différentes façons [El Mansouri, 2006]: spectroscopie UV [Zakis, 1994], spectroscopie de résonance magnétique nucléaire protonique [Lundquist, 1992] et du carbone treize [Robert et al. 1984].

D'autres techniques comme la méthode au periodate de sodium, la méthylation avec du diazométhane, la conductimétrie ou la potenciométrie, ont également été utilisées pour l''étude des lignines.

II.3.2.b. Hydroxyles aliphatiques

Chen [1992], a observéé que la lignine du bois a un taux de groupements hydroxyles aliphatiques généralement supérieur à 1.1 mol/C9. Ce taux est déterminé par soustraction des hydroxyles phénoliques aux hydroxyles totaux, ces groupes étant caractérisés par RMN ^{1}H [Lundquist, 1992] et RMN [Robert et al. 1984].

II.3.2.c. Metoxiles

Le taux de groupements méthoxyles dépend de l'origine de la plante et de la méthode d'extraction. Leur proportion dans les résineux et les feuillus est respectivement de l'ordre de 14-16% et 17-22%. Pour les quantifier, les méthodes les plus communément utilisées sont la méthode Ziel, et la quantification du méthanol libéré après une hydrolyse acide [Balogh et al. 1992].

II.3.2.d. Carbonyles

Ils constituent une famille de groupes réactifs rencontrés dans la lignine. La proportion des différents types de carbonyles (totaux, quinones, cétones et aldéhydes) dépend de la méthode utilisée pour leur détermination [El Mansouri, 2006]: l'oximacion modifiée [Faix et al. 1998], la spectroscopie UV différentielle [Zakis, 1994], la methode ^{19}F RMN qui permet la distintion entre differents groups carboniles comme les aldehydes, cétones y quinones (Sevillano et al. 2001).

II.3.2.e. Carboxyliques

Les groupes carboxyliques sont présents en concentrations extrêmement petites dans des lignines issues de feuillus, et en quantités plus importantes dans les lignines soumises à des traitements biologiques ou biochimiques avec la rupture des noyaux aromatiques de la structure qui conduit à une augmentation de ces groupes. Leur quantification fournit une information supplémentaire quant à la solubilité [El Mansouri, 2006]. Parmi les méthodes existantes pour déterminer les groupes carboxyliques on compte : la méthode de détermination d'indice d'acidité [Gosselink et al. 2004], la potentiometrie en phase aqueuse [Gosselink et al. 2004] et non aqueuse [Dence 1992; Gosselink et al. 2004].

II.3.2.f. Sulfonates

Les lignosulfonates produits dans le processus au bisulfite sont un mélange de polymères dérivés solubles dans l'eau et de carbohydrates dégradés. Les acides sulfoniques aliphatiques forment une partie de la lignine en assurant sa solubilité dans l'eau en présence d'ions [Na^{+}, Ca^{2+}, Mg^{2}+, NH^{4+}, etc.]. Il existe des méthodes basées sur la mesure directe des sulfonates par conductimétrie [Kalts, 1989], ou potentiométrie [Pobiner, 1983].

II.3.3. **Réactivité**

Certaines caractéristiques de la lignine, telles que son hétérogénéité en lien avec sa distribution de poids moléculaire, la présence ou non de groupes fonctionnels et le type de liaison entre ses unités structurelles peuvent restreindre son utilisation [Gonçalves et Benar, 2001].

La réactivité de la lignine est déterminée par sa structure particulière avec des groupes fonctionnels spécifiques. Cependant, il existe des traitements abondamment étudiés qui augmentent les sites actifs des lignines traditionnelles. Ces traitements sont : l'hydroxyméthylation [Benar et al. 1999], la phénolation [Ysbrandy et al. 1992], l'oxydation [Gonçalves et Benar, 2001], la diméthylation [Alonso et al. 2001; Alonso et al. 2005], et la technique de fractionnement. Les deux premiers sont les plus efficaces dans la formulation de colles PF [Sellers et al. 1994]. Plus récemment, la dépolymérisation en milieu alcalin a été proposée pour une obtention plus facile de la lignine présentant des propriétés adaptées en vue de son utilisation dans les colles pour panneaux bois [El Mansouri et al. 2006. Chacune de ces méthodes est décrite par la suite.

II.3.3.a. Hydroxymetilation

L'hydroxyméthylation reste la manière la plus commune pour activer la lignine. Le processus consiste à introduire des groupes hydroxyméthyles (CH_2OH) dans le noyau aromatique de la lignine pour augmenter sa réactivité avec le formaldéhyde [Wooten, 1988 ; Calvé, 1990].

L'hydroxyméthylation est un mécanisme parallèle à celui des résines phénol- formaldéhyde. Toutefois, il requiert un temps de réaction beaucoup plus grand pour l'établissement des groupes méthylols. Ceci est dû à la faible réactivité de la lignine avec le formaldéhyde comparée à celle du phénol [Lei, 2009]. Le processus est effectué en milieu basique, à des températures entre 40-60 °C pour produire des résols. Les unités phényl propane réagissent avec le formaldéhyde. Selon Wooten [1988] et Calvé [1990], trois réactions se produisent. La principale réaction est la réaction Leadere-Manasse où les groupes hydroxyméthyles sont incorporés dans le noyau aromatique de la lignine en position C_5 des unités guaïacyles ce qui augmente la réactivité de la molécule.

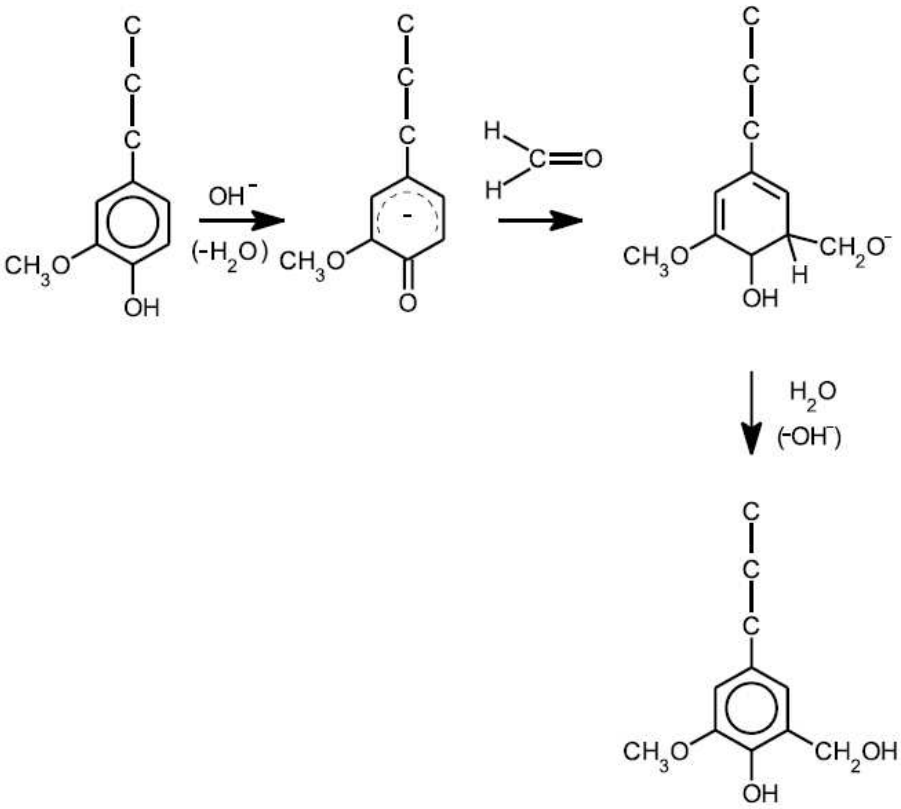


Figure 29: Hydroxymetylation d'noyaux phénoliques d'unités de lignine type guayacyl. (Bernar et al. 2001).

La réaction de Canizarro (figure 30) et celle de Tollens sont indésirables dans le processus. La première consiste en la réaction du formaldéhyde avec lui-même à températures élevées et en milieu basique en produisant de l'acide formique et du méthanol. La deuxième réaction (Tollens) consiste en l'introduction de groupes hydroxyméthyles dans la chaîne latérale dans la position voisine des groupes carbonyles ou des doubles liaisons [Chen et Wu 1994; 1994; Alonso et al. 2001].

$$2\ HCOH \xrightarrow{\Delta,\ OH^-} HCOONa + CH_3OH$$

$$2\ HCOH \xrightarrow{\Delta,\ OH^-} HCOOH + CH_3OH$$

Figure 30: Réaction de Canizarro.

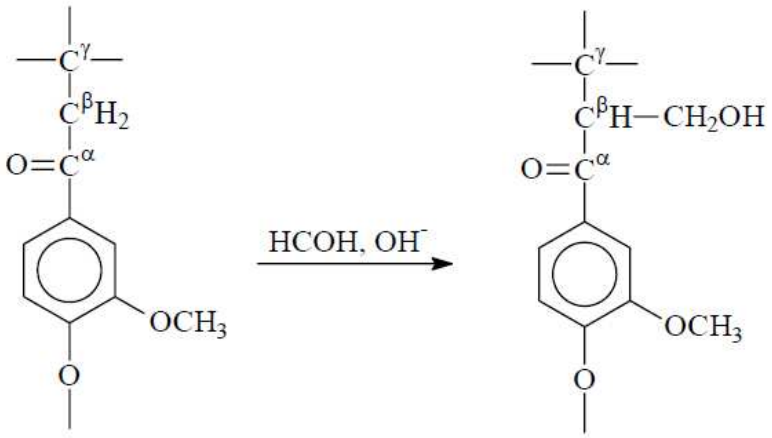


Figure 31: Réaction de Tollens.

La lignine hydroxyméthylée conduit plus facilement à une condensation et à la formation ultérieure de ponts méthylène, ce qui favorise la réticulation des différentes molécules de résine entre elles. Toutefois la réactivité de la lignine dans les processus d'hydroxyméthylation ou de réticulation dépend de (i) sa source: résineux, feuillus ou herbes; (ii) des conditions d'obtention de la pulpe: pH, température, pression; et (iii) des conditions de réaction [Benar et. al 1999; Gonçalves et Benar 2001].

II.3.3.b. Phenolation

Cette réaction a lieu en milieu acide, à haute température**.** La première étape implique la protonation du groupe d'hydroxyle benzylique, suivie d'une déshydratation d' α-carbone, pour donner un ion carbonium. La molécule du phénol subit une attaque électrophile par l'ion carbonium qui donne lieu à un produit de condensation du phénol. La fragmentation des produits d'addition se déroule après l'incorporation de substituts en positions ortho ou para-phényles et α des groupes hydroxyles de la chaîne propane de la lignine, [Nada et al.1987; Ysbrandy et al. 1992; Vasquez et al. 1997].

Cette réaction produit une augmentation des sites actifs et de cette manière augmente la réactivité de la lignine modifiée avec le formaldéhyde. Ainsi la présence d'un groupe hydroxyle phénolique dans un noyau aromatique conduit à la création de deux centres actifs (ortho et para) capables de réagir sur le formaldéhyde.

La phénolation de la lignine est accompagnée de sa fragmentation, ce qui réduit son poids moléculaire. Ceci améliore la compatibilité entre la taille de la lignine phénolée et le pré-polymère entrant dans la formulation de la résine, et améliore les caractéristiques finales de la résine formulée [Calvé et al. 1988, 1991]. De même, on a vérifié que la phénolation est

accompagnée d'une déméthylation de la lignine ce qui entraine une augmentation des groupes hydroxyles phénoliques [Nada et al. 1987]. Alonso et al. [2005] ont trouvé que les conditions optimales de phénolation sont 120 °C ; 160 min et un taux de lignosulfonates de lignine de sapin de 30 % dans la formulation.

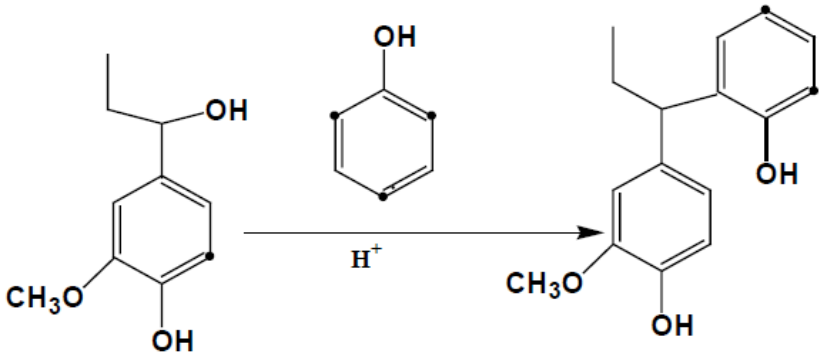


Figure 32: Accroissement des centres actifs dans les fragments de la lignine par phenolation. (Gerrit et al. 1983).

II.3.3.c. Démethylation

La déméthylation est une méthode de modification structurelle de la lignine qui consiste à provoquer la rupture de ses groupes méthoxyles en milieu acide, produisant de nouveaux groupes hydroxyles phénoliques dans sa molécule. On parvient à améliorer ainsi sa réactivité avec le formaldéhyde, parce que celui-ci peut être incorporé au noyau aromatique dans des positions ortho et para du groupe hydroxyle phénolique initialement présent [Alonso 2002; El Mansouri 2006]. Cette méthode est peu viable en raison du coût important des réactifs utilisés comme le dichromate potassique [Hayashi et al. 1997], le periodate sodique [Marton et al. 1963], l'acide chlorhydrique [Gupta et Sehgal 1978] et l'éthanolamine [Wallis 1976].

II.3.3.d. Fractionnement

Ce processus permet d'obtenir des fractions de lignine dans différents intervalles de poids moléculaires. Ainsi, il est possible de choisir la gamme de poids moléculaire la plus adéquate et donc augmenter l'homogénéité du composé. Le fractionnement peut être effectué à travers trois techniques: la méthode de précipitation différentielle, l'extraction sélective avec des solvants et l'ultrafiltration.

La première méthode consiste à précipiter la lignine de la liqueur suite à une diminution progressive du pH de la solution [Sun et Tomkinson 2001; Mussato et al. 2007]. Cette méthode est la plus utilisée parce que l'addition simple d'un acide minéral suffit à atteindre l'objectif. Toutefois cette méthode a un inconvénient majeur : la formation de colloïdes

pendant la précipitation, qui peuvent compliquer les processus de filtration et de séparation, et conduire à l'obtention d'une lignine de pureté médiocre [Toledano et al. 2010].

Sun et Tomkinson [2001] ont montré que la production et la pureté des lignines isolées de la liqueur noire après élimination des produits de dégradation du polysaccharide, augmentent avec une diminution du pH de précipitation.

La méthode d'extraction sélective consiste à utiliser des solvants pour extraire la lignine. L'utilisation d'enzymes, de catalyseurs biomimétiques et de liquides ioniques permet d'obtenir des structures de lignine de bas poids moléculaire, utilisées dans la synthèse de produits de grande valeur ajoutée, mais avec des coûts de production très élevés. Quelques recherches effectuées avec cette méthode sont décrites dans la suite.

Mörk et al. [1986], ont fractionné la lignine de résineux et de feuillus par des extractions successives avec des solvants organiques. La caractérisation des fractions a montré que celles de poids moléculaires les plus faibles présentent de faibles dispersions tandis que celles de poids moléculaires plus élevés sont davantage polydispersées. Avec l'augmentation du poids moléculaire, la teneur des groupements hydroxyle phénoliques et le rpport syringyl/guaïacyl diminuent alors que la stabilité thermique et la température de transition vitreuse augmentent [Mörck et al. 1988].

Thring et al. [1996] et Yuan et al. [2009] ont obtenu des résultats semblables en utilisant la lignine de résineux d'Alcell et kraft d'Eucalyptus pellita. Les premiers ont fractionné la lignine en trois fractions observé un acroissement de la polydispersité ainsi qu'un taux plus élevé de structures guaïacyles avec des chaînes latérales saturées pour les fractions de plus haut poids moléculaire. Les seconds ont observé que les fractions de lignine contiennent de plus grandes quantités d'hémicelluloses et d'unités non condensées de syringyl/guaïacyl. En outre, de petites quantités de liaisons β-aryl ether (β-O-4) restent inaltérées dans le processus d'obtention de la pulpe.

Sun et Tomkinson [2001] ont fractionné la lignine dans quatre parts et ont observé quant à eux, une augmentation de la capacité à former des liaisons hydrogène, du nombre des groupements carbonyles et de la stabilité thermique et une diminution nette du rapport des unités non-condensées syringyl/guaïacyl avec l'augmentation de poids moléculaire.

Quatre processus de filtration peuvent être distingués : microfiltration [MF], ultrafiltration [UF], nanofiltration [NF] et osmose inverse [RO] qui ont chacun son domaine d'utilisation [Toledano et al. 2010]. Ces processus de séparation ont été largement étudiés et utilisés dans le domaine industriel de l'alimentation, de la pharmacie, de la chimie et de la biologie [Kovacs et al. 2009; Toledano et al. 2010]. L'ultrafiltration est un processus efficace pour

fractionner la lignine et consiste à séparer de la liqueur noire, au moyen d'une membrane spécifique, des fractions de lignine avec des distributions de poids moléculaires définis, de les concentrer puis de les purifier.

L'analyse structurelle a révélé que la lignine de M. sinensis obtenue par procédé kraft est composée d'unités H:G:S avec un important taux de G. On n'a pas trouvé de différence significative dans la composition des fractions, les structures présentes dans les fractions ultrafiltrées étant de type β-O-4 et β-5, guaïacyles, syringyles, ferulates et acide p-coumarique (Figure 33).

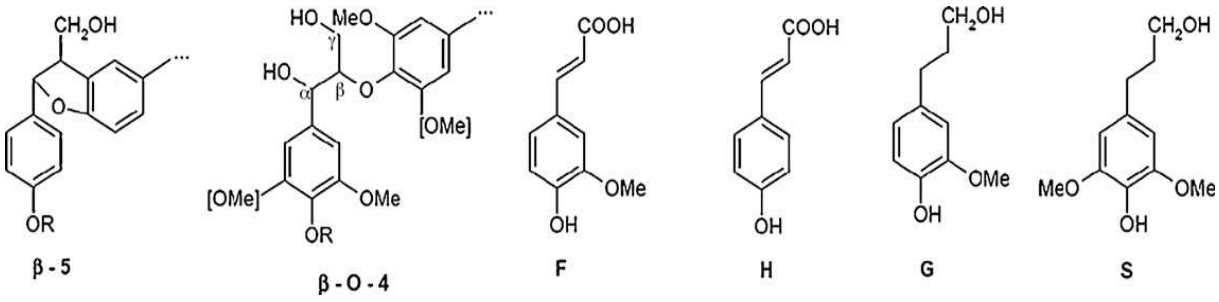


Figure 33: De principales structures présentes dans des fractions de la lignine kraft obtenues à travers ultrafiltration. Structures β-5; structures β-O-4 ; F: ferulates ; H: acide p-coumaric ; G: alcool du guaiacyl ; S: alcool du syringyl. (Toledano et al. 2010).

En ce qui concerne l'utilisation de lignines, Forss et Fuhrmann (1976), privilégient l'emploi de fractions de lignine de haut poids moléculaire, purifiées par ultrafiltration, dans les résines phénoliques. Forss et Fuhrmann [1976] assurent que la condensation entre lignines de bas poids moléculaires et une colle PF ne peut pas contribuer à la formation du réseau tridimensionnel.

Toutefois, Shen [1980] a confirmé l'efficacité des molécules de lignine de bas poids moléculaire lors de l'utilisation des lignosulfonates.

Olivares et al. [1998] ont observé que les lignines kraft fractionnées offrent de meilleurs résultats que ceux obtenus avec la même lignine non fractionnée.

II.3.3.e. Dépolymérisation

Les lignines de bas poids moléculaire sont les plus adaptées dans la préparation de colles en raison de leur taux d'hydroxyles phénoliques et des protons aromatiques (plus grand nombre de centres actifs dans le noyau aromatique des molécules de la lignine). En effet, dans ces lignines la rupture des liaisons éthers α et β permet de produire des hydroxyles phénoliques [El Mansouri, 2006].

La dépolymérisation alcaline de la lignine, proposée récemment, est une méthode facile pour obtenir une lignine présentant des propriétés adaptées en vue de son utilisation dans la fabrication de colles pour panneaux de particules [El Mansouri et al. 2006].

L'hydrolyse de la lignine dans un milieu alcalin dégrade ses molécules de sorte que de nouveaux groupes hydroxyle phénolique sont produits. Dans des conditions appropriées, les produits réactifs de dégradation peuvent être directement employés dans la réaction de condensation des colles phénoliques [El Mansouri et al. 2006; Mancera et al. 2011].

Le traitement alcalin des lignines fortement condensées cause une augmentation du taux des divers groupements fonctionnels, tels que les hydroxyles phénolique et hydroxyles aliphatiques et une diminution des impuretés, telles que les sucres et les extractibles, comme le montre la diminution de la teneur en cendres des lignines traitées. Ces changements structurels rendent les lignines alcalino-traitées plus appropriées à la formulation de résines phénoliques ou à l'usage direct dans les panneaux de bois [Mancera et al. 2011].

II.3.4. **Applications**

Le potentiel de la lignine est actuellement sous-estimé. Son utilisation se limite à celle de combustible pour produire de l'énergie. Or il serait envisageable de récupérer les substances chimiques lors du processus de la transformation en pulpe dont seule une quantité restreinte est utilisée dans diverses applications.

La substitution partielle du phénol par la lignine apparait comme l'alternative la plus attrayante, étant donné les similitudes structurelles existantes entre ce polymère naturel à caractère phénolique et les résines phénol-formaldéhyde [Forss et Fuhrmann, 1979]. Bon nombre de recherches portent sur ce sujet.

Des colles à bois à base de lignine, sans formaldéhyde, ont satisfait aux conditions des normes internationales pour la fabrication de panneaux destinés à des utilisations intérieure et extérieure. Toutefois, pour l'utilisation commerciale de ces formulations, l'addition d'une colle synthétique comme une phénol-formaldéhyde ou un polymère iso-cyanate (PMDI), dans des quantités qui varient entre 20 et 40%, est nécessaire.

La lignine peut également être utilisée dans la production de biomatériaux, d'engrais, de charges pour peintures, vernis, colorants, émulsifiants [Boeriu et al. 2004], d'agents séquestrants, d'additifs dans l'asphalte ou sous forme de dispersants dans les ciments [Buranov et Mazza, 2008]. On la retrouve en particulier, à cause de ses caractéristiques anti-

oxydantes, comme liant dans les additifs alimentaires ou précurseur de la fabrication de la vanilline [Quelenis, 2007] mais aussi dans les aliments pour animaux, comme plastifiant et additif dans les granulés combustibles basés sur la biomasse [Kluko, 2009].

La valorisation de la lignine dans le secteur des carburants porte sur un processus de conversion en deux temps : une première étape de dépolymérisation de la lignine et une seconde étape d'hydrotraitement des composés obtenus. Les produits résultants ont un indice d'octane supérieur à 100 et sont utilisables comme additifs dans les carburants.

Dans l'agriculture animale, la lignine est en grande partie considérée comme une barrière à la digestibilité nutritive. Vraisemblablement pour cette raison, le potentiel de la lignine purifiée en tant qu'additif alimentaire n'est pas très reconnu. Dans la fabrication d'aliments, la lignine sous forme de lignosulfonate, sert de liant dans les granulés d'alimentation. Cependant, son utilisation reste assez peu répandue [Baurhoo et al. 2008].

Les fortes liaisons carbone-carbone et éther de la lignine la rendent résistante à la dégradation. Pour cette raison, la lignine est acceptée comme l'organisme primaire responsable pour la limitation de l'alimentation de fourrage en réduisant sa valeur nutritionnelle [Besle et al. 1995]. La lignine n'est pas digérée par les animaux monogastriques, mais la flore bactérienne des ruminants est capable de dégrader les liaisons benzyle éther de la lignine [Kajikawa et al. 2000]. Ainsi, des différences dans la composition et la proportion de lignine, en fonction de l'espèce animale peut contribuer à l'obtention de résultats différents [Baurhoo et al. 2008].

La lignine purifiée, comme la lignine d'Alcell, montre des effets pré-biotiques sur les animaux monogastriques en favorisant la croissance de bactéries bénéfiques et en améliorant les structures morphologiques dans les intestins. En outre, les propriétés bactéricides des fragments de la lignine peuvent aider dans le contrôle de pathologies intestinales, sécurisant d'une certaine manière, des produits d'origine animale destinés à la consommation humaine. De nombreuses recherches sont encore nécessaires pour étudier les effets de cet ajout dans l'alimentation sur le régime des animaux, sur les consommateurs, mais aussi sur l'environnement [Baurhoo et al. 2008].

La décomposition de la lignine en ses constituants élémentaires est un domaine de recherche assez récent, axé sur l'étude de la microflore intestinale des ruminants, ou l'utilisation de l'appareil enzymatique de certains champignons ou d'insectes xylophages, [Quelenis, 2007].

La lignine peut également servir comme précurseur pour la production de charbons actifs (AC). En effet, la lignine contient un taux important de carbone et une structure moléculaire

similaire à des charbons bitumineux. Elle peut donc être un précurseur idéal et les données qui sont actuellement disponibles confirment que la lignine est une matière particulièrement intéressante à utiliser [Carrott 2007].

Les résidus ligno-cellulosiques, les sous-produits de la conversion à l'éthanol, sont reconnus comme des matières premières pour absorbants. On a observé que ces résidus possèdent des capacités d'absorption élevées envers les phénols et les composés aromatiques azotés. La modification des résidus de l'hydrolyse par un tensio-actif d'ammonium quaternaire augmente leurs propriétés d'absorption car la microporosité des résidus est augmentée suite à cette modification [Dizhbite et al. 1999].

II.4. GLUTEN

Les protéines sont des polymères naturels qui possèdent une structure complexe. Le soja, le sang, la caséine ont été largement utilisés comme sources de protéines pour la fabrication de colles pour bois [Lambuth, 1994].

Depuis les années 60, les colles de protéine ont été remplacées par les produits dérivés du pétrole qui présentent une meilleure résistance à l'eau. Toutefois, étant donné la préoccupation environnementale croissante, les colles à base de protéines apparaissent actuellement plus attractives [Nordqvist et al. 2010]. Dans ce contexte, des études portent sur l'utilisation éventuelle du gluten.

Le gluten est un sous-produit de la production de l'amidon de blé. Les raisons de sa production commerciale découlent d'un besoin industriel d'amidon de blé sans gluten. Il est utilisé principalement dans le secteur boulanger [Nordqvist et al. 2010; Day et al. 2006]. Il peut trouver d'autres applications et a été utilisé avec succès dans la fabrication de films biodégradables alimentaires ou non [Guilbert, 2002] ou de composites à base de bois [El-Wakil et al. 2007].

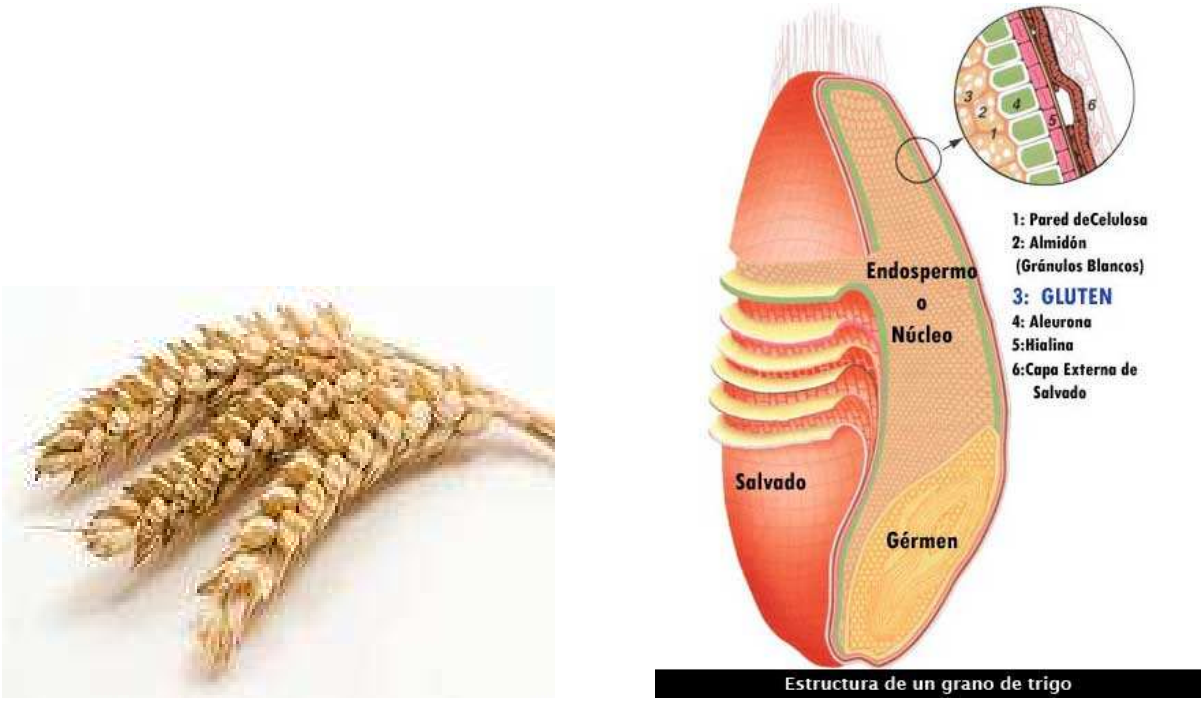


Figure 34: Structure d'un grain de blé.

C'est la plus grande fraction de protéine dans le blé et représente environ 8-15% du poids sec du grain [Kim Sanghoon, 2008]. Approximativement 80% de gluten de froment

composent la protéine du blé [Guilbert, 2002]. Le reste est constitué de traces de polysaccharides, de lipides, et de minéraux. Le gluten est un mélange de plusieurs protéines parmi lesquelles la gliadine, la glutenine sont les formes prédominantes. Les gliadines sont solubles dans l'alcool, alors que les glutenines son dispersibles dans l'acide dilué ou les solutions alcalines. Les deux classes de protéines sont riches en proline et glutamine [Nordqvist et al. 2010].

Les matériaux composites à base de gluten montrent des propriétés fonctionnelles intéressantes en termes de viscoélasticité, de résistance à l'eau ou de perméabilité aux gaz. Les propriétés mécaniques dépendent de la proportion en gluten dans la protéine, qui dépend elle-même des paramètres de processus de séparation, et particulièrement de la température appliquée, qui doit être plus élevée que la température de transition vitreuse, dans la limite de la température de dégradation de la protéine. La résistance à la compression est comparable à celle du polypropylène [Kim Sanghoon, 2008]. Des fibres naturelles sont employées pour améliorer les propriétés mécaniques du matériau. Cependant, dans ce cas l'amélioration est non seulement le résultat d'un effet de renfort, mais aussi d'un abaissement du caractère plastique de la matrice [Kunanopparat et al. 2008]. Les fibres qui contiennent de la lignine peuvent fortement interagir avec le gluten ce qui entraine une réticulation chimique [Papadopoulou et Frazier 2004]. Le gluten de froment a été utilisé avec des fibres naturelles dans des matériaux composites [Kunanopparat et al. 2008].

Le gluten a été aussi employé pour la préparation de panneaux de particules [Khosravi et al. 2011], comme alternative aux colles synthétiques qui contiennent du formaldéhyde.

Jusqu'ici les colles à base de protéines n'ont jamais conduit aux mêmes performances que celles obtenues avec les colles à base de formaldéhyde. Par exemple, une colle au gluten et du bois de hêtre ont été utilisés pour la préparation de panneaux contreplaqués selon les normes EN 204 et 205. Les résultats trouvés ont été jugés satisfaisants dans la mesure où ils sont en accord avec les exigences imposées par les normes. Toutefois, la résistance à l'eau s'est avérée beaucoup trop faible [Nordqvist et al. 2010].

Aussi, des recherches sont encore à effectuer pour parvenir à fabriquer des panneaux de particules aux caractéristiques satisfaisantes et plus respectueux de l'environnement car sans formaldéhyde libre [Khosravi et al. 2011; Nordqvist et al. 2010].

II.5. EMISSIONS DE FORMALDEHYDE ET DE COV

II.5.1. **Antécédents**

Les composés organiques volatils (COVs) sont un groupe d'agents polluants qui sont associés à bon nombre de problèmes de santé, incluant notamment le cancer [WHO, 1987]. Ils sont émis dans l'atmosphère de manière naturelle, comme dans les environnements marins et terrestres, [Fehsenfeld et al. 1992; Seco et al. 2008]. Mais l'industrialisation, le nombre croissant d'automobiles et l'utilisation de nouveaux matériels et de produits synthétiques, principalement dans le domaine de la construction, ont eu pour conséquences l'accroissement en nombre de ces substances dans l'atmosphère durant ces dernières années [Ilgen et al. 2001].

Il est important de comprendre les caractéristiques des sources et les mécanismes de transport de ces composés et c'est ce qui est représenté sur la figure 35.

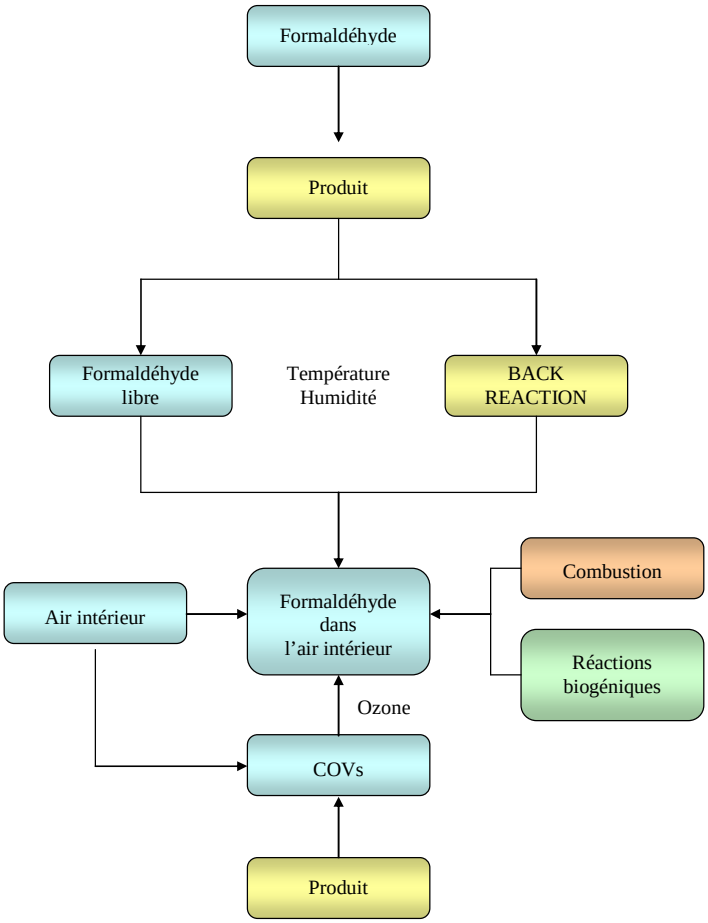


Figure 35: Sources extérieures et intérieures possibles de libération de formaldéhyde. (Salthammer et al. 2010).

Les COVs biogéniques représentent la majorité en contribuant à environ 90% des émissions globales (en termes de COVs) [Guenther et al. 1995]. La majorité de ceux-ci, incluant les isoprènes, les mono- et les sesquiterpènes, les alcools, les aldéhydes et les cétones sont émis par la végétation. Le facteur d'émission de tels composés dépend de nombreux paramètres, principalement la température et l'intensité du rayonnement solaire [Roffael, 2006]. Les composés carbonyles proviennent de la production industrielle, d'émissions du trafic et du chauffage domestique. Par ailleurs, ils peuvent être des produits secondaires issus de processus atmosphériques suite à l'oxydation d'hydrocarbures de sources naturelle et anthropogénique [Müller et al. 2006]. Parmi les COVs, un groupe de composés oxygénés à chaines carbonées courtes (C1-C3) comme le méthanol, l'éthanol, le formaldéhyde, l'acétaldéhyde, et les acides formiques et acétiques, atteignent des concentrations considérables dans l'atmosphère [Seco et al. 2008]. Certains COVs naturels ne sont pas nocifs mais apparaissent dans le calcul des COVs totaux (TCOVs) [Kim, 2010]. Il est important de déterminer et de quantifier les COVs dans la mesure où les TCOVs peuvent être influencés fortement par la présence de composés naturels comme les monoterpènes avec notamment l'α-pinène [Yrieix et al. 2010].

Le formaldéhyde est le composé carbonylé le plus abondant dans l'atmosphère [Müir et Shirazi, 1996]. Ce composé est obtenu à partir de la déshydratation du méthanol et il a été largement introduit dans la formulation de colles pour le bois et les composites entre 1900-1930 [Salthammer et al. 2010]. Les conséquences sur la santé et l'environnement ont été et continuent d'être au centre de nombreuses recherches. C'est un cancérigène humain suspect qui est connu [Kim et Kim 2004, 2005a; Kim et al. 2006]. En France, le forladehyde est classé cancérigène certain (C1) pour l'homme depuis juin 2004 par le CIRC, la commission d'union européenne le place en catégorie 3 : cancérigène probable. Partant de ces considérations, des efforts ont été faits afin de modifier la formulation chimique des colles dans le but de réduire les émissions de formaldéhyde [Kim et al. 2010]. Le formaldéhyde libéré des produits à base de bois a diminué pendant les cinq dernières décennies avec le développement de nouvelles colles et de nouvelles technologies d'assemblage [Roffael 2006]. Certaines d'entre elles consistent à ajouter un produit chimique, comme les acétates polyvinyliques [Kim et Kim, 2005]. Le polyuréthane, le sulfite d'ammonium, et les composés du soufre et du sodium sont aussi employés dans les contreplaqués pour réduire les émissions [Wang et al. 2008; Kunaver et al. 2010]. Une autre façon efficace de réduire les émissions de formaldéhyde se trouve dans l'utilisation de composés comme l'ammoniaque, les sels d'ammonium, l'urée/chlorure d'ammonium, ou les chlorhydrates d'amines [Kim et

al. 2006d]. Ainsi, des poudres de verre et des sels inorganiques ont été employés comme extracteurs de formaldéhyde [Kim et al. 2006d]. L'application de composés naturels tels que l'urée [Uchiyama et al. 2007; Wang et al. 2008; Kunaver et al. 2010], et la vanilline pourraient fournir une approche simple et efficace pour supprimer l'émission de formaldéhyde dans l'environnement intérieur [Uchiyama et al. 2007]. Le Pezzolan, un matériau avec une surface rugueuse, irrégulière et poreuse, a été ajouté à la colle UF pour réduire l'émission de formaldéhyde des panneaux de particules. Ce matériau absorbe les émissions de formaldéhyde et réduit les émissions de TCOV [Kim et al. 2007c].

Les hydrocarbures non saturés comme les terpènes se retrouvent de plus en plus dans l'atmosphère intérieure en raison de leur large présence dans les matériaux de construction qu'ils soient neufs ou recyclés. Les phénomènes en jeu dans l'atmosphère restent un domaine complexe. Les réactions chimiques entre les agents polluants intérieurs et leur impact sur la santé nécessitent encore de nombreuses recherches aussi bien sur le plan chimique que médical [Uhde et Salthammer, 2007].

Les COVs peuvent facilement s'incorporer dans le corps humain et par conséquent, ils entrainent un risque pour la sante même a de basses concentrations si le temps d'exposition est long.

Les COVs comme le benzène, le formaldéhyde, l'acétaldéhyde, le toluène et les xylènes sont des agents polluants particulièrement nocifs dont on essaie de réduire les émissions en priorité. Mais d'autres substances chimiques comme le limonène et l'α-pinène nécessitent aussi une attention particulière car on ne connait pas forcément leurs effets sur la santé et la limite d'exposition [Missia et al. 2010].

Les principales sources d'aldéhydes dans les ménages incluent les matériaux de construction, le bois, les panneaux, les colles, les peintures et les vernis. Dans certains cas, ce sont des produits de réaction avec l'ozone [Weschler et al. 1992].

Le formaldéhyde est libéré par les produits de base utilisés dans la construction, comme les panneaux fabriqués principalement avec des colles urée-formaldéhyde [Kelly et al. 1999].

Les concentrations de COVs montrent une diversité considérable étant donné les multiples sources d'émissions dans l'air intérieur et extérieur, qui peuvent dépendre par exemple des conditions environnementales et climatiques [Missia et al. 2010].

Une alternative est l'utilisation de tanins et de lignine pour la préparation de colles naturelles. Les colles de tanin sont obtenues par le durcissement des polymères de flavonoïdes, particulièrement de tanins condensés [Pizzi, 1994]. Mais, les extractibles du bois jouent un rôle double: ils émettent le formaldéhyde et peuvent agir en tant qu'extracteur de

ce composé [Schäfer et Roffael, 2000]. Pour cette raison, ils doivent être étudiés individuellement.

La lignine provenant de divers processus et d'espèces différentes a été évaluée comme alternative naturelle et efficace dans la colle de bois [Alonso et al. 2005; Khan et al. 2004; Chum et al. 1993a, b]. Ce matériau a une faible réactivité et doit être la plupart du temps traité avant la synthèse de la résine qui sera utilisée comme colle à bois [Doering, 1993; Cetin et Ozmen, 2002]. Ainsi, on a proposé divers traitements [El Mansouri et al. 2006; Alonso et al. 2002]. Toutefois, dans certaines circonstances, la lignine peut donner lieu à la production de formaldéhyde. Le formaldéhyde est ainsi libéré directement des principaux composés du bois [Schäfer et Roffael, 2000].

Schäfer et Roffael [2000] ont publié un travail de recherche très intéressant sur la contribution de quelques composants du bois dans la libération de formaldéhyde en utilisant la méthode du bocal [EN-717-3], avec des températures de 40, 100 et 150 °C pendant 3h. Ainsi, ils ont étudié la cellulose de coton, l'amidon, l'arabinose et la xylose, le glucose et le galactose, la lignine, les acides gras, l'acide abiétique, les extractibles solubles dans l'eau chaude.

Les résultats ont montré que la cellulose soumise à une hydrolyse thermique émet des quantités très faibles de formaldéhyde. La température n'a pas d'influence significative dans les émissions [<6.0 mg/1000 g de fibre de coton]. Un comportement similaire a été observé pour l'amidon sous les mêmes conditions expérimentales [<1.0 mg/1000g d'amidon], et pour les hexosas, glucose et galactose [<5 mg/1000 g d'hexoses].

L'arabinose et la xylose libèrent une plus grande quantité de formaldéhyde [>11 mg/1000g pentoses] que la cellulose et l'amidon aux températures élevées [150°C]. Le procédé thermohydrolytique permet les émissions de formaldéhyde à partir de polysaccharides. Dans des conditions acides, les hexoses se dégradant en oxyméthyl-furfural, lequel se décompose en formaldéhyde et furfural (figure 36).

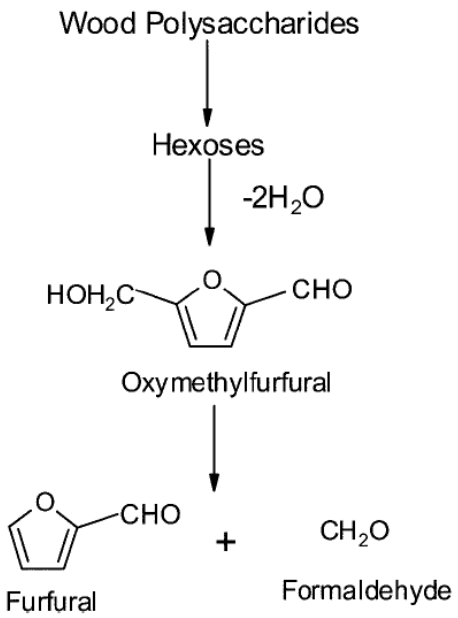


Figure 36: Possible formation de formaldéhyde à partir de les polysaccarides de bois.
(Shäfer et Roffael, 2000).

La composition de la lignine et son traitement sont des facteurs très importants qui influencent la libération de formaldéhyde [Schäfer et Roffael, 2000; Freudenberg et Harder, 1927]. Le lignosulfonate de sodium traité par thermohydrolyse à des températures de l'ordre de 150°C libère du formaldéhyde en quantités très importantes [<38 mg/1000g de lignosulfonate de sodium]. Les voies possibles pour la formation de formaldéhyde à partir de la lignine sont montrées sur la figure 37.

Pour déterminer la participation des extractibles dans les émissions de formaldéhyde, Schäfer et Roffael [2000] ont utilisé du bois avec et sans extractibles. Les résultats ont montré que le bois sans extractibles émet une quantité de formaldéhyde plus importante et la température ainsi que l'espèce de bois jouent un rôle fondamental. Quelques extractibles peuvent agir comme extracteur de formaldéhyde, mais ils peuvent également émettre du formaldéhyde.

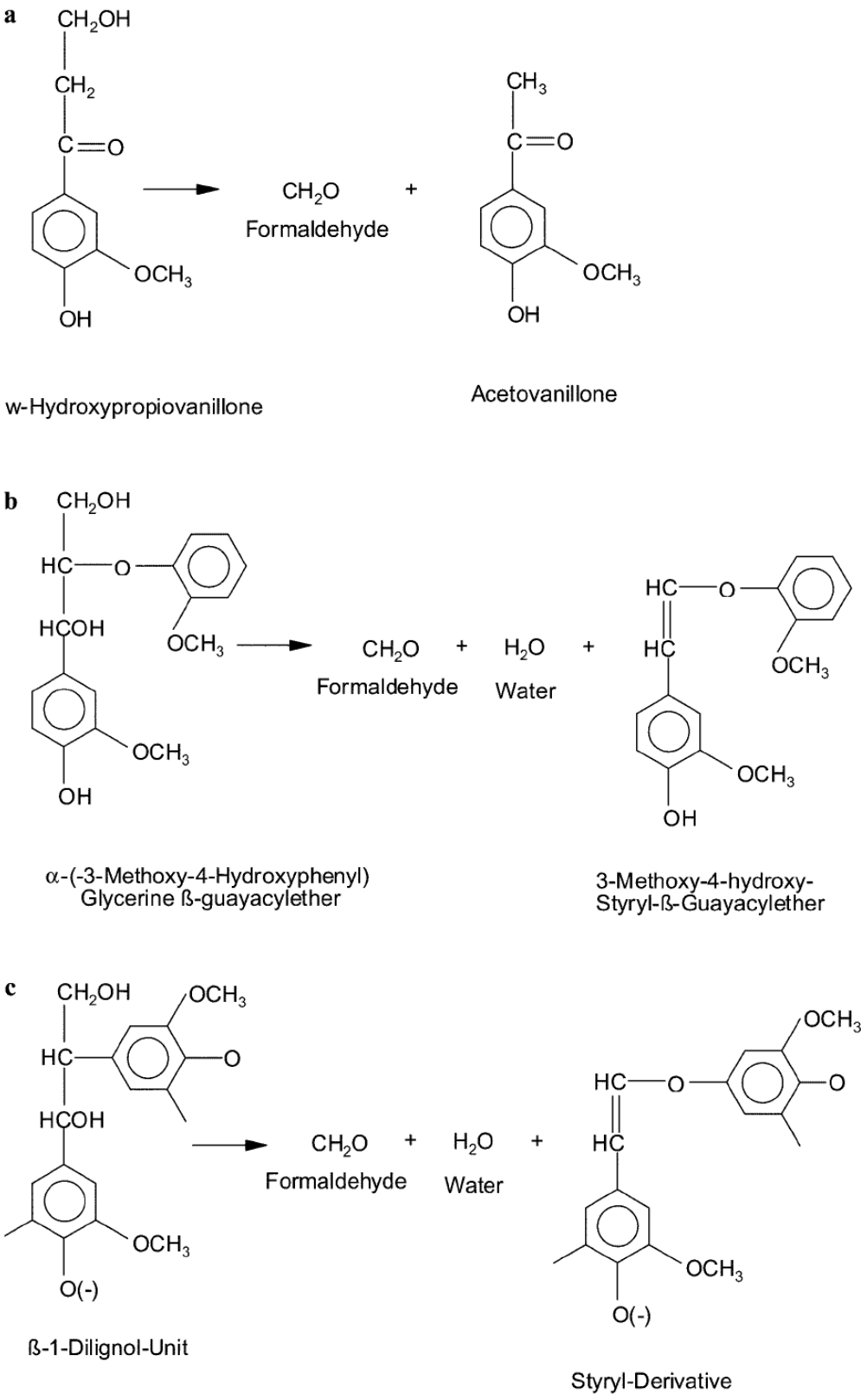


Figure 37: Voies possibles de formation de formaldéhyde à partir de la lignine.
(Shäfer et Roffael, 2000).

II.5.2. **Méthodes pour la détermination d'émissions de formaldéhyde et autres COVs**

Les méthodes typiques pour l'évaluation des émissions de formaldéhyde sont les méthodes dites du dessiccateur, WKI et du perforateur. Il y a aussi la méthode par analyse de gaz. Toutes celles-ci ont en commun l'estimation de la quantité de formaldéhyde émis par spectrométrie [Kim et Kim, 2004]. Le principe pour déterminer la concentration du formaldéhyde se base dans la réaction de Hantzsch d'après laquelle le formaldéhyde réagit avec les ions ammonium et l'acétylcétone pour produire de la diacétyldihydrolutidine [Kim et al. 2006].

Les méthodes utilisées pour la détermination du formaldéhyde et des COVs sont décrites brièvement dans la suite (figure 38).

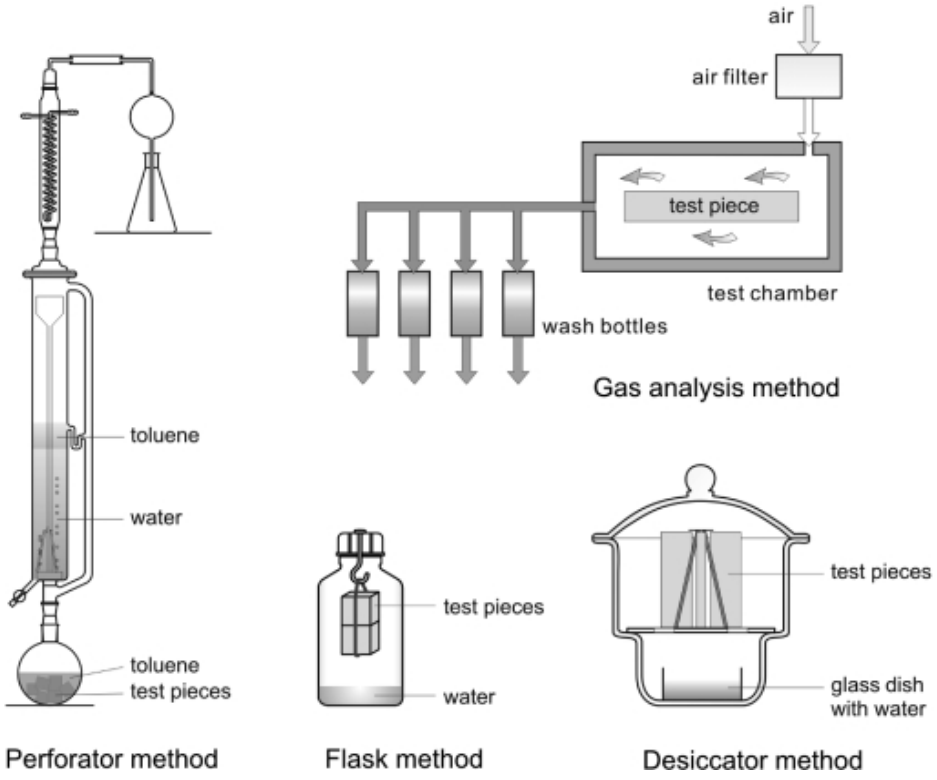


Figure 38: Méthodes standard pour la détermination de formaldéhyde libéré à partir de matériaux à base de bois. (Salthammer, 2010).

II.5.2.a. Méthode dessiccateur (A 5905)

La méthode du dessiccateur, décrite par la norme japonaise JIS (Japanese Industrial Standard A 5905), est utilisée pour mesurer les émissions de formaldéhyde libre. Les émissions de formaldéhyde sont déterminées en plaçant des échantillons de panneaux de surface connue au desus d'eau distillée à l'intérieur d'un dessiccateur, à une température contrôlée pendant

un temps déterminé. L'eau piège le formaldéhyde libéré des échantillons, lequel est analysé en utilisant un spectrophotomètre.

II.5.2.b. Méthode WKI ou bocal (EN 717-3)

La méthode du bocal a été développée par Roffael en 1975. Elle est utilisée pour mesurer les émissions de formaldéhyde libre C'est une méthode très facile à mettre en œuvre qui consiste à placer à l'intérieur d'une bouteille de polyéthylène un à trois échantillons de bois ou de panneaux de bois de dimensions 2.5 cm x 2.5 cm avec l'épaisseur nécessaire pour obtenir une masse totale de 20 g. Les échantillons sont suspendus à 50 cm au-dessus du niveau de l'eau distillée. Les bouteilles sont fermées et sont ensuite portées à une température de 40°c pour un temps déterminé. Le formaldéhyde libéré est absorbé par l'eau. La teneur est déterminée photométriquement à 412 nm par la méthode de l'acétylcétone.

II.5.2.c. Méthode perforateur (EN 120)

La méthode du perforateur a été développée en 1960 par la Fédération Européenne des panneaux de particules. Elle mesure le formaldehyde libre plus le formaldehyde hidrolisé. Elle consiste à couper de petits échantillons de panneaux de bois de masse 110 g. Ensuite, 600 ml de toluène sont placés avec les échantillons dans un ballon et le perforateur est rempli avec 1000 ml d'eau distillée. Pendant 2 h, tandis que le toluène en ébullition passe par l'eau distillée, le formaldéhyde est absorbé par cette eau. La teneur en formaldéhyde est déterminée photométriquement à 412 nm par la méthode de l'acétylcétone comme pour les méthodes précédentes.

II.5.2.d. Méthode de gaz (EN 712-2)

Un échantillon de surface connue est mis dans une chambre fermée à une température de 60°C, sous une certaine humidité, avec une circulation d'air et une pression contrôlées. Le formaldéhyde libéré de l'échantillon se mélange avec l'air dans la chambre. Cet air est extrait de la chambre et passe continuellement à travers les bouteilles de lavage du gaz, contenant l'eau distillée qui absorbe le formaldéhyde libéré. Les bouteilles sont changées chaque heure sur une durée totale de quatre heures. Au terme de l'essai, la concentration du formaldéhyde est déterminée photométriquement. Les valeurs d'émissions sont obtenues sans prendre en compte la première heure.

II.5.2.e. La méthode de la chambre (EN 717-1)

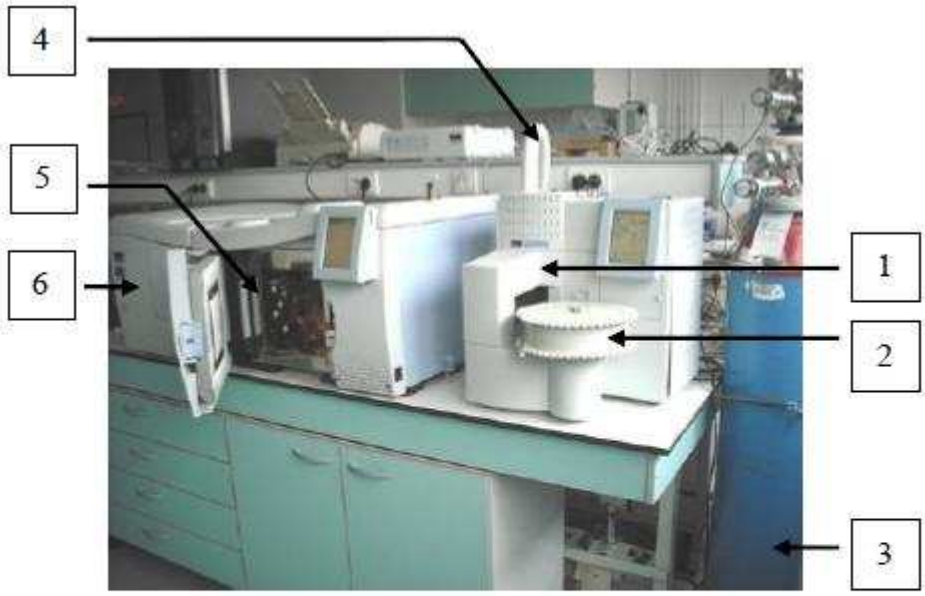
L'émission spécifique du formaldéhyde provenant de produits à base de bois peut être quantifiée dans des conditions contrôlées de température et d'humidité. La méthode de la chambre est décrite par la norme EN 717-1. Il existe différentes tailles de chambres pour lesquelles les paramètres et les conditions opératoires fondamentales ont été fixés : température, surface des échantillons exposés, vitesse et débit d'air dans la chambre.
Les résultats obtenus sont très voisins de la situation réelle, mais cette méthode nécessite des temps d'analyse longs et coûteux. Pour ces raisons, plusieurs pays européens ont mis au point des méthodes d'analyse avec des chambres de volume au plus égal à 1 m^3, les résultats étant corrélés à ceux obtenus dans des grandes chambres.

II.5.2.f. Méthode de la chambre d'essai (NF ISO 16000-9)

Elle détermine la performance environnementale des produits de construction en général en atmosphère intérieure. La température à l'intérieur de la chambre «Climpack» est maintenue à 23 °C (±2°C) et l'humidité à 50 % (±5). Ces conditions climatiques sont imposées par la norme NF EN 16000 – 9. Les échantillons sont placés dans la chambre de prélèvements. Les prélèvements d'air sont réalisés après 3 et 28 jours de conditionnement en chambre sur cartouches DNPH et sur adsorbant Tenax TA pour mesurer les émissions de formaldéhyde (ou autres aldéhydes et cétones) et autres COV par Chromatographie Liquide Haute Perfomance (HPLC) et Chromatographie en phase Gazeuse couplée à une Spectromètre de Gaz (GC-MS).

La méthode GS MS (figure 39) est une technique de détection extrêmement sensible qui permet de déterminer des structures moléculaires. Le spectromètre de masse est souvent couplé avec un système de chromatographie en phase gazeuse.
Les échantillons gazeux prélevés sur des tubes adsorbants sont disposés dans un désorbeur thermique où il est soumis à une température de 330 °C pendant 20 minutes pour volatiliser tous les gaz prélevés au cours de l'essai. Le gaz vecteur utilisé est l'hélium afin d'acheminer les gaz vers un piège froid à -30 °C contenant un support adsorbant. Une fois l'ensemble des gaz désorbé du tube et envoyé dans le piège froid, la température de celui-ci s'élève rapidement à 330°C. La caractérisation et la quantification des les composés organiques volatils sont réalisées par spectrométrie de masse dont le principe est décrit ci-après.

Le composé, organique ou non, introduit dans le spectromètre de masse est ionisé par bombardement électronique. L'ion ainsi obtenu, appelé ion moléculaire, permet la détermination de la masse molaire du composé. Les échantillons prélevés dans des tubes sont placés dans un désorbeur thermique pour les volatiliser et les envoyer dans une colonne chromatographique. Les gaz en sortie de la colonne sont envoyés dans le compartiment d'ionisation du spectromètre de masse.



1. *Injecteur (désorption thermique)*
2. *Porte échantillon*
3. *Bouteille d'hélium : gaz vecteur*
4. *Ligne de transfert*
5. *Colonne dans le four*
6. *Spectromètre de masse*

Figure 39: Système d'analyse chromatographique GC – MS

Au niveau de la source à ionisation électronique, les molécules séparées heurtent un faisceau d'électrons et se trouvent ainsi en excès d'énergie qu'il faut évacuer. Les molécules se fragmentent alors en différents ions caractérisés par un rapport masse/charge que l'appareil filtre et analyse. Les ions sont repoussés vers des lentilles pour focaliser le flux et le diriger vers un quadripôle porté à une certaine tension électrique. Pour une combinaison de potentiels donnée, tous les ions de rapport m/z correspondant peuvent traverser le quadripôle sur toute sa longueur. Pendant un temps extrêmement court, le spectromètre balaie l'ensemble des rapports m/z et comptabilise le nombre de détections de chaque rapport sur un spectre de masse. Chaque composé pur détecté par la spectrométrie de

masse se caractérise par son temps de rétention et possède un ion majoritaire avec un rapport m/z spécifique lequel apparaît dans un spectre de masse. Les pics de chaque composé (masses majoritaires) sont intégrés suivant une méthode d'intégration manuelle. Ensuite, est établi le facteur de réponse de chaque composé pour procéder à la quantification des prélèvements effectués dans la chambre (après étalonnage de l'appareil). Les composés organiques volatiles possibles d'identifier sont montrés dans le tableau 1.

Tableau 1: Composés organiques volatiles et temps de rétention.

Composé	T_R (min)	Intervalle de détection		(m/z)
		Début (min)	Fin (min)	
3 - Méthylpentane	5.45	5	6.5	57
Benzène	7.05	6.5	8	78
Triméthypentane	7.51	6.6	8.1	57
Toluène	10.25	9.6	11.1	91
Ethylbenzène	13.35	12.3	14.3	106
m – xylène	13.62	13.2	14.7	106
o – xylène	14.36	14	15.5	106
α pinène	15.39	15	16.2	93
β pinène	16.73	16.4	17.3	93
Triméthylbenzène	17.32	17	18	105
3-Carène	17.57	17.3	18	93
Limonène	18.70	17.8	19	68
Naphtalène	22.35	22.10	23.6	128

La méthode HPLC (figure 40) permet de séparer des composés en phase liquide. Elle a l'avantage de pouvoir séparer facilement une grande variété de mélanges chimiques.

Elle est constituée de deux circuits indépendants de circulation des liquides : la boucle d'échantillonnage pour l'introduction de la solution à analyser et la boucle de circulation du solvant vecteur (acétonitrile).

En fonction du solvant, il existe une phase stationnaire et une phase inverse. La phase stationnaire dans la colonne est constituée d'un gel de silice sur lequel sont greffées des chaines aliphatiques octadécyl (C18). Lors de la phase inverse, les composés polaires ne sont pas retenus par la phase stationnaire et sortent avant les composés apolaires qui sont adsorbés plus fortement.

Après introduction de l'échantillon, il suffit de basculer l'injecteur pour «connecter» les deux circuits et le solvant entraîne alors l'échantillon dans la colonne.

Il existe plusieurs manières pour la détection quand une substance est passée à travers la colonne. Le plus commun est l'absorption sous rayons ultraviolets.



1. *Injecteur*
2. *Réglage de la température du four où se trouve la colonne.*
3. *Pompe d'entrainement de liquides et de régulation du débit.*
4. *Enceinte chauffée contenant la colonne*
5. *Détecteur UV réglé à 360 nm.*

Figure 40: Système d'analyse HPLC.

Comme dans le cas précédent, la HPLC est alors étalonnée. Les pics de chaque composé sont intégrés suivant une méthode d'intégration manuelle. Ensuite, le facteur de réponse de chaque composé est établi pour procéder à la quantification des prélèvements effectués dans la chambre. Les carbonyles potentiellement identifiables sont montrés dans le tableau 2.

Tableau 2: Composés carbonylés et temps de rétention.

Numéro du pic	Composé carbonylé	Formule chimique	Temps de rétention (min)
1	Formaldéhyde	CH_2O	4.5
2	Acétaldéhyde	C_2H_4O	5.97
3	Acroléine et Acétone	C_3H_4O et C_3H_6O	8.14
4	Propionaldéhyde	C_3H_6O	9.11
5	Crotonaldéhyde	C_4H_6O	11.6
6	Méthacrylaldéhyde	$C_8H_{16}O_4$	12.63
7	2 – Butanone	C_4H_8O	13.17
8	Butyraldéhyde	C_4H_8O	13.67
9	Benzaldéhyde	C_7H_6O	15.79
10	Valéraldéhyde	$C_5H_{10}O$	21.01
11	p – Tolualdéhyde	C_8H_8O	24.08
12	Héxaldéhyde	$C_6H_{12}O$	33

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III RESULTATS

Les 13 publications présentées dans ce travail de thèse comportent des informations qui permettent d'évaluer dans le détail les caractéristiques des différentes colles préparées avec de la lignine, du tanin et du gluten pour lesquelles différentes techniques analytiques ont été utilisées afin d'identifier les propriétés.

Dans le but de mieux présenter les travaux de cette thèse, les publications sont regroupées en quatre sections:

- Colles pour panneaux à base de tanin
- Colles pour panneaux à base de tanin et de lignine
- Colles pour panneaux à base de tanin et de gluten
- Emissions de formaldéhyde et autres COVs

III.1. COLLES POUR PANNEAUX DE BOIS A BASE DE TANIN

III.1.1. **Caractérisation de tannin industriel de pin maritime par 13C RMN et Maldi-tof**

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Les extractibles de tanins polyflavonoïdes industriels sont composés dans leur majorité d'unités répétées de flavan-3-ols, et de plus petites fractions de polysaccharides et sucres simples. Ce sont des matériaux polymères naturels qui peuvent être utilisés pour diverses applications industrielles [Pizzi, 1994], comme par exemple, la fabrication de colles [Pizzi, 1994], de base pour vernis inhibiteur de la corrosion pour les métaux [Rahim et al., 2007], ainsi que d'agents pour le contrôle de la pollution de l'eau [Oo, 2007].

Les pins maritimes sont très répandus dans les forêts mono-essence de la Région du Pays Basque (France), où ils constituent une richesse artificielle énorme. Ceci constitue aussi un grand potentiel de réserve d'écorce à partir de laquelle les tanins commercialement utilisables peuvent être extraits.

La structure de tanins purifiés utilisés dans des applications nutritionnelles ou pharmaceutiques, comme le pycnogenol, sont principalement extraits à l'aide de solvants organiques comme l'éthanol. En outre, les procédés de purification des tanins nécessaires en vue d'une consommation humaine altèrent souvent beaucoup leur composition initiale, leur structure et plus particulièrement leur degré de polymérisation.

La véritable structure des principaux composants monomères du tanin de pin maritime n'est pas réellement connue. De même, son degré de polymérisation n'a pas été clairement défini. Si quelques structures sont modifiées au cours de la purification, celles-ci n'ont pas encore été déterminées et il est nécessaire de définir ses caractéristiques en vue de leur industrialisation.

On a étudié le tanin de pin maritime provenant de la région des Landes, en France. Ce matériau est obtenu par un processus industriel d'extraction avec une solution aqueuse à 2% de bisulfite de sodium et 0.5% de bicarbonate de sodium et de séchage avec un vaporisateur.

Les méthodes utilisées pour la caractérisation des tanins ont été la spectrométrie de masse MALDI-ToF (Matrix-assisted laser desorption/ionization time-of-flight) et ^{13}C-RMN. La première méthode permet d'examiner les oligomères des polyflavonoïdes comme les tanins et de déterminer les aspects de leur structure et de leurs caractéristiques. La deuxième méthode peut indiquer rapidement et simplement les caractéristiques de leur structure et des propriétés potentiellement intéressantes en vue de leur utilisation dans la formulation de colles à bois.

D'après l'analyse par MALDI-ToF, dans le tanin non purifié, les oligomères prédominants correspondent à des unités de monoflavonoïdes, de type dimère de catéchine galate qui a perdu des résidus d'acide gallique et un groupe hydroxyl (528-529 Da), répétées 20-21 fois. Des oligomères de proanthocyanidines formés par catéchine/épicatéchine, épilogalocatéchine et monomères d'épicatéchine galate ont également été trouvées. La présence d'épigalocatéchine galate et d'autres structures a été confirmée par ^{13}C RMN.

De petites proportions d'unités de fisétidine semblent aussi être présentes dans le tanin de pin maritime. Toutefois, l'existence d'une structure de ce type n'a jamais été mentionnée auparavant pour un tanin de pin.

Une structure inconnue, appelée D, a aussi été trouvée. Aucun flavonoïde connu ne correspond à cette nouvelle structure, mais il semble qu'elle participe de façon prédominante dans la formation du tanin de pin maritime.

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MALDI-TOF and ^{13}C NMR characterization of maritime pine industrial tannin extract

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ABSTRACT

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry is a suitable method for examining polyflavonoid tannin oligomers as it is capable to determine aspects of their oligomeric structure and characteristics, which are otherwise too difficult to determine by other techniques. It has been possible to determine by MALDI-TOF for non-purified industrially extracted maritime pine polyflavonoid tannin that: (i) procyanidins oligomers formed by catechin/epicatechin, epigallocatechin and epicatechin gallate monomers are present in the tannin. The presence of epicatechin gallate and of the other structures has been confirmed by ^{13}C NMR; (ii) oligomers up to 20–21 repeating monoflavonoid units in which the repeating unit at 528–529 Da is a catechin gallate dimer that has lost both the gallic acid residues and a hydroxygroup are the predominant species, all procyanidin-related; (iii) oligomers of the two types covalently linked to each other also occur; (iv) small proportion of fisetinidin units also appear to be present in this tannin fisetinidin.

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1. Introduction

Polyflavonoid tannins are natural polyphenolic materials which can be used for a variety of industrial applications (Pizzi, 1994). Industrial polyflavonoid tannin extracts are mostly composed of flavan-3-ols repeating units, and smaller fractions of polysaccharides and simple sugars. Polyflavonoid tannins have been promoted both as wood adhesives (Pizzi, 1994), as the basis of corrosion inhibiting varnishes on metals (Rahim et al., 2007) as well as heavy metal complexating agents for pollution control in water (Oo, 2007).

Maritime pine trees are extensively cultivated for their timber in the basque region of France where they constitute a huge manmade, single-species forest. This also constitutes a huge potential reserve of bark from which commercially usable tannins can be extracted. Recently, awareness of this potentially huge resource has come to the fore with the extraction of a few industrial batches of maritime pine bark tannin extract. While the structure of heavily purified tannin extracts used for nutritional applications or pharmaceutical use, such as for instance pycnogenol, has been described (Jerez et al., 2006, 2007a,b; Weber et al., 2007) these are mostly on laboratory extractions, mostly done with organic solvents such as ethanol, thus rather different of water extractions done in industrial conditions at the level of several tons per batch. Furthermore, the process of purification of tannins to render them suitable for human consumption often alters markedly their initial composition, structure and particularly their degree of polymerisation. Just one such case that has recently come to attention is that of the structure of one of the most studied tannins, a hydrolysable tannin, chestnut wood tannin extract (Pasch and Pizzi, 2002; Pizzi et al., 2009). Industrial maritime pine bark tannin extracts obtained in bulk, and still containing consistant amounts of carbohydrates, as for all commercial flavonoid tannins for leather or for adhesives applications (Pizzi, 1983), are not purified at all and their structures are much closer to reality, no further treatments having been applied capable of somewhat influencing their structure. Thus, the real structures of the main monomers constituting the maritime pine tannin oligomers are not known, and their degree of polymerisation distribution too has not been as a consequence defined. If some structures have been altered in purification this has not been determined either. As different polyflavonoid tannins present different structures, different average molecular mass distribution, and different degrees of polymerisation (Pasch et al., 2001), and nothing of these is known in the case of maritime pine bark tannins, it is necessary to define their characteristics to understand which of the existing

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adhesive technologies can be used for their industrial exploitation.

Since its introduction by Karas and Hillenkamp in 1987 (Karas et al., 1987), matrix-assisted laser desorption/ionization (MALDI) mass spectrometry has greatly expanded the use of mass spectrometry towards large molecules and has revealed itself to be a powerful method for the characterization of both synthetic and natural polymers (Bahr et al., 1992; Ehring et al., 1992; Danis et al., 1992; Danis and Karr, 1993; Pasch and Gores, 1995; Pasch et al., 2001). Fragmentation of analyte molecules upon laser irradiation can be substantially reduced by embedding them in a light absorbing matrix. As a result intact analyte molecules are desorbed and ionized along with the matrix and can be analysed in a mass spectrometer. This soft ionization technique is mostly combined with time-of-flight (TOF) mass analysers. This is so as TOF-MS presents the advantage of being capable to provide a complete mass spectrum per event, for its virtually unlimited mass range, for the small amount of analyte necessary and the relatively low cost of the equipment. Its usefulness in the elucidation of the distribution of different oligomers in the case of polyflavonoid tannins in general has already been demonstrated (Pasch et al., 2001).

This paper investigates the structure of the oligomers present and their distribution as well as the degree of polymerisation of maritime pine polyflavonoid tannins by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry and ^{13}C NMR solid phase spectra.

2. Experimental

2.1. Samples

Maritime pine (*Pinus maritimus*) bark from the Landes region of France was obtained. The bark was dried and ground to coarse chips followed by further drying until a constant weight was obtained. The extraction of tannin from the pine bark was carried out in an industrial reactor of the company Biolandes (Le Sen, France) by total immersion under continuous stirring of the ground bark in a water solution containing 2% sodium bisulphite and 0.5% sodium bicarbonate. The extract so obtained, after spray-drying was used for analysis.

2.2. MALDI-TOF-MS

The spectra were recorded on a KRATOS Kompact MALDI 4 instrument. The irradiation source was a pulsed nitrogen laser with a wavelength of 337 nm. The length of one laser pulse was 3 ns. The measurements were carried out using the following conditions: polarity-positive, flight path-linear, mass-high (20 kV acceleration voltage), 100–150 pulses per spectrum. The delayed extraction technique was used applying delay times of 200–800 ns.

2.3. MALDI-TOF sample preparation

The samples were dissolved in acetone (4 mg/mL). The sample solutions were mixed with an acetone solution (10 mg/mL acetone) of the matrix. As the matrix 2,5-dihydroxy benzoic acid was used. For the enhancement of ion formation NaCl was added to the matrix. The solutions of the sample and the matrix were mixed in equal amounts and 0.5–1 μL of the resulting solution was placed on the MALDI target. After evaporation of the solvent the MALDI target was introduced into the spectrometer.

2.4. Solid state ^{13}C NMR

Solid state CPMAS (cross-polarisation/magic angle spinning) ^{13}C NMR spectra were recorded on a Bruker MSL 300 spectrometer at a frequency of 75.47 MHz. Chemical shifts were calculated relative to TMS. The rotor was spun at 12 kHz on a double-bearing 4 mm Bruker probe. The spectra were acquired with 5 s recycle delays, a 90° pulse of 5 μs and a contact time of 1 ms. The number of transients was 3000. The spectra were run with suppression of spinning sidebands.

3. Results and discussion

Pine tannins are generally almost exclusively composed of procyanidins. Examination of the ^{13}C NMR spectra in Fig. 1 indicates however that variations on the expected standard procyanidin pattern are present in the case of maritime pine. Thus the peak 176 ppm is the indication of the presence of a gallic acid residues linked in C3 to the heterocycle ring of a flavonoid structure. It represents the C=O of a gallic residue linked to catechin or epicatechin gallate. While gallic acid is generally observed at 173–174 ppm (Oo et al., 2008) and the peak at 176 ppm is in the low range of a quinone band, which is generally observed at 176–180 ppm, the considerable width of the band (170–180 ppm) indicates that it is composed of different superposed signals. The band appears to be divided in 170–176 ppm part and in a 177–181 ppm part, the latter belonging to quinone structures due to phenolic hydroxy groups oxidation. The former portion appears to belong to a catechin or epicatechin gallate. This structure has already been observed in procyanidins from other tree species (Oo et al., 2008). While several peaks in the MALDI-TOF spectrum can be interpreted not only by including this structure, but also by other combination of structures, its presence is confirmed by the MALDI-TOF spectra peaks at 966 Da, 1073 Da, 1340.9 Da, 1665 Da, 2158 Da and 2817 Da which cannot be explained otherwise than by the presence of a structure of type C. This is not an unusual structure in itself but it is in pine bark procyanidins. The other bands in Fig. 1 are that at 154 ppm belonging to the flavonoid C5, C7 attached to phenolic –OH groups on the A-ring; the 144 ppm band belonging to the C3′ and C4′; at 130.7 ppm for the C1′; 116 ppm for the C5′ the interflavonoid bond C4–C8 and C4–C6 at 105 ppm and the unreacted C6, C8 and C10 at 97.7 ppm (Waver et al., 2006). The C3 band is at 70.7 ppm: indication that this band could correspond to C3 sites in the chain interior and upper chain-ending positions was given by Lorenz and Preston (2002). Its higher intensity is also due to the superposition of the oligomeric carbohydrates in the industrial extract, the band being rather large. The band at 36.5 belongs to the C4 involved in the interflavonoid bond, indicating that this tannin is quite polymerised (Pizzi and Stephanou, 1993; Waver et al., 2006) and that at 19.6 ppm to the free C4. The bands for C2 are covered by the C3 band but are visible as shoulder of the latter, especially the C2(*cis*) at 81 ppm. The absence of the C4–C6 interflavonoid band at 95 ppm and the presence of the C4–C8 interflavonoid band at 105 ppm seem to indicate that the units are exclusively linked to C4–C8 (Pizzi and Stephanou, 1993; Newman and Porter, 1992), a classical pattern for a procyanidin. The characteristic of the flavonoid B-ring can also be deduced from Fig. 1. Thus, the band at 116.4 ppm is an indication of the proportion of pyrogallol-type B-ring: the greater its intensity the lower the proportion of pyrogallol ring in the tannin. In Fig. 1 the intensity of the band is intermediate indicating that both pyrogallol and catechol B-rings coexist in the structure of maritime pine tannin. Listing of flavonoid bands is reported in a number of publications and can be consulted (Pizzi, 1994).

The MALDI-TOF spectra indicate that the most common monomer constituents are catechin, epicatechin, epigallocatechin and epicatechin gallate with molecular weights (MW), respectively of 290.3 Da, 290.3 Da, 306.3 Da and 442.4 Da (Fig. 1).

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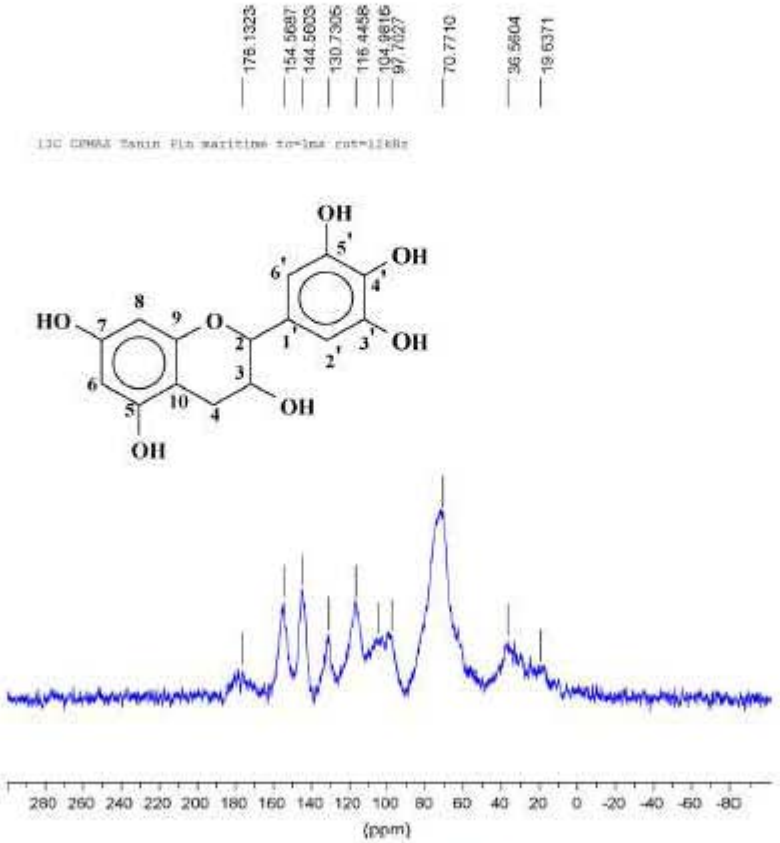
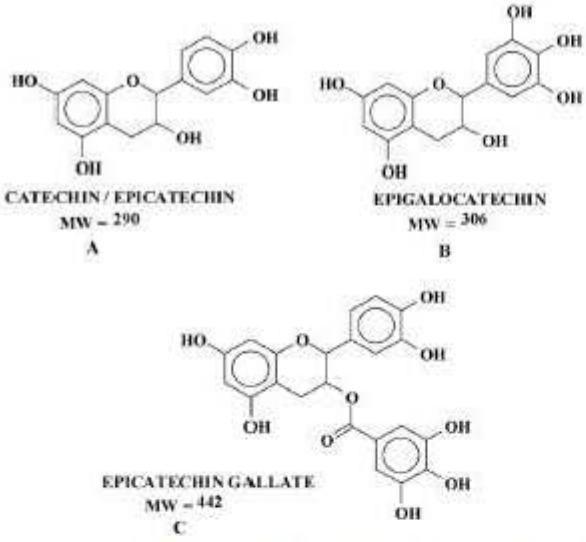
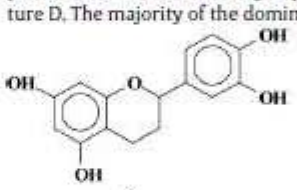


Fig. 1. CP MAS ^{13}C NMR spectrum of maritime pine.



The MALDI-TOF spectra in Fig. 2a and b indicate clearly that alternate repeating units with mass increments of 264–264.9 Da occur, indicating possibly the presence also of other monomers than those shown in Fig. 1 and/or different combinations of various structures.

Combination of the masses of the catechinin monomers can be used to calculate the masses of the oligomer peaks in the spectra in Fig. 2a and b according to the expression $M+Na^+ = 23(Na)+2$ (endgroups, $2 \times H$)+290.3 (−2H)A+306.3 (−2H)B+442.4 (−2H)C (Table 1). The only problem about this is the presence of a repeating structure the MW of which is regular at 264.0–264.9 Da. This unit has not been identified before, and we will call it here structure D. Calculation of the MALDI masses indicates that certain peaks can only be explained by the presence of epicatechin gallate units in which the gallic acid residue has been removed at 274.3 Da (structure E), these being related to the unknown structure. The equation then becomes $M+Na^+ = 23(Na)+2$ (endgroups, $2 \times H$)+290.3 (−2H)A+306.3 (−2H)B+442.4 (−2H)C+274.3 (−2H)E. In Table 1 are shown the results of the combination of monomer units forming the different oligomers observed by MALDI-TOF. It must be noticed that only few of the dominant peaks (Fig. 2) can be explained only on the basis of the catechinic structures A, B and C. Some mass peaks however are not easily explained without the use of structure D. The majority of the dominant peaks are shown in Table 1.



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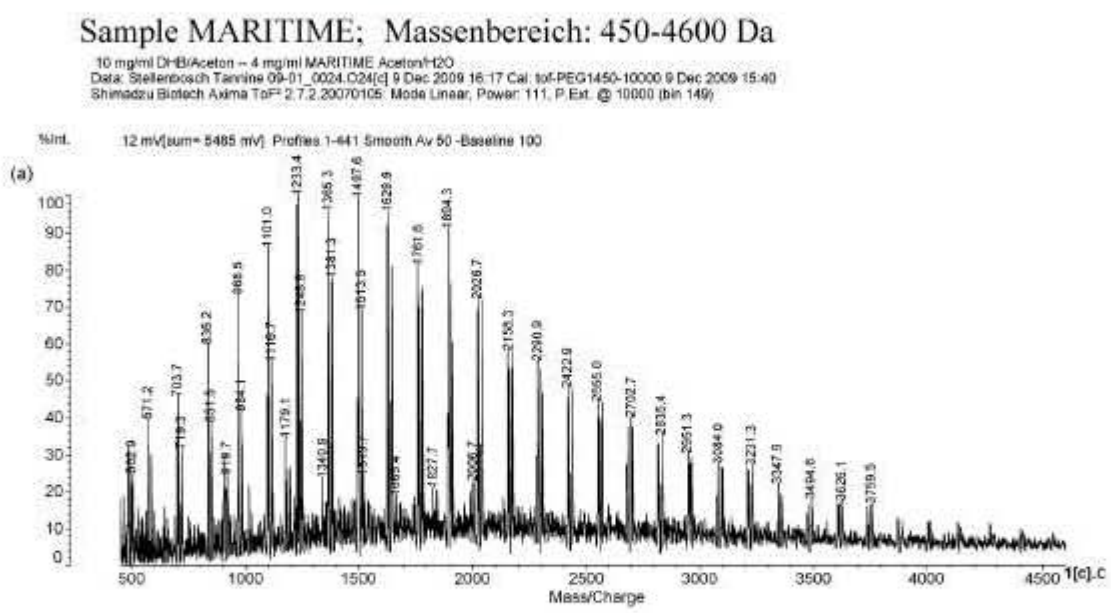


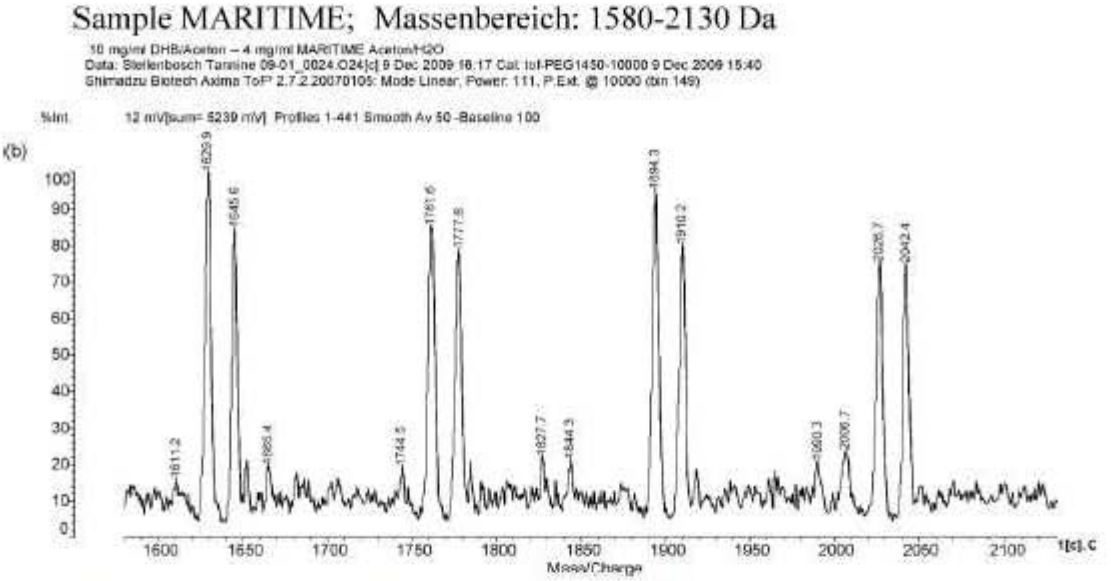
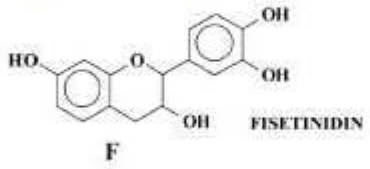


Fig. 2. (a) MALDI-TOF of maritime pine in the 450–4600 Da range and (b) detailed MALDI-TOF of maritime pine in the 1580–2130 Da range.

Alternatively a fisetinidin structure (structure F) could also correspond to the 274.3 mass value although the existence of a structure of type F in a pine tannin is relatively unlikely.



FISETINIDIN

F

Structure D cannot be inserted in the equation simply because no known flavonoid structure can be found with such a molecular weight. However, this structure participates markedly. From peak intensities in Fig. 2 its participation being predominant to the formation of the maritime pine tannin oligomers. Two series of the most intense MALDI mass peaks rely on the repetition of this 264 Da structure (see Table 2). These are separated by the 264 Da motive recurring several times. Table 2 shows the interpretation of the two predominant 264 Da series present. In the detail of the MALDI spectra in Fig. 2b the double peaks are simply due to the difference of the presence or absence of one –OH group (Fig. 2b).

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Table 1
MALDI fragmentation peaks for mixed maritime pine bark tannin extract. Note that repeat units in this tannin indicate that this tannin is a mixed prorobinetinidin/profisetinidin/procyanidin.

M+Na⁺ (exp.) Da	M+Na⁺ (calc.)	Peak intensity (%)	Unit type				
			A 290.3	B 306.3	C 442.4	D 264/265	E (C-gallic) or F 274.3
836.2	841.9	60	–	–	–	–	3
919.7	921.9	.30	1	2	–	–	–
966.5	967.7	73	–	–	1	1(−2 × O)	1
1073.4	1074		–	2	1	–	–
1101.0[a]	1094.1 or	85	1	–	2	–	–
	1098.6		–	–	–	2	2
1179.1	1178.2	34	4	–	–	–	–
1233.4[a]	1226.2	100	1	3	–	–	–
1248.4	1242.2	68	–	4			
1340.9	1346.2 or	24	–	–	3		
	1346.3 or		2	1	1		
	1346.3		–	2	1	–	1
1365.3[a]	1362.3 or	95	1	2	1		
	1362.3		–	–	–	2	3
1381.5	1378.3	76	–	3	1		
1497.6	1498	98	1	3	–	1	–
1629	1629	93	–	–	–	3	3
1665.4	1666.3	17	1	3	1		
1761.6	1762	82	1	3	–	2	–
1894.3[a]	1890.9 or		4	–	1	–	1
	1894	89	–	–	–	4	3
2026.7	2026	72	1	3	–	3	–
2158.3	2158	58	4	–	1	1	–
2290.9	2290	56	1	3		4	–
2555.0	2556.2 or	45	–	6	1	1	
	2554		1	3	–	5	–
2817.2	2819.7 or	35	3	2	3		
	2819.6		1	1	5		
2951.3	2940.0 or	32	–	6	–	–	4
	2940.0		2	5	–	–	3
3084	3082	28	1	3	–	7	–
	3076.1 or		4	3	1	–	2
	3076.0		2	2	3	–	2
	3076.0		–	3	3	–	3
3347.9	3346	24	1	3	–	8	–

[a] Dominant fragment.

It is of interest to find out what structure corresponds to 264 Da. No known monoflavonoid corresponds to such a structure. If one takes structure E however, for a E repeating unit at 272.3 Da, the difference with 264 Da is always of 8 Da, that does not correspond to the mass of any leaving functional group. However, if one considers that there is an –OH group less in a dimer formed by two joined E structures this will give the loss of 16 Da, hence of an oxygen. It means that the repeating unit of the system is not 264 Da but appears to be 264 × 2 = 528 Da. Thus, the repeating unit is a dimer of structure E with a –OH group missing. The unit has a single phenolic –OH group which has been lost, as shown in the structure, the alcoholic –OH groups in C3 having already been lost at the separation of the gallic acid.

Table 2
Subseries in D in the oligomers pattern of maritime pine tannin.

M+Na⁺ (exp.) Da	M+Na⁺ (calc.)	Unit type				
		A 290.3	B 306.3	C 442.4	D 264/265	E (C-gallic) or Basic F 274.3
1233.4	1226.2	1	3	–	–	–
1497.6	1498	1	3	–	1	–
1761.6	1762	1	3	–	2	–
2026.7	2026	1	3	–	3	–
2290.9	2090	1	3		4	–
2555.0	2554	1	3	–	5	–
3084	3082	1	3	–	7	–
3347.9	3346	1	3	–	8	–
571.2	571.6	–	–	–	–	2
836.2	841.9	–	–	–	–	3
1101.0	1098.6 or	–	–	–	2	2
		–	–	–	1	3
1365.3	1362.3	–	–	–	2	3
1629	1629	–	–	–	3	3
1894.3	1894	–	–	–	4	3
2158.3					5	3
2422.9					6	3
2951.3					8	3

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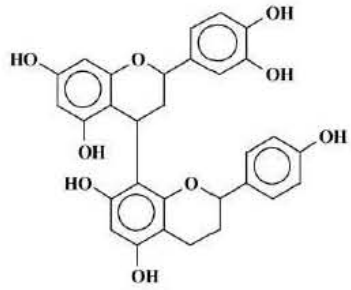

Dimer structures such as F–E and E–E have also been considered but none of these corresponds to the period of 528 Da separating the various oligomers of the MALDI-TOF spectra. Both F–E and E–E would have periods of 548 Da, which is not what is observed.

Thus, in Table 2 is shown the main series of dominant MALDI masses indicating that oligomers of this unit appear to occur in maritime pine tannins. This is the most likely case seeing the regular progression to bigger oligomers in Tables 1 and 2. Thus, mixed oligomers where a procyanidin oligomer formed by structures of types A, B and C (1363.6 Da) is linked to progressively increasing number of D structure oligomers are possible. The results in Table 2 of the second most important series of recurrent MALDI peaks clearly confirm that mixed procyanidin and D oligomers covalently linked do exist in pine tannins because none of the masses of the series in Table 2 can be explained without having units of structures A, B and C linked to the D oligomers (first series where up to 8 units at 264 Da are linked to tetramers of structures A and B yielding up to dodecamers). It appears most likely then that both pure oligomers of the two types as well as linked mixed oligomers do coexist in this tannin. The possibility that maritime pine tannin, while being mainly a procyanidin as other pine tannin types presents also some units of fisetinidin in its structure (structure F) are indeed remote as the NMR results confirm the existence of structure C and thus of its decomposition product, structure E. Equally important is to discuss the second series of peaks in Table 2. Here to three E structures are linked up to 8 264 Da units, up to undecamers. Moreover, observing the spectra in Fig. 2a it is possible to see that the 264 Da pattern continues to form even higher oligomers, although these are in much lower relative proportion (between 6% and 10% relative peak intensities). Thus, peaks corresponding to oligomers in which up to 9 more 264 Da units are linked, thus 17 repeating units at 264 Da linked to trimers or tetramers of the other types of unit, it is possible to reach oligomers containing up to 20–21 repeating units. Such higher molecular mass tannins would have a very fast gel time, they would autocondense relatively easily, and they might have problems of solubility or early precipitation.

In conclusion the maritime pine tannin is formed exclusively of monomer of different types but all presenting a phloroglucinol-type A-ring, indicating that these are almost pure procyanidins with some segments in the form of prodelphinidins bound to the procyanidin. To these are mixed a minority of units of type C linked to short oligomers composed of A and B units. High molecular mass tanins, in appearance formed by 20–21 linked flavonoid units have also been observed.

It must also be noticed that the original thought that marked purification for materials for pharmaceutical and nutritional applications can alter some aspects of the structure of the originally roughly extracted tannin, which appears to be correct (Pasch and Pizzi, 2002; Pizzi et al., 2009). Thus, the presence of structures of type C and of type D has not been observed in purified ethanol extracted materials in relation to the present water-based, rough industrial extractions. The present work supports the presence of gallate esters already found by other authors (Weber et al.) in laboratory water extracted maritime pine bark tannins, with the highest oligomers up to dodecamers. It also defines in detail the combination of monomers present in each oligomers composing the industrial water-based extract.

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III.1.2. **Caracterisation de deux tanins de pin maritime pour leur utilisation comme colle de bois**

Journal of Adhesion Science and Technology. Soumis, le 07 d'Octobre 2011.

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Dans ce travail, on a utilisé du tanin de pin maritime provenant de deux processus industriels: l'un est fourni par l'entreprise BIOLANDES et le deuxième par DRT (Dérivés Résiniques & Terpéniques).

Le processus d'extraction par immersion de l'écorce dans une solution d'eau contenant 2% de bisulfite de sodium et 0.5% de bicarbonate de sodium a été effectué. Le tanin BIOLANDES a été séché dans un séchoir spray et le tanin DRT a été purifié avant de subir ce même traitement.

Les tanins DRT et BIOLANDES ont été caractérisés par spectrométrie MALDI-ToF (Matrix-assisted laser desorption/ionization time-of-flight) et ^{13}C-RMN.

La réactivité a été évaluée d'après le temps gel mesuré sous différentes conditions de pH et en présence de divers durcisseurs. Finalement grâce à des analyses thermomécaniques (TMA) et de cohésion interne (IB), on a déterminé le comportement de deux préparations de colle à base des deux tanins.

Dans le tanin DRT, on a trouvé des oligomères de proanthocyanidines formés par catéchine/épicatéchine, épilogalocatéchine et des monomères d'épicatéchine galate. La présence d'épigalocatéchine galate et d'autres structures a été confirmée par les analyses de ^{13}C RMN.

De petites proportions d'unités de fisétidine semblent aussi être présentes dans le tanin de pin maritime. Toutefois, une telle structure n'a jamais été isolée auparavant pour ce type de matériau. Une structure inconnue, appelée D, a également été trouvée. Cela recoupe les observations précédemment effectuées pour le tanin BIOLANDES.

Les analyses de ^{13}C-RMN ont montré que le tanin DRT a une réactivité plus importante que le tanin BIOLANDES. A 98 ppm, les carbones C6 et C8 n'ayant pas réagi apparaissent. L'intensité de C6 est plus élevée dans le tanin DRT que dans le BIOLANDES ce qui révèle une réactivité plus importante.

Les panneaux préparés avec la colle au tanin DRT présentent toujours des valeurs plus importantes de cohésion interne. Un panneau pressé à partir d'une formulation à base de tanin DRT et de glyoxal utilisé comme durcisseur et dont la teneur en eau a été augmentée (24%) a une valeur d'IB de 0.51 (MPa).

CHARACTERIZATION OF TWO MARITIME PINE TANNINS AS WOOD ADHESIVES

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ABSTRACT

Maritime pine tannins from the two suppliers BIOLANDES and DRT were used in combination with paraformaldehyde (5%), hexamine (6%) and glyoxal (9%) as hardener in the preparation of adhesives for wood based panels. A high reactivity has been checked by gel time measurements and the rat of viscosity increase. Already the BIOLANDES and the DRT tannin alone registered an internal bond (IB) of 0.4 (MPa). The BIOLANDES tannin with paraformaldehyde could reach the European standard EN 312. In general, DRT tannin obtained the best mechanical results. However, the pH and the moisture content play a fundamental roll for the final IB. Thus, DRT tannin with glyoxal as hardener at high pH and moisture content reached an IB of 0.51 (MPa). The mechanical resistance differences between BIOLANDES and DRT tannin were confirmed by the ^{13}C NMR spectra. DRT tannin presents a lower polymerization degree than BIOLANDES, and more reactive sites are available.

Key words: Tannins, structures, autocondensation, internal bond, mass spectrometry, MALDI-TOF, ^{13}C NMR spectra.

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1. INTRODUCTION

Tannins are polyphenolic components used by the plants to defend themselves from insects, fungi and animals (Robbins et al. 1987). Historically, they have been associated with the preservation of hides to leather (Van Langenberg et al. 2010).

Two types of tannins exist in nature: hydrolysable and condensed tannins. The last ones are the most interesting economically for adhesives and resins preparation (Pizzi, 2003), and for the production of wood based panels (Tondi and Pizzi, 2009; Lei et al. 2008; Pichelin et al. 2006; Pizzi, 2003, 2006).

The condensed tannins are soluble in water; they are constituted mainly of flavan 3-ols and flavanes-3,4-diols (Drewes and Roux, 1963; Roux and Paulus, 1961; Bahr at al. 1992), smaller fractions of polysaccharides and of simple sugars (Garcia, 1997). Two types of phenolic rings having different reactivity with formaldehyde are presented in each flavan 3-ol repeating unit: A-ring and B-ring (Pasch et al. 2001). They contain a high number of phenolic hydroxyls, and their structure characteristics lend themselves to the stabilization of radicals not only by mechanisms characteristic for all phenols but also by the existence of side reactions, such as heterocycle pyran ring opening and others (Noferi et al. 1997). Four types of repeating units predominate in the various tannin types (Pizzi and Stephanou, 1993). Although such differences in structure appear to be minor, they induce rather well-defined and marked characteristics behaviour to the tannin (Masson et al. 1997).

Condensed tannins have been used since 1970s (Pizzi, 1983); various tannins and hardeners have been investigated in order replace phenol and to decrease or eliminate the formaldehyde release from wood base panel using others hardener. In order to promote the use of tannins, two lines of investigation have been pursued: the use of hardener without formaldehyde emission (Pizzi, 2006); and the tannins autocondensation (Pizzi et al. 1995; Pizzi, 2006). Methylolated nitroparaffins (trishydroxymetyl nitromethane TN), hexamethylentetramine (hexamine), and glyoxal (Lei et al. 2008; Ballerini et al. 2005; Trosa et al. 2001) are only some hardeners used in the wood tannin adhesives preparation. Methylolated nitroparaffins function well as hardener of a variety of tannin-based adhesives while affording considerable side advantages to the adhesive and to the bonded wood joint (Trosa and Pizzi, 2001); their use is associated with a marked reduction in formaldehyde emission from bonded wood panels with heated wood material as only source for formaldehyde emission (Trosa and Pizzi, 2001; Pizzi, 2006). Main disadvantage of this

hardener is its high costs. A cheaper alternative is hexamethylentetramine (Pizzi, 2006; Pichelin et al. 2006). In the presence of fast-reacting species, hexamine is not a formaldehyde-yielding compound. It neither decomposes to formaldehyde and ammonia in acid environment nor to formaldehyde and trimethylamine in an alkaline environment (Pizzi and Tekely 1995, 1996; Pizzi et al. 1996; Kamoun et al. 2003). Hence, the use of hexamine as a hardener is a very environmentally-friendly proposition (Pizzi, 2006). The emission measured in a large chamber test (EPA, 1999) has been shown to be so low to be limited exclusively to that generated by the heating of wood itself (Pizzi, 2006), and now this hardener has been accepted by JIS A5908 as not being a formaldehyde source in presence of polyflavonoid condensed tannin. Glyoxal is a non-volatile non-toxic aldehyde that has been tested in lignin (El Mansouri et al. 2007; Lorenz et al., 2006), tannin (Ballerini et al. 2005) and other adhesives as soy (Amaral, 2008) for application to wood panels such as particleboards. Glyoxal as hardener has been used not only for tannin, but also for lignin (Lei et al. 2008; El Mansouri, et al. 2007). Glyoxal presents several advantages compared to formaldehyde; it is readily biodegradable and quickly transformed enzymatically by bacteria and fungi. Glyoxal is a non-toxic (LD50 rat $\geq$ 2960 mg/kg; LD50 mouse $\geq$ 1280 mg/kg, NIOSH, 2000), non-volatile aldehyde; but it is less reactive than formaldehyde which is toxic (LD50 rat $\geq$ 100 mg/kg; LD50 mouse $\geq$ 42 mg/kg, NTIS, 2005), and as a consequence, no noxious gas emission either during board manufacturing or service occurs (Ballerini et al. 2005).

Hardening by autocondensation is a reaction characteristic for polyflavonoid tannins, and it has been used to prepare adhesive polycondensates (Meikleham et al. 1994). In the case of pine tannin with its content of procyanidin and prodelphinidin, cellulose catalysis is more than enough to cause hardening and to produce boards of strengths satisfying the relevant standards for interior grade panels (Meikleham et al. 1994).

Thus, in this work, we used maritime pine tannin obtained from two processes and two suppliers. Paraformaldehyde, hexamine, and glyoxal were used as hardeners; also hardening by autocondensation was studied in order to confirm the performance of the panel manufactured under these conditions.

2. EXPERIMENTAL

Maritime pine (*Pinus maritimus*) bark from the Landes region of France was dried and ground to coarse chips followed by further drying until a constant weight was obtained. Two different modes of preparation of the maritime pine tannin were used. One extraction was

performed in an industrial reactor at BIOLANDES (Le Sen, France) by total immersion under continuous stirring of the ground bark in a water solution containing 2% sodium bisulphite and 0.5% sodium bicarbonate. The extract so obtained was spray-dried and used without further treatment. The second type of tannin was obtained from DRT (Dérivés Résiniques & Terpéniques, France); this tannin was prepared under the same conditions, but was purified in an additional step.

2.1. MALDI-TOF-MS

Since its introduction by Karas and Hillenkamp in 1987 (38), matrix-assisted laser desorption/ionization (MALDI) mass spectrometry has greatly expanded the use of mass spectrometry towards large molecules and has revealed itself to be a powerful method for the characterization of both synthetic and natural polymers (39-44). Fragmentation of analyte molecules upon laser irradiation can be substantially reduced by embedding them in a light absorbing matrix. As a result intact analyte molecules are desorbed and ionized along with the matrix and can be analysed in a mass spectrometer. This soft ionization technique is mostly combined with time-of-flight (TOF) mass analysers. This is so as TOF-MS presents the advantage of being capable to provide a complete mass spectrum per event, for its virtually unlimited mass range, for the small amount of analyte necessary and the relatively low cost of the equipment.

The spectra were recorded on a KRATOS Kompact MALDI 4 instrument. The irradiation source was a pulsed nitrogen laser with a wavelength of 337 nm. The length of one laser pulse was 3 ns. The measurements were carried out using the following conditions: polarity: positive, flight path: linear, mass: high (20 kV acceleration voltage), 100–150 pulses per spectrum. The delayed extraction technique was used applying delay times of 200–800 ns.

The samples were dissolved in acetone (4 mg/mL). The sample solutions were mixed with an acetone solution (10mg/mL acetone) of the matrix (2,5-dihydroxy benzoic acid). For the enhancement of ion formation NaCl was added to the matrix. The solutions of the sample and of the matrix were mixed in equal amounts and 0.5–1 µL of the resulting solution was placed on the MALDI target. After evaporation of the solvent the MALDI target was introduced into the spectrometer.

2.2. Solid state ^{13}C NMR

The BIOLANDES and DRT tannin were prepared for Solid state CP MAS (cross-polarisation/magic angle spinning) ^{13}C NMR spectra analysys. The tests were recorded on a Bruker MSL 300 spectrometer at a frequency of 75.47 MHz. Chemical shifts were calculated relative to TMS. The rotor was spun at 12 kHz on a double-bearing 4 mm Bruker probe. The spectra were acquired with 5 s recycle delays, a 90° pulse of 5 s and a contact time of 1 ms. The number of transients was 3000. The spectra were run with suppression of spinning sidebands.

2.3. Gel time and viscosity

Gelation is defined as the point at which the resin ceases to be a viscous liquid and becomes a soft, elastic, rubbery solid (Pizzi and Stephanou, 1993). Triplicate samples from each adhesive system resins were prepared. Gel time tests were conducted at 100° C. Five (g) of the adhesive mix were weighed in a glass test tube, and then placed in a boiling water bath. The wire spring was manually moved rapidly upwards and downwards, and the time for gelation was measured. The viscosity was determined in a Brookfield viscometer at 20 rpm using spindle N 27 and 25 °C.

The tannin solution in water was prepared at 45% concentration and its pH adjusted with 33% water solution of NaOH. The pHs of each formulation are indicated in the table 3. The tannin solution then was mixed with the various hardeners: paraformaldehyde, hexamine or glyoxal in natural pH tannin (4.5 and 3.5 for BIOLANDES and DRT, respectively) and the maximal pH possible to increase. Increase the pH, the viscosity of the tannin solution increased considerably to try to increase the pH (Pizzi et al. 1995; Pizzi, 2006). Paraformaldehyde was used to determine the reactivity of the tannin solution as a control. It was used in solid state in a 5% w/w of tannin.

The hexamine was used in a 6% (on solids tannin base contain) as a 30% hexamine solution in water. In general, the hexamine solution is used in mixes with the tannin solutions adjusted to pH = 10 in according to the best perform at such a pH (Hillis and Urbach 1959; Pichelin et al. 2006; Theis and Grohe, 2002). However with our tannins, the maximal pH used were 6.5 and 5.3 for BIOLANDES and DRT tannin with all the hardeners, due they increase the viscosity easily.

With respect to glyoxal, this was used in a 9% (on solids tannin base contain) as a 40% glyoxal solution in water.

2.4. Thermomechanical analysis (TMA)

The hardening reaction of a single resin system or mixtures can be evaluated by TMA, by monitoring the rigidity of a bonded wood joint as a function of temperature. Thus, tannin adhesives systems were analysed by TMA. All experiments were conducted under the same conditions: heating rate = 10 °C / min, application rate of the adhesive 30 mg, in the temperature range 25-250 °C.

Triplicate samples of beech wood alone, and of two beech wood plies each 0.6 mm thick bonded with each adhesive system, for sample dimensions of 21 mm × 5 mm × 1.2 mm in three-point bending on a span of 18 mm. A force varying continuously between 0.1 N, 0.5 N and back to 0.1 N was applied on the specimens with each force cycle of 12 s (6 s/6 s). The classical mechanics relation between force and deflection $E = [L^3/(4bh^3)][\Delta F/(\Delta f)]$ (where L is the sample length, b and h are the sample width and thickness, ΔF is the variation of the force applied, and Δf is the deflection obtained) allows the calculation of the modulus of elasticity (MOE) E for each case tested. The thermomechanical analyzer used was a Mettler Toledo TMA40. The software used for data treatment was STARe. The MOE of the wood joints bonded with different resin systems gave a good indication of the final strength of the adhesive system tested.

2.5. Wood panels manufacture and internal bond

Duplicate one-layer laboratory particleboards of dimensions 350 x 300 x 14 mm were prepared using a mixture of core particles of beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*) woods. The wood particles before resin addition had a moisture content of 2%. The resin solids loading on dry wood were maintained at 10%. The operational conditions are composed of three stages of different pressure applied to the panel: 3.5 MPa, 1.2 MPa, and 0.7 MPa. The total press time as 7.5 minute, and the press temperature 195° C. After sanding by hand the panels were cut into specimens of 50 × 50 × 13 mm. A total of 10 samples from each panel were obtained. All particleboards were tested for dry internal bond (IB) strength in agreement with the EN 319 standard.

3. RESULTS AND DISCUSSION

3.1. Solid state ^{13}C NMR

In the figure 1 is showed a typical tannin structure. The ^{13}C NMR examination are indicated the in Figures 2 and 3. They show very similar structures in both tannins.

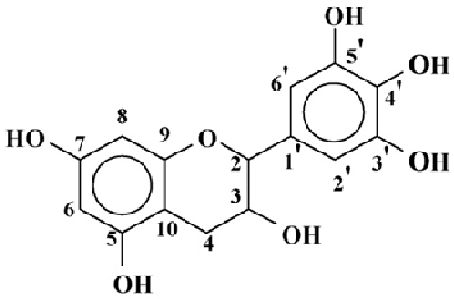

Figure 1: Tannin structure.

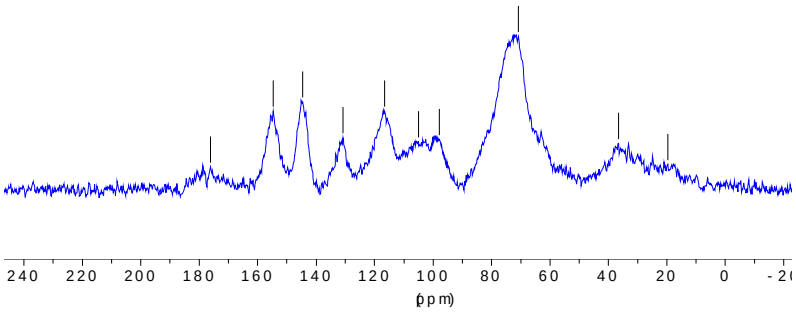

Figure 2: CP MAS ^{13}C NMR spectrum of BIOLANDES maritime pine.

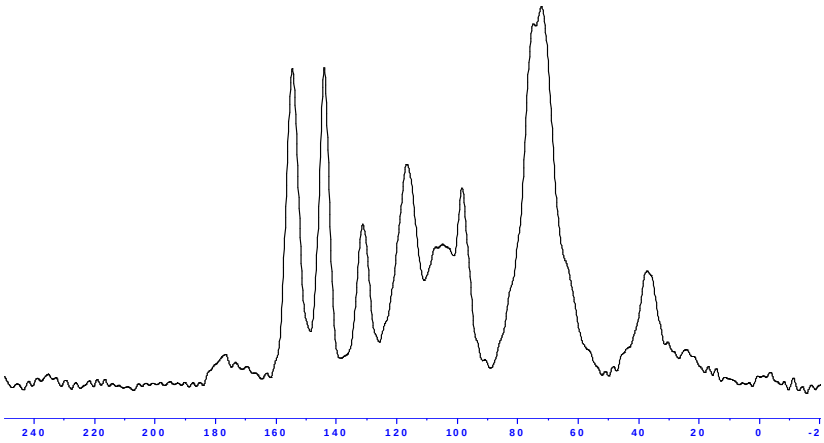

Figure 3: CP MAS ^{13}C NMR spectrum of DRT maritime pine.

The differences between them are present in the intensity of some peaks. The gallic acid residue is observed at approx. 176 ppm, represented by its C=O group and linked in C3 to the heterocyclic ring of the flavonoid structure (catechin or epicatechin). While gallic acid is generally observed at 173-174 ppm (Oo, et al., 2008), and the peak at 176 ppm is in the low range of a quinone band, the considerable width of the band (170 - 180 ppm) indicates that it is composed of different superposed signals.

It is interesting to notice always higher intensities in the DRT tannin in the various bands at 154-155 ppm, 144 ppm, 131 ppm, 116 ppm, 105 ppm, and 98 ppm and in the band between 60 and 80 ppm compared to the BIOLANDES tannin. The peak at 154 ppm belongs to the flavonoid C5, C7 attached to phenolic –OH groups on the A-ring. In this region we found C9 band at 155 ppm. The higher intensity in DRT tannin is a consequence of a higher proportion of non opened heterocyclic ring structured. This is an indication of very little or no opening of their heterocyclic ring (Pizzi, 1994), as a consequence of the extraction cleaves in the interflavonoid link in procyanidin-prodelphinidin tannins preferentially (Hemingway et al. 1985; Hemingway and McGraw, 1983). At 144.11 ppm belongs to C3’ and C4’. The 131.34 ppm band belongs to C1. At 131 ppm it found C1 and 116 ppm belongs to C5’ and C2’ from B ring. The B-ring reaction with formaldehyde is favoured in tannins where the pyrogallol units are present (Pizzi 1994). The band at 105 ppm belongs to C4-C8 and C4-C6 and the unreacted C6, C8 and C10 at 97.7-98.57 ppm (Waver et al. 2006). The higher intensity of free C6 (98.54 ppm) in DRT tannin indicates that this tannin has a lower polymerization degree than BIOLANDES tannin, and more reactive sites are available. The C6 free proportion would indicate furthermore the predominance of C4-C8 interflavonoides linkage but that absence of C4-C6 at 95 ppm seem to indicate that the units are exclusively linked to C4-C8. The

region between 90 and 60 ppm mask the C2 (75 ppm) and C3 (72.25 ppm). Their high intensity is also due to the superposition of oligomeric carbohydrates in the industrial extract, the band being rather large (Pizzi, 1994). The peak at 37 ppm belongs to the C4 involved in the interflavonoid band, indicating that both tannins are quite polymerized (Pizzi and Stephanou, 1993; Waver et al. 2006).

3.2. MALDI-TOF-MS

The MALDI-TOF spectra indicate that the most common monomeric constituents in DRT and BIOLANDES tannins are similar. These constituents are catechin, epicatechin, epigallocatechin and epicatechin gallate, with molar masses of 290.3 Da, 290.3 Da, 306.3 Da and 442 Da (Figure 4). The figures 5 and 6 show repeating units with mass increments of 264-264.9 Da. It could indicate the possible presence of other monomers and/or different combinations of various structures (Oo et al. 2008).

To calculate the oligomeric masses in both tannins, the same expression as follows was used:

M + Na = 23*(Na) + 2 (end groups, 2xH) + 290.3 (-2H)*A + 306.3 (-2H)*B + 442.4 (-2H)*C (Oo et al. 2008). The structures of each monomer that compose the expressions are shown in the figure 4.

The problem is the presence of a repeating unit of the molar mass of 264.0–264.9 Da. This unit has not been identified before, and it was called structure D (Oo et al. 2008). Calculation of the MALDI masses indicates that certain peaks can only be explained by the presence of epicatechin gallate units in which the gallic acid residue has been removed (Figure 7), hence the molar mass is 274.3 Da (structure E), these being related to the unknown structure. The equation then becomes M + Na = 23(Na) + 2 (end groups, 2×H) + 290.3 (−2H)*A + 306.3 (−2H)*B + 442.4 (−2H)*C + 274.3 (−2H)*E (Oo et al. 2008).

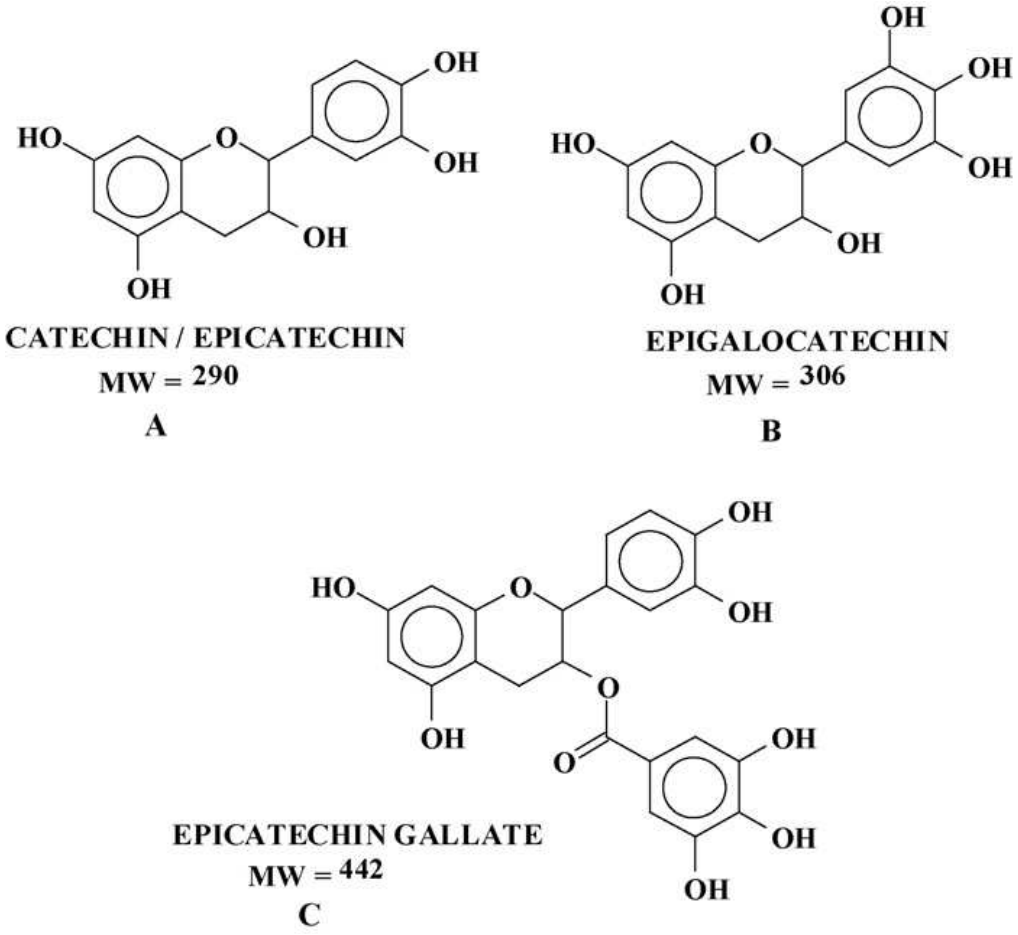

Figure 4: Monomers constituents of BIOLANDE and DRT tannin.

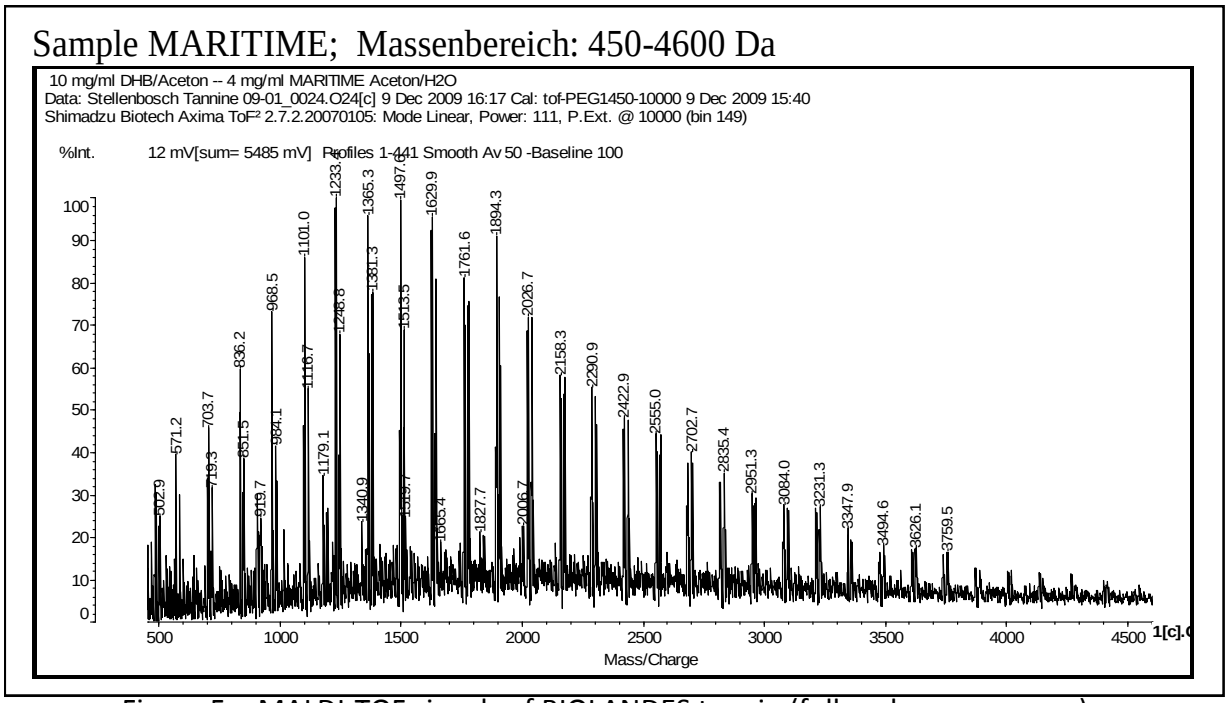

Figure 5a: MALDI-TOF signals of BIOLANDES tannin (full molar mass range).

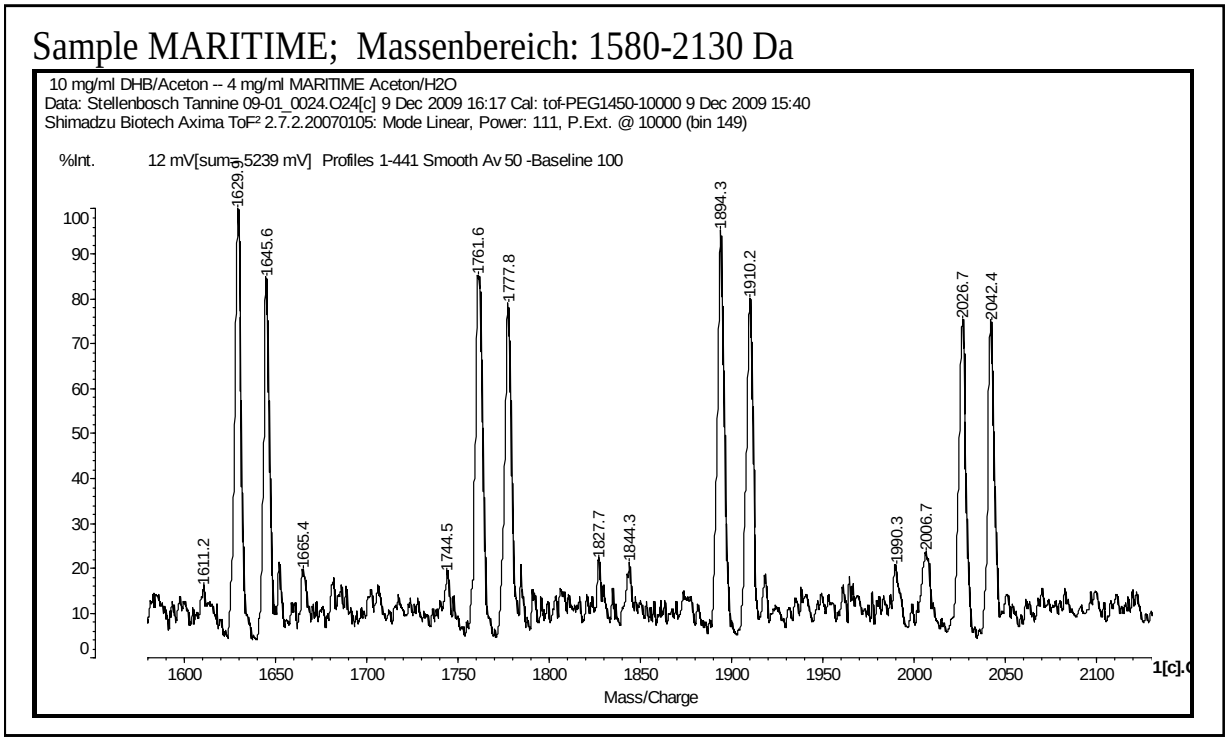

Figure 5b: MALDI-TOF signals of BIOLANDES tannin (selected molar mass range).

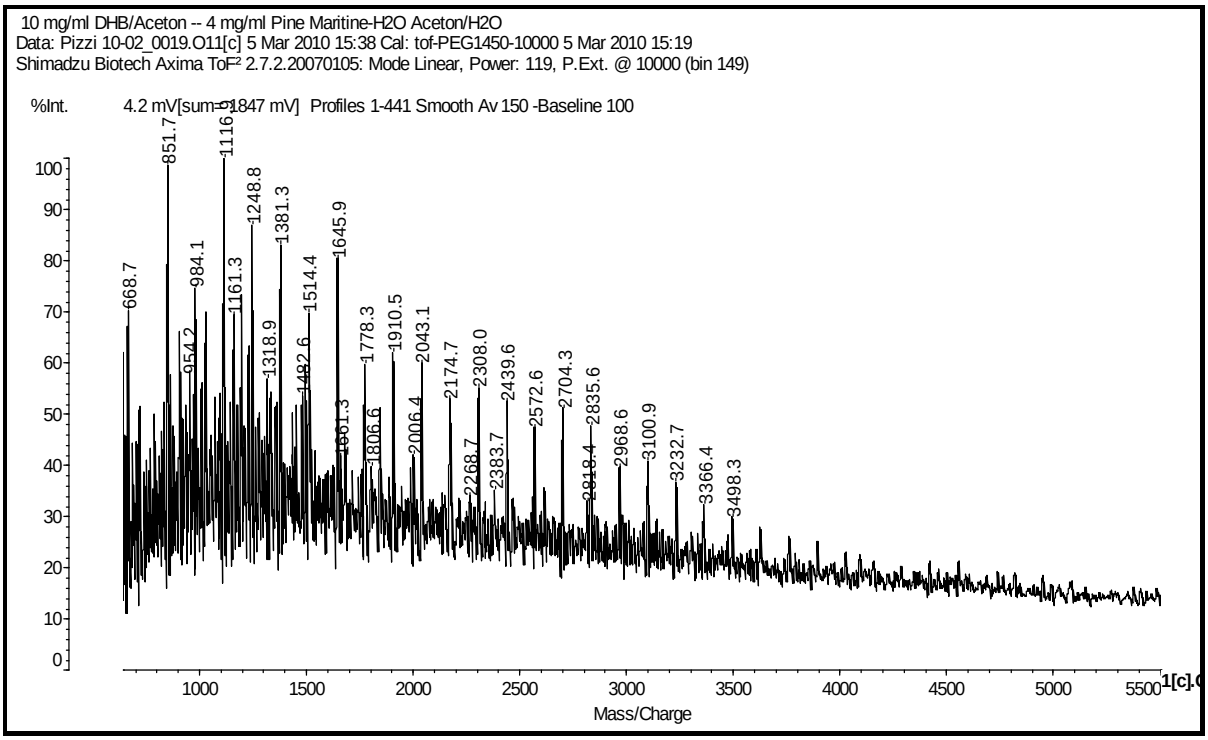

Figure 6: MALDI-TOF signals of DRT tannin (full molar mass range).

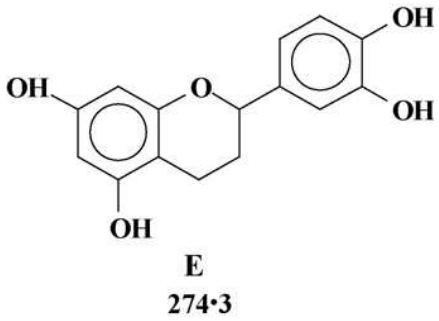

Figure 7: Possible epicatechin gallate units in which the gallic acid residue has been removed.

Alternatively a fisetidin structure (F) could also correspond to the molar mass of 274.3, although the existence of a structure of type F (Figure 8) in pine tannin is relatively unlikely (Oo et al. 2008).

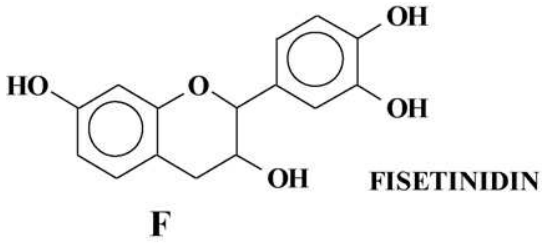

Figure 8: Fisetidin structure unlikely in pine.

Structure D cannot be inserted in the equation simply because no known flavonoid structure can be found with such a molecular weight. However, this structure participates markedly. From peak intensities in Fig. 8 and 9 its participation being predominant to the formation of the maritime pine tannin oligomers. Two series of the most intense MALDI mass peaks rely on the repetition of this 264 Da structure. These are separated by the 264 Da motive recurring several times. Table 2 shows the interpretation of the two predominant 264 Da series present. In the detail of the MALDI spectra in the double peaks are simply due to the difference of the presence or absence of one –OH group (Fig. 8b).

It is of interest to evaluate which structure corresponds to 264 Da. For structure E as repeating unit of 272.3 Da, the difference with 264 Da is always 8 Da this, however does not correspond to the mass of any leaving functional group. However, considering that there is an –OH group less in a dimer formed by two joined E structures, this causes the loss of 16 Da. It appears that the repeating unit of the system is not 264 Da but a relevant dimer with the molar mass 264 x 2 = 528 Da. Thus, the repeating unit is a dimer of structure E with a –

OH group missing. The unit has a single phenolic –OH group which has been lost, as shown in the structure, the alcoholic –OH group in C3 having already been lost at the separation of the gallic acid.

Dimer structures such as F-E and E-E (Figure 9) have also been considered but none of these corresponds to the period of 528 Da separating the various oligomers of the MALDI-TOF spectra. Both F-E and E-E would have periods of 548 Da, which is different from observation.

The maritime pine tannin is formed exclusively of monomers of different types but all presenting a phloroglucinol-type A-ring, indicating that these are almost pure procyanidins with some segments in the form of prodelphinidins bound to the procyanidin.

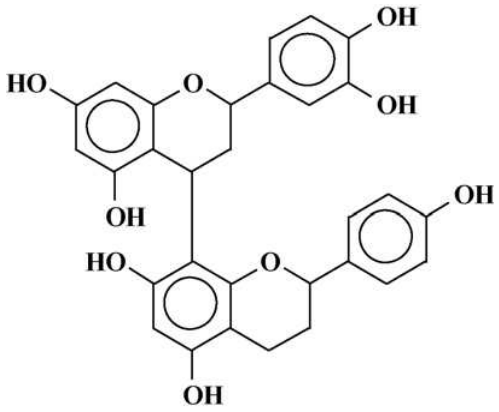

Figure 9: Dimer E-E.

Table 1: MALDI-TOF fragmentation peaks for BIOLANDES maritime pine bark tannin extract.

Experimental	Calc.	Peak intensity	A	B	C	D		E or F
M+Na+ (Da)	M+Na+ (PM23)	(%)	290,3	306,3	442,4	264	265	274,3
836,2	841,9	60						3
919,7	921,9	30	1	2				
966,5	969,7	73			1	1(-2*O)		1
1073,4	1074			2	1			
1101	1194,1	85	1		2			
	1093,6					2		2
1179,1	1178,2	34	4					
1233,4	1226,2	100	1	3				
1248,4	1242,2	68		4				
1340,9	1346,2	24			3			
	1346,3		2	1	1			
	1346,3			2	1			1
1365,3	1362,3	95	1	2	1			
	1365,9					2		3
1381,5	1378,3	76		3	1			
1497,6	1488,2	98	1	3		1		
1629	1627,9					3		3
1665,4	1666,6	17	1	3	1			
1761,6	1752,2	82	1	3		2		
1894,3	1890,9		4		1			1
	1889,9					4		3
2026,7	2012,2	72	1	3		3		
2158,3	1880,6	58	4		1	1		
2290,9	2274,2	56	1	3		4		
2555	2553,2	45		6	1	1		
	2536,2		1	3		5		
2817,2	2819,7	35	3	2	3			
	2819,6		1	1	5			
2951,3	2940	32		6				4
	2940		2	5				3
3084	3060,2	28	1	3		7		
	3076,1		4	3	1			2
	3076		2	2	3			2
	3076			3	3			3
3347,9	3322,2	24	1	3		8		

Table 2: MALDI-TPF fragmentation peaks for DRT maritime pine bark tannin extract.

Experimental	Calc.	Peak intensity	A	B	C	D		E o F
M+Na+. (Da)	M+NA+ (PM23)	(%)	290,3	306,3	442,4	264	265	274,3
851,7	857,9	100	1					2
954,2	25	58						
984,1	989,4	75						
1116,9	1114,2	100						4
1161,3	1162,2	70	3					1
1248,8	1242,2	88		4				
1318,9	1322	84	1	1	1	1		
1381,3	1378,3	82		3	1			
1482,6	1482,6	58	4	1				
1514,4	1514,5	70	2	3				
	1514,4			2	2			
1645,9	1650,5	80		1	3			
	1650,6		2		2			
	1650,6			3	1			1
1661,3	1666,6	46	1	3	1			
1778,3	1770,7	60	3		2			
	1770,8		4	1				
	1770,7		1	1	2			2
	1778,5			4		1		1
1806,6	1802,7	39	1	2	2			
	1802,8		3	3				
	1810,3				1	2		3
1910,5	1906,9	62	5		1			
	1914,6		2	2	1	1		
	1914,5			1	3	1		
2006,4	2011,1	42	5					2
	2011,1		1	2				4
	2012,2		1	3		3		
2043,1	2038,5	60		4		2		1
	2043,1		5	1				1
	2043,1		7					
2174,7	2173,1	54	1		3			2
	2179,2		5		1			1
	2179,2		1	2	1			3

Experimental	Calc.	Peak intensity	A	B	C	D		E o F
M+Na+. (Da)	M+NA+ (PM23)	(%)	290,3	306,3	442,4	264	265	274,3
	2267,4		2	1				5
2268,7	2267,4			2				6
	2267,4		4					4
	2306,6		3		2	1		1
2308	2307,1	56	7			1		
	2307,1		5	1		1		1
	2379,2		1	1	4			
	2379,4		5	3				
2383,7	2379,4	36	1	5				2
	2387,1		2	5		1		
	2387,5		1		1			6
	2435,5		2	1	1			4
2439,6		53						
	2443,2		5		1	1		1
	2571,7			3				6
	2578,6				4	3		
2572,6		48						
	2579,3		3		2	1		2
	2579,5		1	6	1			
	2699,7			7				2
2704,3	2707,2	52	5		1	2		1
	2707,5			3	4			
	2819,7		3	2	3			
2818,4		33						
	2819,8		5	3	1			
	2835,6			2	5			
2835,6	2835,7	47	6			1		3
	2835,8			6	1			2
	2971,2		5		1	3		1
	2971,3		2	4	2			1
2968,6		40						
	2971,7				3	1		5
	2971,9		4	3	2			
	3097,8			6	1	1		2
	3107,8				7			
3100,9								
	3107,9		2	1	5			
	3100,2		1		2			7
	3228		1		6			1
	3228,2		1	4	2			3
3232,7		37						
	3228,3		9	2				
	3236			7		1		3

Experimental	Calc.	Peak intensity	A	B	C	D		E o F
M+Na+. (Da)	M+NA+ (PM23)	(%)	290,3	306,3	442,4	264	265	274,3
3366,4	3364,4	32	9	1	1			
	3366,4		7	2	1			1
3498,3	3500,3	30	1		6			2
	3500,5		1	1	1	1		8
	3500,6		11	1				
	3500,6		1	6				5

3.3. Gel time and viscosity

The gel time results are shown in Table 3. They indicate a high reactivity for both marine pine tannin, BIOLANDES and of DRT. The pH was very difficult to increase in both cases. Immediately autocondensation seems to occur. Meikleham et al. (1994), Pizzi et al. (1995), Pizzi and Meikleham (1995), Merlin et al. (1996), and Masson et al. (1996 a,b, 1997) have already reported about this behaviour. This reaction can be induced by bases and by weak Lewis acids (Pizzi, 1994). The autocondensation reaction in tannin based adhesives can, under certain circumstances, contribute to the network formation as well as to the increase of the solution viscosity (Pizzi et al. 1995; Pizzi, 1994).

Table 3: Gel time results of the various adhesives resins prepared with the two maritime pine tannins.

Maritime pine tannin	Hardener	pH	Viscosity (MPa*s)	Gel time (min') (s")	Observations
BIOLANDES	Paraformaldehyde	4.5	14.000	45"	The viscosity increased very quickly.
		6.5	--	--	
	Hexamine	4.5	20.000	2'22''	
		6.5	30.000	1'38'	The viscosity increased very quickly.
	Glyoxal	4.5	10.200	2'44''	
		6.5	15.200	1'38''	The viscosity increases quickly. After some minutes, the mix is transformed into a gel, even at room temperature.
DRT	No hardener	3.5	9.600	9'45''	
	Paraformaldehyde	3.5	9.900	52"	
		5.3	--	--	
	Hexamine	3.5	2.050	39"	With hexamine, apparently the tannin solution seems precipitate. However, with stirring the viscosity decrease.
		5.3	4.150	35"	
	Glyoxal	3.5	10.600	1'10''	
		5.3	91.000	53"	

3.4. Thermomechanical analysis (TMA)

The values obtained by the thermo-mechanical tests enable a good prediction of the behaviour of the adhesive mix when boards are produced (Figures 10 – 13). Both maritime pine tannins reached TMA values higher than 3.500 MPa at lower pH with the various hardeners used; only glyoxal in combination with the BIOLANDES tannin remained at a thermomechanical resistance of approx. 3.050 MPa.

When the pH was increased in the two maritime pine tannin solutions, the TMA values decreased to approx. 3.050 MPa with the BIOLANDES tannin and to 3.250 MPa with the DRT tannin. The DRT tannin at pH 5.3 using glyoxal showed even lower results of approx. 2.770 MPa.

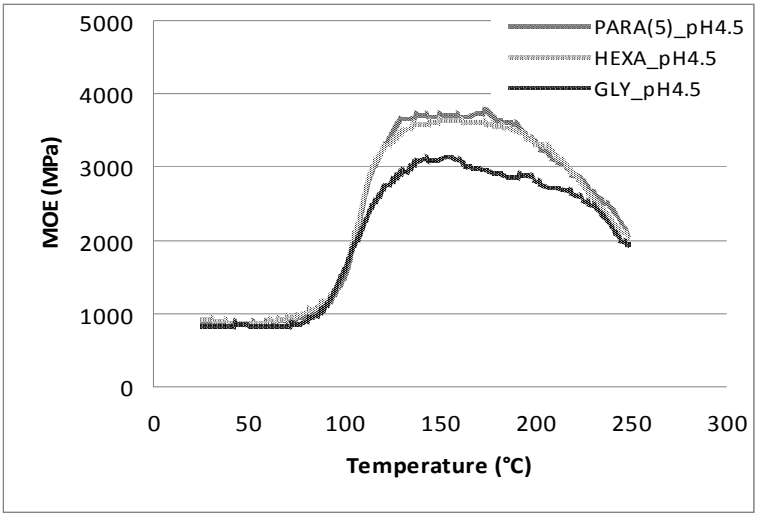

Figure 10: Thermomechanical analysis (TMA) of BIOLANDES maritime pine tannin at pH = 4.5 with various hardeners.

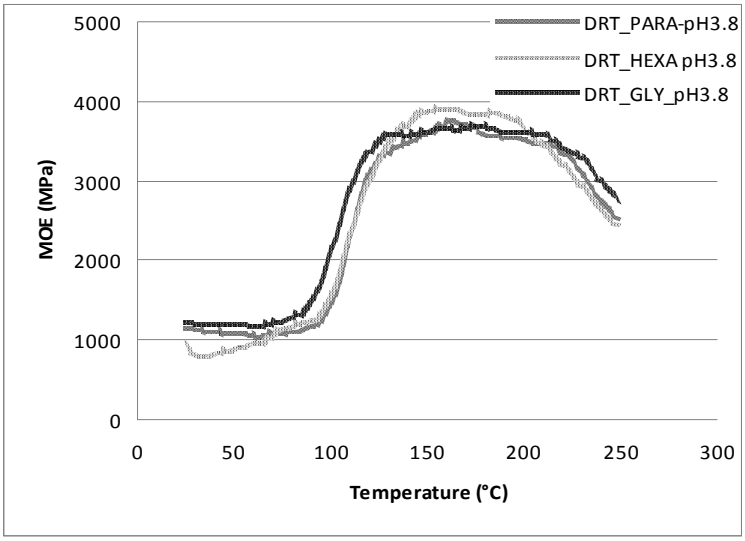

Figure 11: Thermomechanical analysis (TMA) of DRT maritime pine tannin at pH = 3.8 with various hardeners.

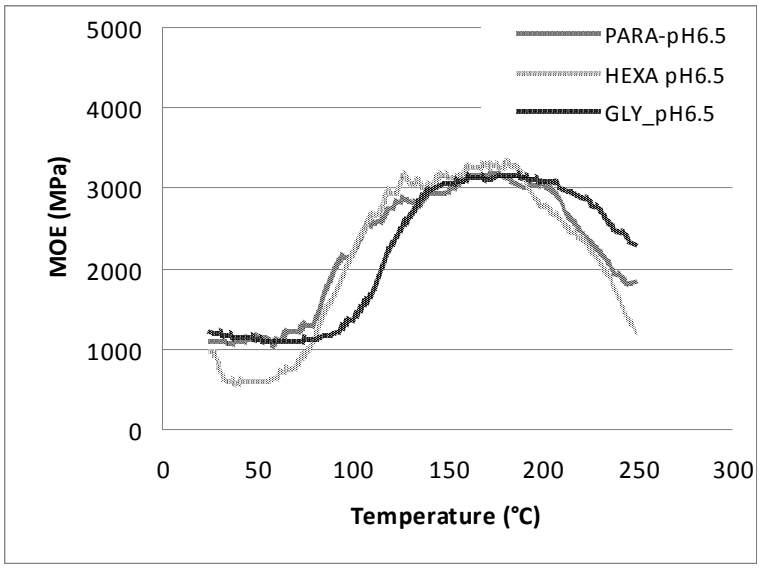

Figure 12: Thermomechanical analysis (TMA) of BIOLANDES maritime pine tannin pH 6.5 and different hardeners.

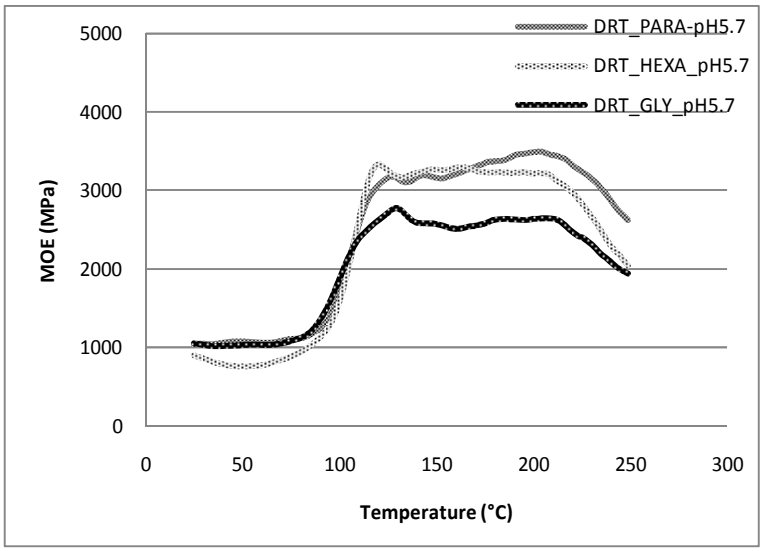

Figure 13: Thermomechanical analysis of DRT maritime pine tannin at pH = 5.3 with various hardeners.

3.5. Wood panels manufacture and internal bond

The internal bonds (IB) are shown in the Figures 14 and 15. The IB from the panels prepared with the BIOLANDES and the DRT tannin alone obtained 0.4 MPa, in both cases under the same conditions. This behaviour seems to be caused by autocondensation (Pizzi, 1994).

Paraformaldehyde obviously has no significant effect on the IB in case of the BIOLANDES tannin (0.46 MPa), whereas for the DRT tannin a remarkable increment of the IB was registered (0.6 MPa).

According to Meikleham et al. (1994), the autocondensation reactions or hardening in absence of aldehydes is characteristic of polyflavonoids. These reactions are based on opening under alkaline or acid conditions of the O1-C2 bond of the flavonoid unit and subsequent condensation of the reactive centre formed at C2 with the free C6 or C8 sited on another flavonoid unit (Meikleham et al., 1994; Pizzi and Stephanou, 1993; Pizzi and Meikleham, 1995; Pizzi et al. 1995). Pizzi et al. (1995) and Garcia et al. (1997) have revealed that notwithstanding the differences in structure and behaviour of different polyflavonoid tannins, good strength in particleboards could be obtained just based on the autocondensation reactions of the tannins. In some cases it even can happen that the use of a hardener (coreactant) might impede the autocondensation reaction (Garcia et al. 1997). The combination of the two effects caused by autocondensation and by crosslinking reactions initiated by hardeners (crosslinking agents) rather does not offer a synergistic advantage (Garcia and Pizzi 1998a, b); this also was confirmed in the case of paraformaldehyde and tannin of BIOLANDES in the work reported here.

BIOLANDES tannin in combination with hexamine and glyoxal reached low internal bond of the laboratory boards. In general, the standard requirements of EN 312 were not satisfactory DRT tannin in combination with hexamine and glyoxal used at the initial pH = 3.8 could fulfil the minimum requirements of EN 312. An increase of the pH, however, always causes a decrease of the internal bond as also recognized with the TMA results.

Due to these insufficient results the moisture content of the particles after application of the adhesive mix was increased. It is known from past that higher moisture content positively influences the mobility (flowing behaviour) of tannins (Sedano et al. 2010; Pichelin et al. 2001).

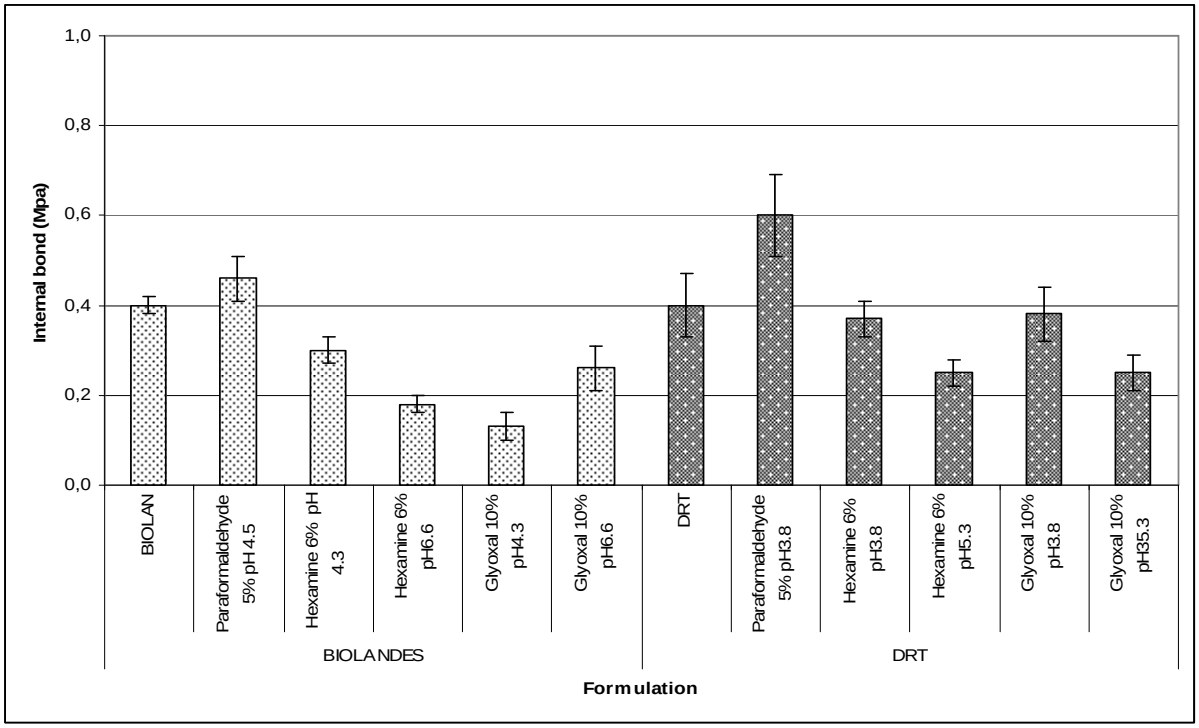

Figure 14: Internal bond results for particleboards prepared with BIOLANDES and DRT maritime pine and different hardeners between 12-14% of moisture content after application of the adhesive mix onto the particles.

Increasing the moisture content to approx. 24 %, however, caused a further reduction in mechanical properties of the lab boards bonded with the BIOLANDES tannin. None of the various formulations were able to reach the minimum standard request of EN 312. Contrary to this, the DRT tannin in combination with hexamine and glyoxal at pH = 5.3 resulted in IB above the requirements. DRT tannin shows a similar behaviour as the DITECO pine radiata tannin (Ballerini et al. 2005), which also was used for OSB (Pichelin et al. 2001).

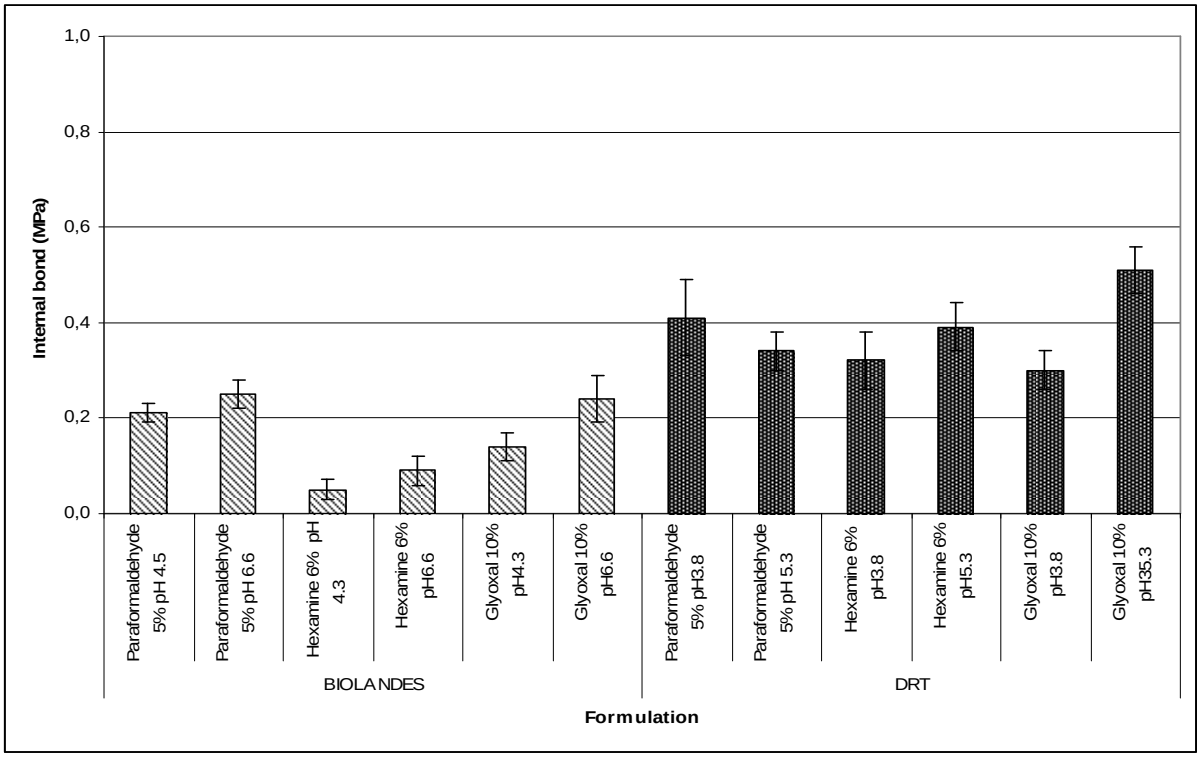

Figure 15: Internal bond results for particleboards prepared with BIOLANDES and DRT maritime pine and different hardeners at 24% of moisture content after application of the adhesive mix onto the particles.

4. CONCLUSIONS

The ^{13}C NMR and MALDI-TOF results confirm the same oligomeric structures in both maritime pine tannins. However, the DRT tannin presents a higher intensity of free C6 (98.54 ppm) compared to the other tannin type; the DRT tannin has a lower degree of polymerization compared to the BIOLANDES tannin, and, hence, more reactive sites are available in the DRT flavonoid unit.

In general, both tannin types, BIOLANDES and DRT, are very reactive and an autocondensation behaviour seems to be observed when increasing the pH.

The highest mechanical results were yielded with DRT tannin using (i) paraformaldehyde as hardener with an IB of 0.6 MPa, and (ii) glyoxal as hardener and a high moisture content of the particles before pressing (24 %) with 0.51 MPa.

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III.1.3. **Effet de la teneur en humidité sur la cohésion interne au sein de panneaux de particules fabriqués avec des colles à base de tannin de pin radiata**

Eur. J. Wood Prod. (2010) 68: 355–357

Auteurs: M. Sedano-Mendoza, **P. Navarrete** et A. Pizzi

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Des tanins de pin radiata DITECO ont été utilisés pour la fabrication de panneaux. Leur cohésion interne a été évaluée en fonction du pourcentage d'humidité présente dans les couches de particules avant pressage. Il existe en effet un gradient d'humidité entre les couches superficielles et le centre d'un panneau. Ce différentiel a des conséquences sur la cohésion interne.

Dans le cas d'une utilisation de colles à base de tanins, ces différences peuvent être plus prononcées que dans celui des colles synthétiques et l'effet est plus facilement observable.

Pour les panneaux collés avec du tanin, comme l'OSB, le temps de pressage, la perméabilité de la couche et l'application de l'effort pendant la densification ont été des paramètres déterminants qui influencent la performance du panneau (Pichelin et al. 2001). La température influe aussi sur le temps de transfert de vapeur dans la couche centrale (Pichelin et. al 2001). Toutefois, le pourcentage d'humidité relative en eau en surface et au cœur avant le pressage du panneau est certainement le paramètre prépondérant. Aussi, nous avons fait varier le pourcentage d'eau avant le pressage des panneaux à base de tanins et observé des résultats très différents. Les tanins, contrairement au phénol, ont besoin d'eau pour être mobiles. Ainsi un bon résultat d'IB dépend du pourcentage en eau présent dans le panneau au moment du pressage. Les résultats en termes de valeurs d'IB obtenus sont compris entre 0.53 et 055 MPa avec des teneurs en humidité entre 10 et 15%.

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BRIEF ORIGINALS KURZORIGINALIA

Effect of layers relative moisture content on the IB strength of pine tannin bonded particleboard

M. Sedano-Mendoza · P. Navarrete · A. Pizzi

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Abstract Relative percentage of moisture content of surface and core layers in pine tannin-bonded particleboard influences markedly the internal bond (IB) strength of the board. This is due to the differential compression exercised on the layers related to their moisture content. The moisture content differential between surface and core layers in particleboard during pressing is a determining parameter for the internal bond (IB) strength of particleboard. In the case of tannin adhesives where these differences can be more pronounced than for synthetic adhesive the effect is more easily observable.

1 Introduction

Pressing conditions of particleboard and other panel products are as much responsible for the good IB strength results as the performance of the binder itself. In this regard the density profile along the thickness of the panel is one of the most determining factors. The density profile along the thickness of the panel can be changed according to different techniques and manipulates different parameters, with large effects on the IB strength of the panel. The higher the moisture content of inner layer the higher the internal bond (IB) strength of the panel. For tannin-bonded panels, such as OSB, press closing time, mat permeability and strength development during densification have already been found to be determining parameters for board performance (Pichelin et al. 2001). The press temperature will also influence the steam front transfer time to the core layer (Pichelin et al. 2001). However, the relative moisture content percentage of surfaces and core of the furnish before panel pressing is perhaps the parameter the manipulation of which causes a more marked effect. While this is known in particleboard and other wood panels manufacture the same applies, but even to a more extreme extent, to tannin-bonded particleboard. This is the case as such adhesives allow the use, both in the laboratory and in the factory, of much higher moisture content percentages. Thus, the difference in results observed by manipulating the relative moisture content percentage before pressing of the board surfaces and core in tannin-bonded boards is particularly telling. In this paper, this effect is investigated for pine tannin-bonded particleboard.

2 Materials and methods

Triplicate one layer laboratory particleboards of $350 \times 300 \times 14$ mm^3 dimension were prepared using a mixture of core particles of beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*) wood particles at 28 kg/cm^2 maximum pressure and 190–195°C press temperature. The resin used was composed of a 40% solution of pine (*Pinus radiata*) tannin extract in water, at pH 5, to which 5% by weight on tannin content of paraformaldehyde powder was added. The resin solids load on dry wood was maintained at 10% of pine tannin extract solids. The moisture content was varied in core and surfaces according to what is indicated in Table 1. The total pressing time was maintained at 7.5 minutes. All particleboards were tested for dry internal bond (IB) strength according to EN 312 (EN 312 1995). The IB strength test

This article is dedicated to Gerd Wegener on the occasion of his retirement as professor at the Technische Universität München.

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was done on five board specimens and is a tension test perpendicular to the plane of the board. Thus, each IB strength and panel density result in the tables is the average of 15 specimens. The panel density reported in the tables is the average of the density of the series of specimens for each series of panels.

Table 1 Interral bond (IB) strength results for one layer and three layer particleboards at different moisture contents at the moment of pressing
Tab. 1 Querzugfestigkeit einschichtiger und dreischichtiger Spanplattem in Abhängigkeit der Holzfeuchte beim Pressen

Moisture content Surface/core/surface (%)	Ave. board density (kg/m^3)	Dry IB strength (MPa)
One layer boards		
15	704	0.53 ± 0.05
13	700	0.50 ± 0.05
10	717	0.41 ± 0.05
9	706	0.39 ± 0.05
Three layer boards		
25–15–25	702	0.36 ± 0.06
15–15–15	704	0.53 ± 0.05
10–15–10	704	0.55 ± 0.05

The panel density profiles were done on all the specimens tested for internal bond, before destructive testing, by using a Grecon Da-X (Greten, Germany) density profiler.

3 Results and discussion

The results of the series of one layer particleboard reported in Table 1 indicate clearly the action of moisture content on a tannin adhesive. As tannins contrary to phenol do not melt, they need water to be mobile, thus a good IB result depends on the amount of moisture content percentage present in the board at the moment of pressing. This is valid for most types of panels and has been clearly demonstrated for OSB panels (Pichelin et al. 2001). In general, in industrial panels it is a compromise between improving the mobility of the tannin during pressing and to limit the excess of water to avoid vapor blisters in the panel. The latter is generally not a problem in laboratory particleboard, where elimination of excess vapor is greatly facilitated by the more convenient board edge perimeter to board volume ratio. This is true for one layer boards in Table 1. The higher the moisture content of the one layer the higher the internal bond (IB) strength of the panel as already shown for OSB (Pichelin et al. 2001). For tannin-bonded panels, such as OSB, press closing time, mat permeability and strength development during densification have also been found to be determining parameters for board

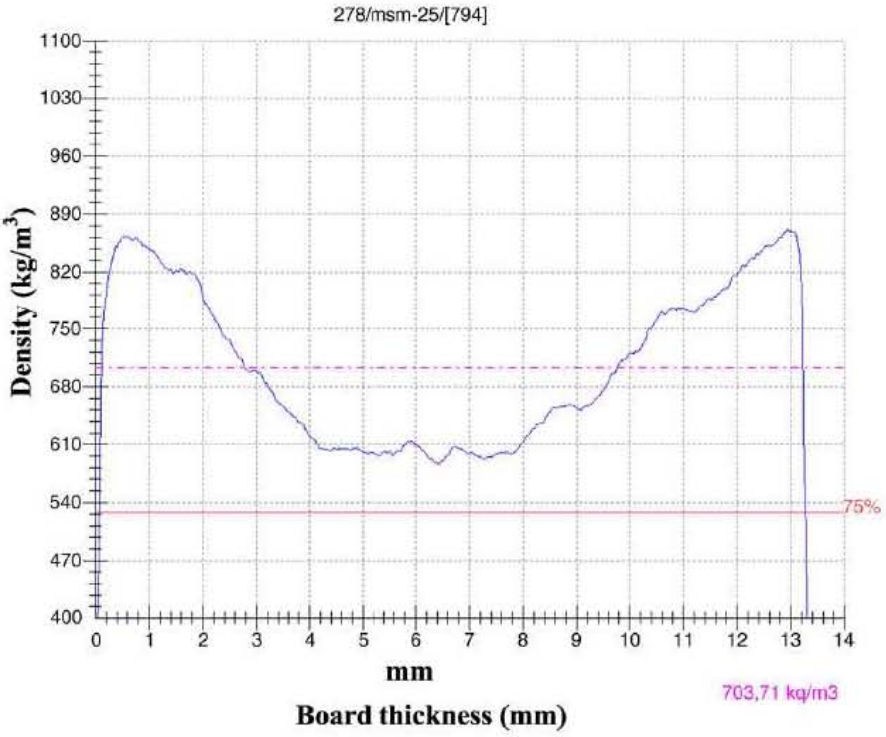

Fig. 1 Density profile of a three layer particleboard bonded with a pine tannin adhesive and presenting surfaces/core moisture contents at the moment of pressing of 25% and 15%, respectively
Abb. 1 Dichteprofil einer dreischichtigen, mit Kieferntanninklebstoff verklebten Spanplatte mit einer Holzfeuchte beim Pressen von 25% in den Deckschichten und 15% in der Mittelschicht

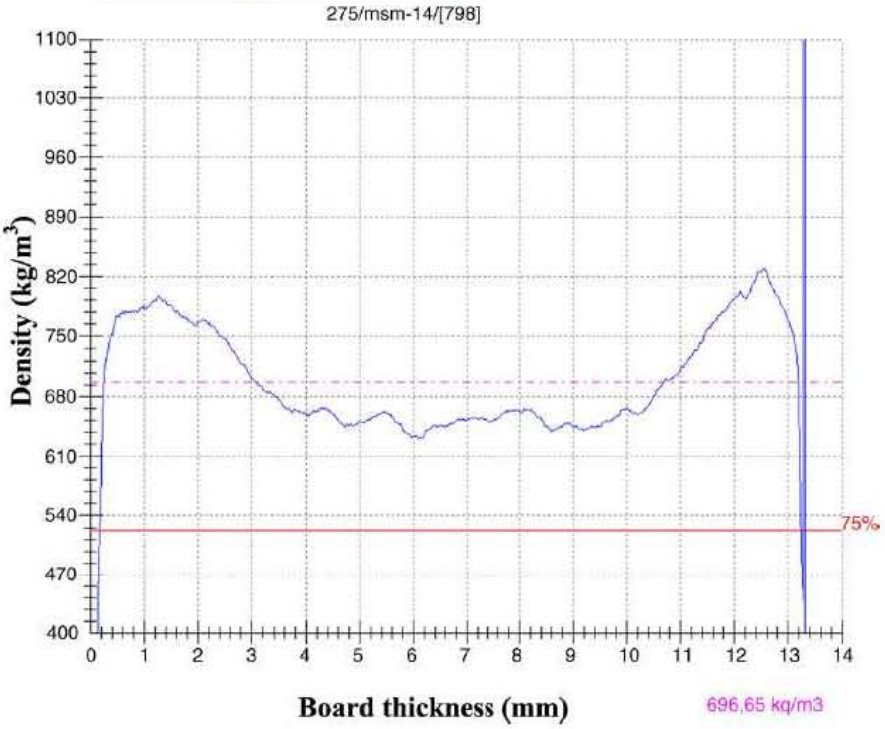

Fig. 2 Density profile of a three layer particleboard bonded with a pine tannin adhesive and presenting surfaces/core moisture contents at the moment of pressing of 10%/15%, respectively
Abb. 2 Dichteprofil einer dreischichtigen, mit Kieferntanninklebstoff verklebten Spanplatte mit einer Holzfeuchte beim Pressen von 10% in den Deckschichten und 15% in der Mittelschicht

performance (Pichelin et al. 2001). The press temperature will also influence the steam front transfer time to the core layer (Pichelin et al. 2001).

Passing to three layer boards and playing on the differential in percentage moisture content between board surfaces and core confirms that the relative compression ratio of surfaces and core is also a determining parameter for IB strength. As compression depends on the moisture content of the layer, be it surface or core, the wetter the more compressible it is, while the drier the less compressible it is, hence the less densifiable is the layer. Thus, a board in which the moisture content of the surfaces is much higher than that of the core, presents surfaces compressed to much higher density. In Fig. 1, an example of one of these board density profiles is given as a function of board thickness indicating the high densification of the moister surfaces in relation to the core, core at a density minimum of 580 kg/m^3. The IB strength is correlated with the lower density through the panel thickness. The board at moisture content percentage of 25% surface/15% core has then a lower IB strength than both those at 15%/15% moisture content and 10%/15% moisture content (Table 1). In Fig. 2, an example of the density profile of a 10%/15% board is given in which the moister core has had a greater chance to densify in relation to the drier surfaces, with a consequent increase of the minimum board density to 628 kg/m^3 in the core with a higher corresponding IB strength (Table 1) and a lower densification of the surfaces (Fig. 2). In conclusion, relative compression ratio of surface and core layers can be added as another determining parameter for the IB strength of a tannin-bonded particleboard.

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Pichelin F, Pizzi A, Frühwald A, Triboulot P (2001) Exterior OSB preparation technology at high moisture content, part 1: transfer mechanisms and pressing parameters. Holz Roh- Werkst 59(4):256–265

III.1.4. **Inhibition de la réactivité de tannins condensés à travers des réarrangements interneS : Détection CP-Mas ^{13}C RMN**

Maderas, ciencia y tecnologia 13(1): 59-68. 2011

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La pureté de tanins végétaux est très différente d'un polymère à l'autre ce qui rend assez complexe l'utilisation de ces polyflavonoïdes dans la préparation de colles thermostables pour produits bois. Dans ce contexte, différents travaux ont été développés sur l'extraction de tanins, dans le but d'améliorer leur qualité et leur production. Ainsi, différents additifs ont été utilisés comme des sulfites, qui limitent leur autocondensacion, ainsi que la formation de phlobaphènes (Sealy-Fischer and Pizzi, 1992), et favorisent la rupture de liaisons interflavonoïdes ce qui rend les polymères de tanins plus solubles (Pizzi, 1983). Par exemple, l'emploi d'urée permet d'augmenter la production de l'extraction (Sealy-Fischer and Pizzi, 1992), et celui de l'ammoniaque améliore les performances du tanin dans la colle (Panamgama, 2006).

Les extraits de tanin ont été préparés à partir d'écorce de pin maritime (*P. maritimus*), de pin d'Alep (*P. halepensis*), d'épicéa (*P. abies*) et d'un mélange d'écorce de différentes espèces (*Pinus spp*., à savoir 20% pin noir (*P. thunbergii*)+ 30% pin écossais (*P. sylvestris*) + 20% pin maritime (*Pinus maritimus*) + 10% pin d'Alep (*P. halepensis*) + 5% sapin de Douglas (*Pseudotsuga menziesii*) + 15% sapin de Norvège (*Picea abies*)). L'extraction de pin maritime a été effectuée industriellement par Dérivés Résiniques et Terpéniques (France). Les autres espèces ont été extraites par une méthode standard (Sealy-Fischer et Pizzi 1992) avec une solution aqueuse à 2% de Na_2SO_3 + 0.5% $NaHCO_3$, à 75°C pendant 1 heure. Les échantillons ainsi produits ont été séchés durant 20 jours à une température de 45-50 °C et à un pH 8-9. Ce procédé permet un réarrangement interne des tanins condensés qui inhibe leur réactivité.

Les tanins ainsi produits ont été analysés par la technique ^{13}C RMN, et des colles à 5% de paraformaldéhyde ont été préparées. Leur réactivité a été évaluée à partir du temps de gel et la résistance mécanique des panneaux collés a été caractérisée par analyse thermomécanique (TMA) et mesures de la cohésion interne selon la norme européenne EN-312.

D'après l'analyse par RMN, les déplacements chimiques à 163 ppm et l'augmentation des petites bandes entre 20 et 65 ppm indiquent clairement que l'extraction de proanthocyanidines n'a pas été correcte avec pour preuve une production relativement importante de structures d'acide catéchinique et de dimères d'acide diarylpropanol-catéchine causée par un réarrangement interne. Cette situation conduit au blocage des sites réactifs C6 et C8, ce qui rend inutilisable le tanin comme colle pour applications bois. Par conséquent, les temps gel sont très longs sauf dans le cas de la colle à base de tanin de pin maritime qui n'est que de 51s. Les résistances mécaniques des panneaux analysés par TMA sont très faibles sauf pour le panneau à base de colle de tanin de pin maritime. Ces faibles résultats ont été confirmés par les valeurs de cohésion interne. Seul le pin maritime conduit à des résultats de résistance mécanique satisfaisant les exigences de la norme EN-312 avec des valeurs de 0.6 (MPa).

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CONDENSED TANNIN REACTIVITY INHIBITION BY INTERNAL REARRENGEMENTS: DETECTION BY CP-MAS ^{13}C NMR

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ABSTRACT

Tannin extracts were prepared from barks of maritime pine (*Pinus maritimus*), aleppo pine (*Pinus halepensis*), Norway spruce (*Picea abies*) and from a mixture of barks of different pine species (*Pinus spp.*, namely 20% black pine (*Pinus thunbergii*)+ 30% Scots pine (*Pinus sylvestris*) + 20% maritime pine (*Pinus maritimus*) + 10% Aleppo Pine (*Pinus halepensis*) + 5% Douglas fir (*Pseudotsuga menziesii*) + 15% Norway spruce (*Picea abies*)) at a paper mill. The 13C-NMR spectra were carried out from these tannins. Blending systems adhesives were prepared with 5% paraformaldehyde to measure the reactivity by gel time, and to determinate the mechanical resistance by thermomechanical analysis (TMA) and the internal bond strength (IB) according to the European Norm EN-312. The CP MAS ^{13}C NMR bands at 163 ppm and the increase number of smaller bands between 20 and 65 ppm clearly indicate that the extraction of procyanidin-type tannins has been incorrect yielded high relative proportions of catechinic acid structures and diarylpropanol-catechinin acid dimer caused by internal flavonoid rearrangement, and causing the blockage of the C6 and C8 reactive sites, rendering unusable the tannin for wood adhesive applications. Thus, the gel times are very long except for the maritime pine tannin with a value of 51 (s). The TMA analysis gave very low MOE values for almost all the tannin extract samples, except for maritime pine. This situation was confirmed by the IB test. Only maritime pine tannin presented a good mechanical resistance with a value of 0.6 (MPa). Only this sample fulfils the standard requested.

Keywords: Catechinic acid, tannins, rearrangements, reactivity inhibition, NMR, wood adhesives.

INTRODUCTION

Natural adhesives were used a lot before the1930's, and then, they have been almost entirely replaced by synthetic adhesives (Wang *et al.* 2008). However, wood adhesives from renewable raw materials have been a topic with considerable interest for many years. This interest, already present in the 1940's, became bigger with the first world oil crisis in the early 1970's but subsided as the cost of oil decreased. Since the beginning of the 21st century this interest intensified again for a lot of reasons. The main stimulus for today's renewed interest in bio-based adhesives is the acute sensitivity from the general public toward anything concerning the environment and its protection (Pizzi 2006). Health problems that can present symptoms such as the most common of which are eyes irritation and the upper respiratory tract are attributed to the emission of formaldehyde gases (Kim 2009). Also, formaldehyde has been found to produce nasal carcinomas in mice and rats after a prolonged exposure at 14.1 and 5.6 mg/L level, respectively. These findings have led to an intensified interest in the indoor environment (Kim 2009, Kim and Kim 2004, Brown 1999). Consumer products, especially construction materials, are the main source of formaldehyde emissions in the indoor environment. On the other hand, the limited fossil fuels pool and the broad availability of renewable resources are especially appealing as a source

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of materials and energy (Vazquez *et al.* 2010). For all these reasons, it is necessary to find new natural alternatives to produce wood adhesives.

While the majority the literature on this subject focuses on already commercially available tannins such as mimosa and quebracho, work on pine tannins has yielded perfectly workable formulations some of which have already been used industrially for almost ten years at Masisa Chiguayante plant facility in Chile (Pizzi *et al.* 1993, Pizzi *et al.* 1996) as adhesives for particleboards. The scarcity of commercially available mimosa and quebracho tannins in a period where the interest in adhesives from renewable materials, and the very positive Chilean experience with *Pinus radiata* bark tannins, has prompted a number of investigations in the suitability for wood adhesives application of tannins obtained from the bark of other pine species (Pizzi 1998). Thus also, hybrids adhesives such as tannin/PVAc have been studied as environment-friendly adhesives (Kim 2009) to decrease the formaldehyde emissions.

The purity of vegetable tannin extracts is very different from one to an other. The non-tannin fraction, mainly sugars and hydrocolloid gums, generally does not participate in resin formation with formaldehyde (Pizzi 1982), and there are some differences between different poliflavonoid tannins that can be used for thermosetting wood adhesives. Flavonoide units in such tannin present phloroglucinol o resorcinol A–rings and catechol or pyrogallol B-rings is presented in the figure N°1.

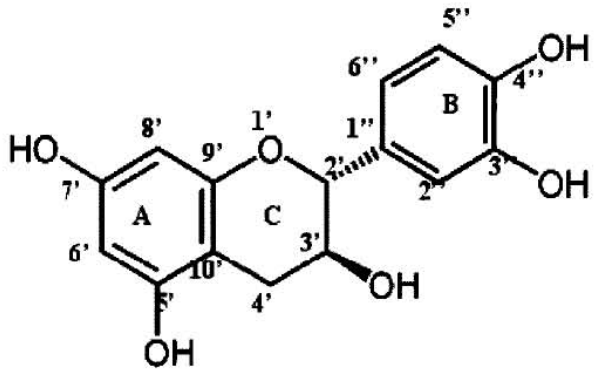

Figure 1. Flavonid structure.

Since tannin molecules are generally large, the rate of molecular growth in relation to the rate of linkage is high; thus tannin adhesives can tend to have short pot-lives (Pizzi 1982). From the point of view of both molecular size and reactivity, phloroglucinolic tannins are worse than mainly resorcinolic tannins as shown by their relative gel times at their pH's of lower reactivity: 950 (s) for wattle tannin extract (resorcinolic) against 65 (s) for pine tannin extract (phloroglucinolic). Molecular size reduction is difficult and therefore it does not offer a practical solution (Pizzi 1982).

Different works have been developed about the tannin extraction with the aim of improving their quality and yield. Thus, different additives have been added to improve some properties, such as sulphites, allow limiter the tannin auto-condensation and the phlobaphenes formation (Sealy-Fischer and Pizzi 1992), as well as to break the interflavonoides link, or to do the tannin polymers more soluble because they are smaller (Pizzi 1983). The urea allows coverall to increase the yield extraction (Sealy-Fischer and Pizzi 1992), and the amoniac allow the tannin use with a better adhesive aptitude (Panamgama 2006). However, some manipulation errors in extraction, drying conditions generally could cause this unusual behaviour, and this paper sets out to explain what rearrangement occurs in the tannin to inhibit its reactivity to the point of even inhibiting gelling altogether.

EXPERIMENTAL

Extractives preparation

Tannin extracts were prepared from the barks of maritime pine (*Pinus maritimus*), aleppo pine (*Pinus halepensis*), Norway spruce (*Picea abies*) and from a mixture of barks of different pine species (*Pinus spp.*, 20% namely black pine (*Pinus thunbergii*)+ 30% Scots pine (*Pinus sylvestris*) + 20% maritime pine (*Pinus maritimus*) + 10% Aleppo Pine (*Pinus halepensis*) + 5% Douglas fir (*Pseudotsuga menziesii*) + 15% Norway spruce (*Picea abies*)) at a paper mill. All these are composed of procyanidin-type tannins. The tannin samples were extracted in the standard manner (Sealy-Fischer and Pizzi 1992) by extracting 100 grams bark with 500 ml water solution of 2% Na_2SO_3 + 0.5% $NaHCO_3$ at 75°C for 1 hour, with the exception of the maritime pine for which an industrially water extracted material was used produced by DRT (Dérivés Résiniques et Térpeniques, France). The samples for which a catechinic acid rearrangement was required to study the appearance of the ^{13}C NMR spectra were dried in an oven for 20 days at 45-50°C and pH 8-9 after extraction prompting the catechinic acid rearrangement described for pure catechin model compounds under these conditions by Sears *et. al.* (1974) and Kiatragrajai *et. al* (1982), according to figure N°2. The industrial sample was obtained after spray-drying to ensure minimization or absence of the same rearrangement.

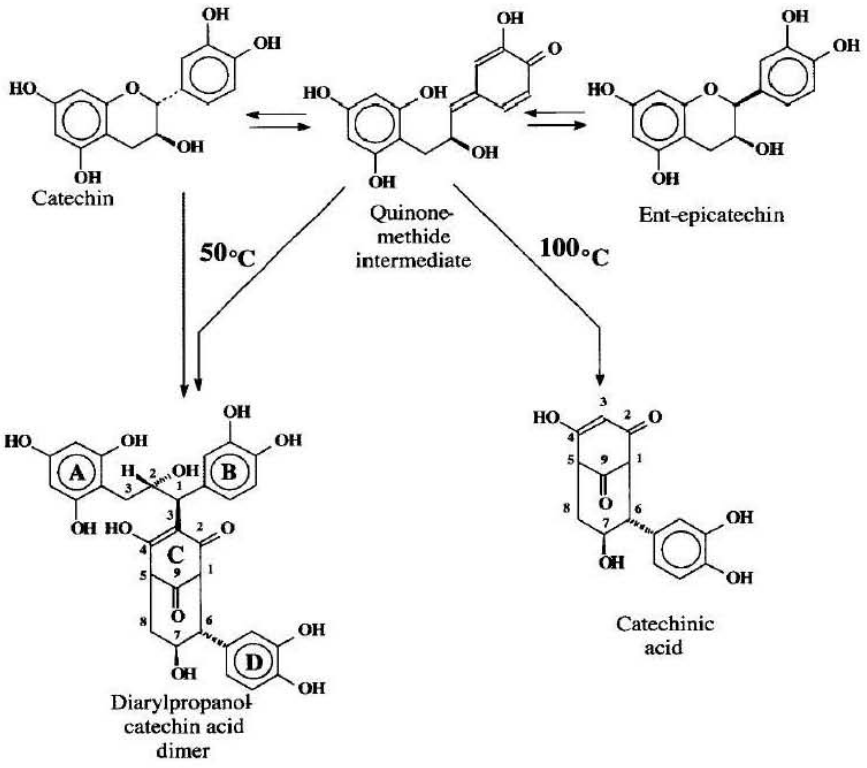

Figure 2. Base catalyzed reactions of (+) catachin at pH12. (Kiatragrajai *et al.* 1982).

^{13}C NMR analysis

The dried powder sample were analysed by solid state CP MAS (cross-polarisation/magic angle spinning) ^{13}C NMR spectroscopy. Spectra were obtained on a Bruker AVANCE II 400 MHz spectrometer operating at a frequency of 100.6 MHz and a sample spin of 12 kHz, using a recycling delay of 1s and a contact time of 1 ms. Number of transients was about 15000, and a 53 kHz decoupling field. Chemical shifts were determined relative to tetramethyl silane (TMS) used as control. Spectra, accurate to 1 ppm, were recorded with suppression of spinning side bands.

Gel time

The gelation is defined as the point at which the resin ceases to be a viscous liquid and become a soft, elastic, rubbery solid (Pizzi and Stephanou 1993). Triplicate samples at 45% tannin solution in water to which 5% paraformaldehyde on tannin extract solids content had been added were prepared. The samples initial pH is indicated in the table N° 1. In the Norway spruce, Aleppo pine and the mixed pine species had high initial pH compared with Maritime pine, for this reason the initial pH was dimishes to pH 5. Gel time tests were conducted at 100 °C, Five (g) of liquid was weighed in a glass test tube, and then placed in a boiling water bath. The wire spring was manually moved rapidly upward and downward, and the time for gelation was measure using a stopwatch.

Table 1. Gel times for different procyanidin type tannins extracted under different conditions.

Specie	Hardener	pH	Get time (s)
Control: Commercial *Pinus maritimus*	Paraformaldehyde	5.2	51
Control: Commercial *Pinus radiata*	Paraformaldehyde	5	80
Norway Spruce *(Picea abies)*	Paraformaldehyde	10	3060
		8,2	2160
	Hexamethylenetetramine	10	6000
		8,2	4200
Aleppo pine *(Pinus halepensis)*	Paraformaldehyde	4,95	3360
		9	1740
		10,2	2490
	Hexamethylenetetramine	10,2	2754
Mixed pine species *(Pinus spp)*	Paraformaldehyde	5	3300
		9	2280
		10	2352
	Hexamethylenetetramine	9,7	2610

Thermomechanical analysis TMA

The hardening reaction of one resin system or glue mixes can be evaluated by TMA, by the study of the rigidity of the wood–resin joint as a function of temperature. Thus, the different system adhesives tannins were thermomechanically analyzed under the same conditions. The formulations were identical to gel time formulations. Triplicate samples of beech wood alone, and of two beech wood ply each 0.6 mm thick, bonded with each system, for a total samples dimension of 20×5×1.1mm were tested in non-isothermal mode between 25 °C and 250 °C at a heating rate of 10 °C/min, 30 mg of resin, with a Mettler 40TMA apparatus in three points bending on a span of 18 mm exercising a force cycle of 0.1 0.5 N on the specimens with each force cycle of 12 seconds (6 s/6 s). The classical mechanics relation between force and deflection $E = [L^3/(4bh^3)]\ [\Delta F/(\Delta f)]$ allowed the calculation of the Young's modulus E for each case tested. Deflection curves that allow MOE determination are obtained by the three-points bending TMA mode. The MOE of the wood joints bonded with different resin systems give a good indication of the final strength of the adhesive system tested and the possible end performance of the adhesive system tested.

Internal bond strength IB

Duplicate one-layer laboratory particleboards of dimensions 350 mm×310 mm×14 mm were prepared, using only enter particles of beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*) wood mixture. The resin solid load was maintained at 10% of the tannin resin on dry wood particles. All particleboards were tested for dry internal bond (IB) strength according to the European Standard EN-312.

RESULTS AND DISCUSSION

The gel time results in table N° 1 indicates that for industrially extracted samples the gel times with paraformaldehyde hardener at pHs of around 5 were fast as expected of procyanidin-type tannin extracts (Pizzi 1994). However, procyanidin-type tannins that were laboratory extracted and dried in an oven first for a number of days at a temperature of 50°C, followed by further days of drying at 45°C, even if the pH was low at around 4-5, as their natural pH (8-9) thus in non ideal conditions for maximum to catechinic acid rearrangement and diarylpropanol-catechinic acid dimer, showed gel times which were far too long. Moreover they did not gel well but remained exceedingly soft. The difference was major, between the 50-90 seconds recorded for the normally extracted and spray-dried commercial tannin extracts (Pizzi 1994), and the rest at 50-80 minutes gel time (Table 1). In addition, at very high pHs the reactivity of normally extracted procyanidin-type tannins is of around 20-30 seconds. This result indicated that a great proportion of the available C6 and C8 reactive sites of the flavonoid structure were blocked, and thus unavailable for reaction with paraformaldehyde. The catechinic acid rearrangement shown in figure N° 2 shows that blocking a great proportion of C6 and C8 sites available for reaction, thus slowing down considerably and sometime even stopping altogether gelation of the tannins with hardeners. It was then of interest to examine these tannins by ^{13}C NMR spectroscopy to observe whether catechinic acid and apparently diarylpropanol-catechinic acid dimer had been formed Hashida and Ohara 2002).

About the base-catalyzed reactions of (+)-catechin, (Figure N° 2), several investigations have been reported (Hashida and Ohara 2002, Sears *et al.* 1974). At 25 °C, ent-epicatechin is formed by epimerization at C-2 position of pyran-ring, whereas catechin acid is obtained in high yield in the reactions at 100 °C (Hashida and Ohara 2002; Kiatgrajai *et al.* 1982, and Sears *et al.* 1974). These reactions are supposed to proceed through opening of the pyran-ring to give the quinine-methide intermediate, followed by reclosure of pyran-ring or by nucleophilic attack of carbanion at C-8 position on the C-2 position of quinine-methide intermediate. A radical reaction mechanism is also proposed because these reactions are inhibited by the exclusion of oxygen (Kennedy *et al.* 1984, Hashida and Ohara 2002). Previosly (Ohara and Hemingway 1991) showed that at intermediate temperature (40 °C), diarylpropanol-catechin acid dimer is produced together with catechin acid. There are other compounds are produced in low yield on that condition but they are not been identified (Hashida and Ohara 2002).

The ^{13}C NMR spectra in figure N°3 show a maritime pine tannin extract (Fig. N° 3a), and the rearranged of Norway spruce extract (Fig. 3b), Aleppo pine extract (Fig.N° 3c), and mixed pine species extract (Fig. N° 3d) samples. The ^{13}C NMR chemical shift assignments for the tannins are indicated on the spectra themselves corresponding to the relevant notations on the catechin and catechinic acid formulas. The rearranged tannin samples show signals for the catechinic acid C2 (176-180 ppm), C4 (163 ppm), C6, C3 (around 110 ppm), C7, C1, C5 and C8 (37-40 ppm). Only the spectra in Figures 3c, 3d, show presence of the C9 position (190-200 ppm). The majority of these peaks are relatively small although clearly indicating the presence of noticeable proportions of catechinic acid (Pretsch *et al.* 1998, Werhli and Wirthlin 1978). This is explained by the fact that only some of the flavonoid structures have converted to catechinic acid and diarylpropanol-catechinic acid dimer rearranged due to the lower temperature of drying and high pH of the samples (Figure N° 2). However, one peak is very sensitive to the presence of catechinic acid and is always present, very sharp and evident, where no other equivalent peaks are present for non-rearranged flavonoid structures. In normal flavonoid tannins the two peaks at 155 and 144 ppm are characteristic and clearly visible. But only where a consistently

high proportion of catechinic acid is high peak at 163 ppm does appear transforming the peak couple in a three peaks set (Figs. 3b, c and d). The second best indication of the presence of catechinic acid is the series of peaks between 20 and 65 ppm. In this range are generally found the C3, C6' and the unreacted C4 of normal flavonoids. However, in the case of the catechinic acid rearrangement, some sharp peaks appear corresponding to the C1, C7, C6, C5, C8 (Fig. 3b and 3d) and 1', 2' and 3' (Figure N° 2 and N° 3c) where these ones are clearly present (Figure N° 3b and 3d). The third indication of the presence high proportions of catechinic acid structures is its C2 peak presenting a sharp peak at around 176-177 ppm (clearly visible in Figures N° 3b and 3d) (Pretsch *et al.* 1998, Werhli and Wirthlin 1978). This is not always clearly obvious and thus cannot be totally relied on. As for the causes of the rearrangement, they appear to be the very long time exposure during the drier (20 days), at low temperature (50-45°C) and high pH samples that caused the rearrangement to occur under these relatively mild conditions. Differently from Hashida and Ohara (2002), while the shifts around 65 ppm as they find indicate the presence of catechinic acid-like molecules, the more obvious band at 163 ppm is an even clearer and more noticeable indication of such flavonoid rearrangements (table N°2).

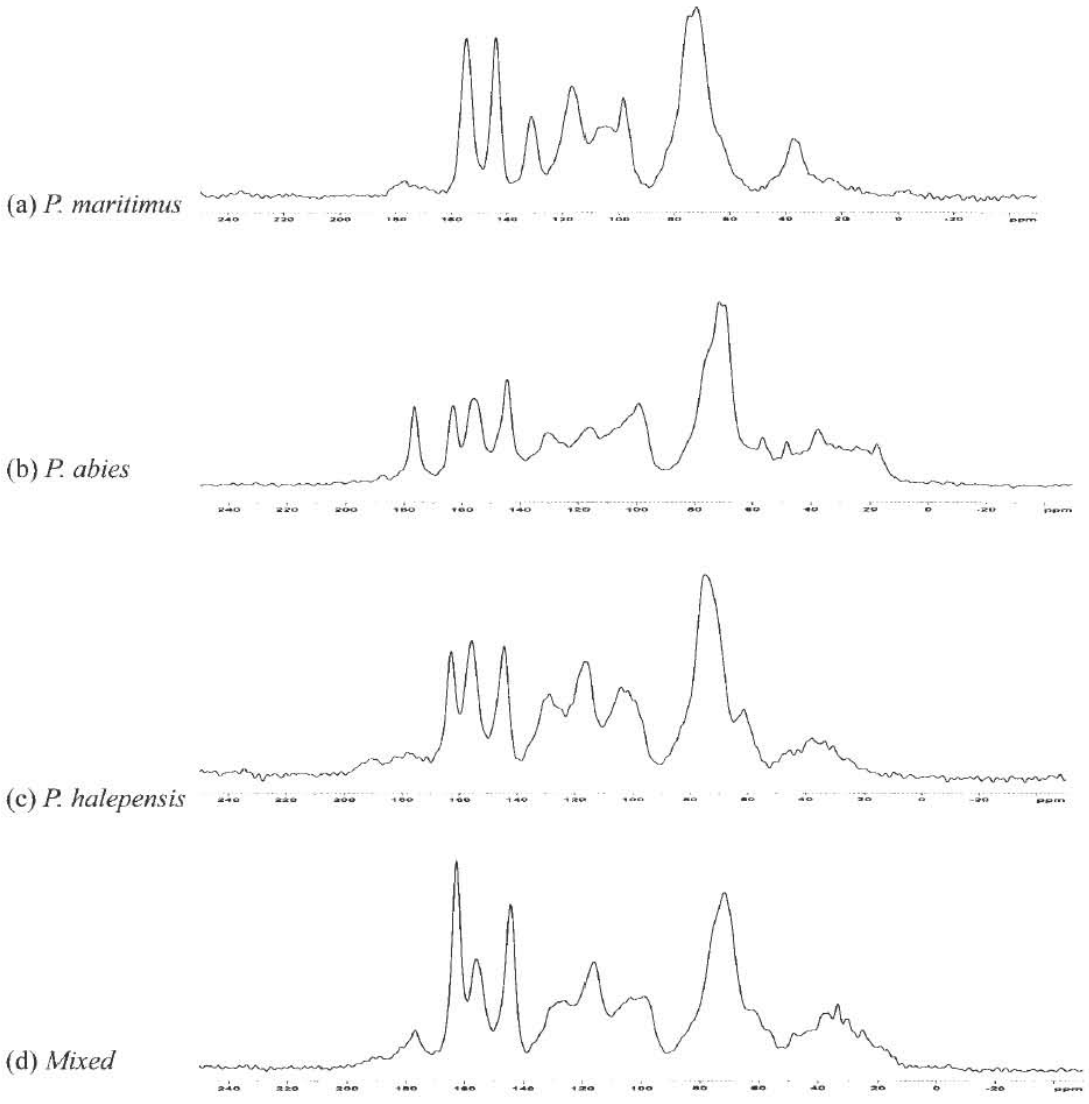

Figure 3. CP MAS 13C NMR solid state spectra of (a) *P. maritimus*, (b) *P. abies*, (c) *P. halepensis*, and (d) mixture tannin extract species showing catechin acid rearrangement.

Table 2. ^{13}C NMR Chemical shifts to the rearranged samples results.

Chemical shifts		*P. maritimus*	*P. abies*	*P. halepensis*	*Mixture*
C9	190-120	Absent			
C2	176-180				
C4	163	Absent			
C6, C3	110				
C8, C7, C5, C1	37-40	Absent			
C5', C7'	156-157				
C9'	155				
C3'', C4''	145-146				
C1'	131				
C6''	120-121				
C5'', C2''	115-117				
Phloroglucinol interflavonoid	110		Absent	Absent	Absent
C10'	101				
C6'	96-98				
C8'	95-96				
C2'	91-82				
C3'	67-68				
Catechinic acid	31-32				
C4'	27-28				

The TMA analysis showed low Elasticity Modulus (MOE) for *P. halepensis, P. abies* and the bark mixture tannin adhesives (Figure N° 4). The maximal value registered with these tannins was hardly 2.500 (MPa). Nevertheless, the maritime pine situation was different. The elasticity modulus for this specie presented a maximal value of 3.744 (MPa). The MOE of the wood joints bonded with different resin systems gives a good indication of the final strength of the adhesive system tested and the possible final performance of the adhesive system tested.

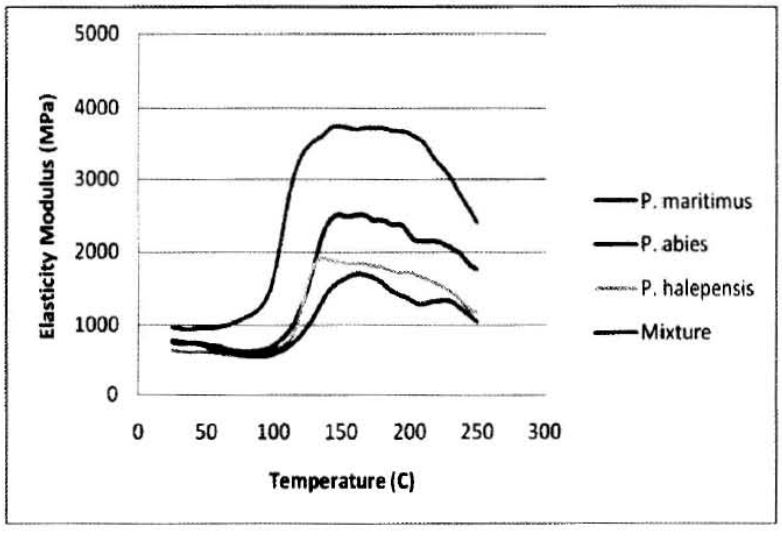

Figure 4. Thermomechanical test comparison by elasticity's modulus (MOE).

The results of the TMA tests were confirmed by preparing and testing laboratory particleboard panels. Thus, in table N° 3 the IB results from particleboard prepared using the different extracts are shown. The IB results from particleboards tannins are in direct relation with the TMA analysis. The registered value didn't reach the minimal requirements demanded by the European Norm, except for the particleboard prepared with maritime pine tannin. This result was able to satisfy the dry IB strength requirement of >0.35 (MPa) of the relevant European Norm EN 312 for interior grade panels, to press time of 7.5 (min), and at 195 (°C). There are not available reactive sites in *P. abies, P. halepensis* and the bark mixed tannin studied. Then, there was not to cross linking between the adhesive and the wood, even using paraformaldehyde as a hardener.

Table 3. Internal bond results.

Formulation	Density (kg/m^3)	IB (MPa)
P. maritimus	700	0,6±0,09
P. abies	700	0,08±0,01
P. halepensis	692	0,03±0,02
Mixed	690	0,01±0,00

CONCLUSIONS

The CP MAS ^{13}C NMR bands at 163 ppm, and the increase number of smaller but clearly defined bands between 20 and 65 ppm can be taken as a clear indication that the extraction of procyanidin-type tannins has been incorrect yielding high relative proportions of catechinic acid structures, caused by internal flavonoid rearrangement, and causing the blockage of the C6 and C8 reactive sites, rendering unusable the tannin for wood adhesive applications. This was confirmed by the gel time and the mechanical tests (TMA and IB). Only maritime pine with a strength of 0.6 (MPa) satisfy the European Norm EN-312 request.

It is important to note that alkaline pHs of the tannins extracted (8-9) noticeable proportions of flavonoid units to catechinic acid rearranged and other compounds have been formed if the temperature is sufficiently low (around 45-50 °C), and the time at which the tannin extract is maintained at such a temperature is sufficiently long, such as for days.

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III.1.5. **Extraction de tannins condensés à partir de pulpe de raisin pour utilisation comme adhésifs**

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Les sous-produits agricoles représentent une source abondante et provisoire de stockage pour la production industrielle d'énergie et de matériaux.

Le raisin est l'un des fruits le plus récolté dans le monde (après l'orange). En France, autour de 700 000 tonnes de sous-produits secs naturels ont été produites en 2009 par les industries vinicoles. Après l'éthanol, des graines et des anthocyanes sont récupérées dans les distilleries. Le résidu contient principalement la peau du raisin et est souvent utilisé comme aliments pour animaux ou fertilisants du sol. Une faible extraction lors du procédé de production de vin conduit à des niveaux importants de tanins condensés dans les résidus, principalement des proanthocyanidines composées d'unités procyanidines et prodelphinidines qui sont liées par une liaison C4-C8. Ainsi, le sous-produit n'est pas intrinsèquement dangereux mais son taux important de polyphénols et le fait que sa production soit concentrée sur une courte période de l'année entrainent potentiellement des problèmes de pollution lors de son épandage (Morthup et al., 1998). Depuis les premières observations du «paradoxe français» (Renaud et de Lorgeril, 1992), beaucoup d'études récentes ont souligné les effets bénéfiques des polyphénols du raisin ou du vin pour la santé humaine (Shrikhande, 2000). Par conséquent, des efforts considérables ont été consacrés à l'optimisation de l'extraction des composés phénoliques de haute pureté à partir des sous-produits vinicoles pour applications comme additifs alimentaires (Monagas et à., au 2006), mais aussi pour l'industrie cosmétique et pharmaceutique. (Ozkan et al., 2004; Torres et al., 2002). Les techniques généralement utilisées pour l'extraction incluent l'utilisation de

solvants organiques comme MeOH, EtOAc (Guerrero et al., 2008; Negro et al., 2003; Ruberto et al., 2007; Spigno and De Faveri, 2007; Pinelo et al., 2005; Makris et al., 2007; Monrad et al., 2010) ou supercritiques CO_2 (Pinelo et al., 2007; Vatai et al., 2008), pour la production de faibles fractions d'extraits mais de très grande pureté.

Les colles à bois à base de matières premières renouvelables sont étudiées depuis plusieurs années (Pizzi, 2006) et des travaux ont montré que les tanins condensés sont chimiquement et économiquement intéressants pour la préparation de colles et de résines destinées à la production de panneaux de particules et qu'ils pourraient également être utilisés comme substituts du phénol dans la production de colles de phénol-formaldéhyde (Tondi et Pizzi, 2009; Lei et al., 2008; Pichelin et al., 2006, Pizzi, 2003). Les tanins utilisés ici sont généralement extraits de l'écorce des arbres comme par exemple le mimosa, le québracho, le pin, etc., (Fradinho et al., 2002; Panamgama, 2007; Vazquez et al., 2000). Les tanins condensés extraits à partir de pulpe de raisin n'ont jamais été, à notre connaissance, étudiés pour la production de colles. Le but de ce travail est de proposer une utilisation économique et environnementalement viable de la pulpe de raisin. Ainsi l'extraction de tanins à partir de cette source naturelle a été réalisée en utilisant une solution aqueuse d'hydroxyde de sodium à différentes concentrations à 120°C et en absence de solvants organiques. Les fractions de tanins ont été caractérisées par analyse ^{13}C RMN et la réactivité avec le formaldéhyde a été évaluée à partir de la détermination du temps gel. Leur utilisation potentielle dans la formulation de colles vertes a été estimée à partir de la résistance mécanique d'échantillons de panneaux collés soumis à une analyse thermomécanique en flexion.

Les tanins obtenus par extraction de la pulpe de raisin avec une solution aqueuse d'hydroxyde de sodium à 10%, présentent des propriétés prometteuses et laissent présager leur application dans la formulation d'adhésifs.

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Short communication

Extraction of condensed tannins from grape pomace for use as wood adhesives

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ABSTRACT

The extraction of condensed tannins from grape pomace was examined using a mixture water–sodium hydroxide at 120 °C. The extracts were characterized by solution ^{13}C NMR and have showed characteristic consistent with that of condensed tannins with dominant procyanidin units. The tannin fractions reactivity toward formaldehyde was studied by gel time analysis and thermomechanical analysis in bending. It has been demonstrated that the extracts obtained by using 10% of NaOH (w/w) displayed promising properties for adhesive applications.

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1. Introduction

Agricultural by-product stream is an abundant and promising feedstock for industrial production of energy and materials since it pursues two major goals: environment protection and economic profit. Grape is one of the world's largest fruit crops (after orange). In France, about 700 000 tons of natural dry grape by-product, commonly called pomace, were produced in 2009 by the wine industries. After ethanol, seed and anthocyans recovery in the distilleries, the residue mainly consists in grape skins and is often used for animal feed or soil fertilizer. Because of a low extraction during winemaking, pomace retains high levels of condensed tannins mainly proanthocyanidins composed by procyanidin and prodelphinidin units that are linked together by a C4–C8 bond in grapes (Fig. 1). Thus, this by-product is not intrinsically hazardous but its high polyphenols content and also the fact that its production is concentrated in a short period of the year poses potentially pollution problems when it is used as soil fertilizer (Morthup et al., 1998). Since the first observations of the "French paradox" (Renaud and De Lorgeril, 1992), many recent studies have highlighted the beneficial effects of grape or wine polyphenols for human health (Shrikhande, 2000). As a consequence, considerable efforts have been devoted to optimizing the extraction of high purity phenolic compounds from winery by-products for food additive applications (Monagas et al., 2006) but also for cosmetic and pharmaceutical industries (Ozkan et al., 2004; Torres et al., 2002). The techniques generally employed used organic solvents (MeOH, EtOAc) (Guerrero et al., 2008; Negro et al., 2003; Ruberto et al., 2007; Spigno and De Faveri, 2007; Pinelo et al., 2005; Makris et al., 2007; Monrad et al., 2010) or supercritical CO_2 (Pinelo et al., 2007; Vatai et al., 2008) for the isolation of low yield/high purity extract fractions which were mainly studied as natural antioxidants.

Wood adhesives from renewable raw materials have been a topic of considerable interest for a long time but since few years this interest has intensified again (Pizzi, 2006). In previous works, we demonstrated that condensed tannins are both chemically and economically interesting for the preparation of adhesives and resins destined to the production of particleboards and that they could be successfully used as substitutes for phenol in the production of phenol–formaldehyde resins (Tondi and Pizzi, 2009; Lei et al., 2008; Pichelin et al., 2006, Pizzi, 2003). Condensed tannins used in the previously described studies are very generally extracted from the bark of various trees (mimosa, quebracho, pine, etc.; Fradinho et al., 2002; Panamgama, 2007; Vazquez et al., 2000). To the best of our knowledge, condensed tannins extracted from grape pomace have never been studied for the production of adhesives.

The aim of our study is to propose an economically and environmentally valuable utilization of grape pomace which is currently underexploited. Thus extraction of tannins from this resource was investigated using a process the solvent of which is water, without any organic solvent or reagent. The tannin extracts were characterized by ^{13}C NMR and we verified the possible use of these fractions as a component of a green adhesive formulation.

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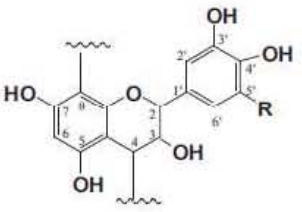

R=H : procyanidin ; R=OH : prodelphinidin

Fig. 1. Chemical structure of proanthocyanidins.

2. Materials and methods

The samples correspond to mixtures of by-products of the wine-making process of red grape variety (*Vitis vinifera*), growing in south-east of France and were provided by a French distillery (Franck Le Net, UDM). The air-dried grape pomace was composed by grape skin, seeds and stalks manually separated. It was stored at room temperature during the course of this study.

2.1. Soda treatments

Twenty grams (oven-dry matter) of grape pomace were treated with an aqueous solution of sodium hydroxide (NaOH) at 120 °C during 2 h (see Table 1 for treatment conditions), following the process depicted in Fig. 2. The solid-to-liquid ratio used was 1:8. The treatments were carried out in a 4560 Mini Bech Top reactors with a Parr 4836 temperature controller (Parr Instrument Company, Moline, IL). The reaction mixture was heated at a heating rate of ~5 °C/min with continuous stirring. At the end of the treatment, the pomace was cooled, washed and filtered through filter paper. In path 1, 3 N HCl was added to adjust the pH at a 1.5 value in order to precipitate the acidified tannins, which were collected by centrifugation and then air-dried. In path 2, the washed liquid was directly lyophilized to yield lyophilized tannins. A sample of each solid residue and liquid phase was separated and stored in a freezer at −5 °C before analysis.

2.2. Analytical procedures

Moisture content was determined using KERN MRS 120-3 infra-red moisture analyser. Carbohydrate and lignin contents were measured on extractive-free material (soxhlet extracted with dichloromethane overnight), ground to pass a 40-mesh screen, according to the laboratory analytical procedure (LAP). Samples were hydrolyzed with 72% sulfuric acid for 1 h and then autoclaved after being diluted to 3% sulfuric acid through the addition of distilled water. The autoclaved samples were filtered and the dried residue was weighed to give the Klason lignin content. Monosaccharide contents in the filtrate were quantified using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Pronto, 1998). The acid soluble lignin content was determined from absorbance at 205 nm according to Lin and Dence (1992). Total soluble polyphenols were spectrophotometrically determined using gallic acid as standard. 1 ml of dilute sample solution, 1 ml of 10% Na_2CO_3 (w:v) were added to a glass tube (10 ml) sealed with a PTFE screwcap. The tube was placed in a water bath at 38 °C for 10 min. Afterward, 1 ml of Folin reagent (1:2, v:v) was added, shaken, rested 15 min at room temperature in darkness and finally measured at 660 nm (UV-2500 PC series spectrophotometer).

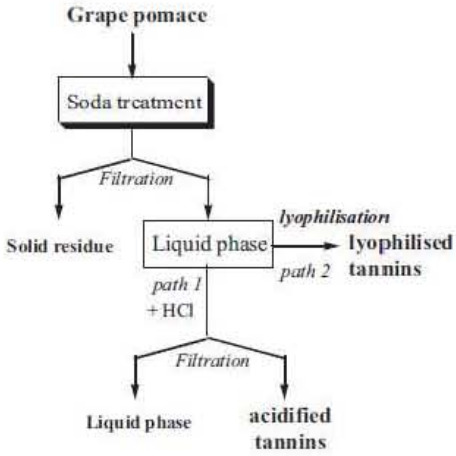

Fig. 2. Schematic of the treatment.

2.3. Stiasny number analyses

The Stiasny number reaction was used to determine the reactivity of tannins towards formaldehyde. According to Yazaki and Collins (1994) about 0.2 g (oven-dry mass) of tannins sample, 5 ml of 37% aqueous formaldehyde and 5 ml of 10 M hydrochloric acid solution (HCl) were mixed together and heated under reflux for 30 min. At the end of this reaction, the mixture was filtered through a sintered glass filter (filter no. 3) while it was still hot. The precipitate was dried to a constant weight in an oven at 105 °C. The Stiasny number was determined as a percentage of the weight of the precipitate to the weight of the starting sample.

Table 1
Experimental conditions for the treatment of grape pomace in water at 120 °C. Yields and Stiasny number of acidified (path a) and lyophilized (path b) tannins.

	NaOH %[a]	Acidified tannins (path 1)			Lyophilized tannins (path 2)			
		Yield[b] %	Stiasny number	Polyphenol % in solution[b,c]	Yield[b] %	Stiasny number	Gel time (pH)	MOE[d] (MPa)
Essay 1	5	4.85	95	4.7	23.8	46	174 (6.7)	2073
Essay 2	10	21.67	89	5.8	45.2	55	53 (8.7)	2996
Essay 3	15	31.4	97	10.7	58.8	50	100 (9.2)	1960
Essay 4	20	29.1	98	12.6	71.1	49	138 (9.6)	–
Essay 5	30	31.8	97	21.8	88.4	39	1111 (10.7)	–
Essay 6	40	25.8	98	23.2	101.5	32	∞ ((12.9)	–

[a] % based on weight of dry pomace.
[b] Yield calculated from the raw material (oven dry mass).
[c] Polyphenol content in the liquid phase.
[d] Modulus of elasticity.

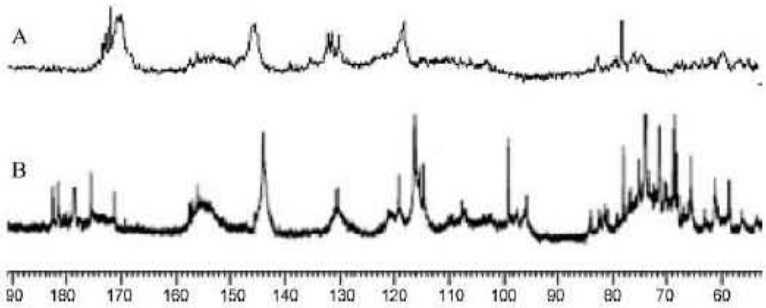

Fig. 3. ^{13}C NMR spectrum of acidified tannin (A) in d_6 DMSO and lyophilized tannin (B) in D_2O extracted from grape pomace.

2.4. Gel-time analyses

About 10 g of a 30% (w/w) of lyophilized tannins solution, 5% of powdered paraformaldehyde on a dry and solid tannins matter content basis were added to a test tube and placed in a water bath, which was maintained at boiling temperature (just below 100 °C) at normal atmospheric pressure. The time taken to reach the gel point was recorded during constant stirring with the aid of a wire spring and a stopwatch. The test was duplicated and the average value was reported. The pH of the mixture was measured using a microprocessor pH meter (Hanna pH 211). 33% of sodium hydroxide solution (NaOH) and hydrochloric acid solution (HCl 4 M) were used to adjust different pH of the mixture.

2.5. Modulus of elasticity analyses

Modulus of elasticity (MOE) of the tannins formulations were studied using thermal mechanical analyzer with TA controller (Mettler TMA 40). About 5 g of a 30% (w/w) of lyophilized tannins solution, 5% of powdered paraformaldehyde on a dry and solid tannins matter content basis were added and mixed together. 30 mg of the mixture was coated in one side of two small wood chips (5 mm × 20 mm), which were tested by three points bending TMA mode. The test condition: heating rate of 10 °C/min from 25 to 250 °C.

2.6. ^{13}C NMR analyses

^{13}C NMR experiments were performed on a Bruker Avance-400 spectrometer operating at a ^{13}C frequency of 100.59 MHz. Acidified tannins (100 mg) were dissolved in DMSO-d_6 (0.5 ml) and lyophilized tannins (100 mg) were dissolved in D_2O (0.5 ml) with slight heating. ^{13}C NMR spectra were acquired at 50 °C in order to reduce viscosity. 10,000 scans were collected with a pulse delay of 12 s. ^{13}C data were processed offline using XWinNMR processing software (Acorn NMR Inc).

2.7. Error analyses

The composition of the raw material and the fraction after soda treatments were established from two independent experiments performed using the same conditions. For all the samples, Klason lignin, acid soluble lignin and Stiasny number were calculated from analysis of duplicate samples. Error values <0.6% (standard deviation), <0.1% (standard deviation) and <2.5% (standard deviation) were, respectively, determined for the Klason lignin, acid soluble lignin and Stiasny number.

3. Results and discussion

Total polyphenol content of grape pomace was gravimetrically determined as Klason lignin (48.7%) and as soluble polyphenol (4.3%) by UV quantification. According to Rebole (1994) components such as tannins (condensed tannins) and proteins (as protein-tannins complexes or as Maillard products) are in the Klason lignin fraction. The pomace used in this study also contains 14.6 (±0.6)% of glucans, 6.14 (±0.5)% of other sugars and 7.0 (±0.2)% ash.

The tannins were extracted from grape pomace in basic medium at 120 °C according to Fig. 2.

Two different processes were used for tannins recovery:

- Path 1: addition of dilute HCl to precipitate the tannins which are collected by centrifugation and then air-dried.
- Path 2: lyophilization of the liquid phase to yield a crude residue.

The influence of soda concentration on the composition of the solid residue and on the yield and properties of extracted tannins was examined. Table 1 presents the results obtained including the yield of crude tannins recovered following paths 1 and 2 and the Stiasny numbers of the tannins fractions isolated which are an indication of the purity of the polyphenol extracts (Stiasny, 1906). As shown in Table 1, high yields of crude lyophilized tannins (higher than 100% for test 6) were obtained according path 2 because of the high sodium hydroxide content recovered after lyophilization.

The solution ^{13}C NMR spectra of the acidified and of the lyophilized tannins were performed in d_6 DMSO and D_2O, respectively. The acidified tannins spectrum was performed in DMSO because of its low solubility in water. The spectra are presented in Fig. 3A and B and were interpreted according to previous studies (Fu et al., 2007; Zhang et al., 2010). Lyophilized tannins (Fig. 3B) shows characteristic ^{13}C peaks consistent with that of condensed tannins with dominant procyanidin units and with a minor amount of prodelphinidin. Specifically, C5, C7, and C8a carbons of procyanidins appear between 160 and 150 ppm. Peaks at 145.3 and 145.5 belong to C3′ and C4′ of procyanidin units. A small amount of prodelphinidin is also detected as its C4′ peak at 131 ppm, overlapping with the chemical shifts of C1′. The cluster of peaks between 110 and 90 ppm is assigned to C8, C6, C6′, and C2′ of procyanidins. Relatively high carbohydrate content could also be deduced by the high intensity of the peaks at 65–85 ppm, the range characteristic of carbohydrates in tannin extracts. The acidified tannin (Fig. 3A) displayed a different pattern: a lower carbohydrate content and an intense signal at about 170 ppm assigned to carbonyl groups. The presence of carbonyl groups in acidified tannins could be rationalized by the well known catechinic acid rearrangement (Sears et al., 1974; Kiatgrajai et al., 1982) which would occur during the acidification step, according to Fig. 4. The formation of catechinic

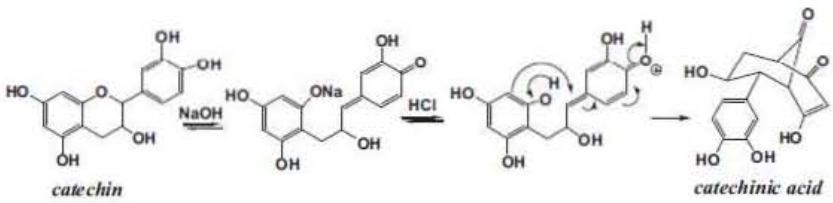

Fig. 4. Mechanism of formation of catechinic acid.

acid could also explain the low water solubility of these tannins fractions.

Regarding path 1, extracts bearing high Stiasny number values (95–98%) were recovered indicating a high phenolic content. In addition, analysis of the resulting liquid phases (see Fig. 2) reveals:

- Very low monosaccharides contents in the water effluents (<0.3%, w/w determined by ionic chromatography, data not shown) suggesting an extensive degradation of sugars in during the process.
- The presence of soluble phenolic compounds; as seen in Table 1, the gradual increase in NaOH concentration of the medium causes a gradual increase in the soluble phenol concentration in the liquid phase.

For lyophilized tannins (path 2) Stiasny numbers between 32 and 55% were observed. The low polyphenol content of these extracts is rationalized by (1) the high sodium hydroxide content of the crude hydrolysate and (2) the important quantity of non-tannin fractions extracted from pomace and recovered. This observation is in agreement with the NMR results regarding the important amount of sugars (or sugar derivatives) detected in the lyophilized tannins samples. As a result, for high sodium hydroxide concentration (tests 5 and 6) large non-tannin fractions were extracted which did not react with formaldehyde (Pizzi, 1994). Apparently tannins yield is affected by the sodium hydroxide concentration. Table 1 shows that reacting the pomace in NaOH 5% (w/w) generated low amounts of tannins. When the concentration was increased to 15%, the tannin yield markedly increased to about 30% (w/w) of pure polyphenols.

Gel time is defined as the point at which the polycondensates formed by the reaction of a tannins solution in water and formaldehyde become an elastic, rubbery solid. This is an indication of the reactivity of the sites of tannins molecules toward formaldehyde. Gel time is highly dependent on the pH of the tannins solution: the gel time generally decreases when the pH of the tannin solution is increased.

Concerning acidified tannins (path 1), because of the low solubility in water of the fractions and the low reactivity of these tannins toward paraformaldehyde, it was not possible to establish gel times. This behavior is in agreement with the catechinic rearrangement previously proposed which should affect the more reactive A aromatic ring of the catechin moiety and consequently should decrease the water solubility (as previously noticed) and the reactivity toward paraformaldehyde. Concerning lyophilized tannins (path 2) the gel time results of tannins formaldehyde-based glues are given in table accompanied with the pH of the tannins solutions in water (30%, w/w tannin/water). It appears that the gel times are directly correlated with the Stiasny number of the tannins fractions and a dramatic increase in the gel time was observed when high sodium hydroxide concentrations were employed. Fig. 5 gives the gel times of 3 lyophilized tannins extracts (tests 1, 2 and 3) as a function of pH of the tannins solution. The pH of each tannins solution was adjusted by addition of sodium hydroxide or HCl solution (see Section 2). The best result was observed for test 2 using a concentration of NaOH of 10% (w/w) during the extraction process: in these conditions, extracts bearing short gel times were isolated (72 s at pH 8 and 32 s at pH 10). Compared to previously described results, this tannins fraction cannot reach the gel times yet obtained at pH 8 for pine tannins and pecan nut tannins, which are equal to 26 and 22 s, respectively (Pizzi, 1994). However, despite their lower purity (about 50% of active phenolic ingredients), this grape pomace extract displays gel time values lower than the ones of mimosa and quabracho tannins, demonstrating the good reactivity of these crude tannins toward paraformaldehyde. Compared to acidified tannins, the higher reactivity of the lyophilized extract is probably due to the low catechinic acid content observed by NMR.

The three formulations of lyophilized tannins solutions (tests 1–3)—paraformaldehyde were scanned by thermomechanical analysis in bending according to a technique already reported (Laigle et al., 1998). The curves of the modulus of elasticity (MOE) as a function of increasing temperature for the three resins are given in Fig. 5. The maximum MOE values are also reported in Table 1. The higher modulus of elasticity (MOE) was reached for test 2 (NaOH = 10%, w/w). The value indicates that the tannins fraction isolated in these conditions gave relatively high wood-joint strength. This is in accordance with gel time results, demonstrating a good correlation between Stiasny number of the tannins extracts and the gelation properties of the resulting resins. Moreover, concerning test 2, a signal is observed at about 100 °C (Fig. 6) which corresponds to a first cross-linking reaction through formation of a non-stable cross-linker. This behavior is a characteristic of several phenolic resins (Garcia and Pizzi, 1998). In this precise case, the resin quickly reticulated involving the more reactive tannins molecules with formation of unstable methylene–ether cross-linkers ($-CH_2OCH_2-$). Because of their instability and the

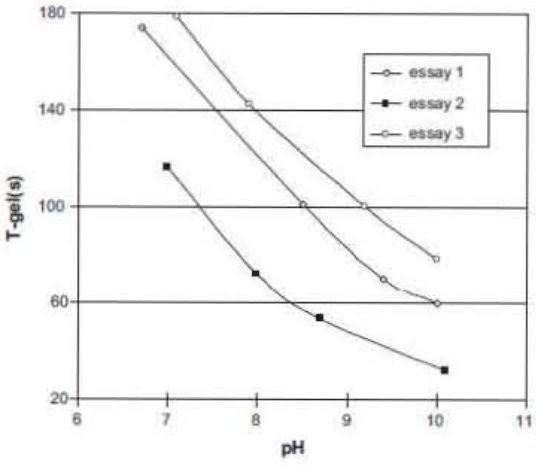

Fig. 5. Gel time of lyophilized grape pomace tannin extracts reacted with paraformaldehyde at pH ranged from 7 to 10 based on 40% concentration.

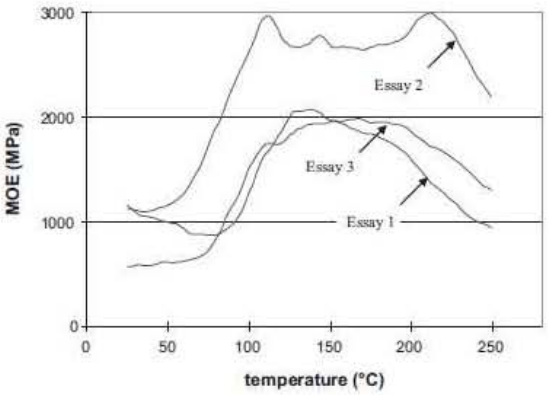

Fig. 6. TMA measuring MOE as a function of temperature to describe the curing of resins of lyophilized grape pomace tannins/paraformaldehyde.

increase of the temperature, they break to form a more stable methylene cross-linker (–CH_2–) with emission of formaldehyde able to react with phenolic material.

To conclude, we examined for the first time the extraction of condensed tannins from grape pomace for the production of adhesives. The process, which only used water–sodium hydroxyde at 120 °C, produced relatively high extract yields. The condensed tannins extracted were characterized by ^{13}C NMR and showed characteristics consistent with that of condensed tannins with dominant procyanidin units. The tannins fractions reactivity toward formaldehyde was studied by gel time analysis and thermomechanical analysis in bending. We demonstrated that (1) the extracts obtained by precipitation in acidic conditions exhibit a high phenolic content but a low water solubility and a low reactivity toward formaldehyde because of the formation of catechinic acid during the acidification step; (2) the tannins extracts obtained by lyophilization of the liquid, despite of their lower phenolic contents, displayed promising properties for adhesive applications. The best result was observed for a NaOH concentration of 10% (w/w). Extension of these first results and their application to the production of wood panels in which the binder is composed of grape pomace extracts is actively being pursued. The final goal of this study is to propose an economically valuable utilization of grape pomace which is currently largely underexploited and to allow an improved use of this resource.

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III.2. COLLES POUR PANNEAUX A BASE DE TANNIN ET LIGNINE

III.2.1. **Colles pour panneaux bois à base de tannin et lignine de bas poids moléculaire sans colle synthétique**

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Les tanins, la lignine puis plus récemment les protéines de soja sont utilisées dans la préparation de colles naturelles depuis plusieurs années (Pizzi, 2006). Toutefois, dans les deux derniers cas, les matières premières nécessitent l'ajout de 20 à 40% de durcisseurs tels le pMDI ou une colle phénol-formaldéhyde pour satisfaire aux exigences des normes internationales en termes de performances (Pizzi, 2006; Lei et. al, 2008; Pizzi et Stephanou, 1993; El Mansouri et al., 2007; Lorenz et al., 2006; Wescott et al. 2006; Amaral-Labat et al. 2008). Cet ajout de durcisseur limite l'utilisation de ce type de colles du fait des contraintes environnementales de plus en plus pressantes.

En considérant ces faits, l'objectif de ce travail a été de préparer une colle à base de matériaux d'origine naturelle (lignine et tanin), intéressants d'un point de vue économique et environnemental et qui soit capable de répondre aux exigences des normes.

La matière première utilisée est une lignine de faible poids moléculaire obtenue industriellement à partir de paille de blé par un processus organosolv avec des acides formique et acétique. Cette lignine, provenant de l'entreprise CIMV (France), a été utilisée en l'état après purification. Le tanin utilisé est un produit commercial provenant du mimosa et conditionné par l'entreprise SILVA (Italie).

Des formulations de colles à partir de mélanges de tanin et de lignine (à hauteur de 50%) ont été mises au point. Les matériaux polymères respectueux de l'environnement, d'origine naturelle et non toxiques constituent ainsi plus de 94% de la colle du panneau. Le panneau lui-même est constitué par 99.5% de matériaux naturels en tenant compte de la part des particules. Les 0.5% restant correspondent au glyoxal, un aldéhyde non toxique et non volatil, et à l'hexamine qui ne produit pas de formaldéhyde en présence d'espèces très réactives comme les flavonoïdes du tanin par exemple.

Dans les formulations développées lors de ce travail, on a montré que deux types de panneaux remplissent les conditions énoncées par les normes pour panneaux de bois d'utilisation intérieure.

La lignine organosolv non purifiée, qui présente un moindre coût, conduit à des résultats semblables à ceux obtenus à partir de la lignine purifiée, plus onéreuse.

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Wood Panel Adhesives from Low Molecular Mass Lignin and Tannin without Synthetic Resins

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Abstract

Mixed interior wood panel tannin adhesive formulations were developed in which lignin was in considerable proportion, 50%, of the wood panel binder, and in which no 'fortification' with synthetic resins, such as isocyanates or phenol-formaldehyde resins as used in the past, was necessary to obtain results satisfying relevant standards. A low molecular mass lignin obtained industrially by formic acid/acetic acid pulping of wheat straw was used. Environment-friendly, non-toxic polymeric materials of natural origin constitute up to 94% of the total panel binder. The wood panel itself is constituted of 99.5% natural materials, the balance 0.5% being composed of glyoxal, a non-toxic and non-volatile aldehyde, and of hexamine already accepted as a non-formaldehyde-yielding compound when in presence of very reactive chemical species such as a flavonoid tannin. Particleboards and two types of plywoods were shown to pass the relevant interior standards with such adhesive formulations. Moreover, the much cheaper non-purified organosolv lignin showed the same level of results as the more expensive purified type.
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Keywords

Wood adhesives, tannin, lignin, particleboard, plywood, wood panels

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1. Introduction

Biocomposites have been an area of growing interest and a subject of active research for quite some time. This is due to both environmental concerns as well as anticipated future scarcity of oil and oil-derived products. A class of composites in which resins of natural origin have already had a commercial/industrial impact is in the field of rigid wood panels, such as particleboards. Natural-origin resins have already been used commercially for the last 30 years for these wood panels, and their use is still growing, although still relatively slowly [1]. In such an application, the binder is never more than 10% by weight of the whole composite panel. This is sufficient to conform to the performance and costs required by the wood panels industry and their respective product standards.

The main natural resins used as wood panel binders are vegetal tannin adhesives, lignin adhesives and more recently also soy protein adhesives [1]. Of these, tannin-based adhesives have been used commercially the longest, since 1971. They offer the advantage over the other two types of not needing any reinforcement with an oil-derived synthetic resin [1]. Lignin [2–5] and soy binders [1, 6–8], however, still require between 20% and 40% of the total resin to be either phenol-formaldehyde or most often PMDI (polymeric isocyanate) to satisfy the requirements of relevant board standards.

The use of synthetic resins limits somewhat the environmental attractiveness of such adhesives based on purely natural materials, while the use of tannins alone is limited at present by the relatively limited supply of these materials [1]. Thus, the aim is to prepare an adhesive based on materials of natural origin, satisfying international standards for both performance and emission, which does not emit or even better does not contain any formaldehyde, the composition of which does not include any synthetic resins, and that is less costly and uses widely available materials. This will render wood panel adhesives based on natural materials much more acceptable both economically and environmentally.

This paper, thus, deals with the preparation of lower cost wood panel adhesives with good performance based on natural materials and needing no fortification by any synthetic resins.

2. Experimental

2.1. Preparation of Glyoxalated Lignin Resin

The lignin used was a low molecular mass lignin obtained industrially as a byproduct of pulp and paper production from wheat straw by an acetic acid-based organosolv process [9]. Two types of lignins were used, one washed and purified and the other without washing. In this article we call this second type 'impure' lignin. These two lignins were supplied by their industrial manufacturer, CIMV (Compagnie Industrielle de la Matiere Vegetale, Reims Factory, France). The characteristics of these two lignins were determined by the FCBA (Table 1) and by CIMV (Table 2).

Table 1.
Characteristics of wheat straw organosolv lignin

	Formyl (mmol/g)	Acetyl (mmol/g)	Free hydroxyls (mmol/g)	Total (mmol/g)
Total –OH groups	0.6	0.5	2.90	4.00
Phenolic –OH groups	0.20	0.05	0.85	1.10
Aliphatic –OH groups	0.40	0.45	2.05	2.90

Table 2.
Additional characteristics of wheat straw organosolv lignin

	Klason lignin		Total lignin (%)	Ashes (%)	Glucan (%)	Xylan (%)	Mannan (%)	Galactan (%)	Arabinan (%)	Total without glucan (%)
	Insoluble (%)	Soluble (%)								
Non-purified	79.3	3.1	82.4	3.49	5.57	5.80	0.15	1.01	1.58	8.54
Purified	82.7	2.22	84.9	1.83	4.49	5.98	0.38	0.97	1.45	8.78

295 parts by mass of this lignin powder (96% solid) were slowly added to 477 parts of water while sodium hydroxide solution (30%) was added from time to time, thus, keeping the pH of the solution between 12 and 12.5 for better dissolution of the lignin powder which was also facilitated by vigorous stirring with an overhead stirrer. A total of 141 parts by mass of 30% sodium hydroxide aqueous solution were added which resulted in a final pH close to 12.5.

A 2-liter flat-bottom flask equipped with a condenser, a thermometer and a magnetic stirrer bar was charged with the above solution and heated to 58°C. 87.5 parts by mass glyoxal (40% in water) were added and the lignin solution was then continuously stirred with a magnetic stirrer/hot plate for 8 h. The solids content for all glyoxalated lignins was around 31%. Glyoxal is a non-volatile non-toxic aldehyde that has been tested in lignin [5, 6], tannin [10] and other adhesives [8] for application to wood panels such as particleboards. Glyoxal is a non-toxic (LD50 rat $\geqslant$ 2960 mg/kg; LD50 mouse $\geqslant$ 1280 mg/kg) [11], non-volatile aldehyde but less reactive than formaldehyde which is toxic (LD50 rat $\geqslant$ 100 mg/kg; LD50 mouse $\geqslant$ 42 mg/kg) [12].

2.2. *Tannin, Final Resin and Glue-Mix*

Mimosa tannin extract (origin: Tanzania. Supplied by: Silva, S. Michele Mondovi', Italy) of Stiasny value of 92.2 [13–16] was used. The tannin solution in water was prepared at 45% concentration and its pH adjusted to 10.4 with 33% water solution of NaOH. The high pH was chosen as the hardener used performs best at such a

pH [15, 17–19]. The glue-mix was composed of (i) the tannin extract solution to which was added as hardener 5% hexamethylenetetramine (hexamine) (hexamine solids on tannin solids) as a 30% hexamine solution in water and (ii) the glyoxalated lignin solution. The proportion of tannin solids: glyoxalated lignin solids was 50:50 by weight. Hexamine hardener has now been accepted by JIS A5908 as not being a formaldehyde source in presence of a polyflavonoid condensed tannin [17, 21–23]. The glue mix consisted of the solution of the 50:50 tannin/hexamine: glyoxalated lignin resin, adjusted to pH = 10.0, to which was added 200 mesh olive stone flour (38% on total resin solids) as filler.

The total amount of natural material on dry resin solids was almost 94% and the adhesive so prepared did not contain any synthetic resins.

2.3. Wood Panels (Particleboards and Plywoods) Manufacture and Testing

Duplicate one-layer laboratory particleboards of dimensions 350 × 300 × 14 mm were prepared using a mixture of core particles of beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*) woods at 28 kg/cm^2 maximum pressure and 190°–195°C press temperature. The wood particles before resin addition had a moisture content of 2%. The resin solids loading on dry wood was maintained at 10% of the total mix of tannin + glyoxalated lignin. The total press time was maintained at 7.5 min. All particleboards were tested for dry internal bond (IB) strength. The IB strength test is a relevant international standard test [24] done on 5 board specimens and is a tension test perpendicular to the plane of the board. Thus, each IB and panel density result in the tables is the average of 10 specimens.

Three-ply triplicate wood panels of dimensions 500 × 300 × 14 mm and of 400 × 400 × 6 mm were prepared. The former were composed of pine wood (*Pinus sylvestris*) veneers of, respectively, 3.5 mm and 7 mm thicknesses for the two lower plys and of an oak (*Quercus* spp.) veneer of 3.5 mm thickness for the upper veneer giving 14 mm total plywood thickness. These panels were suitable for floor covering. The second type of plywood panels were composed of three 2 mm thick beech (*Fagus sylvatica*) veneers.

The liquid glue-mix spread used was 380 g/m^2 d.g.l. (double glueline), press temperature was 130°C, and pressure was 15 kg/m^2 for both types of panels. Press time was 5 min for the thicker panel type, this being rather short, and 4 min for the thinner one. These press times were chosen because industrial plywood panels are manufactured with press times calculated as 1 min/mm panel thickness to the furthest glueline from the press hot platen + 2 min. The panels were tested for dry tensile strength according to European Norm EN 314 [25].

2.4. CP-MAS ^{13}C NMR Spectroscopy

The glyoxalated organosolv lignin resins were hardened at 105°C for 2 h in an oven before being ground finely for NMR analysis. The hardened lignin resins were analyzed by solid state CP MAS ^{13}C NMR. Spectra were obtained on a Bruker AVANCE II 400 MHz spectrometer at a frequency of 100.6 MHz and a sample spin

of 12 kHz, using a recycling delay time of 1 s and a contact time of 1 ms. Number of transients was about 15 000, and the decoupling field was set at 78 kHz. Chemical shifts were determined relative to tetramethylsilane (TMS) used as control. The spectra were accurate to 1 ppm. The spectra were recorded with suppression of spinning side bands.

2.5. Thermomechanical Analysis (TMA)

The hardening reaction of a single resin system or mixtures can be evaluated by TMA, by monitoring the rigidity of a bonded wood joint as a function of temperature. Thus, glue-mixes of glyoxalated purified and non-purified lignins with tannin/hexamine were analysed by TMA. The compositions of different glue-mixes are given in the tables. All experiments were conducted under the same conditions: heating rate = 10°C/min, 30 mg resin, in the temperature range 25–250°C. The thermomechanical analyzer used was a Mettler Toledo TMA40. The software used for data treatment was STARe. Deflection curves that allow modulus of elasticity (MOE) determination were obtained in the three-point bending TMA mode. The MOE of the wood joints bonded with different resin systems gave a good indication of the final strength of the adhesive system tested.

2.6. Molecular Weight and Molecular Weight Distribution

The number average molecular masses of the two lignins studied were determined by GPC. These were obtained as 1034 and 1028 for the non-purified and purified lignins, respectively. The effluent of the industrial CIMV pilot plant in Reims, France, after washing in water was monitored at 280 nm wavelength with a Beckman UV detector. The column (TSKgel G3000 PWXL) was calibrated with poly(ethylene glycol) in the 138–40 000 g/mol range. The flow rate of the $NaNO_3$ solution (0.1 M at pH 7) was 0.6 ml/min, and the samples were dissolved in $NaNO_3$ at a concentration of 3 mg/ml. The detected signals were digitalized at a frequency of 2 Hz, and the molecular weight distribution was calculated from the recorded signals with normal GPC calculation procedures.

Examination by MALDI-TOF (Matrix assisted laser desorption/ionisation time-of-flight) mass spectrometry showed, however, that GPC determination of the lignin molecular mass produces much higher values than the real values, thus, the values of Mn from GPC result also from molecular associations as already shown for other water soluble polymers [26]. MALDI-TOF results are shown in Fig. 1, indicating that molecular masses only as high as that of tetramers are present and, thus, are lower than those obtained by GPC. The oligomers composing the non-purified and purified lignins are the same and in the same proportion. Their types are listed in Table 3.

The MALDI-TOF spectra were recorded on a KRATOS Kompact MALDI AXIMA TOF 2 instrument. The radiation source was a pulsed nitrogen laser with a wavelength of 337 nm. The time period of one laser pulse was 3 ns. The measurements were carried out using the following conditions: polarity: positive; flight path:

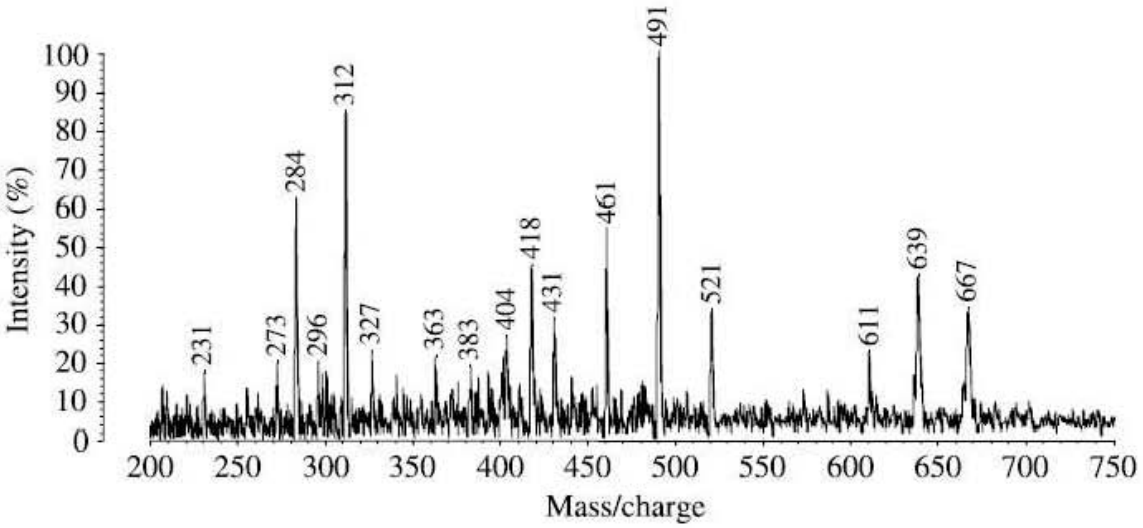


Figure 1. MALDI-TOF mass spectrum in the 200–700 Da range showing distribution of main oligomers in the low mlecular weight organosolv lignin used.

linear; mass range: high (this meant using a high acceleration voltage = 20 kV), and 100–150 pulses per spectrum. The delayed extraction technique was used by applying delay times of 200–800 ns.

For sample preparation, the lignin water solution samples were dissolved in acetone (4 mg/ml, 50/50 vol%) and mixed with an acetone solution (10 mg/ml in acetone) of the matrix. As the matrix 2,5-dihydroxy benzoic acid was used. For the enhancement of ion formation, NaCl was added to the matrix (10 mg/ml in water). The solutions of the sample and the matrix were mixed in the proportion 3 parts matrix solution + 3 parts sample solution + 1 part NaCl solution, and 0.5 to 1 µl of the resulting solution mix were placed on the MALDI target. After evaporation of the solvent the MALDI target was introduced into the spectrometer. The dry droplet sample preparation method was used.

2.7. Phenolic Hydroxyl Groups Determination by UV Spectroscopy (Δε Method)

The concentrations of the various phenolic units in the lignin samples were determined by UV spectroscopy, as described by Zakis [27]. This method is based on the difference in the absorptions at 300 and 360 nm between phenolic units in neutral and alkaline solutions. The concentration of ionizing phenolic hydroxyl groups can be quantitatively evaluated by comparing with the respective model compounds with a free ortho position in their aromatic ring.

3. Results and Discussion

Organosolv lignin obtained with a mixed formic and acetic acids pulping technique [9] is bound to present a number of formylated and acetylated phenolic and aliphatic hydroxyl groups. The results in Table 1 confirm this by the evidence of esterification by formyl and acetyl groups mainly of the aliphatic but also of the phenolic

Table 3.
MALDI-TOF derived distribution of oligomers in the organosolv lignin used for the adhesive

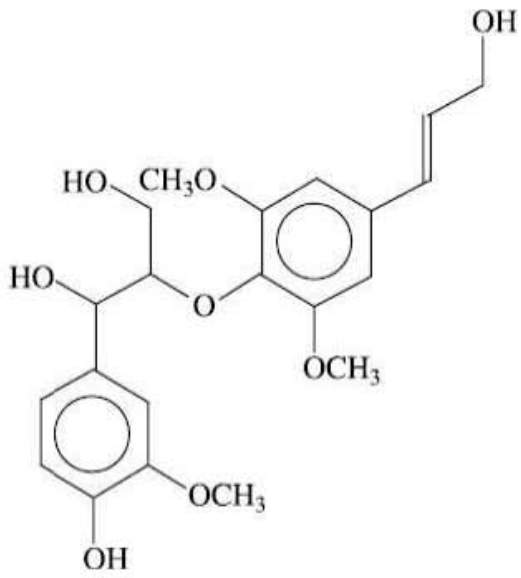
	Structure	Calculated MW	Calculated MW + Na	Experimental MW + Na	Peak intensty (%)
S	monomer	210	233	231	18
H-β1-H-1x-OH	dimer	261	284	284	63
H-β1-G G-β1-H	dimers	273	296	296	18
326-1x-CH_2– (demethylation)	dimers	289	312	312	85
H-β1-S G-β1-G S-β1-H	dimers	303	326	327	22
H-βO_4-S G-βO_4-G S-βO_4-H	dimers	360	360	383	18
G-$\beta\beta$-G G-β5-G G-55′-G	dimers	394.4	417.4	418	43
G-βO_4-S G-αO_4-S	dimers	407	430	431	32
S-βO_4-S S-αO_4-S	dimers	437	460	461	54
S-βO_4-S + 2x-OH S-αO_4-S + 2x-OH	dimers	468	491	491 521	100
S-βO_4-S-$\beta\beta$-H G-βO_4-G-$\beta\beta$-S G-βO_4-G-β5-S G-βO_4-S-55′-G	trimers	585	608	611	21
G-βO_4-S-$\beta\beta$-S G-βO_4-S-β5-S G-βO_4-S-55′-S	trimers	615	638	639	40
S-βO_4-S-$\beta\beta$-S S-βO_4-S-β5-S S-βO_4-S-55′-S	trimers	644.7	667.7	667	34

G = lignin guaiacyl units; S = lignin siringyl units; H = lignin phenolic units. Linkage between units indicated as βO_4, αO_4, $\beta\beta$, β5, 55′ [30].

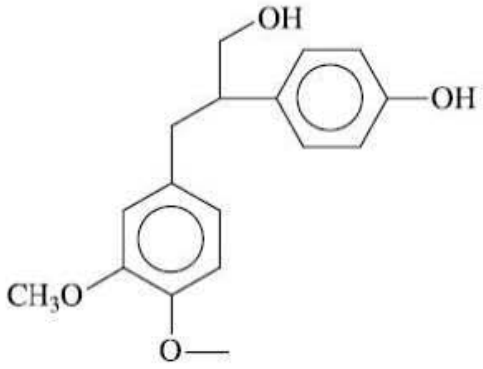
hydroxyl groups of lignin. These aliphatic and aromatic formic and acetic acid ester groups are of interest as the presence of esters, even in small amounts, has been shown to accelerate the reaction and curing of phenolic/aldehyde resins [3, 4, 28] and this was found also in the case of slow curing lignin-based resins [3, 4].

Their presence in this type of organosolv lignin is, therefore, of some benefit. The proportions of acid soluble and Klason lignin shown in Table 2 indicate that in the case of both the purified and non-purified wheat straw organosolv lignins the level of total lignin is high. Not much differences are seen between the two lignins, and even the values of their number average molecular masses are very similar at 1028 and 1034 g/mol. The percentage distribution of residual sugar oligomers is also similar (Table 2) with glucans and xylans from hemicelluloses and also from cellulose predominating. The major difference being observed is in the percentage of inorganic ashes. In these cases the ashes are the cations of residual acetic and formic acid salts which derive from the precipitation and washing of the residual lignin with an aqueous NaOH solution [9].

A word of caution must be given on the GPC results regarding molecular masses. MALDI-TOF examination of the same two lignins (Fig. 1) showed that the masses of the highest oligomers are lower than those observed by GPC. Associations of molecules by secondary forces often cause molecular aggregates to form. This leads to finding higher mass values, which are incorrect, when using GPC for mass determination [26, 29]. It is nonetheless of interest to observe the type and relative proportion of the oligomers existing in this organosolv lignin as listed in Table 3. From this one can see that dimers and trimers of different types predominate, but that no oligomers of higher masses are present, confirming that the Mn results obtained by GPC are too high and are due to association. As lignin is composed of guaiacyl (G), siringyl (S) and phenolic (H) repeat units, so in Table 3 the interpretation of the compounds detected is indicated as G for guaiacyl units, S for siringyl units, and H for phenolic units not containing methoxy groups. The linkages between them are indicated with the 'classical' nomenclature [30] as βO_4, αO_4, $\beta\beta$, $\beta 5$, $55'$. Thus, as an example, the G-βO_4-S and G-αO_4-S peak at 431 Da in Fig. 1 corresponds to the structure



and the H-β1−G peak at 296 Da corresponds to the structure



The ^{13}C NMR analysis confirms the similarity of the two lignins. In Fig. 2 the NMR spectra show that the differences between the two lignins exist but they are rather small. Thus, in Fig. 2 the band at 55 ppm characteristic of the methoxy groups ($-OCH_3$) of lignin bound to the aromatic ring is slightly less intense for the purified lignin. As this was purified at 50°–60°C some demethoxylation occurred,

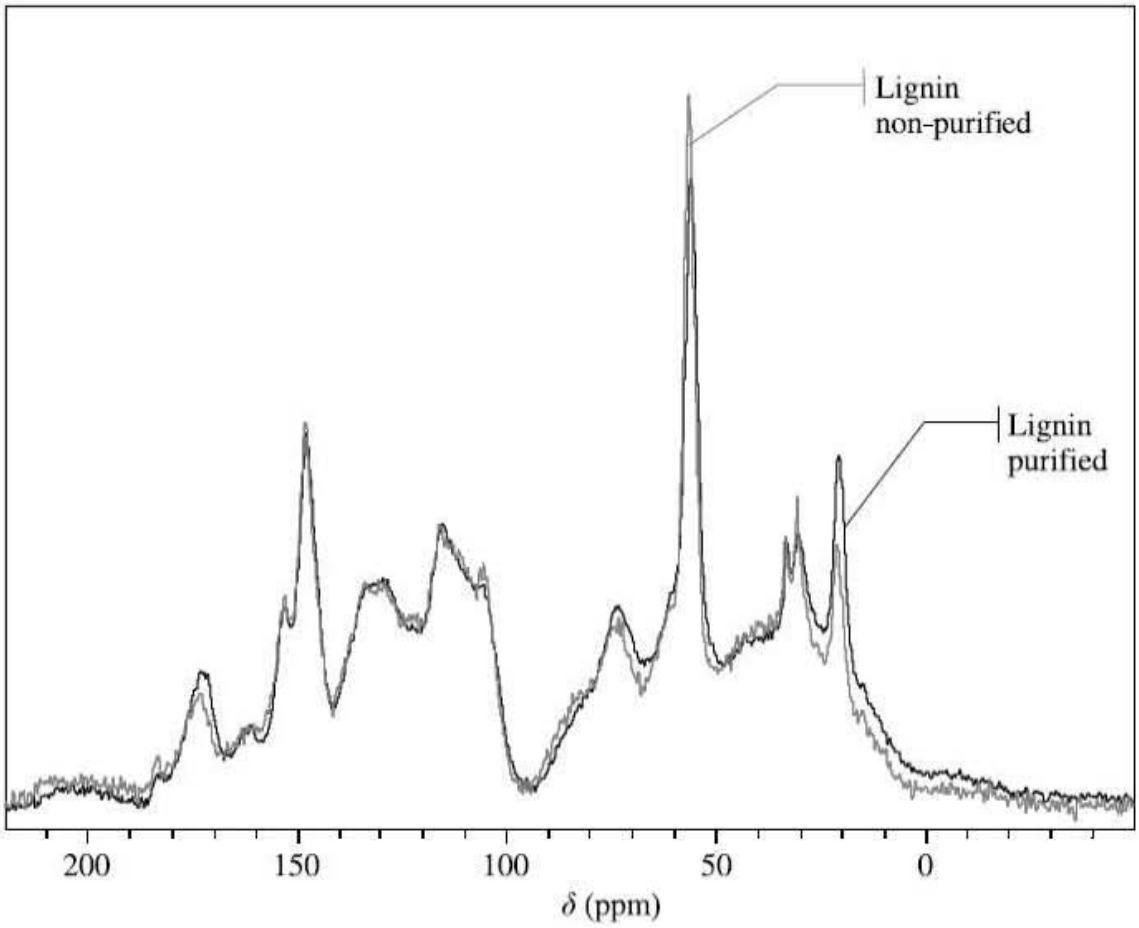


Figure 2. Comparative CP-MAS ^{13}C NMR spectra of purified and non-purified organosolv lignins derived from formic acid/acetic acid wheat straw pulping.

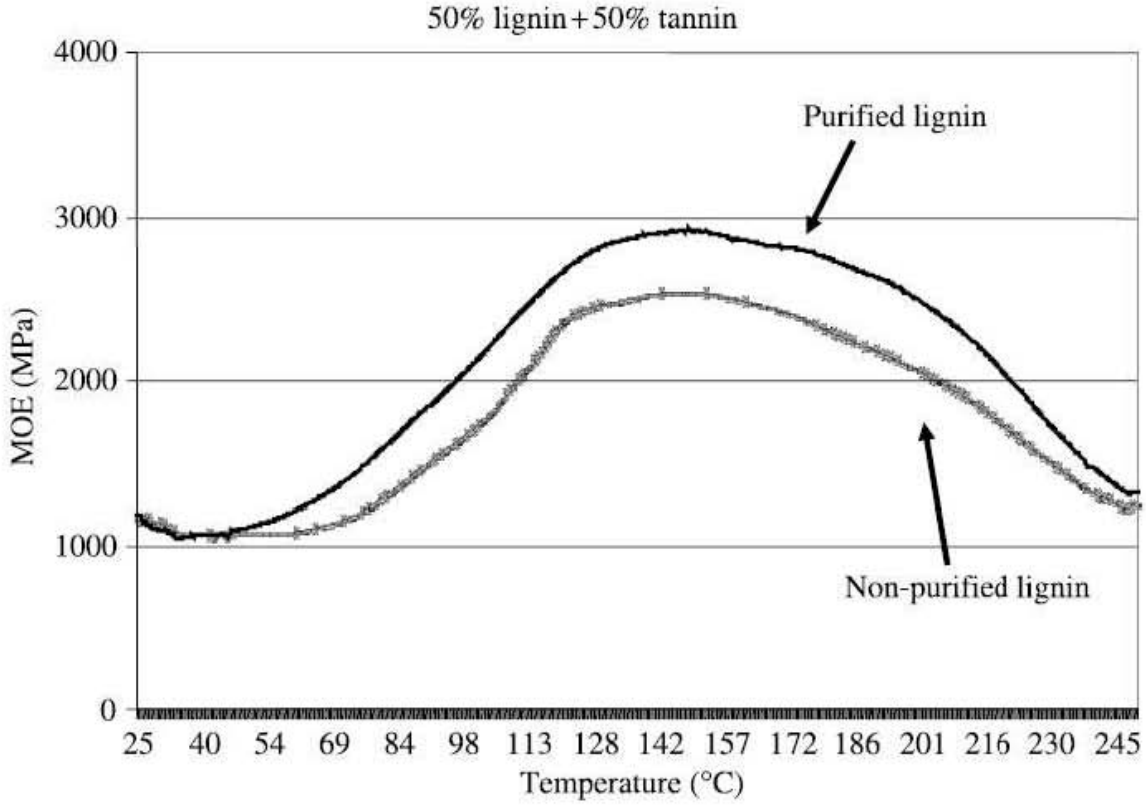


Figure 3. Comparison of thermomechanical analysis curves of beech wood joints bonded with tannin/lignin 50/50 wood adhesive. Variation of the joint MOE as a function of temperature.

a very common reaction in lignin [30]. Equally, the higher relative proportion of acetic/formic acid esters in purified lignin are different as shown by the peaks at 21 and 30 ppm of the carbon of the methyl group $-CH_3$ of, respectively, the acetic acid esters and free acetic acid carboxyl ion, and the less noticeable increase of the C=O peak of the formic acid esters at 161 ppm and of the free formic acid carboxyl ion at 172 ppm.

The results of the thermomechanical analysis indicate that the purified organosolv lignin gives higher Modulus of Elasticity (MOE) values than the non-purified one (Fig. 3). However, the result for non-purified lignin is only lower in appearance. In reality, the impurities present in the non-purified lignin decrease the amount of actual binding material on the beech laminae in the TMA tests. Such a slightly lower amount of actual binding resin yields a lower TMA maximum MOE value for the non-purified lignin, due to the well-known effect of TMA to exaggerate the differences in results [31, 32]. That this is indeed the case is shown by the results of actual wood panels bonded with these adhesives (Tables 4 and 5). The MOE starts to decrease after 170°C in the TMA curves (Fig. 3) due to the start of the degradation of wood constituents, as shown previously [23]. This does not affect board strength as at the very short press times used for panel manufacture the maximum temperature reached in the board core, which determines IB strength, is no higher than 120°C.

Table 4.
Results on particleboards bonded with tannin/lignin adhesives

	Proportions	Density (kg/m^3)	Dry IB strength (MPa)
Tannin/hexamine + glyoxalated lignin (non-purified)	50/50	702	0.52 ± 0.05
Tannin/hexamine + glyoxalated lignin (purified)	50/50	700	0.45 ± 0.04
Tannin/hexamine + glyoxalated lignin (non-purified)	40/60	710	0.35 ± 0.04
Tannin/hexamine + glyoxalated lignin (non-purified)	30/70	700	0.18 ± 0.03
European Norm EN 314-2 requirement			$\geqslant 0.35$

IB: Internal bond.

Table 5.
Results on plywoods bonded with tannin/lignin adhesives

	Proportions	Dry tensile strength (MPa)	Wood failure (%)
Beech Plywood			
Control tannin/hexamine	100	$1.4 + 0.10$	0
Tannin/hexamine + glyoxalated lignin (non-purified)	50/50	$1.2 + 0.20$	10
Thick floor plywood			
Tannin/hexamine + glyoxalated lignin (non-purified)	50/50	1.0 ± 0.19	46
EN 314-2 requirements		$\geqslant 1.0$	$\geqslant 0$
		$0.6 \leqslant$ and < 1.0	$\geqslant 40$

The results for wood particleboard prepared with the mixed formulations of tannin/hexamine + glyoxalated lignin are shown in Table 4. The internal bond (IB) strength of a panel is a direct measure of the performance of the adhesive. The results in Table 4 indicate that no significant difference exists as regards internal bond (IB) strengths of the panels prepared using the two lignins according to relevant interior panel standards [24]. When the two resins are mixed 50/50 by weight the results are adequate to satisfy international standard requirements for interior grade boards (Table 4). However, a decrease in the relative proportion of tannin/hexamine results in a rapid decrease in the IB strength of the panels (Table 4). As the panel at tannin: lignin = 40:60 has an IB strength equal to the minimum value required by the standard, the indications are that to use formulations in which the amount of tannin is less than about 45% of the total would not be feasible at the industrial level. One consideration that is of particular interest is that the results obtained with the

non-purified lignin are comparable and equally good as those of the formulations using the purified one. This is a considerable advantage as the unpurified, unwashed lignin is considerably cheaper to produce.

The results for two types of plywoods, 6 mm thick standard beech plywood, and 14 mm thick flooring type mixed veneers plywood are reported in Table 5. The European Norm EN 314-2 [25] for interior grade plywood specifies that the percentage wood failure and dry tensile strength obtained on testing of the panel be evaluated on a scale. Thus, the percentage wood failure for a dry tensile strength can be 0% for any strength results $\geqslant$ 1.0 MPa. Equally, if the dry tensile strength is higher than 0.6 MPa but lower than 1.0 MPa the percentage wood failure required is $\geqslant$40%. Table 5 shows that both plywoods pass well such standard requirements, and that the tensile strength value of tannin/lignin bonded panel is only slightly lower than that obtained with the tannin alone.

It is of interest to understand the main mechanisms according to which the formulations work. As lignin is of much lower reactivity than a flavonoid tannin, a series of parallel reactions occur to yield coreaction of tannin with lignin. Thus, tannin reacts very rapidly in the hot press with hexamine to form a network based on the known reactions of tannin with hexamine [20–23]. Lignin, much slower, is prereacted in a reactor with glyoxal [1, 2]. As some condensation of glyoxalated lignin occurs during the reaction, so if the reaction is prolonged it will increase unduly the viscosity of the glyoxalated lignin, so condensation needs to be minimized and addition of glyoxal in the lignin maximized. The glyoxalated lignin is, thus, rich in methylol-type groups obtained by glyoxal addition, but the aromatic nuclei of lignin are still too slow for glyoxalated lignin alone to condense to a sufficiently hardened network in just the very brief period the board remains in the hot press. The methylol-type groups obtained by glyoxal addition to lignin are, thus, forced to react with the much more reactive flavonoid tannin, thus, introducing the lignin in the final copolymer network. A simplified scheme explaining in brief the reactions involved is shown in Fig. 4. It must also be pointed out that at pH 10 and higher

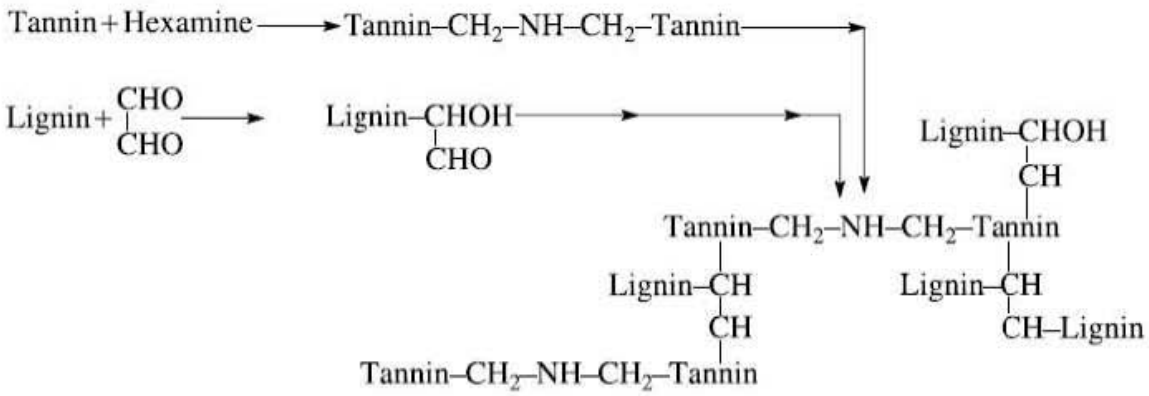
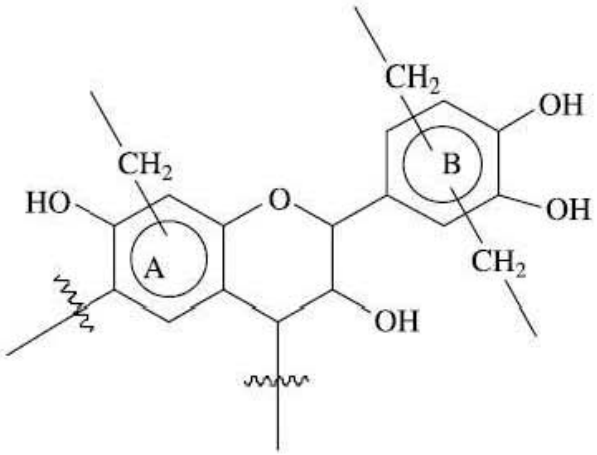


Figure 4. Schematic representation of the series of reactions occurring in the formation of the tannin/lignin hardened network.

both A- and B-rings of a flavonoid unit are able to react and cross-link [15, 18], as shown in the following formula,



not only the A-ring, thus, giving better board strength. Hardening is then controlled by the decomposition of the hexamine into reactive species [17, 20–22]. Such a system is usable with prorobinetinidin/profisetinidin flavonoid tannins such as mimosa and quebracho tannins [17], but yields poor results for the more reactive procyanidin tannins such as pine tannin [34], where the A-ring of the flavonoid unit is so reactive as to prevent reaction of the less reactive B-ring with hexamine.

In Table 4 the decrease of board IB strength when decreasing the relative proportion of tannin in the formulation is due to the decrease of the reactive sites at which the glyoxalated lignin can react in the brief period the panel is in the hot press. In the hot press, the maximum temperature reached in the board core is no higher than 120°C, rendering the reaction even more sensitive to reagents proportions.

4. Conclusions

1. For the first time mixed wood panel adhesive formulations for interior-grade applications were developed in which lignin is in considerable proportion, 50%, of the wood panel binder.
2. For the first time wood adhesives in which no 'fortification' with synthetic resins, such as the isocyanates or phenol-formaldehyde resins used in the past, is necessary to obtain results satisfying relevant standards were developed.
3. In the resins developed environment-friendly, non-toxic polymeric materials of natural-origin constitute as much as 94% of the total panel binder formulation. The wood panel itself is, thus, constituted of 99.5% natural materials.
4. The 0.5% non-natural material is composed of glyoxal, a non-toxic and non-volatile aldehyde, and of hexamine that has already been accepted as a non-formaldehyde-yielding compound when in presence of a flavonoid tannin [17].

The tannin/hexamine binders alone produced panels with zero formaldehyde emission when tested by the desiccator method [33].

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ORIGINALS ORIGINALARBEITEN

Synthetic-resin-free wood panel adhesives from mixed low molecular mass lignin and tannin

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Abstract Mixed interior wood panel tannin adhesive formulations were developed in which lignin is in considerable proportion, 50%, of the wood panel binder and in which no "fortification" with synthetic resins, such as isocyanates and phenol-formaldehyde resins as used in the past, was necessary to obtain results satisfying relevant standards. A low molecular mass lignin obtained industrially by formic acid/acetic acid pulping of wheat straw was used. Environment-friendly, non-toxic polymeric materials of natural origin constitute up to 94% of the total panel binder. The wood panel itself is constituted of 99.5% natural materials, the 0.5% balance being composed of glyoxal, a non-toxic and non-volatile aldehyde, and of hexamine already accepted as a non-formaldehyde-yielding compound when in presence of condensed tannin. Both particleboard and two types of plywood were shown to pass the relevant interior standards with such adhesive formulations. Moreover, the much cheaper non-purified organosolv lignin showed the same level of results as the more expensive purified type.

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Kunstharzfreie Klebstoffe für Holzwerkstoffe aus niedermolekularem Lignin und Tannin

Zusammenfassung Es wurden verschiedene Tannin-Klebstoff-Rezepturen mit einem hohen Ligninanteil von 50% für plattenförmige Holzwerkstoffe für den Innenbereich entwickelt. Dabei konnte auf eine „Verstärkung" mit Kunstharzen, wie zum Beispiel Isocyanaten und Phenolformaldehyd, wie sie bisher zur Erfüllung der Anforderung relevanter Normen erforderlich war, verzichtet werden. Verwendet wurde ein niedermolekulares Lignin, das industriell durch Ameisensäure-/Essigsäureaufschluss von Weizenstroh gewonnen wurde. Umweltfreundliche, ungiftige polymerische Stoffe natürlichen Ursprungs machen bis zu 94% des gesamten Bindemittels aus. Die Holzplatte selber besteht zu 99,5% aus natürlichen Materialien und zu 0,5% aus Glyoxal, einem nicht toxischen und nicht flüchtigen Aldehyd, sowie aus Hexamin, das in Verbindung mit kondensiertem Tannin als nicht Formaldehyd emittierende Substanz gilt. Es wurde gezeigt, dass sowohl eine Spanplatte als auch zwei Sperrholzarten, die unter Verwendung dieser Rezepturen hergestellt wurden, entsprechende Normen für Platten zur Verwendung im Innenbereich erfüllten. Zudem führte das wesentlich billigere, ungereinigte Organosolv-Lignin zu gleichen Ergebnissen wie der viel teurere, gereinigte Typ.

1 Introduction

Biocomposites are and have been an area of growing interest and a subject of active research for quite some time now. This is due to both environmental concerns as well as the foreseen future scarcity of oil and oil-derived products. One class of composites in which natural origin resins already have commercial/industrial impact is in the field of rigid wood panels, such as particleboard. Natural origin resins are already used commercially for the last 30 years for these wood panels, and their use is still growing, although still relatively slowly (Pizzi 2006). In such an application, the binder is never more than 10% by weight of the whole composite panel. This is sufficient to stay in line with the performance and costs required by the wood panel industry and their respective product standards.

The main natural resins used as wood panel binders are vegetal tannin adhesives, lignin adhesives and more recently also soy protein adhesives (Pizzi 2006). Of these tannin-based adhesives have been commercial the longest, since 1971. They have the advantage of not needing any reinforcement with an oil-derived synthetic resin of some kind in comparison to the other two types (Pizzi 2006). This is, however, still the case for the already commercial use of lignin (Lei et al. 2008; Pizzi and Stephanou 1993a, 1993b; El Mansouri et al. 2007) and soy binders (Lorenz et al. 2006; Wescott et al. 2006; Amaral-Labat et al. 2008; Pizzi 2006), where either phenol-formaldehyde or very often PMDI (polymeric isocyanate) is till needed in amounts between 20% and 40% of the total resin to satisfy the requirements of relevant board standards.

The use of synthetic resins limits somewhat the environmental attractiveness of such adhesives based on natural materials, while the use of tannin alone is limited at present by the relatively limited supply of these materials (Pizzi 2006). Thus, the aim is to prepare an adhesive based on materials of natural origin, satisfying international standards for both performance and emission, which does not emit or even better does not contain any formaldehyde, the composition of which does not include any synthetic resins, and which uses cheap and widely available materials. This will render wood panel adhesives based on natural materials much more accepted both economically and environmentally.

This paper deals with the preparation of lower-cost wood panel adhesives of good performance based on natural materials and without fortification by any synthetic resins.

2 Experimental

2.1 Preparation of glyoxalated lignin resin

The lignin used was a low molecular mass lignin obtained industrially as a by-product of pulp and paper production from wheat straw by an acetic acid-based organosolv process US patent 7,402,224 (Avignon and Delmas US patent 7,402,224-2000). Two types of lignin were used, one washed and purified and the other prior to washing. In this article, the second type of lignin is called "impure" lignin. These two lignins were supplied by the industrial manufacturer, CIMV (Compagnie Industrielle de la Matiere Vegetale, Reims factory, France). The characteristics of the two specimens were determined by the FCBA (Table 1) and by CIMV (Table 2). 295 parts by mass of this lignin powder (96% solid) were slowly added to 477 parts water while sodium hydroxide solution (30%) was added from time to time thus keeping the pH of the solution between 12 and 12.5 for better dissolution of the lignin powder which was also facilitated by vigorous stirring with an overhead stirrer. A total of 141 parts by mass of 30% sodium hydroxide water solution was added which resulted in a final pH close to 12.5.

A 2 litre flat bottom flask equipped with condenser, thermometer and magnetic stirrer bar was charged with the

Table 1 Characteristics of wheat straw organosolv lignin
Tab. 1 Eigenschaften des Organosolv-Lignins aus Weizenstroh

	Formyl (mmol/g)	Acetyl (mmol/g)	Free hydroxyls (mmol/g)	Total (mmol/g)
Total -OH groups	0.6	0.5	2.90	4.00
Phenolic -OH groups	0.20	0.05	0.85	1.10
Aliphatic -OH groups	0.40	0.45	2.05	2.90

Table 2 Characteristics of wheat straw organosolv lignin
Tab. 2 Eigenschaften des Organosolv-Lignins aus Weizenstroh

	Klason lignin		Total lignin (%)	Ashes (%)	Glucan (%)	Xylan (%)	Mannan (%)	Galactan (%)	Arabinan (%)	Total without glucan (%)
	insoluble (%)	soluble (%)								
Non-purif.	79.3	3.1	82.4	3.49	5.57	5.80	0.15	1.01	1.58	8.54
Purified	82.7	2.22	84.9	1.83	4.49	5.98	0.38	0.97	1.45	8.78

above solution and heated to 58°C. 87.5 parts by mass glyoxal (40% in water) were added and the lignin solution was then continuously stirred with a magnetic stirrer/hot plate for 8 hours. The solids content for the glyoxalated lignin was around 31%. Glyoxal is a non-volatile non-toxic aldehyde that has been tested in lignin (El Mansouri et al. 2007; Lorenz et al. 2006) and tannin adhesives (Ballerini et al. 2005) and other adhesives (Amaral-Labat et al. 2008) for application to wood panels such as particleboard. Glyoxal is a non-toxic aldehyde (LD50 rat $\geq$ 2960 mg/kg; LD50 mouse $\geq$ 1280 mg/kg) (NIOSH 2000), non-volatile but less reactive than formaldehyde which is toxic (LD50 rat $\geq$ 100 mg/kg; LD50 mouse $\geq$ 42 mg/kg) (NTIS).

2.2 Tannin and final resin

Mimosa tannin extract (ex Tanzania, supplied by Silva, S.Michele Mondovi', Italy) with a Stiasny value of 92.2 (Stiasny 1905a, 1905b; Hillis and Urbach 1959; Suomi-Lindberg 1985) was used. The tannin solution in water was prepared at 45% concentration, and its pH was adjusted to 10.4 with NaOH 33% water solution. The high pH was chosen as the hardener used performs best at such a pH (Pichelin et al. 2006; Hillis and Urbach 1959; Pizzi 1978; Theis and Grohe 2002). Hexamine was dissolved in water to form a 30% solution. It was added to the tannin extract solution on the basis of 5% hexamine solids on tannin extract solids to which then the glyoxalated lignin solution was added to obtain resin solids to resin solids proportion of tannin:glyoxaleted lignin of 50:50 by weight. Hexamine hardener is now accepted by JIS A5908 as no formaldehyde source in presence of a polyflavonoid condensed tannin (Pichelin et al. 2006; Kamoun and Pizzi 2000a, 2000b; Kamoun et al. 2003).

The total amount of natural material on dry resin solids was almost 94% and the adhesive so prepared did not contain any synthetic resins.

2.3 Wood panels (particleboard and plywood) manufacture and testing

Duplicate one layer laboratory particleboard of 350 × 300 × 14 mm^3 dimension were prepared using a mixture of core particles of beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*) wood particles at 28 kg/cm^2 maximum pressure and 190–195°C press temperature. The wood particles prior to resin addition had a moisture content of 2%. The resin solids load on dry wood was maintained at 10% of the total mix of tannin + glyoxalated lignin. The total pressing time was maintained at 7.5 minutes. All particleboards were tested for dry internal bond (IB) strength. The IB strength test is a relevant international standard test (EN 312 1995) done on five board specimens and a tension test perpendicular to the plane of the board. Thus, each IB and panel density result in the tables is the average of ten specimens. The panel density reported in the tables is the average of the density of the series of specimens for each series of panels.

Three ply triplicate wood panels of 500 × 300 × 14 mm^3 and 400 × 400 × 6 mm^3 dimension were prepared. The former was composed of pine wood (*Pinus sylvestris*) veneers of respectively 3.5 mm and 7 mm thickness for the two lower plies and an oak (*Quercus spp.*) veneer of 3.5 mm thickness for the upper veneer. The assembly was then 3.5 × 7 × 3.5 mm^3 for a total of 14 mm total plywood thickness. These were panels apt for floor covering. The latter were of 2 mm thick beech (*Fagus sylvatica*) veneers. The following glue mix was used in parts by weight: a solution of the Tannin/Hexamine + Glyoxalated lignin resins described above in proportion 50/50 by dry weight, at pH = 10.0, to which 38% of 200 mesh olive stone flour on total resin solids was added as filler.

The glue spread used was 380 g/m^2 double glue line, press temperature was 130°C, pressure was 15 kg/m^2 for both types of panel. Pressing time was 5 minutes for the thicker panel type, this being rather fast, and 4 minutes for the thinner one (1 minute/mm until the furthest glue-line from the hot platen + 2 minutes). The panels were tested for dry tensile strength according to European Standard EN 314 (1993).

2.4 ^{13}C CP-MAS NMR spectra

Glyoxalated organosolv lignin resins were hardened at 105°C for 2 hours in an oven before being finely ground for NMR analysis. The hardened lignin resins were analyzed by solid state CP MAS ^{13}C NMR. Spectra were obtained on a Bruker AVANCE II 400 MHz spectrometer at a frequency of 100.6 MHz and at sample spin of 12 kHz, using a recycling delay of 1s-depending on ^{1}H spin lattice relaxation times (t_1) estimated with the inversion-recovery pulse sequence-, a contact time of 1 ms. Number of transients was about 15000, and the decoupling field was 78 kHz. Chemical shifts were determined relative to tetramethyl silane (TMS) used as control. The spectra were accurate to 1 ppm. The spectra were run with suppression of spinning side bands.

2.5 Thermomechanical analysis (TMA)

The hardening reaction of one resin system or glues mixes can be evaluated by TMA, studying the rigidity of a bonded wood joint as a function of the temperature. Thus, glues mixes of glyoxalated purified and non-purified lignin with tannin/hexamine were analysed by TMA. The composition of different glue mixes is given in the tables. All experiments were conducted under the same conditions: heat rate = 10°C/min, 30 mg of resin, in the temperature range of 25–250°C. The thermo-mechanical analyzer used was a Mettler Toledo TMA40. The software used for data treatment

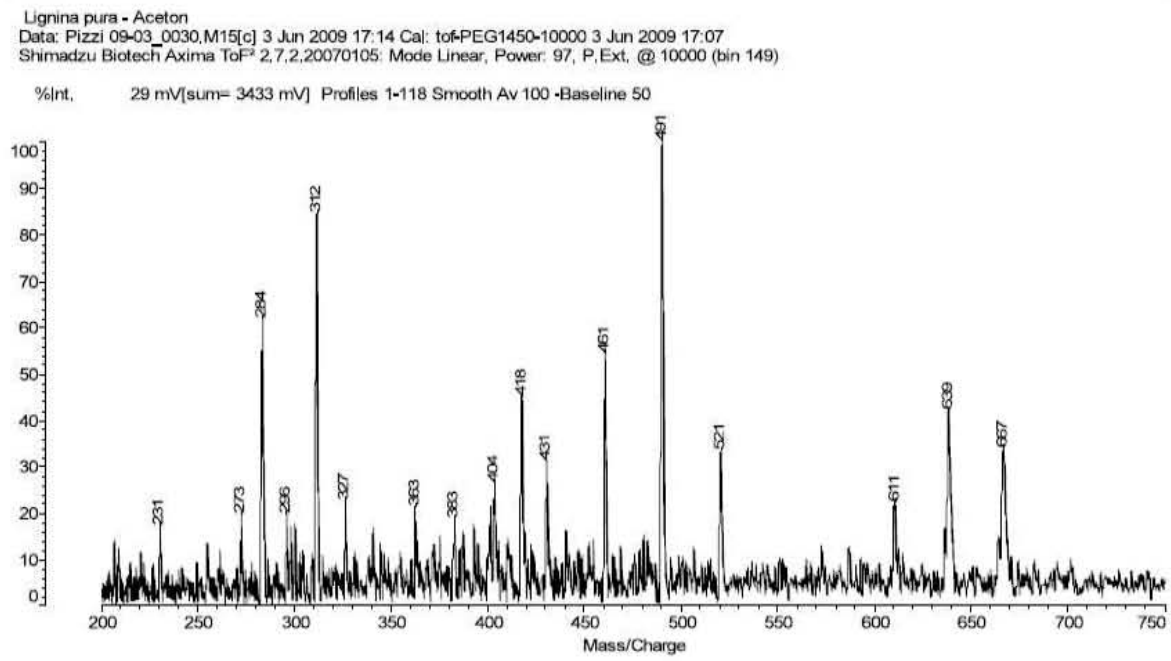


Fig. 1 MALDI-TOF mass spectrum in the 200–700 Da range indicating the main oligomers distribution in the low molecular weight organosolv lignin used

Abb. 1 MALDI-TOF Massenspektrum im Bereich von 200–700 Da, das die Verteilung der Hauptoligomere im verwendeten niedermolekularen Organosolv-Lignin zeigt

was STARe. Deflection curves that allow MOE determination were obtained by the three-point bending TMA mode. MOE of the wood joints bonded with different resin systems gave a good indication of the final strength of the adhesive system tested.

2.6 Molecular weight and molecular weight distribution

The number average molecular mass of the two lignins studied was determined by GPC. They were obtained as 1034 and 1028 for the non-purified and purified lignin, respectively. The effluent of the industrial CIMV pilot plant in Reims, France, after washing in water was monitored at 280 nm with a Beckman UV detector. The column (TSKgel G3000 PWXL) was calibrated with poly(ethylene glycol) in the 138–40,000 g/mol range. The flux of the $NaNO_3$ solution (0.1M at pH 7) was 0.6 mL/min, and the samples were dissolved in $NaNO_3$ at a concentration of 3 mg/mL. The detected signal was digitalized at a frequency of 2 Hz, and the molecular weight distribution was calculated from the recorded signal with normal GPC calculation procedures (Pichelin et al. 2006).

Examination by MALDI-TOF (Matrix assisted laser desorption/ionisation time-of-flight) mass spectrometry showed however that GPC determination of the lignin molecular mass indicates much higher values than what is real, thus that the values of Mn from GPC result also from molecular associations as already shown for other water soluble polymers (Despres and Pizzi 2006). MALDI-TOF results are shown in Fig. 1 indicating that molecular masses as high as those of tetramers occur, and no more, thus lower than what was obtained by GPC. The oligomers composing of the non-purified and purified lignin are the same and in the same proportion. Their types are listed in Table 3.

The MALDI-TOF spectra were recorded on a KRATOS Kompact MALDI AXIMA TOF 2 instrument. The irradiation source was a pulsed nitrogen laser with a wavelength of 337 nm. The length of one laser pulse was 3 ns. The measurements were carried out using the following conditions: polarity-positive, flight path-linear, mass-high (20 kV acceleration voltage), 100–150 pulses per spectrum. The delayed extraction technique was used applying delay times of 200–800 ns.

Sample preparation: the lignin water solution samples were dissolved in acetone (4 mg/mL, 50/50% volume). The sample solutions were mixed with an acetone solution (10 mg/mL in acetone) of the matrix. As the matrix 2,5-dihydroxy benzoic acid was used. For the enhancement of ion formation NaCl was added to the matrix (10 mg/ml in water). The solutions of the sample and the matrix were mixed in the proportions 3 parts matrix solution + 3 parts polymer solution + 1 part NaCl solution and 0.5 to 1 µL of the resulting solution mix were placed on the MALDI target. After evaporation of the solvent the MALDI target was introduced into the spectrometer. The dry droplet sample preparation method was used.

Table 3 MALDI-TOF derived distribution of oligomers in the organosolv lignin used for the adhesive

Tab. 3 Mittels MALDI-TOF Massenspektrometrie bestimmte Verteilung der Oligomere in dem für den Klebstoff verwendeten Organosolv-Lignin

	Structure	Calculated M.W.	Calculated M.W.+Na	Experimental peak	Peak relative proportion (%)
S	monomer	210	233	231	18
				273	
H-β1-H–1x-OH	dimer	261	284	284	63
H-β1-G	dimers	273	296	296	18
G-β1-H					
326–1x-CH_2-(demethylation)	dimers	289	312	312	85
H-β1-S	dimers	303	326	327	22
G-β1-G					
S-β1-H					
H-βO4-S	dimers	360	360	383	18
G-βO4-G					
S-βO4-H					
G-$\beta\beta$-G	dimers	394.4	417.4	418	43
G-β5-G					
G-55′-G					
G-βO4-S	dimers	407	430	431	32
G-αO4-S					
S-βO4-S	dimers	437	460	461	54
S-αO4-S					
S-βO4-S+2x-OH	dimers	468	491	491	100
S-αO4-S+2x-OH					
				521	
S-βO4-S-$\beta\beta$-H	trimers	585	608	611	21
G-βO4-G-$\beta\beta$-S					
G-βO4-G-β5-S					
G-βO4-S-55′-G					
G-βO4-S-$\beta\beta$-S	trimers	615	638	639	40
G-βO4-S-β5-S					
G-βO4-S-55′-S					
S-βO4-S-$\beta\beta$-S	trimers	644.7	667.7	667	34
S-βO4-S-β5-S					
S-βO4-S-55′-S					

2.7 Phenolic hydroxyl groups by UV spectroscopy ($\Delta\varepsilon$ method)

The concentrations of the various phenolic units in the lignin samples were determined by UV spectroscopy, as described by Zakis (1994). This method is based on the difference in the absorption at 300 and 360 nm between phenolic units in neutral and alkaline solutions. The concentration of ionizing phenol hydroxyl groups can be quantitatively evaluated by comparison of the $\Delta\varepsilon$ values of substances studied at certain wavelengths with the values of $\Delta\varepsilon$ of the respective model compounds presenting a free ortho position in their aromatic ring.

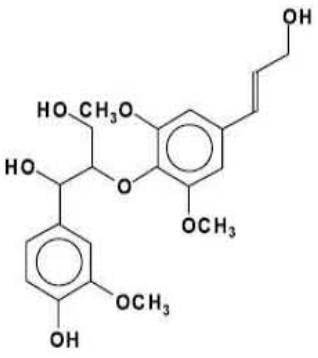
3 Results and discussion

Organosolv lignin obtained with a mixed formic and acetic acids pulping technique (Avignon and Delmas 2000) is bound to present a number of phenolic and aliphatic hydroxyl groups formylated and acetylated. The results in Table 1 confirm this by the presence of formyl and acetyl group present on mainly aliphatic but also phenolic hydroxyl groups of lignin. These aliphatic and aromatic formic and acetic ester groups are of interest as the presence of esters, even in small amounts, has been shown to accelerate the reaction and curing of phenolic/aldehyde resins (Zhao et al. 1999; Pizzi and Stephanou 1993a, 1993b) and this also in the

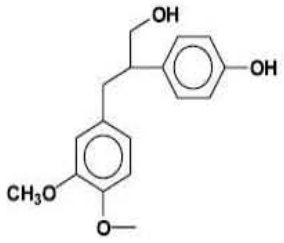
case of slow curing lignin-based resins (Pizzi and Stephanou 1993a, 1993b).

Their presence in this type of organosolv lignin is therefore of some benefit. The proportions of acid soluble and Klason lignin shown in Table 2 indicate that in the case of both the purified and non purified wheat straw organosolv lignins the level of total lignin is high. Not much difference occurs between the two lignins, and even the values of their number average molecular mass are very similar at 1028 and 1034 g/mol. The percentage distribution of residual sugar oligomers is also similar (Table 2) to glucans and xylans from hemicelluloses and also cellulose predominating. The major difference being observed is the percentage of inorganic ashes. In this case the ashes are the cations of residual acetic and formic acid salts which derive from the precipitation and washing with a NaOH water solution of the residual lignin (Avignon and Delmas 2000).

A word of warning must be given on the GPC results of molecular mass. MALDI-TOF examination of the same two lignins (Fig. 1) showed that the masses of the highest oligomers are lower than what was observed by GPC, indicating that even in this case, like for other water soluble polymers, colloidal molecular associations often falsify the results of GPC mass determination (Despres and Pizzi 2006; Roux 1976). It is nonetheless of interest to observe the type and relative proportion of the oligomers existing in this organosolv lignin as listed in Table 3. From this one it can be seen that dimers and trimers of different types predominate, but that no oligomers of higher mass are present, confirming that the Mn results obtained by GPC are too high and due to association. In Table 3 the interpretation of the compounds involved are indicated as G for guaiacyl units, S for siringyl units, and H for phenolic units not containing methoxy groups. The linkages between them are indicated with the "classical" nomenclature (Fengel and Wegener 1983) as βO4, αO4, $\beta\beta$, β5, 55′. Thus, as an example, the G-βO4-S and G-αO4-S peak at 731 Da in Fig. 1 corresponds to the structure



and the H-β1-G peak at 296 Da corresponds to the structure



The ^{13}C NMR analysis confirms the similarity of the two lignins: in Fig. 2 the NMR spectra show that differences between the two lignins exist but that they are rather small. Thus, in Fig. 2 the band at 55 ppm characteristic of the methoxy groups (-OCH_3) of lignin bound to the aromatic ring is slightly less intense for the purified lignin. As this was purified at 50–60°C some demethoxylation has occurred which is a very common reaction in lignin (Fengel and Wegener 1983). Equally, the higher relative proportion of acetic/formic esters in purified lignin are different as shown by the peaks at 21 and 30 ppm of the carbon of the methyl group -CH_3 of the acetic acid esters and free acetic acid carboxyl ion, respectively, and the less noticeable increase of the C=O peak of the formic acid esters at 161 ppm and of the free formic acid carboxyl ion at 172 ppm. These small differences in ester content are relative to the higher purity of purified lignin.

The results of the thermo-mechanical analysis indicate that the purified organosolv lignin gives higher Modulus of Elasticity (MOE) values than the non-purified one (Fig. 3). However, the result is low only in appearance. In reality, the impurities present in the non-purified lignin decrease the

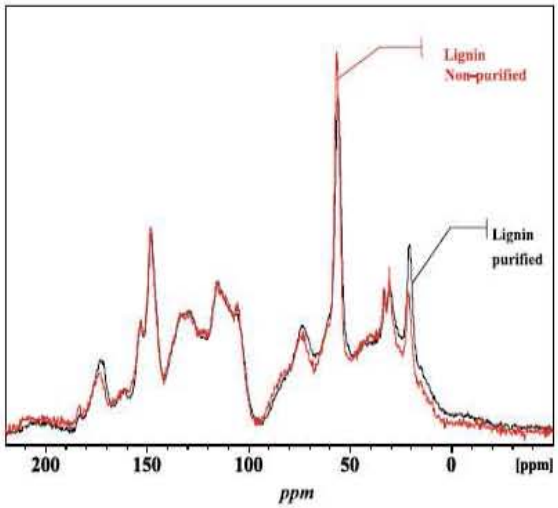


Fig. 2 Comparative CP-MAS ^{13}C NMR spectra of purified and non-purified organosolv lignin derived from formic acid/acedic acid wheat straw pulping

Abb. 2 Vergleichende CP-MAS ^{13}C NMR Spektren von gereinigtem und nicht gereinigtem Organosolv-Lignin aus Essigsäure-/Ameisensäureaufschluss von Weizenstroh

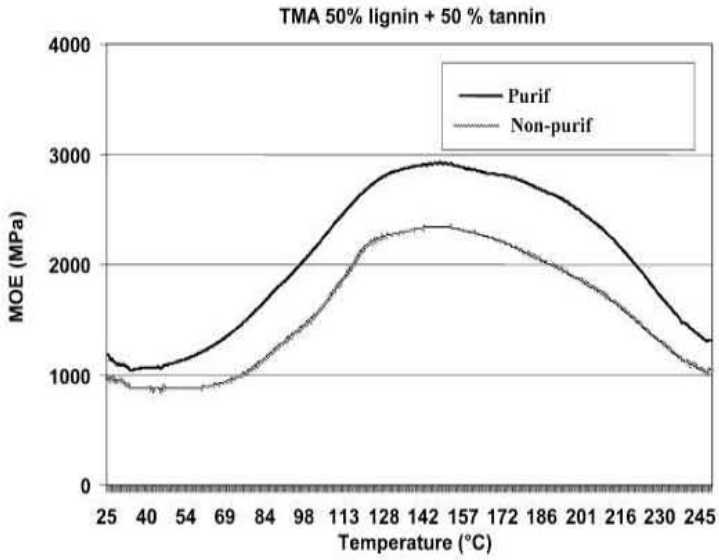


Fig. 3 Comparison of thermo-mechanical analysis curing curves of beech wood joints bonded with tannin/lignin 50/50 wood adhesives. Variation of the joint MOE as a function of temperature
Abb. 3 Vergleich der Ergebnisse einer thermomechanischen Analyse von Buchenholzverbindungen, die mit Tannin/Lignin (50/50) Klebstoffen verklebt wurden. Variation des E-Moduls der Verbindung in Abhängigkeit der Temperatur

Table 4 Particleboard bonded with tannin/lignin adhesives. Results
Tab. 4 Eigenschaften der mit Tannin/Lignin-Klebstoffen hergestellten Spanplatten

Proportion	Density (kg/m^3)	Dry I.B. strength (MPa)
Tannin/hexamine + glyoxalated lignin(non-purified) 50/50	702	0.52 ± 0.05
Tannin/hexamine + glyoxalated lignin(purified) 50/50	700	0.45 ± 0.04
Tannin/hexamine + glyoxalated lignin(non-purified) 40/60	710	0.35 ± 0.04
Tannin/hexamine + glyoxalated lignin(non-purified) 30/70	700	0.18 ± 0.03
European Norm EN 314-2 requirement		≥ 0.35

amount of actual binding material on the beech lamina in the TMA tests. Such a slightly lower amount of actual binding resin yields a lower TMA max MOE value result for the non-purified lignin due to the well known effect TMA has to exaggerate the differences in results (Pizzi et al. 2003; Zanetti et al. 2004). That this is indeed the case is shown by the results of actual wood panels bonded with these adhesives (Tables 4 and 5). The MOE starts to decrease after 170°C on the TMA diagrams (Fig. 3) due to the start of the degradation of wood constituents, as previously shown (Kamoun et al. 1998). This does not affect board strength as at the very short press times used for panel manufacture the max temperature reached in the board core, which determines IB strength, does not reach a temperature higher than 120°C.

The results for wood particleboard prepared with the mixed formulations of tannin/hexamine +glyoxalated lignin are shown in Table 4. The internal bond (I.B.) strength of a panel is a direct measure of the performance of the adhesive. The results in Table 4 indicate that no significant difference exists as regards internal bond (I.B.) strength of the panels prepared using the two lignins according to relevant interior panel standards (EN 312). When the two resins are mixed 50/50 by weight solids content to solids content, the results are enough to satisfy international standard requirements for interior grade boards (Table 4). However, a decrease in the relative proportion of tannin/hexamine entails a rapid decrease in the IB strength of the panels (Table 4). Because the panel at tannin: lignin = 40:60 has an IB strength equal to the minimum value required by the standard, the indications are that to use formulations where the amount of tannin is less that around 45% of the total would be unfeasible at industrial level. One consideration of particular interest is that the results obtained with the non-purified lignin are comparable and equally good as those of the formulations using the purified one. This is a considerable advantage as the unpurified, unwashed lignin is considerably cheaper to produce.

The results for the two types of plywood, 6 mm thick standard beech plywood and 14 mm thick flooring type mixed veneers plywood are reported in Table 5. The European Standard EN314-2 for interior grade plywood specifies that the percentage wood failure and dry tensile strength obtained by testing of the panel are to be evaluated on a

Table 5 Plywood bonded with tannin/lignin adhesives. Results
Tab. 5 Eigenschaften der mit Tannin/Lignin-Klebstoffen hergestellten Sperrholzplatten

	Proportions	Dry tensile strength (MPa)	Wood failure (%)
Beech Plywood			
Control Tannin/hexamine	100	1.4 ± 0.10	0
Tannin/hexamine + glyoxalated lignin(non-purified)	50/50	1.2 ± 0.20	10
Thick floor plywood			
Tannin/hexamine + glyoxalated lignin(non-purified)	50/50	1.0 ± 0.19	46
EN314-2 requirements		if ≥ 1.0 then	No requirement
		if $0.6 \leq$ and < 1.0 then	≥ 40

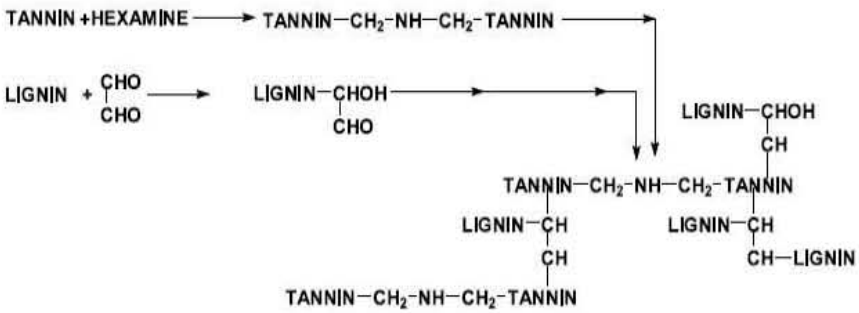


Fig. 4 Schematic representation of the series of reactions occurring in the formation of the tannin/lignin hardened network of the formulation
Abb. 4 Schematische Darstellung der Reaktionen beim Aushärten des Tannin/Lignin-Klebstoffes

reciprocal sliding scale. Thus, the percentage wood failure for dry tensile strength can be of 0% for any strength results ≥ 1.0 MPa. Equally, if the dry tensile strength is higher than 0.6 MPa but lower than 1.0 MPa the percentage wood failure required is $\geq 40\%$. The results in Table 4 indicate that both plywoods pass well such a standard requirement, and that the tensile value of tannin/lignin bonded panel is only slightly lower than what was obtained with the tannin alone.

It is of interest to understand the main mechanisms according to which the formulation works. As lignin is of much lower reactivity than flavonoid tannin, a series of parallel reactions occurs to yield coreaction of tannin with lignin. Thus, tannin reacts very rapidly in the hot press with hexamine to form a network based on the known reactions of tannin with hexamine (Kamoun and Pizzi 2000a, 2000b; Pichelin et al. 2006). Lignin, much slower, is prereacted in a reactor with glyoxal (Lei et al. 2008; Pizzi 2006). Although some condensation of glyoxalated lignin occurs during the reaction, that if the reaction is protracted for longer times will increase unduly the viscosity of the glyoxalated lignin, condensation needs to be minimized and addition of glyoxal on the lignin maximized. The glyoxalated lignin is then rich in methylol-type groups obtained by glyoxal addition, but the aromatic nuclei of lignin are still too slow for glyoxalated lignin alone to condense to a sufficiently hardened network in just the very brief period the board remains in the hot press. The methylol-type groups obtained by glyoxal addition to lignin are then forced to react with the much more reactive flavonoid tannin introducing then the lignin in the final copolymer network. A simplified scheme explaining in brief the reactions involved is shown in Fig. 4.

In Table 4 the decrease of board IB strength when decreasing the relative proportion of tannin in the formulation is then due to the decrease of the reactive sites in which the glyoxalated lignin can react in the brief period the panel is in the hot press. In the hot press, the max temperature reached in the board core does not reach a temperature higher than 120°C rendering the reaction even more sensitive to reagents proportions. It is not excluded that varying glyoxalation level and times of the lignin the proportion of tannin could be somewhat decreased but the correct conditions to achieve this have not been explored as yet.

4 Conclusion

For the first time, mixed wood panel adhesive formulations for interior-grade applications were developed in which lignin is in considerable proportion, 50%, of the wood panel binder and in which no "fortification" with synthetic resins, such as isocyanates and phenol-formaldehyde resins used in the past, is necessary to obtain results satisfying relevant standards. Furthermore, environment-friendly, non-toxic polymeric materials of natural origin constitute as much as 94% of the total panel binder formulation. The wood panel itself is then constituted of 99.5% natural materials. The 0.5% non natural material is composed of glyoxal, a non-toxic and non-volatile aldehyde, and of hexam-

ine that has already been proven and accepted as a non-formaldehyde-yielding compound when in presence of a condensed tannin adhesive (Pichelin et al. 2006). As for the tannin/hexamine binders alone these panels also present an effective zero formaldehyde emission when tested by the dessicator method (JIS A5908).

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III.2.2. **Faibles emissions de formaldehyde a partir de bio materiaux de bois fabrique avec de colles a base de tanins/lignine glyoxalée**

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De la lignine kraft et de la lignine de paille de blé glyoxalées ont été melangées à de la colle à base de tanin contenant de l'hexamine comme durcisseur pour fabriquer des panneaux de particules. Les colles étudiées contenaient les proportions lignine/tanin 40/60 et 50/50. Les temps de gel ont été déterminés pour connaître les temps de polymérisation des mélanges dans des conditions contrôlées. Il s'avère que la polymérisation est plus lente dans le cas d'un mélange lignine kraft/tanin de mimosa que dans celui d'un mélange lignine de paille de blé/tanin de mimosa. Des essais thermomécaniques (TMA) ont été effectués pour caractériser la résistance finale de la colle à partir du module d'élasticité (MOE). Des valeurs de 3.422 et 3.347 (MPa) ont été déterminées pour les mélanges de lignine de paille de blé/tanin et lignine kraft/tanin dans les proportions lignine/tanin de 40/60. Ce module n'atteint que 2.122 (MPa) pour les proportions lignine/tanin 50/50. La cohésion interne des panneaux fabriqués avec ces colles a été évaluée et les émissions de formaldéhyde ont été

quantifiées selon les normes européennes EN 312 et EN 717-3. Les résultats de cohésion interne confirment ceux des analyses thermomécaniques. Les valeurs les plus importantes, ont été reportées pour les panneaux fabriqués avec les mélanges aux proportions lignine/tanin 40/60, soit 0.43 et 0.53 (MPa) avec la colle lignine de paille de blé/tanin et lignine kraft/tanin respectivement. Ces panneaux ont obtenu un classement intérieur type P2. Par ailleurs, les panneaux génèrent une émission de formaldéhyde d'au moins 87% et 75% plus faible que celle d'une colle comerciale urée-formaldéhyde et mélamine-urée-formaldéhyde et reçoivent de ce fait un classement E0.

LOW FORMALDEHYDE EMITTING BIOBASED WOOD ADHESIVES MANUFACTURED FROM MIXTURES OF TANNIN AND GLYOXYLATED LIGNIN

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ABSTRACT

Kraft (LN-T-CO_2-2) and wheat straw (CIMV) glyoxalated lignin mixed with mimosa tannin and hexamine as a hardener were used as wood adhesive resins in particleboards fabrication. The adhesive systems proportion used were respectively 40/60 and 50/50 w/w for lignin and tannin,. The gel time test was determined to know the polymerization time between the different mixes under controlled conditions. It has showed a slower polymerization with the kraft lignin/mimosa tannin blending than with the wheat straw lignin/mimosa tannin one. Thermomechanical analyses (TMA) tests were carried out as an indication of the final strength of the adhesive systems revealed by the elasticity modulus (MOE). The MOE results have demonstrated the best mechanical resistance values in 40/60 lignin/mimosa tannin proportion with respectively 3.422 and 3.347 (MPa), for CIMV and LN-T-CO_2-2, and 2.122 (MPa) for 50/50 proportion. Particleboards were prepared and the internal bond (IB) tests were carried out according to the European Standard EN 312. The IB confirmed the TMA results. The higher mechanical results of the IB were 0.43 and 0.53 (MPa), for CIMV and LN-T-CO_2-2 lignin in a 40/60 lignin/mimosa tannin proportion. They were classified as interior

panel P2 in according with the standard request EN-312. Free-formaldehyde was determined through the flask method EN 717-3. Particleboards prepared with these natural adhesive resins registered emissions at least 87% and 75% lower than the commercial UF and MUF adhesive resins. The panels were classified as E0.

Keywords: lignin, tannin, wood adhesives, particleboard, free-formaldehyde.

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1. INTRODUCTION

The main stimulus for today's renewed interest in bio-based adhesives is the acute sensitivity from the general public toward anything concerning the environment and its protection (1). However, use of synthetic resins limits somewhat the environmental attractiveness of such adhesives based on purely natural materials, while the use of some natural material such as tannins alone is currently affected by the relatively low supply of these materials (1).

Woody biomass primarily consists of hemicellulose, cellulose and lignin. Lignin is the second most abundant and important natural product coming from plants (2). When plants need to develop mechanical support of their fibrous tissues, they produce lignin, and deposit it as a reinforcing agent. In addition, lignin performs as a sealant and decay retarder, to name only a few of its many service functions to the woody plant (3). Lignin is a polymer synthesized from phenolic compound of phenylpropane type and occurring under well defined molecular associations (4). The structural lignin research has been great challenge, due to its heterogeneous and complex molecular structure, to its deficiency of stereo regularity and to the molecular weight depending of the species of the plant and the sample origin (5). Lignin is a three dimensional natural polymer highly ramified with a very big quantity of functional groups that they provide active centres to chemical and biological (6). The main lignin's groups include phenolics hydroxyls, alinphatics hydroxyls, metoxiles, carbonyls, carboxyles and sulfonates (6). The amount of lignin in wood depends on several factors, including tree species, climate and soil conditions (2). Typically it represent between 19 and 40% of the dry wood weight (7). They are low cost waste product available in large amounts (6). Different waste lignins produced in pulp mills have made such materials an attractive proposition in converting it to phenolic precursors for phenol-based resins (6), and for the preparation of

natural adhesives (8). It has been motivated because there are readily available waste from the debarking of wood performed by many panel manufactures, and also because it is a relatively high in lignin and phenolic extractives (6). Much has been written about and many studies have been made regarding the use of lignin for wood panel adhesive, some of them are based in the simple substitution of phenol in PF resins (9). Some researchers have demonstred the natural phenols sources can be an alternative in order to replace phenol (10), but always in using some synthetic products or pMDI combinations (8**,** 11) in order to obtain a more reactive and resistant adhesive, or to produce more reactive phenolic precursors lignin, they can be processed prior to resin synthesis (12-13).

On another hand, formaldehyde emission is an important factor in the evaluation of the environmental, and healthy effects of wood based board materials (14). This product is the most abundant (15) and the most important carbonyl in the atmosphere (16). It is an ubiquitous gas which reaches high concentrations in polluted areas and can have great impact on human health (15). Although main sources of formaldehyde are burning process, chipboards production and chemical industry (17), the formaldehyde is one of the two short chain aldehydes emitted by plants, but its origin within plants remains unclear (18).

To reduce formaldehyde emission, several studies have been carried out. The possibility of using replacements materials for UF and PF adhesives has been studied for a long time (19). An other alternative has been the tannin use for example, which gives very good properties and lower emissions even in substituting a small proportion in the normal synthetics resins (20). However, the hardeners use can cause formaldehyde emissions even when the tannin adhesives are used (21-22). This depends on the wood extractives composition, and this situation implies the different natural raw material used to prepare adhesives resins must be studied individually.

In this work, the goal was to use mixtures of mimosa tannin and glyoxalated lignin from kraft and organosolv process from wheat straw for the preparation of lower cost wood panel adhesives with good performances, without needing any fortification with synthetic resins. These natural resins emit lower levels of free formaldehyde than commercial synthetic resins-adhesives. The adhesive resins formulations we used were between 40 and 50% of kraft and wheat straw glyoxalated lignin and between 60 and 50% of mimosa tannin in the adhesive formulation.

2. EXPERIMENTAL

2.1. Lignin and tannin preparation

Wheat straw lignin was obtained industrially as a product of pulp and paper by an acetic acid-based organosolv process (23). This lignin was supplied by the industrial manufacturer, CIMV (Compagnie Industrielle de la Matiere Vegetale, Reims Factory, France). A second lignin obtained industrially like by-product of production of pulp and paper corresponding to black liquor lignin by the factory Tembec Tarascon. This was precipitated by the FCBA (Forêt Cellulose Bois-Construction Ameublement, France) with CO_2 and then washed several times in the acid until pH 2-4. This last lignin called LN-T-CO_2-2 is of low molecular weight because the rupture of polymers takes place in the saw mill process, but it has often a ring carbon ortho not occupied that can react with formaldehyde in estate of synthesis (10).

The both lignins were subjected to the glyoxalation process according to the procedure described by El Mansouri (8). 295 Parts by mass of this lignin powder (96% solid) were slowly added to 477 parts of water while sodium hydroxide solution (30%) was added from time to time thus keeping the pH of the solution between 12 and 12.5 for better dissolution of the lignin powder which was also facilitated by vigorous stirring with an overhead stirrer. A total of 141 parts by mass of 30% sodium hydroxide aqueous solution were added.

A 2-liter flat bottom flask equipped with a condenser, a thermometer and a magnetic stirrer bar was charged with the above solution and heated to 58 °C. 17,5 parts by mass glyoxal (40% in water) were added and the lignin solution was then continuously stirred with a magnetic stirrer/hot plate for 8 hours. The final pH glyoxalated lignin was 6.6 and 12.3 for CIMV and LN-T-CO_2-2, respectively.

Glyoxal was used because it is an aldehyde that has been tested in lignin (8, 24), tannin (25) and other adhesives as soy (1, 26) for application to wood panels such as particleboards. Glyoxal is a non-toxic (LD50 rat $\geq$ 2960 mg/kg; LD50 mouse $\geq$ 1280 mg/kg) (27), non-volatile aldehyde but less reactive than formaldehyde which is toxic (LD50 rat $\geq$ 100 mg/kg; LD50 mouse $\geq$ 42 mg/kg) (28).

Mimosa tannin extract (origin: Tanzania. Supplied by: Silva, S. Michele Mondovi', Italy) of Stiasny value of 92.2 (29-30) was used. The tannin solution in water was prepared at 45% concentration and its pH adjusted to 10 with 33% water solution of NaOH. At high pH level (pH: 10), the hardener used present the best performs at such conditions (31-32).

The glue-mix was composed of: the tannin extract solution to which was added as hardener 6% hexamethylenetetramine (on solids tannin base contain) as a 30% hexamine solution in

water, and the glyoxalated lignin solution. The proportions of tannin solids/glyoxalated lignin were 60:40 and 50:50 w/w (%). The adhesives specifications and the viscosity are shown in the table N° 1.

Table N° 1: Initial lignin glyoxalated and tannin solutions conditions.

Solution	Total solids percentage (%)	Viscosity (spin 27, 20 rpm, 25 °C) (cp)	pH
LN-T-CO_2-2: Tarascon lignin	35.28	362	12,3
CIMV: Wheat straw lignin	31.42	100	6.6
MIMOSA: Mimosa tannin	45	1.100	10

2.2. Gel time

The gelation is defined as the point at which the resin ceases to be a viscous liquid and become a soft, elastic, rubbery solid (33-34). Triplicate samples from each adhesive system resins were prepared. Gel time tests were conducted at 100 °C. Five grams of each mixed was weighed in a glass test tube, and then placed in a boiling water bath. The wire spring was manually moved rapidly upward and downward, and the time for gelation was measure using a stopwatch.

Table N° 2: Adhesives specifications.

Formulation	Specifications	Viscosity (spin 27, 20 rpm, 25 °C) (cp)
40%LN-T-CO_2/60%MIM	40% lignin LN-T-CO_2-2/60% mimosa tannin.	3.100
40%CIMV/60%MIM	40% lignin CIMV/60% mimosa tannin.	613
50%CIMV/50%MIM	50% lignin CIMV/50% mimosa tannin.	325

2.3. Thermomechanical analysis (TMA)

The hardening reaction of a single resin system or mixtures can be evaluated by TMA, by monitoring the rigidity of a bonded wood joint as a function of temperature. Thus, glue-mixes of glyoxalated lignin with tannin/hexamine were analysed by TMA. All experiments were conducted under the same conditions: heating rate = 10 °C / min, 30 mg resin, in the temperature range 25-250 °C.

Triplicate samples of beech wood, and of two beech wood plies each 0.6 mm thick bonded with each adhesive system, for sample dimensions of 21 mm × 5 mm × 1.2 mm in three-point bending on a span of 18 mm. A force varying continuously between 0.1 N, 0.5 N and back to 0.1 N was applied on the specimens with each force cycle of 12 s (6 s/6 s). The classical mechanics relation between force and deflection $E = [L^3/(4bh^3)][\Delta F/(\Delta f)]$ (where L is the sample length, b and h are the sample width and thickness, ΔF is the variation of the

force applied, and Δf is the deflection obtained) allows the calculation of the modulus of elasticity (MOE) E for each case tested. The thermomechanical analyzer used was a Mettler Toledo TMA40. The software used for data treatment was STARe. Deflection curves that allow modulus of elasticity (MOE) determination were obtained in the three-point bending TMA mode. The MOE of the wood joints bonded with different resin systems gave a good indication of the final strength of the adhesive system tested.

2.4. Wood panels manufacture and internal bond

One-layer laboratory particleboards of dimensions 350 x 300 x 14 mm were prepared using a mixture of core particles of beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*) woods. The wood particles before resin addition had a moisture content of 2%. The resin solids loading on dry wood were maintained at 10% of the total mix of glyoxalated lignin and tannin/hexamine. The operational conditions are composed of three stages different of pressure applied to the panel: 35 kg/cm^2, 12 kg/cm^2 and 7 kg/cm^2. The total press time was maintained at 7.5 minute, and the press temperature at 195 °C. The panels, after a sandpapering of surface, are cut out in samples of 50 × 50 × 13 mm. All particleboards were tested for dry internal bond (IB) strength, and after two hour in boiling water tested dry (35-37).

2.5. CP-MAS ^{13}C NMR spectroscopy

The glyoxalated organosolv (CIMV) and kraft lignin resins in solid state were prepared for NMR analysis. The hardened lignin resins were analyzed by solid state CP MAS ^{13}C NMR. Spectra were obtained on a Bruker AVANCE II 400 MHz spectrometer at a frequency of 100.6 MHz and a sample spin of 12 kHz, using a recycling delay time of 1s and a contact time of 1 ms. Number of transients was about 15000, and the decoupling field was set at 78 kHz. Chemical shifts were determined relative to tetramethyl silane (TMS) used as control. The spectra were accurate to 1 ppm. The spectra were recorded with suppression of spinning side bands.

2.6. Maldi-tof

Since its introduction by Karas and Hillenkamp in 1987 (38), matrix-assisted laser desorption/ionization (MALDI) mass spectrometry has greatly expanded the use of mass spectrometry towards large molecules and has revealed itself to be a powerful method for the characterization of both synthetic and natural polymers (39-44). Fragmentation of

analyte molecules upon laser irradiation can be substantially reduced by embedding them in a light absorbing matrix. As a result intact analyte molecules are desorbed and ionized along with the matrix and can be analysed in a mass spectrometer. This soft ionization technique is mostly combined with time-of-flight (TOF) mass analysers. This is so as TOF-MS presents the advantage of being capable to provide a complete mass spectrum per event, for its virtually unlimited mass range, for the small amount of analyte necessary and the relatively low cost of the equipment.

The MALDI-TOF spectra were recorded on a KRATOS Kompact MALDI AXIMA TOF 2 instrument. The radiation source was a pulsed nitrogen laser with a wavelength of 337 nm. The time period of one laser pulse was 3 nanoseconds. The measurements were carried out using the following conditions: polarity: positive; flight path: linear; mass range: high (this meant using a high acceleration voltage = 20 kV), and 100-150 pulses per spectrum. The delayed extraction technique was used by applying delay times of 200-800 ns.

For sample preparation, the lignin water solution samples were dissolved in acetone (4 mg/mL, 50/50 vol. %) and mixed with an acetone solution (10 mg/mL in acetone) of the matrix. As the matrix 2,5-dihydroxy benzoic acid was used. For the enhancement of ion formation, NaCl was added to the matrix (10 mg/ml in water). The solutions of the sample and the matrix were mixed in the proportion 3 parts matrix solution + 3 parts sample solution + 1 part NaCl solution, and 0.5 to 1 µL of the resulting solution mix were placed on the MALDI target. After evaporation of the solvent the MALDI target was introduced into the spectrometer. The dry droplet sample preparation method was used.

2.7. Free-Formaldehyde

In the last years, several approaches to produce wood-based panels with low formaldehyde emission using these wood adhesives have been developed and several different methods have been used (19). Formaldehyde emission is an important factor in the evaluation of the environmental, healthy and comfort effects of wood-based board materials (14, 45). Formaldehyde is in the gaseous form at room temperature. It can be transformed into paraformaldehyde through polymerization and readily form methylenglycol through dissolution in water (46).

In this work, the free-formaldehyde was determined by the flask method (47). This method developed by Roffael, is based on storing one to three board pieces with a total mass close to 20 g in a close polyethylene or glass bottle (48), with 50 ml distilled water and under a constant temperature of 40 °C during 180 min. The formaldehyde released by the test

samples during this period of time is absorbed by the water. Then, the formaldehyde content in water photo metrically is determined by the acetilcetone method (Hantzsch's reaction) at 412nm.

A total of three natural adhesives from lignin/tannin base, and two synthetic (UF and MUF) adhesive resins were analyzed. The goal was to compare the formaldehyde emissions of theses resins, taking the synthetic resins ones as a reference.

3. RESULTS AND DISCUSSION

3.1. Gel time

The gel time results are showed in table N° 3. Comparing the same proportions in each adhesive system to 40/60 (w/w), the CIMV/MIM tannin formulation is more reactive than the LN-T-CO_2-2/MIM, with a gel time of 507 and 700 seconds, respectively. The blending system with a higher CIMV proportion (50%), presented a gel time of 480 seconds.

Table N° 3: Gel time results.

Adhesive formulations	Gel time (mn/s)
40%LN-T-CO_2/60%MIM	11'40''
40%CIMV/60%MIM	8'27''
50%CIMV/50%MIM	8'
Reference: Urea formaldehyde	2'07"

In general the shorter gel time by the CIMV/MIM presented is because CIMV lignin has lower pH than the LN-T-CO_2-2. The mix of CIMV lignin (pH=6) with mimosa tannin solution and hexamine (PH=10) reduces the final adhesive system pH. The hexamine is a monoprotic base and to react it has first of all to decompose itself. This decomposition is more difficult at higher pH, thus it is slower and, as a consequence, there is a slowing availability of the reactive species to cross-link the tannin. The hardening time of the mimosa tannin with hexamine lengthens with increasing pH (49-50). In our case, the system pH decreased as a consequence of the lower CIMV glyoxalated lignin pH. This condition makes the gel times shorter than if kraft glyoxalated lignin is used.

3.2. Thermomechanical analysis TMA

The thermomechanical analysis (TMA) results are shown in Figure N° 1. The 40/60 CIMV/MIM and LN-T-CO_2-2/MIM formulations showed very similar MOE values. There were

no registered significative differences in both of the case. The maximal MOE values were 3.422 and 3.347 (MPa), for the CIMV/MIM and LN-T-CO_2-2/MIM blending, respectively. In the second case, the polymerization starts at lower temperature (around 60°C) than in the first one (around 80 °C) before to reach the maximal value. In the 50/50 CIMV/MIM, the results were the lowest, with a MOE of 2.122 (MPa). This was expected because mimosa tannin is more reactive than lignin and in this case there is a lower tannin proportion in the blending adhesive system.

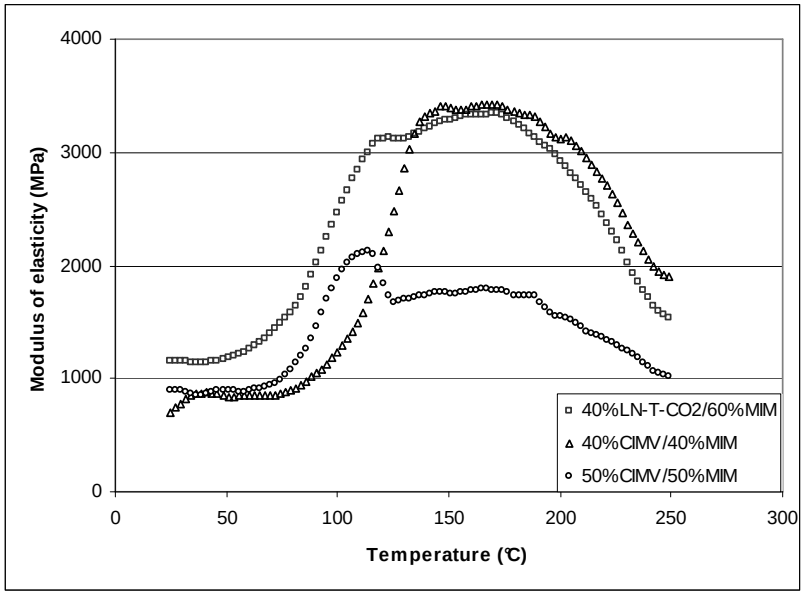

Figure N° 1: Thermomecanical resistance.

3.3. Internal bond strength IB and swelling

TMA tests results were confirmed by the internal bond strength results (table N°4). These analyses showed the higher values with the formulations containing 40% lignin proportion. All the formulations tested reached the standard requirements.

Table N° 4: Internal bond and swelling results.

Adhesives	Density (kg/m3)	IB strength dry (MPa)
40%LN-T-CO_2/60%MIM	712	0,53 ± 0,10
40%CIMV/60%MIM	708	0,43 ± 0,06
50%CIMV/50%MIM	707	0,39 ± 0,04

IB results obtained in this work registered the best mechanical resistance with the kraft lignin LN-T-CO_2/MIM at 40/60 (w/w) with 0.53 MPa, and the wheat straw lignin CIMV/MIM at the same proportion with 0.43 MPa. To increase the wheat straw lignin proportion, the values decreased to 0.39 MPa for 50/50 (w/w).

To compare the CIMV/MIM 50/50 (w/w) results with those obtained by Mansouri (51) at the same proportions, our results with CIMV were lower for two reasons: on the first hand, the glyoxalated lignin pH was much lower and finally this condition makes the final pH system decreasing the mimosa tannin pH reached lower mechanical resistances (32). On another hand the ^{13}C NMR results showed unreacted lignin presence in wheat straw lignin.

About the boil water resistance, none of these formulations was able to resist to the drastic conditions, and very high water absorption and swelling were registered. The high pH level would increase the water absorption and the thickness swelling (32). Therefore, and in accordance with the IB results, the particleboards prepared with kraft and wheat straw lignin were classified as boards type P2.

3.4. CP-MAS ^{13}C NMR spectroscopy

The ^{13}C NMR from glyoxalated lignins shows some functional groups that confirm the reaction lignin-glyoxal. In the CIMV and LN-T-CO_2-2 is possible to see similarities and some differences could explain the mechanical results (Figures N° 2 and 3). In both cases the bands at 183-182, 148-151, 125-135 and 54-55 ppm are present in the lignins. The 183-181 ppm belongs to the aromatics quinones generated by oxidation in the site of phenolic hydroxyl groups of lignin. Between 148-151 ppm the aromatic carbons are linked to phenolic hydroxyl groups indicating reactivity of aromatic nuclei of lignin being slightly more marked peak in the LN-T-CO_2-2 lignin than the CIMV. The 125-135 ppm belongs to the ArC-C linkages form from methylene and methylene ether bridges.

There is not peak at 120 ppm which could be a signal that the ArC-H has disappeared indicating extensive introduction of substituted groups on the aromatic rings of phenyl propane unit lignin (9).

In the LN-T-CO_2-2 there is not 105-115 ppm band presence as in the CIMV case, which would means that in the second one there is unreacted lignin presence of *ortho* and *para* sites on the aromatic lignin (9, 52). This could be a consequence of the low pH in CIMV glyoxalated lignin. Equally noticeable is the presence of the band between 54.2-55.2 ppm characteristic of the metoxy groups (-OCH_3) on the aromatic nuclei of lignin coupled with of the 148.5-151.9 ppm band of aromatic carbons linked to phenolic hydroxyl groups due the reactivity of the aromatic nuclei of lignin.

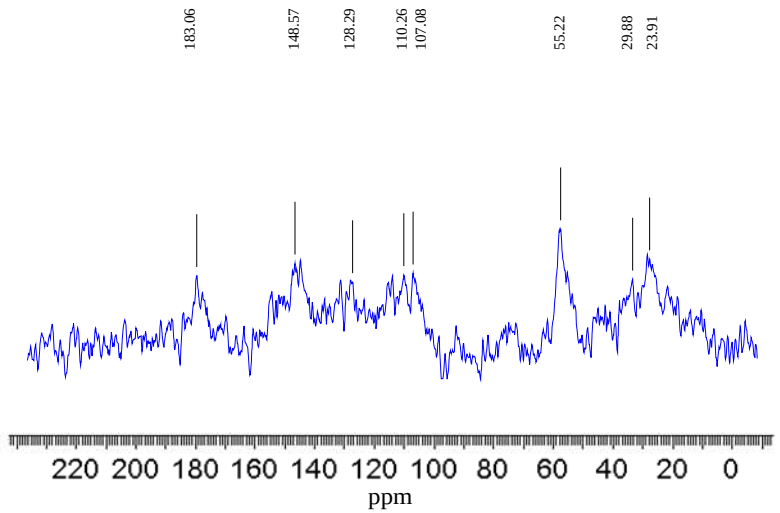

Figure N° 2: ^{13}C NMR lignin CIMV pH 6.6.

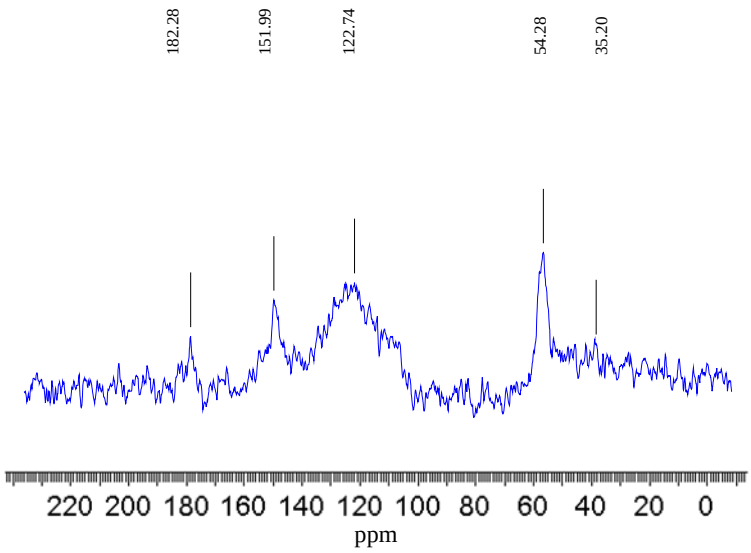

Figure N° 3: ^{13}C NMR lignin LN-T-CO_2-2.

In CIMV lignin appears a peak at 29.88 ppm due –CH- groups of the glyoxylene bridges linking the two aromatic rings in structure as figure N° 3. The 84.5 ppm peak absence is notice in both lignins. This means, there is not formaldehyde presence in the glyoxalated lignin (9).

3.5. Maldi-tof analysis

Glyoxalated lignin presented internal rearrangements of the lignin itself and by glyoxal reaction (Figures N° 4 and 5). The interpretation assignments of the compounds involved in the glyoxalated lignin are indicated as G for guaiacyl units, S for siringyl units and H for phenolic units (Table N° 5 and 6). H_F, G_F and S_F fractions appear (Figure N° 7), indicating demethylation of methoxy groups as well as cleavages have occurred of the aliphatic C3

chain from aromatic ring. The –β1- linked dimmers, in which one of three fractions H_F, G_F and S_F (Figure N° 6) are linked to an uncleaved lignin have been observed. This type of rearrangement is well known in lignin chemistry and is one of the main mechanisms for the cleavage and recombination of such polymers (7). As regards the lignin alone, the heat treatment also leads to further types of recombination by autocondensation. Thus, compounds such as G-G-G-G, a tretramer at 750 Da appears to have been formed by this route (52).

As a consequence of glyoxalation, a number of different condensates seem to have been formed. The simple phenol represented by structures H_F, G_F and S_F have easily reacted with the aldehyde to give a variety of different compounds. Then compounds still containing a reactive hydroxyglyoxyl group are formed in abundant proportions. Oligomers are also present; such as the hydroxyglyoxylated trimers and tetramers in different proportions (table N° 5 and 6). The oligomers present are very similar in both lignins.

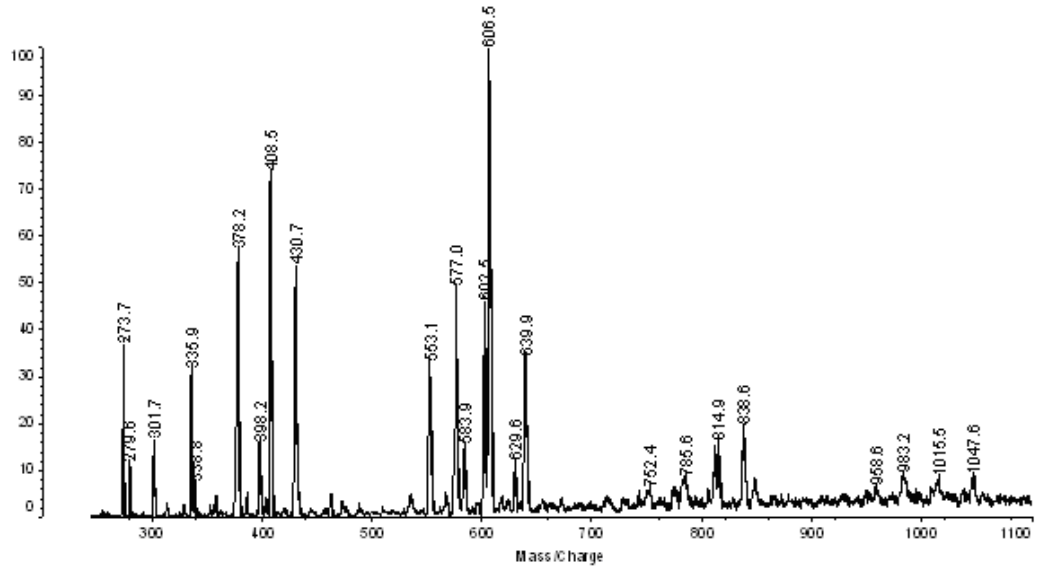

Figure N° 4: Maldi–Tof glyoxalated lignin LN-T-CO_2-2.

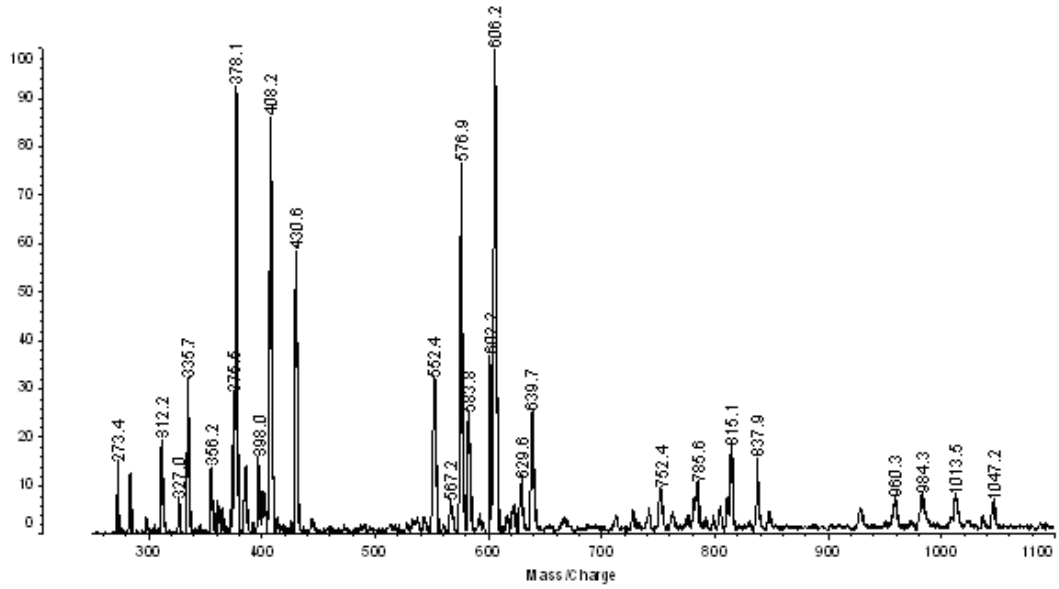

Figure N° 5: Maldi–Tof glyoxalated lignin CIMV pH 6.6.

Table N° 5: LN-T-CO_2-2 Glyoxalated lignin interpretation.

Experimental	Calc.		Peak intensity	Interpretation
M+Na+. (Da)	M+NA	M+NA+ (PM23)	(%)	
273,7	248	271	38	G-H_F
279,6	261	284	12	H-G_F
301,7	274	297	16	H-G_F or/and G-H_F
335,9	313	336	32	H-G_F (minus a CH_3) = H-H_F -OH
338,8	318	341	8	G-glyox-H_F
378,2	348	371	57	S-glyox-H_F
398,2	373	396	16	S-H_F –OH and/or HO-H_F -H–glyox-OH**
408,5	382	405	73	H-H_F-glyox-H_F
430,7	406	429	53	G-glyox-G and/or S-glyox-S_F, and/or G-βO4-S, and/or G-βO4-S
553,1	529	552	34	G-glyox-G-G_F and/or G-S-glyox-S_F
577	551	574	50	G-G-glyox-H
583,9	566	589	18	H_F-G-glyox-H-H_F
602,5	579	602	46	G-S-H_F –OH and/or HO-H_F –H-G–glyox-OH**
606,5	584	607	100	G-G-glyox-G
629,6	607	630	12	S-G-glyox-H
639,9	614	637	32	G-G-glox-S or S-S-glox-H
752,4	728	751	8	G-G-G-G and/or S_F -S-S-S_F
785,6	764	787	9	G-G-glyox-G-G or S-H-glyox-H-S or G-G-glyox-G-G
814,9	791	814	16	**(HO-glyox-)G-H-S-H_F –OH and/or HO-H_F –H-H-G(–glyox-OH)$_2$**
838,6	808	831	20	S-H-glyox-H-S-glyox
958,6	928	951	6	S-S-glyox-S-S-glyox
983,2	962	985	6	G-G-glyox-G-G-glyox-S_F
1015,5	988	1011	10	S-G-glyox-G-S-glyox-S_F
1047,6	1022	1045	6	S-G-glyox-G-S-glyox-S_F

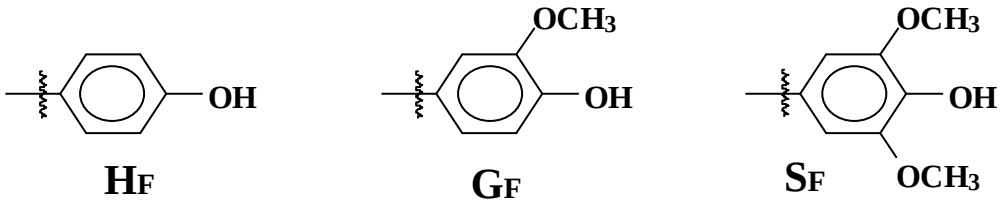

Figure N° 6: H_F, G_F and S_F fractions appear.

Table N° 6: CIMV Glyoxalated lignin interpretation.

Experimental	Calc.		Peak intensity	Interpretation
M+Na+. (Da)	M+NA	M+NA+ (PM23)	(%)	
273,7	248	271	38	G-H_F
312,2	290	313	20	G_F -glyox-G_F and/or 327 (minus1x-CH_3)
327	304	327	6	H-S_F and/or G-G_F and/or S-H_F, and/or H-β1-S, and/or G-β1-G, and/or S-β1-H
335,7	313	336	32	H-G_F (minus a CH_3)) = H-H_F -OH
356,2	334	357	14	G-S_F
375,5	350	373	28	S_F -glyox-S_F
378,2	348	371	57	S-glyox-H_F
398	373	396	15	S-H_F –OH and/or HO-H_F -H–glyox-OH**
408,2	382	405	85	H-H_F-glyox-H_F
430,6	406	429	58	G-glyox-G and/or S-glyox-S_F, and/or G-βO4-S, and/or G-βO4-S
552,4	529	552	32	G-glyox-G-G_F and/or G-S-glyox-S_F
567,2	540	563	6	G-G-G
576,9	551	574	76	G-G-glyox-H
583,8	566	589	24	H_F-G-glyox-H-H_F
602,2	579	602	46	G-S-H_F –OH and/or HO-H_F –H-G–glyox-OH**
606,2	584	607	100	G-G-glyox-G
629,6	607	630	11	S-G-glyox-H
639,7	614	637	25	G-G-glox-S or S-S-glox-H
752,4	728	751	10	G-G-G-G and/or S_F -S-S-S_F
785,6	764	787	11	G-G-glyox-G-G or S-H-glyox-H-S or G-G-glyox-G-G
815,1	791	814	18	**(HO-glyox-)G-H-S-H_F –OH and/or HO-H_F –H-H-G(–glyox-OH)$_2$**
837,9	808	831	14	S-H-glyox-H-S-glyox
960,3	928	951	6	S-S-glyox-S-S-glyox
984,3	962	985	6	G-G-glyox-G-G-glyox-S_F
1013,5	988	1011	6	G-G-glyox-G-G-glyox-G
1047,2	1022	1045	6	S-G-glyox-G-S-glyox-S_F

3.6. Formaldehyde emissions

The formaldehyde emission test showed a great difference between the natural and the traditional commercial synthetic adhesives resins we used (Figure N° 7). These results were different from some reported in other publications for several reasons, but always the

natural adhesives have reported lower formaldehyde releases. Different methods and the adhesive resins systems formulations have been studied. For example, Sukhbaatar et al. (10) determined free formaldehyde in adhesives resins in which a part of phenol had been replaced by lignin in phenol formaldehyde adhesive. Their results indicated that the amounts of formaldehyde used in resins synthesis were not in excess and no formaldehyde odour problem will result in using the resins in boards manufacturing, but in this case, test was made directly in the resins and not in the panel prepared with lignin phenol formaldehyde resins.

As an other example, Mansouri et al. (51) research reported zero emission by dessicator method using glyoxalated lignin and tannin blending as adhesives, and Pichelin et al. (32) did not find formaldehyde emission in panels bond with mimosa tannin adhesives using hexamine. However, Ballerini et al. (25) found very small emissions with glyoxal in radiate pine tannin adhesive using the same method.

In our results, to compare the particleboards prepared with UF and the natural adhesives, these adhesives presented at least more than seven times higher emission formaldehyde than in particleboards prepared with lignin and tannin. The MUF particleboards registered more than four times higher formaldehyde emissions than the natural adhesives. The higher values found in the natural adhesives blending was 1.12 mg formol/kg panel in CIMV/MIM for 40/60 (w/w) and the lower 0.92 mg formol/kg panel in LN-T-CO_2/ MIM for 40/60 (w/w). All the results with natural adhesive resins obtained could be classified as E0 panel (14). The formaldehyde present in the particleboards prepared with lignin and tannin could be the formaldehyde from wood. The ^{13}C NMR results at 84.5 ppm peak showed not formaldehyde emission from glyoxalated lignins has been found, and the hexamine using as hardener with tannin has been accepted by JIS A5908 as not being a formaldehyde source in presence of polyflavonoid condensed tannin.

We found the same tendency than Eom et al. (19), for which the natural adhesives were used in the particleboards preparation. Although different methods were evaluated, the natural adhesives always presented the formaldehyde emission lower than commercial synthetics adhesives as UF and MF resins. The formaldehyde presence in the natural material could be explained because the emission of volatile organics compounds starts in the forestry (53), the natural wood composition is a natural source of formaldehyde emissions although it is in low amounts as it has been demonstrated by some researchers, and due the lignin decomposition (48). The oxidation of terpene compounds simple aldehydes like formaldehyde can be formed (53), and this compound is directly released in

the atmosphere. Of course, there are a great amount of factors that take part direct or indirectly in the formaldehyde emissions and in general the COVs emission has a multiple atmospheric implications (18).

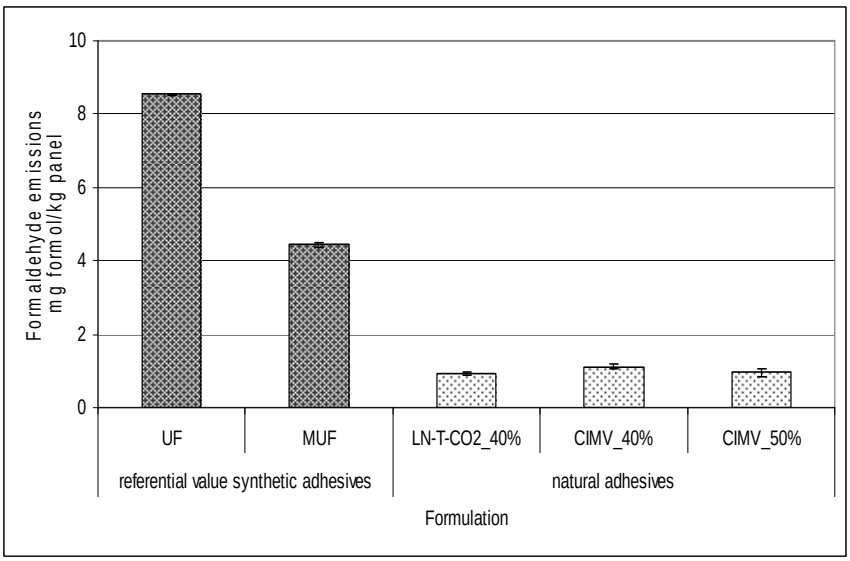

Figure N° 7: Formaldehyde emissions by EN-717-3 standard method.

Finally, it is possible to mention in according to Schäfer and Roffael (54) work that extractives play a dual role. On the one hand the release formaldehyde and by another one they react with formaldehyde and hence act as a formaldehyde scavenger. This dependance of the wood extractives composition, pH, and this behaviour make the different natural raw material must be studied individually.

4. CONCLUSIONS

1. The kraft and wheat straw organosolv lignin showed very smooth differences between them in their chemical structure according to the ^{13}C NMR and Maldi-tof results. Both presented internal rearrangements by themselves and by glyoxal reaction. The simple phenol represented by structures H_F, G_F and S_F have easily reacted with the aldehyde to give a variety of different compounds. Thus oligomers such as the hydroxyglyoxylated trimers and tetramers are present in different proportions.

2. The different formulations of lignin and mimosa tannin studied in this work have showed a very good performance to be used for interior-grade applications according to the standard EN 312 exigencies. The internal bond obtained were 0.53 MPa for LN-T-CO_2/MIM at 40/60 (w/w) and 0.43 MPa for CIMV/MIM at the same proportions. At 50/50 proportion the internal bond decreased to 0.39 MPa. All of them reached the P2 grade (EN 312). That

means the lignin from mills and the wheat straw provenance used here with mimosa tannin have a great potential to be used in the natural adhesives for panel industry. The difference IB results between the kraft and wheat straw lignin found are because a proportion of wheat straw lignin did not react with the glyoxal at low pH conditions as the kraft lignin and the results with the first lignin were lower and it was possible to see with the Maldi-tof results.

3. The formaldehyde release from boards prepared with natural adhesives must be studied in each particular case. In our case, a great difference between the natural and the traditional commercial synthetic adhesives resins was found. The free-formaldehyde emissions from particleboards prepared with lignin and tannin were very low. According to the flask method (EN 717-3), the particleboards could be classified as E0 with a formaldehyde release between 0.92-1.12 mg formol/kg panel. The free-formaldehyde could be the formaldehyde release from wood because the ^{13}C NMR at 84.5 ppm peak showed no formaldehyde emission from glyoxalated lignins and the hexamine using with tannin has been accepted by JIS A5908 as not being a formaldehyde source in presence of polyflavonoid condensed tannin.

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III.2.3. **Etude de la réaction entre la lignine el le glyoxal àu moyen de la spectrometrie de masse MALDI-ToF et de la ^{13}C-NMR**

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La réaction du glyoxal avec une lignine de faible poids moléculaire engendre diverses réactions: une dépolymérisation et une recombinaison de la lignine ainsi qu'une condensation avec le glyoxal pour former des ponts de glyoxalène qui relient principalement les fractions des unités de la lignine issues de la dégradation subie lors de la dépolymérisation. La lignine hydroxyglyoxalée réactive est une espèce qui peut être utilisée comme colle à bois. Les réactions précédemment citées augmentent la viscosité de la lignine et aboutissent à la formation d'une espèce de masse moléculaire plus importante. L'analyse par ^{13}C RMN a confirmé l'existence de groupes fonctionnels relatifs aux structures qui se forment lors de la glyoxalation, de même l'analyse par la spectrométrie de masse MALDI-TOF. Cette dernière technique s'avère une méthode incontournable pour étudier les réactions entre la lignine et le glyoxal.

STUDY ON LIGNIN-GLYOXAL REACTION BY A MALDI-TOF AND CP-MAS 13C NMR

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ABSTRACT

To elucidate the lignin-glyoxal reactions, MALDI-TOF mass spectrometry was used. Reaction of glyoxal with a low molecular weight lignin involves a number of different reactions in particular lignin depolymerisation and recombination through condensation reactions with glyoxal to form glyoxalene bridges linking both lignin units and mainly fractions of lignin units derived from degradation during depolymerization. The reactive hydroxyglyoxalated lignin is the one still available for coreaction to the formation of wood adhesives. The reactions described increase the viscosity of the lignin as the reaction proceeds towards higher molecular mass species and the condensed species grow in relative proportions. NMR analysis confirmed the existence of functional groups pertaining to structures that are formed from glyoxalation of the lignin observed by some results obtained by Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight (MALDI-TOF). MALDI-TOF appears to be a suitable method for examining lignin-glyoxal reactions.

Keywords: lignin, glyoxalation, adhesives, MALDI, lignin-glyoxal reaction, oligomer species, oligomers distribution.

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1. INTRODUCTION

Biocomposites are and have been an area of growing interest and a subject of active research for quite some times now. This is due to both environmental concerns as well as to the foreseen future scarcity of oil and oil-derived products. A class of composites in which natural origin resins have already had a commercial/industrial impact is in the field of rigid wood panels, such as particleboard. Natural origin resins are already commercially used for the last 30 years for these wood panels, and their use is still growing, although still relatively slowly (1). In such an application, the binder is never more than 10% by weight of the whole composite panel. This is enough to stay in line with the performance and costs required by the wood panels industry and their respective product standards.

The main natural resins used as wood panel binders are vegetal tannin adhesives, lignin adhesives and more recently also soy protein adhesives (1). Of these, tannin-based adhesives have been commercial the longest, since 1971. They present the advantage over the other two types of not needing any reinforcement with an oil-derived synthetic resin of some kind (1). This is however still the case for the already commercial use of lignin (2-5) and soy binders (1, 6-8) where either phenol-formaldehyde or most often PMDI (polymeric isocyanate) are still needed in amounts between 20% and 40% of the total resin to satisfy the requirements of relevant board standards. Recently, mixed tannin/lignin adhesives without any synthetic resin added, and of good performance, were reported (9-11). These were based on the use of lower molecular weight lignins prereacted with a non-volatile, non-toxic aldehyde, namely glyoxal. Glyoxalated lignin was then mixed 50/50 by weight with mimosa or quebracho tannin added of a hexamethylenetetramine as hardener, this mixture constituting the total adhesive (9-11).

Since its introduction by Karas and Hillenkamp in 1987 (12), Matrix-Assisted Laser Desorption/Ionization (MALDI) mass spectrometry has greatly expanded the use of mass spectrometry towards large molecules and has revealed itself to be a powerful method for the characterization of both synthetic and natural polymers (13 - 18). Fragmentation of analyte molecules upon laser irradiation can be substantially reduced by embedding them in a light absorbing matrix. As a result intact analyte molecules are desorbed and ionized along with the matrix and can be analysed in a mass spectrometer. This soft ionization technique is mostly combined with time-of-flight (TOF) mass analysers. This is so as TOF-MS present the advantage of being capable to provide a complete mass spectrum per event, for its virtually unlimited mass range, for the small amount of analyte necessary and the relatively

low cost of the equipment. Its usefulness in the elucidation of the distribution of different oligomers in the case of polyflavonoid tannins in general has already been demonstrated (18). Of particular interest has been the MALDI-TOF analysis of the structure of grass lignins conducted by Banoub and Delmas (19) and of eucalyptus wood by Evtuguin et al (20-21).

The goal of this paper is to explain what occurs during the preparation of glyoxalated lignin, to be used as cost wood panel adhesives of good performance based only on natural materials and devoid of fortification by any synthetic resins.

2. EXPERIMENTAL

2.1. Preparation of glyoxalated lignin resin

The lignin used was a low molecular mass lignin obtained industrially as a by-product of pulp and paper production from wheat straw by an acetic acid-based organosolv process US patent 7,402,224 (22). Lignin from wheat straw was supplied by their industrial manufacturer, CIMV (Compagnie Industrielle de la Matiere Vegetale, Reims factory, France). The characteristics of this sample were determined by the FCBA (Centre Technique Fibres, Cellulose, Bois et Ameublement) (Table 1) and by CIMV (Table 2). 295 Parts by mass of this lignin powder (96% solid) were slowly added to 384 parts water while sodium hydroxide solution (30%) was added from time to time thus keeping the pH of the solution between 12 and 12.5 for better dissolution of the lignin powder which was also facilitated by vigorous stirring with an overhead stirrer. A total of 181 parts by mass of 30% sodium hydroxide water solution was added which resulted in a final pH near to 12.5.

Table N° 1. Characteristics of wheat straw organosolv lignin.

	Formyl (mmol/g)	Acetyl (mmol/g)	Free Hydroxyls (mmol/g)	Total (mmol/g)
Total -OH groups	0.6	0.5	2.90	4.00
Phenolic -OH groups	0.20	0.05	0.85	1.10
Aliphatic -OH groups	0.40	0.45	2.05	2.90

Table N° 2. Characteristics of lignin.

Klason lignin			Ashes	Glucan	Xylan	Mannan	Galactan	Arabinan	Total without glucan
Insoluble (wt %)	Soluble (wt %)	Total (wt %)	(wt %)	(wt %)	(wt %)	(wt %)	(wt %)	(wt %)	(wt %)
82.7	2.22	84.9	1.83	4.49	5.98	0.38	0.97	1.45	8.78

A 2 litres flat bottom flask equipped with a condenser, thermometer and magnetic stirrer bar was charged with the above solution and heated to 60 °C. 175 parts by mass glyoxal (40% in water) were added and the lignin solution was then continuously stirred with a magnetic stirrer/hot plate for 8 hours. The solids content for all glyoxalated lignin were around 31-33%. Glyoxal is a non-volatile non-toxic aldehyde that has been tested in lignin (5-6) and tannin adhesives (23) and other adhesives (8) for application to wood panels such as particleboard. Glyoxal is a non-toxic aldehyde (LD50 rat $\geq$ 2960 mg/kg; LD50 mouse $\geq$ 1280 mg/kg) (24), non-volatile but less reactive than formaldehyde which is toxic (LD50 rat $\geq$ 100 mg/kg; LD50 mouse $\geq$ 42 mg/kg) (25).

Viscosity was measured with a Brookfield RV viscometer; spindle Nr. 4, at 25°C for all the samples.

2.2. MALDI-TOF Mass Spectrometry

The spectra were recorded on a KRATOS Kompact MALDI AXIMA TOF 2 instrument. The irradiation source was a pulsed nitrogen laser with a wavelength of 337 nm. The length of one laser pulse was 3 ns. The measurements were carried out using the following conditions: polarity-positive, flight path-linear, mass-high (20 kV acceleration voltage), 100-150 pulses per spectrum. The delayed extraction technique was used applying delay times of 200-800 ns.

2.3. MALDI-TOF Sample Preparation

The lignin samples were dissolved in acetone (4 mg/ml, 50/50% volume). The sample solutions were mixed with an acetone solution (10 mg/ml in acetone) of the matrix. As a matrix, 2,5-dihydroxy benzoic acid was used. For the enhancement of ion formation NaCl was added to the matrix (10mg/ml in water). The solutions of the sample and the matrix were mixed in the proportions 3 parts matrix solution + 3 parts polymer solution + 1 part NaCl solution and 0.5 to 1 µL of the resulting solution mix were placed on the MALDI target. After evaporation of the solvent the MALDI target was introduced into the spectrometer. . To each peak value in the resulting positive mode spectrum must be subtracted the 23 Da of the Na^+ of the matrix to obtain the molecular weight of the chemical species of the peak.

2.4. CP-MAS ^{13}C NMR

The glyoxalated organosolv lignin resin was either dried at 103°C for 2 hours in an oven before being grounded finely for NMR analysis or first precipitated with either acetic acid or

phosphoric acid before being dried for NMR analysis. The hardened lignin resins were analysed by solid state CP MAS ^{13}C NMR. Spectra were obtained on a Bruker AVANCE II 400 MHz spectrometer at a frequency of 100.6 MHz and at sample spin of 12 kHz, using a recycling delay of 1s -depending of ^{1}H spin lattice relaxation times (t_1) estimated with the inversion-recovery pulse sequence-, a contact time of 1 ms. Number of transients was about 15000, and the decoupling field was 78 kHz. Chemical shifts were determined relative to tetramethyl silane (TMS) used as control. The spectra were accurate to 1 ppm. The spectra were run with suppression of spinning side bands. Only the spectra of the unreacted lignin and of the lignin dried directly at 103°C for two hours are reported, as the latter spectrum was the same to that of the glyoxalated lignin precipitated in the two other different manners.

3. RESULTS AND DISCUSSION

It is of interest to observe the type and relative proportion of the oligomers existing in this organosolv lignin before reaction with glyoxal (Table 3, Figure N°. 1) and compare them with those after glyoxalation (Table 4, Fig. 2a,b). From Table 3, one can see that dimers and trimers of different types predominate, but that no oligomers of higher mass are present. The interpretation of the compounds involved are indicated as G for guaiacyl units, S for siringyl units and H for phenolic units not containing methoxy groups. The linkages between them are indicated with the "classical" nomenclature (26) as βO4, αO4, ββ, β5, 55'. Thus, as an example, to the G-βO4-S, and G-αO4-S peak at 431 Da in Fig. 1 corresponds the structure (407+23 = 430 Da).

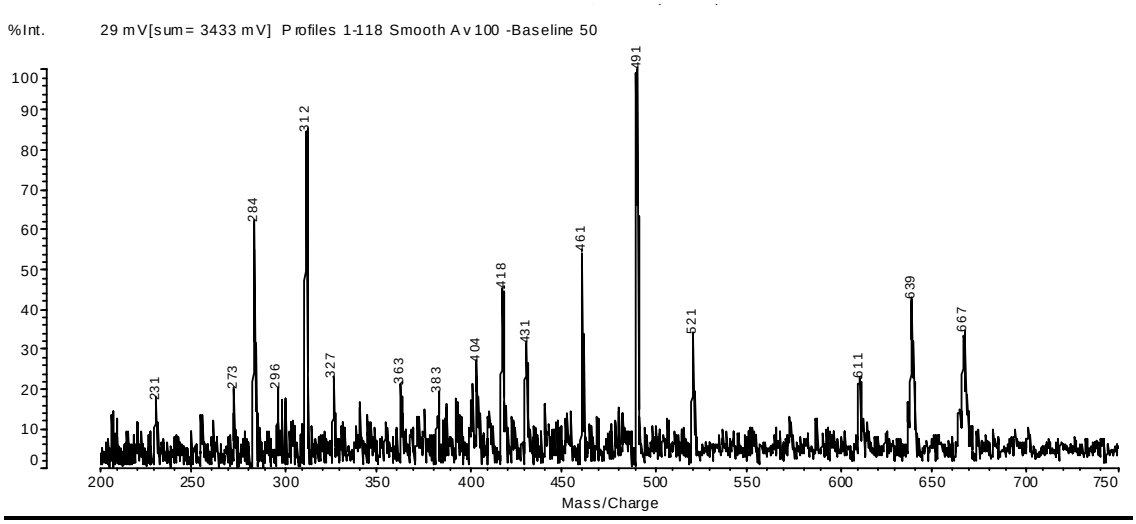


Fig. 1: MALDI-TOF of unreacted wheat straw organosolv lignin.

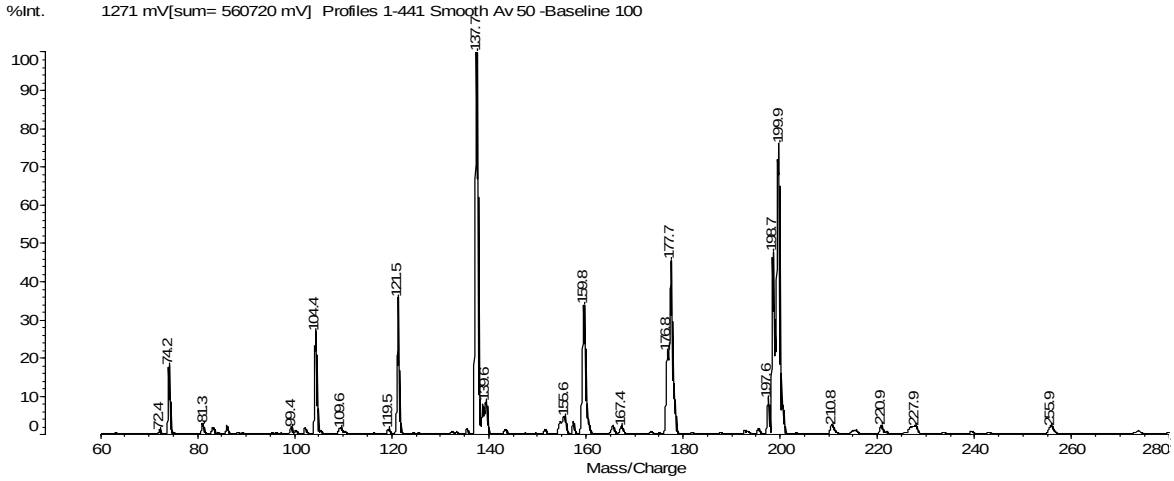


Fig. 2a: MALDI-TOF of glyoxalated wheat straw organosolv lignin: (a) 60 Da - 280 Da range.

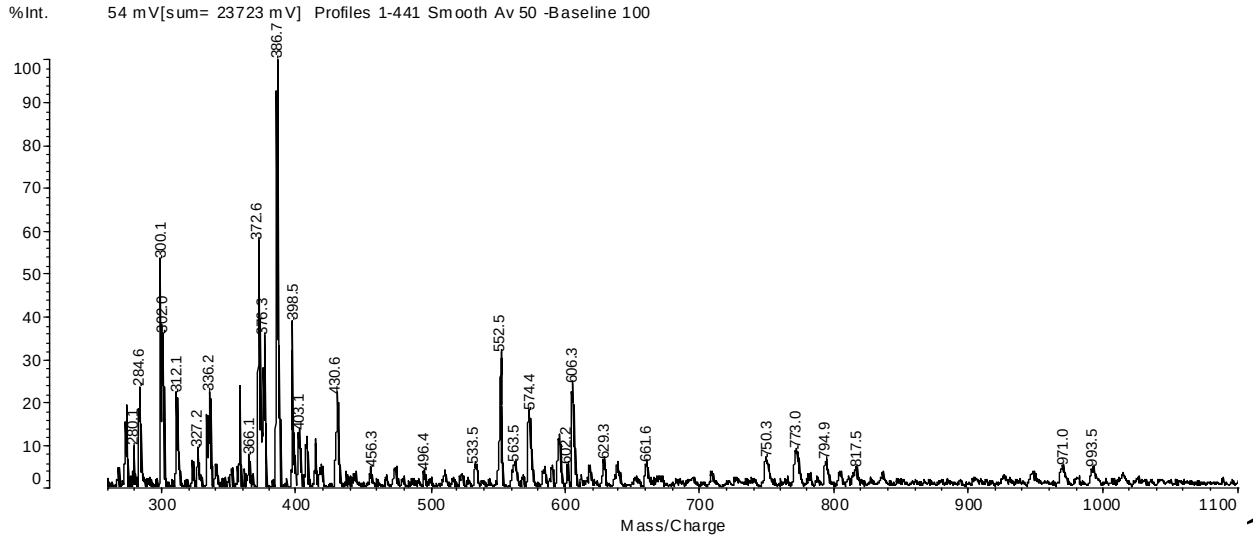
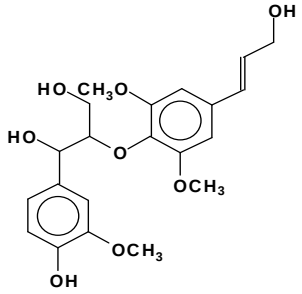
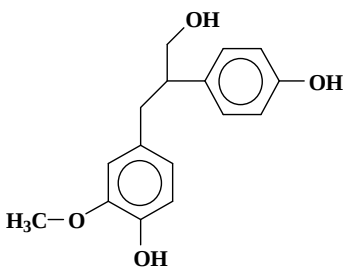


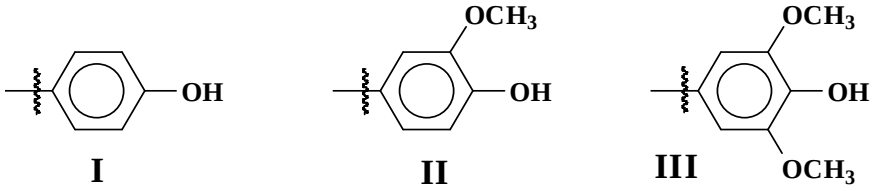
Fig. 2b: MALDI-TOF of glyoxalated wheat straw organosolv lignin: (b) 280 Da - 1100 Da range.



And to the H-β1-G peak at 296 Da corresponds the structure (274 +23 = 296 Da).



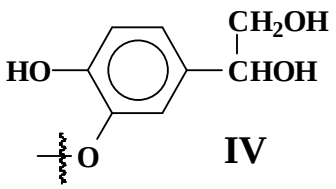
Such a structure is more simply indicated according to the nomenclature used in Table 4 as H_F, G_F and S_F are indicated the three fractions (I), (II) and (III) linked -β1- to another lignin unit or fraction of lignin unit.



I II III

Comparing the results of Table 4 and Figs. 2a,b with Table 3 and Fig. 1, it can be noticed that major changes have occurred during the glyoxalation of lignin at 60°C for 8 hours and alkaline pH. First of all, some of the original lignin compounds disappeared due to both the effect of the long period of heating at very alkaline pH. Thus the S monomer at 231 Da disappeared and at its place appeared the H_F, G_F and S_F fractions, indicating that both demethylation of the methoxy groups as well as cleavage of the aliphatic C_3 chain from the aromatic ring occurred. These changes are not due to any reaction with the glyoxal, but are internal rearrangements of lignin itself. The -β1- linked dimers, in which one of the three fractions H_F, G_F and S_F are linked to an uncleaved lignin unit have greatly increased: such a type of rearrangement is well known in lignin and is one of the main mechanisms of cleavage and recombination of such a polymer (26). As regards the lignin alone, the heat treatment also leads to further types of recombination by autocondensation. Thus, compounds such as G-G-G-G, a tetramer at 750.3 Da, appears to have been formed by this route because tetramers were not present in the original lignin.

As for the reaction of lignin with glyoxal, a number of different condensates seem to have been formed. Thus, the simple phenols represented by structures (I), (II) and (III) have easily reacted with the aldehyde to give a variety of different compounds. Thus, compounds still containing a reactive hydroxyglyoxyl group are formed in abundant proportions, as can be seen from the relative peak.



IV

Intensities in Table 4 and Figs. 2a, b, such as those at 197.6 Da, 198.7 Da and 199.9 Da correspond to structures of type (IV) with the second aldehyde moiety of glyoxal in the form it generally aldehydes exist in water.

Thus, functional groups that could belong to structures of type (IV) are observed in the CP-MAS ^{13}C NMR of glyoxalated lignin simply precipitated by pH variation after glyoxalation. In Fig. 3 the band at 61.9 ppm belongs to the -CH_2OH of structure (IV) while a split in the band a 72.9 ppm belonging to the aliphatic -CH_2OH characteristic of the structure of unreacted lignin (Fig. 4 unreacted lignin) giving rise to an additional band at 70.3 ppm indicates the presence of the >CHOH groups of the reacted glyoxal of structure (IV).

Table N° 3: MALDI-TOF derived distribution of oligomers in the original organosolv lignin.

Experimental peak (Da)	Calculated M.W. (Da)	Calculated M.W.(+23,5Na+) (Da)	Peak relative proportion (%)	Structure	
231	210	233	18	S	monomer
273					
284	261	284	63	H-β1-H – 1x-OH	dimer
296	273	296	18	H-β1-G G-β1-H	dimers
312	289	312	85	326–1x-CH_2- (demethylation)	dimers
327	303	326	22	H-β1-S G-β1-G S-β1-H	dimers
383	360	360	18	H-βO4-S G-βO4-G S-βO4-H	dimers
418	394.4	417.4	43	G-ββ-G G-β5-G G-55'-G	dimers
431	407	430	32	G-βO4-S G-αO4-S	dimers
461	437	460	54	S-βO4-S S-αO4-S	dimers
491	468	491	100	S-βO4-S+2x-OH S-αO4-S+2x-OH	dimers
521					
611	585	608	21	S-βO4-S-ββ-H G-βO4-G-ββ-S G-βO4-G-β5-S G-βO4-S-55'-G	trimers
639	615	638	40	G-βO4-S-ββ-S G-βO4-S-β5-S G-βO4-S-55'-S	trimers
667	644.7	667.7	34	S-βO4-S-ββ-S S-βO4-S-β5-S S-βO4-S-55'-S	trimers

The glyoxalated lignin -CHOH peak at 61.9 ppm indicating the reaction of glyoxal with lignin could be confused with the hydroxilated form of the aldehyde that is present in water namely HO-CH2-CH2-OH. However, the two carbons of the latter are expected to appear at 63.6 ppm and not at a lower shift as it is the case in Figure N° 3. Some doubts about this

subject could have subsisted if only the NMR analysis have been done. The difference in NMR shift coupled with the increasing viscosity reported in Table 5 and the MALDI-TOF show a clear indication of higher molecular weight oligomers formation by reaction of lignin with glyoxal and so the interpretation of the NMR 61.9 ppm peak is correct.

Table N° 4: Peak distribution and assignments for MALDI TOF spectra of glyoxalated lignin.

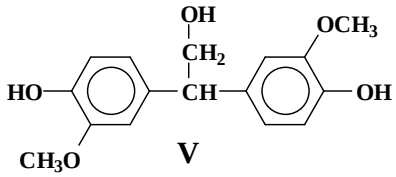
Experimental peak (Da)	Calculated M.W.(Da)	Calculated M.W.(+23,5Na+) (Da)	Peak relative proportion (%)	Structure
101-104.4	78	101	28	benzene ring from degradation
119.5-121.5	94	117.5	37	H_F
137.7	112	135	100	G_F (minus-CH_3) diprotonated
176.8	152	175	24	H
177.7	154	177	48	S_F
197.6	174	197	10	(G_F minus CH_3)(–glyox-OH)* diprotonated
198.7	176	199	50	(G_F minus CH_3)(–glyox-OH)** diprotonated
199.9	176	199	78	(G_F minus CH_3)(–glyox-OH)**
255.9	232	255	3	G_F (–glyox-OH)$_2$ and/or H_F -glyox-H_F
284.6	262	285	24	G_F -glyox-H_F and/or H-β1-H(minus1x-OH)
300.1	274	297	54	H-G_F or/and G-H_F
312.1	290	313	22	G_F -glyox-G_F and/or 327 (minus1x-CH_3)
327.2	304	327	11	H-S_F and/or G-G_F and/or S-H_F, and/or H-β1-S, and/or G-β1-G, and/or S-β1-H
336.2	313	336	24	H-G_F (minus a CH_3)) = H-H_F -OH
366.1	343	366	8	G-H_F -OH
372.6	350	373	57	S_F -glyox-GS_F
386.7	364	387	100	S-S_F and/or G-G (=Gβ-O-4G), or H-S
398.5	373	396	40	S-H_F –OH and/or HO-H_F -H–glyox-OH**
430.6	406	429	24	G-glyox-G and/or S-glyox-S_F, and/or G-βO4-S, and/or G-αO4-S
456.3	433	456	5	G-S
552.5	529	552	32	G-glyox-G-G_F and/or G-S-glyox-S_F
563.5	540	563.6	7	G-G-G
574.4	551	574	20	G-G-glyox-H
602.2	579	602	6	G-S-H_F –OH and/or HO-H_F –H-G–glyox-OH**
606.3	584	607	25	G-G-glyox-G
661.6	639	662	6	**(HO-glyox-)G-S-H_F –OH and/or HO-H_F –H-G(–glyox-OH)$_2$**
750.3	728	751	6	G-G-G-G and/or S_F -S-S-S_F
773.0	750	773	10	G-G-G-S
794.9	772	795	6	G-G-glyox- G-G and/or S_F-S-glyox-S-S_F

817.5	791	814	4	**(HO-glyox-)G-H-S-H_F –OH and/or HO-H_F –H-H-G(–glyox-OH)$_2$**
971.0				773-glyox-H
Experimental peak (Da)	Calculated M.W.(Da)	Calculated M.W.(+23,5Na+) (Da)	Peak relative proportion (%)	Structure
993.5	967	990	5	G-G-glyox- G-G-glyox-H and/or S_F-S-glyox-S_F –S-glyox

* = 59 Da
** = 61 Da

Higher hydroxyglyoxalated oligomers are also present such as the hydroxyglyoxylated trimers at 602.2 Da and 661.6 Da and the hydroxyglyoxalated tetramers at 817.5 Da and 971.0 Da. Their relative proportions are relatively small, as it can be seen by their peaks relative intensity. Hydroxyglyoxalated monomers such as those at 197.6 Da, 198.7 Da, 199.9 Da and 255.9 Da, as well as hydroxyglyoxalated dimers such as those at 398.5 Da predominate in quantities (see intensities in Table 2 and Figs. 2a,b). Di-hydroxyglyoxalated species also occur (255.9 Da) but they appear to be relatively rare.

The other species that occur are those in which the glyoxal has succeeded not to just react with a lignin unit aromatic ring but to form a glyoxylene bridge between two different lignin aromatic rings. Among these, the glyoxylene bridges between the different cleaved fractions (I), (II) and (III) predominate as can be seen from the relative intensities of the peaks at 284.6, 312.1, 372.6 and 430.6 Da. The example of the 312.1 Da peak is structure (V).



V

Functional groups of structure of type (V) are also observed first of all by the increase of the band at 32.7-33.3 ppm of the unreacted lignin to higher intensity than the 30 ppm band due to the -CH- groups of the glyoxylene bridge linking the two aromatic rings in structure (V) also being observed at the same ppm. The -CH_2OH group of structure (V) also contributes to the 70.3 ppm band (Fig. 3). However, structures in which full scale lignin units are obtained by condensation with glyoxal and linked by one or more glyoxylene bridges are also present such as: (i) the trimers formed by a glyoxylene bridge between a lignin dimer and a lignin monomer such as those at 552.5 Da, 574.4 and 606.3 Da, also confirmed as for structure (V) by the NMR analysis, which are present in noticeable proportion by the functional groups, and (ii) the less abundant tetramers formed by a glyoxylene bridge between either two lignin dimers or between a lignin trimer and a lignin monomer such as those at 794.9 Da, and (iii)

the pentamers formed presenting two glyoxylene bridges between the lignin units such as those at 993.5 Da.

The CP-MAS ^{13}C NMR of glyoxalated lignin also confirms other aspects of the intermediate structures formed by the reaction of lignin with glyoxal. Thus the relative intensities of the bands the unreacted *ortho* and *para* sites on the lignin aromatic rings at 105-115 ppm decreases markedly in relation to the 125-135 ppm bands after glyoxalation, especially the 105 ppm band of the unreacted *ortho* sites (cf. Figs. 3 and 4). This is coupled with the marked increase of the intensity of the 125-135 ppm bands characteristic of reacted *ortho* and *para* sites indicating some reactions on particularly in *ortho* sites of the aromatic lignin ring. Thus reactions linking aromatic nuclei of lignin are confirmed supporting what found by MALDI-TOF. The pattern of methylene groups is also different after glyoxalation with the apparition of a small band at 13.9 - 14.3 ppm this belonging to a sterically crowded -CH_2- group. Of interest is also the very intense peak at 181.8 ppm, which is not present in unreacted lignin. This band belongs to aromatic quinones generated by oxidation on the site of the phenolic hydroxyl group of lignin. The acetic acid residue band at 173 ppm characteristic of the acetic acid from the organosolv process used to prepare this lignin (22) is markedly decreased after the hot glyoxal treatment (compare Figs 3 and 4). Equally noticeable is the marked decrease of the 55.3-55.5 ppm band characteristic of the methoxy (-OCH_3) groups on the aromatic nuclei of lignin (Figs. 3 and 4) coupled with the moderate increase of the 148-150 ppm band of aromatic carbons linked to phenolic hydroxy groups indicating an increase reactivity of the aromatic nuclei of lignin.

Thus, reaction of glyoxal with a low molecular weight lignin involves a number of depolymerisation and recombination reactions proper to just the lignin structure itself, the condensation of glyoxal with both fraction of cleaved lignin to form hydroxyglyoxylated species, as well as condensation through glyoxylene bridges both between cleaved fractions of lignin like between uncleaved lignin units. To this corresponds invariably an increase of viscosity of the reaction mixture as the reaction proceeds towards higher molecular mass species. Thus, progressive lengthening of the reaction leads to higher viscosity glyoxalated lignins as the species condensed through the formation of glyoxalene bridges grow in relative proportions. The viscosity results in Table 5 confirm the increase in viscosity of the same lignin when the glyoxalation time is progressively longer.

4. CONCLUSION

Reaction of glyoxal with a low molecular weight lignin involves a number of reactions. These are: (1) depolymerisation and β1 recombination reactions yielding β1-linkages, reactions proper to just the lignin structure itself; (2) the formation of simple phenolic species derived from the cleavage of lignin units; (3) the condensation of glyoxal with both fraction of cleaved lignin to form reactive hydroxyglyoxalated species still free for further condensation, as well as (4) condensation through glyoxylene bridges both between cleaved fractions of lignin as well as (5) between uncleaved lignin units. The reactive hydroxyglyoxalated are those still available for co-reaction either with a diisocyanate or with a reactive flavonoid tannin for the formation of wood adhesives. To the reactions described correspond invariably an increase of viscosity of the reaction mixture as the reaction proceeds towards higher molecular mass species. Thus, progressive lengthening of the reaction leads to higher viscosity glyoxalated lignins as the species condensed through the formation of glyoxylene bridges grow in relative proportions.

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III.2.4. **Étude par spectrométrie de masse MALDI-ToF de la distribution d'oligomères dans la lignine glyoxalée à stabilité durable**

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Lors de travaux précédents, on a montré qu'une colle contenant de la lignine (provenance CIMV) glyoxalée mélangée à du tanin de mimosa, avec un durcisseur de type hexamine en proportions lignine/tanin 50/50, répond aux exigences des normes internationales pour son utilisation dans la fabrication de panneaux de particules d'usage extérieur et intérieur. Cependant, pour être acceptable d'un point de vue commercial, la lignine glyoxalée doit être stockée et donc rester stable pour un temps suffisamment long. Pendant son stockage à l'état liquide, elle tend comme toutes les colles à la polycondensation ce qui se traduit par une augmentation de sa viscosité et une diminution de ses performances.

A la suite de ces observations, le séchage par atomiseur semble être une technique appropriée pour obtenir une stabilisation de la colle sous forme solide.

La lignine glyoxalée, séchée dans un sécheur «spray», a été analysée par la technique de MALDI-TOF pour étudier la distribution d'oligomères. Les résultats ont été comparés à ceux correpondant à la lignine glyoxalée fraîche.

Au moment de son utilisation, la colle est préparée en dissolvant dans l'eau de la lignine glyoxalée et séchée jusqu'à un taux de solides de 33%. Cette solution de lignine est ensuite mélangée avec une solution de tanin de mimosa à 45% de solides dans une proportion

lignine/tanin de 50/50. Le module d'élasticité d'échantillons de panneaux collés avec cette colle a été déterminé par des essais TMA. Les résultats ont été comparés avec ceux obtenus à partir d'une colle à base de lignine glyoxalée fraîche, et stockée pendant 3 mois à l'état liquide.

On a montré que la lignine glyoxalée séchée conserve sa réactivité sans diminution des performances de la colle. Ceci indique que la déshydratation par atomisation est une méthode efficace pour un stockage de longue durée sans altération du matériau. Un effet clair a été observé dans la distribution d'oligomères après séchage de la lignine. D'après l'analyse de MALDI-ToF, le séchage entraine plusieurs conséquences:

Une rupture de certains composés tels que les oligomères de sucres sous l'effet de l'atomisation ce qui entraine une baisse de la viscosité de la colle obtenue (après dissolution de la lignine séchée)

Une rupture et des réarrangements possibles suite au chauffage de la lignine conduisant à la formation de plusieurs oligomères

Une polymerisation du glyoxal avec les monomers et les dimères qui conduit à des oligomères de plus haut poids moléculaire (condensation et formation de ponts de glyoxylène).

MALDI-TOF STUDY OF OLIGOMERS DISTRIBUTION FOR STABILITY DURABLE SPRAY-DRIED GLYOXALATED LIGNIN FOR WOOD ADHESIVES

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ABSTRACT

Spray-drying of glyoxalated lignin used in tannin/lignin adhesives for exterior and interior-grade wood particleboard and other types of panels has been shown to be capable of maintaining its reactivity after spray-drying with no decrease in perfomance of the adhesive once the spray-dried material is re-dissolved in water. This indicates that spray-drying is a very suitable method for long term storage of the adhesive without loss of performance. A clear variation in the distribution of oligomers in the material after spray-drying has been observed by Matrix Assisted Laser Desorption Ionisation Time of Flight (MALDI-TOF) analysis. One effect of the reaction that has been noticed with aging is the assembly of the monomers and lower molecular mass oligomers such as dimers into structures of greater molecular weight as a consequence of their condensation with glyoxal and the formation of glyoxylene bridges between them.

Keywords: lignin, glyoxalation, spray-drying, adhesive, distribution of oligomers, MALDI.

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1. INTRODUCTION

Rigid wood panels, such as particleboards, represent a class of composites in which natural origin resins have already had a commercial/industrial impact. Natural origin

resins/adhesives have already been used commercially for the last 30 years for these wood panels, and their use is still growing, although relatively slowly [1]. Of these, tannin-based adhesives have been commercially available since 1971. Lignin adhesives, however [2-5] need addition of either phenol-formaldehyde or polymeric diphenyl methane diisocyanate (PMDI) in amounts between 20 and 40% of the total resin to satisfy the requirements of relevant board standards. Recently, however, mixed tannin/lignin adhesives without any synthetic resin added, and with good performance, were reported [6-7]. These were based on the use of lower molecular weight lignins pre-reacted with a non-volatile, non-toxic aldehyde, namely glyoxal. Glyoxalated lignin was then mixed 50/50 by weight with mimosa or quebracho tannin with hexamethylenetetramine added as hardener, this mixture constituting the total adhesive [6-7].

However, to be acceptable commercially glyoxalated lignin needs to be stable in time and capable of being stocked for a long time. In its liquid solution form, as all polycondensation resins, glyoxalated lignin tends to increase in viscosity, beyond a certain value of which the performance of the tannin/lignin wood particleboard resin decreases. Among several techniques that were considered to improve stability of the resin upon storage, such as glyoxalation in fine powder suspension [8] but the reproducibility of which proved to be inconsistent [9], spray-drying appeared to be the most suitable solution to the stability need of the resin over time.

Since its introduction by Karas et al in 1987 [10], Matrix-Assisted Laser Desorption/Ionization (MALDI) mass spectrometry has greatly expanded the use of mass spectrometry for large molecules and has revealed itself to be a powerful method for the characterization of both synthetic and natural polymers [11-16]. Its usefulness in the elucidation of the distribution of different oligomers in the case of polyflavonoid tannins in general has already been demonstrated [14-16]. Of particular interest has been the MALDI-TOF analysis of the structure of grass lignins conducted by Banoub and Delmas [17], of eucalyptus wood by Evtuguin and Amado [18] and Evtuguin et al [19] and of glyoxalated lignin in solution [20].

This paper then deals with investigating what occurs after the preparation of glyoxalated lignin, hence of lower cost wood panel adhesives with good performance. They are based only on natural materials and devoid of fortification by any synthetic resins, once the glyoxalated lignin is spray-dried and then re-dissolved in water before application as an adhesive for wood panels.

2. EXPERIMENTAL

2.1. Preparation of glyoxalated lignin resin

The lignin used was a low molecular mass lignin obtained industrially as a by-product of pulp and paper production from wheat straw by an acetic acid-based organosolv process [21]. The lignin was supplied by its industrial manufacturer, CIMV (Compagnie Industrielle de la Matiere Vegetale, Reims Factory, France). Its characteristics have already been reported [7, 20]. 295 Parts by mass of this lignin powder (96% solid) were slowly added to 477 parts water while sodium hydroxide solution (30%) was added from time to time thus keeping the pH of the solution between 12 and 12.5 for better dissolution of the lignin powder which was also facilitated by vigorous stirring with an overhead stirrer. A total of 141 parts by mass of 30% sodium hydroxide water solution was added which resulted in the final pH near 12.5.

A 2 liters flat bottom flask equipped with a condenser, thermometer and magnetic stirrer bar was charged with the above solution and heated to 70°C. 175 parts by mass glyoxal (40% in water) were added and the lignin solution was then continuously stirred with a magnetic stirrer/hot plate for 5 h. The solids contents for all glyoxalated lignin were about 31% by weight. Glyoxal is a non-volatile non-toxic aldehyde that has been tested in lignin [5, 22] and tannin adhesives [23] and other adhesives [24] for application to wood panels such as particleboards. Glyoxal is a non-toxic aldehyde (LD50 rat $\geq$ 2960 mg/kg; LD50 mouse $\geq$ 1280 mg/kg) [25], non-volatile but less reactive than formaldehyde which is toxic (LD50 rat $\geq$ 100 mg/kg; LD50 mouse $\geq$ 42 mg/kg) [26].

Viscosity was measured with a Brookfield RV viscometer, spindle Nr. 4, at 25°C for all the samples.

2.2. Spray-drying of glyoxalated lignin

The glyoxalated lignin sample after 60 days aging to check that viscosity had increased was spray-dried with a laboratory bench spray-drier ICF & WELKO (Special plant, Maranello (Mo), Italy) under the following conditions:

Inlet air temperature = initial 154°C, final 151°C

Outlet air temperature = initial 92°C, final 86°C

Temperature of the collected spray-dried powder = 54°C constant throughout spray-drying.

Sample injection rate = 100 cm^2/hour

The spray-dried glyoxalated lignin powder was then cooled and sent for MALDI-TOF analysis. The spray-drying yield was in excess of 97%.

2.3. Thermomechanical analysis (TMA)

The hardening reaction of a resin system or resins mixes can be evaluated by TMA by studying the rigidity of the bonded wood joint as a function of temperature. Thus, mixes of glyoxalated lignin with tannin/hexamine were prepared and analysed by TMA [7]. Mimosa tannin extract (origin: Tanzania. Supplied by: Silva, S. Michele Mondovi, Italy) was used. The tannin solution in water was prepared at 45% concentration and its pH adjusted to 10 with 33% water solution of NaOH. The high pH = 10 was chosen as the hardener used performs best at such a pH [30, 31]. The glue-mix was composed of: (1) the tannin extract solution. (2) 6% hexamethylenetetramine (on tannin solids content) hardener, which was added to the tannin as a 30% aqueous solution. (3) Glyoxalated spray dried powder lignin was re-disolved 60 days after spray drying as a 31% solution in water. The proportions of mimosa tannin/glyoxalated lignin were 50:50 (w/w). This result was compared with mimosa tannin/fresh glyoxalated lignin and with the mimosa tannin/aged glyoxalated lignin (after 90 days) in the same proportions. All experiments were conducted under the same conditions: heat rate = 10°C/min, 30mg of resin, in the temperature range 25-250°C. The thermomechanical analyzer used was a Mettler Toledo TMA40. The software used for data treatment was STARe. Deflection curves that allow MOE (Elasticity Modulus) determination were obtained by the three-point bending TMA mode. The MOE of the wood joints bonded with different resin systems gave a good indication of the final strength of the adhesive system tested.

2.4. MALDI-TOF Mass Spectrometry

The spectra were recorded on a KRATOS Kompact MALDI AXIMA TOF 2 instrument. The irradiation source was a pulsed nitrogen laser with a wavelength of 337 nm. The length of one laser pulse was 3 ns. The measurements were carried out using the following conditions: polarity-positive, flight path-linear, mass-high (20 kV acceleration voltage), 100-150 pulses per spectrum. The delayed extraction technique was used by applying delay times of 200-800 ns.

2.5. MALDI-TOF Sample Preparation

The glyoxalated dried lignin samples were dissolved in acetone (4 mg/ml, 50/50% volume). The sample solutions were mixed with an acetone solution (10 mg/ml in acetone) of the matrix. As the matrix 2,5-dihydroxy benzoic acid was used. For the enhancement of ion formation NaCl was added to the matrix (10 mg/ml in water). The solutions of the sample

and the matrix were mixed in the proportions 3 parts matrix solution + 3 parts polymer solution + 1 part NaCl solution and 0.5 to 1 μL of the resulting solution mix were placed on the MALDI target. After evaporation of the solvent the MALDI target was introduced into the spectrometer. From each peak value in the resulting positive mode spectrum the 23 Da of the Na^+ of the matrix must be subtracted to obtain the molecular weight of the chemical species of the peak.

3. RESULTS AND DISCUSSION

Glyoxalated lignin has been used in 50/50 tannin+hexamethylenetetramine/glyoxalated lignin adhesives without synthetic resin according to recent results for wood panel adhesives [7]. Thermomechanical analysis is used to comparatively predict the strength results of adhesives for wood panel products [27-31]. In Fig. 1 are shown the thermomechanical analysis (TMA) curves of adhesives composed of freshly glyoxalated lignin, aged (60 days) spray-dried and re-dissolved glyoxalated lignin, and glyoxalated lignin still in solution but aged for 90 days. The maximum value of the modulus of elasticity (MOE) found indicates that there is no major difference in strength performance between freshly glyoxalated lignin and spray-dried glyoxalated lignin, indicating that spray-drying is a good way to maintain such an adhesive component active for a long time. Spray-drying does allow long term storage of the adhesive before the need to re-dissolve it for use by blocking the progress of polycondensation due to the absence of water in the spray-dried powder. The maximum MOE value of glyoxalated lignin still in solution but aged for 90 days is instead much lower indicating that glyoxalated lignin in solution has a limited storage time span. The increase in viscosity of the glyoxalated lignin solution as a function of storage time is shown in Table 1. This indicates that the resin continues to condense at ambient temperature when in solution, slowly proceeding towards inactivation. On the contrary, not only the spray-dried glyoxalated lignin is still active as shown by the TMA curves in Fig. 1 but spray-drying has decreased somewhat its viscosity once re-dissolved in water at the same concentration. This indicates that the relative abundance and type of the molecular species present have changed due both to aging and to the action of the spray-drier. The MALDI-TOF analysis can then give some idea of the changes that have occurred by spray-drying glyoxalated lignin.

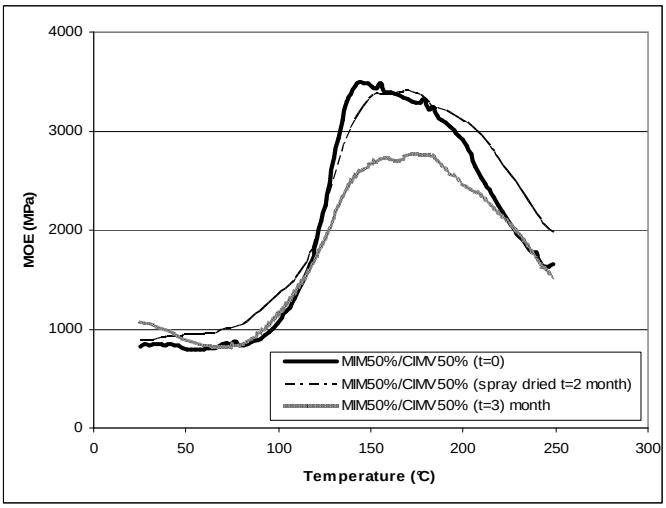


Fig. 1. Thermomechanical analysis (TMA) curves of tannin/glyoxalated lignin adhesives with gyoxalated lignin maintained in solution (freshly prepared and after 90 days aging) and with glyoxalated lignin after spray-drying and redissolving in water.

Table 1. Viscosity variation as a function of time of glyoxalated lignin solution

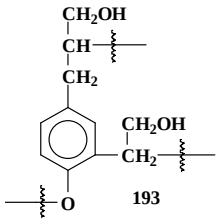
Time from glyoxalation	Temperature (°C)	Viscosity (cPs)
t=0	25	110
t=60 days	25	175
t=90 days	25	387
Lignin spray dried, re-dissolved	25	75

A previous study had examined in detail by both MALDI-TOF and ^{13}C NMR the oligomers distribution of the original lignin and of glyoxalated lignin in solution a short time after its preparation. The oligomers were identified and reported in detail for both cases [20]. The glyoxalated lignin was used succesfully for the preparation of tannin/lignin 50/50 adhesives for wood panels with good performance and this was done without the aid of any synthetic, oil-derived resins [7].

Spray-drying of any natural polymer often induces modifications in viscosity and other properties. Thus, several effects may occur during spray-drying operation. These are: (i) the heated resin continues to polymerise, it "advances" during spray-drying, this being favoured by the higher temperature. If spray-drying conditions are not appropriates this can even result in total or partial curing and deactivation of the resin. Hardening can occur in extreme cases. (ii) in natural resins certain components such as carbohydrate oligomers (like hemicelluloses) are cleaved by the action of the spray-drier with consequent decrease of viscosity of the resin and often some small improvement in resin performance [32]. (iii)

when the temperature is too high, thus the conditions used are too drastic, then the resin can also be partially burned, and thus totally useless for any further application. In the case of lignin, the additional effect of (iv) lignin cleavage and rearrangement can also occur, lignin being quite prone to this type of reactions when heated [33].

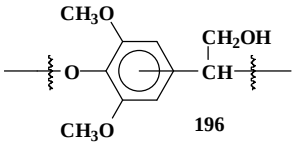
In Figs 2a,b and c are reported the MALDI-TOF spectra and details of the same glyoxalated lignin that was examined in solution much earlier [20]. Spray-drying was done only almost three months after preparation of the resin, i.e. after the resin was "advanced". The first features that are noticeable in Fig. 2a are (i) the regular incremental mass steps of 198-199 Da of the peaks, and (ii) the increase in width of the bases of the peaks. This latter can be ascribed to the "advanced" polymerisation of the resin due to aging, and the base constituent peaks will be discussed in more detail later. More interesting is the repeating 198-199 Da. This can be attributed to two different repeat units due to the product of the reaction of lignin with glyoxal, one at 193 Da, namely:



(I)

Which when deprotonated on the -OHs could reach 195 Da, due to the reaction of a H-unit (a unit without methoxy groups on the aromatic ring) of lignin with glyoxal, the second aldehyde group of glyoxal being in -CH_2OH form like all aldehydes in water solution during polycondensation reactions.

The second possible repeat unit at 196 Da could be:



(II)

obtained by cleavage of a lignin syringyl unit that has subsequently reacted with glyoxal to give such a repeat unit, and with one -OH protonated this would give 197 Da. That some units of this type exist is confirmed by the MALDI-TOF work on freshly glyoxalated lignin already reported [20].

It is, however, of interest to consider that the major peak at 602 Da can be due to different oligomers. Thus, as a first consideration it could be due to three 193 Da units linked together, thus:

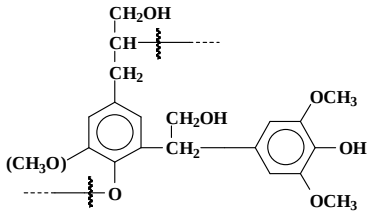
3x(193) +23 (Na^+ of the matrix) = 602 Da as well as being due either to

2x(syringyl units)+H_F+$(glyoxal)_1$+23 (Na^+ of the matrix) = 598 Da and with 3 -OHs protonated = 601 Da, or x(guajacyl units)+H_F+$(glyoxal)_2$+23 (Na^+ of the matrix) = 598 Da and with 3 -OHs protonated = 601 Da

All three possibilities are likely. However, in the freshly prepared glyoxalated lignin in solution the 602 Da peak is relatively small (not presented here) at 6% peak intensity, while in the aged, spray-dried glyoxalated lignin it is the dominant peak at 100% intensity (Fig. 2a). It is unlikely that such an increase in intensity is due exclusively to the last two proposed oligomers, especially considering that the viscosity of the glyoxalated lignin has increased over time indicating a certain degree of polymerisation. Furthermore, in Fig. 2b a clear peak at 598.2 Da is present, indicating that the 602 peak is most likely a combination of the three compounds, the last two protonated. Thus, the greatest contribution to the 602 Da peak is from by the first oligomer, namely 3x(193) +23 (Na^+ of the matrix) = 602 Da. This implies that in the process either the lignin has partially depolymerized or that a high proportion of lignin monomers are present, with the consequence that the more reactive of these, the H-type units, do react and polymerize by polycondensation with glyoxal. The lignin used has effectively a low degree of polymerisation, and thus a high proportions of lignin monomers and dimers are present [7]. The function of glyoxal is then to polymerize these monomers and dimers to give higher molecular weight oligomers much in the same way of phenol-formaldehyde resins [31]. Such type of reaction would fit well with the series of peaks (Fig. 2a) at 619.7 Da (602 +1x-OH of the last glyoxal), 818.9 Da, 1017.3 Da, 1216.3 Da, 1414.4 Da and subsequent, but smaller, peaks at 1612 Da, 1810 Da, 2009 Da, 2208 Da and higher. This would imply the formation through structure (I) of up to octamers (1414.4) by polycondensation of lignin monomers and glyoxal, and of smaller proportions of oligomers up to dodecamer and higher. It is not really possible to define if these oligomers formed by such a polycondensation route are branched or linear, but the repeat unit implications from Fig. 2a are that they are linear.

It is worthwhile to investigate the possibilities that structure (II) would participate in the lignin/glyoxal polycondensation, after all at 196-197 Da its molecular weight is much nearer the repeating 198-199 Da observed in Fig. 2a. In reality structure (II) can only participate by linking onto existing lignin oligomers. Thus, the phenolic oxygen is bound to be an -OH and thus reaction of the unit could be exclusively through the glyoxal part of structure (II) if its 197 Da molecular weight has to be retained. Thus reaction through the second aldehyde function of the glyoxal is excluded otherwise the molecular weight would decrease to 179

Da, which does not correspond to the reality of the repeating motive in Fig. 2a. This would be the case while the polymer is still soluble and has not hardened as in the present, soluble spray-dried powder. Thus, structure (II) is present in structures of type (III):



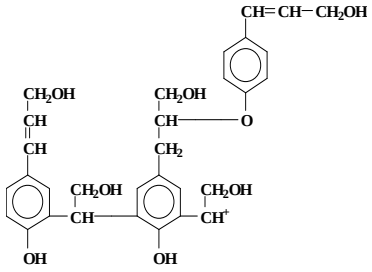
(III)

The evidence for this can be found (i) in the peak at 398.7 Da, formed by the reaction of structure (II) with a single guayacyl unit of lignin (398-400 Da), and in the peak at 431.3 Da formed by the reaction of structure (II) with a single syringyl unit (calculated 430-431 Da), and in the 371 Da peak due to the reaction of a coumaryl-type lignin monomer with structure (II) (calculated 371 Da). (ii) in the peak at 775.9 Da, formed by the reaction of two structures of type (II) with a guayacyl dimer that would give theoretically 777 Da. (iii) by the 954.0 Da and 1152.8 Da peaks that correspond respectively to a guaiacyl trimer to which are linked two structures of type (II) (calculated 955 Da) and three structures of type (II) (calculated 1151 Da). Nonetheless the low percentage intensity of these peaks indicates that participation of structure (II), while definitely occurring is minor and does not contribute to the major intensity peak series at 602 Da, 619.7 Da (602 +1x-OH of the last glyoxal), 818.9 Da, 1017.3 Da, 1216.3 Da, 1414.4 Da formed by polycondensation of glyoxal with structures of type (I). The absence of peaks corresponding to the polymerisation of guayacyl and syringyl moieties in the same manner is explained by the much higher reactivity of the ortho free sites in coumaryl-like lignin monomers to reaction with an aldehyde.

The possibility of course exists and is quite likely that mixed oligomers due to the polycondensation of both structures (I) and (II) with glyoxal would exist and participate in the series of major peaks shown in Fig. 2a.

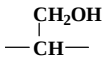
Around each major peak there are a host of smaller peaks, mainly at lower Da, separated from the main one by intervals of 22-23 Da (Fig. 2a and Fig. 2c). These could be attributed to a number of different reasons, which are difficult to define. It is known that each peak defines an oligomer molecular weight plus 23 Da of a Na^+ attached. Thus, elimination of this would give a peak that is around 22-23 Da lower than the main one. These peaks are very small, but accompany all the main peaks; thus, the peaks at 796.5 Da, 994.5 Da, 1193.6 Da are possibly due to the loss of Na^+ from the major peaks at 818.9 Da, 1017.3 Da, and 1216.3 Da, respectively the peak at 577.5 (Fig. 2b) could be the 602.9 peak that has lost Na^+.

However, for this peak a structure exists that can better explain it. It is composed of three coumaryl-type lignin units linked with themselves and by glyoxalation as the structure IV.

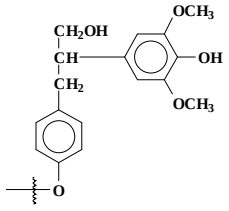


(IV)

Equally, there are small peaks at 44-46 Da lower than the 4 main peaks. These are oligomers of the type exemplified by structure (I) and structure (III) where in both cases either a glyoxylene group of the type:



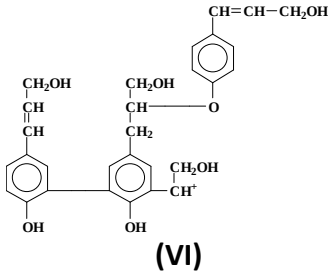
has been eliminated and the structure has rearranged, for instance, from structure (III), to became as the structure (V).



(V)

or simply from dimers to tetramers of lignin linked with each other have reacted with only glyoxal to became in a structure type (VI) which have forms of the types for the 562.9 or 553.3 Da with Na^+ included.

These peaks are those at 562.9 or 553.3 Da both with Na^+ included, and 775.9 Da (although all these can be explained in a different way, see above), at 978.2 Da, 1177.7 Da.



(VI)

4. CONCLUSIONS

Spray-drying of glyoxalated lignin used in tannin/lignin adhesives for exterior and interior-grade wood particleboards and other types of panels has been shown to be capable of

maintaining its reactivity after spray-drying with no decrease in performance of the adhesive once the spray-dried material is re-dissolved in water, according to the termomechanical analysis indicated there is no major difference in the strength performance between freshly glyoxalated lignin and spray-dried glyoxalated lignin. However, some important effects could occur in the lignin according to the MALDI-TOF analysis, namely:

1. Certain components such as carbohydrate oligomers are cleaved by the action of the spray-drier with consequent decrease of viscosity of the resin, once this has been re-dissolved in water.
2. Cleavage and rearrangement can also occur when the lignin is heated given rise to different oligomers possibilities.
3. The glyoxal polymerize monomers and dimmers to give higher molecular weight oligomers as a consequence of their condensation with glyoxal and the formation of glyoxylene bridges between them.

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III.2.5. **Extraction, caractérisation et utilisation de lignine organosolv de miscanthus pour l'elaboration de colles pour bois erespectueuses de l'environnement**

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La lignine a été obtenue à partir de miscanthus à travers deux processus d'extraction : un traitement organosolv eau/éthanol (A), et un processus en deux étapes comprenant une dilution sous forme d'un pré-trempage suivi d'un traitement organosolv eau/éthanol (B). La lignine organosolv a subi une caractérisation structurelle par ^{13}C RNM et par spectrométrie de masse MALDI-ToF. Cette lignine a été utilisée pour la préparation de colles naturelles.

La meilleure formulation avec la lignine de miscanthus organosolv comprend 40% de lignine glyoxalée et 60% de tanin de mimosa. Cette formulation, utilisée dans la fabrication de panneaux de particules, a donné de bons résultats de cohésion interne, permettant de remplir les spécifications exigées par la norme pour leur utilisation comme panneaux d'usage intérieur.

EXTRACTION, CHARACTERIZATION AND UTILIZATION OF ORGANOSOLV MISCANTHUS LIGNIN FOR THE CONCEPTION OF RESIN FOR WOOD ADHESIVES

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Abstract

Ethanol Organosolv Lignin was extracted from Miscanthus x giganteus using a 2 steps process: (1) a dilute acid presoaking step and (2) an ethanol organosolv treatment catalysed with sulphuric acid. The organosolv lignin was subjected to a comprehensive structural characterization by ^{13}C NMR and MALDI TOF MS and was used for the formulation of a green wood adhesive in which a nonvolatile nontoxic aldehyde (glyoxal) is substituted for formaldehyde in its preparation. The best formulation which was composed of 100% of natural resins (60% of mimosa tannin and 40% of glyoxalated lignin) yielded good internal bond strength results for the panels, which was good enough to pass relevant international standard specifications for interior-grade panels.

Key words: lignin; organosolv; miscanthus x gigantheus, adhesives; wood, glyoxal; nontoxic.

I. INTRODUCTION

Lignin is a three-dimensional phenylpropanoid polymer that represents the second most abundant natural polymeric material on earth (Argyropoulos et al., 1998, Fengel and Wegener, 1989). Large amounts of lignin are currently produced each year by the pulp and paper industry and are expected to be produced in future biorefineries as a by-product of biofuel production. Among all the pretreatment methods currently studied for the production of pulp and/or ethanol (Pu et al., 2008), the ethanol organosolv process seems to be very promising. It was very recently studied and optimized in the literature for different raw materials (poplar (Pan et al. 2006), pine (Pan et al., 2007, 2008), sugar cane bagasse (Mesa et al., 2010). This procedure has ability to cause extensive removal of hemicelluloses under mild conditions, with no significant cellulose degradation and generates a large amount of a high-quality lignin with valuable properties, including high hydrophobicities, low glass transition temperatures, low polydispersity and high functionalization (El Hage et al.,

2009, Kubo and Kadla, 2004). Thus, these organosolv lignins could find applications in the fields of fibers, films (Pereira, 2007), biodegradable polymers (Vasquez, 1999) and adhesives. The literature on the use of lignins to prepare wood adhesives is very extensive, and good reviews of it exist (Nimz, 1983, 2002). In contrast to the great number of articles regarding its utilization, the record of its industrial use for wood adhesives is instead rather poor. Recently, we described lignosulfonate lignin-based wood adhesives satisfying the requirements of relevant international standards for the manufacture of wood particleboard (Lei et Al., 2008, Mansouri et al., 2007). These lignin-based wood adhesives did not use any formaldehyde in their formulation; formaldehyde was substituted with a nonvolatile nontoxic aldehyde, namely, glyoxal. The adhesives yield good internal bond strength results for the panels, which are good enough to comfortably pass relevant international standard specifications for exterior-grade panels. The adhesives also show sufficient reactivity to yield panels in press times comparable to that of formaldehyde-based adhesives.

In previous a paper, we described the pretreatment of Miscanthus x Giganteus, a dedicated energy crop, using an ethanol organosolv process (Brosse et al., 2009). We demonstrated in this paper that a presoaking step prior to the organosolv treatment not only allowed a better recovery of xylans but also enhanced the dissolution and recovery of lignin and the digestibility of the remaining cellulose by enzymes. In the present study, we investigated the structure of the organosolv lignin extracted from miscanthus straw using this 2 steps protocol (EOL2SP) using ^{13}C nuclear magnetic resonance spectroscopy (NMR) and MALDI TOF spectrometry. We also verified the possible use of these lignin fractions as a component of a green adhesive formulation in which glyoxal is substituted for formaldehyde in its preparation.

II. MATERIAL AND METHODS

2.1. Material

The raw *Miscanthus x giganteus* (M×G) was harvested in spring 2008 from Trier (Germany). The air-dried Miscanthus was milled to a particle size of 1–3 mm using a Wiley mill and stored at −5°C during the course of this study. The untreated feedstock contained 25% Klason lignin, 37% cellulose and 36% hemicellulose. All chemical reagents used in this study were purchased from VWR International and used as received, with the exception of p-dioxane which was distilled over $NaBH_4$ prior to use.

2.2. Presoaking

The scheme for isolation of organosolv lignin fraction is illustrated in Figure 1. 55 g (dry weight, Dry Matter Content about 90 %) of Miscanthus, 500.0 mL of water and 40.0 mL of sulfuric acid 2 M (concentration of H_2SO_4 = 0.15 mol.L^{-1}) were mixed in a flask and heated to reflux for 17 h. At the end of the reaction, the residue was filtered and air dried. A portion of the solid residue and of the liquid was separated and stored in a freezer at -5 ^{o}C before analysis.

2.3 Organosolv lignin extraction

25 g (dry weight, Dry Matter Content about 90%) of Miscanthus was treated with aqueous ethanol (EtOH/H_2O 0.5 or 0.65, see table 1) with sulfuric acid as a catalyst (0.5 or 1.2 %w/w, see table 1) at 170°C or 190°C (see table 1) during 60mn. The solid to liquid ratio used was 1:8. The pretreatments were carried out in a 1.0 L glass-lined pressure Parr reactor equipped with a 4842 temperature controller (Parr Instrument Company, Moline, IL). The pretreated Miscanthus was washed with warm (60 ^{o}C) ethanol/ water (8:1, 3 x 50.00 mL). The washes were combined and 3 volumes of water were added to precipitate the Ethanol Organosolv Lignin (EOL), which was collected by centrifugation and air dried.

Table 1. Experimental conditions

	2-steps protocol (with presoaking)	1-step protocol (without presoaking)
1st step Presoaking	H_2SO_4, O.15 mol.L^{-1} 17h, 100°C	_
2nd step Organosolv	170°C, 1h, H_2SO_4 0.5% EtOH/H_20= 0.5	190°C, 1h, H_2SO_4 1.2% EtOH/H_20= 0.65
EOL %[a]	55 %	51 %

[a] Ethanol Organosolv Lignin isolated, % based on weight of the initial lignin contend of dry Miscanthus x Giganteus (25.1%)

2.4 Characterization of lignins

EOL was extracted with pentane (in a Soxhlet apparatus) to remove remaining low molecular weight impurities prior to NMR analysis. All NMR experiments were performed on a Bruker Avance-400 spectrometer operating at a frequency of 100.59 MHz for ^{13}C and MWL (82.6 mg) and EOL (100 mg) were dissolved in DMSO-d6 (0.50 mL) with slight heating and stirring with a micro stir bar. ^{13}C NMR spectra were acquired at 50°C using an inverse-gated decoupling pulse sequence. 10, 000 scans were collected/sample with a pulse delay of 12 seconds[20]. Data were processed offline using XWinNMR processing software.

2.6 Maldi Tof Mass Spectrometry

The spectra were recorded on a KRATOS Kompact MALDI AXIMA TOF 2 instrument. The irradiation source was a pulsed nitrogen laser with a wavelength of 337 nm. The length of one laser pulse was 3 ns. The measurements were carried out using the following conditions: polarity-positive, flight path-linear, mass-high (20 kV acceleration voltage), 100-150 pulses per spectrum. The delayed extraction technique was used applying delay times of 200-800 ns.

The lignin samples were dissolved in acetone (4 mg/mL, 50/50% volume). The sample solutions were mixed with an acetone solution (10 mg/mL in acetone) of the matrix. As the matrix 2,5-dihydroxy benzoic acid was used. For the enhancement of ion formation NaCl was added to the matrix (10mg/ml in water). The solutions of the sample and the matrix were mixed in the proportions 3 parts matrix solution + 3 parts polymer solution + 1 part NaCl solution and 0.5 to 1 µL of the resulting solution mix were placed on the MALDI target. After evaporation of the solvent the MALDI target was introduced into the spectrometer. To each peak value in the resulting positive mode spectrum must be subtracted of 23 Da of the Na^+ of the matrix to obtain the molecular weight of the chemical species of the peak.

2.7 Glyoxalation of lignin and resin adhesive preparation

To prepare the lignin, process described for El Mansouri et al. (2007) was used. Then, the miscanthus lignin powder (29.5 parts by mass) was gradually added until 38.4 parts water while sodium hydroxide solution (30%) was added periodically to maintain the pH of the solution between 12 and 12.5 for better dissolution of the lignin powder, which was also facilitated by vigorous stirring. A 250-mL flat-bottom flask equipped with a condenser, thermometer and magnetic stir bar was charged with the above solution and heated to 58°C. After the lignin is totally dissolved, 17.5 parts by mass glyoxal (40% in water) was added and the lignin solution was maintained to pH 12-12.5, with a magnetic stirrer on a hot plate for 8 hours. The total solids contents were about 37%. The glyoxal is a non-toxic aldehyde (LD50 rat $\geq$ 2960 mg/kg; LD50 mouse $\geq$ 1280 mg/kg) (NIOSH, 2000), non-volatile but less reactive than formaldehyde which is toxic (LD50 rat $\geq$ 100 mg/kg; LD50 mouse $\geq$ 42 mg/kg) (NTIS) , that has been tested in lignin (Lei et al, 2008; El Mansouri, 2007) and tannin adhesives (Ballerini; et al. 2005) and other adhesives (Amaral et al., 2008) for application to wood panels such as particleboard.

Mimosa tannin solution in water was prepared with 45% concentration and its pH adjusted to 10 with NaOH 33% water solution. It was added 6% (solids on mimosa tannin) hexamine

at 33% solution in water as a hardener, which it is accepted by the Japanese standard JIS A5908 (1994) as not being a formaldehyde source in presence of a polyflavonoid condensed tannin (Pichelin et al. 2006; Kamoun et al. 2000). Then the glyoxalated lignin was mixed with this tannin solution. In a first stage, we worked with the EOL2SP and a second one with EOL1SP.

The TMA, study the rigidity of a bonded wood joint in function of the temperature. The experiments were conducted under the same conditions: heat rate = 10°C / min, 30mg of resin, in the temperature range 25-250°C. The thermomechanical analyzer used was a Mettler Toledo TMA40. The software used for data treatment was STARe. Deflection curves that allow MOE determination have been obtained by the three-point bending TMA mode. The maximum MOE value and its increase as a function of time or temperature for wood–resin systems is a good indication of the possible end performance of the adhesive system that was tested. The composition of different glue mixes to make this test was 50/50: lignin/tannin proportion in base to the solids content.

2.8 Particleboard manufacture and testing

One layer laboratory particleboards of 350×300×16mm^3 dimension were prepared using only core particles of beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*) wood particles at 35,7 kg/cm^2 maximum pressure and 190°-195°C press temperature. The total adhesive resin solids load on dry wood was 10% of the total mix of glyoxalated + lignin tannin. The total pressing time was 7.5 minutes. The particleboards were tested for dry internal bond (IB) strength test, which is a relevant international standard test (EN 312).

III. RESULTS AND DISCUSSION

The Ethanol Organosolv Lignin (EOL2SP) was extracted from miscanthus straw according a procedure given in the Figure 1 and Table 1. The protocol involved (1) a dilute acid presoaking step and (2) an ethanol organosolv treatment catalysed with sulphuric acid; this procedure allowed a fractionation of the three main constituents of miscanthus with high recovery yields. In the Table 1 are also given the experimental conditions of a 1-step organosolv treatment of Miscanthus previously described.

Using SEM, the microscopic surfaces of untreated, presoaked and organosolv treated pulp fibers were compared. Examination showed great differences between fibers. Untreated pulp fibers had an intact surface and well-defined folds (Figure 2A) whereas presoaked

material exhibited fibers with a disrupted surface with small tears and holes (Figure 2B). This observation could be attributed to a preferential removal of hemicelluloses during the presoaking step. Concerning the pulp fibers, as seen in Figure 2C after organosolv treatment, the removal of a large part of the lignin results in a fibrillar material mainly composed of cellulose fibers. These observations confirmed the hypothesis previously proposed about the removal of a part of the hemicelluloses during the presoaking step (Brosse et al., 2009) which disrupted enough of the remaining polymers to enhance the hydrolysis of lignin during the organosolv process. As a consequence, using the 2 steps protocol, high lignin yield was obtained and low severity was required for the second step (lower reaction time, temperature, sulphuric acid concentration and higher ethanol/water ratio, see Table 1).

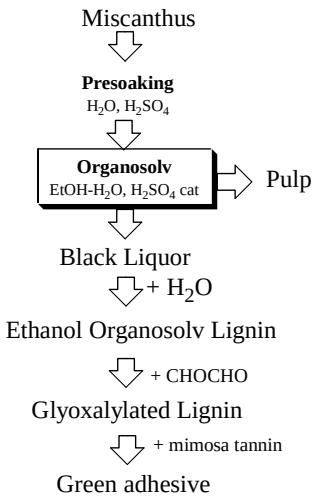

Figure 1 : Schematic of the two-step presoaking and ethanol organosolv pretreatment

The quantitative ^{13}C NMR spectrum of Miscanthus EOL2SP is given in Figure 3A. The integral at δ 160-102 ppm was set as the reference, assuming that it includes 6.12 aromatic carbons (representing all aromatic carbons plus a contribution of 0.12 from the unsaturated side chain of coniferic and coumaric moieties). Therefore, the integral values for the structural moieties are reported per aryl group. Based on a previous publication (El Hage et al., 2009), Table 2 gives the signal assignments in the spectrum and the quantification of the signals for the lignin (EOL2SP) compared to EOL1SP, the lignin fraction recovered using the one step protocol previously described and characterized (El Hage et al., 2009). EOL1SP was recovered without presoaking (thus a higher severity treatment, see Table 1). A comparison of the integration values of the three aromatic regions can provide some information on the degree of condensation of the lignin fractions. As can be seen in Table 2, compared to EOL1SP, EOL2SP displayed a lower number of condensed aromatic carbons (δ 142-124 ppm)

and a higher number of protonated aromatic carbons (δ 124-102 ppm) per aromatic ring. This observation translates to a lower degree of condensation.

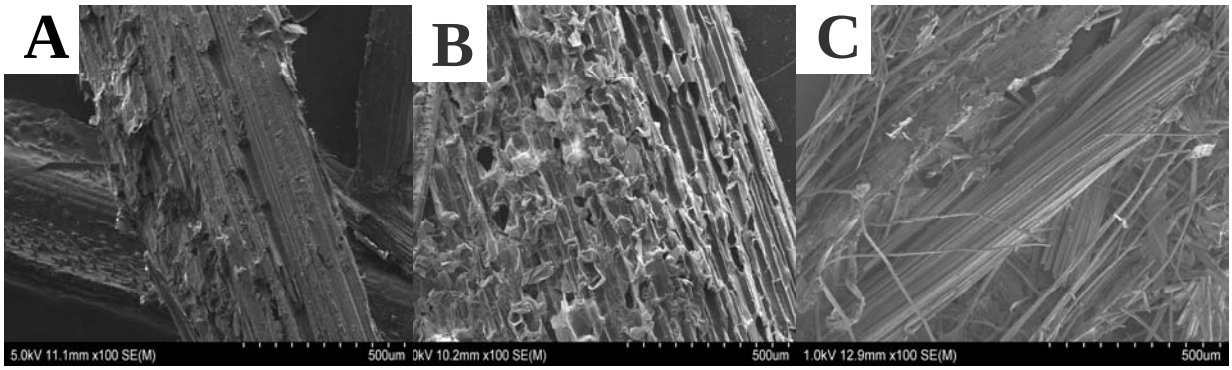

Figure 2 : Scanning electron micrographs of **(A)** Untreated **(B)** Presoaked **(C)** Organosolv miscanthus pulp

Table 2. Signal assignment in ^{13}C NMR spectra of EOL of Miscanthus and results from quantification of functional groups. Values presented are in number of functional groups per aromatic unit.

Chemical shift	Assignment	EOL2SP[a]	EOL1SP[b]
		This study	reference x
155-142	Arom C-O	1.84	1.51
142-124	Aromatic C-C	1.94	2.00
124-102	Aromatic C-H	2.39	2.27
89-58	Aliphatic C-O	1.06	0.97
89-78	Cβ in βO4, Cβ in β 5 and β β	0.31	0.19
73-71	Cα in β-O-4	0.10	0.23
64-61	Cγ in β-O-4	0.65	0.55
56	OMe	1.10	1.08

[a] Ethanol Organosolv Lignin recovered using a two-steps protocol (Presoaking + organosolv) ; [b] Ethanol Organosolv Lignin recovered using a one-step protocol (organosolv).

It is possible to distinguish the signals assigned to the 3 carbon atoms (Cβ, Cα and Cγ) of the propyl chain respectively at 86.5 ppm, 73 ppm and 61 ppm. According to Capanema et al. (2005), it is possible to estimate the total amount of β-*O*-4 structures from the integral at 88-82.5 ppm (which also includes β-5 and β -β structures) from which is subtracted the integral at 54-53 ppm. Therefore, the amount of β -*O*-4 structures is estimated to ~0.27/Ar for EOL2SP and 0.17/Ar for EOL1SP. These modifications are in accordance with a more important scission of aryl-ether linkages and subsequent dehydratation reactions using the 1 step protocol. Thus, examination of the ^{13}C spectra clearly showed that the two steps protocol degraded the macromolecule structure to a lower significant extent.

MALDI TOF MS analysis of EOL2SP indicated that this was a very low molecular weight lignin composed almost exclusively of dimers and trimers. The MALDI-TOF Mass Spectrum of the lignin is shown in Fig. 4 for the interval 200-750 Da. The MALDI-TOF spectrum between 60 and 200 Da was recorded but is not reported here. However, the relevant assignment of the

MALDI-TOF peaks and their distribution are reported in Tables 3. In Table 3 are shown all the relevant peaks in the spectrum in Fig. 4 with the calculated molecular mass + 23 Da (for the Na^+ matrix) for the relevant corresponding oligomers and their relative intensity expressed as a percentage of the highest peak.

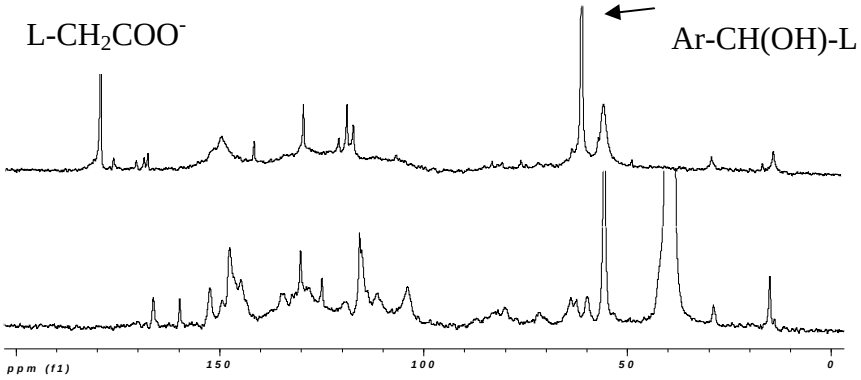

Figure 3. Quantitative ^{13}C NMR spectra of EOL2SP in DMSO (A) and of EOL2SP after glyoxalation in D_2O (B)

To explain the shortened nomenclature used in Table 3, lignin units are of hydroxyphenyl-type (H-type), guayacil-type (G-type) and syringyl-type (S-type). As examples, peaks at 113 Da and 181 Da indicated as $H_{FRACTION}$ and $S_{FRACTION}$ correspond to fragments, respectively, of H-type and S-type monomers, the calculated peak values of which are given by the molecular weight of the oligomer plus that of the Na matrix, thus peaks at 94+23(Na^+) Da = 117 Da and 154+23(Na^+) Da = 177 Da. These correspond to structures (I) and (II) (see Figure 5). Equally as an example the dimer H+S at an actual peak 385 Da corresponds to an oligomer of molecular weight 362 that plus the 23 Da of the Na^+ gives 362+23(Na+) Da = 385 Da; this could correspond to structure (III) of formula given in Figure 5 where the H and S type units are linked with the most common β-O-4 linkage. As a further example the peak at 355-356 Da corresponds to a dimer G+$S_{FRACTION}$ or to a S+$G_{FRACTION}$, the calculated molecular weight of which is 333, thus giving 332 + 23(Na+) Da = 355 Da.

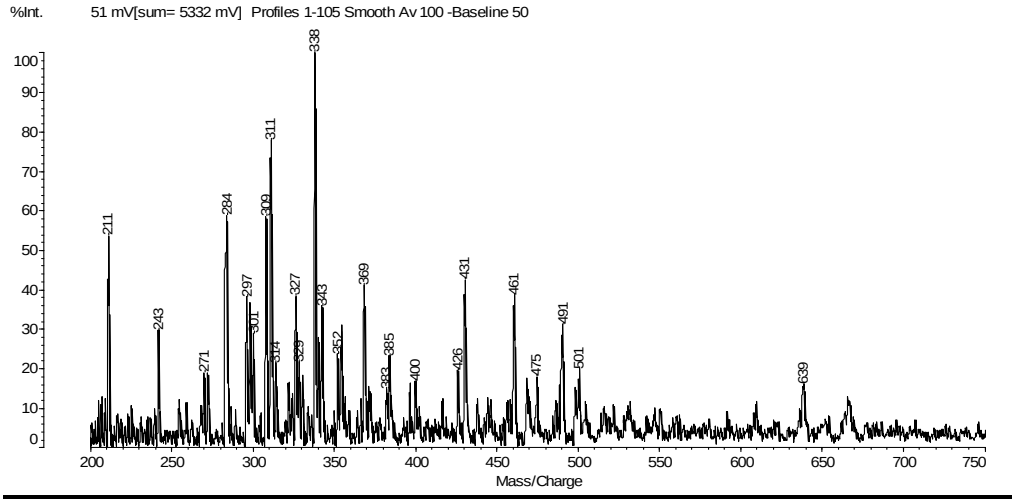

Figure 4. MALDI-TOF MS of Ethanol Organosolv Lignin of Miscanthus

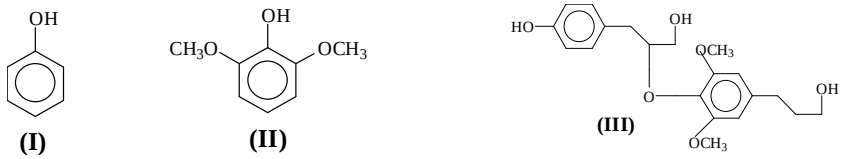

Figure 5. Molecular formula of $H_{FRACTION}$, $S_{FRACTION}$ and H+S dimer

Thus, while it is quite possible to identify monomers, dimer and trimer fragments and which lignin units are involved, it is not strictly possible to define which types of linkages tie the units together, although these types are relatively few in lignin.

Table 3. Observed and calculated masses of lignin fragments by MALDI-TOF MS.

Dimer/trimer	Molecular weight +Na	Peak (Da)	Relative peak height* (%)
$H_{FRACTION}$	117	121	18
$G_{FRACTION}$	147	147	25
$S_{FRACTION}$	177	177	18
G	203	198	10,5
$H + H_{FRACTION}$	266	271	20
$H + G_{FRACTION}$	296	297	38
$G + H_{FRACTION}$			
H + H	327	327	38
$S (-CH_3) + H_{FRACTION}$	337	338	100
$H + S_{FRACTION}$	326	327	38
$G + G_{FRACTION}$			
$S + H_{FRACTION}$			
$G + S_{FRACTION}$	356	352	24,5
$S + G_{FRACTION}$			
$G + S_{FRACTION}$	356	355	32
$S + G_{FRACTION}$			
H + S	383	383	15,5
G + G			
$S + S_{FRACTION}$	386	385	23,6
H +H +H	473	475	19
$G + G + H_{FRACTION}$	477		
H +H + G	503	501	21

The glyoxalation of EOL1SP and EOL2SP was performed in a basic medium by addition of glyoxal (see experimental section). The ^{13}C-NMR spectrum of EOL2SP after glyoxalation (Figure 3B) indicates that the conditions used for the reaction modified the polymer in a large extend; aliphatic region (89-58 ppm) of the ^{13}C NMR spectrum displays very weak signals arising from the aliphatic C-O on the lignin propyl side; this observation indicates that the aryl ether bonds (β-O-4 and α-O-4) bonds were highly affected by the treatment. Few peaks appeared after glyoxalation: the most important one is the 61-ppm peak that corresponds to the benzyl alcohol type group formed by the reaction of glyoxal with the ortho site of the aromatic ring of a phenolic type lignin. As previously proposed, the signal at

181 ppm was assigned to HO-CH_2-COO_2Na and the same form attached to lignin namely L–CH_2-COO_2Na due to the Cannizzaro reaction of glyoxal in water (Salomaa, 1956, Arcus, 1964).

As it was previously demonstrated (Lei, 2009), the glyoxalated lignin by itself gave relatively low wood-joint strength and it can't be used alone as wood adhesive unless it can be cross-linked furtherly. Thus, two adhesive formulations based on a mixture glyoxylated organosolv lignin (EOL1SP and EOL2SP) and mimosa tannin (50/50) were prepared and evaluated by TMA by the study of the rigidity of the wood–resin joint as a function of temperature. The results of the thermomechanical analysis given in the Figure 5 indicate a comparative performance for both of the two adhesive formulations with maxima Elasticity's Modulus very similar. However, concerning EOL1SP, an additional signal is observed at ~100°C which corresponds to a first reticulation reaction through formation of a non stable cross linker. This behavior is a characteristic of several phenolic resins. In this precise case, the resin quickly reticulated involving the more reactive tannin molecules with formation of unstable methylen-ether cross linkers (-CH_2OCH_2-). Because of their instability and the increase of the temperature, they break to form a more stable methylen cross linker (-CH_2-) with emission of formaldehyde able to react with phenolic material.

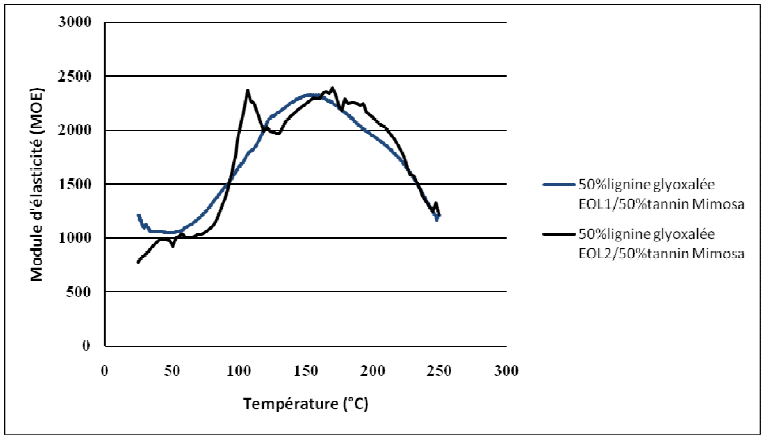

Figure 6. TMA measuring MOE as a function of temperature to describe the curing of the two resins of glyoxalated miscanthus organosolv lignins.EOL1SP and EOL2SP

Finally, organosolv lignin based adhesives were tested for application to wood panels such as particleboard. The internal bond (I.B.) strength of a panel is a direct measure of the performance of the adhesive. In this work, two different formulations given in the Table 4 were analyzed.

From these it can be seen that glyoxalated EOL2SP/ tannin 50/50 formulation does not satisfy the IB strength results required (‡0.35 MPa) but is slightly lower. However,

glyoxalated EOL1SP/ tannin 60/40 formulation gives a good result and satisfies the relevant requirements of standard specifications for interior type wood particleboard (Pizzi, 1993).

Table 4. Results of Wood Particleboard Bonded with Glyoxalated Organosolv Lignin – Mimosa Tannin Formulation

Formulation	Press Time (mn)	Temperature	Density (kg/m3)	IB N/mm² = Mpa
EOL2SP (50%) + tannin (50%)	7,5 min	195 °C	*695*	*0,33 ± 0,02*
EOL1SP (40%) + tannin (60%)	7,5 min	195 °C	*696*	*0,41 ± 0,05*

IV CONCLUSION

The extraction of miscanthus Ethanol Organosolv Lignin (EOL) was performed with a good yield using a two steps procedure involving a presoaking step prior to the organosolv treatment. The recovered EOL was characterized by MALDI TOF MS and by ^{13}C NMR before and after glyoxalation. Glyoxalated organosolv lignin based adhesives for wood particleboard without any addition of either formaldehyde or formaldehyde based resin were prepared. The best formulation which was composed of 100% of natural resins (60% of mimosa tannin and 40% of glyoxalated lignin) yielded good internal bond strength results for the panels, which was good enough to pass relevant international standard specifications for interior-grade panels. These results show that boards in which the binder is 100% composed of natural material are possible.

Acknowledgements

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III.3. COLLES A BASE DE GLUTEN

III.3.1. **Colles A base de protéine de glutens pour panneaux de bois**

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Les colles pour les panneaux de bois composées de matériaux naturels sont pour certaines déjà utilisées dans les secteurs commerciaux et industriels, dans certains pays. De nombreuses recherches ont été effectuées sur des colles naturelles notamment les colles à base de tanins [Pizzi, 1983; Pizzi, 1978; Pichelin et al. 2006], de tanins et de lignine [Mansouri et al. 2011; Pizzi, et al. 2009; Pizzi et Walton, 1992], de protéines de soja, de farines et autres composés divers [Liu et Li, 2007; Geng et Li, 2006; Amaral-Labat et al. 2008]. Dans toutes ces colles, les polymères synthétiques sont présents en petites quantités voire totalement absents.

Le gluten est une protéine dérivée du blé [Shewry et al. 2002; Lindsay et Skerritt, 1999]. Les groupes amide de la liaison peptide dans la protéine du gluten sont capables de réagir avec les aldéhydes. Les groupes aminés tels que la lisine ou l'arginine (le gluten est riche en ces aminoacides) présentent une réactivité forte avec les aldéhydes, semblable à celle de la mélamine et des phénols [Pizzi, 1983].

Dans ce travail, on étudie des formulations de colles pour panneaux de particules à base de protéines de glutens hydroxyméthylées ou hydrolysées glyoxalées contenant ou non une proportion égale de colle de tanins/hexamine. Les panneaux fabriqués à partir de ces colles présentent des caractéristiques satisfaisantes pour permettre leur utilisation à l'intérieur selon les spécifications des normes. Les résultats sont améliorés avec l'ajout de faibles proportions d'isocyanate (pMDI) que ce soit avec les formulations à 90-95% ou à 70-80% de

matériaux naturels. L'utilisation de la formulation 30/70 pMDI/gluten hydroxyméthylate dans la fabrication de panneaux de particules a entrainé une diminution du temps de pressage. Les valeurs de cohésion interne obtenues sont en accord avec celles de la norme EN-312 pour des panneaux d'utilisation intérieure.

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Gluten Protein Adhesives for Wood Panels

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Abstract

Adhesives for wood particleboards based on hydroxymethylated or glyoxalated hydrolysed gluten protein with addition of an equal proportion of tannin/hexamine resin or without any addition of synthetic resins are shown to yield results satisfying the relevant standard specifications for interior wood boards. Even better are resins based on hydroxymethylated or glyoxalated hydrolysed gluten protein to which has been added a small proportion of an isocyanate (pMDI). Adhesive resins formulations based on the latter in which the total content of natural material was either 70% or 80% of the total resin solids content gave good results. Resins based on the former in which the natural material content was approximately 90–95% also gave good results. The 30/70 pMDI/hydroxymethylated lignin adhesive formulation was tried at progressively shorter pressing times yielding internal bond strength results acceptable for relevant interior grade standards at industrially significant pressing times.

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Keywords

Wood adhesives, gluten, NMR, wood, particleboard, panel products

1. Introduction

Recent regulations and environmental legislation have put pressure to stop the use of traditional synthetic adhesives for wood panels. Higher costs of oil from which traditional adhesives are derived have also contributed to this trend. As a consequence, the interest in natural adhesives for wood panels has intensified. This has translated in the revival of the traditional approach of partially substituting in the formulations a small proportion of the synthetic adhesive with a natural material [1]. This approach is rather old and is revived now to conserve traditional synthetic adhesives that might well not be available in the future. Its main disadvantages are

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(i) still the presence of too high a proportion of synthetic oil-derived polymers in the adhesive formulation, with its consequences on costs, and (ii) the negative environmental impact of the presence of a high proportion of synthetic polymers. This approach, as simple as it may first appear to be, is not a short, or medium or long term solution to the problems being faced now by the wood panels industry.

Wood panel adhesives predominantly, or even exclusively, composed of natural materials already exist. Some of these have now been in commercial/industrial use in a few countries for a rather long time. Among these it is sufficient to briefly mention tannin adhesives [1–3], commercial since the early 1970s, variations on tannin/lignin adhesives that are coming to the market at present [4, 5], some types of lignin adhesives [6–9] and different adhesives based on soy protein flour and hydrolysates [10–13] also on the way to early industrialisation. In all these adhesives, synthetic polymers are present in very small amounts or are totally absent.

Gluten is a protein derived from wheat [14, 15]. The amide groups of the peptide links in gluten proteins are capable to react with aldehydes in the same manner as other amides, e.g., urea [1]. Amine groups in lysine and arginine (aminoacids in which gluten is rich) [14] also present even more marked reactivity with aldehydes, similar to those presented by melamine [1] and phenols [1].

As human allergy to flour products containing gluten is rather widespread, gluten is extracted in great quantities from wheat flour to prepare wheat meal and other gluten-free products. Great quantities of gluten are thus available for other applications. Gluten adhesives for wood panels have already been prepared. However, they need to be applied in powder form to the panel furnish. This is, in general, unsuitable for the industrial application in wood particleboard factories where the equipment can only apply liquid adhesives [16].

This paper thus deals with different approaches to the formulation of liquid wood adhesives predominantly based on gluten for wood particleboards and other particulate wood panels, in an easily applied liquid form.

2. Experimental

The three types of gluten used in this study were donated by Syral (Aalst, Belgium). Two of the gluten types used were gluten protein powder hydrolysates, one produced by chemical and the other by enzymatic hydrolysis, while the third sample was gluten flour. The gluten protein powder hydrolysate obtained by chemical hydrolysis was of the type PX705, which is a partially hydrolyzed functional wheat protein for food application. It appears as a fine, slightly yellowish powder. It contains 86 wt% protein and 4.9% water. Its solubility at pH 7.0 is 96%. This material was used throughout the experiments. The resin preparation procedures described were applied to all three types of gluten.

2.1. Resin Preparation

A gluten-formaldehyde resin, a gluten-glyoxal resin, and gluten that was treated with alkali without forming a resin were prepared.

2.1.1. Gluten-Formaldehyde Resin and NaOH-Treated Gluten

In a three-neck reaction vesssel equipped with a mechanical stirrer, a thermometer and a condenser were charged water (709 g), NaOH (28 g), a phase transfer agent such as ethylene glycol (5.3 g), and silicone oil (10 drops) and the mixture heated to 70°C under continuous mechanical stirring. Gluten protein powder hydrolysate (350 g) was then added to the rapidly stirring solution. The mixture was then heated to 90°C over 15 min, under rapid mechanical stirring, and held between 88°C and 92°C for 1 h. This mix constituted the alkali-treated gluten (GB) used in the formulations as indicated in the tables.

To GB, formaldehyde as a 37% formalin solution (134 g) was added in the hot reaction mixture over a 5 min period. The mixture was maintained under continuous mechanical stirring between 88°C and 92°C for an additional 55 min. The mixture was then cooled to 35°C in an ice bath. This product constituted the gluten-formaldehyde resin GA used in the formulations as indicated in the tables.

2.1.2. Gluten-Glyoxal Resin

In a three-neck reaction vesssel equipped with a mechanical stirrer, a thermometer and a condenser were charged water (709 g), NaOH (28 g), a phase transfer agent such as ethylene glycol (5.3 g), and silicone oil (10 drops) and the mixture heated to 70°C. Gluten protein powder hydrolysate (350 g) was then added to the rapidly stirring solution. The mixture was then heated to 90°C over 15 min, under rapid mechanical stirring, and held between 88°C and 92°C for 1 h. Glyoxal (40% in water) (239 g) was added to the hot mixture over 5 min. The mixture was maintained under continuous mechanical stirring between 88°C and 92°C for an additional 2 h and 55 min. The mixture was then cooled to 35°C in an ice bath. This product constituted the gluten-glyoxal resin GC used in the adhesive formulations as indicated in the tables.

2.1.3. Tannin, pMDI and Final Resins

Mimosa tannin extract (supplied by Silva, S. Michele Mondovi', Italy) of Stiasny value of 92.2 [17–19] was used. The tannin solution in water was prepared at 45% concentration and its pH adjusted to 10.4 with a 33% NaOH aqueous solution. The high pH was chosen as the hardener used performs best at such a pH [3]. The hardener used was 5% hexamethylenetetramine (hexamine) on tannin solids. This was added to the tannin extract solution as a 30% aqueous solution.

To the tannin/hexamine solution were added the modified GA, GB and GC lignin solutions. The proportion of tannin: modified lignin was 50:50 by weight as shown in Table 1. The pMDI (polymeric 4,4′-diphenylmethane diisocyanate) used was Desmodur 44VK from Bayer. The PF resin used in one of the formulations in Table 1 was prepared according to the procedure already presented [20].

2.2. Thermomechanical Analysis

The resins were tested dynamically by thermomechanical analysis (TMA) on a Mettler 40 apparatus. Triplicate samples of beech wood alone, and of two beech wood

Table 1.
Internal bond (IB) strength results of particleboards prepared with different gluten protein adhesive formulations. GA = gluten-formaldehyde. GB = gluten alkali treated. GC = gluten-glyoxal

		Board density (kg/m^3)	IB strength (MPa)
A	Tannin(6% hexamine)/GA-50/50	692	0.28
B	Tannin(6% hexamine)/GB-50/50	689	0.53
C	Tannin(6% hexamine)/GC-50/50	679	0.34
D	pMDI/PF/GA-25/20/55	663	0.42
E	pMDI/GA-30/70	678	0.71
F	Tannin(6% hexamine)/HA-50/50	680	0.28
H	GA:11% resin loading	–	–
EN 312			$\geqslant$0.35

plies each 0.6 mm thick bonded with each adhesive system, for sample dimensions of 21 mm × 6 mm × 1.2 mm were tested in non-isothermal mode between 40°C and 220°C at a heating rate of 10°C/min in three-point bending on a span of 18 mm. A force varying continuosly between 0.1 N, 0.5 N and back to 0.1 N was applied on the specimens with each force cycle of 12 s (6 s/6 s). The classical mechanics relation between force and deflection $E = [L^3/(4bh^3)][\Delta F/(\Delta f)]$ (where L is the sample length, b and h are the sample width and thickness, ΔF is the variation of the force applied, and Δf is the deflection obtained) allows the calculation of the modulus of elasticity (MOE) E for each case tested.

2.3. Solid State NMR

GA, GB and GC resins where examined by freeze drying without curing, while the GA + pMDI reaction product was first hardened and then examined. Solid state CP–MAS (cross-polarisation/magic angle spinning) ^{13}C-NMR spectra were recorded on a Bruker Avance II MSL 300 MHz spectrometer at a frequency of 75.47 MHz. Chemical shifts were calculated relative to tetramethylsilane (TMS). The rotor was spun at 12 kHz on a 4 mm Bruker probe. The spectra were acquired with 5 s recycle delays, a 90° pulse of 5 μs and a contact time of 1 ms. The number of transients was 5000.

2.4. Particleboard Preparation and Testing

Duplicate one-layer laboratory particleboards of dimensions 350 mm × 310 mm × 16 mm were prepared by adding 10% total resin solids on dry wood particles. The boards were pressed at a maximum pressure of 28 kg/cm^2 followed by a decrease in pressure first to 15 kg/cm^2 and last to 5 kg/cm^2, at a press temperature of 195°C and for a total press time of 7.5 min. Shorter press times were also used (see Table 3). The target average density for all the panels was 680–700 kg/m^3. The panels, after light surface sanding, were tested for dry internal bond (I.B.) strength according to European Norm EN 314 [21].

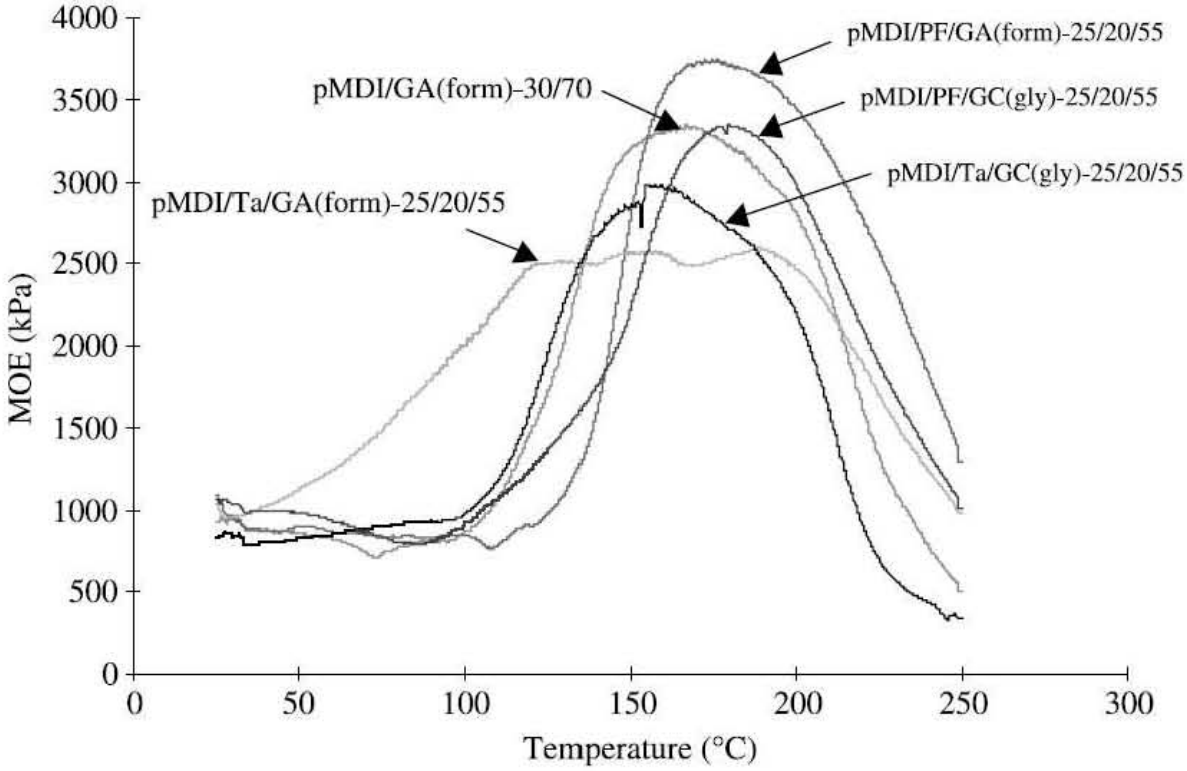

Figure 1. Modulus of elasticity (MOE) variation as a function of temperature obtained by thermomechanical analysis (TMA) of different gluten protein-formaldehyde and gluten protein-glyoxal adhesive formulations. Gluten protein hydrolysate produced chemically (G). GA(form) = gluten reacted with formaldehyde. GC(gly) = gluten reacted with glyoxal. Ta = tannin.

3. Results and Discussion

The thermomechanical analysis results for the PX705 gluten protein powder hydrolysate (prepared by chemical route) are shown in Fig. 1. All the formulations appear to give good results but some of them perform better. Of these the pMDI/PF/GA formulation, pMDI/PF/GC formulation and the pMDI/GA formulation appear to give the highest possible modulus of elasticity (MOE) of the joints between 3300 and 3600 MPa. However, the pMDI/GA 30/70 formulation appears to have two advantages over the other two formulations: (i) the relative proportion of gluten, hence of natural material, is markedly higher, and (ii) the rise of the curve on curing is shifted to lower temperature indicating this to be a faster curing resin, hence more suited for particleboard application. The other two formulations in which the synthetic PF resin is substituted with a tannin(+hexamine hardener) yield a lower strength of the joint. However, they are as fast curing (the pMDI/Ta/GC formulation) or considerably faster curing (the pMDI/Ta/GA formulation) than the pMDI/GA formulation. Thus, they also appear to be suitable for fast pressing particleboard application, although their maximum possible strength may not be up to standard.

Tannin/gluten resins without any isocyanate added were also tried. The gluten protein powder hydrolysate (prepared by enzymatic route) was used as this did

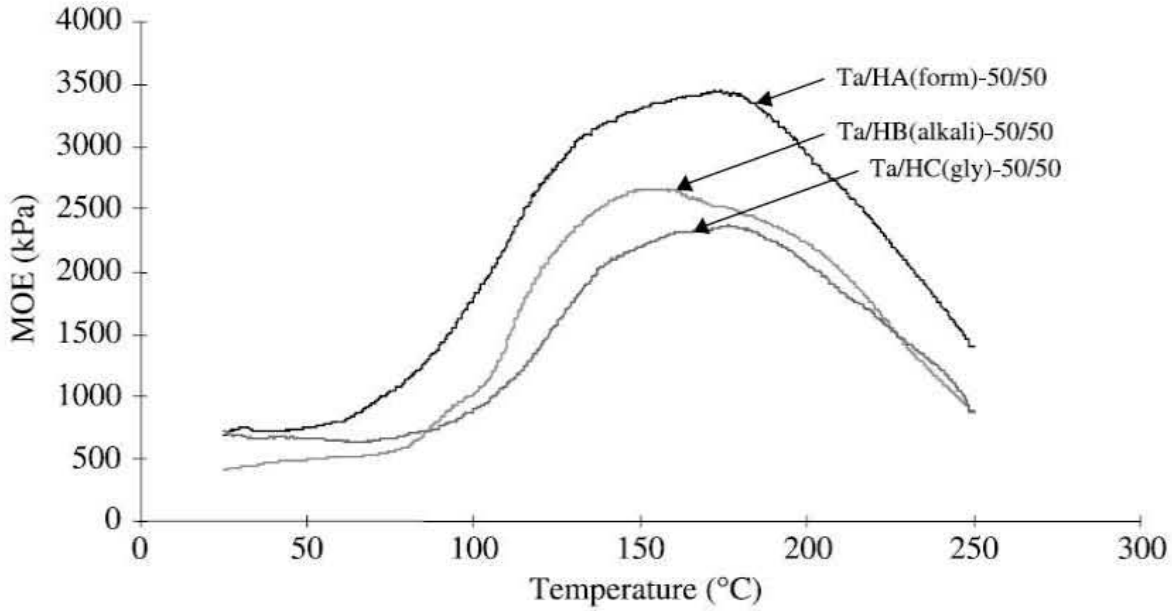

Figure 2. Modulus of elasticity (MOE) variation as a function of temperature obtained by thermomechanical analysis (TMA) of different gluten protein-formaldehyde and gluten protein-glyoxal adhesive formulations. Gluten protein hydrolysate produced enzimatically (H). HA(form) = gluten reacted with formaldehyde. HB(alkali) = gluten pretreated with alkali. HC(gly) = gluten reacted with glyoxal. Ta = tannin.

not appear to be much different from the chemically hydrolysed one. The thermomechanical analysis results are shown in Fig. 2. While the maximum possible stength results appear to indicate that the tannin/gluten-formaldehyde resin yields the best results of the three tested; if compared to the results of the other formulations in Fig. 1 it is clear that all these formulations are slower-curing than those tested in Fig. 1. For completeness, the more interesting formulations of Figs 1 and 2 were tested with the non-hydrolysed gluten flour. The results obtained are shown in Fig. 3. The faster curing formulations appear again to be the pMDI/GA 30/70, and the pMDI/Ta/GA 25/20/55. However, notwithstanding such promising results from gluten flour, its problem is that it is not soluble hence it is rather difficult to handle and apply with the equipment generally available in particleboard factories.

The results of the TMA tests were confirmed by preparing and testing laboratory particleboard panels. Thus, in Table 1 are shown the results of laboratory particleboard panels prepared using a number of different formulations. Of the different formulations tried, at the very long press time of 7.5 min at 195°C, only three are able to satisfy the dry IB (internal bond) strength requirement of >0.35 MPa of the relevant European Norm EN 312 [21] for interior grade panels. These are:

(i) The pMDI/GA 30/70 which presents an IB strength of 0.71 MPa which is double of that required by EN 312 [21]. This implies that the pMDI/GC 30/70 would be suitable and with no formaldehyde, thus with no emission;

(ii) The tannin + hexamine/GB 50/50;

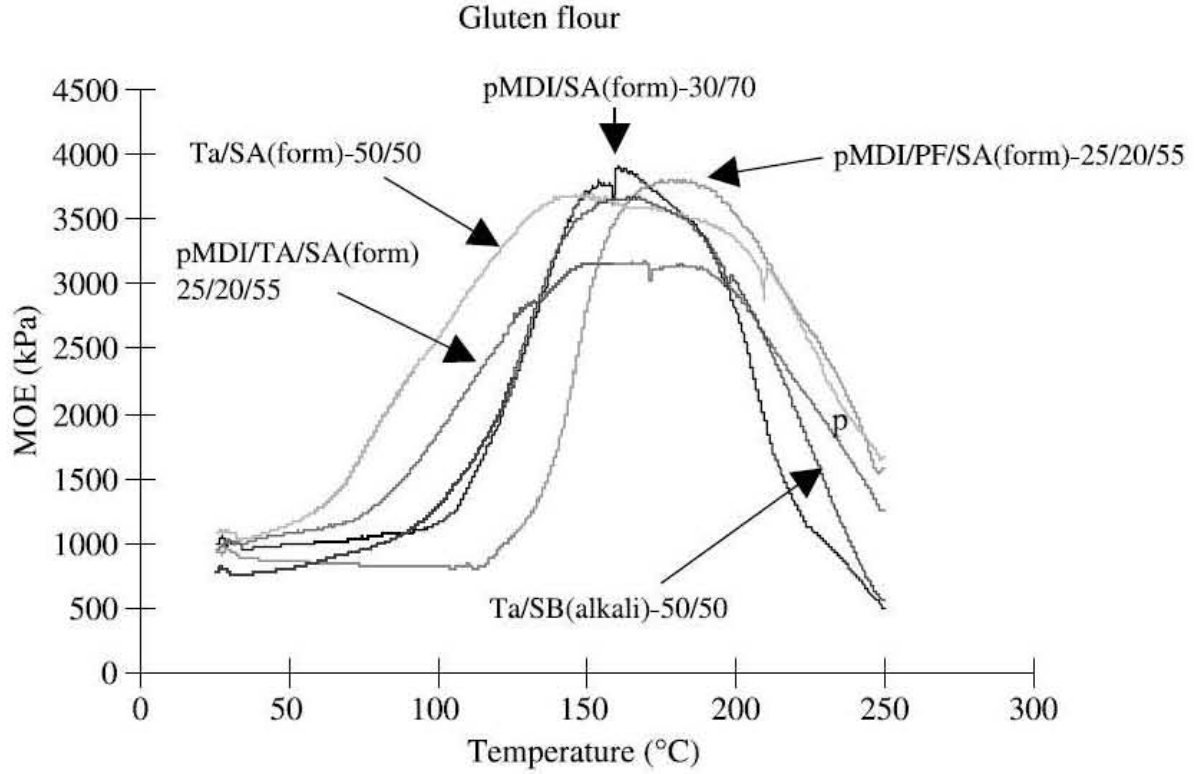

Figure 3. Modulus of elasticity (MOE) variation as a function of temperature obtained by thermomechanical analysis (TMA) of different gluten protein-formaldehyde and gluten protein-glyoxal adhesive formulations. Gluten flour (S). SA(form) = gluten reacted with formaldehyde. SB(alkali) = gluten pretreated with alkali. Ta = tannin.

(iii) The pMDI/PF/GA 25/20/55, the results of which would be better than the 0.42 MPa in Table 1 if the average board density is close to the 680–700 kg/m^3 considered as industrially acceptable. The results of this last formulation imply from Figs 1 and 3 that the pMDI/Ta/GA 25/20/55 would also satisfy the EN 312 [21] requirements.

As pMDI is relatively expensive and is the only synthetic material present in the best formulation a series of particleboards in which the proportion of pMDI was reduced in relation to the gluten (both GA and GC) were prepared and tested. In Table 2 are shown the results of the pMDI/GA and pMDI/GC formulations when progressively decreasing the proportion of pMDI from 30% to 20% and lower. The results in Table 2 indicate that while IB strength decreases with a decreasing proportion of pMDI it is still possible to decrease the pMDI proportion down to 20% while still comfortably satisfying the dry IB strength requirements. However, at pMDI proportions lower than 20%, starting at 15% pMDI on total adhesive formulation the results no more satisfy the relevant interior grade requirements of the standard. This is true for both the pMDI/GA and pMDI/GC formulations, the latter of these being favorite if a total absence of formaldehyde is required. The pMDI/GB 20/80 formulation gives such poor particleboards that they have no strength (Table 2). This is expected as the reaction of pMDI with any aldehyde-based resin has been

shown to be due to the reaction of the isocyanate group with the methylol group introduced in the resin by the previous reaction with an aldehyde [6]. In GB this group is not present and thus the presence of pMDI is of no benefit at all.

As the press time used is one of the most important parameters in particleboard manufacture, the shorter it is the more acceptable is the adhesive, so a series of particleboards prepared by using progressively shorter press times were tested. Thus, in Table 3 the press time of the pMDI/GA 30/70 formulation was progressively decreased from the long 7.5 min down to 5.5, 4.5, 3.5 and 2.5 min at 195°C. The results in Table 3 indicate that even at 2.5 min press time (10.7 s/mm board thickness) and at a board density on the low side, the results are well above the requirements

Table 2.
Internal bond (IB) strength results of particleboards prepared with different gluten protein adhesive formulations. Effect of the decrease in pMDI proportion

		Board density (kg/m^3)	IB strength (MPa)
A	pMDI/GA-30/70	661	0.71
B	pMDI/GA-25/75	659	0.69
C	pMDI/GA-20/80	670	0.45
D	pMDI/GC-30/70	664	0.61
E	pMDI/GC-25/75	659	0.51
F	pMDI/GC-20/80	659	0.45
	pMDI/GB-20/80	–	–
G	pMDI/GC-15/85	669	0.32
H	pMDI/GC-10/90	666	0.15
EN 312			$\geqslant$0.35

Table 3.
Internal bond (IB) strength results of particleboards prepared with a gluten protein/formaldehyde adhesive formulation. Effect of the decrease of press time

	Press time (min)	Board density (kg/m^3)	IB strength (MPa)
A	7.5 min, pMDI/GA-30/70	661	0.60
B	5.5 min, pMDI/GA-30/70	659	0.62
C	4.5 min, pMDI/GA-30/70	670	0.58
	4.5 min, pMDI/GA-20/80	672	0.30
	4.5 min, pMDI/GA-15/85	685	0.16
D	3.5 min, pMDI/GA-30/70	684	0.58
E	2.5 min, pMDI/GA-30/70	662	0.51
F	7.5 min, pMDI/GA-30/70, +10%$(NH_4)2SO_4$	667	0.43
EN 312			$\geqslant$0.35

of the relevant interior grade boards standard [21]. A press time of 10.7 s/mm is still long, and just on the border of the industrially useful range for interior panels, where press times between 3 and 6 s/mm are the norm today. However, this was achieved at 195°C while modern presses function at the much higher temperature of 220°–230°C. Thus, the effective press time of such a formulation will be much shorter than that achieved in the laboratory and will be in the industrially useful press time range. However, lowering the proportion of pMDI to 20% decreases already at 4.5 min press time the IB strength to values lower than that required (Table 3). Thus, the conclusion is that if industrially viable press times on modern presses are required, the pMDI/GA and pMDI/GC 30/70 formulations are the most convenient to use. Only when longer press times are allowed then one can decrease the proportion of pMDI in the adhesive.

To better understand what occurs in the reaction of gluten with an aldehyde, the resins were examined by solid state CP–MAS ^{13}C-NMR analysis. GA, GB and GC were freeze dried without curing, while the GA + pMDI reaction product was first hardened and then examined (Figs 4–7). The spectrum of GB is not reported as it

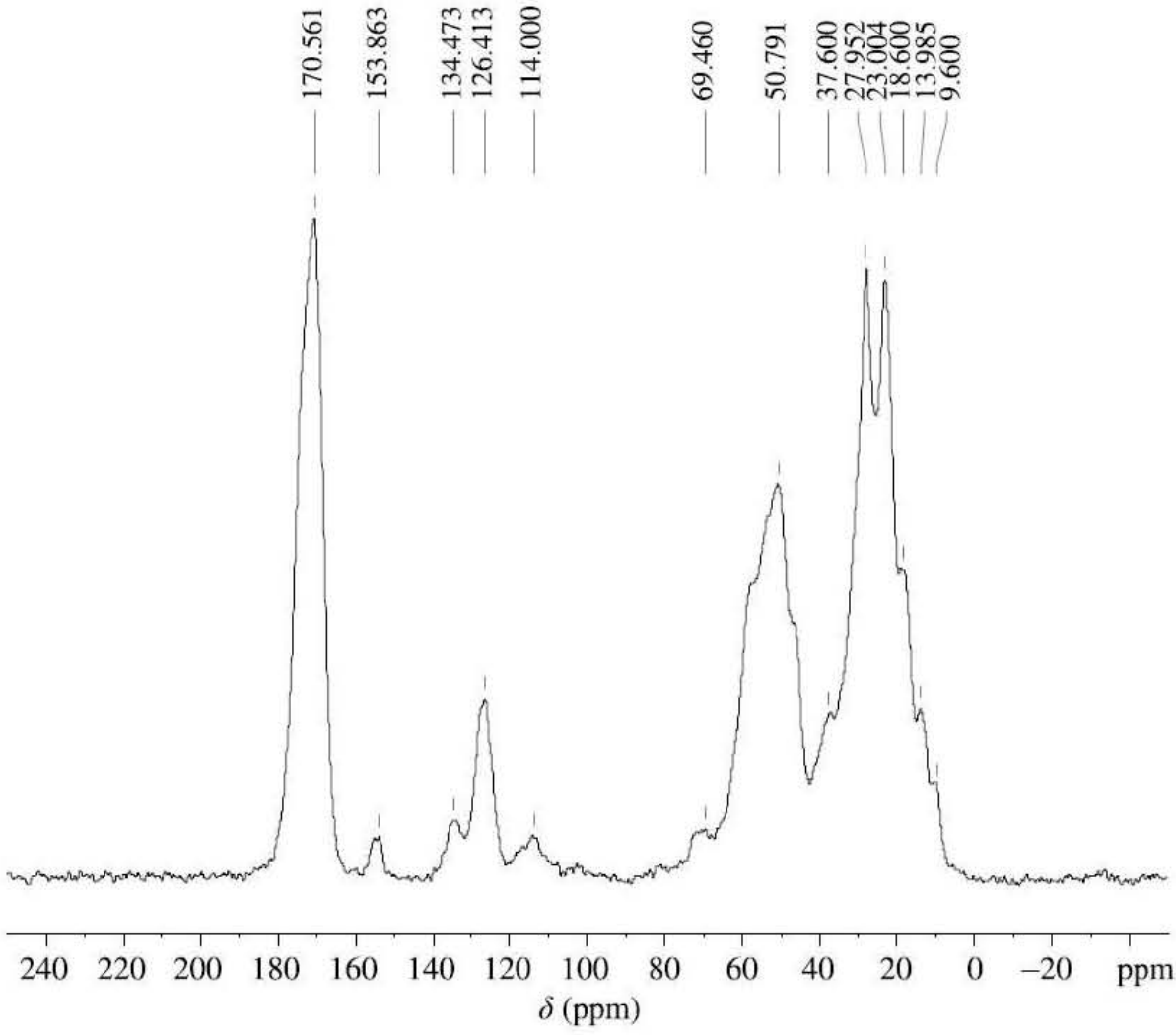

Figure 4. CP–MAS ^{13}C-NMR spectrum of glutein protein hydrolysate.

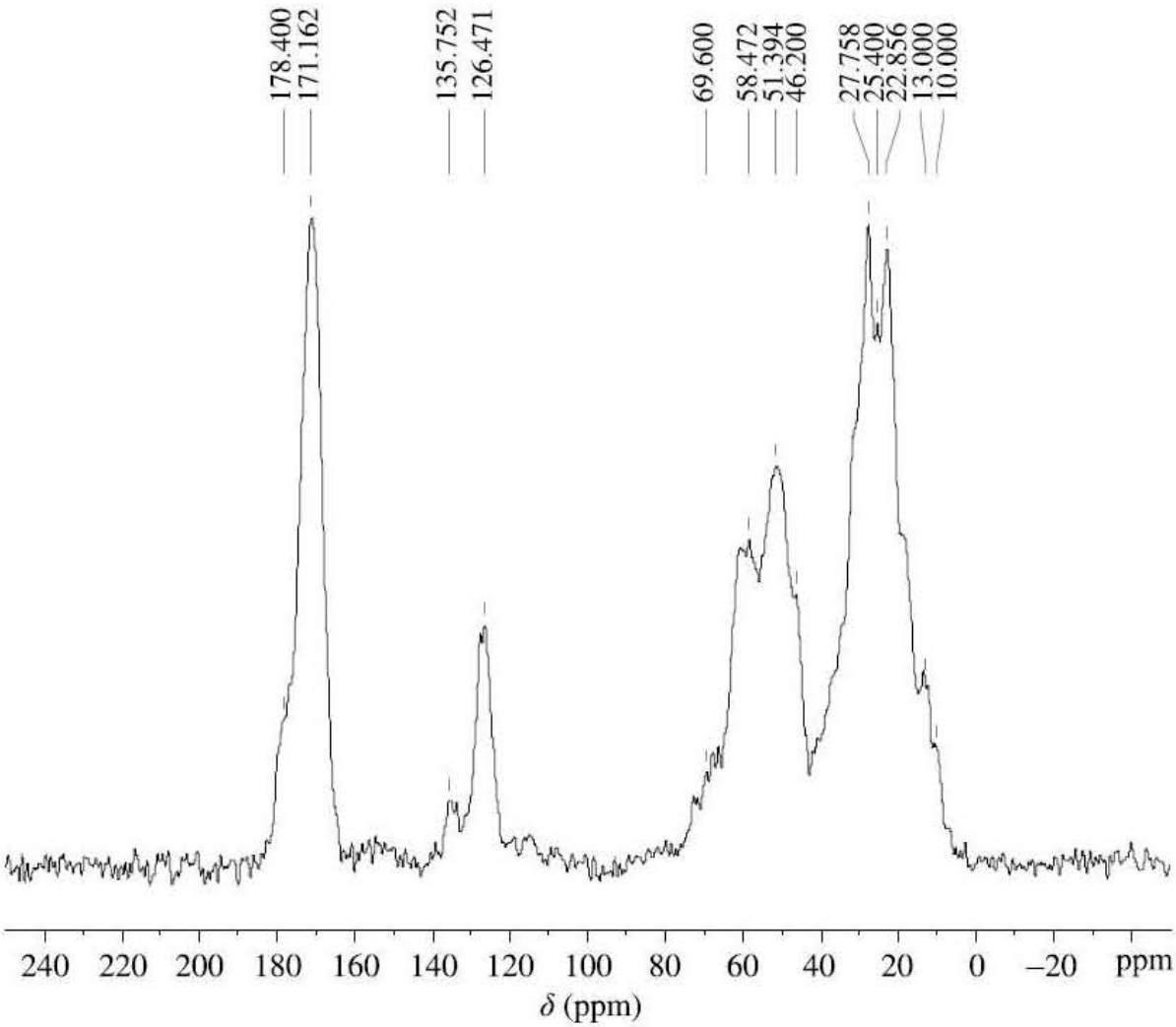

Figure 5. CP–MAS ^{13}C-NMR spectrum of glutein protein hydrolysate-formaldehyde reaction product.

is practically identical to that of the unmodified gluten hydrolysate. Comparing the unmodified gluten hydrolysate (Fig. 4) with GA (Fig. 5) the most evident features differentiating the two are the appearance of a definite peak at 58.5–59 ppm which is due to the formation of the methylol group as a consequence of the reaction of formaldehyde with the protein [1, 22–24]. As the reaction was carried out under alkaline conditions there is no evidence of methylene groups in the 35 to 45 ppm range [23, 24], as it would be expected. However, their presence could be masked by the dominant peaks belonging to the protein in this same region of the spectrum. Some condensation has, however, occurred as can be noticed by the increase in the 69.5–70 ppm band corresponding to the appearance of methylene ether bridges ($-CH_2OCH_2-$) [23, 24] and is even more evident in the spectrum of GC (Fig. 6) as the 57.5–58.5 peak, although appearing quite clearly, is less pronounced than for GA (Fig. 5). This is also expected as the reactivity of formaldehyde is about five time higher than that of glyoxal [24]. A third feature of interest is the disappearance of the unmodified gluten band at 153.8 ppm (GA and GC) confirmed by the disappearance (GA) or marked decrease (GC) of the 113–114 ppm band. These small

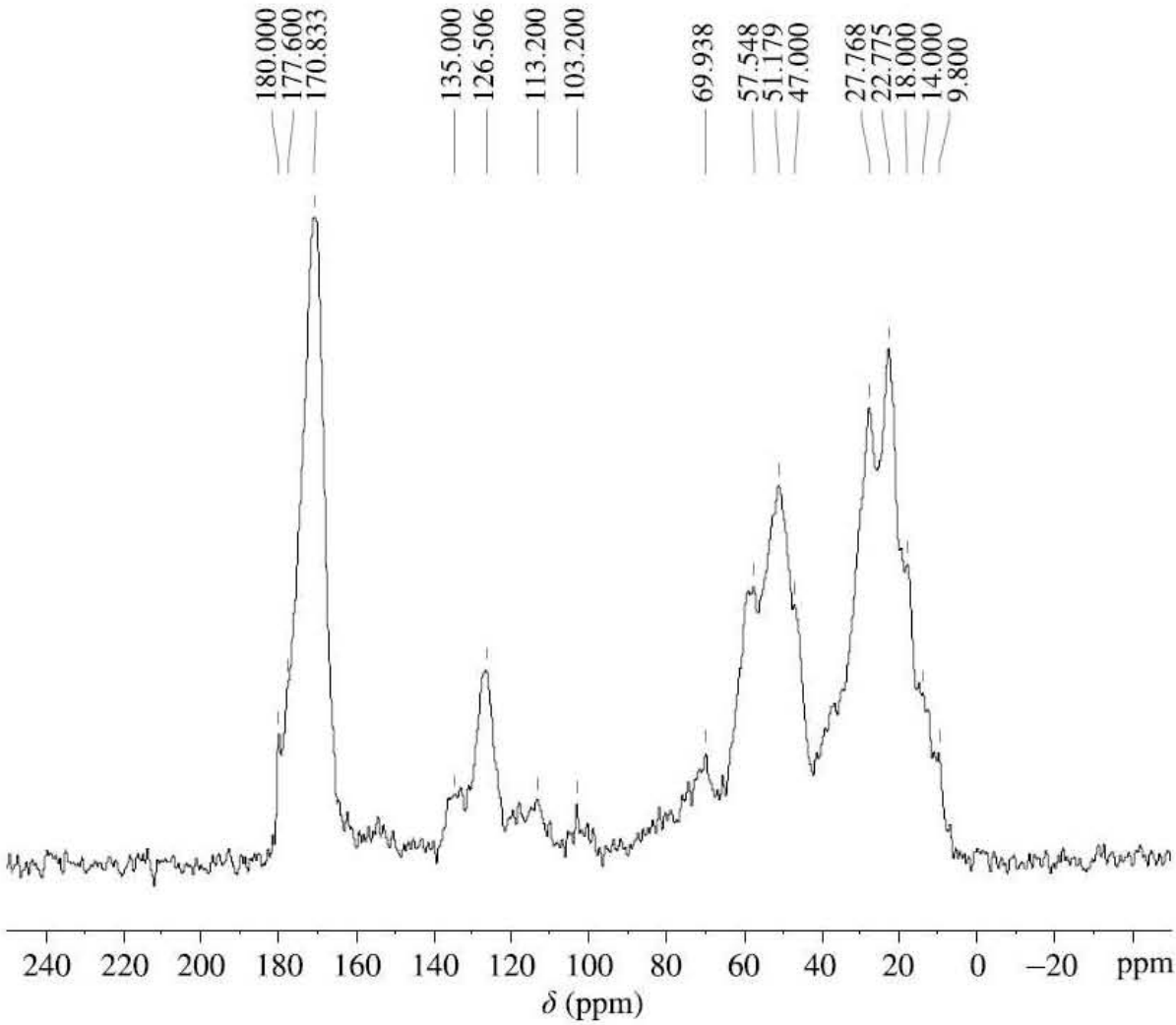

Figure 6. CP–MAS ^{13}C-NMR spectrum of glutein protein hydrolysate-glyoxal reaction product.

bands indicate reaction with the aldehyde of the few aromatic rings present in the protein.

The most interesting NMR spectrum is that of the pMDI/GA resin after hardening (Fig. 7). The features of this are the dominant peaks at 117, 126.8 and 134 ppm [23, 24]. These are all due to the pMDI used, but the most important of these is the 126–127 ppm peak that corresponds to the unreacted isocyanate group (–N=C=O) [23, 24]. The 134 ppm peak belongs to the aromatic carbon of pMDI reacted with the secondary amide group of the ureas obtained by the reaction of pMDI with water. The peak at 117 ppm indicates the presence of the relatively unstable carbamic acid [23, 24], its presence indicating the reaction between isocyanate and methylolated protein is diffusion-controlled, hence limited, possibly due to the too high viscosity of the system. Lower viscosity would favour cross-linking. Thus, even after curing there appears to be a marked proportion of unreacted isocyanate (126 ppm). This might be due to a too fast immobilisation of the network during hardening. This confirms the TMA analysis showing that low proportions of pMDI can be used in the adhesive. The peak at 152 ppm in Fig. 7 is due to the carbonyl

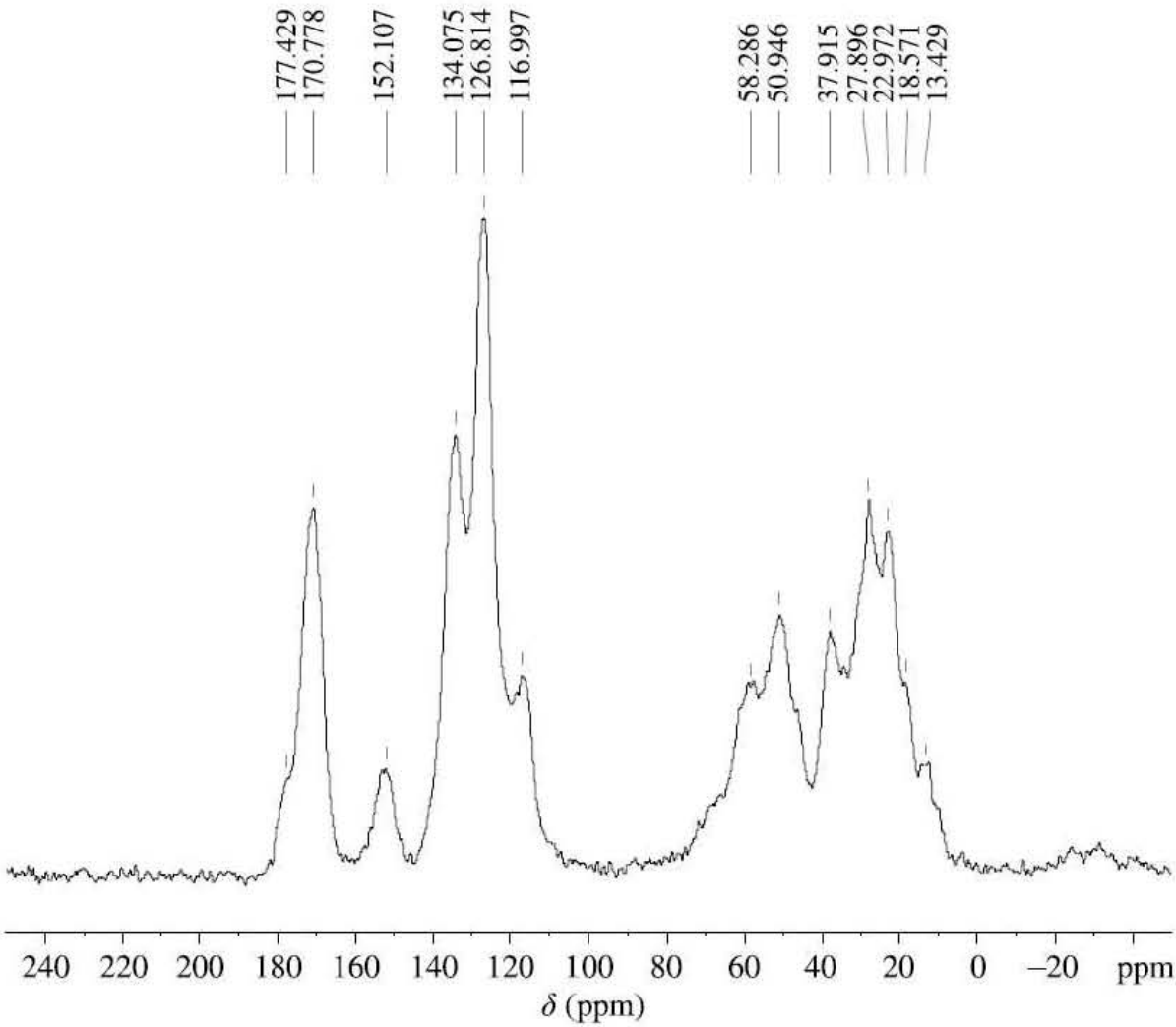

Figure 7. CP–MAS ^{13}C-NMR spectrum of the reaction product of pMDI with glutein protein hydrolysate-formaldehyde intermediate.

groups from the polyureas, biuret, urethane and intermediate carbamic acid produced by the reaction of the isocyanate with both methylolated protein hydrolysate as well as with water. Two further peaks in Fig. 7 are of interest: The higher peak at 51 ppm and the appearance of the peak at 37.9 ppm. The latter is due to the $–CH_2-$ bridge between the aromatic rings of the pMDI [23, 24]. The former is the $–CH_2-$ that belonged to the methylol group on the gluten that has now reacted with the isocyanate to give a urethane bridge (R–NHCOO–$\underline{C}H_2$–R′). The increase of this peak shows that the urethane cross-links have effectively formed and that the modified gluten has indeed, at least partially, reacted and cross-linked with the isocyanate.

4. Conclusion

Adhesives for wood particleboards were obtained giving results satisfying the relevant standard specifications for interior grade wood boards and the following were found:

(1) Hydrolysed gluten protein reacted with formaldehyde or with glyoxal to which was added an equal proportion of tannin/hexamine resin and without any addition of synthetic resins. The natural material content was approximately 90–95%.

(2) Hydrolysed gluten protein reacted with formaldehyde or with glyoxal to which was added a smaller proportion (20–30%) of an isocyanate (pMDI). The natural material content was approximately 70–80%.

(3) Shorter, industrially significant press times were obtained for the 30/70 pMDI/hydroxymethylated gluten formulation.

Compared to previous gluten based adhesives the present resins are applicable in liquid form, thus without any need for modifications of the application systems in particleboard factories. Relative to other protein adhesives such as soy-based adhesives based on reaction with formaldehyde some of the resins presented here have several advantages: (i) they cannot and do not produce any aldehyde emission as neither formaldehyde nor any other volatile aldehyde was used in some of the formulations; (ii) the percentage of natural materials was increased up to 70% for one type of formulation and up to 95% for others. Furthermore, in relation to resin formulations based on different cross-linking reactions other than those with formaldehyde the resins presented here have other advantages: they are competitive with alternate natural resin systems such as those based exclusively on tannins and/or lignins.

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III.4. EMISSION DE FORMALDEHYDE ET COVS

III.4.1. Impact sanitaire et dégagement de formaldéhyde

Impact sanitaire et dégagement de formaldéhyde

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2009 / 2010

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1. INTRODUCTION

L'une des préoccupations sanitaires majeures actuelles concerne la qualité de l'air intérieur, puisque nous passons en moyenne 85% de notre temps dans des environnements clos. L'intérêt de cette problématique est pourtant apparue tardivement et notamment, bien après le souci de la qualité de l'air extérieur. Nombre de polluants peuvent être présents dans un environnement intérieur et ceux-ci peuvent avoir diverses origines et effets sur la santé. Les conséquences de ces pollutions ne sont d'ailleurs pas toujours quantifiables puisqu'il est difficile de déterminer quelle part affecter à quels facteurs: génétiques, sociaux et environnementaux. Les effets pathologiques peuvent être des irritations, des maladies allergiques, des pathologies broncho-pulmonaires, des intoxications aiguës, des cancers, etc.
Cette préoccupation tardive explique un manque de valeurs de référence permettant de situer les niveaux de concentrations mesurés dans les environnements clos et d'instaurer les mesures de réduction des émissions. Ce manque impacte aussi la qualification, en termes sanitaires, des émissions par les produits de construction, de décoration ou d'usage courant. On commence néanmoins aujourd'hui à voir quelques valeurs guides pour la qualité de l'air intérieur. De plus, suite au Grenelle de l'environnement et aux travaux de l'OQAI[1], de l'Afsset[2] ou encore de l'INERIS[3], la surveillance de la qualité de l'air intérieur a fait son apparition dans la réglementation française. La loi Grenelle 1 du 3 août 2009 prévoit en effet que des systèmes de mesures et d'informations sur la qualité de l'air intérieur seront mis en place.
Les polluants les plus courants dans les espaces clos sont les composés organiques volatiles (COV) issus de produits d'usage courant. En particulier, le mobilier intérieur est très souvent composé de panneaux de particules qui émettent ces COV. Ce problème, qui touche la qualité d'air intérieur, concerne aussi les industries de fabrication de panneaux de particules et d'adhésifs.
En rapport direct, le 18 décembre 2006 était adopté par le parlement européen REACH[4] un règlement qui met en place un système intégré unique d'enregistrement, d'évaluation et d'autorisation des substances chimiques dans l'Union Européenne. Son objectif est

[1] Observatoire de la Qualité de l'air intérieur
[2] Agence Française de Sécurité Sanitaire de l'Environnement et du Travail
[3] Institut National de l'Environnement Industriel et des RISques
[4] Registration, Evaluation, Authorisation and Restriction of CHemicals

d'améliorer la protection de la santé humaine et de l'environnement tout en maintenant la compétitivité et en renforçant l'esprit d'innovation de l'industrie chimique européenne.
On s'intéresse ici aux émissions de formaldéhyde des panneaux de particules composés de différents types de colles et on les compare en termes d'impacts environnemental et sanitaire.

2. GENERALITES

2.1 Impact sanitaire du dégagement de formaldéhyde

Le formaldéhyde est un composé qui est naturellement présent dans notre organisme. Il est aussi présent dans de nombreux produits de construction et de consommation d'usage courant (produits de bricolage, meubles, produits d'entretien, cosmétiques…). Il est également dégagé lors des combustions incomplètes (fumée de tabac, bougie, feux de cheminée…). L'exposition de la population par inhalation provient donc pour la majeure partie du milieu intérieur (98%). Dans les habitats en France, les concentrations en formaldéhyde dans les chambres varient de 1,3 à 86,3 $\mu g.m^{-3}$. Les concentrations relevées dans les écoles, les crèches et autres lieux publics sont également très variables, avec des valeurs moyennes de 3 à 60 $\mu g.m^{-3}$.
Voici quelques informations générales concernant le formaldéhyde (Tableau 1 et 2).

Tableau 1: Identification de la substance.

Numéro CAS, EINEICS, etc.	50-00-0
Nom	Formaldéhyde
Synonymes	Fr : Formaline, méthanal, aldéhyde formique, formol En : Formalin, formic aldehyde, methanal, methyl aldehyde, methaldehyde, methyloxide, methylene oxide
Formule brute	CH_2O
Formule développée	$H_2C=O$

Tableau 2: Propriétés physico-chimiques.

Forme physique	Gaz incolore à température ambiante, odeur «piquante»
Poids moléculaire	30,026 $g.mol^{-1}$
Point d'ébullition	-19,1 °C
Point de fusion	-92 °C
Pression de vapeur	440 kPa à 20 °C ; 516-519 kPa à 25 °C
Densité	1 ?03 à 1,06 (air = 1)
Facteurs de converision	1 PPM = 1,23 $mg.m^{-3}$ à 25 °C
Solubilité	Très soluble dans l'eau (400-500 $g.L^{-1}$). Soluble dans les solvants organiques: éther, alcool, etc.
Log Kow	0,35 à 25°C
Koc	11,75 g/g
BCF	1,07 $L.kg^{-1}$
BAF	3,2 $L.kg^{-1}$
Produits de dégradation environnementale	Essentiellement acide formique, méthylene glycol, H_2O et CO_2
Point d'éclair	85 °C
Température d'auto-inflamation	424-430 °C
Limites d'explosivité dans l'air	Inférieure: 7% Supérieure: 73%

2.2. Impact sur la santé

Comme ce composé est très soluble dans l'eau et qu'il a une forte réactivité avec les macromolécules, une exposition par voie respiratoire entraîne une toxicité locale sur des sites d'exposition directe comme l'œil. Des expositions aiguës et chroniques entrainent des irritations au niveau des yeux et des voies aériennes supérieures. Par ailleurs, le formaldéhyde est cancérigène (cancer du nasopharynx) ainsi, il est classé catégorie 1[5] par le Centre International de Recherche sur le Cancer (CIRC) qui dépend de l'OMS[6]. Il est enfin de plus en plus suggéré que de faibles expositions au formaldéhyde pourraient accroître, à long terme, le risque de développer des pathologies asthmatiques et des sensibilisations allergiques, bien que les études soient encore peu nombreuses pour permettre d'identifier un lien de causalité notamment dû à l'émergence d'un syndrome : le "syndrome des bâtiments malsains" (SBM) (Menzies et Bourbeau, 1997).

[5]Le CIRC classe les agents, mélanges et expositions en cinq catégories de risque du plus au moins cancérigène: C1, C2A, C2B, C3, C4.

[6] Organisation Mondiale de la Santé.

Ainsi l'Afsset (2009) recommande, concernant l'exposition au formaldéhyde: une exposition à court terme de 0,5 $mg.m^{-3}$ sur 15 minutes maximum et une exposition à long terme de 0,25 $mg.m^{-3}$ sur 8 heures maximum.

2.3. Réglementation

Comme indiqué précédemment, il existe aujourd'hui des règlementations pour la classification du formaldéhyde en tant que substance chimique. Mais ces règlementations ne sont pas toujours en accord et notamment en ce qui concerne le formaldéhyde. Si, en France, il est classé cancérigène certain (C1) pour l'homme depuis juin 2004 par le CIRC, la commission d'union européenne le place en catégorie 3: cancérigène probable. Une demande de reclassement devait être étudiée en 2007 mais la part de lobbying des industries du panneau semble avoir une influence non négligeable dans ce domaine.

2.4. Les facteurs impactant sur la concentration de formaldéhyde

Le dégagement de formol augmente de pair avec l'humidité relative, la température, la charge de formol et inversement avec le taux de renouvellement d'air. La qualité des matériaux et le process ont également une influence sur le dégagement de formaldéhyde.
Il faut faire très attention aux autres sources de HCHO (formol) lorsque l'on veut déterminer une concentration en formaldéhyde.

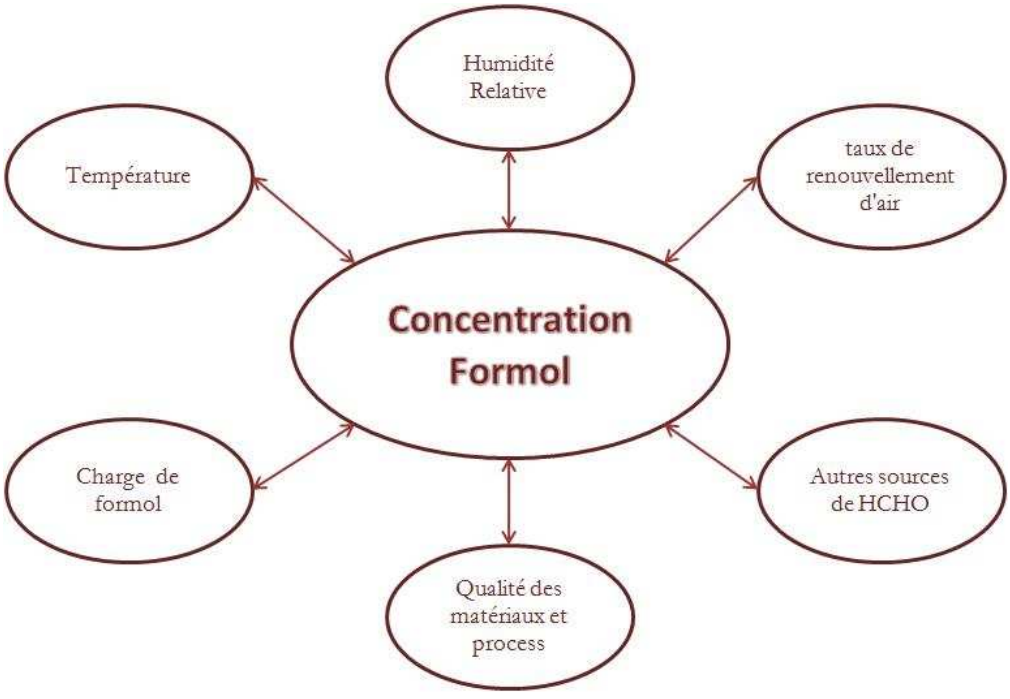

Figure 1: Les facteurs impactant sur la concentration de formaldéhyde.

2.5. Méthode de mesures et classes d'exigence

Il existe une multitude de méthodes de mesures répertoriées ci-après (Tableau 3).

Du fait de l'impact sanitaire avéré du formaldéhyde, sont apparues des classes d'exigences concernant les émissions de formol des panneaux de particules. Ces classes, originaires de divers pays, présentent des valeurs garde-fous disparates et s'appuient sur des méthodes différentes: il est donc parfois très difficile de les comparer. Il est important de remarquer que ce sont les grands groupes commerciaux qui obligent leurs fournisseurs à atteindre telles classes d'exigences. D'autres part certains pays ont des valeurs garde-fous bien plus drastiques que d'autres : le Japon qui est très en avance sur la limitation du taux de formol propose des valeurs F**** plus exigeantes que la valeur E1 européenne.

Tableau 3: Catalogue des méthodes existantes.

Méthode	unité	norme	type de produit	localisation
Chambre d'essai	ppm	ASTM E 1333	contreplaqué	USA
Chambre d'essai	$mg/m^2/hr$	JIS A 1901	tout type	Japon
Chambre d'essai	$mg/m^2/hr$	NF EN ISO 1600-9	tout type	Europe
Dessiccateur	mg/L	JIS A 1460	tout type	Japon
Dessiccateur	mg/L	ISO 12460-4:2008	tout type	France
Bocal	mg/kg	EN 717-3:1996	tout type	Europe
Perforateur	mg/100g	EN 120:1992	tout type	Europe

Un tableau répertoriant les valeurs trouvées selon les différentes normes est présenté en annexe.

2.6. Les colles pour la fabrication de panneaux

2.6.1. La colle UF

La colle urée formol est une colle traditionnelle très usitée dans le domaine du bois: on l'emploie notamment dans la confection de panneaux de particules, de MDF intérieur, de contreplaqué intérieur, charpentes intérieures, etc. Sa gamme de température d'emploi et son prix en fond un produit très avantageux mais on peut lui reprocher sa faible résistance à l'humidité, à la chaleur et bien sur, son fort dégagement de formol.

La synthèse d'une colle UF est réalisée en deux phases : une étape alcaline de méthylolation qui consiste en (1) la formation de monométhylolurée, (2) diméthylolurée, et (3) triméthylolurée (figure 2); et une seconde de condensation d'acide. Cette phase de

condensation et celle de formation du réseau tridimensionnel après durcissement de la résine sont representées par les figures 3 et 4.

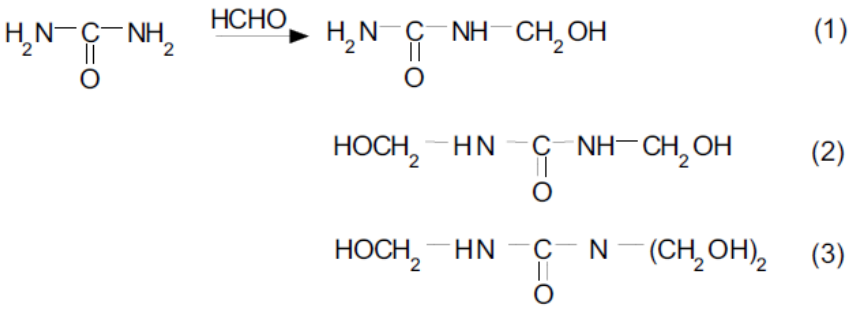

Figure 2 : Réaction de synthèse d'une résine UF : phase de méthylolation.

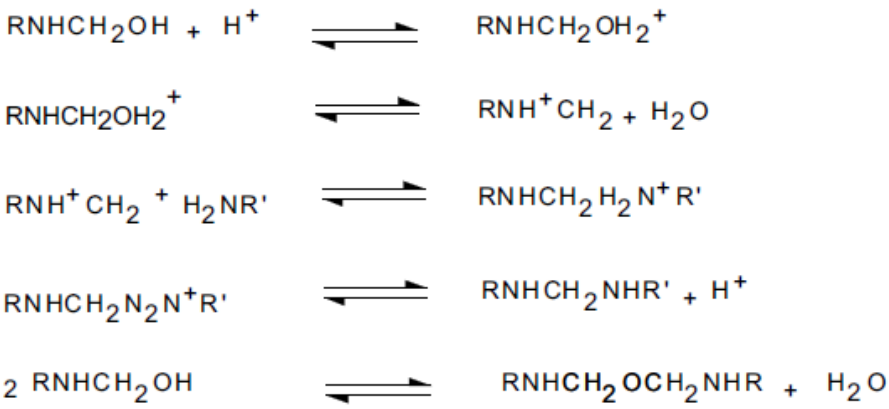

Figure 3: Réaction de synthèse d'une résine UF : phase de condensation.

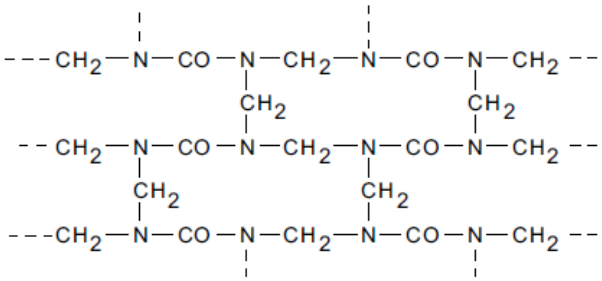

Figure 4 : Réseau tridimensionnel d'une résine UF durcie.

La préparation typique d'une colle UF est représentée sur la figure 5.

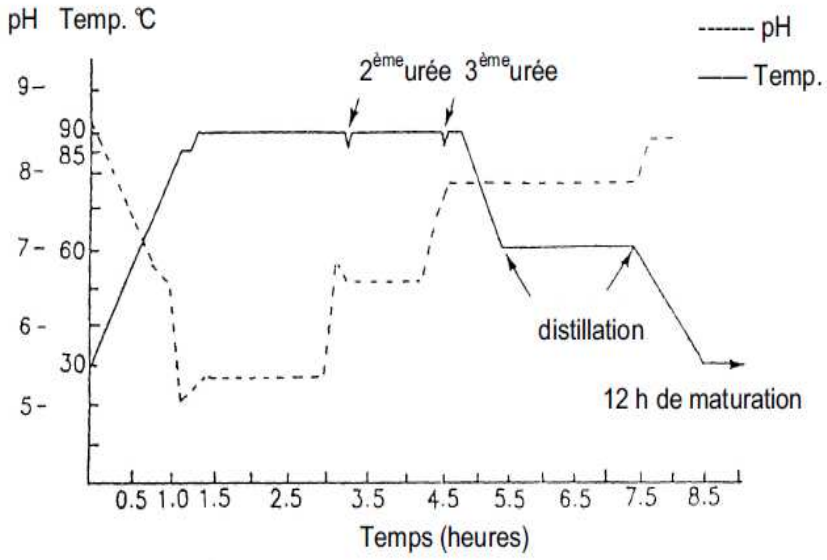

Figure 5: Diagrame de synthèse d'une résine UF. Variation de la temperature, du pH et proportion formaldehyde/urée comme une fonction de temps de reaction. (Pizzi, 1994).

2.6.2. La colle MUF

La colle MUF sert originellement à remplacer la colle MF appréciée pour sa résistance à l'humidité. Elle est donc un compromis entre prix et propriétés. On l'utilise dans la confection de panneaux de particules extérieurs, de MDF résistants à l'humidité, en menuiserie, etc. Son prix est supérieure à la colle UF mais elle résiste mieux à l'eau et dégage beaucoup moins de formaldéhyde. En effet, la mélamine a la propriété de lier le formaldéhyde dans le bois.

Le système MUF est donc composé de trois éléments : mélamine, formaldéhyde et urée. Le mécanisme de réaction de ce système est beaucoup plus complexe que celui d'un système à deux éléments comme pour les résines UF.

Les formulations peuvent être classées par rapport à la séquence d'addition des composants dans le réacteur. La séquence d'addition s'effectue dans l'ordre :

1. mélamine, puis première quantité d'urée et finalement seconde quantité d'urée (MUU);
2. première et deuxième quantités d'urée puis addition finale de mélamine (UUM);
3. réaction initiale de l'urée avec le formaldéhyde, suivie de l'addition de mélamine et enfin d'une deuxième quantité d'urée (UMU).

Le diagramme de fabrication d'une telle résine est reporté sur la figure 6. Cette procedure s'apelle methode sécuentielle.

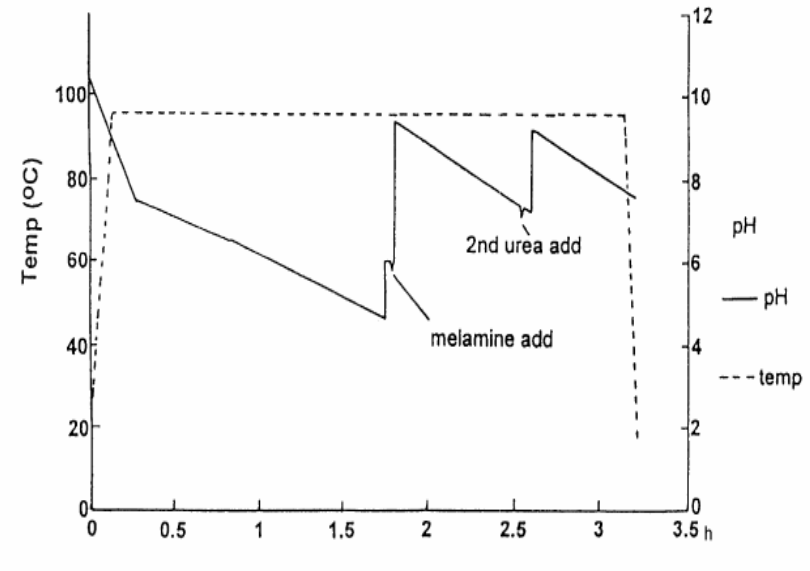

Figure 6: Synthèse de type MUF (Mercer et Pizzi, 1994; Pizzi, 2003).

Comme la mélamine est beaucoup plus réactive que l'urée, il faut d'abord former un premier condensat urée-formaldéhyde et ajouter seulement la mélamine qui réagit avec les chaînes linéaires d'urée-formaldéhyde préformée et donne ainsi des points de réticulation.

L'addition de mélamine, suivie par l'addition d'une deuxième ou d'une troisième quantité d'urée, peut absorber le formaldéhyde restant.

La structure d'une résine MUF est représentée sur la figure 7.

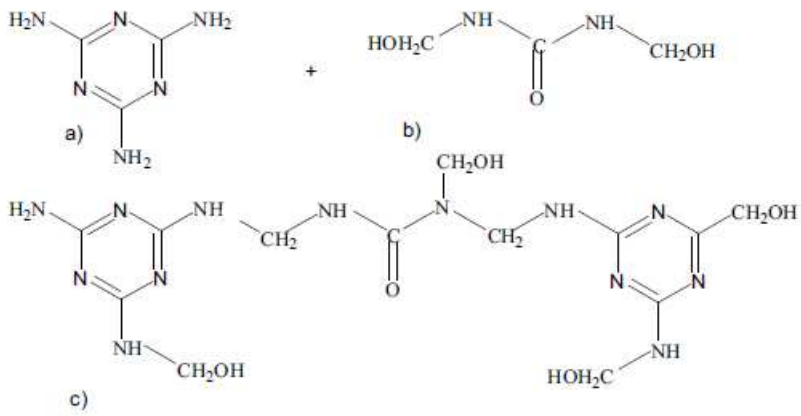

Figure 7 : Produits de condensation entre la mélamine (a) et l'urée méthylolée (b) par le formaldéhyde. (Pizzi, 2003).

Il faut remarquer toutefois que les réactions de synthèse et la structure réticulée des résines MUF n'ont pas encore été étudiées dans le détail comme pour les résines UF et MF (Pizzi, 1994; Mansouri, 2007).

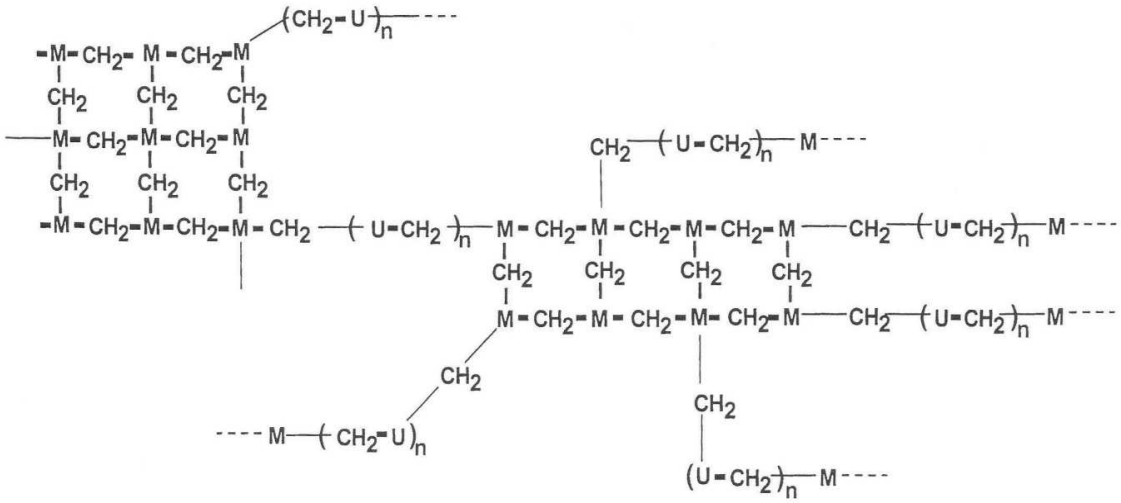

Figure 8 : Représentation schématique d'un réseau formé par une résine MUF standard.

2.6.3. La colle tannin de mimosa + hexamine

Le tannin de mimosa est extrait de l'Acacia. La ressource naturelle dont il est issu ainsi que le fait que l'on n'utilise pas de formaldéhyde lors de la synthèse en font une colle verte. Ses propriétés mécaniques sont tout à fait acceptables mais son utilisation n'est pas encore démocratisée. Nous nous attendons avec cette colle, à n'observer qu'un très faible dégagement en formol.

Les éléments réactifs du tannin sont appelés flavonoïdes. Le tannin d'Acacia est composée de 65 à 80% de ces quatre groupes (figure 9): pyrogallol (I), catechol (II), phloroglucinol-pyrogallol (III), phloroglucinol-catechol (IV).

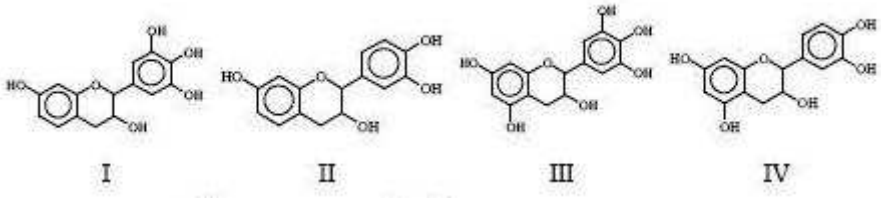

Figure 9 : les flavonoïdes

La préparation de colles phénoliques et hexamine a déjà été étudiée de manière assez détaillée. Ces colles sont utilisées industriellement pour la production de panneaux de type intérieur à base de tanins avec des émissions de formaldéhyde faibles voire inexistantes. [Pizzi, 1977; Pizzi et Stephanou, 1994 a, b; Pichelin et al. 1999].

La réticulation de la colle de tanin se produit par le biais de ponts benzylamines par réaction avec une base imine-amine méthylène (CH_2=N-CH_2^+) obtenue par la décomposition d'hexamine [Pichelin et al. 1999; Kamoun et Pizzi 2000 a, b; Kamoun et al. 2003].

Un mécanisme de la condensation du tannin de mimosa avec l'hexamine comme durcisseur est proposé figure 10.

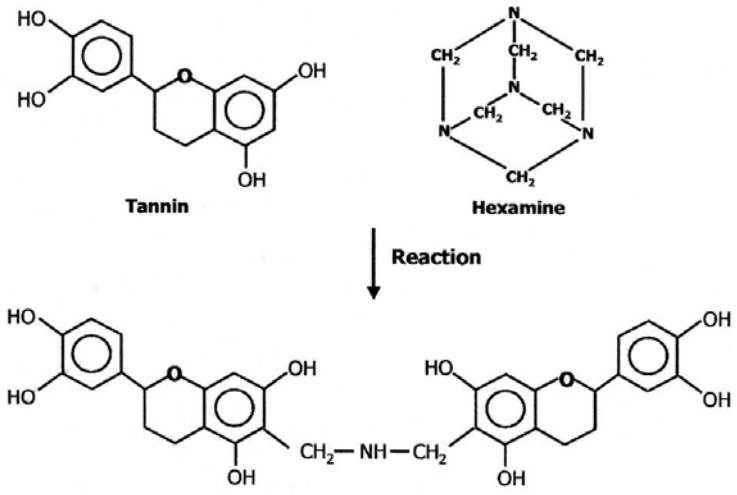

Figure 10 : Exemple de condensation du tannin avec des ponts benzylamines par réaction avec des bases imino-amino méthylène, dérivées de la décomposition de l'hexamine en présence de tannins. (Pichelin et al. 2006).

2.6.4. La colle tannin mimosa + hexamine/lignine + glyoxal

Le principal intérêt de cette colle, est de transformer une matière première perdue, par exemple la lignine de blé, en un produit utile et écologique. En effet la synthèse ne fait pas intervenir de formaldéhyde donc on peut s'attendre à ce qu'elle soit respectueuse de l'environnement. Le process de glyoxalation de la lignine fait la lignine plus réactive et donc favorise sa condensation, en utilisant le glyoxal pour augmenter la reactivite de la lignine.

La colle consiste à préparer un mélange de lignine glyoxalée avec la colle de tannin de mimosa. Ainsi se produisent une série de réactions pendant le durcissement de la colle (figure 11). Ce mélange permet de créer des groupes méthylols (CH_2OH) plus réactifs.

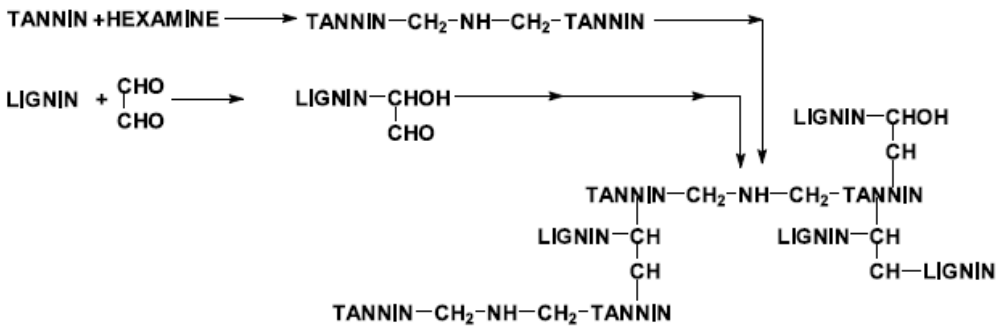

Figure 11 : Représentation d'une série de réactions qui se déroulent pendant le durcissement de la colle tannin/lignine. (Mansouri et al. 2011).

3. MATERIAUX ET METHODES

3.1. Les colles

Les colles synthétiques traditionnelles utilisés dans cet travaille sont l'urée formaldéhyde et la mélanine urée formaldéhyde. Elles ont été fournies par une entreprise de panneaux.

Les colles vertes étudiées sont constituées de tannin de mimosa + hexamine et de tannin mimosa + hexamine/lignine + glyoxal.

La colle de tanin de mimosa: consiste en un mélange de 100 g de tanin de mimosa avec 83 g d'eau distillée et de 19 g de soude (33%) pour avoir un pH égal à 10. Finalement on ajoute la solution d' hexamine (30%) et la solution est agitée jusqu'à obtenir un mélange homogène.

La colle de lignine/tanin de mimosa: dans la première étape, la lignine glyoxalée est preparée à partir de 100 g de lignine de paille de blé, de 132 g d'eau distillée, et de 117 g de soude (30%).

La soude est ajoutée en petites quantités au mélange lignine–eau distillée jusqu'à stabilisation du pH entre 12 et 12.5. Un pH aussi élevé et une agitation vigoureuse du mélange permettent la dissolution de la lignine. La solution est ensuite versée dans un ballon de 1 L maintenu dans un système de reflux régulé à 58-60 °C pendant environ 1 heure. 60 g de glyoxal (40%) sont ensuite versés dans le ballon et le mélange reste sous agitation magnétique pendant 8 heures supplémentaires. Le complexe tanins/hexamine utilisé à la fin pour le mélange est le même que préparé précédemment. La porportion tanin+hexamine/lignine + glyoxal correspond à 50/50.

3.2. La fabrication de panneaux et la préparation d'échantillons

Les particules utilisées pour la fabrication des panneaux sont issues de l'épicéa et sont à une humidité comprise entre 2 et 3 %. Les panneaux monocouches ainsi fabriqués sont de dimensions 350*310*14 mm^3, avec une densité de 700 kg/m^3.

Des particules sèches sont imprégnées par la colle ; soit un taux d'encollage de 10 %. Les particules encollées sont introduites dans un malaxeur automatique pendant 1 min pour homogénéiser la répartition de la colle entre les particules d'Epicéa. Après ce mélange, les particules sont réparties de façon régulière dans un moule pour former un mat. Le panneau est ensuite mis sous presse.

Les réglages de la presse suivent 3 paliers de durées différentes pendant lesquels seule la pression diffère (Tableau 4).

Tableau 4 : Conditions de pressage du panneau de particules.

Les durées (s)	120	150	180
Pression ($kg.cm^{-2}$)	35	24	7
Température (°C)	195	195	195

Les méthodes utilisées pour rendre compte du dégagement de formaldéhyde prévoyaient des dimensions et des masses d'échantillons bien précises (en accord avec chaque norme utilisée). Le plan de coupe est indique ensuite (figure 12):

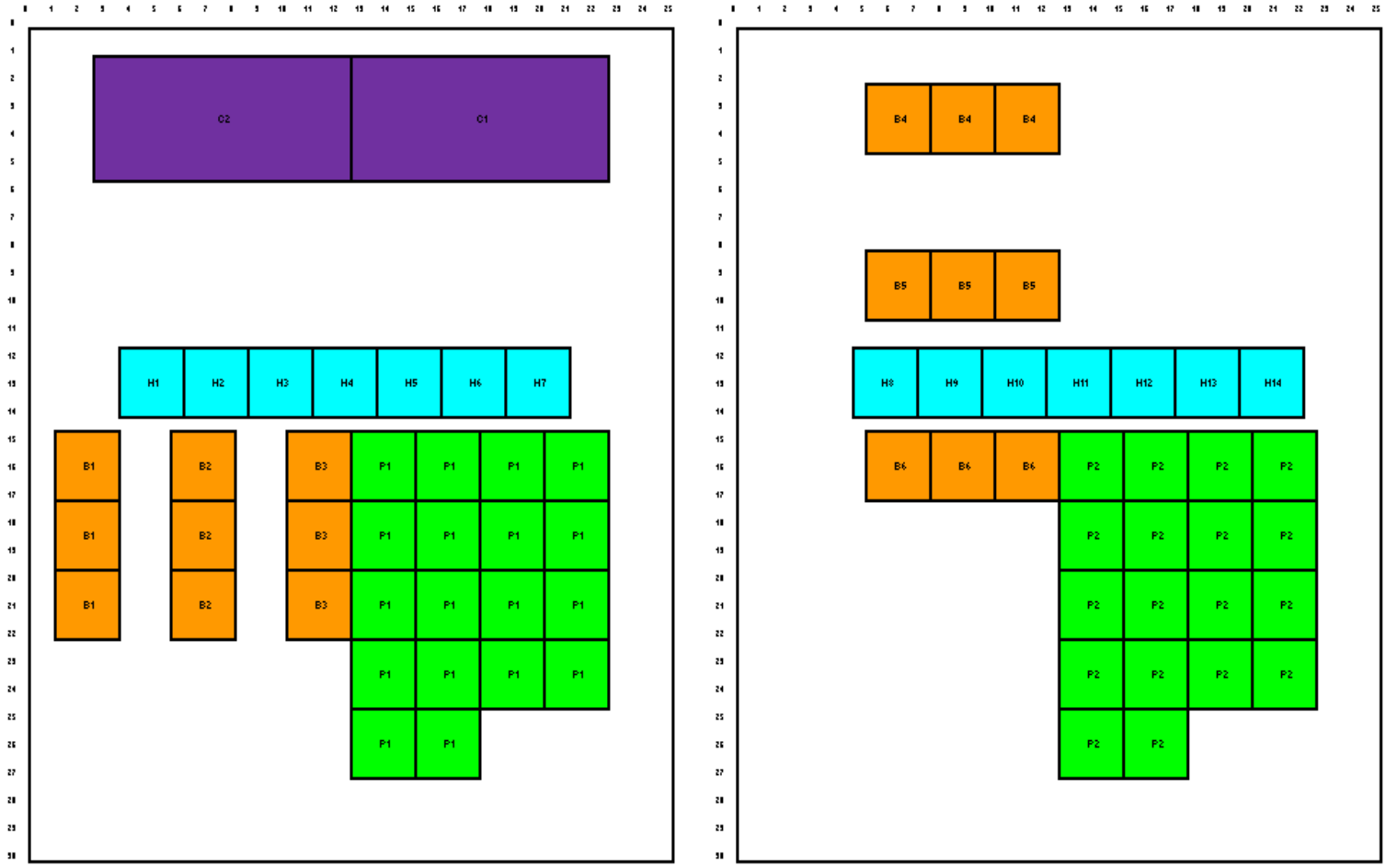

Figure 12 : Plan de coupe pour l'échantillonnage.

Où:

B: Echantillons pour méthode bocal.

P: Echantillons pour méthode perforateur.

C: Echantillons pour méthode climpack.

Les échantillons étaient ensuite conservés selon les exigences des normes utilisées.

3.3. Les méthodes de mesure utilisées

Dans les méthodes bocal et perforateur, il a été déterminé la teneur en formaldéhyde en analysant photométriquement l'extrait aqueux. Le formaldéhyde étant incolore, on ne peut mesurer directement son absorbance. La détermination est en fait basée sur la réaction de Hantzsch dans laquelle du formaldéhyde en solution aqueuse réagit avec des ions ammonium et de l'acétylacétone pour donner de la diacétyldihydrolutidine. Les mesures

d'absorbance ont été effectuées à $\lambda = 412nm$ qui est le maximum d'absorption de la DDL. Cette réaction est spécifique du formaldéhyde. Elle est détaillée ci-dessous (figure 13):

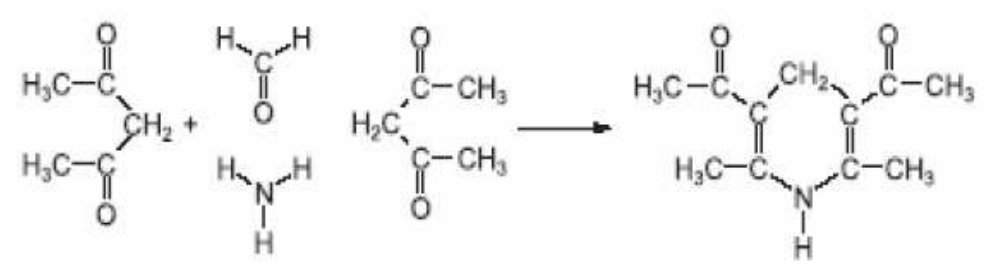

Figure 13: Conversion de formaldéhyde avec l'acétyle acétone à 3,5- diacétyl-1,4- dihydrolutidine, selon la réaction de Hantzsch.

Les méthodes que nous allons effectuer nous donne des valeurs d'absorbance. De ces valeurs, nous désirons obtenir une concentration en $\mu g/ml$. C'est la courbe d'étalonnage qui nous permet de passer de l'absorbance à la concentration.

3.3.a) La méthode au bocal

Cette méthode consiste à laisser des échantillons de bois reposer au dessus d'un lit d'eau dans un bocal (figure 14) à une temperature de 40 °C pendant 180 min. Durant le temps de repos, le formaldéhyde libre qui s'échappe des échantillons est piégé dans l'eau distillée. Il ne reste plus qu'à faire réagir le formaldéhyde piégé selon la réaction de Hantzsch et de déterminer l'absorbance de la solution obtenue. La valeur de l'absorbance obtenue n'est pas exploitable en tant que telle mais doit être introduite dans une formule donnée dans la norme EN 717-3 qui donne la «valeur au bocal F_v , la formule étant:

$$F_v = \frac{(A_s - A_b) * f * 50 * 10 * (100 + H)}{m} mg/kg$$ de panneaux secs anhydre

A_s est l'absorption de la solution analysée provenant des récipients

A_b est l'absorption de l'eau distillée analysée

f est la pente de la courbe d'étalonnage, en milligrammes par millilitre

H est l'humidité des éprouvettes en pourcentage

m est la masse des éprouvettes en grammes

Cette méthode pratique a permis de réaliser de nombreux essais et d'avoir une distribution de résultats plus exploitable que pour les autres méthodes.



Figure 14 : Exemple de test au bocal.

3.3.b) La méthode au perforateur

Cette méthode consiste à extraire le formaldéhyde libre du bois à l'aide de toluène pur. Les échantillons de panneau sont placés dans un ballon dans lequel on ajoute du toluène. Ensuite, il est porté à ébullition. Les vapeurs de toluène chargées en formaldéhyde passe à travers le perforateur et sont condensées au niveau du réfrigérant à bulle (figure 15). Lors de cette étape le formaldéhyde, bien plus miscible à l'eau, passe du toluène à l'eau. Le formaldéhyde est très volatil, alors il est nécessaire de placer un barboteur au dessus du réfrigérant à bulle au cas où certaines vapeurs s'échapperaient. Il ne reste plus qu'à récupérer l'eau dans le corps du perforateur et celle du barboteur, puis de nouveau en réalisant la réaction de Hantzsch, à déterminer l'absorbance de la solution obtenue. Comme précédemment, l'absorbance fournit grâce à la courbe d'étalonnage une concentration en formaldéhyde qui permet d'obtenir la «valeur au perforateur»:

$$\textit{Valeur au perforateur} = \frac{f * (A_s - A_b) * (100 + H) * V}{m_H} mg/100g \text{ de panneaux sec}$$

A_s est l'absorbance de la solution d'extraction analysée

A_b est l'absorbance de la solution d'eau distillée ou déminéralisée

H est la teneur en humidité du panneau à base de bois en pourcentage

f est la pente de la courbe d'étalonnage de formaldéhyde

m_H est la masse des éprouvettes, en grammes

V est le volume de la fiole jaugée (2000ml)

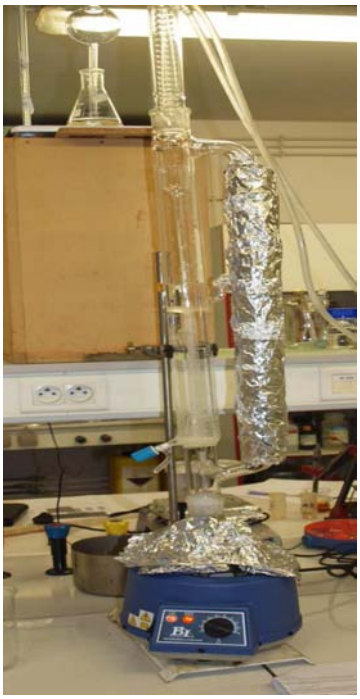

Figure 15 : Exemple d'essai au perforateur.

3.3.c) La méthode au CLIMPACK

Cette méthode est certainement la plus directe et la plus proche de la réalité du dégagement de COV. La CLIMPACK est une chambre d'émissions (figure 16). Le dispositif de mesure du CLIMPACK[7] permet de mesurer en temps réel la concentration de COV émis par un panneau de particules pendant un temps donné. Il faut bien observer que l'analyseur enregistre la quantité de COV totale et non uniquement la quantité de formaldéhyde.

La CLIMPACK ayant un volume de 50 L, il a été préféré de mettre deux échantillons de 120*100*14 mm3 à chaque extrémité de la chambre au lieu d'un seul qui serait grand et au contact des parois de la chambre.

La température à l'intérieur de la chambre «Climpack» est maintenue à 23 °C (±2) et l'humidité à 50 % (±5). Ces conditions climatiques sont imposées par la norme NF EN 16000-9.

[7] Analyseurs de gaz HC51M de Environnement-sa

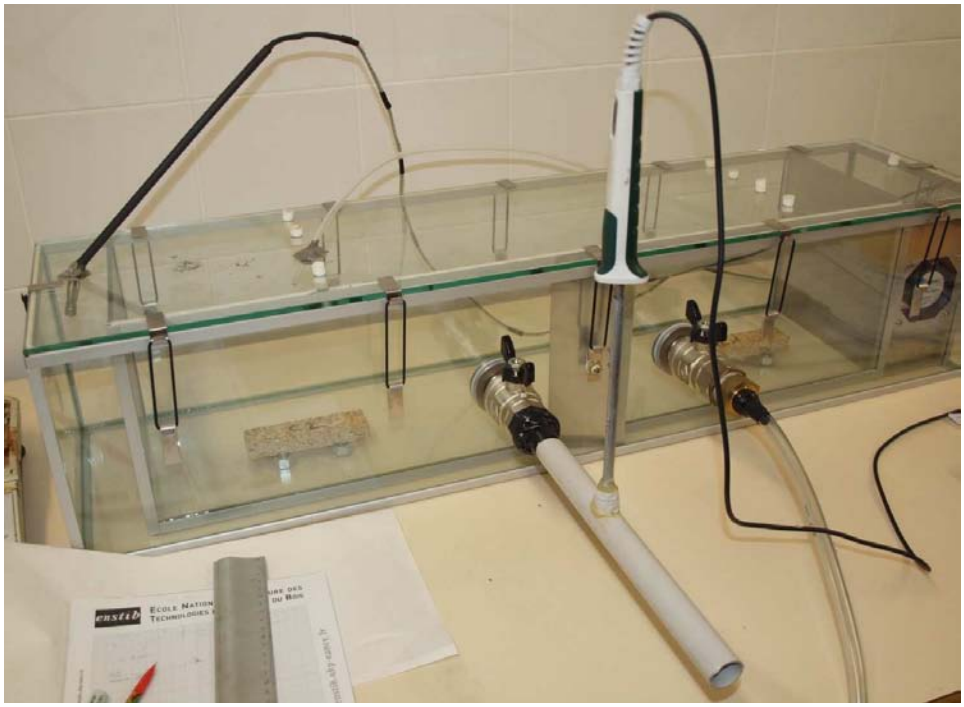

Figure 16 : Exemple d'essais au CLIMPACK.

Cet analyseur utilise un détecteur à ionisation de flamme. Le mécanisme d'ionisation des substances organiques se déroule en deux étapes:

Cracking des composés organiques en $CH^{\bullet}$, $CH_2^{\bullet}$ et $CH_3^{\bullet}$

Ionisation chimique au contact de l'oxygène: $CH^{\bullet} + O \rightarrow CHO^{+} + e^{-}$

Les électrons e^{-} sont récupérés au moyen d'une tension électrique et sont comptabilisés, ce qui permet de remonter au nombre de carbones mesurés. Un four sélectif permet de distinguer le CH_4 des autres hydrocarbures.

Les capteurs donnent directement une concentration en ppm et des ppm, et il est possible obtenir une concentration en mg / Nm^3 :

$1ppm = 1*10^{-6} * \frac{16000}{22.4*10^{-3}} = 0.71 mg/Nm^3$ en équivalent CH_4.

Ensuite il faut appliquer des facteurs correctifs pour obtenir des mesures que nous pourrons comparer.

$q_s = \rho * n/L$

q_s est le débit d'émission spécifique par unité de surface en $\mu g/m^2/h$

ρ est la concentration des COV autres que le méthane en $\mu g/m^3$

n est le taux de renouvellement d'air en h^{-1}

L est le facteur de charge du produit en $m^2 bois/m^3 chambre$

4. RESULTATS

Cette étape est la plus cruciale des essais par mesure d'absorbance. En effet une courbe d'étalonnage correcte est essentielle pour l'obtention de teneurs en formol précises (figure 16 et tableau 5). Le coefficient de corrélation, égal à 0.9992, indique que la concentration et l'absorbance sont bien liées par une relation de linéarité.

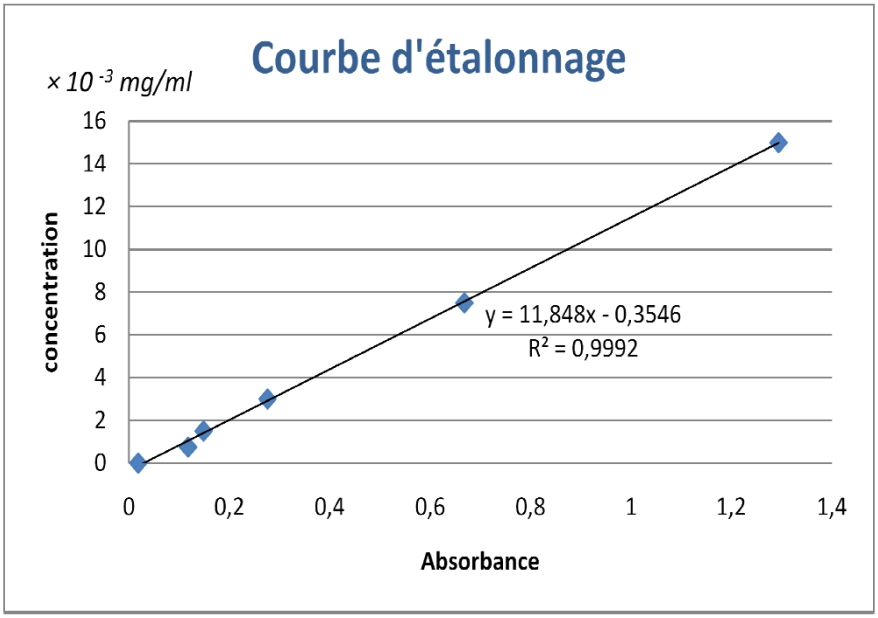

Figure 16: Courbe d'étalonnage.

Tableau 5: Mesures de la courbe d'étalonnage.

n°de fioles	*Absorbance*	*Concentration mg/ml (× 10 -3)*
0	0,0184	0
5	0,1176	0,75
10	0,1483	1,5
20	0,2756	3
50	0,668	7,5
100	1,2939	15

Ensuite à partir des résultats d'absorbance, il a été déterminé la valeur de la pente:

$f = 0{,}011848 \mathrm{mg/ml}$

4.1. Méthode au bocal et perforateur

Les resultat d'emissions de formadehyde à travers des méthodes bocal et perforateur sont montrées dans le tableau 6.

Tableau 6 : Resultats methode bocal et perforateur.

Colle	Méthode	Bocal (mg/kg de panneaux)	Perforateur (mg/100 g panel)
UF	Moyenne	8.54	1.84
	Ecar type	1.01	0,32
MUF	Moyenne	4.44	0.64
	Ecar type	0.57	0.24
Tanin mimosa	Moyenne	1.27	0.32
	Ecar type	0.32	0.03
Lignine-glyoxalée/tannin-mimosa	Moyenne	1.12	0.56
	Ecar type	0.14	0.10

4.1.a) Colle UF

La valeur obtenue classifie le panneau en classe d'exigence F***. Les classes F sont des classes japonaises beaucoup plus drastiques que les classes E utilisées en Europe. La proportion molaire a été indiquée: U: F = 1:1. Il a du se trouver une émission de 8 à $12\,mg/100g$. La formulation de la colle est importante dans les émissions de formaldéhyde mesurées, le process mis en œuvre l'est au moins tout autant.

4.1.b) Colle MUF

Ce résultat nous fait de nouveau passer la barre de l'exigence F****.

4.1.c) Colle tannin mimosa + hexamine

Nous obtenons haut la main la classe F****

4.1.d) Colle tannin mimosa + hexamine et lignine + glyoxal

La méthode perforateur n'était pas forcément adaptée pour analyser les dégagements de formol d'un panneau confectionné avec une colle qui fait intervenir de la lignine. En effet, le toluène qui agit en profondeur dans le panneau peut extraire certaines molécules impactant dans l'étape d'analyse au spectrophotomètre.

La classification de panneaux fabriqués avec cette colle correspond à F****.

4.2 Méthode à la chambre CLIMPACK

4.2.a) Colle UF

La courbe de la figure 17, présente une tendance très particulière difficile à expliquer. Deux hypothèse ont été possible (a) une pollution durant l'essai; ou (b) la présence d'une série de réactions secondaires qui permettent à différents COVs d'être générés ou de réagir en différents temps. Par contre la première action n'a pas été effectuée lors des premières heures de l'essai parce qu'Il n'y a pas de source polluant.

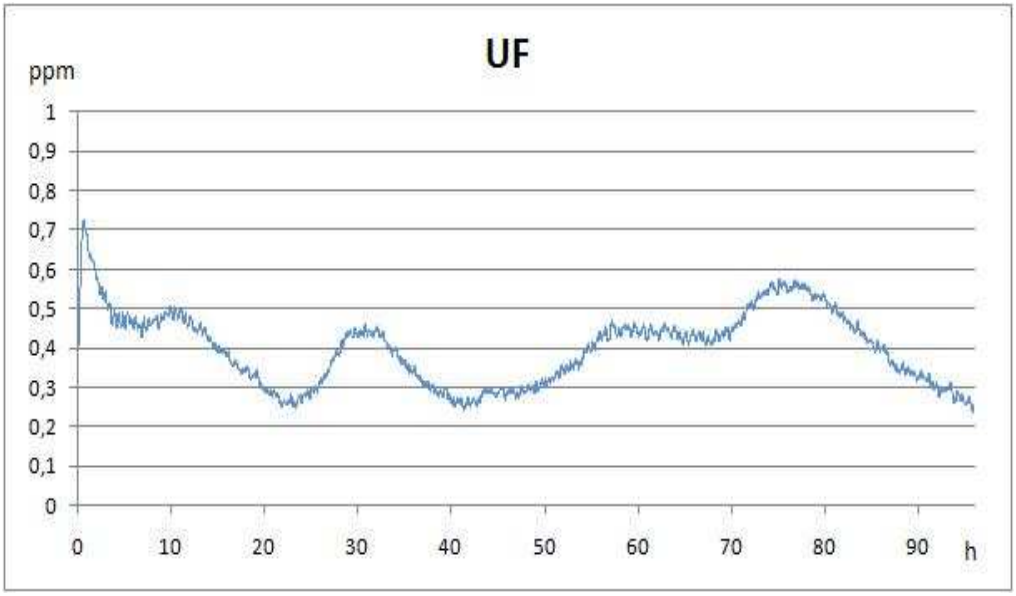

Figure 17 : Tendance des émissions de COV.

Tableau 7: valeurs au CLIMPACK

Echantillon	Masse (g)	ppm max	ρx (µg/m^3)	qs (µg/m^2/h)
Total (g)	212,7	0,80	571,43	172,95

La faible valeur mesurée nous fait atteindre la classe 1 définie par le CTBA.

4.2.b) Colle MUF

La courbe de la figure 18 présente un fort dégagement de COV au début de l'essai suivi d'une atténuation du dégagement. Nous constatons le pic de dégagement à 60 minutes.

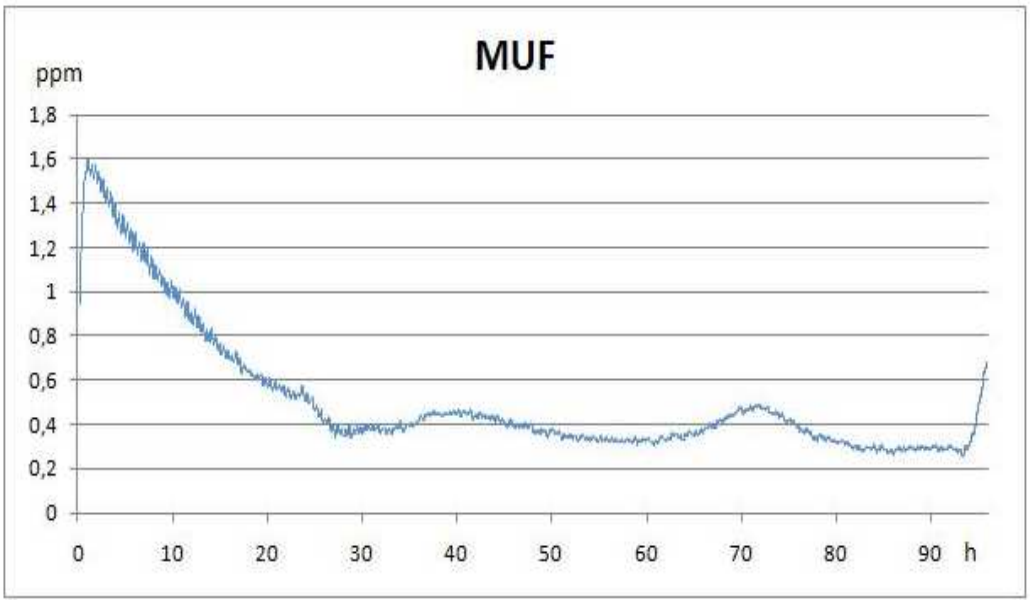

Figure 18 : Tendance des émissions de COV.

Tableau 8: Valeurs de dégagement de COV.

Echantillon	Masse (g)	ppm max	ρx (µg/m^3)	qs (µg/m^2/h)
Total (g)	217,1	1,70	1214,29	149,12

La classe 1 du CTBA est atteinte.

4.2.c) Colle tanin de mimosa + Hexamine

La courbe de la figure 19 a montré une tendance intéressante. Différents pics pendant différents temps sont apparus. Il est très peu probable que ce que nous voyons se dégager ici soit du formaldéhyde. La synthèse d'une colle verte fait participer des molécules carbonées de poids importantqu'il est nécessarie d'identifier.

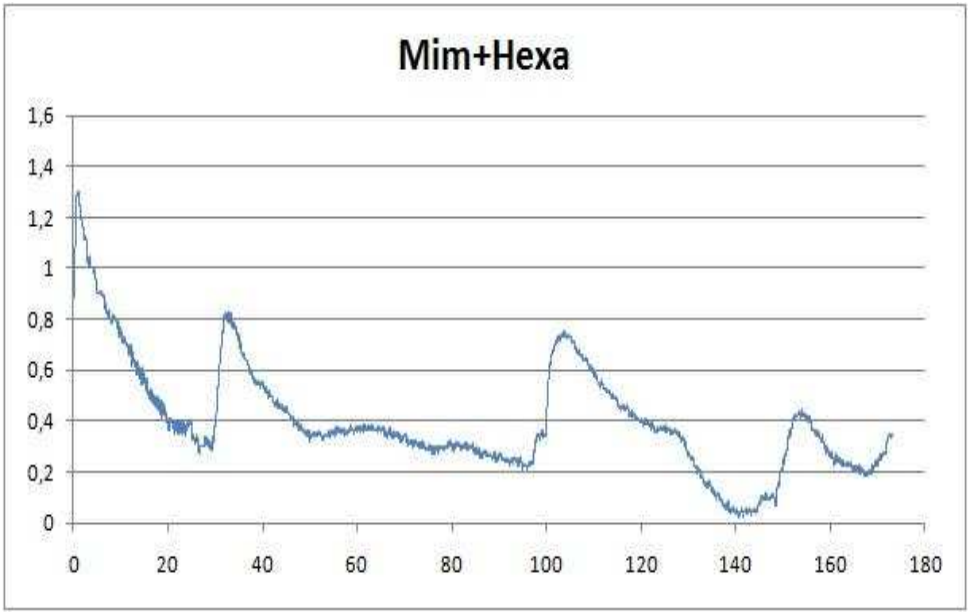

Figure 19 : tendances des émissions de COV.

Tableau 9: Valeur de dégagement de COV.

Echantillon	Masse (g)	ppm max	ρx (µg/m^3)	qs (µg/m^2/h)
Total (g)	180,86	1,40	1000,00	122,80

Faire l'hypothèse que la valeur obtenue ne représente qu'un dégagement de formaldéhyde parait à présent incongrue.

4.2.d) Colle tanin mimosa + hexamine/lignine glyoxalée

Dans la courbe de la Figure 20, on observe un fort pic initial puis d'autres pics successifs. Nous fournirons donc les mêmes analyses que celles vues dans la partie précédente.

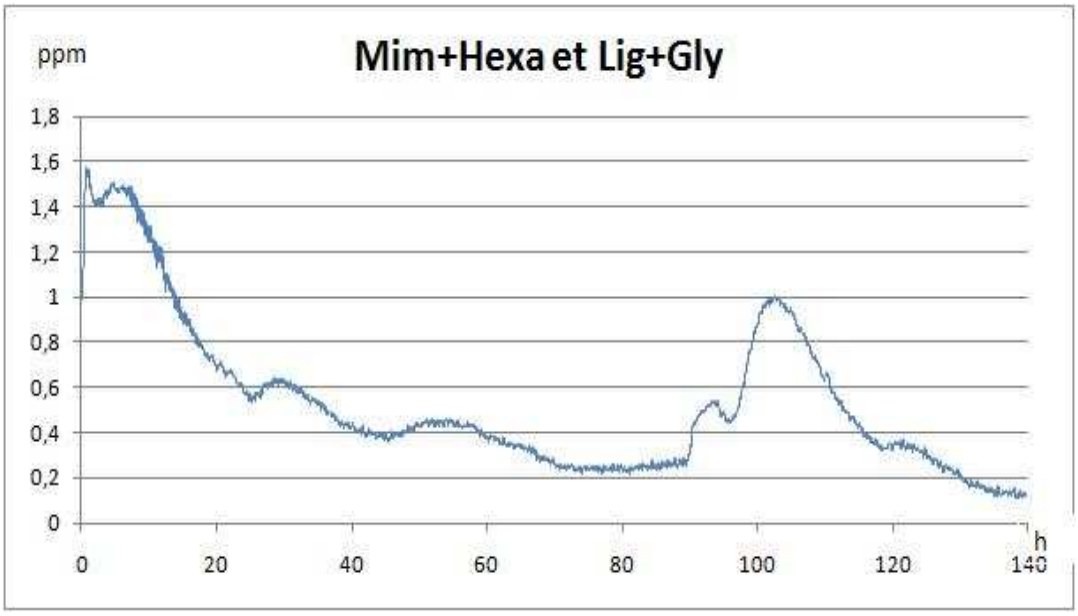

Figure 20 : Courbe de tendance de dégagement de COV.

Tableau 10: Valeurs de dégagement de COV.

Echantillon	Masse (g)	ppm max	ρx (µg/m^3)	qs (µg/m^2/h)
Total (g)	233,9	1,70	1214,29	271,13

5. ANALYSES DE RESULTATS

5.1. Méthode au bocal

D'une manière générale, par la méthode au bocal les colles vertes sont nettement moins polluantes en formaldéhyde par rapport aux colles traditionnelles (Figure 21). Il a été constaté qu'un panneau fabriqué avec de la MUF dégage moins qu'un panneau constitué d'UF. C'est en effet une propriété de la mélamine. Les deux colles vertes présentent des résultats à peu près similaires.

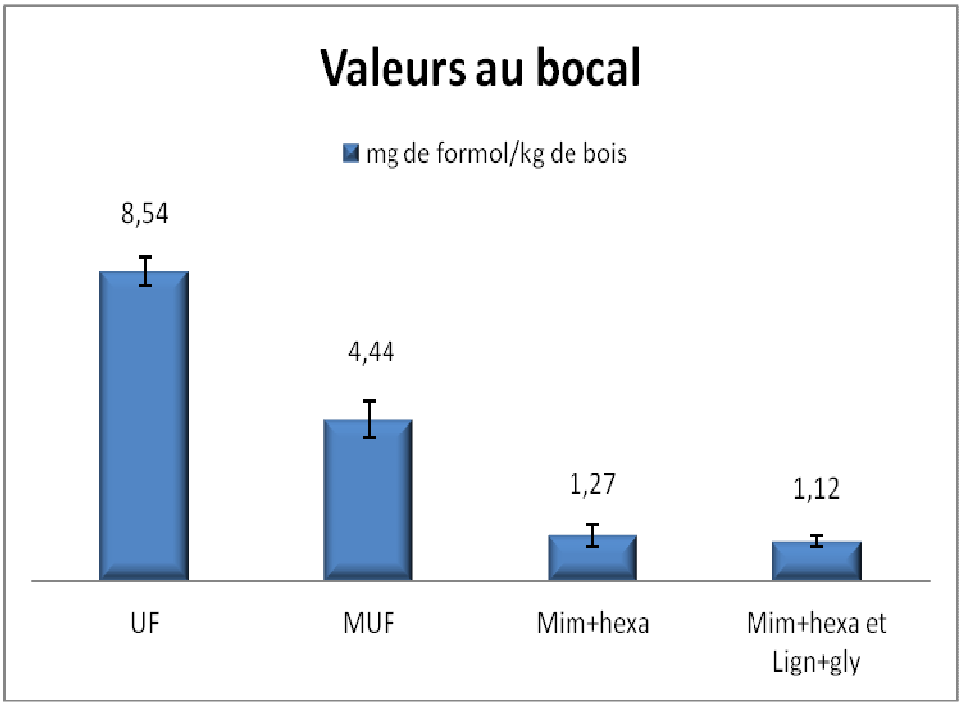

Figure 21 : Comparaison des valeurs au bocal.

5.2. Méthode au perforateur

On constate la même tendance que pour la comparaison des valeurs au bocal à quelques exceptions près (Figure 22). Il a été constaté déjà que l'écart s'est creusé entre un panneau à base d'UF et panneau à base de MUF.

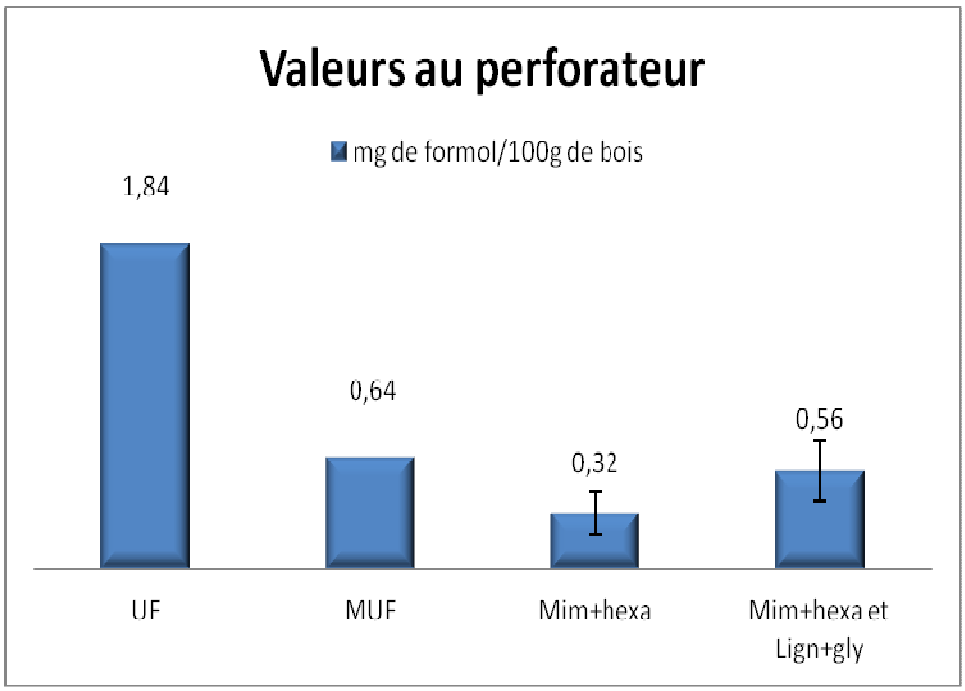

Figure 22: Comparaison des valeurs au perforateur.

La méthode au perforateur extrait le formaldéhyde libre comme le formaldéhyde lié; il est plus difficile d'hydrolyser le HCHO quand la mélamine participe à la constitution du réseau dans le panneau. D'autre part, il existe un décalage significatif entre le résultat du panneau fabriqué avec du tannin Mimosa + hexamine et celui fabriqué avec tannin Mimosa + hexamine/Lignine + glyoxal: ce dernier est supérieur et se rapproche plus du panneau fait avec de la MUF. Ce sont donc des molécules propres à la lignine qui ont été extraites par le toluène et il est possible que ces molécules faussent les mesures finales effectuées au spectrophotomètre.

5.3. Méthode à la chambre CLIMPACK

La méthode au CLIMPACK présente des résultats moins marqués que ceux précédemment énoncés (figure 23).

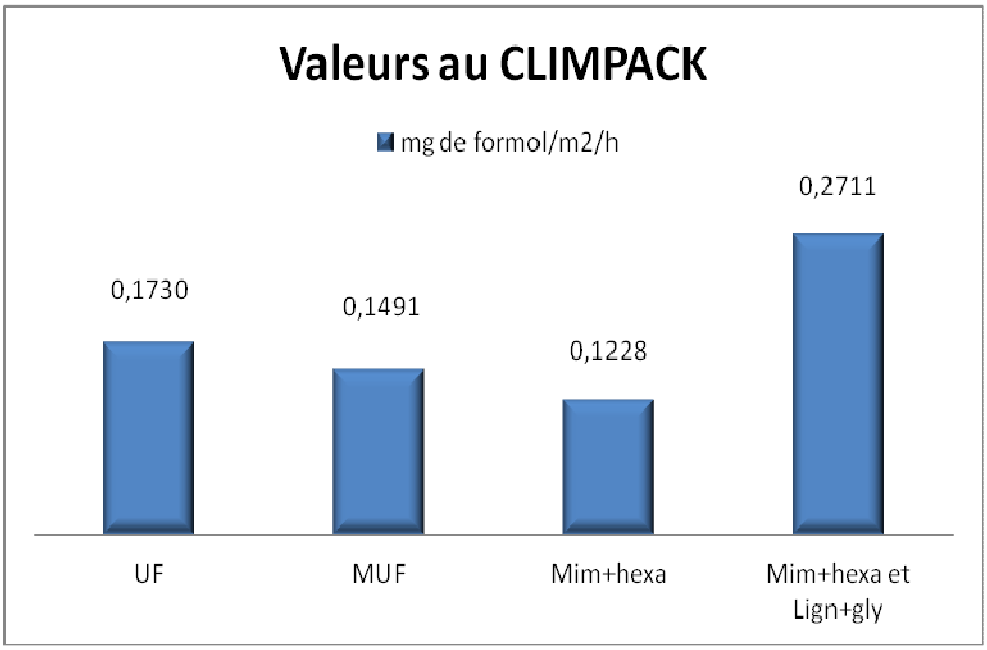

Figure 23 : Comparaison des valeurs au CLIMPACK

Dans les trois premières formulations, les colles traditionnelles dégagent plus de COV que la première colle verte. Il a été constaté d'autre part que la seconde colle verte émet beaucoup plus de COV que toutes les autres colles. Cette observation va dans le sens de celle émise pour la comparaison des valeurs au perforateur, à savoir que la lignine implique le dégagement d'autre molécules que le formol. On peut rajouter cette fois que ces autres molécules ont un nombre d'éléments carbone supérieur à 1.

5.4. Comparaison des différentes méthodes

Les tendances qui ont été observées par la méthode climpack sont les mêmes pour le test au bocal et le test au perforateur, bien qu'elles soient moins marquées (figure 24).

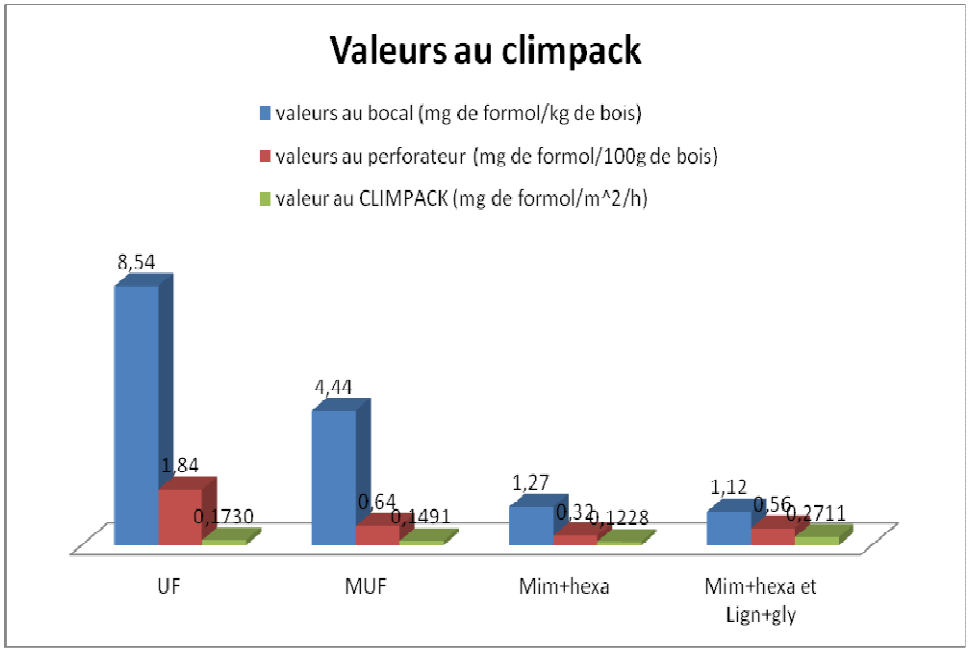

Figure 24: Comparation de tendances.

Au vu de ces observations, il parait difficile de relier d'une manière simple ces trois méthodes.

Un élément commun de toutes ces méthodes est qu'elles extraient du formol du panneau de particulesMais il faut savoir qu'elles n'extraient pas le formol de la même manière. Par exemple, la méthode au bocal, qui est une méthode douce, n'extrait que le formol libre présent dans le panneau. La méthode au perforateur extrait le formol libre et une partie du formol lié du panneau. Enfin l'analyseur CLIMPACK permet de voir l'extraction de tous les COV du panneau, dont le formol libre.

Le taux de formol libre, obtenu par la méthode au bocal, permet de mesurer l'impact sanitaire à court terme du panneau étudié, par exemple dans l'usine de fabrication.

Le taux de formaldéhyde obtenu par la méthode au perforateur, n'est pas très efficace pour tous les types de panneaux fabriqués avec différentes colles.

Enfin l'analyse du taux de COV autre que le formol, obtenue par comparaison des méthodes au CLIMPACK et au perforateur, permet de veiller à ce qu'il n'y ait pas de transfert de pollution. La réglementation visera certainement un jour, à réglementer tout dégagement de COV.

6. CONCLUSIONS

Aux vues de l'impact sanitaire des émissions de formaldéhyde, il est aujourd'hui difficile de faire fi des diverses règlementations et recommandations publiées sur le sujet. Les industries d'adhésifs et de panneaux ont compris qu'il était dans leurs intérêts de diminuer le formol de leurs produits voire mieux, de le remplacer par des produits innovants. Les colles vertes étudiées dans ce rapport semblent être une solution possible pour la fabrication de panneaux de particules d'après les résultats obtenus tout au long du projet. Néanmoins une attention particulière devra être apportée en ce qui concerne le dégagement possible d'autres composés organiques volatiles. Il s'agit en effet de ne pas effectuer un transfert de pollution. Si le formaldéhyde est aujourd'hui pointé du doigt il n'en reste pas moins qu'il existe d'autres COV sans doute moins étudiés dont les effets sur l'environnement et la santé pourraient être encore méconnus. De plus l'attention apportée pour minimiser l'impact environnemental et sanitaire ne doit pas se faire au mépris de performances techniques attendues.

Il convient d'indiquer que les méthodes pour déterminer une émission de formaldehyde étudiées ici ne sont pas comparables entre elles puisqu'elles mesurent le fomaldehyde libre, le formaldehyde hydrolisé ou les COV totaux qui incluent d'autres composés différents du formaldéhyde.

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Impact sanitaire et dégagement de formaldéhyde

Anexo: Tableaux concernant les classes d'exigences

Classe européenne		
Classe	chambre d'essai (ppm)	Perforateur (mg/100g)
E3	2,3	60
E2	1	30
E1	0,1	8
1/2 E1	0,05	4

Classe américaine	
Classe	chambre d'essai (ppm)
CARB1	0,18
CARB2	0,09

Classe japonaise			
Classe	Perforateur (mg/100g)	Dessicateur (mg/l)	chambre d'essai (mg de formol/m^2/h)
F*	non renseigné	5	non renseigné
F**	non renseigné	1,5	de 0.02 a 0.12
F***	3	0,5	de 0.005 a 0.02
F****	1,5	0,3	<= 0.005

Classe française	
Classe	chambre d'essai (mg de formol/m^2/h)
CTBA classe 1	3,5
CTBA classe 2	de 10 à 3,5

III.4.2. **Formalddehyde and COVs emissions from bio-particleboards**

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Des colles naturelles ont été préparées en vue d'une utilisation dans la fabrication de panneaux de particules. Les émissions de formaldéhyde et autres composés organiques volatils ont été mesurées et comparées avec celles d'une colle synthétique traditionnelle à base d'urée-formaldéhyde. Une colle de tanin de mimosa (T) et une colle à base d'un mélange de lignine de paille de blé glyoxalée et de tanin de mimosa (TL) ont été utilisées comme colles naturelles. Les analyses ont été faites selon la norme NF EN ISO 16000. Ainsi, les échantillons de panneaux de particules ont été placés à l'intérieur d'une chambre Climpack® et des prélèvements d'air ont été effectués avec le système Sympac®. Les aldéhydes et les cétones libérés ont été piégés par réaction chimique et analysés par chromatographie liquide haute pression HPLC. Les autres COVs sont piégés par adsorption et leur analyse s'effectue par chromatographie en phase gazeuse couplée spectrométrie de masse TD/GC – MS.

Formaldéhyde, acétaldéhyde, acétone/acroléine et hexanaldéhyde ont été les principaux composés détectés par la technique HPLC. Toluène, éthylbenzene, xylène, et terpènes: α-pinène, β-pinène, 3-carène et limonène ont été déterminés par GC-MS. L'efficacité des colles vertes est incontestable quant à la réduction des émissions de formaldéhyde par rapport à celle des colles synthétiques. Les resultats sont presente dans le tableau suivant:

Table N 1: Emissions de formaldehyde et COV a partir de panneaux de particules fabriques avec UF et colles naturelles.

Formaldehye et COV	Emission (µg/m^{2}h)		
	UF	T	TL
Formaldehyde	240.0	66.0	34.0
Acetaldehyde	29.0	40.0	46.0
Acétone/acroléine	39.5	29.3	53.9
Hexanaldehyde	11.7	14.6	13.7
Toluene	19.4	9.4	9.4
Ethylbencene	17.4	7.6	7.6
m-Xylene	69.11	26.6	26.6
o-Xylene	16.0	5.6	5.6
α-Pinene	8.5	19.7	14.8
β-Pinene	1.8	3.6	2.6
3-Carene	3.6	4.9	4.9

FORMALDEHYDE AND COVs EMISSIONS FROM BIO-PARTICLEBOARDS

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ABSTRACT

Formaldehyde and others volatile organic compounds (VOCs) emissions were determined from particleboards manufactured with natural adhesives. Thus, mimosa tannin with hexamine solution (T), and a blending of glyoxalated wheat straw lignin solution mixing with mimosa tannin and hexamine (TL) in 50/50 proportion were used as resins. These resins were compared with a traditional synthetic adhesive of urea formaldehyde (UF). Formaldehyde and other VOCs were determined and quantified through high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry methods (GC-MS). Formaldehyde, acetaldehyde, acetone/acroleine and hexanaldehyde were the main compounds detected by HPLC. Toluene, ethylbenzene, xylene, and following terpens: α-pinene, β-pinene, 3-carene and limonene were determined by MC-GS. Results were obtained according to the EN ISO 16000-9. After three days, emission results were: (a) formaldehyde UF: 240>T:66>TL:34 µg/m^{2}h; (b) acetaldehyde TL:46>T:40>UF:29 µg/m^{2}h; (c) acetone/acroleine TL:53.9>UF:39.5>29.3 µg/m^{2}h; (d) hexanaldehyde T:14.6>TL:13.7>UF:11.7 µg/m^{2}h; (e) toluene UF:19.4> TL and T: 9.4 µg/m^{2}h; (f) ethylbenzene UF:17.4> TL and T: 7.6 µg/m^{2}h; (g) m-xylene UF 69.11> TL and T 26.6 µg/m^{2}h; (h) o-xylene UF:16.0> TL and T: 5.6 µg/m^{2}h; (i) terpens were emitted with lower rate than all the other compounds. In general, higher emissions have been noticed in natural adhesives than in the UF. Thus, the main composes organic volatiles were α-pinene: UF:8.5<TL:14.8<T:19.7; β-pinene: UF:1.8<TL:2.6<T:3.6, and 3-carene UF:3.6<TL and T: 4.9. Limonene did not registered changes when natural adhesives were used.

Key words: Natural adhesives, formaldehyde, VOCs, mimosa tannin, glyoxalated wheat straw lignin, terpenes.

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1. INTRODUCTION

Natural raw material to wood adhesive manufacture is known for a long time. There are lot's of articles published about this subject. Casein, tannins, lignin, soy proteins, etc…, have been used in formulation of different wood adhesive systems by many authors (Pizzi, 1983; Hemingway, 1985; Cetin and Osmen, 2002 a, b; Kim, 2003; Muller, 2007; Lei et al. 2008; Lei, 2009; Amaral-Labat, 2008; El Mansouri, 2006; El Mansouri et al. 2007, etc.). The main stimulus for today's renewed interest in bio-based adhesives is the acute sensitivity from the general public toward anything concerning the environment and its protection (Pizzi, 2006). However, the use of the synthetic resins limits somewhat the environmental attractiveness of such adhesives based on purely natural materials, while the use of some natural materials such as tannins alone is currently affected by the relatively low supply of these materials (Pizzi, 2006).

Aminoplastic resins, especially UF resins, are the main binders used in the industry of wood panels. These adhesive resins are fast curing resins, with good performance and low costs (Roffael et. al, 2010; Kim, 2009), but they emit detectable amounts of formaldehyde and other volatile organic compounds (VOCs) (Roffael, 2006; Roffael et al., 2010), considered to have negative health effects on humans by the IARC (International Agency for Research on Cancer) (Roffael, 2006).

VOCs are emitted into the atmosphere from natural sources (Seco et al. 2008). They also exist throughout the different processes and ultimately in the products (Roffael, 2006).

Biogenic VOCs represent the most important part of volatile organic compounds and seem to contribute to about 90% of global VOC emissions (Guenther et al. 1995). Large variety of these including isoprene, mono and sesquiterpens, alcohols, aldehydes and ketones are known to be emitted by vegetation into the atmosphere. The emission factor of such compounds depends on many variables, especially on temperature, intensity of solar radiation as well as forest coverage (Roffael, 2006). Carbonyl compounds also have anthropogenic sources from industrial production, solvent applications, traffic emissions and domestic heating. Moreover, they are secondary products formed by atmospheric oxidation processes of hydrocarbons from natural and anthropogenic sources (Müller et al. 2006). Among VOC, a group of short-chained oxygenated compounds includes C1-C3 like methanol, ethanol, formaldehyde, acetaldehyde, acetone, and formic and acetic acids, reach considerable concentrations in the atmosphere (Seco et al. 2008). About this, there are

some natural VOC not harmful but they are included in the TVOC calculation (Kim, 2010). It is important to determinate and quantify the VOCs because the TVOCs can be strongly influenced by the presence of natural compounds as monoterpenes, in particular α-pinene as well it has been showed by Yrieix et al. (2010).

Formaldehyde gas is emitted from a natural range in the troposphere by oxidation of hydrocarbons (Possanzini et al. 2002), and human-made materials (Young, 2004). Combustion of many products and heating wood during drying process (Young, 2004), pressed-wood products used in home construction (Kim and Kim 2004, 2005a; Kim et al. 2006a), produce formaldehyde. But the most important source of formaldehyde is the automotive exhaust (Young, 2004; Uchiyama et al. 2007).

Formaldehyde is a very reactive product. Due to its chemical reactivity, it participates in a huge number of chemical reactions; it can be degraded in the atmosphere leading to the formation of free radicals. Oxidation of formaldehyde in the atmosphere to formic acid is also well known in the literature especially in areas with high humidity. Furthermore, formaldehyde can form bisulphite compounds with sulphite and bisulphite compounds usually present in the atmosphere, especially in industrial areas (Roffael, 2006).

Formaldehyde is the most abundant carbonyl in the atmosphere (Muir and Shirazi, 1996). It is a suspected human carcinogen that is known (Kim and Kim 2004, 2005a; Kim et al. 2006a), for short and long term exposure to the gas. Efforts have been made to modify the chemical formulation of resins to reduce formaldehyde emissions (Kim et al. 2010). Thus, the formaldehyde releasing from wood-based products has been declined over the 5 last decades with the development of new resins and new resin technologies have been proposed in order to obtain best results (Roffael, 2006). Polyurethane and sulphur compounds of sodium have also been used as a treatment of plywoods (Wang et al. 2008; Kunaver et al. 2010). Another effective way to reduce the released formaldehyde is based in compounds like ammonia, ammonium salts, urea/ammonium chloride, or amines hydrochlorides (Kim et al. 2006b, Kim et al. 2007b). The application of natural compounds such as urea (Uchiyama et al. 2007; Wang et al. 2008; Kunaver et al. 2010), and vanillin could provide a simple and effective approach for eradicating formaldehyde emissions into the surrounding indoor environment (Uchiyama et al. 2007). Pezzolan has been added to UF resin to reduce formaldehyde emission from particleboards. This material has a rough and irregular surface with porous forms; its addition to UF resin increases the formaldehyde absorption and reduces TVOC emission (Kim et al. 2007b).

Another solution proposed has been the natural raw material in the adhesives preparation. Tannin and lignin for example have been studied.

Tannin formaldehyde adhesives are obtained by hardening of polymeric flavonoids from natural origin, especially from condensed tannin (Kim et al. 2007a; Pizzi, 1994). Mimosa, quebracho and radiata pine tannin are excellent resources which can be used for replacing petroleum-derived phenolic compounds (Kim, 2009). Tannin/PVAc hybrid adhesive systems have been prepared (Kim, 2009). The role of PVAc was to avoid formaldehyde emission, by replacement of tannin adhesive with formaldehyde-based hardener.

Hardeners such as hexamine have been successfully used to reduce the formaldehyde emission in tannin-based adhesives (Kim and Kim 2004). Some extractions can drag terpene compounds which are susceptible to ozone oxidation in the atmosphere which seems to have significant influence on the monoterpene released from vegetation. Oxidation of terpene compounds can lead to the formation of simple aldehydes like formaldehyde (Roffael, 2006). Wood extractives play a dual role; they emit formaldehyde and can act as a formaldehyde scavenger (Schäfer and Roffael, 2000). For this reason they most be studied individually, and different hardeners can be used in the adhesives preparation (Pichelin et al. 1999; 2006; Trosa and Pizzi, 2001).

On the other hand, the lignin from different processes and tree species as adhesive resins has been evaluated as a natural and effective alternative for the wood adhesive preparation (Alonso et al. 2005; Khan et al., 2004; Chum et al. 1989, 1993). This material has a low reactivity and most be processed prior to the resin synthesis to be used as a wood adhesive (Cetin and Ozmen, 2002a, b). Thus, different treatments have been proposed (El Mansouri et al. 2006; Alonso et al. 2002). However, under certain conditions, the lignin can lead to a formaldehyde production (Schäfer and Roffael, 2000). In according to all the facts previously exposed, it is necessary to study precisely each case.

Different methods have been used to measure the formaldehyde and VOCs emissions: perforator (EN 120), desiccator (JIS A 1460, ASTM D 5582-00) and flask (EN 717-3), methods which are the most used for free-formaldehyde measure. All these methods have common formaldehyde level estimation by spectrometry (Kim and Kim, 2004). The way to determine the formaldehyde concentration is based on the Hantzsch reaction in which the formaldehyde reacts with ammonium ions and acetylacetone to yield diacetyldihydrolutidine (Kim et al. 2006). Chamber methods (EN 717-1, ISO 16000, ASTM D 7143-05, etc.) have been proposed too. Techniques as HPLC and GC-MS have also been used furthermore to measure formaldehyde and even other VOC emitted by wood based panels

(Kim and Kim, 2004). Some researches have been carried out to compare these different methods (Risholm-Sundman et al. 1999; Kim and Kim 2005a; Kim et al. 2006).

Although no standard exists, various state government and private companies are attempting to incorporate design and operational features into the selection of materials and furnishings to improve indoor air quality (Kim et al. 2010).

The aim of this work is to measure and compare formaldehyde emission and other VOCs from particleboard prepared with three different natural adhesive formulations: One commercial urea formaldehyde and two natural adhesives systems: mimosa tannin and organosolv lignin from wheat straw were used as raw material to prepare the adhesives. An emission chamber and the HPLC and GC-MS techniques were used to quantify and determine the different VOCs emitted (EN ISO 16000-9). Thus, formaldehyde, acetaldehyde, acroleine/acetone, hexaldehyde were identified and quantified by HPLC; and toluene, ethylbenzene, xylene and some natural compounds which are non-harmful VOCs such as terpenes (Kim 2010b, Kim 2006) were determined by GC-MS.

2. EXPERIMENTAL

2.1. Adhesives preparation

Three adhesives resins were used. Commercial UF adhesive, and two natural resins from mimosa tannin, and a wheat straw organosolv glyoxalated lignin mixing with mimosa tannin were prepared. The natural adhesive preparations are described as following.

Tannin adhesive preparation

The natural mimosa tannin adhesive (origin: Tanzania. Supplied by: Silva S. Michele Mondovi, Italy) was prepared in water solution at 45% concentration and its pH was adjusted to 10 with a sodium hydroxide water solution (33%). Finally, it was added 6% (w/w) of hexamine solution (30%) on the tannin solids base. The final viscosity was 1100 m.Pa.s (Brookfield RV viscometer; spindle Nr. 27, at 25°C and 20 rpm). It was showed by Pichelin et al. (2006), that the internal bond (IB) strengths of the tannin-hexamine adhesives are good and increase with increasing pH, but it is necessary to characterize the gases emitted by the particleboard prepared in this condition.

Preparation of glyoxalated lignin

The wheat straw organosolv lignin was supplied by CIMV (Compagnie Industrielle de la Matière Végétale, Reims Factory, France). It is a lignin with a low molecular mass obtained industrially as a by-product of pulp and paper production by an acetic acid-based organosolv process US patent 7,402,224 (Avignon et Delmas, 2000).

To increase its reactivity the lignin was modified by the glyoxalation process described by El Mansouri et al. (2007) as following: 295 Parts by mass of this lignin powder (96% solid) were slowly added to 384 parts water while sodium hydroxide solution (30%) was added from time to time thus keeping the pH of the solution between 12 and 12.5 for better dissolution of the lignin powder which was also facilitated by vigorous stirring with an overhead stirrer. A total of 181 parts by mass of 30% sodium hydroxide water solution was added which resulted in a final pH near to 12.5.

A 2 liters flat bottom flask equipped with a condenser, thermometer and magnetic stirrer bar was charged with the above solution and heated to 60 °C. 175 parts by mass glyoxal (40% in water) were added and the lignin solution was then continuously stirred with a magnetic stirrer/hot plate for 8 hours. The solids content for all glyoxalated lignin were around 33%, and the final viscosity around 4200 m.Pa.s. Glyoxal is a non-volatile non-toxic aldehyde that has been tested in lignin (El Mansouri et al. 2007; Lorenz et al. 2006), tannin adhesives (Ballerini et al. 2005), and other adhesives as soy protein (Amaral-Labat et al. 2008) for application to wood panels such as particleboard. Glyoxal is a non-toxic aldehyde (LD50 rat $\geq$ 2960 mg/kg; LD50 mouse $\geq$ 1280 mg/kg) (NIOSH, 2000), non-volatile but less reactive than formaldehyde which is toxic (LD50 rat $\geq$ 100 mg/kg; LD50 mouse $\geq$ 42 mg/kg) (NTIS, 2005).

Blending system adhesive lignin/tannin:

The lignin/tannin adhesive was prepared in mixing the tannin extract solution with the glyoxalated lignin in a 50/50 solids proportion (w/w) (Mansouri et al. 2011). The adhesive properties are shown in the table N° 1.

Table N° 1: Adhesives properties.

Adhesive	Total solids percentage (%)	Viscosity (spin 27, 20 rpm, 25 °C) (mPs)
UF	67.0	150
Mimosa tannin + hexamine	45.0	1.100
Lignine CIMV/Mimosa tannin	31.4	100

2.2. Particleboards manufacture and samples preparation.

One-layer laboratory particleboards with dimensions 350 x 300 x 14 mm^3 with a 680-700 kg/m^3 density were prepared using a mixture of core particles of beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*) woods. The wood particles before resin addition had a moisture content of 2%. The gluing factor was 10%. The operational conditions are composed of three stages of pressure applied to the panel: 35, 12 and 7 kg/cm^2. The total press time was maintained at 7.5 minutes, and the press temperature at 195 °C.

Samples of 120x100x14 mm^3 were obtained from the particleboards. They were kept in aluminium and plastic bags in order to avoid any reaction or contamination with the environment before introduction in the emissions chamber.

2.3. Emission chamber (Climpack).

The climpack is in a conditioned small emission chamber of 50 L. This chamber is connected to a computer system to control the environmental conditions inside at 23°C (±2) and HR 50% (±5) in accordance with the EN ISO 16000-9, and a flowmeter is used to control the air change. From each formulation two samples were placed inside the climpack. The time of measurement was 3 hours/day during seven days.

The air flow in the climpack was 2 L/min and the total volume collected for aldehydes and the others VOC were 91.98 and 9.198 L, respectively.

The aldehydes and cetones were determined through HPLC (high pressure liquid chromatography), and the others VOC such as toluene, ethylbencene, xylene and terpenes through MS-GS (gas chromatography mass spectrum detector). Each compound leaves after a specific interval time, which is detected and quantified through a chromatogram.

The standard method EN-16000-9 requires to take the air samples 72 (±2) and 28 (±3) days after initializing the test (Yrieix, et al. 2010). However the standards methods for wood panel formaldehyde emission requires to measure during the 72 hours after preparing the panel. According to this fact, the air sampling was daily made during one week and in agreement with the works made for other researchers (Kim et al., 2006; Kim, S. et al. 2010; Kim, K-W. et al. 2010).

2.4. High performance liquid chromatography (HPLC) method.

The performance liquid chromatography (HPLC) method was used to determine and quantify the aldehydes emitted by the different particleboards formulations.

A mixture of acetonitrile and water 60/40 v/v proportion was used as a vector solvent. The flow solvent circulation was 1 mL/min. The compounds detection is directly made and it is based on the aqueous solution absorptive through the Beer-Lambert Law.

The DNPH calibration solution contains 13 compounds belonging to the aldehydes and cetones family: Formaldehyde, acetaldehyde, acroleine acetone, propionaldehyde, crotonaldehyde metacrylaldehyde, 2-butanone, butiraldehyde, benzaldehyde, valeraldehyde, p-tolaldehyde and hexaldehyde. DNPH derivative concentrations were calculated by specific calibration. Each DNPH cartridge was treated with 3 mL of acetonitrile pure with a flow of 1mL/min. This solution was placed in a glass container. Finally the solution sample was manually injected in the HPLC with a volume of 20 µl to separate and identify the compounds.

2.5. Gas chromatography-mass spectrometry (GC-MS) method.

The gas chromatography mass spectrometry (GC-MS) was used for the separation and determination of compounds of a given gaseous mixture.

The VOC cartridge contains an adsorbent Tenax. It is a product composed of a porous polymer containing hydroxide of 2,6-diphenilene and 30% of carbon graphite. The air circulates inside the cartridge, and then, compounds are trapped in the different sizes pores.

The cartridge must be prepared before the measure. A standard solution is injected through a system at 250°C and under a flow of nitrogen of 50 $mL.min^{-1}$. This way let the standard solution in gaseous form arrive in the cartridge. Then, the cartridges are placed to the GC-MS to make the measure.

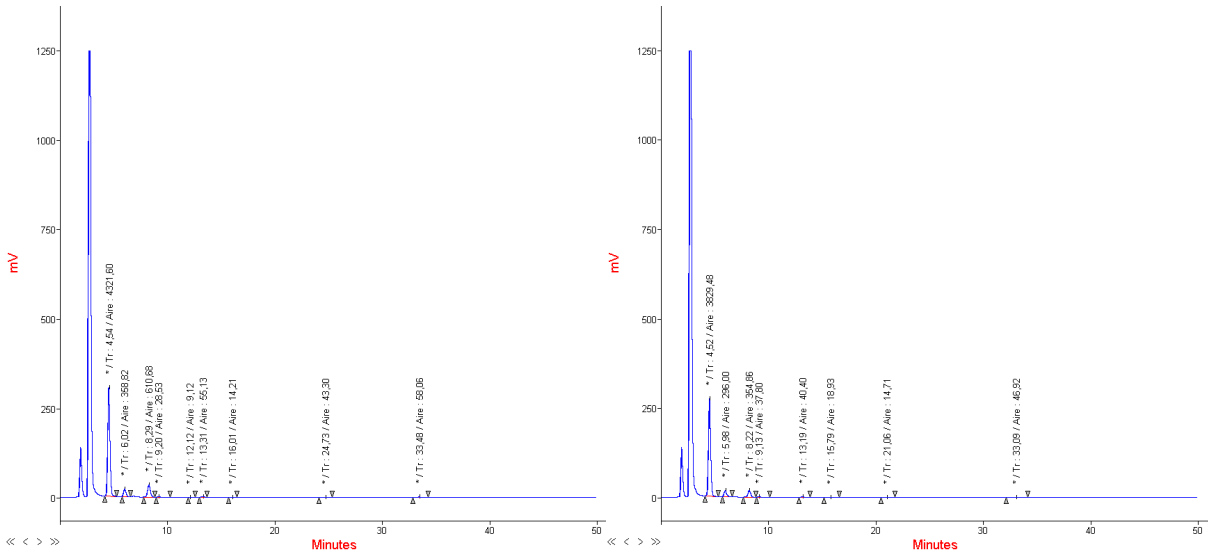
Helium was used as gas vector with a flow of 0.8 mL/min. The GS-MS length column is 60m with 250µm of internal capillary diameter. The stationary phase inside has a thickness of 0.25 µm, it is made up of 5% of diphenyl and 95% of dimethylpolysiloxane. In the first stage the substance were identified and in the second one they were quantified. The column is in an oven with a temperature first maintained at 35 °C during 7 min, secondly set up at 185 °C (7.5 °C/min), then increasing until 335 °C (15°C/min) finally maintained during 6 min.

The principal VOCs analyzed were toluene, ethylbenzene and o-xylene. They are the main VOCs found in indoor environments (Risholm-Sundman and Wallin, 1999), with the terpenes such as α-pinene, β-pinene, 3-carene, and limonene. The results were calculated in toluene equivalents and by specific calibration to natural terpenes (Yeonjung et al., 2011).

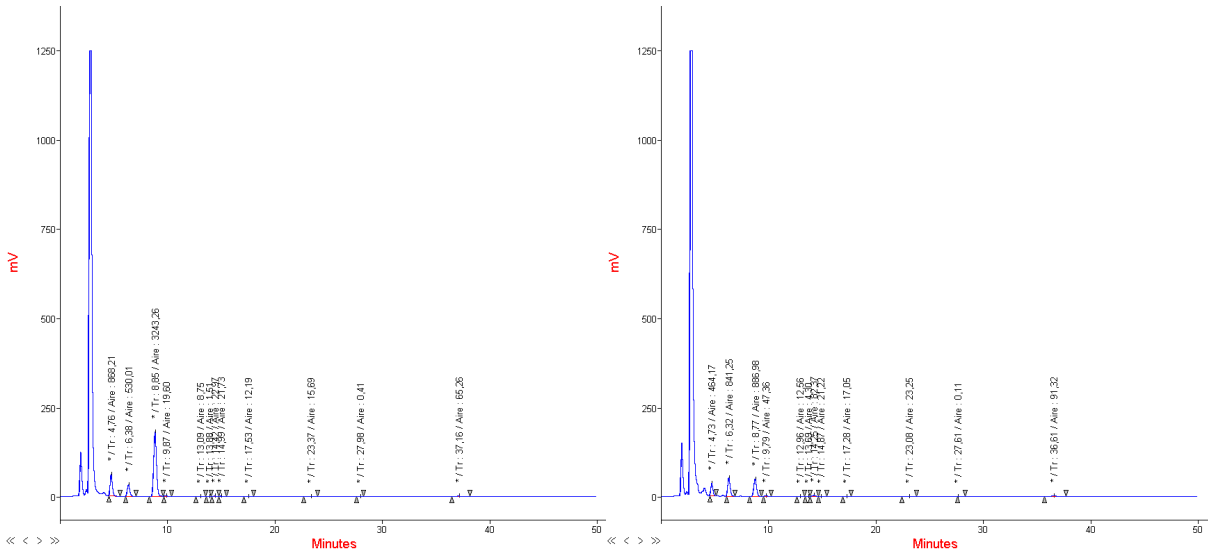
3. RESULTS AND DISCUSSION

3.1. Aldehydes and cetone emissions:

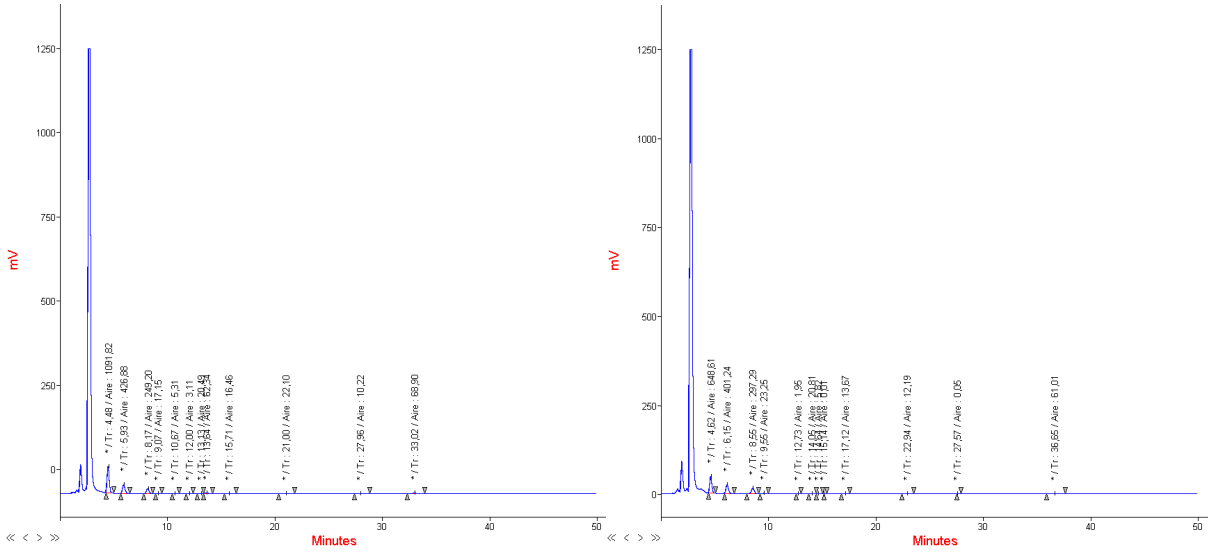
Formaldehyde, acetaldehyde and acetone/acroleine were the principal compounds detected by HPLC emitted from the particleboards studied with the different resins. Haxanaldehyde was also found, but in lower concentrations. The particleboards did not emit the maximal rate emission the first day. The samples need time to balance to environmental conditions (relative humidity and temperature); and on the other hand, the different compounds are emitted at different time. The HPLC chromatograms from first and seventh day of each formulation are shown in the figure N° 1.



a) Urea formaldehyde UF: Day 1 UF day 7



b) Lignin/tannin: Day 1 UF day 7



c) Tannin: Day 1 UF day 7

Figure N° 1: HPLC chromatograms from board prepared with (a) UF, (b) wheat straw lignin/mimosa tannin and (c) mimosa tannin adhesives resins during the first and the seventh day.

Formaldehyde:

The formaldehyde (HCHO) emissions are showed in the figure N° 2. The maximal rate was found during the second day and after it begins to decrease for all the adhesives used. However, the formaldehyde emitted from UF decrease is less pronounced because of the hydrolysis of this resin. The UF chemical reaction depends on the humidity (Yrieix et al. 2010).

The maximal emission values reported were 250, 44 and 77 µg/m^2h for UF, TL and T adhesive resins during the second day. To compare the results after 3 days as the EN-16000 standard request, the rates decrease until 240, 34 and 66 µg/m^2h for UF, TL and T adhesive resins, respectively. The UF value is higher than the results reported by Yrieix et al. (2010) because the initial samples conditions were different. They conditioned the samples for 4 weeks before the test to reduce the concentration of natural wood compounds, mainly terpenes and hexanal. But, this situation makes the other compounds emissions as formaldehyde decrease.

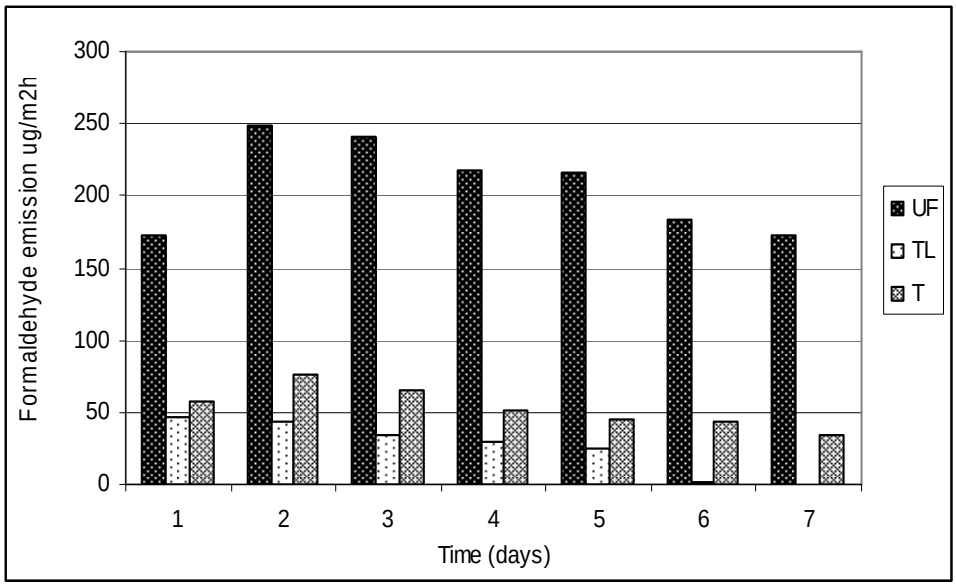


Figure N° 2: Formaldehyde emission from urea formaldehyde (UF), mimosa tannin and wheat straw lignin and mimosa tannin (TL) particleboards during 7 days by the climpack method.

The formaldehyde presence in all the formulations can be explained because this volatile compound is not only emitted from UF adhesive. The emission of volatile organics compounds starts in the forestry (Roffael, 2006). The formaldehyde can be formed from the main components of wood (cellulose, hemicelluloses and lignin) as well as from its extractives (Schäfer and Roffael, 2000). The natural wood composition is a natural source of formaldehyde emissions although it is in low amounts as it has been showed by some

researchers, and due to the lignin decomposition (Schäfer and Roffael, 2000; Salthammer et al. 2010). Moreover, the formaldehyde released from wood increases under thermal treatment and during drying and pressing processes (Marutzky and Roffael 1977, McDonald et al. 2004).

The oxidation of terpene compounds in simple aldehydes like formaldehyde can be formed (Roffael, 2006), and then this compound is directly released in the atmosphere. Of course, there are a great amount of factors that take part in the formaldehyde emissions and in general the VOCs emission has a multiple atmospheric implications (Seco et al., 2007).

In according to Schäfer and Roffael (2000), the extractives play a dual role. On the one hand the release formaldehyde and by another one, they react with formaldehyde and hence act as a formaldehyde scavenger. This depends of the wood extractives composition, pH, etc., which makes it possible to find formaldehyde even in the green adhesives used in very small proportions.

Acetaldehyde:

In the acetaldehyde (CH_3CHO) case, the results are not the same. With the UF samples (figure 3), the maximal value was obtained during the third day (29 µg/m^2h). This value was higher than those reported by Yrieix et al. (2010). With the natural adhesives, at the third day the values reported were 46 and 40 (µg/m^2h) for TL and T, respectively.

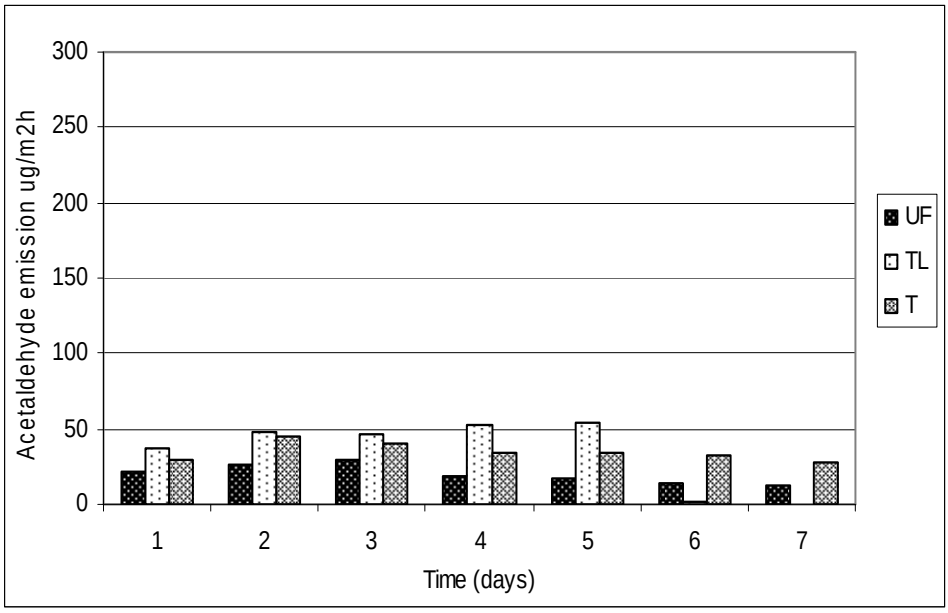


Figure N° 3: Acetaldehyde emission from urea formaldehyde (UF), mimosa tannin and wheat straw lignin and mimosa tannin (TL) particleboards during 7 days by the climpack method.

The higher acetaldehyde presence in the natural adhesive is explained because of its formation within plants which occurs at least by root metabolism, and by sunflecks and leaf pyruvic acid decarboxylation (Seco et al. 2007). However, according to Seco et al. (2007), the mechanism for acetaldehyde metabolism has not been studied yet, although there is evidence that enzymatic pathways take acetaldehyde into the general metabolism (Rottenberger et al. 2004). It can be supposed that the greatest acetaldehyde part in the natural adhesives found is due to the natural interactions within the different compounds present in source origin. However, this part has not been studied in detail before.

Acetone/Acroleine:

The acetone (CH_3COCH_3) and/or acroleine (C_3H_4O), are difficult to differentiate from the analysis of fixed quantity when they are analyzed by HPLC. This due to the similar amounts of molecules and retention times (Kim et al., 2009).

To analyse the UF adhesives (figure 4), the maximal values of acetone and/or acroleine emission has been found at the second day (54.3 µg/m^2h) and the same tendency was observed for T adhesives (49.8 µg/m^2h). Despite the emission level in TL particleboard, acetone and/or acroleine emission reached higher levels than the others within 24 hours (265.1 µg/m^2h). However, it begins to decrease very quickly until it disappears at the end of the week. At the third day, the emission in decreasing order were 53.9, 39.5 and 29.3 (µg/m^2h) for TL, UF, and T adhesives resins.

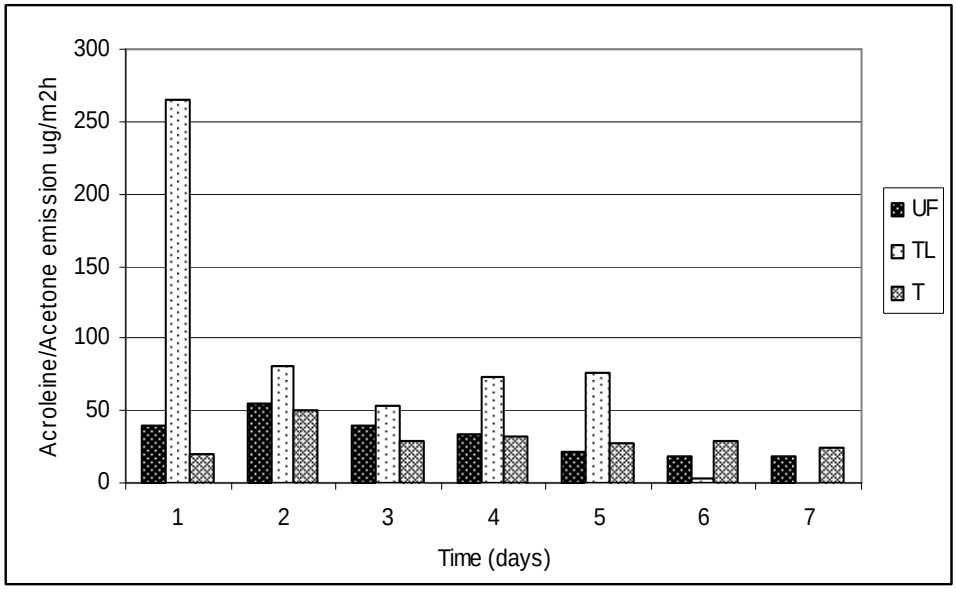


Figure N° 4: Acetone/Acroleine emission from the urea formaldehyde (UF), mimosa tannin and wheat straw lignin and mimosa tannin (TL) particleboards during 7 days by the climpack method.

The acetone is generally recognized as a substance of relatively low toxicity to human and environment (Tunç et al. 1997). It is absorbed through the skin, but lungs and kidneys eliminate the absorbed acetone in a short period of time (Grossel and Bricker, 1990). It can be found in the nature. Sources of acetone released into soil include disposal of agricultural and food wastes. Natural emissions from a variety of tree species contain acetone vapour, animal waste, atmospheric wet deposition, household septic tank effluents and chemical waste disposal sites Acetone is an emission product from the combustion of wood, refuse and plastics. It is also emitted in exhaust from automobile, diesel and turbine engines (OMS, 1998).

The acroleine is one compound with an intensively acrid odor, relatively nonpersistent and does not bioaccumulate in the organism (Pourmiza et al. 2008). It is a volatile obtained from the incomplete combustion or organic matter automobile exhaust, smoking tobacco products and other common sources (EPA, 2009; Canadian environmental protection, 1999), and oil combustion of vegetables or biomass (CEP, 1999). It is formed from carbohydrates, vegetable oils and animal fats, amino acids during heating of foods (Stevens et al., 2008). The EPA integrated risk information system has classified acroleine in group C, i.e. a potential carcinogen (K-D. Kim et al. 2011)**.**

Thus, acetone and acroleine have natural origin since it has been described previously. This could be one reason to find them in the TL adhesive. On another hand, the pressing process at high temperature could produce acroleine and/or acetone which were detected by the method described. Finally, it is possible that an unknown reaction between lignin and glyoxal occurs, which must be studied.

Hexanaldehyde:

In general the hexanaldehyde ($C_6H_{12}O$) emissions detected were very low in all the cases (figure N° 5). The first day, the maximal value was registered by the UF samples reaching 15.7 µg/m^2h. At the third day, in decreasing order the emission were T>TL>UF, with 14.6, 13.7 and 11.7 µg/m^2h, respectively. The hexanaldehyde results obtained here were lower than those obtained by Yrieix et al. (2010). This was expected because the hexanaldehyde is a natural compose that it comes from the wood and not from the adhesives used. On another hand, they used just maritime pine in the particleboard preparation.

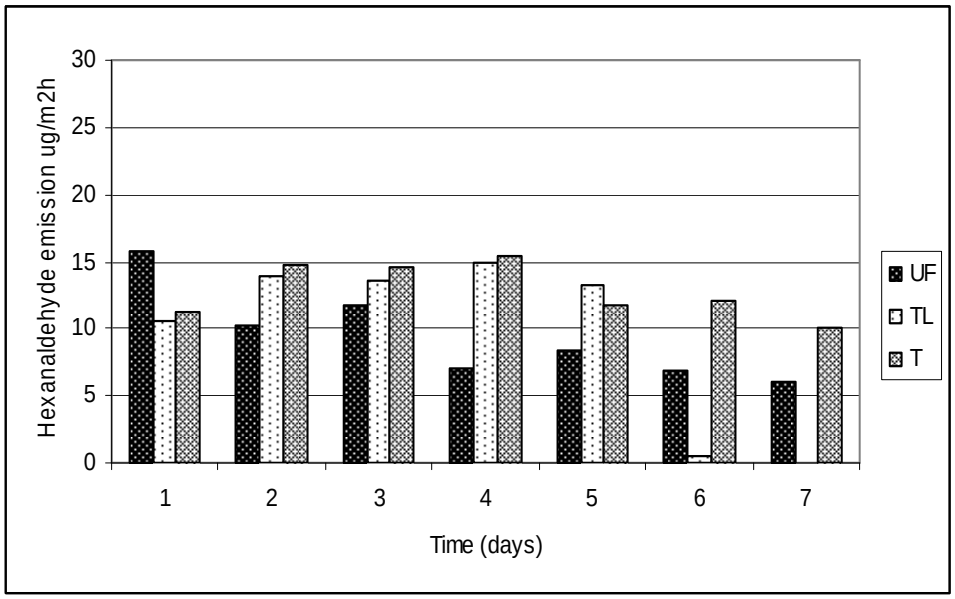


Figure N° 5: Hexanaldehyde emission from the urea formaldehyde (UF), mimosa tannin, and wheat straw lignin and mimosa tannin (TL) particleboards during 7 days by the climpack method.

3.2. Others VOCs emissions

In general, higher emissions were registered during the first days. The principal VOCs registered by the GC-MS methods were the aromatic hydrocarbures gases such as: toluene, ethylbenzene, xylene, and the terpenes: α-pinene, β-pinene, 3-carene and limonene.

The emission begins to stabilize since the fifth day (figure N° 6). Xylene was the highest VOC detected in all the samples, especially in the UF panels. Similar tendencies were found by Kim (2009, 2010) who used green adhesives to decrease the formaldehyde and VOC emissions in wood-based flooring. Toluene and ethylbenzene were compounds with lower emission detected.

Toluene:

The toluene emissions (C_7H_8) in the TL and T adhesives did not undergo big changes during the seven days. The values found were between 11.4-8.2 y 8.5-6.6 μg/m^2h, for TL and T, respectively. Contrarily, in the UF panels, the toluene emission reached a value of 51 μg/m^2h the first day, but it begins to decrease at the second day. At the third day the values registered were 19.4, 10.5 and 8.3 μg/m^2h for UF, TL and T panels. In our work, toluene emissions were lower in the samples with natural adhesives. However, although no

biochemical pathway for the production of toluene is known, the Heiden et al. rechearche (1999) indicated that there are some plants from which toluene is synthesized.

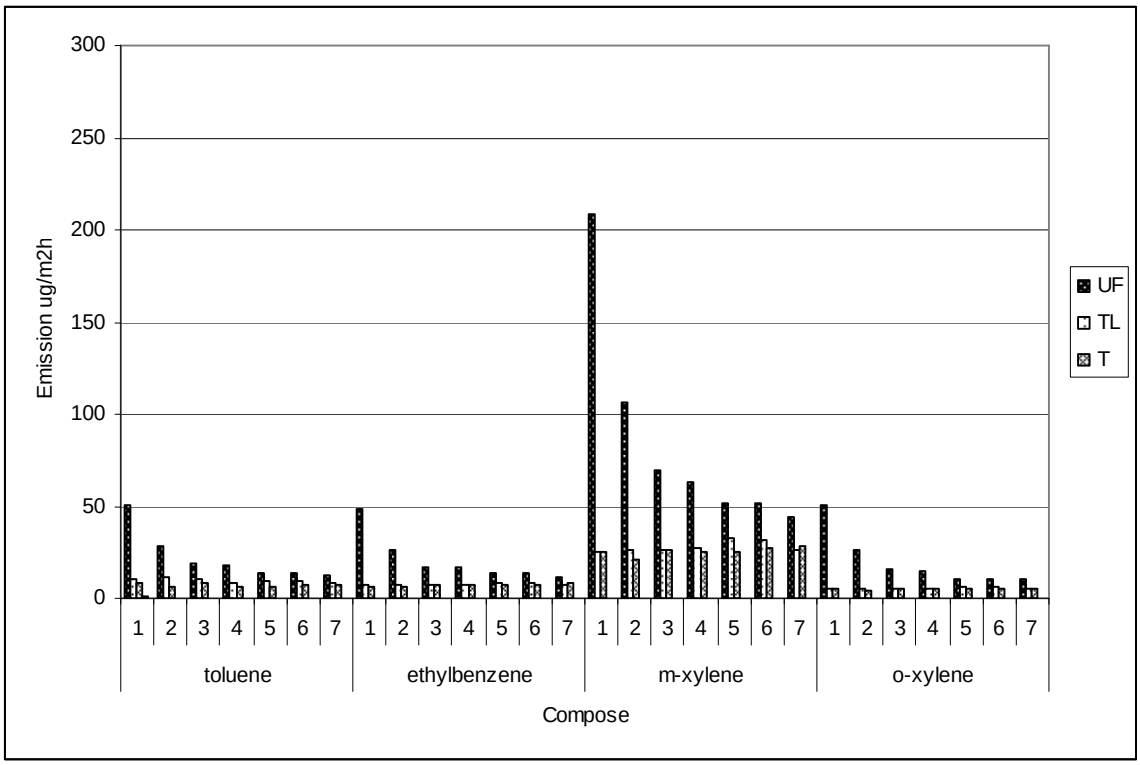


Figure N° 6: Toluene, ethylbenzene, m-xylene and o-xylene emission from particleboards prepared with urea formaldehyde (UF), mimosa tannin and wheat straw lignin (TL), and mimosa tannin (T) during 7 days using the climpack method

Ethylbenzene:

UF adhesive registered the highest emission factor. At first day the ethylbenzene (C_8H_{10}) emissions registered 48.4 µg/m^2h in the UF samples. To compare with the third day, the emission factor reached 17.4 µg/m^2h. In the TL and T samples, the emissions factors were between 7.0-8.6 and 5.9-8.1 µg/m^2h, respectively.

m-o-Xylene:

Highest m-o-xylene [$C_6H_4(CH_3)_2$] emission is detected with high rates at the first day, but it decreases quickly in the UF samples. These values reache 208 and 50.9 µg/m^2h for m-xylene and o-xylene, respectively. The natural adhesives present almost the same behaviour during whole the test time but much lower that the UF samples, being the m-xylene higher rates than o-xylene. At the third day, the UF samples reported emission factors more than 2.5 times higher than the natural adhesives. In the m-xylene case, each adhesive present emissions factor of 69.1, 26.7 and 26.3 µg/m^2h for UF, TL and T, respectively. In the o-xylene

case, the emission rates registered were 16, 5.3-5.7 μg/m^{2}h for UF and the natural adhesives, respectively.

Terpenes: α-pinene, β-pinene, 3-carene and limonene:

Terpenes are always present in particleboards tested (figure N° 7). The emissions were higher when a natural adhesive resin was used, with exception of limonene, which has no change comparing to UF with natural adhesives. The α-pinene shows the highest rates. TL and T samples emissions are between 23.9 and 27.9 μg/m^{2}h, respectively at the first days. After the third day, the emission rates become between 14.8 and 19.7 μg/m^{2}h for each of them. These values were around twice for the UF registered results. UF samples emission were constant during whole the test time, with values around 7-10 μg/m^{2}h.

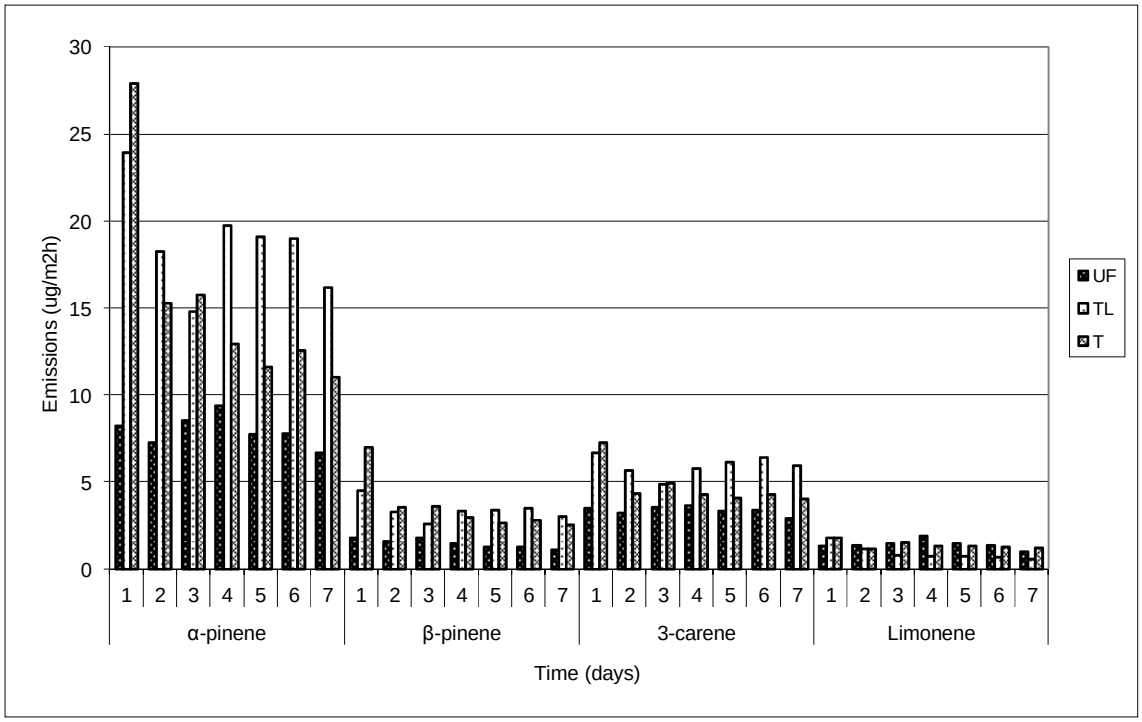


Figure N° 7: Terpenes emission factors from the urea formaldehyde (UF), mimosa tannin and wheat straw lignin and mimosa tannin (TL) particleboards during 7 days in the climpack method.

β-pinene and 3-carene emission factor increase slightly with the natural adhesive use. β-pinene are more than 2.5 higher samples used with natural adhesives than UF at the first day. 4.5 and 7 μg/m^{2}h of β-pinene emission rates were found in TL and T samples, respectively. At the third day it decreases to 2.6 and 3.6 μg/m^{2}h for TL and T, respectively, and it begins to be constant until the end of the test.

3-carene emissions were constant during the whole test duration for TL samples, with values between 6.7-4.9 μg/m^{2}h. T samples registered rates between 4.0-7.0 μg/m^{2}h. The UF samples presented a rate almost constant around 3.4 μg/m^{2}h.

These results were expected. Terpenes are emitted by deciduous and coniferous trees, agricultural crops and grass in various amounts depending on different conditions (Müller et al. 2006). Although, emissions of some terpenes present in the natural material have been detected, the formaldehyde emissions were not influenced by them as it has been observed in other studies (Yrieix et al. 2010).

4. CONCLUSION

Different aldehydes, cetones and others VOCs emitted from particleboards prepared with synthetic and natural adhesives were found. The proportion of these compose vary according to the adhesive. Thus, it is possible to conclude the following:

From aldehydes and cetones, the main carbonyl compounds emitted from UF were in sequential order was formaldehyde>acroleine/cetone>acetaldehyde>hexanaldehyde (240.8>39.5>28.9>11.6 μg/m^{2}h).

In the TL adhesives the situation was different. The higher carbonyl detected was acroleine/cetone, but it begins to decrease quickly. Then the in this case the sequential order for the carbonyl emissions was acroleine/cetone > acetaldehyde > formaldehyde > hexanaldehyde (53.9>46.3>34.4>13.6 μg/m^{2}h).

For T adhesives, the most important compound released was formaldehyde but in much lower proportions than the UF. Thus, the emission levels sequence was formaldehyde > acetaldehyde > acroleine/acetone > hexanaldehyde (65.7>40.8>29.6>14.6 μg/m^{2}h).

From the others VOCs studied, the emission sequence at the third day were m-xylene > toluene > ethylbenzene > o-xylene in all the cases. However the emission levels are particular for UF and the natural adhesives. UF adhesives registered: 69.11>19.4>17.4>16.0 μg/m^{2}h, while TL and T registered in average: 26.6>9.4>7.6>5.6 μg/m^{2}h.

Finally, the terpenes in according to the emission levels registered, were α-pinene > 3-carene > β-pinene. Limonene did not registered changes with the use of natural adhesives.

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IV CONCLUSION

A travers ce travail, les tannins et lignine d'origines diverses ont été étudiées et des formulations de colles intéressants pouvant être utilisées dans la fabrication de panneaux ont été mises au point. Des expériences avec la protéine de gluten dans le but d'augmenter sa réactivité et de trouver une application dans le domaine de la colle ont également été menées.

Du point de vue de l'environnement, on a reporté des différences en termes d'émissions de formaldéhyde, en fonction de la méthode utilisée. D'autres émissions comprenant les composés organiques volatiles interviennent aussi.

Les conclusions de chacun des travaux effectués apparaissent ci-après :

1. Colles pour panneaux de particules à base de tannin.

Dans le tannin de pin maritime on a trouvé des oligomères de proanthocyanidines formés par les catéchine/epicatechine, l'epilogalocatechine et les monomères d'epicatechine galate. La présence d'epigalocatechine galato, des petites proportions d'unités de fisetidine et d'autres structures a été confirmée par les analyses MALDI-TOF. Toutefois, l'existence d'une structure appelée structure D jamais rencontrée auparavant dans le pin a été dévoilée. Celle-ci ne s'apparente pas un à flavonoïde mais sa participation prédomine dans la formation de pin maritime.

Les analyses de ^{13}C-RMN ont montré que le tannin de pin maritime purifié a une réactivité plus importante que le tannin non-purifié.

Les conditions d'humidité dans les particules jouent un rôle important dans la résistance de cohésion interne des panneaux. Ainsi, on a montré que les meilleurs résultats de cohésion interne sont obtenus avec les panneaux fabriqués avec une colle de tannin de pin maritime purifié et de glyoxal avec une importante quantité d'eau.

En ce qui concerne les tannins de pin d'Alep (*P. halepensis*), d'épicea (*P. abies*) et de mélange d'écorce, un réarrangement a été produit, en donnant lieu à la formation d' acide catéchétique et d'acide diaryl propanol catechinique. Cette situation a rendu impossible l'utilisation de ces tannins dans la préparation de colles.

Les tannins de pulpe de raisin paraissent être une autre alternative économiquement et environnementalement viable. Ces tannins ont montré une importante réactivité au

formaldéhyde, et les analyses thermomécaniques ont indiqué un futur prometteur pour son utilisation comme matériel primaire dans la fabrication de colles.

2. Colles pour les panneaux à base de lignine et tannin.

La glyoxalation est une bonne méthode pour augmenter la réactivité de la lignine utilisée pour la préparation de colles pour la fabrication de panneaux. La lignine glyoxalée est riche en groupes methylol lesquels réagissent avec des flavonoïdes plus réactifs comme ceux des tannins.

La lignine de paille de blé obtenue à partir de procès organosolv, la lignine kraft en provenance de l'industrie papetière et la lignine de miscanthus représentent une alternative intéressante pour la préparation de colles naturelles pour l'industrie de panneaux.

On a ainsi trouvé des formulations pour applications dans les panneaux de particules à usages intérieur et extérieur contenant jusque 50% de lignine, et sans ajout d'une quelconque autre colle synthétique.

Le proces de glyoxalation conduit a une série de changements dues aux réactions de réarrangement de la lignine en elle-même, telles des réactions d'autocondensation produit par le chauffage. La réaction avec le glyoxal produit différentes structures qui contiennent un groupe réactif d'hydroxylglyoxyl en abondantes proportions, lesquelles sont disponibles pour la co-réaction avec un flavonoïde de tannin ou une colle de di-isocyanate pour la formation de résine.

La glyoxalation de lignine produit aussi un accroissement de la viscosité accompagné d'une augmentation du temps de réaction, ce qui indique une élévation de la masse molaire à travers la formation de ponts glyoxylene.

Pour être stable dans le temps, la lignine glyoxalée peut être soumise à un séchage spray. A travers cette technique, une série de réactions se produisent (réarrangement, polymérisation, clivage de certains components comme les oligomères de carbohydrates en diminuant sa viscosité, etc.). Il devient ainsi possible de stocker cette lignine glyoxalée sans altérer ses propriétés mécaniques. Ces résultats ont été obtenus par des analyses thermo-mécaniques effectuées sur de paille de blé gyoxalée.

3. Colles à base de protéine de glutens et de tannin pour panneaux de bois.

Les expériences avec le gluten hydrolysé traité avec une solution basique ne permettent pas la réaction avec la colle pMDI à cause de l'absence de groupes methylol.

La protéine de gluten hydrolysée, traitée par une solution basique et ensuite traitée avec une solution de formaline ou de glyoxal puis mélangée avec une colle pMDI en proportions de 20-30% (de la colle totale), a montré les meilleurs résultats de cohésion interne dans les panneaux de particules. Les exigences de la norme internationale ont été respectées avec une préference pour l'utilisation du glyoxal pour l'absence de formaldéhyde dans sa formulation. Par ailleurs, une diminution de pMDI dans la colle engendre une diminution immédiate du coût total de la colle.

Les temps d'opérations pour la fabrication de panneaux de particules ont été réduits pour la formulation de pMDI/gluten hydroxymethylée avec une proportion 30/70.

4. Emissions de formaldéhyde et d'autres COVs à partir de panneaux fabriqués avec des colles naturelles.

L'efficacité des colles vertes est incontestable quant à la réduction des émissions de formaldéhyde par rapport à la colle synthétique. Néanmoins une attention particulière devra être apportée en ce qui concerne le dégagement possible d'autres composés organiques volatiles. Il s'agit en effet de ne pas effectuer un transfert de pollution.

L'identification et la quantification d'autres aldéhydes/cetones différentes du formaldéhyde incitent à comprendre leurs provenances. C'est particulièrement le cas de l'acroléine ou de l'acétone qui apparaissent dans les émissions du panneau à base de lignine de paille de blé. Toutefois, ces deux composés disparaissent rapidement.

Apres trois jours, les résultats d'émissions de formaldéhyde étaient 240> 66 > 34 $\mu g/m^2h$ pour les colles urée formaldéhyde (UF), mimosa tannin (T) et lignine/mimosa tannin (TL), respectivement.

On a trouvé des COV naturels émis qui ne sont pas nocifs pour la santé, en plus faibles taux d'émission que les autres composés, mais toujours présents surtout avec les panneaux fabriqués avec des colles à base de tannin et de lignine. Ainsi, on a trouvé de l'α-pinène: UF:8.5<TL:14.8<T:19.7; du β-pinène: UF:1.8<TL:2.6<T:3.6, et du 3-carène UF:3.6<TL et T: 4.9.

La quantification de formaldéhyde et d'autres COVs dépend fortement de la méthode utilisée. Ainsi, la méthode du bocal reste la plus facile et la plus rapide alors que la méthode de climpack couplée avec HPLC et GC-MS donne des résultats d'une plus grande précision.

V PERSPECTIVES

D'une manière générale, les colles dites naturelles sont très peu utilisées dans le domaine industriel pour plusieurs raisons. Si l'intérêt écologique apporté par ces résines est indubitable, des recherches sont encore nécessaires sur les plans technique et économique avant de passer sereinement à l'étape d'industrialisation. L'utilisation de la ressource de sous-produits d'origine naturelle (comme le tanin ou la lignine) va clairement dans le sens d'un développement durable. Cependant, elle nécessite la mise au point de protocoles spécifiques dans le but d'obtenir des produits qui soient intéressants sur les deux plans en question. La suite de cette partie propose les perspectives qui sont apparues à la suite de ce travail.

Concernant les colles à base de tanin, le glyoxal montre de bonnes caractéristiques en tant que durcisseur sous certaines conditions (humidité, pH,…). La nécessité actuelle est de connaître précisément le mécanisme de réactions de ce composé avec le tanin dans le but de mieux comprendre les interactions entre eux.

Les résultats obtenus avec les résines à base de mélanges tanin de mimosa + lignine de paille de blé glyoxalée sont prometteurs à condition de ne pas dépasser les 50% de lignine. Il serait alors intéressant de mener des essais à partir de matériaux de différentes provenances (tanin de pin maritime, lignine d'eucalyptus ou de pin radiata, par exemple) afin d'élargir la source. L'aspect géographique entre ici en jeu puisque ce potentiel sera largement influencé par les espèces présentes dans telle ou telle région ainsi que par les activités industrielles du site. Dans le cas de la France, par exemple, il semble plus judicieux de développer des résines à partir de tanin de pin maritime étant donnée la répartition territoriale de celui-ci.

Sur le point normatif, les textes concernant les émissions de composés issus des panneaux de bois mettent uniquement en avant les problèmes relatifs au formaldéhyde. Certaines études donnent des résultats sur les composés organiques volatiles totaux (COVT) qu'ils soient d'origine naturelle ou non, nocifs ou non. A l'avenir, il sera alors indispensable d'être capable de faire ces distinctions en connaissant précisément la nature et les caractéristiques des composés émis afin de dresser un bilan révélateur des impacts potentiels sur la santé et/ou l'environnement. L'étude des facteurs influençant ces émissions (température, humidité…) sera également d'un intérêt capital.

RESUME

Lors de cette étude, différentes recherches ont été effectuées sur l'utilisation de divers matériaux naturels comme source de matière premières pour la fabrication de colles vertes pour l'industrie de panneau. Dans ce contexte, le travail a consisté à :
(i) Evaluer le potentiel de différents tannins en provenance de déchets d'écorces et développer des colles à base de tanins avec différents durcisseurs.
(ii) Evaluer le potentiel de lignines de différentes origines et développer des colles à base de tannin de mimosa et de lignine.
(iii) Développer des colles à base de tannin et de protéine de gluten.
(iv) Evaluer les émissions de formaldéhyde et d'autres composés organiques volatiles (COV) à partir de panneaux de particules fabriqués avec les colles naturelles mises au point précédemment.
Les techniques d'analyses thermomécaniques, de spectroscopie CP-MAS ^{13}C NMR, de matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF) et de temps de gel ont été utilisées comme méthodes de travail pour l'évaluation et la caractérisation de ces colles. Les essais de cohésion interne pour l'évaluation de la résistance mécanique de la colle selon la norme EN -312 ont également été effectués.
En ce qui concerne les émissions de formaldéhyde et des autres COV à partir de panneaux contentant des colles vertes, le recours à des techniques normalisées par chromatographie en phase gazeuse associée à un spectromètre de masse (GC-MS) et par chromatographie en phase liquide (HPLC) ont été privilégiées.

Mots clés: Colles, produits naturels, procédés de fabrication, formaldéhyde, composés organiques volatils

ABSTRACT

This study deals with some researches carried out about the use of various natural materials as a source of raw material for the manufacturing of green adhesives for the industry of panel. In this context, work consisted in:
(i) Evaluation of the potential of various tannins coming from barks wastes and development of adhesives containing tannins and various hardeners.
(ii) Evaluation of the lignin potential from various origins and development of adhesives containing tannin from mimosa and lignin.
(iii) Development of adhesives containing tannin and gluten protein.
(iv) Evaluation of formaldehyde and other volatile organic compounds (VOC) from particleboards manufactures with the natural adhesives previously developed.
Thermomechanical analyses, spectroscopy CP-MAS 13C NRM, matrix- assisted laser desorption/ionization mass spectroscometry (MALDI-TOF) and gelling time techniques were performed for evaluation and characterization of these adhesives. Internal bond test for the evaluation of adhesives mechanical resistance according to the standard EN-312 were also carried out.
Concerning formaldehyde and other VOC emissions from panels with green adhesives, the recourse to standardized techniques which are gas chromatography associated with a mass spectrometer (GC-MS) and high performance liquid chromatography (HPLC) were privileged.

Key words: Adhesives, natural products, fabrication process, formaldehyde, volatile organic compounds.