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Starlin Péguy Engozogho Anris

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Par

Starlin Peguy ENGOZOGHO ANRIS

ÉTUDE DES PRODUITS CONNEXES DE LA TRANSFORMATION INDUSTRIELLE DU BOIS D'OKOUME (*Aucouméa Klainéana* Pierre) DU GABON : ANALYSE ET MISE EN ŒUVRE DE NOUVEAUX MATÉRIAUX

Sous la direction du Professeur **Bertrand CHARRIER**
A Mont de Marsan, le 21 février 2020

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« Le meilleur fétiche pour une bonne récolte,
c'est une calebasse de sueur ».
Proverbe Fang du Gabon

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INTRODUCTION GÉNÉRALE

Ce travail de thèse, dont le sujet m'a été proposé par le Pr. Bertrand Charrier et le Dr. Rodrigue Safou Tchiama en relation avec le programme de diversité économique (plan stratégique GABON émergent : Vision 2025 et orientations stratégiques 2011-2016). Il a pour titre **: Etude des produits connexes de la transformation industrielle du bois d'Okoumé (*Aucoumea klaineana* Pierre) du Gabon : Analyse et mise en œuvre de nouveaux matériaux.**

Cette thèse s'est déroulée à l'Université de Pau et des Pays de l'Adour (France) au sein du laboratoire IPREM (Institut des Sciences Analytiques et de Physico-Chimie pour l'Environnement et les Matériaux) et plus précisément au niveau du plateau technique Xylomat, spécialisé dans la mise en œuvre et le développement de matériaux bio-sourcés situé à *Mont de Marsan***,** sous la direction du Professeur des Universités Bertrand Charrier, directeur du réseau Xylomat.

Ce plateau technique, fait partie des six plateaux de la plateforme de recherche appelée Xyloforest qui a été lauréat en janvier 2011 de l'appel à projets Equipex Investissements d'Avenir (ANR-10-EQPX-16).

L'objectif principal de cette démarche consiste à valoriser la biomasse lignocellulosique issue de la forêt gabonaise.

Les travaux réalisés lors de cette thèse s'effectuent dans le cadre de la démarche de développement durable mise en place par le gouvernement gabonais le 06 Novembre 2009 et à l'issue de lquelle a été décidé l'interdiction d'exporter du bois sous forme de grume. Cette décision, l'industrie de la transformation du bois a connu une croissance exponentielle, tant économique qu'industrielle, avec la création de la zone économique spéciale (N'Kok). En revanche, cette décision a eu comme impacts majeurs (i) l'augmentation de la transformation et de l'utilisation locale du bois (ii) l'augmentation du volume de déchets de bois non-valorisé.

En effet, la transformation du bois au niveau du Gabon se limite à la première transformation (65%) ainsi qu'à la deuxième transformation (27%). Ces activités génèrent beaucoup de déchets qui correspondent à 50 % des grumes transformées. 80% de ces déchets sont incinérés à ciel ouvert (Figure 1). Les 20% restant sont utilisés par les petites scieries artisanales et certains ménages.

Figure 1: Incinération à ciel ouvert du bois d'Okoumé (Aucoumea klaineana Pierre): Société Equatoriale de Déroulage (Libreville/Gabon, février 2016)

Ce travail de thèse a deux objectifs principaux :

- Améliorer la connaissance sur les extraits du bois d'Okoumé,
- Mettre en œuvre de nouveaux matériaux à base d'extraits de bois d'Okoumé.

Ces objectifs permettront de pallier non seulement l'absence des stratégies valorisation des déchets de bois, mais aussi celle des déchets plastiques qui restent un fléau pour les pays en voie de développement à l'instar du Gabon.

La première partie de ce travail sera consacrée à l'étude bibliographique. Celle-ci s'interessera au matériau bois, et en particulier à l'essence d'Okoumé utilisée tout au long de nos recherches. Par ailleurs, la composition chimique de la résine et l'environnement économique de cette essence seront également évoqués, sans oublier le taux d'extractibles relatif aux parties non-résiniques du bois d'Okoumé. Pour finir, une brève présentation des travaux antérieurs portant sur la mise au point de composites bois d'Okoumé-polymère sera proposée.

Dans la deuxième partie, nous présenterons le protocole expérimental et les résultats obtenus au cours de cette thèse, sous forme de publications soumises ou acceptées dans différentes revues scientifiques à comité de lecture. Des données complémentaires ainsi que des liens entre les publications ont également été intégrés.

La troisième et dernière partie sera consacrée à la conclusion générale et aux perspectives associées.

PREMIÈRE PARTIE

ÉTUDE BIBLIOGRAPHIQUE

État de l’art sur le bois

I. Le matériau bois

L’arbre est un organisme végétal appartenant à l’embranchement des spermaphytes qui se répartit en deux grandes catégories : les résineux ou conifères appelés gymnosperme et les feuillus appelés angiosperme. Le bois constitue le volume principal de l’arbre et se défini comme étant un amas de plusieurs tissus secondaires résistants. En plus du rôle de conduction et de stockage de réserves, le bois dans l’arbre joue également le rôle de soutien. Par ailleurs, les réserves stockées sont utilisées pour la formation du tronc, des branches et des racines des plantes ligneuses.

II. Anatomie du bois : de l’arbre à la cellule

Le matériau bois est un matériau anisotrope, de structure tridimensionnelle c’est-à-dire que ses caractéristiques physiques, technologiques et mécaniques évoluent selon l’orthotropie considérée (plans ligneux). De plus, le plan ligneux d’une espèce est déterminé grâce aux caractéristiques morphologiques et à la disposition des cellules.

II.A. Observation macroscopique

L’étude de l’anatomie des bois se fait selon trois plans d’orthotropie : LT, LR, TR (longitudinal tangentiel, radial et transversal), perpendiculaires. Ils correspondant aux trois directions d’anisotropie du bois pour la majorité de ses propriétés. La figure 2 indique les trois directions d’anisotropie. Le plan LT correspond au débit sur dosse, le plan LR correspond quant à lui au débit sur quartier et le plan TR correspond à une utilisation dite en bois « de bout ».

Le plan tangentiel est parallèle à l’axe de la tige et tangent aux cernes annuels d’accroissement, le plan transversal est perpendiculaire à l’axe de la tige et le plan radial passe par l’axe de la tige. Ces trois directions, radiale (R), tangentielle (T) et longitudinale (L) représentent les directions anisotropes du bois.

L’arbre se définit macroscopiquement comme étant un ensemble de couches cylindroconiques concentriques présentées de l’intérieur vers l’extérieur du tronc (Figure 2). Au centre de l’arbre se retrouve un tissu spongieux formant une partie importante de l’arbre quand celui-ci est jeune, il s’agit du xylème primaire. Le bois est généralement constitué de l’aubier et du duramen ou bois parfait. L’aubier est la partie fonctionnelle de l’arbre, il renferme les cellules

vivantes et les matières de réserve (amidon). Au fil des années, il se transforme en bois parfait, à la suite de réactions enzymatiques, en passant par la zone de transition que l'on peut distinguer selon les essences, car elle est variable au cours d'un cycle de duraminisation (Bergström, 2000).

Le bois parfait peut avoir une teinte distincte de l'aubier (on parle généralement de couleur foncée) : c'est le cas chez l'Okoumé et le chêne (*Quercus sp*). Parfois, le bois (dit de cœur) ne se duraminise pas et il ressemble à un prolongement de l'aubier tel qu'observable chez le Doussié (*Afzelia africana*) et chez le Sapin (*Abies sp*) (Dirol et Deglise, 2001). De plus, la duraminisation engendre généralement une diminution de l'imprégnabilité mais une augmentation de la résistance aux organismes de dégradation. En d'autre termes, l'aubier est plus imprégnable que le duramen mais présente une plus grande sensibilité face aux organismes de dégradation.

Le cambium, ou assise cambiale intérieure, se défini comme étant une fine couche de cellules méristématiques secondaires qui assurent la croissance de l'arbre. Ce sont des cellules indifférenciées mais qui ont la faculté de se diviser afin de produire vers l'intérieur des cellules du xylème secondaire ou du bois différencié et vers l'extérieur, des cellules de phloème secondaire ou liber. Le phloème joue principalement le rôle de conducteur de la sève élaborée. Par ailleurs, il peut aussi servir de réserve comme pour les parenchymes et de tissu de soutien comme pour les fibres libériennes.

L'écorce est un revêtement périphérique du tronc, des branches et des racines, constituée d'un ensemble de tissus protecteurs qui sont produits par l'assise SUBERO-PHELLODERMIQUE. L'écorce est constituée également de cellules génératrices, méristème secondaire, qui produit du liège ou suber vers l'extérieur, et du phelloderme vers l'intérieur. L'écorce est souvent riche en toxines qui la rend plus protectrice.

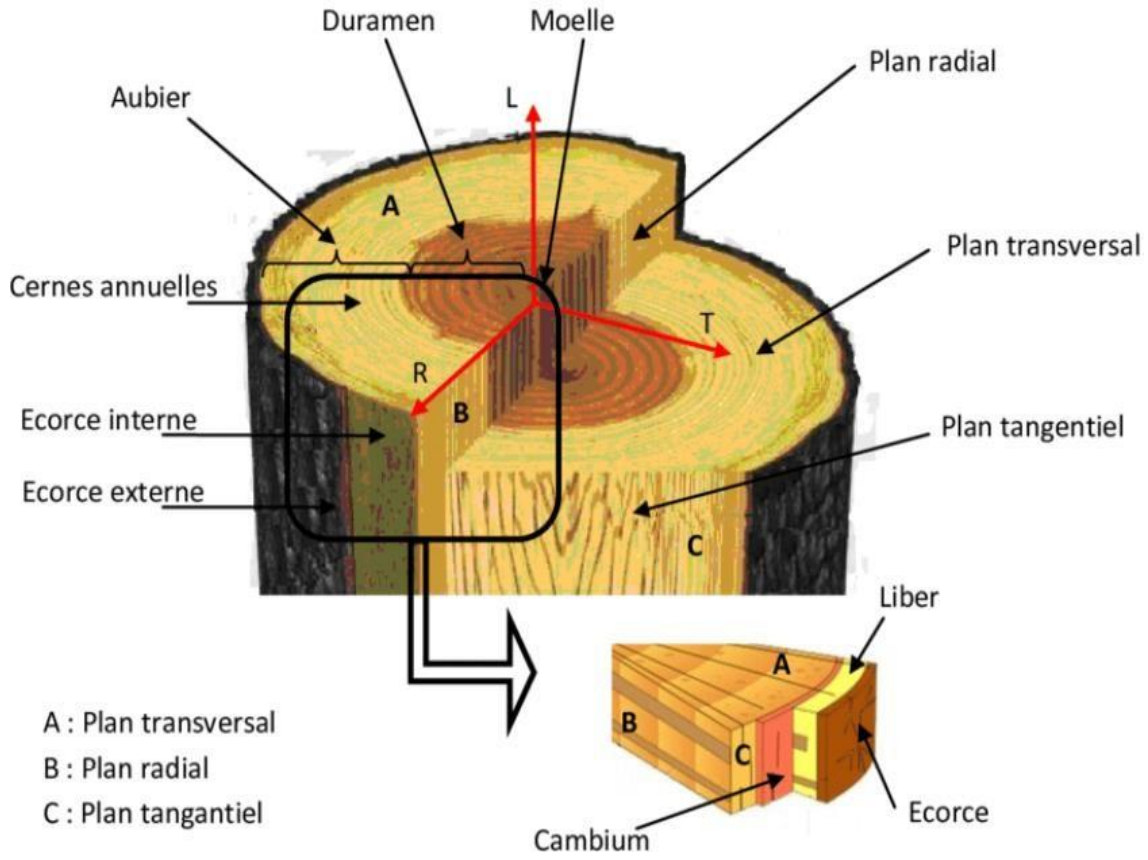

Figure 2: : La structure anatomique du bois et les différentes directions (A) plan transversal, (B) plan radial et (C) plan tangentiel (Candelier, 2013)

II.B. Observation microscopique

II.B.1. Microscope optique : structuration du bois

Les études microscopiques du bois selon les trois plans définis, ont permis d'observer les cellules du bois, leurs caractéristiques intrinsèques et ainsi de distinguer une espèce d'arbre d'une autre : pour une espèce précise, et dans l'ensemble de la structure des branches et du tronc, le plan ligneux reste similaire.

L'observation microscopique en coupe transversale de résineux et feuillus provenant des zones tempérées (Figure 3) réalisée au niveau des accroissements annuels (cernes) montre la présence de deux zones anatomiques distinctes. Ces deux zones caractérisent le bois de printemps ou bois initial et le bois d'été appelé bois final qui se différencient par une variation des dimensions et des épaisseurs des parois des fibres. Le bois initial se caractérise par les trachéides chez les résineux et les vaisseaux chez les feuillus. Les parois cellulaires du bois deviennent en fonction de la saison, soit plus épaisses et dures (fin de l'été) soit plus fines (printemps). En effet, à la fin de

l'été, la conduction de sève se réduit.

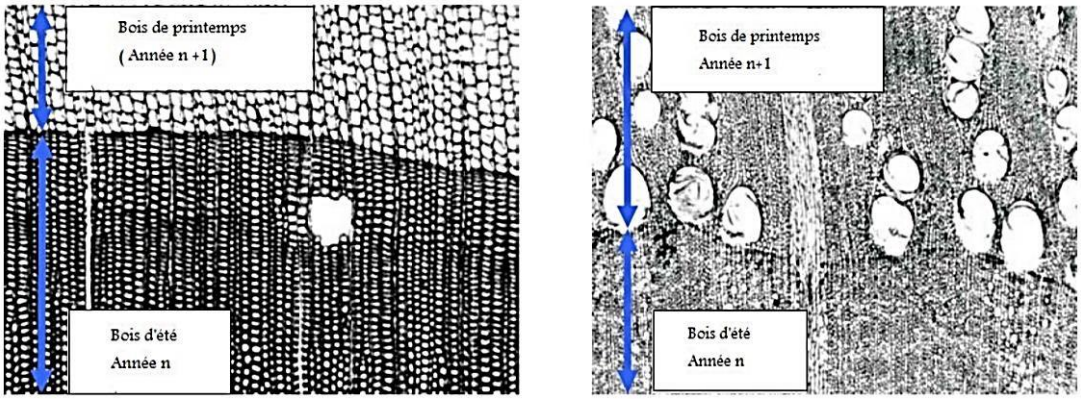

Figure 3: Observation de coupes transversales de bois de chêne (droite) et de Pin sylvestre (gauche) (Pignolet, 2008)

Généralement, les trachéides dans le bois de résineux sont réparties de façon homogène. On peut aussi observer des rayons médullaires et des canaux résinifères axiaux, comme ils ont été décrits et observés par Détienne en 2011 (Figure 4). Selon Détienne, les trachéides sont obliques à leurs extrémités et sont reliées entre elles par des ponctuations aréolées. Les rayons ligneux quant à eux sont des éléments horizontaux associés aux canaux résinifères horizontaux qui communiquent avec les trachéides par les ponctuations aréolées au niveau des champs de croisement.

Les angiospermes (ou bois des feuillus) ont une anatomie plus complexe que celle des gymnospermes. Le bois d'angiosperme a un lumen presque inexistant, des vaisseaux et des fibres très épaisses. Chez les feuillus, les vaisseaux de gros diamètres sont reliés par de nombreuses ponctuations et construisent un réseau complexe vertical avec les fibres (Figure 5). Chez les résineux, le parenchyme axial est inclu dans le réseau ainsi constitué. De plus, les rayons ligneux des angiospermes sont soit constitués d'une simple rangée de cellules soit de plusieurs rangées selon l'essence.

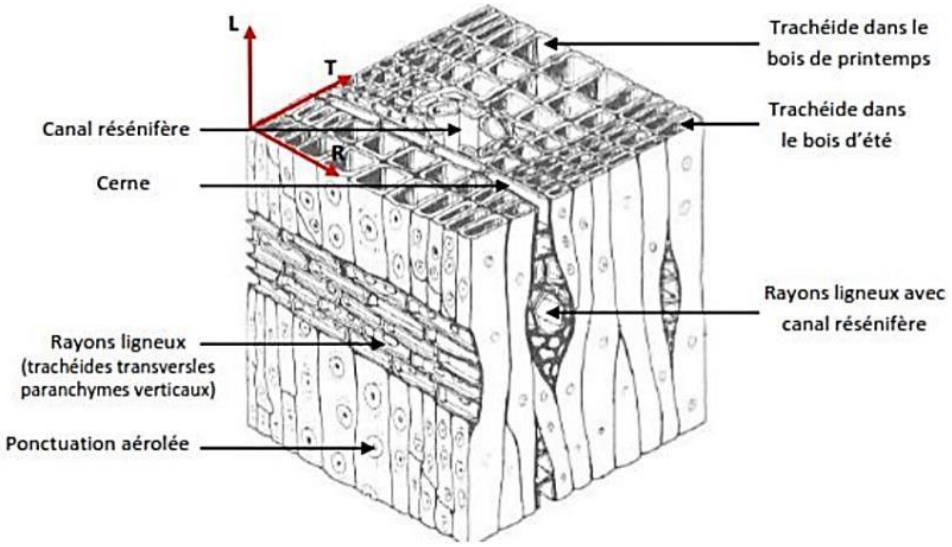

Figure 4: Organisation cellulaire des résineux (Detienne, 2011)

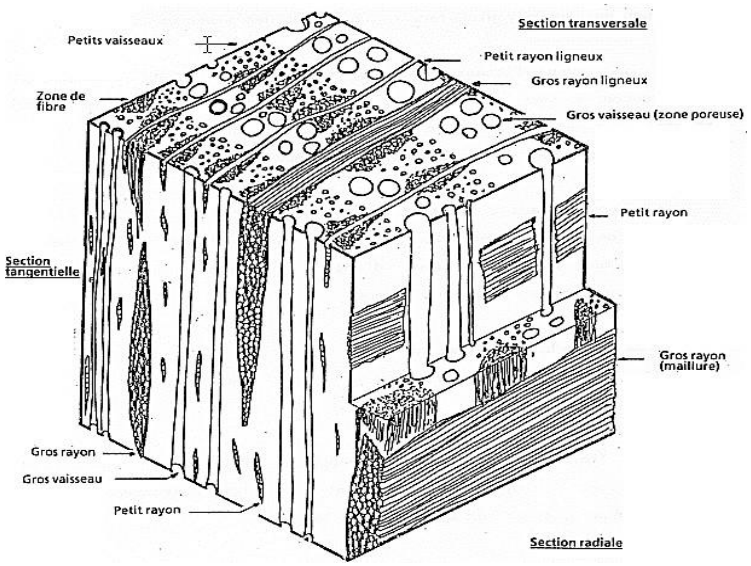

Figure 5: Organisation cellulaire des feuillus (Detienne, 2011)

II.B.2. Microscrope électronique : structure cellulaire des tissus des végétaux

II.B.2.a. Généralité

La microscopie électronique permet la visualisation des démarcations existantes entre les couches composant la paroi cellulaire, ainsi que la lamelle mitoyenne (LM), région ou se joignent deux cellules (Figure 6). La composition chimique et l'orientation des fibres permettent de différencier une paroi d'une autre, formée par diverses couches concentriques (Figure 5). La LM est constituée de lignines et de pectines. Sa teneur en cellulose peut être négligeable.

La paroi cellulaire présente deux sous-divisions : primaire (P) et secondaire (S)

La paroi primaire est une couche mince mesurant entre 0,1 et 0,2 µm (Krezer et *al*., 2002). C'est la première paroi à être achevée lors de la construction de cellule végétale. Son retrait par perte d'eau réduit l'épaisseur de la paroi de 0,03µm (Krezer et *al*., 2002).

La paroi primaire contient un taux de cellulose relativement faible. Elle est constituée de plusieurs couches de microfibrilles de cellulose enchevêtrées dans lesquelles se déposent des lignines, des substances pectiques et des hémicelluloses. La structure dispersée de cette membrane est caractéristique résulterait d'un dépôt de microfibrilles orientées plus ou moins perpendiculairement à l'axe de la cellule (Jodin, 1994).

La paroi secondaire se dépose sur la paroi primaire, c'est une couche d'épaisseur relativement dense et rigide contenant une forte proportion de cellulose. Par sa structure et son volume, elle constitue la partie la plus résistante mécaniquement. Elle présente une structure en couches qui se déposent successivement. Les fibrilles de cellulose s'orientant en une texture parallèle, différente de celle qu'on observe dans la paroi primaire. Comme la paroi primaire, elle comprend des substances pectiques, des hémicelluloses et des lignines qui viennent s'y déposer. Chez les trachéides des résineux et les fibres de feuillus, la paroi secondaire est composée de trois sous couches fibreuses ; la couche S_1 dont l'épaisseur varie de 0,1 à 0,35 µm, la couche S_2 d'épaisseur comprise entre 1 et 10µm et la couche S_3 relativement mince, son épaisseur est de l'ordre de 0,5 à 1,1 µm (Krezer et *al*., 2002).

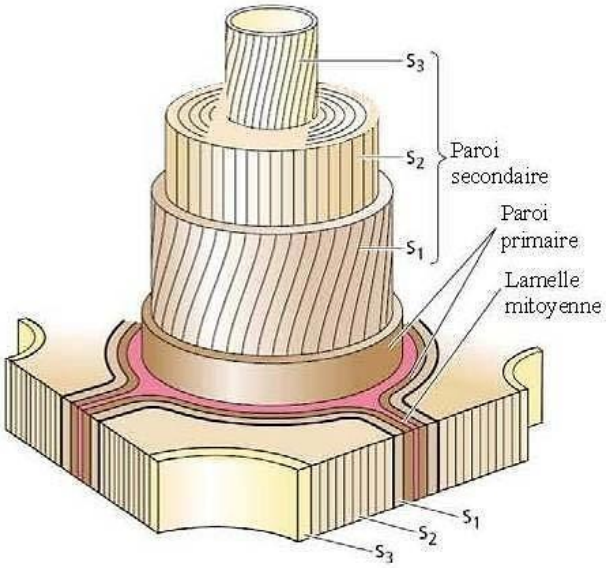

Figure 6: Structure des parois cellulaires (Jaouen, 2007)

II.B.2.b. Structures cellulaires des tissus végétaux des bois tropicaux.

L'appelation « les bois tropicaux » désignent généralement les bois de feuillus ou bois durs. Ils poussent en Afrique, peu en Europe et en Asie.

Compte tenu de cette diversité, il existe de nombreuses études traitant des composants cellulaires des bois des espèces sub-tropicales. Mais, l'essentiel de ces recherches sont réalisées sur les bois tropicaux hors Afrique tropicale, cas des études réalisées par le CIRAD (Centre de coopération internationale en recherche agronomique pour le développement). Par contre, on dénombre quelques données à caractère commercial et technologique faisant mention de la structure anatomique des bois tropicaux d'Afrique c'est le cas des données présentés par l'ATIBT (Association Technique Internationale des Bois Tropicaux) et l'OIBT (Organisation Internationale pour les Bois Tropicaux).

III. Composition chimique du bois

La première classification chimique des polymères constituant le bois et les autres espèces végétales se fait par leur masse moléculaire. On trouve ainsi :

- Des substances de masse moléculaire élevée telles que les hémicelluloses, la cellulose et les lignines présentes en grande quantité dans le bois.
- Des substances de faibles poids moléculaires tels que les extractibles dans lesquels on retrouve les molécules organiques ou des composés minéraux comme le calcium et le magnésium ont des quantités très variables en fonction des essences.

Les macromolécules du bois (cellulose, hémicellulose et lignine) sont majoritaires, soit, 95% de la composition chimique du bois. Chez les essences tropicales, elles représentent 90% et dans certains arbres des zones tempérées, elles représentent plus de 97%. De plus, 96% du carbone présent dans la matière organique vivante est composée de ces macromolécules (Figure 7).

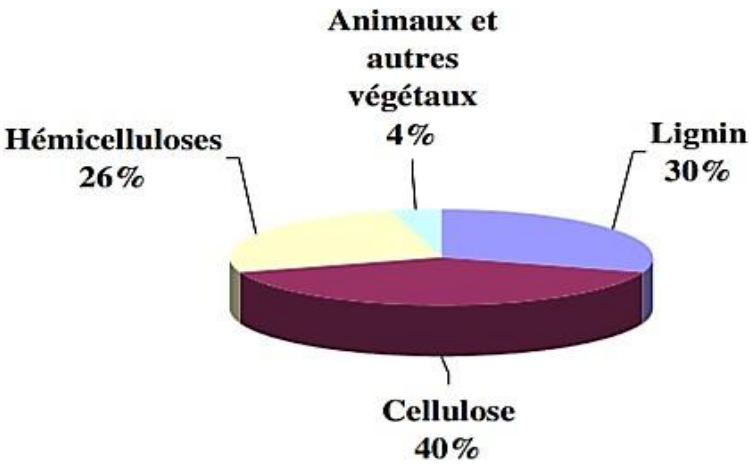

Figure 7:Distribution du carbone dans la matière vivante (Fengel et Wegener, 2011)

III.A. La cellulose

La cellulose est le plus abondant des polymères renouvelables à travers le monde. Des tonnes de celluloses sont produites chaque année par la biomasse soit 50 à 100 milliard (Alfred et *al*., 2003). La cellulose constitue 40 à 50% des parois cellulaires du bois. La Figure 8 montre la structure de la cellulose. C'est un polymère d'unités *β-D-* glucopyranoses liés entre-elles par des liaisons covalentes de type éthers formant ainsi une chaîne polymère entre la fonction hydroxyle (*OH*) du carbone 4 (C_4, position équatoriale) et le carbone 1 (C_1) et dont l'unité répétitive est appelée cellobiose.

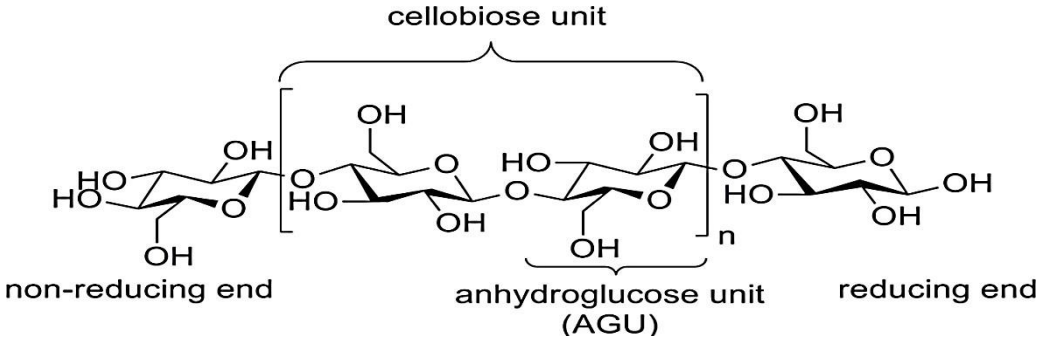

Figure 8: Structure moléculaire de la cellulose (Olsson and Westm, 2013)

Le degré de polymérisation (DP) de la cellulose varie de 5000 à 10 000 et est fonction de l'origine de l'échantillon. De plus, la littérature stipule que la cellulose serait résistante au raccourcissement des fibres dans le processus de broyage pour un DP de 1500. En revanche, pour un DP d'environ ≈750, le raccourcissement des fibres semble être plus facile. La cellulose se présente sous forme d'une maille monoclinique constituée de deux chaînes antiparallèles. La

structure cellulosique schématisée à la Figure 8 montre que la cellulose est constituée de trois parties distinctes : une partie cristalline, une zone amorphe et une fraction de vide (espace vide) qui participent à la pénétration, à la diffusion des solvants et des réactifs à travers les fibres.

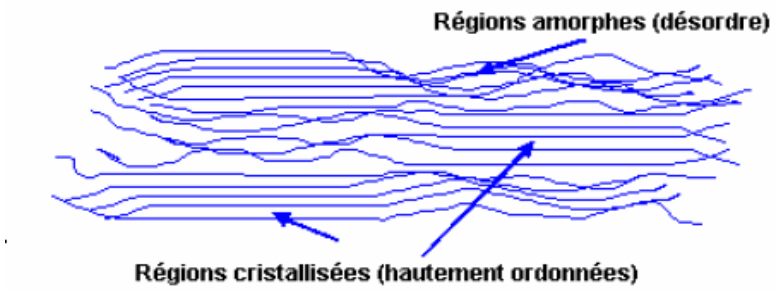

Figure 9: Structure amorphe et cristalline de la cellulose (Davidovic, 2006)

III.B. Les hémicelluloses et les pectines

Les hémicelluloses sont des copolymères de monosaccharides avec des chaînes beaucoup plus courtes, ramifiées, et dont les unités constitutives sont des pentoses, des hexoses, des acides uroniques et des désoxyoses. En moyenne, les hémicelluloses ont un DP (degré de polymérisation) variant entre 200 et 300.

De plus, dans les hémicelluloses, 25 à 40% de ses parois sont constituées de composés amorphes. Au regard de sa structure chimique, on peut distinguer plusieurs monomères de sucres consécutifs dont quatre des plus importants, sont présentés (ci-dessous):

- Les xylanes sont abondamment présents chez les feuillus, tandis que les mannanes sont en grandes proportions chez les résineux. Certains polysaccharides tels que les arabinogalactanes apparaissent en faible quantité dans le bois, soutenant ainsi l'hétérogénéité observée chez les hémicelluloses. Celles-ci sont très réactives, et leur dégradation est généralement rapide lors de la pyrolyse douce du bois (Boiron, 2006 ; Williams et *al*., 1996).

- Les glucanes et les galactanes : ce sont des polysaccharides de faible teneur par rapport aux glycomannanes et aux xylanes. Des espèces telles que *Pinus*, *Acer*, *Fagus* et *Betula* ont en particulier des taux de galactane variant de 0.5 à 3% (Jodin, 1994).

- Les homogalacturonanes représentent plus de 60% des pectines (localisées dans les tissus jeunes et dans les parois primaires). Ce sont des polymères d'acide *α-D*-galacturonique liés en (1→4). La Figure 10 montre deux exemples de chaînes d'hémicellulose.

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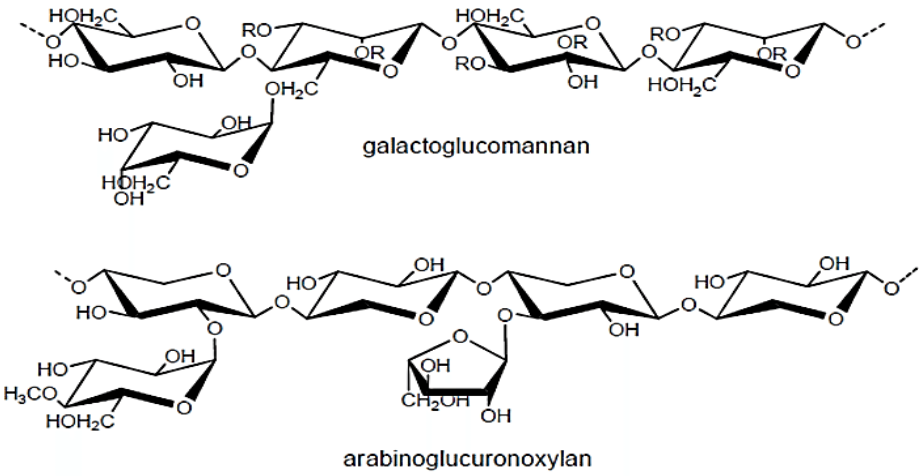


Figure 10: Structure de deux hémicelluloses les plus courantes trouvées dans les résineux (Brandt et al., 2013)

III.C. Les lignines

Les lignines représentent entre 15 à 35% de la masse sèche des matériaux lignocellulosiques selon l'essence : c'est le deuxième polymère le plus abondant sur terre après la cellulose. Les alcools *p*-coumarylique, coniférylique et sinapylique (Figure 11) sont les trois précurseurs phénoliques de la lignine. Les lignines sont biosynthétisées par polymérisation oxydative à partir de ces trois précurseurs, les fonctions alcools phénoliques et allyliques étant à la base de leur réactivité. La polymérisation de ces trois alcools engendre trois types d'unités dans la lignine : G pour guaiacyle, H pour *p*-hydroxyphényle et S pour syringyle. Les études de répartition de la lignine et la nature des monolignols dominants des parois cellulaires, ont montré que les lignines se trouvent abondamment au niveau de la lamelle mitoyenne. De plus, la rigidité du matériau bois est fortement liée à la forte réticulation de la lignine.

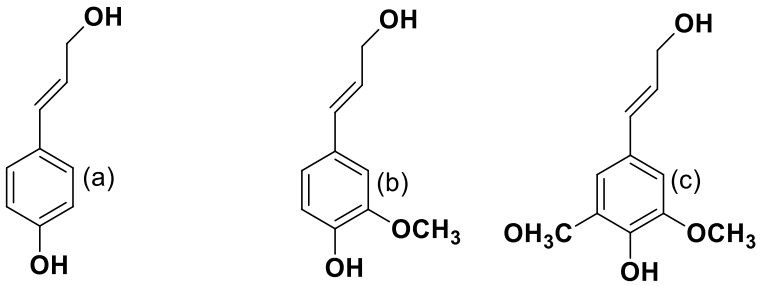


Figure 11: Unités précurseurs de la biosynthèse des lignines : a= alcool coumarylique ; b= alcool coniférylique et c= alcool sinapylique

Les lignines ont une structure chimique qui ne peut être déterminée précisément, elle est très complexe car les unités constitutives sont liées entre-elles de manière désordonnée par de

nombreux types de liaisons. De plus, les liaisons [autres constituants du bois] lignine sont de type benzyléther ou benzylester ce qui, à l'évidence rend leur analyse difficile. La lignine des bois durs est composée d'unités S et G, tandis que celle des bois tendres d'unités G. Les feuillus et les résineux ont des proportions d'unités H très variables. La proportion d'unités H chez les feuillus varierait de 1 à 6% (Camarero et al., 1999) tandis qu'elle atteindrait des taux légèrement plus élevés chez les résineux (Bocchini et al., 1997; Lapierre et al., 1988). Chez les feuillus, la lignine est plus facile à solubiliser car elle présente moins de liaisons hydrogènes et plus de groupements methoxy.

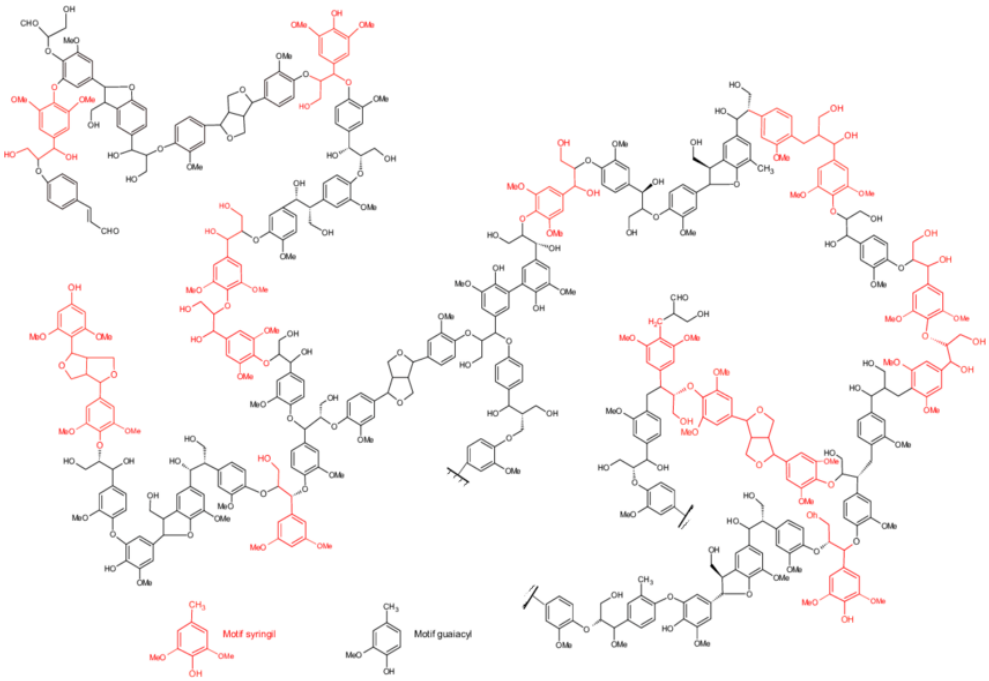


Figure 12: Exemple de structure de la lignine (Candelier, 2013)

III.D. Les extractibles et autres substances de faibles poids moléculaires

Les extractibles sont des composés de faibles poids moléculaires qui ne sont pas intégrés dans la paroi cellulaire du bois mais plutôt dans le duramen. Selon la nature de l'essence, ils peuvent représenter jusqu'à 10% de la masse sèche du bois, et peuvent être extraits par des solvants polaires et apolaires, notamment l'acétone, l'eau, l'éthanol, le toluène, le cyclohexane et le dichlorométhane. Comparativement, le taux d'extractibles contenu dans le bois de feuillus des zones tempérées est moins important que celui provenant des résineux (Fengel and Wegener, 2011). Par ailleurs, les bois tropicaux ont quant à eux un taux d'extractibles beaucoup plus élevé, soit environ 13% de la masse sèche (Bhat et al., 2005; Thulasidas et Bhat, 2007). Les extractibles jouent un rôle important dans le bois : ils sont responsables de son odeur caractéristique, de sa couleur, de sa durabilité sans oublier ses propriétés esthétiques et mécaniques (Aloui et al., 2004;

Amusant et al., 2007). Les travaux de Venäläinen et al., (2004) ont montré que pour certaines essences, les extractibles, malgré leur pourcentage relativement faible, jouent un rôle toxique et répulsif contre les attaques fongiques, bactériennes et d'insectes. Il existe différents types d'extractibles appartenant à des familles chimiques diverses. Les trois grandes familles d'extractibles sont :

- Les terpénoïdes, dans lesquels on retrouve les monoterpènes C10, les sesquiterpènes C15, les diterpènes C20, les sesterpènes C25 et les triterpènes C30 (Figure 13)

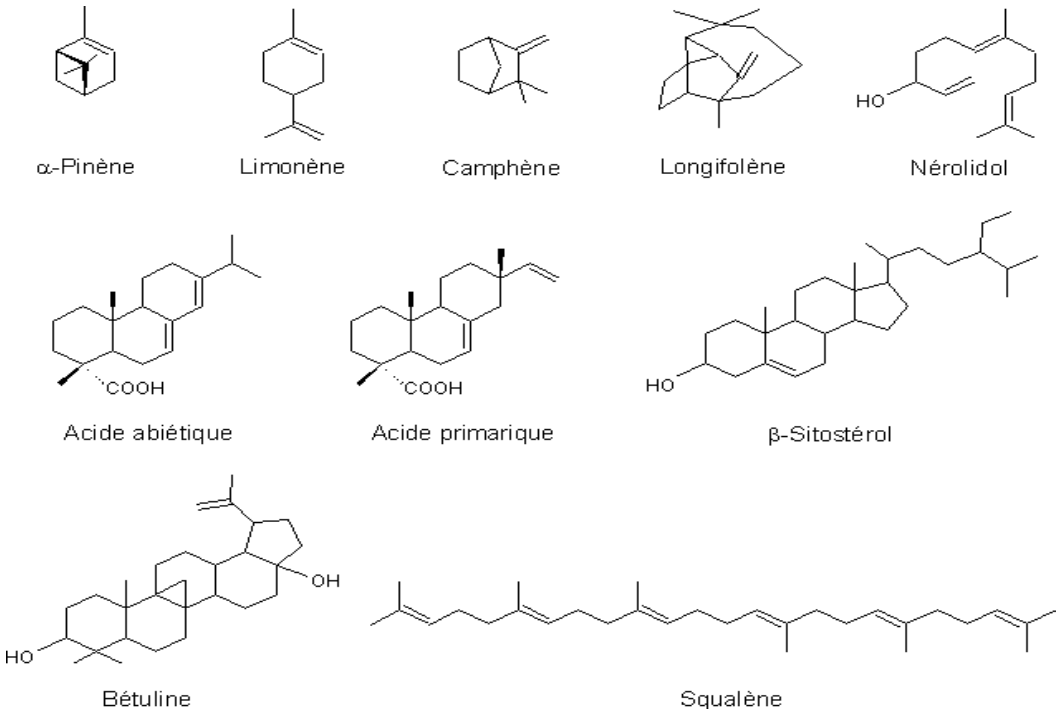


Figure 13: Terpènes et terpénoïdes isolés des bois de résineux et de feuillus (Candelier, 2013)

- Les composés phénoliques et dérivés, qui regroupent, les phénols simples, les tanins hydrolysables et condensés, les flavonoïdes, les stilbènes et les quinones (Figure 14)

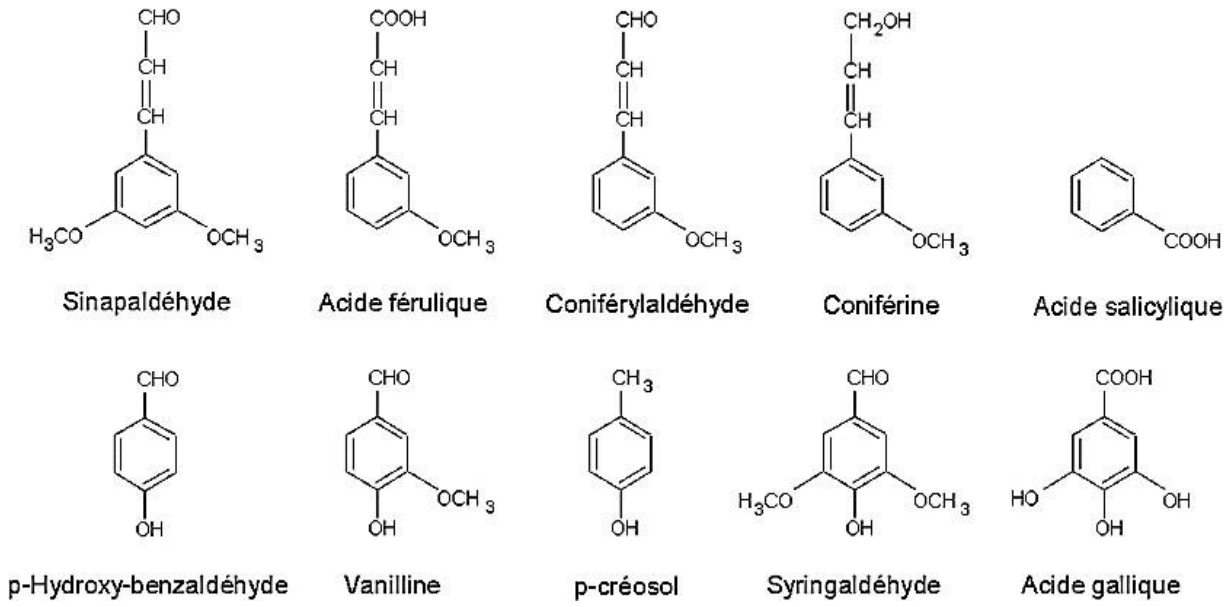


Figure 14: Les composés simples isolés de bois feuillus et de résineux (Candelier, 2013)

- Les composés aliphatiques ou cires et graisses dont l'extraction réduit la durabilité naturelle du bois (Tchinda, 2015). Dans les composés aliphatiques on retrouve généralement, les acides gras saturés et insaturés (triglycérides et diglycérides), les alcanes.

Nous allons nous intéresser plus précisément à trois gammes d'extractibles à savoir : les flavonoïdes, tanins condensés et hydrolysables.

III.D.1. Les flavonoïdes

Les flavonoïdes sont des composés constitués d'une structure tricyclique appelée structure flavane (Figure 15). Il a été montré que les flavonoïdes peuvent se comporter comme étant des antioxydants présents dans beaucoup de plantes en agissant comme récepteurs de radicaux libres, et qui ainsi peuvent protéger des réactions d'oxydations qui ont lieu dans le corps humain (Routray and Orsat, 2012)

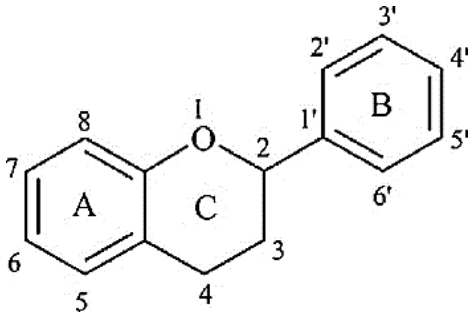


Figure 15: Structure chimique de base du noyau flavane (Ferreira et al., 2015)

III.D.2. Les tanins

Les tanins sont des substances végétales de la famille des polyphénols, les plus souvent hydrosolubles, d'origine végétale à saveur astringente et ayant en commun la propriété de tanner la peau. La masse molaire des tanins se situe entre 500 et 3000g/mol (Bate-Smith, 1973). De plus, à partir de leur solution aqueuse, les tanins ont la capacité de précipiter les protéines, les dérivés d'acide aminé et des polysaccharides (Ozcan et al., 2014). Les tanins protègent les plantes contre les agressions externes en particulier dans l'écorce de certains arbres qui en contiennent une quantité importante. Il existe trois grandes familles de tanins : les proanthocyanidines, les gallotanins et les phlorotanins. Les tanins hydrolysables sont un type de tanin que l'on retrouve dans les bois de chêne et de chataignier mais également les algues brunes et les diatomées. Ces composés sont des oligomères de phloroglucinol (Pavia et al., 1997).

III.D.2.a. Tanins hydrolysables (gallotanins et ellagitanins)

Polyesters de glucides et d'acides phénoliques on les retrouve dans les espèces telles que le tara, le sumac, le castanopsis cuspidata, l'érable à sucre, le bois de chêne et dans les fruits comme les fraises. Les tanins hydrolysables s'hydrolysent facilement en ose et en acides phénoliques par les acides et les enzymes (tannase) mais également par la chaleur (Charrier et al., 1992). Cependant, on distingue deux types de tanins hydrolysables selon la nature de l'acide phénolique : gallotanins, qui libèrent du glucose, de l'acide gallique tandis que les ellagitanins libèrent du glucose, de l'acide hexahydroxydiphénique (HHDP), l'acide ellagique et l'acide chébullique (Figures 16-17).

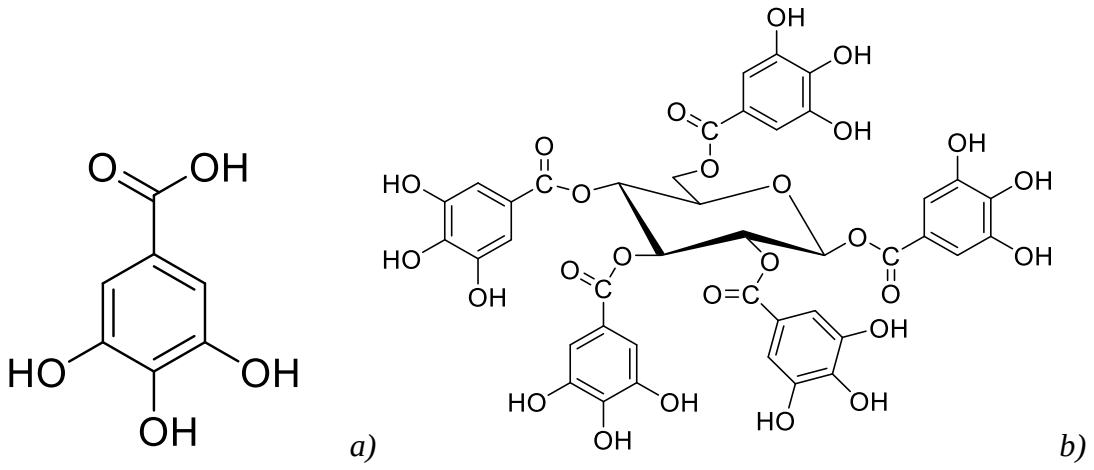


Figure 16: Structures chimiques des gallotanins (a=acide gallique ; b= penta-O-galloyglucose) (Nenonene, 2009)

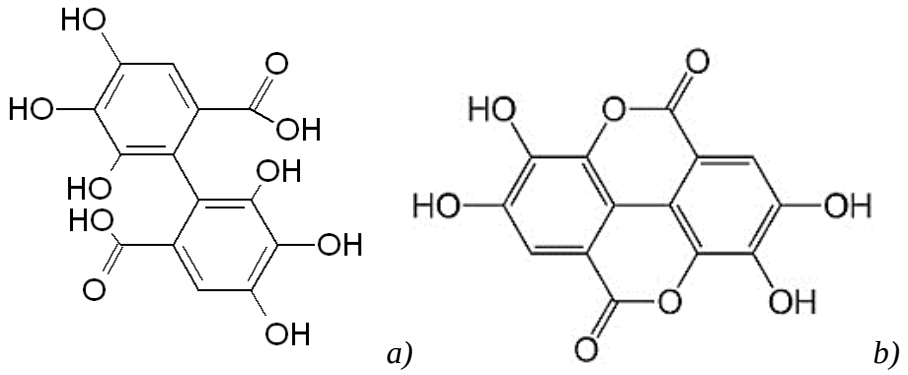
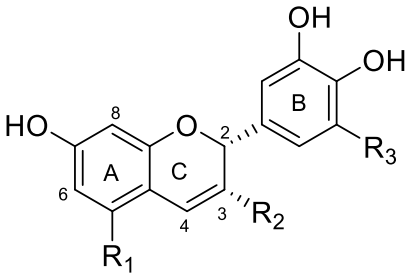


Figure 17: Structures chimiques des unités constitutives des ellagitanins (a= HHDP ; b=acide ellagique)

III.D.2.b. Tanins condensés ou proanthocyanidines

L'unité fondamentale des proanthocyanidines est le noyau flavane. Les proanthocyanidines sont des polyphénols présents dans toutes les plantes, et qui différent des tanins hydrolysables par leur structure qui est très voisine de celle observée chez les flavonoïdes. De plus, les proanthocyanidines n'ont quasiment pas de partie osidique, leurs polymérisations en milieu fortement acide et à chaud donnent des précipités insolubles rouges bruns typiques des phlobaphènes.

Ce sont des oligomères de 2 à plusieurs unités de flavan-3-ols liés par des liaisons interflavonoïdes spécifiques à chaque espèce. Le plus souvent en C4-C8 ou rarement en C4-C6 (Figure 18)



R_1	R_3	Class
OH	H	Proanthocyanidin
OH	OH	Prodelphinidin
H	H	Profisetinidin
H	OH	Prorobinetinidin

Figure 18: Unité de base des tanins condensés (Schofield et al., 2001)

III.D.2.c. Propriétés et utilisation des tanins

En pharmacologie, l'une des premières propriétés connues des tanins est l'astringence. En effet, par voie externe les tanins sont protecteurs de la peau, ils précipitent généralement les glycoprotéines et présentent une activité cicatrisante. Par ailleurs, on peut également les utiliser par voie interne pour leurs propriétés anti-diarrhéiques. D'autres propriétés sont connues des tanins : les propriétés antibactériennes, antifongiques et antivirales. Certains tanins constituent d'excellents compléments alimentaires pour l'homme et pour les animaux : ils présentent des propriétés anti-oxydantes, anti-radicaux libres et anti inflammatoires (Maimoona et al., 2011).

La croissance de bactéries et des champignons peut être inhibée grâce à la fabrication d'hydrogels à base de tanins et d'acides tanniques (Mhessn et al., 2011)

Les tanins trouvent aussi leur utilisation en œnologie. La propriété astringente des tanins condensés conduit en effet à la sensation de sécheresse et de rugosité du palais. Ils participent aussi à la stabilisation de la couleur du vin. De plus, lors de la vinification, les tanins hydrolysables extraits des tonneaux ou ajoutés permettent d'éliminer les protéines indésirables (Sarneckis et al., 2006).

Au cours de ces dernières années, les tanins condensés sont de plus en plus utilisés dans la fabrication de mousses rigides isolantes et dans la fabrication des colles biosourcées (Chupin, 2014; Fuentes, 2011; Santiago-Medina, 2017). D'autre part, les tanins ont la capacité de piéger les métaux lourds tels que le fer, le plomb, le zinc et le cuivre pour former des complexes organométalliques.

III.E. Les cendres

En plus des extractibles, des substances minérales sont également présentes dans le bois. Les minéraux dont l'abondance relative est la plus élevé sont : le potassium, le calcium, le magnésium, le phosphore mais aussi le fer et le manganèse. De plus, la teneur en composés minéraux, n'excède pas 1% de la masse sèche du bois.

IV. Extraction et caractérisation des tanins

IV.A. Méthodes d'extraction des tanins

IV.A.1. Extraction à l'eau chaude

C'est une méthode simple, peu couteuse et qui n'utilise pas de solvant organique, ce qui explique pourquoi elle est beaucoup employée par les industries (Ciesla, 1998 ; Myers, 1998). Les

extractions à l'eau chaude sont très souvent utilisées pour mettre en évidence les propriétés anti-oxydantes et collantes des tanins condensés (Diouf et al., 2009; Jorge et al., 2002). On utilise pour cette méthode des sels tels que le carbonate de sodium, le sulfite de sodium et le bisulfite de sodium. En effet, ces sels sont ajoutés dans la solution d'extraction afin de solubiliser les tanins et empêcher la formation de phlobaphènes (Sealy-Fisher and Pizzi, 1992 ; Panamgama, 2007).

IV.A.2. Extraction au soxhlet

Souvent citée comme extraction témoin dans la littérature, l'extraction au soxhlet est une méthode conventionnelle permettant d'extraire des végétaux, les acides gras, les lipides, les extractibles et la lignine en fonction du solvant utilisé. Cette méthode a le mérite d'être très efficace, simple et peu coûteuse. Mais elle demande toutefois une grande quantité de solvant, et, du fait de la température élevée du solvant, les substances extraites peuvent être dégradées par la chaleur.

IV.A.3. Extraction assistée par micro-ondes (EAM)

Cette méthode est rarement utilisée pour l'extraction des tanins condensés, elle est principalement employée pour la dépollution des sols. L'EAM des matériaux organiques s'emploie essentiellement dans le but d'extraire les polyphénols simples et les flavonoïdes (Routray et Orsat, 2012 ; Jain et al., 2015). Les coûts de l'investissement sont plus élevés que ceux de l'extraction conventionnelle. Les matières thermosensibles peuvent être dégradées. A la fin de l'extraction, une étape de séparation (filtration) est nécessaire pour séparer la matrice du milieu d'extraction.

IV.A.4. Extraction par fluide supercritique

Beaucoup de composés bioactifs sont très souvent extraits par fluide supercritique. Le solvant le plus communément utilisé est le CO_2 supercritique du fait de sa facilité d'obtention, de sa faible température critique (304,1K) et de sa pression modérée (7, 28MPa). L'extraction se fait en deux étapes : (i) extraction du soluté avec le fluide supercritique maintenu au-dessus de ses points critiques, (ii) séparation du fluide supercritique du soluté. En effet, la récupération du fluide à l'état gazeux se fait en réduisant la pression ou en variant la température. Le fluide supercritique perd alors son pouvoir de dissolution et le produit extrait précipite. Cette méthode a pour avantage de n'utiliser aucun solvant toxique résiduel, et présente la possibilité de varier la température et la pression. De plus, elle est plus rapide et la température est faible ce qui permet de récupérer les matières thermosensibles. Par contre, les coûts d'investissements sont très élevés (Figure19).

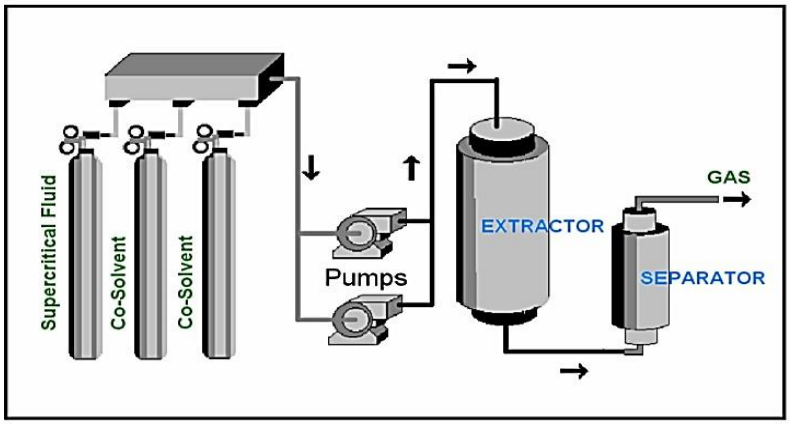


Figure 19: Schéma d'une extraction par lots de fluide supercritique (Mohameda and Mansoorib, 2002)

IV.A.5. Extraction par liquide pressurisé

Cette méthode d'extraction est basée sur l'utilisation de l'eau comme solvant à des préssions et témpératures élévées, généralement dans des conditions sous-critiues, c'est-à-dire entre son point d'ébullition atmosphérique (100°C, 0,1 MPa) et son point critique (374°C, 22,1 MPa) (De Hoyos-Martinez et *al.*,2019). Cette méthode a l'avantage d'être très rapide et les solvants peuvent être portés à haute température et à pression élevée. En effet, la viscosité du solvant se verra diminuer lorsqu'on augmente la température, ce qui occasionnera une augmentation de la diffusivité de ce solvant dans la matière à extraire. De plus, les fortes pressions appliquées permettent au solvant de pénétrer plus facilement. Cette méthode a l'avantage d'être plus rapide qu'une méthode conventionnelle. Et pour éviter toute réaction d'oxydation, l'extraction par liquide pressurisé se fait sous azote et à l'abri de la lumière (Figure 20)

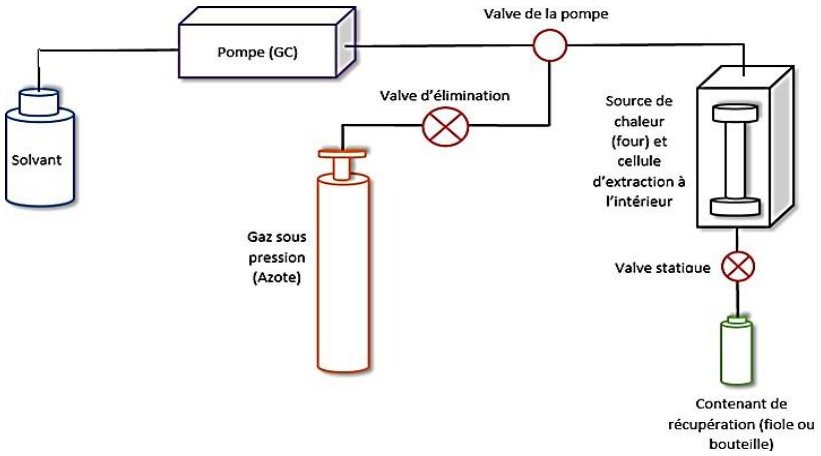


Figure 20: Schéma du principe d'une extraction par liquide pressurisé (Ramos et al., 2002)

IV.B. Méthodes de caractérisation

IV.B.1. Spectroscopie de masse MALDI-TOF

C'est une technique très utilisée au cours de ces dernières années. En effet, la spectroscopie de masse de type Matrix Assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF) a été introduite depuis 1987 par Karas Hillenkamp. Cette technique permet d'évaluer la masse moléculaire avec précision. Introduite pour la caractérisation des flavonoïdes et des polymères taniques en 2001 par Pasch (Pasch et al., 2001), elle permet de fournir un spectre de masse bien complet pour une gamme de masses illimitée à partir de peu d'échantillon pour un coût en équipement relativement faible (Figure 21).

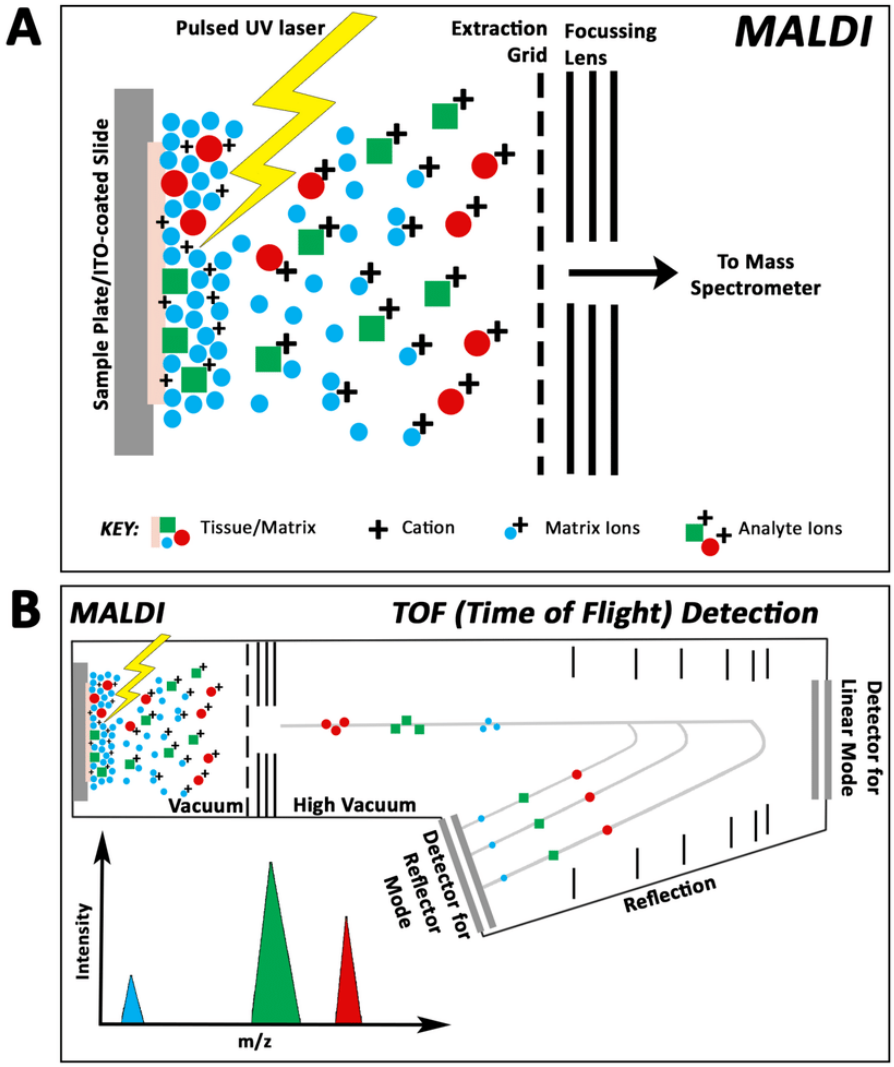


Figure 21: Principe de fonctionnement d'un appareil MALDI-TOF (Lakshini, 2016)

IV.B.2. Chromatographie Liquide Haute Performance (HPLC)

C'est une méthode analytique de séparation ou de préparation de molécules d'un mélange fréquemment utilisée en chimie analytique et en biochimie. Elle a été utilisée pour la caractérisation des proanthocyanidines en 1999 par Cheynier (Cheynier et al., 1999) en se basant sur leur poids moléculaires sans dérivation. Bien que cette méthode convienne dans la plupart des cas, elle peut néanmoins nécessiter des adaptations en fonction de la nature des tanins à extraire et de la présence d'autres constituants de la plante (Figure 22).

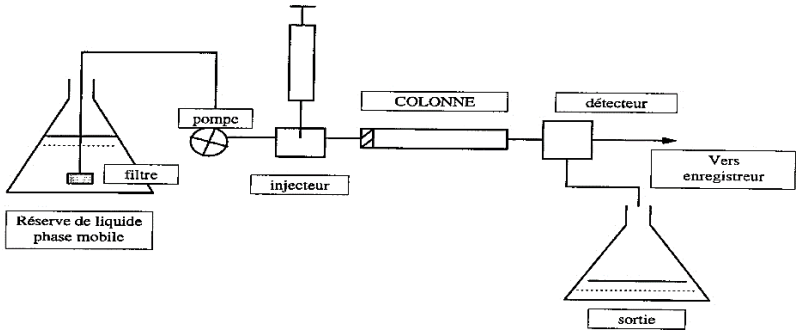


Figure 22: Principe de fonctionnement de la HPLC

IV.B.3. Spectroscopie d'absorption Ultraviolet-Visible (UV-Vis)

Utilisée pour la caractérisation des tanins en 1953, puis en 1997 (Kirby et al., 1953; Muralidharan, 1997), la spectroscopie d'absorption UV-Vis, permet d'identifier globalement la famille d'un tanin en comparant les longueurs d'ondes d'absorption.

IV.B.4. Spectroscopie Infrarouge à Transformée de Fourier (FTIR)

La FTIR est une technique d'analyse qui permet de déterminer la nature des fonctions présentes dans une molécule ou un ensemble de molécules en fonction de la longueur d'onde absorbée dans la gamme de l'infrarouge. Elle permet de qualifier un tanin donné (Nakagawa and Sugita, 1999). Dans une analyse par spectroscopie infrarouge à transformée de Fourier « Fourier Tranform Infrared Spectroscopy », on obtient des gammes spectrales montrant des vibrations moléculaires appartenant à un composé ou une structure chimique bien précise. En effet, la FTIR permet de modifier ou caractériser des matériaux organiques comme les polymères, les lubrifiants, les agents adhésifs et nettoyants. Elle peut aussi être utilisée pour identifier un nombre limite de composés inorganiques (Griffiths and Hesseth, 2007).

Présentation de l'espèce utilisée dans le cadre de l'étude : l'Okoumé (*Aucoumea klaineana* Pierre)

I. Généralités

C'est une essence longévive à croissance rapide dont la durée de vie est estimée entre 150 et 200 ans. En général, la croissance radiale moyenne d'un arbre d'Okoumé est de 10mm/an avec un tronc pouvant atteindre 2m de diamètre au-dessus des nervures saillantes situées à la base du fût au dessus du niveau du sol (Brunck et al., 1990) et dépasser 50 m de hauteur (Brunck et al., 1990). Dans les peuplements dominés par l'Okoumé, il existe une forte compétition pour la lumière. On estime que celle-ci débute à l'âge de 15 ans (Brunck et al., 1990).

L'Okoumé est une essence d'Afrique tropicale, appartenant à la famille des BURSERACEAE. Elle est principalement localisée en Guinée Equatoriale, au Gabon et au Congo. Il y a quelques petits peuplements naturels au sud du Cameroun. Cette essence de nom scientifique *Aucoumea klaineana* Pierre est commercialisée sous le nom Okoumé (nomenclature ATIBT). Par sa diversité de localisation, plusieurs appellations caractérisent cette essence. Au Gabon, on la connait sous le nom Angouma (Fang), Moukoumi (Eschira) et N'koumi (Bavili) ; et au Congo par N'kumi (Brunck et al., 1990). Signalons également que les anglo-saxons dénomment cet arbre « Gaboon », certainement pour signaler la spécificité gabonaise en matière de bois.

II. Ecologie de l'Okoumé

Du point de vue écologique, l'Okoumé est une espèce de lumière dont le comportement et le dynamisme sont ceux des espèces pionnières (Maley, 2001). Son écologie présente cependant certaines particularités qui semblent l'empêcher de pousser ailleurs qu'en Afrique Centrale (Brunck et al., 1990), bien que de petites parcelles expérimentales aient été plantées au Congo, en République Démocratique du Congo, au Ghana, à Madagascar, en Indonésie, en Malaisie, au Surinam et en Guyane française. L'Okoumé a la faculté d'améliorer la fertilité de son support grâce à l'humus issu de sa litière essentiellement composée de feuilles riches en matières organiques. Cette amélioration de son sol sur terrain pauvre favorise la réputation de plasticité édaphique de

cette essence (Figure 23).

Figure 23: Souche d'Okoumé survivant grâce à des anastomoses racinaires avec l'arbre voisin (Doumenge and Louppe, 2002)

III. Environnement économique et utilisation du bois d'Okoumé

La principale utilisation de l'Okoumé a été et reste encore, le contreplaqué : c'est l'un des meilleurs bois d'œuvre pour cette fabrication industrielle. Cette essence représente 60% de la production commerciale du bois au Gabon et de la Guinée équatoriale. Les caractéristiques de cette essence (rectitude et dimension des grumes, faible densité, qualité assez homogène) en font un bois très apprécié au déroulage. Les placages d'Okoumé obtenus après déroulage, peuvent être utilisés autant en face qu'en plis intérieur pour la fabrication de contreplaqués intérieurs ou extérieurs utilisables dans de multiples secteurs (batiment, ameublement, construction navale etc..). En effet, en raison de leur texture, les grumes d'Okoumé sont principalement transformées en placage appliqué sur la surface de contreplaqués de peuplier ou de bouleau. De plus, les marchés d'outre-mer ont été le stimulus de la transformation initiale du bois d'Okoumé, car les consommateurs préféraient les produits en bois rouge (Wenbin et Xiufang, 2013). Le bois d'Okoumé est également utilisé comme bois de chauffage et exploité pour la fabrication artisanale des pirogues. En tant que matériau commun dans les produits ligneux intermédiaires, les produits finaux des grumes d'Okoumé approvisionnent à la fois les marchés extérieurs et chinois. 30% du contreplaqué du bois d'Okoumé est directement exporté, les principales destinations étant les Etats-Unis, l'Union Européenne, le Moyen Orient, l'Amérique Latine et même l'Afrique (Wenbin et Xiufang, 2013). Par ailleurs, les 70% restants sont utilisés dans la fabrication de meubles en bois, de matériaux de décoration. Le bois d'Okoumé peut être utilisé comme combustible et convient pour la production

de pâte à papier (Doat, 1972).

IV. Maladie et ravageurs

L'Okoumé est très sujet aux attaques par différents pathogènes. Selon le CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement), l'utilisation du bois d'Okoumé nécessite un traitement de préservation car il est considéré comme non durable vis-à-vis des agents de dégradation biologique du bois (CIRAD, 2012). En effet, la maladie la plus importante est le chancre noir, qui se définit comme étant une infestation par des *Asterolecanium pustulans* (Cockerell) élevées par les *Crematogaster*, *Oecophylla* (fourmis). Les blessures de l'écorce qui découlent de l'activité des hommes sont surinfectées par une maladie fongique, des pourritures noires (*Botryodiplodia theobromae*).

Par ailleurs, le taux élevé de silice dans le bois d'Okoumé serait responsable de sa résistance aux térébrants marins, avec une concentration en silice de l'ordre de 0,17% Doat (1978).

V. Utilisation de la résine d'Okoumé

Certains auteurs se sont intéressés à la valorisation de quelques molécules extraites de la résine d'Okoumé dans les domaines cosmétiques et pharmaceutiques, notamment en dermatologie (Delaveau et al., 1980; Tessier et al., 1982; Delaveau and Vidal-Tessier, 1988) .

L'arbre d'Okoume, tout comme le pin maritime, produit naturellement de la résine. A l'origine, la résine d'Okoumé était principalement utilisée au Gabon et en Guinée équatoriale pour la fabrication de flambeaux utilisés lors des cérémonies d'initiation et des lampes à huile. Cette utilisation persiste encore de nos jours. Dans les missions en forêt, on l'emploie comme succédané de l'encens, et en médecine indigène, la résine est utilisée pour faire mûrir les abcès et dans le traitement des plaies où elle activerait la cicatrisation (Tessier et al., 1982). De plus, les décoctions d'écorces ou de feuilles sont utilisées comme antiseptique externes, l'écorce quant à elle est utilisée comme astringent et anti-diarrhéique (Brunck et al., 1990).

Les tableaux ci-dessus illustrent les ventes réalisées de la résine d'Okoumé pour des firmes internationales.

Tableau 1 : Projection des ventes de résine d'Okoumé et d'échantillons de plantes entre 1998 et 2001 (Lescuyer, 2006)

En Euros	Année 1	Année 2	Année 3	Total
Vente de la résine d'Okoumé (250kg/an)	27500	27500	27500	82 500
Vente d'échantillons de plantes (2000 puis 400 échantillons/an)	120000	24000		144 000
Total	147 500	51 500	27 500	226 500

Tableau 2 : Ventes réalisées de la résine et du échantillons d'Okoumé entre 1998 et 2001 (Lescuyer, 2006)

DIOR	420 kg de résine d'Okoumé	52 119 €
Aventis, Fabre, Novartis-Syngenta	2100 échantillons	115 586 €

La forêt gabonaise couvre 22 millions d'hectares, dont 14 millions de la superficie permanente de l'Etat et 8 millions d'hectares de zones rurales (zones de forêts communautaires, zones de chasse et de pêche, etc.) (Yang, 2008). Le champ de l'Etat est divisé en 10 millions d'hectares de production de bois d'œuvre résineux et 4 millions d'hectares de zones forestières protégées. Les inventaires des facteurs de développement dans les années 90 ont révélé la présence dans la forêt gabonaise de près de 350 espèces potentiellement exploitables, 60 seulement sont soumises à prélèvement. Il s'agit notamment d'Ozigo, d'Okoumé et d'autres espèces dites « bois divers ». Le potentiel total de bois est d'environ 400 millions de m^3 dont un tiers (130 millions de m^3) est constitué d'Okoumé et les essences dites bois divers représentant le reste (Figure 24).

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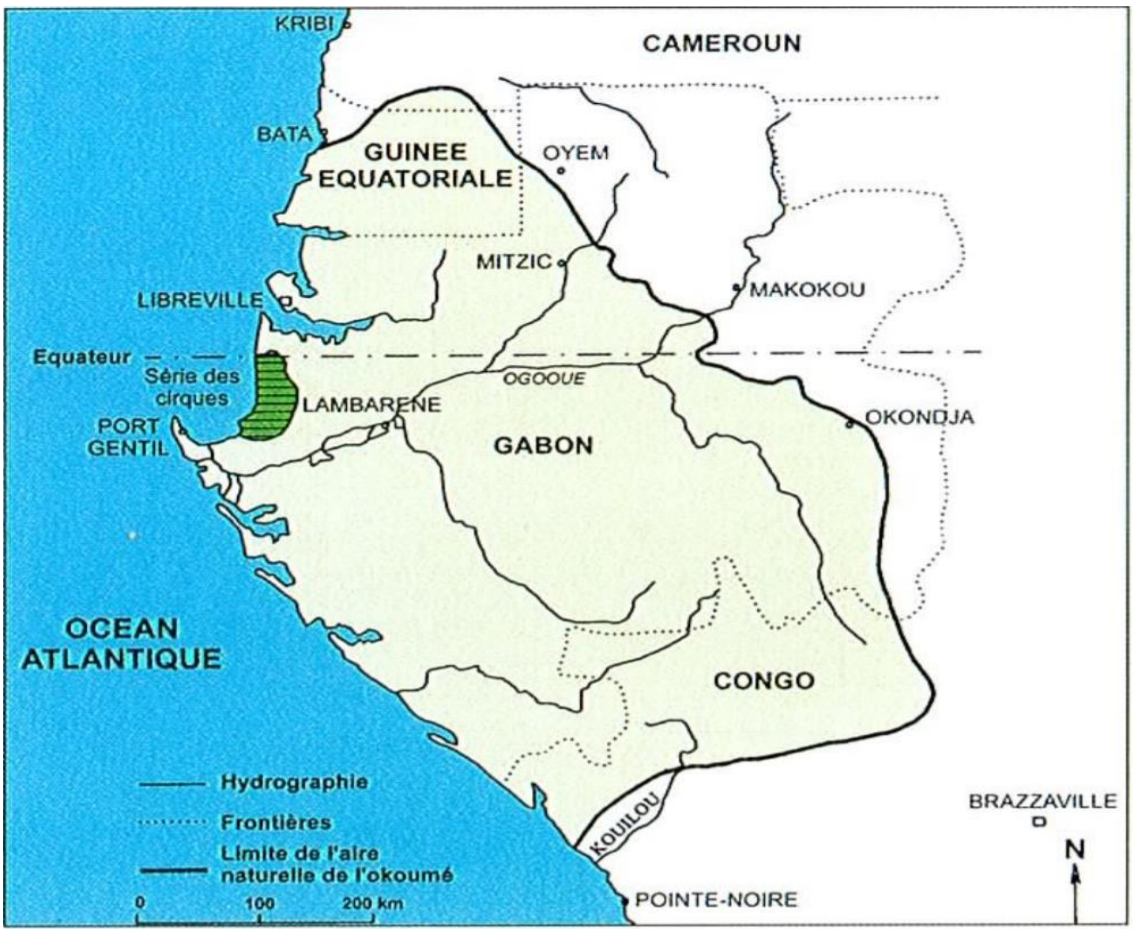

Figure 24: Aire naturelle de l'Okoumé (Doumenge and Louppe, 2002)

VI. Analyse chimique de l'Okoumé

Très peu de travaux existent sur la qualification chimique de la partie non-résinique du bois d'Okoumé, à savoir l'écorce, l'aubier et le bois de cœur. Les quelques études concernant la composition chimique du bois d'Okoumé, se sont limitées à la quantification du taux d'extractibles (lignine, phénols), à l'aspect chimique de la cellulose et l'hémicellulose potentiellement utilisables pour la mise au point de bio-carburants. Par ailleurs, la composition chimique de la résine est bien connue. De plus, un brevet a été déposé par Renimel et Andre pour la parfumerie Christian Dior (Renimel and Andre, 2004). L'objectif de ce brevet visait à utiliser un extrait de la résine d'Okoumé dans les champs cosmétique et pharmaceutique, et notamment dans le domaine dermatologique.

VI.A. Composition chimique de la résine d'Okoumé : travaux antérieurs

Des travaux effectués sur l'Okoumé ont montré que son oléorésine renferme 82% de substances neutres. Des recherches ont permis de détecter et d'isoler certaines d'elles, dont cinq triterpènes. Le composé majoritaire est l'α-amyrine, un triterpène pentacyclique déjà rencontré dans les résines d'autres espèces de la famille des Burseraceae (Tessier et al., 1982) (Figure 25). Ce triterpène peut servir de fonction protectrice contre les attaques microbiennes et insectes pathogènes (Dupont et al. 1930/1948, Tessier et al. 1982, Guang-Yi et al. 1988/1989, Gardrat et al. 2005, Rhourri-Frih, 2009)

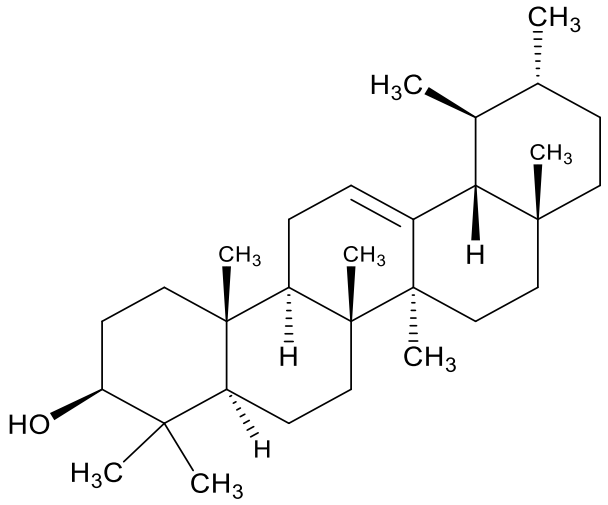

Figure 25: Structure moléculaire de l'α-amyrine

L'examen initial de l'huile essentielle obtenue par hydrodistillation après traitement d'un échantillon d'oléorésine a permis de déceler l'existence de quinze constituants. Dix (10) d'entre eux ont été identifiés, dont, l'α-terpinéol et, le β-phéllandrène qui sont les moléules en quantités plus importantes (Figure 26). Les composés minoritaires sont le *p*-cymène, le *p*-menthène le p-cyménène, l'α-terpinène, Le terpinèn-4-ol, le thymol, le carvacrol et l'alcool cuminique (Tessier et al., 1982).

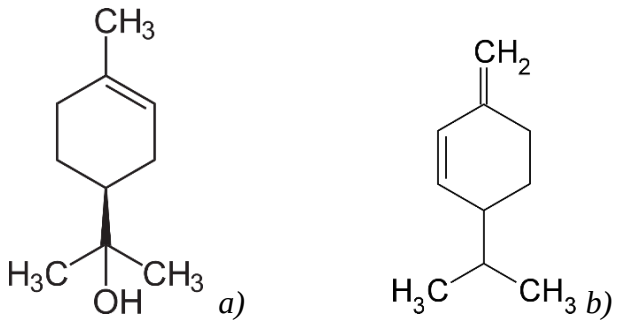

Figure 26: Structures moléculaires des composés extraits des huiles essentielles de l'Okoumé : a=α-terpinéol ; b=β-phéllandrène

Des travaux similaires ont évalué à 5% la quantité d'huile volatile obtenue par distillation à la chaleur de l'oléorésine d'Okoumé. L'analyse de cette partie volatile par chromatographie en phase gazeuse couplée à la spectrométrie de masse (GC/MS) a conduit à l'identification d'un nombre additionnel de monoterpènes : α-pinène, α-phéllandrène, limonène, camphre, 3-carène, 4-carène (Guang-Yi et al., 1988) dont certains avaient été identifiés auparavant par Tessier et al. (1982). Des études complémentaires sur l'oléorésine ont permis d'identifier un certain nombre de triterpènes de la série du tirucallane et de l'oléanane (Guang-Yi et al., 1988).

Un autre échantillon d'oléorésine soluble dans le pentane a été soumis à la même analyse GC/MS, les résultats obtenus ont montré que cette fraction soluble contenait deux composés supplémentaires. Sur les neufs triterpènes isolés, trois sont des composés pentacycliques de la série des oléananes : la β-amyrine, la β-amyrenone et le maniladiol (Figure 27). Les six composés restants sont tous des triterpènes tétracycliques de la série des tirucullanes.

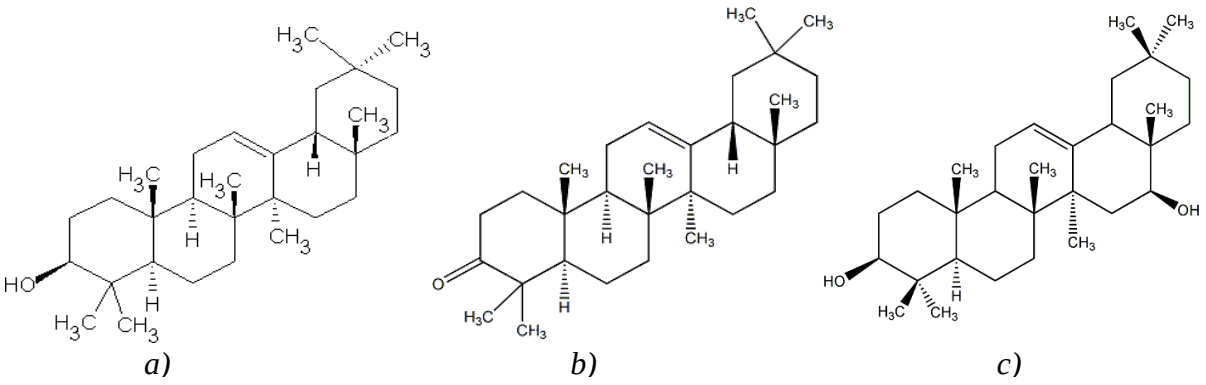

Figure 27: Structures moléculaires des triterpènes pentacycliques majoritaire d'Okoumé : a = β-amyrine b = β-amyrenone et c = maniladiol

VI.B. Composition chimique de la résine d'Okoumé : travaux récents

Les travaux réalisés par Medzegue et al. (2013) en utilisant la chromatographie en phase gazeuse, avec espace de tête (HEAD SPACE) couplée à la spectrométrie de masse ont mis en évidence neuf produits majoritaires déjà identifiés sur la résine d'Okoumé. Ces composés représentent à eux seuls plus de 96% des composés volatils présents dans l'oléorésine d'Okoumé : six mono-terpènes (α-pinène, β-pinène, limonène, α-phéllandrène, β-phéllandrène, 3-carène), un alcool mono-terpénique (α-terpinéol) et deux autres dérivés (p-cymène et eucalyptol).

Dongmo et al. (2010), en se penchant sur les propriétés anti-radicalaire, anti-oxydante et anti- inflammatoire potentielles des huiles extraites de la résine d'Okoumé, ont montré une activité anti-radicalaire à la concentration de 23,7 g/l et, une activité anti-oxydante à la concentration de 9 g/l. Mais aucune activité anti-inflammatoire n'a été observée.

Tableau 3 : Valeurs des produits de composés volatils trouvés dans l'oléorésined'Okoumé en forêt naturelle et en plantation (Gardrat et *al.*, 2005)

Nom du produit	Cocobeach/ oléorésine de forêt naturelle (%)	Ntoum / oléorésine de forêt naturelle (%)	Bokoué1/ oléorésine de plantation (%)	Bokoué2/ oléorésine de plantation (%)
α-pinène	2,7	6	2,6	2,3
β-pinène	0,6	1,5	0,4	0,8
α-phellandrène	11,4	**63**	4	16,6
Δ-3-carène	**37,3**	9	0,2	3
p-cymène	10,5	5	**41,4**	8,1
Limonène + eucalyptol	17,2	13*	28,5	**50,5**
α-terpinéol	12,9	0,8	7,2	12,1
Produits non identifiés	7,4	2,5	15,7	6,6

VI.B.1. Taux d'extractibles présents dans le bois d'Okoumé

Les récents travaux portant sur le taux d'extractibles dans l'écorce, l'aubier et le bois de cœur d'Okoumé ont été réalisés par Minkue M'Eny (2001). Les résultats obtenus à la suite de cette analyse ont montré que la teneur en extraits dans l'hexane est faible quel que soit l'échantillon utilisé : aubier (0,14%) ou cœur (0,15%). Cette teneur augmente lorsqu'on rajoute de l'eau chaude à l'hexane : aubier (5%) ou cœur (3%). Toutefois les rendements obtenus pour une extraction

éthanol/toluène/eau chaude sont supérieurs à ceux des mélanges cités ci-dessus : aubier (6,37%) ou cœur (4,48%). Cela est moins remarquable pour les résultats obtenus par Mounguengui et al. (2016) en utilisant comme solvant le mélange toluène/éthanol et l'éthanol simple pour des résultats respectivement de 2,8% et 0,5%.

VI.B.2. Les éléments minéraux

Les minéraux présentes dans le bois d'Okoumé sont constitués en majorité de calcium et de magnésium. Les travaux rapportés par Minkue M'Eny (2001) ont montré que, la teneur en calcium de l'Okoumé était de 1088 ppm dans l'aubier et de 1546 ppm dans le duramen. Pour le magnésium, l'auteur rapporte une teneur de 871 ppm dans l'aubier et de 691 ppm dans le duramen.

VI.B.3. Taux de lignines dans l'Okoumé

Les différents dosages de la teneur en lignines de l'Okoumé ont montré une valeur moyenne en lignine Klason comprise entre 28,4 et 31,5% (Medzegue et al., 2007; Minkue M'Eny, 2001; Mounguengui et al., 2016; Safou-Tchiama et al., 2016). Toutefois, une teneur en lignine de Klason de 17,2% a été mise en évidence dans des échantillons d'Okoumé d'une autre étude (Safou-Tchiama et al., 2007). Selon Minkue M'Eny, le pourcentage de lignine chez l'Okoumé se situe autour de 30% dans le bois de cœur et de 28% dans l'aubier. Cette variabilité, si elle était confirmée, pourrait permettre d'identifier le diamètre minimum d'exploitabilité de cette essence lors des analyses chimiques, voire de contrôler la traçabilité des échantillons. L'analyse en spectroscopies moléculaires (FT-IR, UV-Vis, Résonances Magnétiques Nucléaires (RMN) et Pyrolyse couplée à la GC/MS) a montré que les lignines dioxane acide de l'Okoumé sont plus riches en unités guaiacyles (G) qu'en unités syringyles (S) (Safou-Tchiama, 2005).

Les composites bois-plastiques

I. Généralités

“Wood polymer composite’’ ou WPC sont des composites bois-polymère ou bois-plastique résultant d’un mélange de particules et ou de fibres de bois à plusieurs polymères thermoplastiques afin de créer un matériau possédant les propriétés inhérentes du bois tout en ayant plusieurs avantages supplémentaires. En effet, les caractéristiques résultantes telles que l’apparence physique, les performances mécaniques en présence ou en absence d’humidité sont liées à la ressource utilisée. De plus, l’obtention des WPC se fait généralement à partir de matière recyclée, ou sur-cyclée (upcycling) avec comme pour renfort le bois et un polymère comme matrice. Le mélange obtenu peut être extrudé ou moulé en une forme cylindrique (comme des granules par exemple).

La densité relativement faible des renforts naturels, est un point essentiel à l’utilisation des matériaux composites bois-polymère aussi bien dans le secteur industrie automobile que dans le secteur de la construction. En effet, la légèreté des pièces est un atout majeur et suscite l’intérêt. De plus le moussage des composites, la conception et le design apparaissent comme des alternatives novatrices à ce sujet. C’est à ces fins que plusieurs entreprises produisent des WPC dont le nombre se multiplie avec le temps.

II. Réactivité chimique et valorisation des refus ou connexes du bois d’Okoumé dans le domaine des composites bois-polymères

II.A. Contrôle de la réaction à l’interface bois-anhydride succinique ou 2-octène- 1-yl d’anhydride succinique

Dans la perspective de valorisation des déchets des industries du bois au Gabon, une étude portant sur le contrôle de la réactivité à l’interface bois-polymères a été réalisée sur les déchets de bois (duramen) d’Okoumé. Les résultats obtenus ont mis en évidence une faible absorption du solvant (DMF/Py) au sein de ses parois cellulaires. La présence de courtes fibres de polysaccharides qui ont tendance à s’agglomérer dans les parois, réduise la capacité de couplage entre les fonctions hydroxyles du bois et la fonction anhydride du copolymère utilisé (Safou-Tchiama et al., 2007).

II.B. Polymérisation par rayonnement gamma de l'acrylamide dans l'Okoumé

Pour formuler des composites bois/plastique, Bakraji et *al*., (2002) se sot intéressés à l'effet synergique des additifs tels que H_2SO_4, la NVP (*N*-vinyle pyrrolidone, le TMPTA (triméthylopropane triacrylate) et l'urée (U) sur l'Okoumé. En effet, l'échantillon d'Okoumé est dans un premier temps imprégné sous vide (6,7 kPa) dans une solution de concentrations différentes en monomères d'acrylamide dans les trois agents de gonflements (méthanol, eau et mélange méthanol/eau) avant d'être irradié à un débit de dose de 8 kGy/ h. De plus, la radiation a lieu en présence ou en l'absence d'additifs (H^+, NVP, TMPTA et U). De ce travail, il peut être déduit dans un premier temps que l'effet du chargement du polymère est plus élevé que lorsque la dose de radiation est plus forte. Dans un second temps, tous les additifs et co-additifs utilisés au cours de cette étude jouent un rôle positif sur le chargement du polymère, et sur la résistance à la traction, à l'exception de H_2SO_4.

De plus l'utilisation d'additifs et de co-additifs a réduit la concentration de la plus grande partie du monomère, ainsi que la dose gamma requise pour la polymérisation totale dans certains cas.

Problématique et Objectifs

L'Okoumé est mondialement connu car il présente des propriétés intéressantes pour une utilisation en papeterie, la production de contreplaqué ainsi que dans les secteurs cosmétiques et pharmacologiques.

En dépit de l'abondante littérature sur la composition chimique de la résine d'Okoumé, de la teneur en extraits de certaines de ses parties solides, ou de la distribution de la lignine, de la cellulose et des hémicelluloses de cette essence, la structure moléculaire des extraits de ses parties solides (écorce, aubier et bois de cœur) ne semble pas avoir été étudiée. En effet, l'analyse bibliographique mentionne des travaux uniquement liés à la chimie de la résine, avec notamment le brevet déposé par Renimel et Andre (2004) pour la célèbre parfumerie Christian Dior. De plus, à notre connaissance, aucune recherche ne fait mention des tanins du bois d'Okoumé, ces polyphénols pourraient sans doute avoir une haute valeur ajoutée à l'instar de sa résine.

Par ailleurs, on assiste depuis un peu plus d'une dizaine d'années maintenant à la recrudescence du développement de produits respectueux de l'environnement, tant dans les secteurs industriels qu'artisanaux. L'utilisation des tanins avec ou sans modification chimique connait un regain d'intérêt. A ce jour, un grand nombre de chercheurs s'accordent à dire que le tanin pourrait, grâce à ses propriétés intrinsèques, limiter l'utilisation des composés d'origine fossile. Le secteur industriel du contreplaqué ou des panneaux agglomérés pourrait tirer un avantage économique et enironnemental si l'on mettait en place une colle bio-sourcée et facilement transposable. Au niveau du Gabon, un tel investissement semblerait nécessaire malgré les coûts qui s'y rattachent. Par ailleurs, le développement de la connaissance de l'essence la plus exploitée du Gabon, permettra une avancée scientifique sur le sujet. En effet, en dehors de son utilisation en placage, seuls quelques applications concernant une valorisation du matériau bois d'Okoumé sont connues : seuls quelques travaux mentionnent les possibilités de son utilisation dans l'industrie du composite (WPC), mais aucun pour la fabrication de colle.

Cette thèse a ainsi trois objectifs principaux :

- Extraire, quantifier, qualifier et caractériser les extractibles du bois d'Okoumé [écorce, aubier et bois de cœur], en particulier les polyphénols et plus précisement les tanins condensés.

- Développer une colle bio-sourcée à partir des tanins d'Okoumé et d'hexamine
- Mettre au point un composite bois d'Okoumé-plastique utilisable dans l'industrie de la construction.

DEUXIÈME PARTIE

RÉSULTATS ET DISCUSSION

Les différents résultats qui permettent de répondre de manière préliminaire aux problématiques initiales sont illustrés sous forme de plusieurs articles d'ores et déjà acceptés pour publication ou en cours d'évaluation ou de soumission. Ils sont regroupés en 3 chapitres :

- **Chapitre 1 : Extraction et caractérisation des tanins d'Okoumé : travaux préliminaires**

Ce premier chapitre concerne le taux d'extractibles en fonction des solvants de la méthode d'extraction et la caractérisation thermique des tanins extraits avec 5% de soude (NaOH). Il correspond à l'article suivant publié dans Journal of Renewable Materials :

Engozogho Anris, S.P., Bikoro Bi Athomo, A., Vidal, M., Denaud, L., Safou-Tchiama, R., Charrier, B. 2019. Extraction and characterization of *Aucoumea klaineana* Pierre (Okoume) extractives. Journal of Renewable Materials. 7(6),519-522.

Des résultats complémentaires concernant une analyse en chromatographie en phase gazeuse ont été ajouté en fin de ce chapitre.

- **Chapitre 2 : Extraction, identification et caractérisation des tanins d'Okoumé : optimisation**

Ce second chapitre est consacré à la caractérisation des tanins et à la mise en place d'une meilleure méthode de séparation des polyphénols de l'Okoumé sans modification chimique. Les résultats sont présentés dans 2 articles:

Engozogho Anris, S.P., Bikoro Bi Athomo, A., Safou-Tchiama, R., Santiago-Medina, F.J., Cabaret, T., Pizzi, A., Charrier, B. Molecular structure and thermal stability of Aucoumea klaineana Pierre (Okoume) condensed tannins: An attempt to African wood wastes valorization (submitted to Scientific Repports. Scientif ReportsScientific Reports, 10(1). 1-14

Engozogho Anris, S.P., Bikoro Bi Athomo, A., Safou-Tchiama, R., Santiago-Medina, F., Pizzi, A., Charrier, B. Maldi-ToF analysis and FTIR characterization of *Aucoumea Klaineana* Pierre (Okoume) sapwood and heartwood condensed tannins from Gabon natural forest (submitted to Journal of wood science and technology, reference number : WSAT-D-19-00269

- **Chapitre 3 : Valorisation**

Ce troisième chapitre est consacré à la valorisation des co-produits de transformation du bois d'Okoumé. Dans un premier temps, il s'agit de la formulation d'un adhésif tanins d'Okoumé/hexamine. Et dans un second temps, la mise au point d'un composite obtenu par thermocompression d'un mélange de sciure de bois d'Okoumé et de broyat (environ 0.5 mm) de bouteilles plastiques. Ce composite pourrait être utilisé dans l'industrie de la construction au Gabon. Ces résultats sont présentés sous forme de 2 articles, soumis dans des journaux à comité de lecture.

Engozogho Anris, S.P.,Bikoro Bi Athomo, A., Safou-Tchiama, R.,Vidal, M.,Charrier, B. Development of green adhesives for fibreboard manufacturing, using Okoume bark tannins and hexamine : Characterization by NMR, TMA, TGA and DSC analysis

Engozogho Anris, S.P.,Bikoro Bi Athomo, A., Safou-Tchiama, R., Eyma, F., Rubini, M., Boucard, M., Charrier, B. *Aucoumea klaineana* Pierre (Okoume) wood plastic panel composite

Chapitre 1

Extraction et caractérisation des tanins d'Okoumé : travaux préliminaires

I. Présentation

Cette première partie décrit les différentes méthodes utilisées pour extraire à la fois les polyphénols et les acides gras présents dans le bois d'Okoumé. Les principaux paramètres étudiés sont le taux d'extractibles et leur caractérisation. En l'occurrence, le pouvoir collant (indice de Stiasny) des tanins condensés extraits a été évalué. Les résulats ont montré une réactivée élévée des tanins face au paraformaldehyde, soutenant la possibilité de l'utilisation des tanins d'Okoumé comme substrat pour le dévéloppement d'un adhésif bio-sourcé.

Par ailleurs, l'analyse GC des extraits au soxhlet nous a permis d'identifier un nombre de molécules différentent de celles déjà identifiés dans la résine d'Okoumé, soutenant ainsi, la variabilité moléculaire de cette essence.

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II. Extraction and Characterization of *Aucoumea klaineana* Pierre (Okoume) Extractives

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II.A. Résumé

Afin de promouvoir des stratégies pratiques d'utilisation des déchets de bois de l'industrie du bois d'*Aucoumea klaineana pierre* (Okoumé), diverses analyses chimiques ont été effectuées sur des échantillons d'origines différentes. La teneur totale en extraits de l'écorce, de l'aubier et du bois de cœur d'Okoumé a été évaluée. Des analyses thermogravimétriques ont été effectuées et l'indice de Stiasny a été calculé. Il a été constaté que l'écorce était plus riche en acide gras de poids moléculaire élevé, tandis que l'aubier était riche en acide gras de faible poids moléculaire. La teneur en tanins condensés variait en fonction de l'origine et de la partie de l'arbre. Ces nouvelles découvertes pourraient ouvrir de nouvelles voies de valorisation, telle que la fabrication d'adhésifs à base de tanins d'Okoumé.

II.B. Abstract

In order to promote convenient strategies for the utilization of wood wastes from aucoumea klaineana pierre (Okoume) timber industry, various chemical analysis were carried out on samples from different origins. Total extractives content of the bark, sapwood and heartwood of Okoume were evaluated. Thermogravimetric analyses were performed and the stiasny number was calculated. it was found that the bark was richer in fatty acid of high molecular weight while the sapwood was rich in fatty acid of low molecular weight. The condensed tannins content varied according to the origin and the part of the tree. These new findings should be useful for green Okoume based tannin adhesives.

Keywords: Extractibles; fatty acids; tannins; polyphenols; stiasny number; TGA

II.C. Introduction

Aucoumea klaineana Pierre (Okoume) is one the best wood species mainly used for its good quality in panels or plywoods industry. This wood presents remarkable capacities for unwinding. Characteristics such as straightness; log dimensions, low density fairly uniform quality and abundance in forest render that wood species popular for peeling indeed.

Since the Gabonese government prohibited exporting logs in 2010, the trade of local transformed wood increased significantly. Therefore, the utilization of local wood species such as Okoume or African mahogany increased in the Gabonese industry. However, the timber industry in this central Africa country is mainly focused on wood cutting, peeling and sawing; that generates a high content of non-valorized wood wastes accounting about for 50% of wood transformed.

Among the 400,000,000 m^3 of exploited woods in Gabon per year, Okoume represented 130,000,000 m^3 (about 31%) in 2016 [1].

Analyzing Okoume wood revealed the presence of resin which pointed out some bactericide properties. These properties are the fact of phenolic compounds located in its essential oil [2]. Additionally, the analysis of Okoume's essential oil revealed antiradical and antioxidant activities [3].

Apart the studies listed above, the chemical characterization of Okoume oleoresin was extensively investigated, and patented by Renimel Isabelle in 1999 [4].

However, the chemical composition of the non-resin parts of Okoume has received little attention.

Our research focused on extracting and characterizing extractible molecules from Okoume wood by Soxhlet apparatus, thermogravimetric analysis (TGA), Stiasny number and condensed tannin quantification.

II.D. Materials and Methods

II.D.1. Materials

The bark, sapwood and heartwood of Okoume were obtained from SED (Société Equatoriale de Déroulage) in different areas of Gabon natural forest. Thus, trees from the West were collected in Nzamaligue (Nzama) forest, and those from the South were obtained in Milolé forest. Trees from the North were sampled in Mitzic forest by the SNBG (Société Nationale des Bois du Gabon). The wood was collected in February 2016 and sampled as follows: One piece of each tree (83 cm × 10 cm diameter x thickness) was taken at 1.3 m from the soil. Samples were

first air dried for one week and then dried in an oven for 24 hours at 105°C. Further, samples were cut and grounded with a grinder (Retsh SK1 rotating knife) at 60 meshes. All the chemicals used in this study were purchased from Fisher Scientific and Sigma-Aldrich. Solvents and reactants were used without further purification

II.D.2. Methods

II.D.2.a. Soxhlet extraction with different solvents

A weighed dried sample (10 g) of the powdered material was extracted at 40-70°C for 4 hours under reflux with 400 mL of solvent (Petroleum ether, hexane and acetone (70%, v/v)) in a round bottomed flask heated. The experiment was repeated three (3) times. After extraction, the content was concentrated with a rotating vapor vacuum pump coupled and the yield of the crude was calculated according to the equation below:

$$Yield\ of\ crude = \frac{mass\ of\ the\ solid\ residue}{dry\ mass\ of\ sawdust} x100$$

II.D.2.b. Tannins Extraction

The tannins were extracted from Okoume wood according to a previously published procedure [5,6] as follows: a sample-water ratio of 1/9 was put in water containing 5% of sodium hydroxide, 0.25 % sodium bisulfite and 0.25% of sodium sulfate. The mixture was maintained under continuous magnetic stirring for 2 hours at 80°C. The obtained tannin extracts were filtered, dried in an oven at 50 °C and yielded as expressed in the equation below:

$$Extraction\ yield\ = \frac{mass\ of\ extract\ recovered}{dry\ mass\ of\ sawdust} x100$$

II.D.2.c. Thermogravimetric Analysis

The thermogravimetric analysis was carried out on a computerized thermobalance (TGA Q50 instrument) using a furnace which allows a heating rate of 10 °C/min. The thermobalance configuration gives a sensitivity of ± 0.4 lg. It allows to use a small sample mass (10-50 mg) which is needed to ensure isothermal conditions in the samples. In order to establish an inert atmosphere (Nitrogen) during all experiments, a controlled air flow (fixed at 60 mL. min^{-1}, 1 atm) sweeps the measurement cell that is purged for 20 min before starting the heating program. The temperature

program was 25 to 600°C.

II.D.2.d. Stiasny Number

The Stiasny number reaction was used to determine the reactivity of tannins toward formaldehyde. According to Hoong et al., (2010), 50 mL of sample (0.4%, w/w) tannin solution was pipetted into a 150 mL flask. Aqueous formaldehyde (37%, 5 mL) and hydrochloric acid solution (10 M, 5 mL) were then added and the mixture was heated under reflux for 30 minutes. At the end of this reaction, the mixture was filtered through a sintered glass filter (filter n° 3) while it was still hot. The precipitate was dried to constant weight in an oven at 105°C. The Stiasny number was performed in triplicate and determined as follows:

$$Stiasna\ Number\ = \frac{Oven - dried\ weight\ of\ precipitate}{extracted\ sample\ dry\ weight} x10$$

II.E. Results and Discussion

II.E.1. Soxhlet Extraction with Different Solvents

In this study, different solvents were used to extract various compounds such as fatty acids with high molecular weight (petroleum ether solvent), fatty acids with low molecular weight (hexane solvent) and tannins (acetone solvent) in order to study the variability of Okoume extracts in the first collection of Nzama site (Nzama 1). The results presented in Tab. 1 pointed out significant differences between the solvents ($p < 0.05$). With the exception of the bark, the highest extracts content was obtained with petroleum ether. Extracts obtained with petroleum ether displayed significant differences between the bark, sapwood and heartwood ($p < 0.0001$). It was noteworthy that fatty acids were more abundant in the bark (12.24 ± 6.5%) than the sapwood (8.72 ± 3.3%) and the heartwood (6.60 ± 2.0%). But, hexane exhibited also significant differences regarding Okoume extracts ($p < 0.0001$), and the highest content on fatty acids of low molecular weight was found in the sapwood (17.66 ± 6.90%) than the heartwood (15.87± 8.70%) and the bark (7.57 ± 5.70%). However, acetone extracts confirmed the presence of tannins in that hardwood species. Although the limited number of trees, Okoume bark and sapwood tannins content didn't exhibit significant ($p > 0.05$) as depicted in Tab. 1, while the heartwood was the least abundant in tannins (1.33 ± 0.60%). The presence of tannins in Okoume was previously described by Mounguengui et al. [8] who found a polyphenol content of 0.64 ± 0.05% for Okoume heartwood. However other authors using acetone (70%, v/v) as solvent found that Okoume extracts were more

abundant in the heartwood than sapwood [9], for samples from Mitzic natural forest. That result underlined the variability and the complexity of wood extracts study depending at least on wood origin and sunshine of heliophilia plant like Okoume. Nevertheless, a deep investigation of Okoume wood extracts variability and other related properties shall concern future studies.

Extractive molecules are assumed to be present in the porous structure of plants [10]. In wood species, polyphenols compounds such as tannins, flavonoids, or other molecules like fatty acids, terpens, fats or oil [10-13] accounted for extracts in the wooden bark.

Table 1: Extracts yield in % of dry matter, obtained by the Soxhlet method of the first collect of Nzamaligue (Nzama 1)

	Petroleum ether	Hexane	Acetone
Sample	Mean scores (± SD)	Mean scores (± SD)	Mean scores (± SD)
Bark	12.24 ± 6.50a	7.57 ± 5.70d	5.51 ± 1.90d,g
Sapwood	8.72 ± 3.30b	17.6 ± 6.90e	4.55 ± 0.50g
Heartwood	6.60 ± 2.00c	15.87 ± 8.70f	1.33 ± 0.60h

N = 3. Means with the same letters are not statistically different at the 0.05 level of significance. Mean± S.D

II.E.2. Extracting Yield of Tannins

Water-soluble extractive yields at 80°C are presented in Tab. 2. These extractives should be mainly composed of condensed tannins and polysaccharide residues. The results showed certain homogeneity in the tannins content of Nzamaligue forest which did not display significant difference on their tannins content ($p > 0.05$), excepted between the sapwood and the heartwood of Nzama 2 which showed a $p < 0.05$. However, no significant difference was found for Milole forest tannins content which showed a $p > 0.05$ between the three part of Okoume (Tab. 2). Nevertheless, the samples collected at Mitzic natural forest suggested significant difference between the bark, sapwood and heartwood condensed tannins ($p < 0.05$). When Nzama 1 and 2 are considered as one group labelled Nzamaligue, a fine analysis based on three groups (Nzamaligue, Milolé and Mitzic) exhibited a clear significant difference between the tannins content of Nzamaligue and Mitzic ($p < 0.05$). However, no difference was found between Milolé and Mitzic

bark ($p > 0.05$); but some differences on tannins content were found between the bark of Milolé and the heartwood of Mitzic, and the heartwood of Milolé and the sapwood of Mitzic, both displayed a $p < 0.05$. In addition, no difference was observed between the tannins content of Milolé and Nzamaligue bark ($p > 0.05$), while Milolé and Nzamaligue heartwood as well as Nzamaligue heartwood and Milolé sapwood exhibited pointed out significant difference on their tannins content ($p < 0.05$).

These results suggested the existence of three distinct forest blocs which should contain differences regarding the tannins content. Taking into account that chemical compounds do not have the same reaction according to their concentration in a solution, the trees origin should have provoked difference regarding their reactivity toward solvent; thus explaining in some extent the variability observed. Similar results pointed out high standard deviation were already observed in wood pine by Chupin [6].

Table 2: Extract yield obtained with water salt solvent at 80°C, expressed in % of dry matter

	Nzama1	Nzama2	Milolé	Mitzic
Sample	Mean scores (± SD)	Mean scores (± SD)	Mean scores (± SD)	Mean scores (± SD)
Bark	29.33 ± 5.00a	28.33 ± 13.80b	50.33 ± 11.84e	35.17 ± 1.44f
Sapwood	31.67 ± 14.15a	19.33 ± 3.62b,c	27.17 ± 14.68e	48.17 ± 2.02g
Heartwood	32.67 ± 4.31a	31.17 ± 4.49b,d	28.83 ± 12.11e	20.00 ± 1.32h

N = 3. Means with the same letters are not statistically different at the 0.05 level of significance. Mean ± S.D

II.E.3. Thermogravimetric Analysis

Fig. 1 showed the TGA curves conducted under nitrogen. According to Galletti et al. [14], tannins pyrolysis could lead to the formation of catechin and catechol moiety. A study conducted by Garro Galvez et al. [15] on the thermal decomposition of gallic acid showed that the degradation occurred mainly in three steps. The first one is at 260°C (26-27%) corresponds to the release of carbon dioxide during heating (decarboxylation). The second one is at 360°C (29%) may be due to an additional loss of hydroxyl groups. The last one occurred at 503°C (45%) corresponded to important residues of carbon oxidation (CO_2, H_2O, CO). First derivative peak observed from 150

to 250°C should correspond to sugars degradation as it is commonly observed during various heat treatments of wood. Phenolic groups may be probably degraded in secondary process after 300°C. The different stages of Okoume phenolic thermal decomposition depends strongly on their structure composition, degree of polymerization and interflavonoids bonds nature as well.

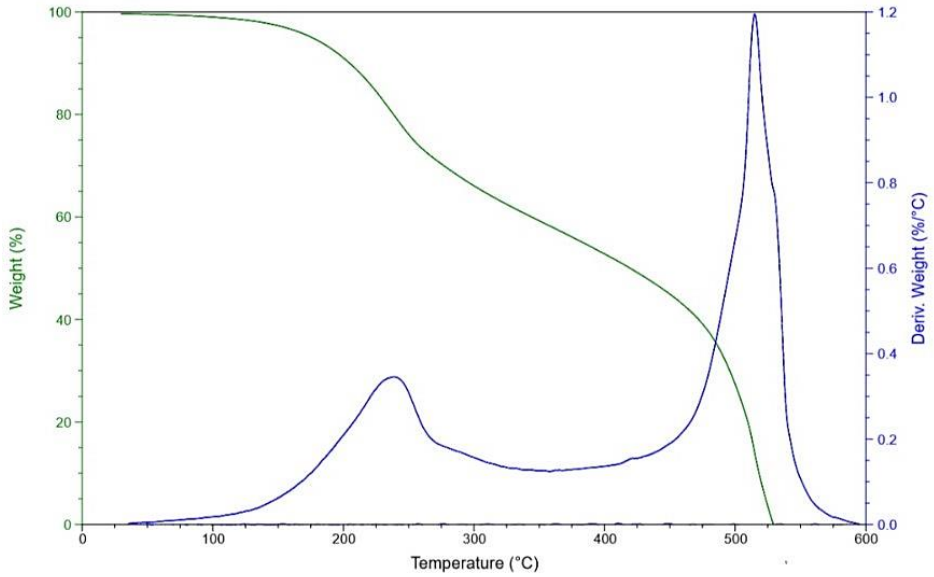

Figure 1: *The TGA of Okoume water extractives at room temperature heated at 10 °C/min under nitrogen*

II.E.4. Stiasny Index

The results are presented in Tab. 3. The Stiasny number gave us informations about our extracts regarding formaldehyde reactivity. This test permit us to appreciate the adhesive capacity of tannins [16, 17]. Yazaki et al. [18] assessed that the minimum Stiasny value to produce high quality adhesives is 65%. However, Ping et al. [15] produced good adhesives quality with a Stiasny number of 45%. In this study, we obtained Stiasny numbers in the range 50% to 93%. So, whatever the origin of Okoume wood wastes, a strong capability for adhesives was obtained through this Stiasny index analysis

Table 3: Stiasny number

	Nzama1	Nzama2	Milolé	Mitzic
Sample	Mean scores (± SD)	Mean scores (± SD)	Mean scores (± SD)	Mean scores (± SD)
bark	83.33 ± 11.55	73.33 ± 37.86	50.00 ± 26.46	76.67 ± 25.17
Sapwood	73.33 ± 15.28	93.33 ± 15.28	66.67 ± 15.28	70.00 ± 10.00
Heartwood	53.33 ± 11.55	66.67 ± 49.33	66.67 ± 5.77	60.00 ± 40

N=3

II.F. Conclusions

The results obtained showed that Okoume is rich in various polar and no polar compounds. The variability of results is high and the maximum amount of extractives through soxhlet extraction method is around 40%. Further work has to be done to analyse the molecular content of each extracts. In addition to its traditional use as panel or plywood, that hardwood wastes revealed good reactivity with formaldehyde with Stiasny test. This property could be used in future, to valorize Okoume extractives as raw material for green adhesives production.

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III. Résultats complémentaires

III.A. Détermination de la structure moléculaire des acides gras par chromatographie en phase gazeuse

L'analyse chimique des fractions extraites au soxhlet, notamment par des extractions à l'éther de pétrole et à l'hexane nous a permis d'identifier des molécules dont la composition chimique et l'abondance sont regroupées dans les tableau 1 et tableau 2. Signalons que la méthode de calibration utilisée ne nous permet d'identifier que les molécules ayant un nombre d'atomes de carbone compris entre 9 et 20. Par ailleurs, la reproductibilité a été contrôlée en effectuant deux mesures successives pour chaque échantillon.

Les indices de Kovats ont été calculés selon la relation ci-dessous :

$$I = 100 \times \left[n + \frac{\log(t'_{\mathrm{r(inconnu)}}) - \log(t'_{\mathrm{r}(n)})}{\log(t'_{\mathrm{r}(N)}) - \log(t'_{\mathrm{r}(n)})} \right]$$

Avec :

I : l'indice de rétention de Kovats ;

n : le nombre d'atomes de carbone dans le plus petit n-alcane ;

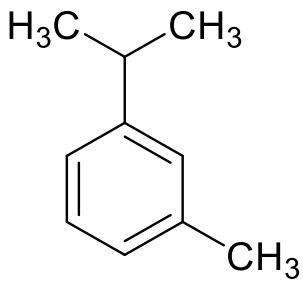
N : le nombre d'atomes de carbone dans le plus grand n-alcane ;

Tr : le temps de rétention.

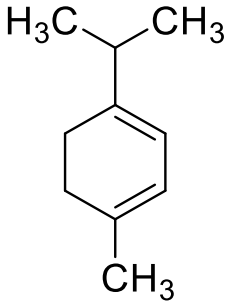
Il apparait à la lumière de cette relation qu'un certain nombre de composés élués à des temps rétention situés au-delà de 60 minutes n'ont pas été identifiés, et cela quelle que soit la nature de l'échantillon analysé. Cette limite imposée par la méthode de calibration ne nous a pas permis d'identifier les composés de grands poids moléculaires, notamment les acides gras que nous espérions identifier au cours de ces analyses.

Mais il apparait clairement que quel que soit le solvant utilisé, la composition chimique de l'écorce, de l'aubier et du cœur d'Okoumé est très différente. L'examen des tableaux 1 et 2 met en évidence la présence majoritaire de monoterpènes (C10), de sesquiterpènes (C15) et de diterpènes (C20) dans les extraits obtenus. Cette composition chimique est très différente de celle obtenue dans les travaux antérieurs (Tessier, 1982 ; Liang Guang et al., 1987 ; Medzegue et al., 2007 et 2013) effectués sur la résine d'Okoumé.

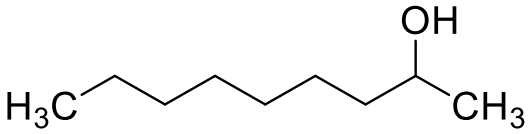
Pour l'écorce, le pourcentage de composés identifiés est plus élevé pour l'extraction à l'éther de pétrole (64,46%) que pour l'hexane (18,22%). Les composés majoritaires sont un mélange de m-cymène et de α-terpinène d'abondance relative 32,57%. Le 2-nonanol représente quant à lui 18% des composés extraits de l'écorce avec l'éther de pétrole (Tableau 3).



m-cymène

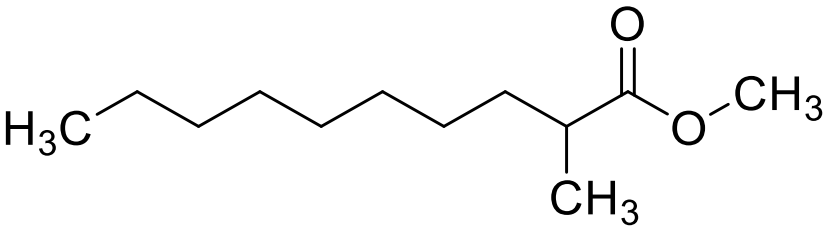


alpha-terpinène



2-nonanol

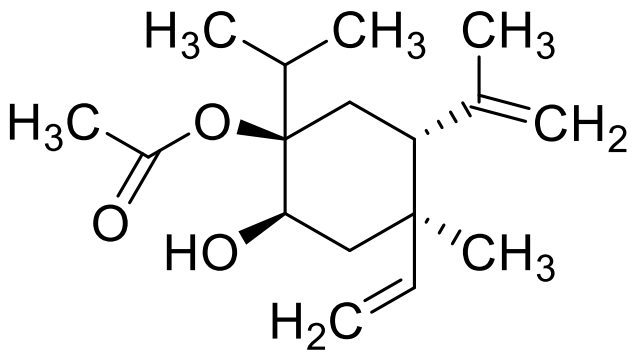
Le 2-méthyl-dodecanoate de methyl d'abondance 9,77% est le composé majoritaire des molécules extraites de l'écorce d'Okoumé en utilisant l'hexane comme solvant (Tableau 2)



Méthyl-2-méthyldécanoate

Certains composés non identifiés apparaissent majoritaires dans l'écorce. C'est le cas du pic d'abondance 34% qui apparaît à 63,48 minutes dans l'extrait à l'éther de pétrole (Tableau 1). Pour l'extrait à l'hexane, nous notons la présence d'un composé majoritaire non identifié et qui est élué au bout de 64,91 minutes pour un pourcentage de 58,42% (Tableau 2).

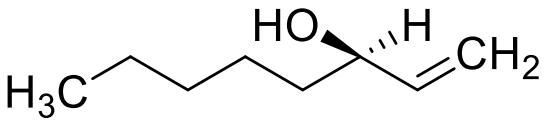
Dans le cas de l'aubier, les résultats consignés dans les Tableaux 3 et 4 montrent que l'abondance des composés identifiés est aussi plus élevée dans les extraits à l'éther de pétrole (70,62%) que dans les extraits à l'hexane (56,38%). Le composé majoritaire de l'aubier dans l'éther de pétrole est le 2-acetoxyfuranoelemene ; ce composé est présent à 59,79% (Tableau 1).



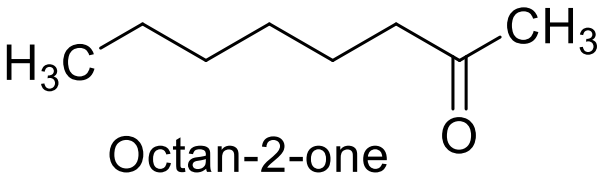
7-acetoxyelema-1,3-dièn-8-ol

L'oxyde de veticadine est par contre le composé majoritaire dans l'extrait à l'hexane de l'aubier, il a une abondance de 41,57% (Tableau 2).

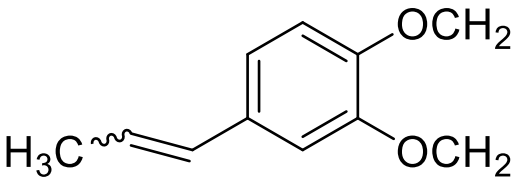
Dans le cœur d'Okoumé, le pourcentage de composés identifiés est plus élevé dans les extraits à l'hexane (73,53%) que dans ceux à l'éther de pétrole (8,54%) (Tableaux 1 et 2). Les composés majoritaires dans l'extrait à l'hexane sont un mélange de : (E)-1,2-diméthoxy-4propenylbenzene, de β-ionone époxyde, de selina-3,7-diene et de rotundene qui est élué au bout de 41,52 minutes avec une abondance relative de 48% (Tableau 2).



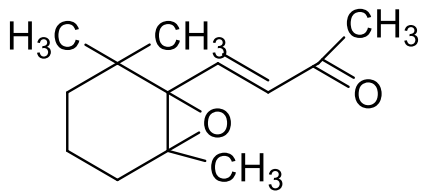
1-octen-3-ol



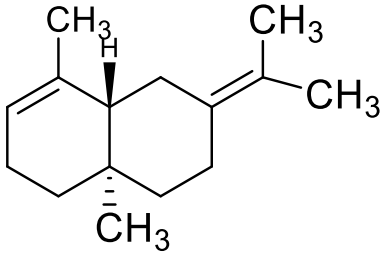
Octan-2-one



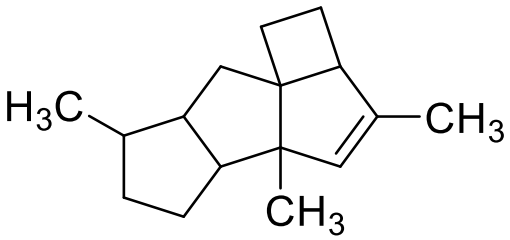
(E)-1,2-diméthoxy-4-propenylbenzene



beta-ionone époxyde :
4-(2,2,6-trimethyl-7-oxabicyclo [4.1.0] heptan-1-yl) but-3-en-2-one



Selina-3,7-diène: 5,8a-dimethyl-3-propan-2-ylidene-1,2,4,4a,7,8-hexahydronaphthalene



Rotundene

Signalons la présence de l'octan-2-one qui représente 25,53% des extraits à l'hexane du bois cœur (Tableau 2). Le composé majoritaire dans l'extrait à l'éther de pétrole est le rimuene d'abondance relative 5,20% (Tableau 1). Cet extrait possède parmi ses composés non identifiés, un pic majoritaire (81,77%) qui est élué au bout de 62,3minutes.

Tableau 1: Composés extraits de l'écorce, de l'aubier et du bois de cœur d'Okoumé à l'Ether de pétrole et identifiés par chromatographie en phase gazeuse.

Ecorce					
N° de pic	Temps de rétention	Indice de Kovats	Nom du composé	Formule brute	Abondance relative (%)
1 2	12,19	1013,68	m-cymène α-terpinène	$C_{10}H_{14}$ $C_{10}H_{16}$	32,57
3	16,46	1084,48	2-nonanol	$C_9H_{20}O$	18,80
4	21,87	1166,42	1-phényléthyl acétate	$C_{10}H_{12}O_2$	05,71
5 6	33,13	1331,98	nepetalactone (isomère) eugénol	$C_{10}H_{14}O_2$ $C_{10}H_{12}O_2$	00,55
7 8 9 10	35,24	1299,99	n-tridecane méthylo-méthoxybenzoate méthyldecanoate dihydronaginataketone	$C_{13}H_{28}$ $C_9H_{10}O_3$ $C_{11}H_{22}O_2$ $C_{10}H_{14}O_2$	04,56
11 12	43,86	1495,77	hinesene eudesma-2,4(15) ,11-triene	$C_{15}H_{24}$ $C_{15}H_{22}$	01,00
13	56,46	1767,36	(z)-g-curcumylacétate	$C_{17}H_{26}O_2$	01,08
14 15	57,22	1793,62	7-acetoxyelema-1,3-dièn-8-ol (z)-nuciferylacétate	$C_{17}H_{28}O_3$ $C_{17}H_{24}O_2$	00,19
16	61,24	1973,02	***	***	06,92
17	63,49	***	***	***	34,00
18	64,38	***	***	***	02,36
19	64,50	***	***	***	04.58
Aubier					
N° de pic	Temps de rétention	Indice de Kovats	Nom du composé	Formule	Abondance relative (%)
20	10,17	0963,12	cis-pinane	$C_{10}H_{18}$	08,83
21	59,23	1877,98	2-acetoxyfuranoelemene	$C_{17}H_{22}O_3$	59,79
22	59,93	1908,86	Rimuene	$C_{20}H_{32}$	02,00
23	62,18	***	***	***	19.89
24	66,47	***	***	***	09,50

Cœur					
N° de pics	Temps de rétention	Indice de Kovats	Nom du composé	Formule	Abondance relative (%)
25	10,10	0961,21	1-octen-3-ol	C_8H1_6O	02,91
26 27	56,74	1777,14	méthylnorpinguisonate drim-8-en-7-one	$C_{15}H_{18}O_4$ $C_{15}H_{24}O$	00,43
28	59,87	1905,91	rimuene	$C_{20}H_{32}$	05,20
29	62,30	***	***	***	81,77
30	63,19	***	***	***	00,78
31	63,63	***	***	***	02,450
32	66,28	***	***	***	06,460

On note toutefois la présence de quelques corps gras. C'est le cas de l'écorce qui possède dans son extrait à léther de pétrole du décanoate de méthyle qui apparait dans un mélange de pics élués à 35,24 min (Tableau1). L'extrait à l'hexane de l'écorce possède cependant un corps gras en C_{14} qui apparait à 47,22 min, c'est du méthyl-2-dodécanoate de méthyle.

Tableau 2: Composés extraits de l'écorce, de l'aubier et du bois de cœur d'Okoumé en utilisant l'hexane comme solvant et identifiés par chromatographie en phase gazeuse.

			Ecorce		
N° de pic	Temps de rétention	Indice de Kovats	Nom du Composé	Formule	Abondance relative (%)
33	14,559	1052,96	trans-sabinene hydrate	$C_{10}H_{18}O$	04,04
34	47,22	1550,37	méthyl 2-méthyldodecanoate	$C_{14}H_{28}O_2$	09,77
35	53,95	1686,99	6a-hydroxygermacra-1(10), 4-diene	$C_{15}H_{26}O$	02,46
36	59,70	1897,83	6b-acetoxyeudesm-4(15)-en-7b-ol	$C_{18}H_{30}O_2$	01,95
37	62,53	***	***	***	03,43
38	63,82	***	***	***	00,74
39	64,40	***	***	***	07,52

40	64,91	***	***	***	58,42
41	66,24	***	***	***	01,68

			Aubier		
N° de pic	Temps de rétention	Indice de Kovats	Nom du composé	Formule	Abondance relative (%)
42 **43** **44**	09,50	942,55	β-citronellence diméthyletrisulfide (E)-2-heptenal	$C_{15}H_{18}$ $C_2H_6S_3$ $C_7H_{12}O$	00,17
45 **46**	10,40	0970,35	3-octanone n-butyle butyrate	$C_8H_{16}O$ $C_8H_{16}O_2$	11,00
47	19,29	1128,60	citronellal	$C_{10}H_{18}O$	01,79
48	43,00	1482,53	oxyde de veticadine	$C_{15}H_{24}O$	41,57
49 **50**	49,21	1582,74	trans-dracunculifoliol thujopsan-2α-ol	$C_{15}H_{26}O$ $C_{15}H_{26}O$	00,50
51	56,82	1780,11	3α-acetoxyamorpha-4,7(11)-diène	$C_{17}H_{26}O_2$	00,39
52	59,92	1908,46	rimuene	$C_{20}H_{32}$	00,96
53	62,38		***	***	14,39
54	63,68		***	***	00,42
55	66,55		***	***	04,78

			Cœur		
N° de pic	Temps de rétention	Indice de Kovats	Nom du composé	Formule	Abondance relative (%)
56	10,22	0964,73	Octan-2-one	$C_8H_{16}O$	25,53
57	19,15	0602,50	***		05,47
58 **59** **60** **61**	41,59	1460,61	(E)-1,2-diméthoxy-4-propenylbenzene β-ionone époxyde selina-3,7-diène rotundene	$C_{11}H_{14}O_2$ $C_{13}H_{20}O_2$ $C_{15}H_{24}$ $C_{15}H_{24}$	48,00
62	62,04		***	***	08,08
63	66,32		***	***	12,82

L'utilisation d'une méthode d'étalonnage adaptée à l'identification des corps gras de chaines supérieures à 14 atomes de carbone, avec une gamme étalon adéquate semble nécessaire pour une meilleure identification des corps gras présents dans l'écorce, l'aubier et le cœur d'Okoumé.

Chapitre 2

Extraction, identification et caractérisation des tanins d'Okoumé : optimisation

I. Présentation :

Cette partie décrit la répartition des polyphénols dans les trois parties du bois en utilisant principalement trois méthodes, à savoir : la spectroscopie de masse MALDI-ToF, la spectroscopie infrarouge et la chromatographie liquide en phase gazeuse couplée à la spectrométrie de masse.

Le premier article a permis une optimisation de la méthode de séparation chromatographie sur des extraits de l'écorce. Initialement, en se basant sur la méthode de séparation des polyphénols décrite par Charrier (1992), il s'est avéré que la séparation des polyphénols d'Okoumé entre 40 et 60 minutes n'a pas donné les résultats espérés. Il a ainsi été developpé une méthode de séparation adaptée à l'Okoumé qui a permis d'obtenir une séparation optimale entre 40 et 60 minutes.

Le second article s'est appuyé sur la méthode optimisée pour analyser des extraits des autres parties du bois, à savoir l'aubier et le bois de cœur.

II. The condensed tannins of Okoume (*Aucoumea klaineana* Pierre): A molecular structure and thermal stability study

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II.A. Résumé :

Afin de promouvoir des stratégies pratiques de valorisation de l'industrie du contreplaqué et des déchets de scierie d'*Aucoumea klaineana* Pierre (Okoumé) dans les domaines des adhésifs et des composites, la teneur totale en phénol de l'écorce, de l'aubier et du bois de cœur d'Okoumé a été mesurée. La structure moléculaire des tanins extraits de l'écorce a été déterminée par spectrométrie de masse par désorption / ionisation au laser assistée par matrice (Maldi-ToF) et par spectroscopie infrarouge à transformée de Fourier (FTIR). La teneur totale en phénol présentait une différence significative ($p = 0,001$) entre l'écorce, l'aubier et le bois de cœur, qui diminuait comme suit : 6 ± 0,4, 2 ± 0,8 et 0,7 ± 0,1%, respectivement. La teneur en pro-anthocyanidines était également significativement différente ($p = 0,01$) entre les trois déchets de bois : l'écorce était la plus riche en tanins condensés (4,2 ± 0,4%) comparée à l'aubier (0,5 ± 0,1%) et au bois de cœur (0,2 ± 0,2%). La spectroscopie de masse couplée à la chromatographie liquide (LC-MS) et l'analyse Maldi-ToF de l'écorce ont montré pour la première fois que les tanins condensés de l'Okoumé sont composés des monomères glycosylés de type fisétinidine, gallocatéchine et trihydroxyflavane. Aucune unité libre de catéchine ou de robitinidine n'a été détectée, tandis que des dimères distinctifs

dihydroxy ou trihydroxyflavan-3-benzoate ont été observés dans les extraits de tanins condensés étudiés. L'analyse FTIR a montré la présence de glucanes et de mannanes dans les tanins condensés et la Maldi-ToF a permis de mettre en évidence une structure oligomérique de type (deux fisétinidines et un trimère de gallocatéchine) glycosylées par dix unités glucidiques. La condensation de ces polyphénols avec du formaldéhyde a conduit à des indices de Stiasny de 83,3, 73,3 et 53,3% pour l'écorce, l'aubier et le bois de cœur, respectivement.

II.B. Abstract

In order to promote convenient strategies for the valorization of *Aucoumea klaineana* Pierre (Okoume) plywood and sawmill wastes industry in the fields of adhesives and composites, the total phenolic content of Okoume's bark, sapwood and heartwood was measured. The molecular structure of tannins extracted from the bark was determined by Matrix Assisted Laser Desorption/Ionization Time-Of-Flight (Maldi-ToF) mass spectrometry and Fourier transform infrared spectroscopy (FTIR). The total phenolic content displayed significant difference (p=0.001) between the bark, sapwood and heartwood which decreased as follows: 6±0.4, 2±0.8 and 0.7±0.1% respectively. The pro-anthocyanidins content was also significantly different (p=0.01) among the three wood wastes, and the bark was the richest in condensed tannins (4.2±0.4%) compared to the sapwood (0.5±0.1%) and heartwood (0.2±0.2%). Liquid chromatography coupled mass spectroscopy (LC-MS) and Maldi-ToF analysis of the bark showed for the first time that Okoume condensed tannins are fisetinidin, gallocatechin and trihydroxyflavan based monomers and complex polymers obtained with glycosylated units. No free catechin or robitinidin units were detected whereas distinctive dihydroxy or trihydroxyflavan-3-benzoate dimers were observed in the investigated condensed tannin extracts. FTIR analysis showed the occurrence of glucan and mannan like sugars in the condensed tannins, and Maldi-ToF highlighted that these sugars should account for ten glycosylated units chemically bonded with two fisetinidins and one gallocatechin trimer. The condensation of these polyphenols with formaldehyde led to Stiasny numbers of 83.3, 73.3 and 53.3% for the bark, sapwood and heartwood, respectively.

Keywords: Okoume, tannins, FTIR, LC-MS, Maldi-ToF, Stiasny number, TGA, DSC.

II.C. Introduction

Okoume is a tropical hardwood from central Africa and mainly located in the Republic of Congo and Gabon. That wood species represents about 130,000,000 m^3 (~31%) of the 400,000,000 m^3 of Gabon forest total reserve[1]. According to Nze Nguema[2], approximately 1,500,000 m^3 of logs are transformed each year in Gabon according to the following distribution : swans (825 000 m^3), veneers (465,000 m^3), plywood (199,500 m^3) and trenching (10,500 m^3). The wood wastes from the timber industry of Gabon estimated to 750,000 m^3/year[1] should be dominated by Okoume which account for 31% of harvested wood in Gabon. Nevertheless, the real content of unexploited Okoume bark, sapwood and heartwood wastes abandoned in forest by companies remained unknown. But a recent study which estimated the added value of unexploited forks to 120,000 FCFA/m3[3] showed the strong financial potential of the 232. 500 m^3 wood wastes produced per year in Gabon[2] . However, 95% of these wood wastes which are unluckily open-air burned or used as energy source for industries could receive a better attention for a suitable valorization.

Therefore, the oleoresin extracted from the trunk of Okoume and used by local populations of Central Africa for several decades as substitute for incense and treatment of wounds[4] exhibited the medicinal properties of Okoume volatile compounds. In modern medicine, Okoume oleoresin was used for skins, nails and hair treatment according to Delaveau and Tessier[5], whereas Delaveau[6] mentioned some bactericide properties of that oleoresin against *Escherichia coli*, and the authors attributed this trend to the presence of particular phenolic compounds in Okoume essential oil. That essential oil exhibited antiradical and antioxidant activities[7] which led to a patent of Okoume essential oils for cosmetic, pharmaceutical and dermatologic applications[8]. Moreover, the works of Rhourri-Frih et al[9] revealed the presence of high content of α and β-amyrin in Okoume essential oil.

Regarding Okoume sapwood and heartwood wastes, many researchers proposed alternatives to bring added value on these lignocellulosic materials as composites. Therefore, Bakraji et al[10] proposed an Okoume/polyacrylamide composite with high tensile strength. Other authors investigated the interfacial bonding between the hydroxyl groups of some tropical hardwoods sawdust and succinic anhydride molecules. They found that Okoume heartwood, holocellulose and cellulose chains were weakly esterified by succinic anhydride[11,12]. This hardwood cellulose displayed short fibers length which tend to aggregate in organic solution, showing the capability of Okoume heartwood wastes to be used as cellulose esters with good micromechanic and microcrystalline stability[12]. Recent study exhibiting significant differences

between Okoume sapwood and heartwood facing enzymatic hydrolysis by *Trichoderma reesei* revealed the potential of that hardwood wastes to be used in biorefinery. However, the differences observed between Okoume sapwood and heartwood should to be controlled by their extractives nature and composition[13].

Despite the abundant literature cited above, the phenolic extracts content and the condensed tannins structure of Okoume have received a little attention except the work of Mounguengui et al[14] who investigated the phenolic content of Okoume heartwood.

The aim of this study was to investigate the phenolic content of the bark, sapwood and heartwood of Okoume while the molecular structure of the bark condensed tannins extracts was studied by Maldi-ToF, LC-MS and FTIR. An attempt was made for the utilization of the condensed tannins in adhesive formulation.

II.D. Materials and methods

II.D.1. Samples.

The bark, sapwood, and heartwood were collected from two Okoume trees of around 80 years old. We collected samples on a section disk of 10 cm of thickness and 85 cm of diameter. The wood was harvested at Nzamaligue in the West of Gabon by the SED (Société Equatoriale de Déroulage) in February 2016. The fresh samples were put in sterilized bags protected from light, air-dried for one week in a refreshed laboratory and oven-dried (105°C) for 48 h. The dried samples were grinded to pass through 180 mesh (≈1mm diameter) with a rotative knife grinder (Retsch SK1).

All the chemicals used in this study were purchased from Fisher Scientific and Sigma Aldrich. Solvents and reactants were used without further purification.

II.D.2. Extraction of polyphenols at room temperature.

350 mg of dried wood powder (M_0) from bark, sapwood and heartwood samples were mixed separately at room temperature with 30 ml of acetone/water solution (7:3, v/v). The mixtures were stirred for 3 h. The supernatant was recovered and acetone evaporated. Each extraction was repeated four times per biological sample (bark, sapwood and heartwood) collected in a tree. However, the extraction was performed on two trees.

II.D.3. Total phenolic content measurement.

The total phenol content was determined by the Folin-Ciocalteu method[15] as follows: 1 ml

of bark, sapwood and heartwood extracts were diluted with 9 ml of distilled water, and 0.5 ml of the diluted extracts was poured into 2 ml of Folin-Ciocalteu reagent (1/10, v/v in distilled water). Then, 2.5 ml of sodium carbonate solution (0.7M) was added to the Folin-Ciocalteu reagent containing the extracts. The final solution obtained was placed into test tubes and left for 5 min in a water bath maintained at 50°C. The absorbance was registered at 760 nm. The results were expressed as gallic acid equivalent (GAE), based on the extracted dry powder amount. The total phenol content was determined according to the following equation 1:

$$Total\ phenol\ (\%) = \frac{C \times D \times V}{1000 \times M_0} \times 100 \qquad (1)$$

C: Total phenol concentration (ppm). D: degree of dilution (10). V: volume of starting solution (30 ml). M_0: mass of dry sawdust (mg)

II.D.4. Determination of proanthocyanidin content

II.D.4.a. Anthocyans measurement by the acid hydrolysis in butanol method.

5 ml of ferrous sulfate solution obtained by dissolving 77 mg of $FeSO_4 \cdot 7H_2O$ previously poured into 500 ml of HCl (37%) in nBuOH (2/3, v/v) were added to 0.5 ml of dried aqueous extracts from bark, sapwood and heartwood extracts. The mixtures obtained were placed for 15 min in a water bath maintained at 95°C. After cooling, the absorbances were recorded at 530 nm and the results were expressed as cyanidin equivalent based on the dried wood extracts content. Proanthocyanidin (PA) was determined[27] according to the following equation 2:

$$[PA] = \frac{A \times V \times D \times V' \times M}{\varepsilon \times v \times m} \times 100 \qquad (2)$$

PA: Proanthocyanidins content (mg cyanidin Equivalent/g dry weight expressed as mg cyaE/g bark); V: volume of reaction (ml). D: dilution factors (10). V': volume of the aqueous extract recovered after extraction with diethyl ether (ml). M: cyanidin molar mass (287g/mol). v: volume of the sample (ml). A: absorbance of the sample. m: mass of dry sawdust samples (g). ε: molar extinction coefficient ($34700 M^{-1} cm^{-1}$) according to Scalbert et al [17].

II.D.4.b. Condensed tannins measurement by the acid condensation of vanillin method.

Condensed tannins in an acid medium were measured according to the vanillin condensation method described by [18] as follows: 0.5 ml of aqueous extracts from bark, sapwood and heartwood contained in a tube were mixed with 3 ml of vanillin reagent dissolved in methanol (4%, w/v). Then, 1.5 ml of concentrated HCl (37%) was added, and the mixture was kept in the dark at 20°C for 15 min. Absorbances were registered at 500 nm. The results obtained were expressed as catechin equivalence (CE) based on the amount of dry extracted samples. The calibration was carried out using an aqueous solution of catechin (30 mg/l). However, the standard solutions contained 0; 15; 25; 35; 45; 55; 65; 75; 85; 95 and 100 ppm of catechin concentration.

II.D.5. Extraction of tannins for thermal and Stiasny number analysis.

Tannin extraction from Okoume bark was carried out in water containing 5% of sodium hydroxide, 0.25% of sodium sulfite and 0.25% of sodium bisulfite, with a sample-to-water ratio equal to 1/9. The bark sawdust was immersed in water maintained under magnetic stirring for 3 h at 80°C. The tannin extracts obtained were filtered and oven-dried at 50°C.

II.D.5.a. Stiasny number determination.

The reactivity of extracts with formaldehyde was determined by measuring the Stiasny number (SI) as described by Voulgaridis et al[19]. A solution of extract of concentration 4g/l was prepared. 25 ml of this solution was put in round bottom flask and 5 ml of formaldehyde 37% and 2.5 ml of HCl 10M were added. The mixture was heated under reflux for 30 min. The residue was filtered through sintered glass n°2 or 4. The precipitate was washed with water and dried at 105°C until constant weight. The reactivity was calculated with the formula below (3):

$$SI=\frac{A}{B}\times 100 \qquad (3)$$

With SI: Stiasny number. *A*: dry weight of precipitate (mg). *B*: dry weight of extracts (mg).

II.D.6. Liquid Chromatogrphy-MassSpectrum analysis.

350 mg of dried wood powder from bark samples were mixed separately at room temperature with 30 ml of methanol/water solution (ratio 4:1, v/v). After 3 h of stirring, the supernatant was recovered and then was analyzed by LC-DAD (Ultimate 3000, Thermo Scientific), equipped with an Acclaim Polar Advantage II (Thermo Scientific) in methanol (A)/water (B) gradient mode at 1 ml/min (injection volume 10 µl). The gradient elution program was set as

follows: 0-9 min. (95% B), 9-16 min. (75% B), 16-25 min. (60% B), 25-35 min. (50% B), 35-52 min. (0.0% B), 52-62 min. (95% B). Then, the elution gradient was linearly ramped down to 60% A for 2 min and so maintained for 9 min to condition the column for the next injection. The column was connected to an electrospray hybrid linear ion Orbitap mass analyzer (LTQ Orbitrap Velos, Thermo-Fisher, Bremen, Germany). The electrospray spray voltage was 3.8 kV. The LC-MS analysis was used in negative ion detection mode. Data were analyzed with Xcalibur software.

II.D.7. Maldi-ToF of bark analysis.

The acetone/water tannin extracts from Okoume bark were oven-dried at 105°C for 24 h and dissolved in acetone/water (1:1, v/v) solution up to 7.5 mg/ml. To increase the ion formation, 1.5 µl of NaCl solution (0.1M) in a methanol/water mixture (1:1, v/v**)** was added and placed on the Maldi target. The sample solution and the matrix were then mixed in equal amounts, and 1.5 µl of the resulting solution was placed on the Maldi target. A matrix of 2,5-dihydroxy benzoic acid was used. Red phosphorous (500–3000 Da) was used as reference for spectrum calibration. Finally, after evaporation of the solvent, the Maldi target was introduced into the spectrometer. The spectra were recorded on a KRATOS AXIMA Performance mass spectrometer from Shimadzu Biotech (Kratos Analytical Shimadzu EuropeLtd., Manchester,UK). The irradiation source was a pulsed nitrogen laser with a wavelength of 337 nm. The length of one laser pulse was 3 ns. Measurements were carried out using the following conditions: polarity-positive, flight path linear, 20 kV acceleration voltages, 100–150 pulses per spectrum. The delayed extraction method was used applying delay times of 200–800 ns. The software Maldi-MS was used for the data treatment and analysis.

II.D.8. Fourier transformation infrared spectroscopy (FTIR).

The acetone/water tannins extracted from Okoume bark were characterized by FTIR analysis. The samples were oven-dried at 105°C for 24 h prior to any analysis and 5 mg of dried tannins powder were place in the crystal device, and the contact was obtained by 150 N applied strength on the sample. 32 scans were used with a resolution of 4cm^{-1} in the range 4000–600cm^{-1}. All the FTIR were carried out in ATR (attenuated total reflection) single bounce mode with a Perkin Elmer Frontier spectrophotometer equipped with a diamond/ZnSe crystal for tannins analysis. The spectra were collected and analyzed using Spectrum software (Perkin Elmer).

II.D.9. Thermogravimetric analysis (TGA analysis).

Thermal decomposition was performed using a TA Instrument (TGA Q50 instrument). The

temperature program was from 25 to 600°C at a heating rate of 10°C/min. the measurement were conducted under nitrogen (40 ml/min). The results were analysed with TA Instruments Universal Analysis 2000 software.

II.D.10. Differential scanning calorimetry (DSC).

The differences in heat exchange between an analysis sample and a reference were carried out on a DSC Q20 (TA instruments) equipped with a rapid cooling system. Samples of Okoume tannins were weighed (~ 7mg) in standard aluminium pans (TA Instruments) and data acquisitions were carried out using the Universal Analysis 2000 program (TA Instruments). The measurements were conducted under nitrogen (40 ml/min) with a standard heating rate of 10◦C/min from room temperature to 400◦C.

II.D.11. Statistical analysis.

All experiments were repeated four times for each part of the wood selected in a tree and the total number of experiments was N=8 for the two trees. We analyzed differences between tissues (sample types) using an ANOVA and a Turkey HSD test with Statview software. Pairwise comparisons were made using Tukey HSD test at the 5% level of significance.

II.E. Results and discussion

II.E.1. Total phenolic content.

The amount of phenolic compounds obtained from Okoume was shown in Table 1. The ANOVA test showed a significant difference (p=0.001) between the phenolic content of the bark, sapwood and heartwood. The bark was the richest in phenolic compounds (6±0.35% of dry wood) while the heartwood was the least abundant (0.74±0.35% of dry wood). Nevertheless, the phenolic content found for the heartwood was close to that published by Mounguengui et al [14] who obtained 0.64±0.05% (of dry wood) of phenolic compounds in Okoume heartwood. Furthermore, the phenolic content obtained for Okoume's bark was higher than that found for the bark of *Khaya ivorensis* [African mahogany] (1.54±0.39% of dry wood) treated in the same experimental conditions [20]. No significant difference was found for their sapwoods phenolic content whereas the African mahogany's heartwood, had a greater phenolic content (1.79±0.11% of dry wood) than Okoume's one.

II.E.2. Condensed tannins content

II.E.2.a. Catechin and proanthocyanidins equivalent content.

The results obtained from the vanillin assay are listed in Table 1. They showed a significant difference (p=0.01) between the condensed tannins content bearing a hydroxyl group at the "meta" of the flavanol unit of the bark, sapwood and heartwood of Okoume. That result which highlighted a high content of anthocyan in the bark was corroborated by those obtained from the ButOH-HCl assay indicating the high content of Okoumes's bark in pro-athocyanidin type condensed tannins. Nevertheless, the dominating condensed tannins content in Okoume bark agreed with that ascertained by Stevanovic and Perrin[21] who defined tannins as polyphenolic compounds present in plants, and the bark of trees may have the highest amount. Moreover, this study has shown the strong condensed tannins content of Okoume's bark (40.19±3.6 mgCE/g of dry bark) compared to *Pinus pinaster* (2.18±0.57 mgCE/g of dry)[22] bark to produce tannins in commercial quantity.

Table 1. Total phenolic, anthocyan (vanillin assay) and pro-anthocyanidin (ButOH-HCl assay) content expressed in % of dry weight and Stiasny number of Okoume expressed in %

Okoume type sample	Total phenolic content (% of dry weight)*	Vanillin assay (% of dry weight)*	ButOH-HCl assay (% of dry weight)*	Stiasny Number (%)*
Bark	6 ± 0.4^{a}	4.2 ± 0.4^{d}	0.6 ± 0.1^{g}	83.3 ± 11.6^{j}
Sapwood	2 ± 0.8^{b}	0.5 ± 0.1^{e}	0.3 ± 0.04^{h}	$73.3\pm15.3^{j,k}$
Heartwood	0.7 ± 0.1^{c}	0.2 ± 0.2^{f}	0.1 ± 0.2^{i}	53.3 ± 11.6^{k}

N=4, represents the number of replicates extraction per sample type from each part of wood (bark, sapwood, heartwood)

*:Means±SD

In a column, value with the same letter was not statistically different at α=0.005 level

II.E.3. LC-MS, Maldi-ToF and FTIR analysis

LC-MS is an analytical method probed to identify and quantify phenolic compounds in plants[23–25], and was used to characterize the phenolic moieties including flavonoid structures of Okoume extracts. The ionization of oligomer structures led to mass fragments of main monomers present in Okoume condensed tannins which appeared in the ionized $[M-H]^-$ form. The results obtained with an output predominance around 40-60 and 60-85 minutes were collected (Figs. 1-2) and compared to those displayed by Maldi-Tof analysis for a further identification of major monomers or oligomers from Okoume extracts. The molecules were identified using MS spectra.

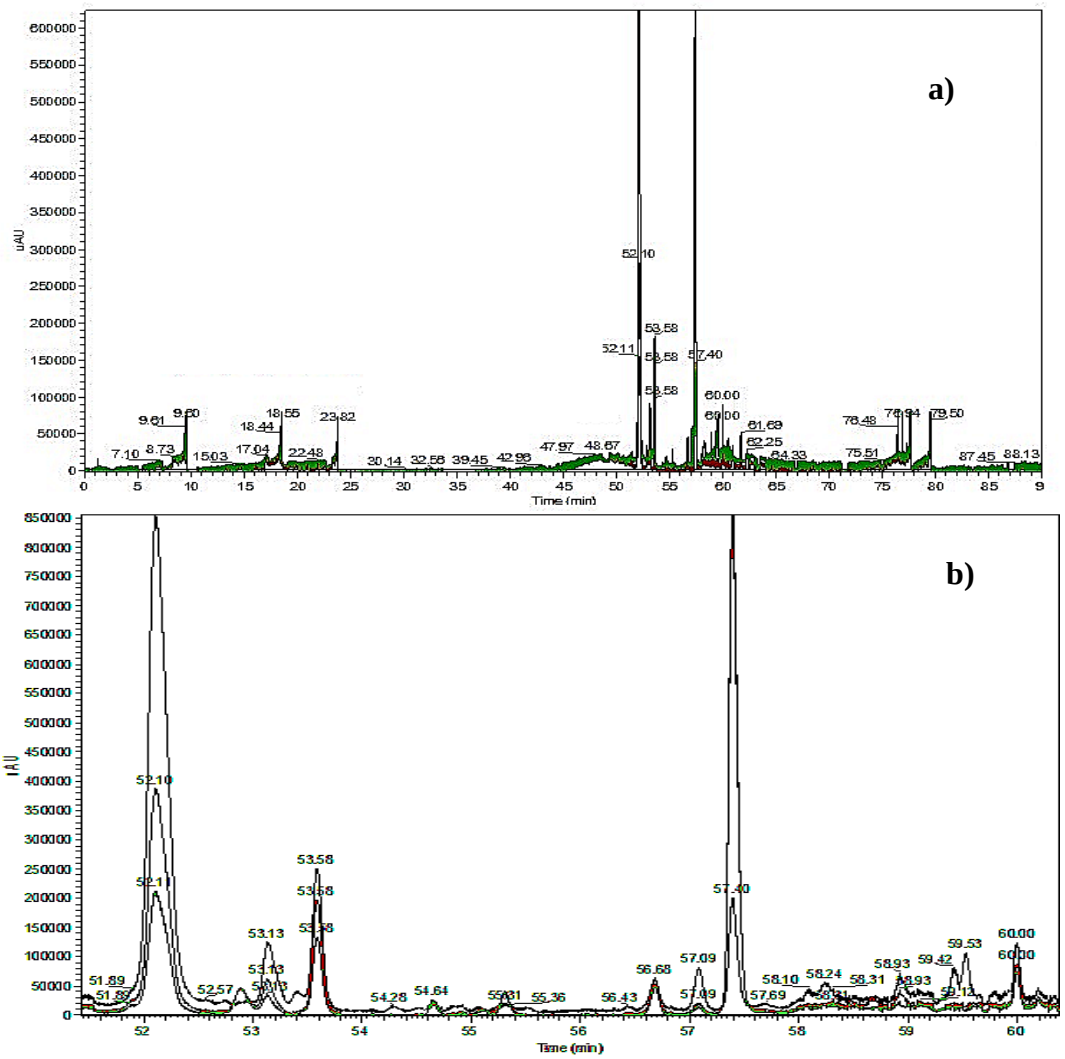


Figure 1. *LC-MS spectra of Okoume bark tannins presented at three different overlaying wavelengths (220=balck;254=red and 280 nm=green. Detected with a Diode Array Detector (DAD) at retention time (a= 0-90 mm; b=50-60 mm)*

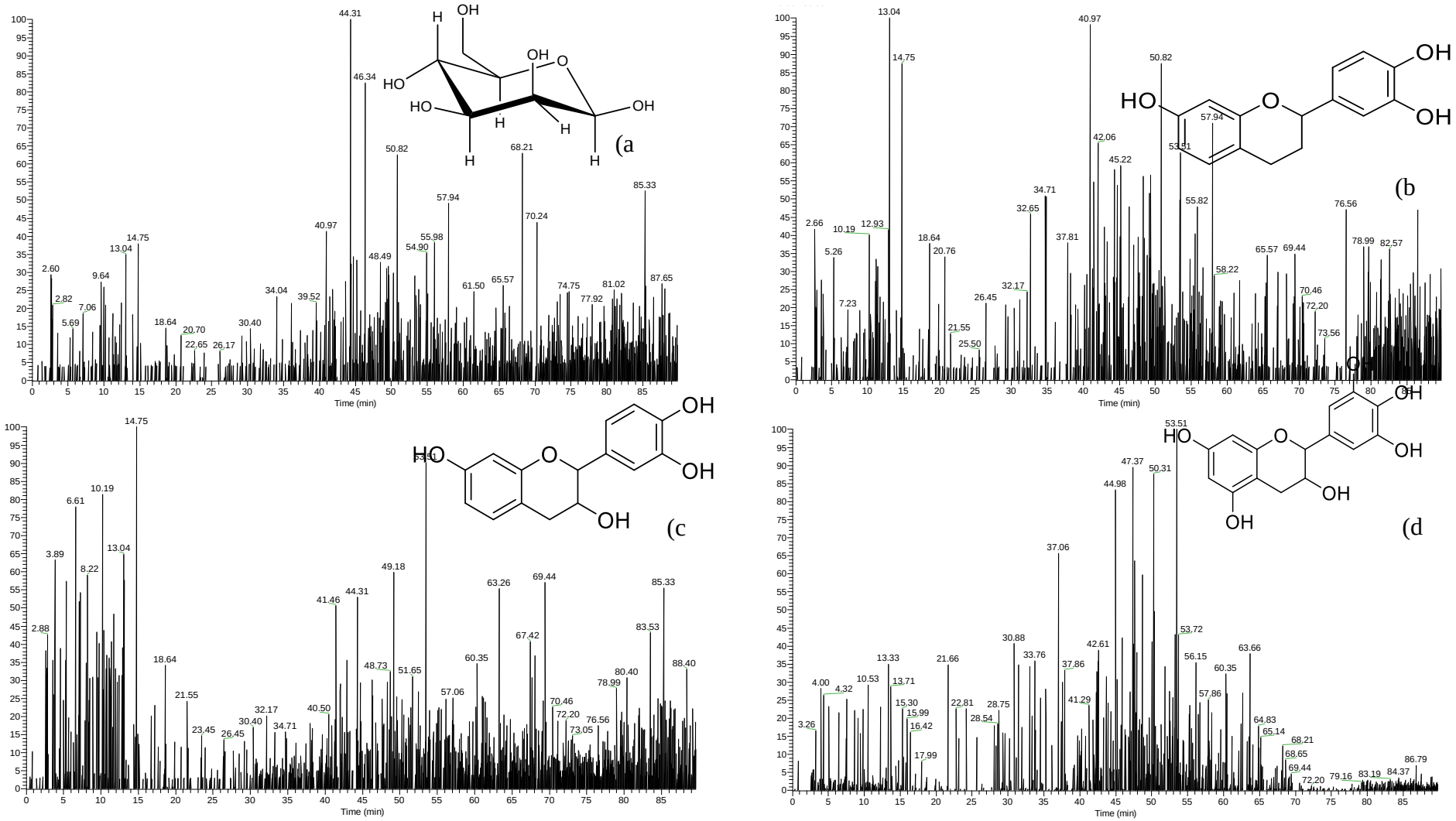
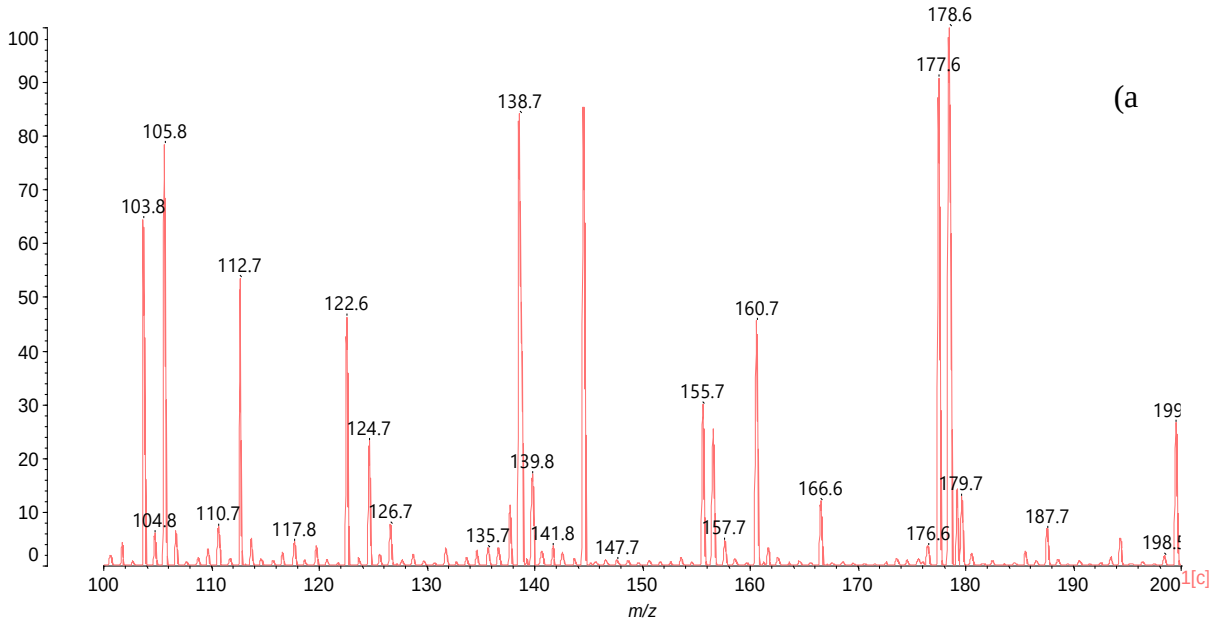


Figure 2. *Mass fragments of the main monomers (a=glucose; b=trihydroxyflavan-3-ol; c=fisetinidin; d=gallocatechin).*

II.E.3.a. Sugar analysis of tannin extracts.

The sugars released in the condensed tannin bark extracts were studied in the range 100-200 Da of Maldi-ToF spectrum (Fig. 3a). It was noteworthy that Okoume's bark displayed peak at 126.7 Da relates to a cleavage mechanisms involving the glucose ring as previously found by Ricci et al[26] in the fragmentation pattern related to polygalloylglucose structures. The presence of glucose or other hexose units should be corroborated by the strong peak at 178.6 Da and the series of peaks at 177.6 and 179.7 Da from glucose or other hexose degradation [11,13,20]. The presence of glucose in Okoume bark extracts was supported by LC-MS results (Fig. 2a) which exhibited masses fragments at 179.9/180 Da assigned to glucose monomer[27]. However, the peaks at 177.6 and 179.7 Da (Fig. 3a) assigned to glucose which has lost 2*x*1H during the Maldi-ToF process[20] should also be attributed to galactose or mannose for *m/z*=180 suggesting, therefore, the occurrence of various sugar units in Okoume bark condensed tannins. Although glucose accounted for the most abundant neutral sugars of Okoume wood[11,12]; recent study showed the presence of strong proportion of water soluble pectin structures like galactose and mannose among the neutral sugars of steam exploded Okoume sapwood. Hence, the occurrence of other sugars than glucose in the acetone/water condensed tannins of the bark cannot completely be ruled out.



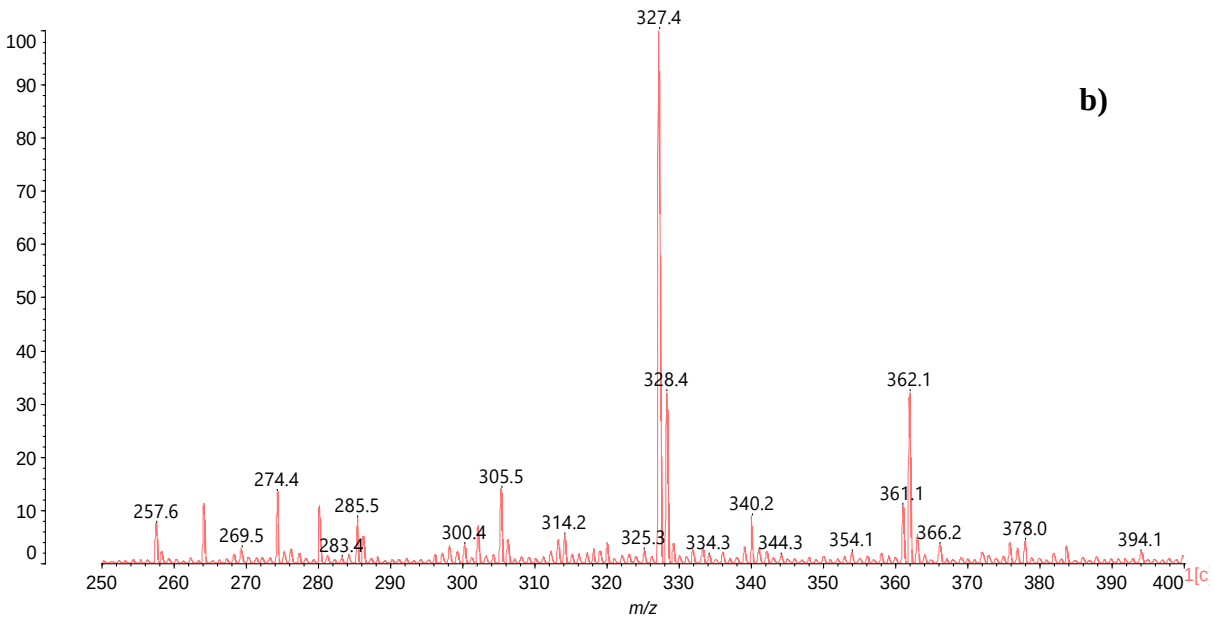


Figure 3. *Maldi-ToF spectrum of Okoume bark tannin extracts in the range 100-200 Da* (a) and 250-400 Da (b).

In addition, the possible mixing of glucose, galactose and mannose as oligosaccharides in Okoume condensed tannins bark was supported by FTIR spectroscopy. A strong signal arising at 975.77 cm^{-1} assigned to glycosidic linkage of mannans[28,29] and C-O stretching (ν_{C-O}) of galactose[29] as well as the one at 880.7 $cm^{-}1$ ascribed to H in the equatorial direction at the position 2 of acetylated mannose[29] agreed with the presence of mannose and galactose units in those condensed tannins. On the other hand, the sharp peak at 1111.6 cm^{-1} assigned to C-OH deformarion of β(1→3)glucanes[28] is in agreement with the presence of glucose units in Okoume condensed tannins. Nevertheless, that peak could also be assigned to ν_{C-O} of exocyclic galactosyl units ring appearing at 1112 $cm^{-}1$[30] which suggested that galactose cannot be ruled out among the sugars bonded to the condensed tannins extracted from Okoume bark. Therefore, the shoulder at 781.1cm^{-1} assigned to β-D-glycopyrannoside structure as stated by Kato et al[29] indicated that oligosaccharide chains should be linked to the condensed tannins extracted from Okoume bark. The peak of weak intensity at 135.7 Da resulting from deoxyribose moiety[20] showed a low content of that sugar in the condensed tannins extracted from Okoume's bark (Fig. 3a).

II.E.3.b. Polypenols analysis of acetone/water extracts.

The acetone/water extracts of Okoume's bark reminded typical structures of condensed tannins as shown in the spectrum collected in Fig. 3b and the corresponding indexations listed in Table 2 and 3. Signals in the range 250-400 Da shown a peak at 274.4 Da previously assigned to fisetinidin (A) in other wood or lignocellulosic materials[16,31] while gallocatechin monomer (D) appeared at 305.5 Da. The presence of these compounds agreed with these obtained by LC-

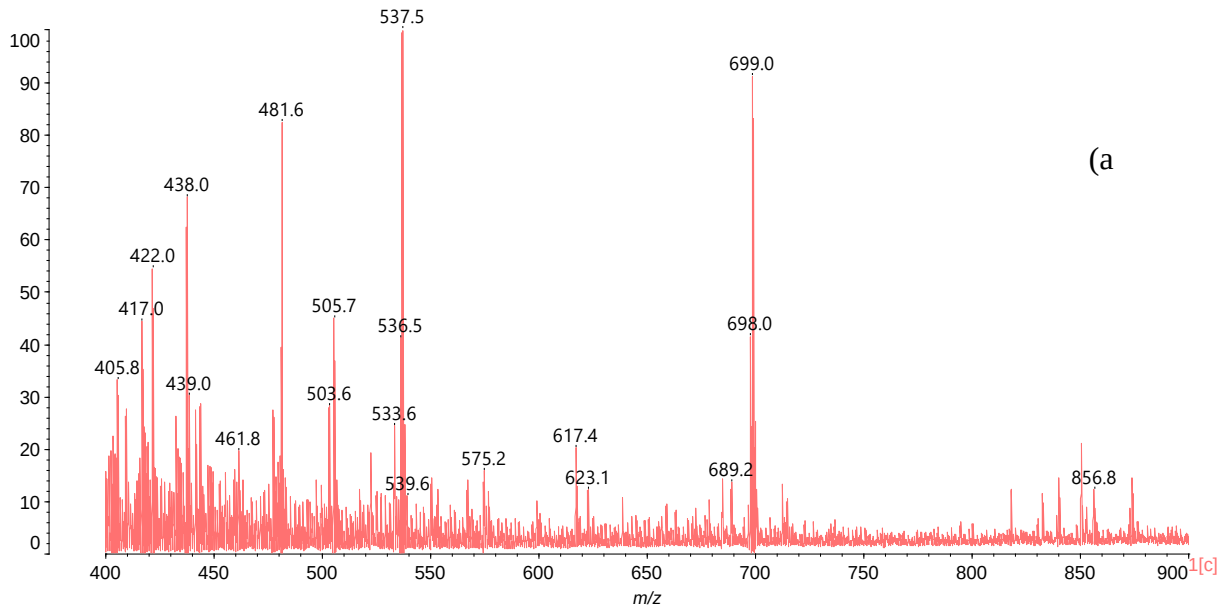
MS where they appear in ionized form $[M-H]^-$ of *m/z*=272 and 304 Da for fisetinidin and gallocatechin, respectively[32,33] (Fig. 2). Any evidence of catechin/robitinidin monomer was found at *m/z*=290 Da in the Maldi-ToF spectra of Fig. 3b. These results agreed with the presence of condensed tannins in Okoume bark as shown by the vanillin acid method. In addition, signal at 257.6 Da assigned to trihydroxyflavan-3-ol (F) in other tannins and accounting for monomers released by LC-MS at $[M–H]^-$=257 Da[34] supported that the acetone/water extracts of the bark are composed with fisetinidin, gallocatechin and trihydroxyflavan-3-ol condensed tannin monomers (Fig. 2).

Table 2. Distribution of polyflavonoid oligomers of acetone/water condensed tannin extract of Okoume bark by Maldi-ToF

Experimental *m/z* (Da)	**Calculated *m/z* (Da)**	**Type unit**	**Oligomer type**
166.6	170.10	E(gallic acid)	Monomer
179.97	180.15	Gly(Glycosyl unit)	Monomer
257.6	258.3	F_1 (trihydroxyflavan)	Monomer
274.4	274.3	A_1(Fisetinidin)	Monomer
305.5	306.3	D_1(Gallocatechin)	Monomer
340.2	342.2	Gly_2 (Cellobiose)	Dimer
362.1	362.0	G_1H_1(Dihydroxyflavan-3-p-hydroxybenzoate) or F_1I_1(Trihydroxyflavan-3-benzoate)	Dimer
405.8	404.0	A_1Gly_1	Dimer
422.0	420.0	F_1Gly_1	Dimer
438.0	436.0	Q_1E_1(Epicatechin-3-gallate)	Dimer
452	452	A_1Gly_1	Dimer
481.6	480.0	P_2 (Dihydroxyflavan)	Dimer
533.6	530	F_1A_1	Dimer
575.2	574.6	A_1D_1	Dimer
617	616.38	R_1E_1 (Isoquercetin-gallate)	Dimer
850.7	850.9	A_2D_1	Trimer
	851.9	P_1D_2	Trimer
1012.6	1012	A_2DGly_1	Tetramer
1174.4	1174	A_2DGly_2	Tetramer
1336.2	1336	A_2DGly_3	Pentamer

1498.1	1498	A_2DGly_4	Hexamer
1660.1	1660	A_2DGly_5	Heptamer
1822.2	1822	A_2DGly_6	Octamer
1984.4	1984	A_2DGly_7	Nonamer
2146.6	2146	A_2DGly_8	Decamer
2309.0	2308	A_2DGly_9	Undecamer
2471.0	2470	A_2DGly_{10}	Dodecamer

The first condensed tannins dimer appeared at 481.6 Da peak, that strong signal was assigned to 2*x*dihydroxyflavan units condensed in C_4-C_8 labeled P_2 as shown in Table 2 and 3. Another dimer was obtained at 533.6 Da for this peak was attributed to a trihydroxyflavan/fisetinidin dimer labeled F_1A_1 in Table 2. In addition, Maldi-ToF spectra of Fig. 4a highlighted a peak at 575.2 Da assigned to a fisetinidin/gallocatechin (A_1D_1) dimer with a loss of 3*x*H as suggested in supplementary Table 1.



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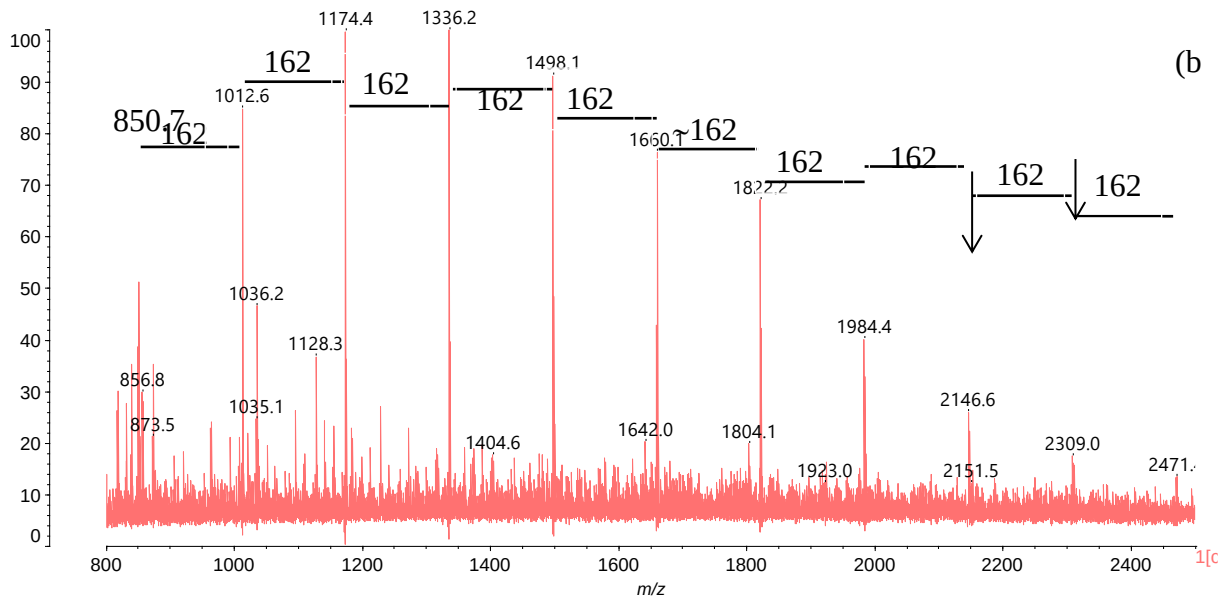


***Figure** 4. Maldi-ToF spectrum of Okoume in the range 400-900 Da (a) and 800-2400 Da (b)*

The acetone/water extracts of the bark exhibited a signal at 850.7 Da assigned to a condensed tannins trimer. That peak should derive from a combination of dihydroxyflavan and two gallocatechins (P_1D_2, *m/z*=851.9 Da) or from the condensation of two fisetinidins coupled with one gallocatechin (A_2D_1, *m/z*=850.9 Da) as proposed in Table 2. Moreover, the presence of condensed tannins in the acetone/water extracts of the bark was corroborated by their typical aromatic C=C- stretching bands at 1605, 1519 and 1446.6 cm^{-1}[35,36] displayed by FTIR (Fig. 5).

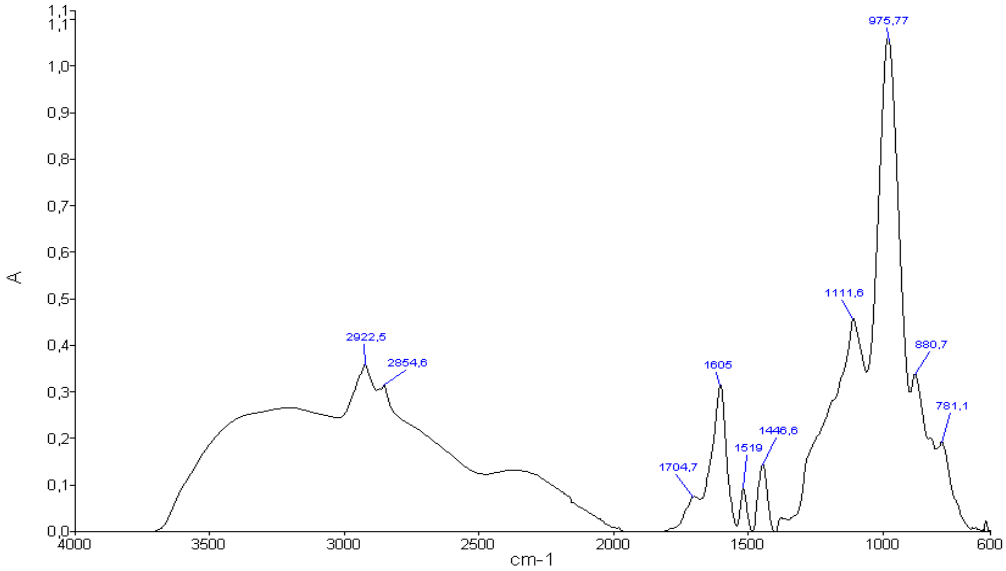


***Figure 5**. Representative FTIR spectra of an Okoume's bark tannins*

However, other dimers including ester moieties were found in the studied

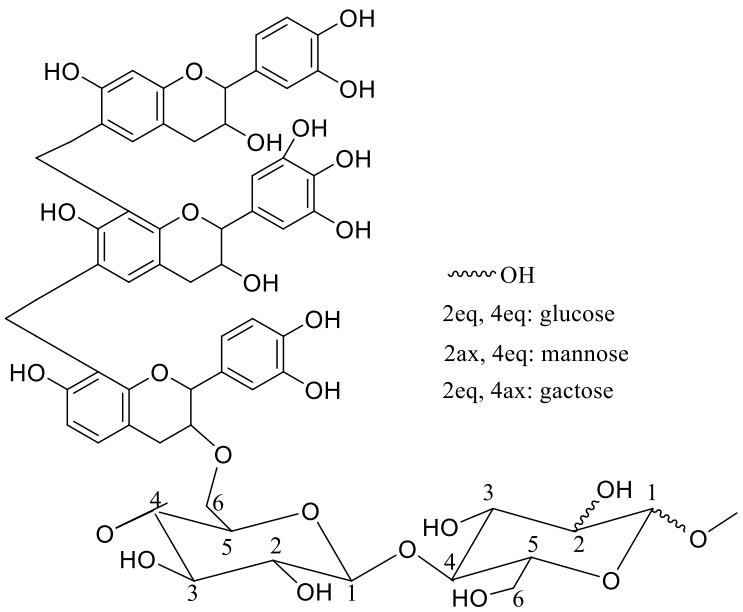
acetone/water extracts. The one arising at 362.1 Da was assigned to dihydroxyflavan-3-p-hydroxybenzoate (G_1H_1) or to trihydroxyflavan-3-benzoate (F_1I_1). These compounds previously described for *Pinus brutia* by Ucar et al[37] put forward to the presence of hydrolysable tannins in the acetone/water extracts of Okoume bark. Therefore, the peak at 166.6 Da of gallic acid (E)[26] depicted in Fig. 3a as well as the strong signal at 438.0 Da assigned to protonated epicatechin-3-gallate (Q_1E_1) based on previous assignment[26], and the one at 461.8 Da (438.0+23 Da) ascribed to epicatechin-3-gallate sodium adducts (Q_1E_1, Na^+) in grape skin[26] should be considered as markers for galloylated units in the bark of Okoume as stated earlier for grape seed extracts[26] (Fig. 4a). It was noteworthy that the peak arising at 617.4 Da in the Maldi-ToF spectrum of Okoume (Fig. 4a) suggested the presence of isoquercetin-gallate (R_1E_1) dimer in the investigated acetone/water extracts. The existence of hydrolysable tannins bearing carbonyl groups (C=O) in these extracts as ascertained by the discussion above was in close agreement with LC-MS analysis. That method showed evidence of gallic acid moiety of $[M–H]^-$=169/170 Da [38–40] in the bark extracts of the studied hardwood species indeed; that result was in close agreement with FTIR which exhibited a specific C=O stretching shoulder of hydrolysable tannins at 1704.7 cm^{-1} [35,36,41,42].

Maldi-ToF analysis displayed a 340.2 Da peak previously assigned to maltose, saccharose or lactose by Sanchez-De Melo[20], this signal supported the presence of glycosyl type dimers in the acetone/water extracts of Okoume's bark. Although the neutral monosides composing saccharose (glucose) or lactose (galactose) dimers were found in Okoume whereas fructose lacked[13], the strong domination of glucose suggested that the peak at 340.2 Da should rather be assigned to cellobiose type dimer as previously found in *Picea abies*[43]. A series of peaks at 405.8 Da, 422.0 Da and 438.0 Da (Fig. 4a) progressively increased in steps of 16 Da starting from the peak at 405.8 Da ascribed to one dihydroxyflavan coupled with a glycosyl unit (P_1Gly_1) dimer of calculated *m/z*=404 Da (Table 2). But, the dimer at 405.8 Da could also result from the loss of *2x*16 Da by a glycosyl-3-fisetinidin (A_1Gly_1) dimer as suggested in Table 1 (Supplementary Table 1). Similar peak of *m/z*=406.4 Da assigned to astringin in *Picea abies* water extracts[43] indicated the possible existence of that stilbene glucoside in acetone/water extracts of Okoume's bark. That hypothesis agreed with the traditional utilization of that endemic hardwood species bark of central African countries like Gabon for stopping diarrhea and hemorrhages[44,45]. Therefore, the peak at 422.0 Da of that glycoside series should also be assigned to a diprotonated isorhapontin, an over stilbene glucoside of *m/z*=420.4 Da previously identified on *Picea abies* water extracts by Bianchi et al[43].

However, the peak at 438 Da that was assigned above to protonated epicatechin-3-gallate (Q_1E_1) and increasing for 16 Da from the peak at 422 Da should also correspond to a

trimer labeled A_1Gly_1 deriving from a fisetinidin/glycoside dimer of calculated *m/z*=452 Da that lost one hydroxyl group (452 Da-1*x*16 Da) during Maldi-ToF treatment as shown in Table 1 (Supplementary Table 1). Similar structures bearing carbohydrate residue linked to the C_3 site of flavonoids have been discussed by various authors[46–48]. Even if the work of Ricci et al[26] endorsed the 461.8 Da peak to epicatechin-3-gallate, the same authors suggested an alternative assignment of that signal to quercetin-3-glycoside (isoquecetin), that agreed with the strong presence of glycosylated condenses tannins in the acetone/water extracts of Okoume's bark.

A fine analysis of the Maldi-ToF spectrum showed a peak series at 850.7 Da; 1012.6 Da; 1174.4 Da; 1336.2 Da; 1498.1 Da; 1660.1 Da; 1822.2 Da; 1984.4 Da; 2146.6 Da; 2309.0 Da and 2471.0 Da which increased for one typical repeating sugar unit of 162 Da period (Fig. 4b). Similar 162 Da repetition was previously observed in water extracts of *Picea abies,* it was assigned to sucrose, raffinose and stachyose like oligosaccharides linked to condensed tannins[43]. Nevertheless, the occurrence of carbohydrates attached to condensed tannins extracted from tropical hardwood's bark such as *Myrotharrmus flabellifofia* or *Parkia biglobosa* was supported by Pizzi and Cameron[49], and Drovou et al[48], respectively. Therefore, the increase of 162 Da starting from the trimer at 850.7 Da composed with two fisetinidins and one gallocatechin labeled A_2D_1 highlighted the existence of glycosylated oligomers including one to ten sugar units chemically bonded to A_2D_1 trimer in Okoume's bark acetone/water extracts. These condensed tannins/glycoside structures led to various oligomers listed in Table 2 and designed $A_2D_1Gly_n$ ($1 \leq n \leq 10$) as proposed below:



$A_2D_1Gly_n$, with $1 \leq n \leq 10$ and Gly: glucose, mannose or galactose unit

Finally, FTIR spectroscopy showed a broad signal between 3500 and 3000 cm^{-1} (Fig. 5) attributed to O-H stretching vibration (ν_{O-H}) of phenols[35,36,42]. The occurrence of these compounds in the investigated extracts was supported by the shoulder at 781.1 cm^{-1} also endorsed to O-H deformation (δ_{O-H}) of phenols[36]. In addition, the bands at 2922.5 and 2854.6 cm^{-1} assigned to C-H stretching (ν_{C-H}) of alkyl groups[35,42,50,51] should arise from flavonoids and sugar units identified in the acetone/water extracts of Okoume's bark (Table 3).

II.E.4. Stiasny number.

The Stiasny number (SI) values collected in Table 1 were between 83.3±11.6 <SI< 53.3±11.6. The ANOVA test did not point out a significant difference ($p>0.05$) between the SI of the bark and sapwood, and between the sapwood and heartwood; however the bark and the heartwood displayed significant difference regarding their SI ($p=0.03$). It is noteworthy that all the SI of this study were above 46% which was defined as good number to produce adhesives of good quality[52]. But the SI of the bark (83%) and the sapwood (73%) were above 65% thought to be the minimum SI value for adhesives of high quality[53]. This result showed clearly the potential of Okoume wood wastes to be used as raw materials for adhesives of high quality. Furthermore, the average SI (83.33±11.6%) displayed by Okoume bark which displayed the highest condensed tannins content of the studied wastes was in the same range with that found for mimosa tannins 92.2%[54], whereas it was to a great extent higher than that published for *Pinus pinaster* bark extracted by three different methods (17.92, 48.97 and 54.98%)[22]. That finding highlighted the strong capability of Okoume bark to be used as raw material for commercial condensed tannins.

II.E.5. Thermal stability control of condensed tannins.

Thermograms of Okoume condensed tannins obtained under nitrogen condition depicted in Fig. 6 exhibited two major steps on tannins degradation. The first step occurred at 110.1; 111.3 and 116.9°C in TGA analysis of the bark, sapwood and heartwood respectively. These first maximum temperatures of degradations (T_{max}) should be associated to residual water removal[55] or some easy-degraded small low molecular materials such as simple sugars and organic acids [56] observed in particular in LC-MS and Maldi-ToF of acetone/water extracts of the bark (Fig. 1, Tables 2, 3). In the second step, DTG thermograms of Fig. 6b denoted T_{max}=243.9 °C for the bark, sapwood and 255.6°C for the heartwood condensed tannins. These second temperatures should correspond to tannins decomposition as previously observed for various condensed tannins[56–58.]

The TG analysis of Okoume condensed tannins at T<100°C showed that the heartwood condensed tannins exhibited a mass loss of 4% for a first onset temperature of degradation or

water loss of T_{d1}≈83.1°C. That temperature was slightly higher than that found for the bark and sapwood tannins (T_{d1}≈81.4°C) which exhibited weak mass losses of 1% (Fig. 6a). The high temperature and mass loss presented by Okoume's heartwood tannins should indicate at least the occurrence of high residual water within the condensed tannins of Okoume's inner wood. Nevertheless, the three condensed tannins did not show difference on their temperature of degradation at 5% mass loss which was $T_{95\%}$~100°C as reported in Table 3; but the heating curves of bark and sapwood condensed tannins showed strong similarity until the second onset of degradation located at T_{d2}≈220°C. Both displayed mass losses of 13.8% against 15% for heartwood condensed tannins (Fig. 6a), that trend of low thermal stability for the heartwood condensed tannins increased with temperature. Although the thermal curves at 80% residual mass supported the least thermal stability of heartwood condensed tannins which degraded at $T_{80\%}$=275°C; the thermal discrimination between the bark and sapwood increasing also for $T>T_{d2}$ highlighted the low thermal stability of the bark condensed tannins which degraded at $T_{80\%}$=362.5; while $T_{80\%}$=425°C for the sapwood tannins (Fig. 6a). The better resistance of Okoume bark and sapwood condensed tannins to thermal degradation was corroborated by their residual rate at the end of decomposition process (T=600°C) which showed residual mass was of 78.5 and 78.9% for the bark and sapwood tannins respectively, while heartwood condensed tannins showed residual mass of 73%. This result showed a strong thermal stability of the three Okoume condensed tannins for temperatures up to 500°C, which suggested similar degree of polymerization as well as interflavonoid bonds in Okoume bark and sapwood condensed tannins. However, the close thermal stability between the bark and sapwood condensed tannins observed in this study suggested that molecular structure and condensed tannins length of these two Okoume wastes should not be very different. Furthermore, the similar thermal behavior between these two condensed tannins was supported by their DTG second step of degradation curves which displayed T_{max}=244.99 for the bark and sapwood tannins (Fig. 6b). It was noteworthy that these T_{max} were slightly higher than that found for quebracho condensed tannin which displayed T_{max}=240°C[57] while Okoume heartwood ones exhibited T_{max}=255.64°C very close to that obtained for *Acacia dealbata* which presented T_{max}=255.77°C [59]. Moreover, the good thermal stability of Okoume condensed tannins was also corroborated by its high T_{d2}≈219.6°C compared to Alepo pine tannins which started to degrade at 179°C[57] or other commercial tannins such as mimosa, quebracho and maritime pine which start to decompose at 146, 145 and 130°C, respectively[60].

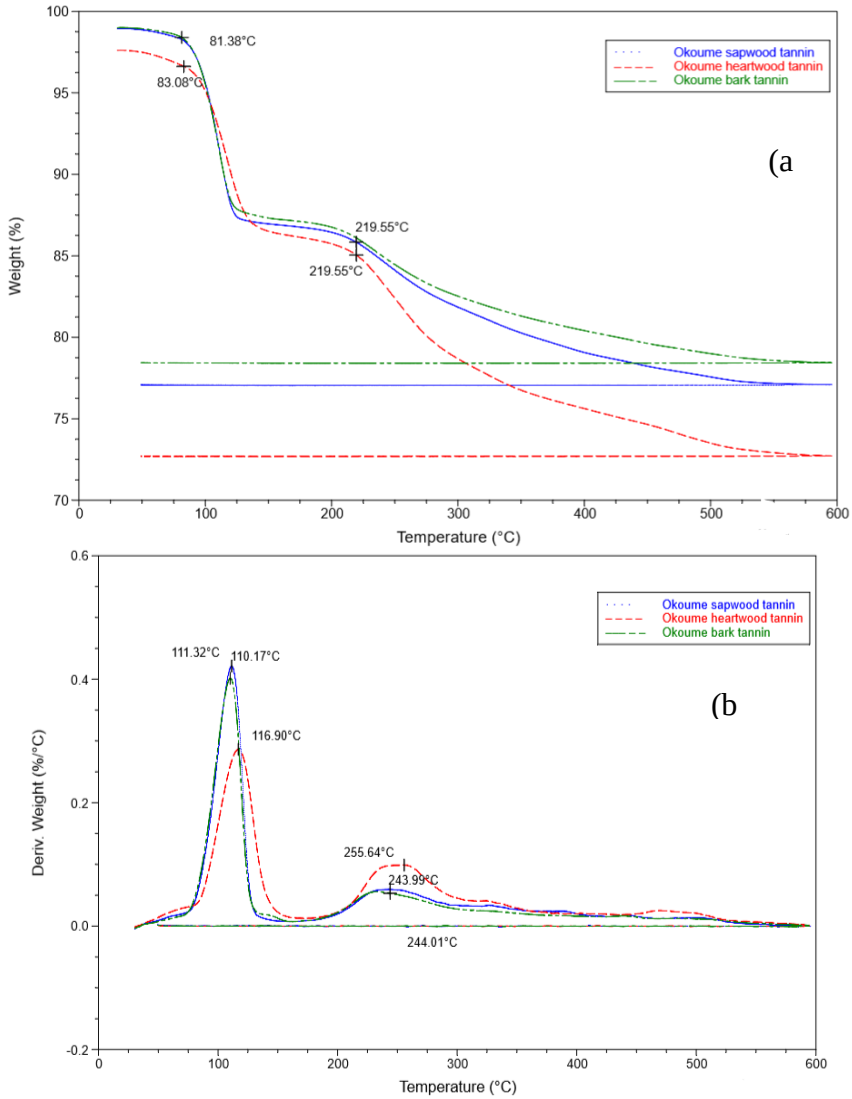


***Figure 6**. TGA (a) and DTG (b) curves of Okoume bark tannins heated at 10°C/ min under nitrogen. With bark tannins= green; sapwood tannins= blue and heartwood tannins=red*

The three Okoume condensed tannins were analyzed by DSC which is the most widely accepted method for determining the glass transition temperature (T_g) of natural or synthetic polymers[59]. That method was carried out at rate of 10°C/min, and the thermograms were depicted in Fig. 7. All the condensed tannins showed typical endothermic peaks of elimination reactions[61,62], and the first endotherm corresponded to changes in the heating curve at 102.4, 91.4, and 97.8°C for the bark, sapwood and heartwood condensed tannins respectively. These peaks should be assigned to the loss of absorbed water as previously observed for other tannins[63,64].

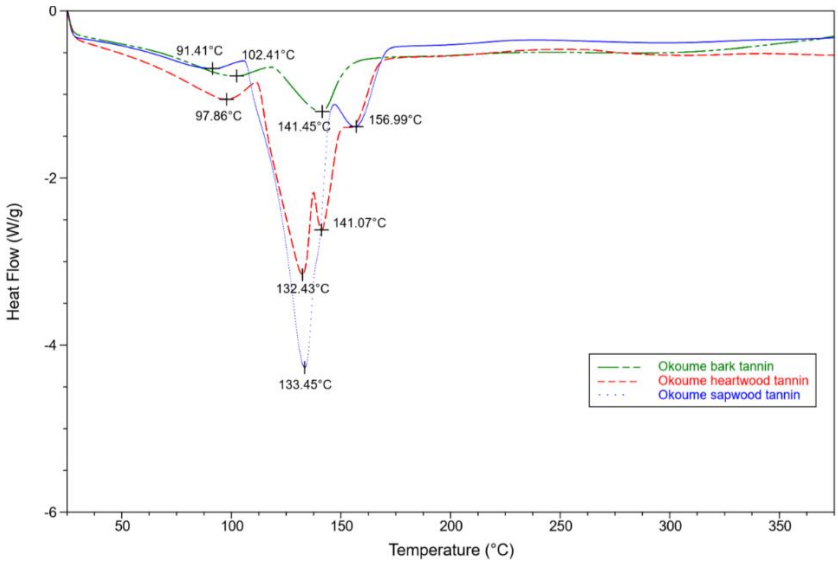


Figure 7. DSC of Okoume tannins heated at 10°C/ min under nitrogen with bark= *green; sapwood tannins= blue and heartwood tannins= red*

That loss of water was also noted in the strong peaks centered at 133.4 and 132.4°C for the sapwood and heartwood condensed tannins (Fig. 7). However, additional endothermic peaks of water loss were observed at 141.4 and 141.07°C for the bark and heartwood condensed tannins although this peak appeared as a shoulder near 141.07°C for the sapwood tannins. However, the thermal curves of Fig. 7 revealed some thermal differences among the studied condensed tannins as shown by the peak at 156.9°C which occurred only in the sapwood and heartwood condensed tannins. Although various studies attributed the four thermal areas of Fig. 7 to the loss of absorbed water in tannins, some authors have found that tannin monomers like catechin showed two endothermic features at 100 and 150°C belonging to the losses of associated water[57] by this polyphenol. That result suggests a strong impact of the molecular structure on the thermal stability of condensed tannins. Thus, the differences observed in the thermal stability of Okoume's condensed tannins should be connected not only to their fisetinidin, gallocatechin, catechin or robitinidin monomers content variability, but also to the condensation of these units in the polyphenol chains. With the exception of the dehydration temperature below 84.43°C, all the Tg of Okoume condensed tannins were above 104, 113, and 126°C respectively ascribed to chestnuts, mimosa and quebracho tannins in previous works[59,63], that finding emphasized the good thermal stability of Okoume condensed tannins than the tannins as cited above.

Table 3. Thermal stability estimators for Okoume bark tannins by TGA (nitrogen 60 ml.min^{-1}, heating rate 10°C.min-1) and DSC (nitrogen 60 ml.min^{-1}, heating rate 10°C.min^{-1})

	TGA					DTG
	Temperature at 95 ($T_{95\%}$) and 80 ($T_{80\%}$) residual weigh		Onset temperature of first (T_{d1}) and second (T_{d2}) degradation		Residual weigh at 600°C ($W_{600°C}$)	Maximum temperatures of degradations (T_{max})
Okoume type condensed tannins sample	$T_{95\%}$	$T_{80\%}$	T_{d1}	T_{d2}	$W_{600°C}$	T_{max}
Bark	101.8	420.8	81.4	220	78.5	243.99
Sapwood	101.8	358.9	81.4	220	77.1	243.99
Heartwood	100.1	276.4	83.1	220	72.7	255.64

II.F. Conclusions

The study of Okoume extract was carried out to achieve three main objectives: (i) to quantify its total polyphenol content according to a colorimetric method; (ii) to characterize for the first time this bark condensed tannin extracts by MALDI-ToF, LC-MS and FTIR; and (iii) to understand the thermal behavior of Okoume condensed tannins. Maldi-ToF spectroscopy revealed the presence of distinctive compounds condensed tannins which contained an extent of hydrolysable tannins bearing galloyl moieties, and oligomer of glycosylated condensed tannins were found. The molecular structure of condensed tannins obtained by maceration of bark in an acetone/water mixture highlighted the presence of fisetinidin, gallocatechin and trihydroxyflavan as major monomers. The highest oligomer chains detected in this condensed tannin were tetramers. A series of oligomers formed by a flavonoid trimer linked to up to ten (10) sugar units was also identified for the first time. Stiasny number and thermal analysis revealed the capability of Okoume wood wastes to be used as raw materials for condensed tannins of strong adhesive properties.

Data availability

Data can be made available for sharing with others

Data generated were analyzed during the current study

II.G. References

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Author Contributions

S.P.E.A. acted as Ph. D student, controlled the hypotheses, led the experimental aspects, analyzed data, highlighted the LC-MS experiments and wrote the manuscript. A.B.B.A and F.J.S.M contributed to the work as Ph. D students and contributed to Maldi-ToF experiment and data analysis. T.C. criticized and corrected the TGA and DSC analysis. A.P. provided Maldi-Tof-MS for tannins structure identification. R.S.T and B.C. acted as scientific directors, they analyzed data and controlled the manuscript. All the authors have approved the final version of the manuscript

Additional Information

Competing Interests: The authors declare no competing interests

III. Maldi-ToF analysis and FTIR characterization of *Aucoumea klaineana* Pierre (Okoume) sapwood and heartwood condensed tannins from Gabon natural forest

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III.A. Résumé :

Les tanins d'*Aucoumea klaineana Pierre* (Okoumé) ont été extraits par une méthode de macération en présence d'un mélange acétone/eau comme que solvant. Les tanins obtenus à partir de l'aubier et du bois de cœur, et caractérisés par MALDI-ToF et FTIR, ne montrent aucune différence en ce qui concerne leurs principales structures oligomériques. Par ailleurs, l'aubier contenait plus de tanins que le bois de cœur, mais tous deux étaient composés de fisétinidine et de gallocatéchine comme principaux monomères des tanins condensés libres. Une faible teneur en monomère trihydroxyflavane a été identifiée, tandis que des tanins condensés non glycosylés allant jusqu'à sept monomères ont été trouvés en forte teneur. Ces résultats, qui ont révélé pour la première fois la structure chimique des tanins condensés d'Okoumé, ont mis en évidence la présence d'une série de tanins condensés glycosylés pouvant contenir jusqu'à six résidus glucidiques liés aux flavonoïdes 2*x*gallocatéchine-1*x*catéchine.

III.B. Abstract

Aucoumea klaineana Pierre (Okoume) tannins have been extracted by an artisanal maceration method in presence of acetone/water as solvent. The tannins obtained from the sapwood and heartwood and characterized by Maldi-ToF and FTIR did not show difference regarding their type of oligomers. The sapwood was more abundant in tannins than the heartwood, but both were composed of fisetinidin and gallocatechin as majors free condensed tannin monomers. A low content of trihydroxyflavan monomer was identified whereas non-glycosylated condensed tannins up to seven oligomers were found in high content in Okoume. These results which revealed for the first time the chemical structure of Okoume's condensed tannins pointed out the presence of a glycosylated condensed tannin series up to six carbohydrate residues attached to 2*x*gallocatechin-1*x*catechin flavonoids.

Keywords: Condensed tannins, carbohydrate, FTIR, heartwood, Maldi-ToF, Okoume, sapwood.

III.C. Introduction

Tannins are natural polyphenolic compounds occurring in plant wood and abundantly localized in the bark of some trees. They can be classified as hydrolysable and condensed or flavonoid tannins (e.g., oligomeric to polymeric proanthocyanidns (PAs)). These natural polyphenolic materials can be used for a variety of industrial applications (Navarrete et al. 2010). Condensed flavonoid tannins received considerable research attention in the last decades for a variety of applications for which environment friendly biosourced polymeric materials can be prepared (Drovou et al. 2015). Industrial condensed tannins are mainly used for these commercial applications.

The chemical analysis of PAs can be somewhat difficult due to the structural heterogeneous of these compounds. Today, different spectroscopic methods such as solid state CP/MAS ^{13}C NMR and Maldi-ToF are used to highlight, identify, differentiate and determine the kind and the number of flavan-3-ol sub-units in the proanthocyanidins structure.

Maldi-ToF Mass Spectroscopy technology has been firstly established for determining the molecular weights of proteins (Karas et al. 1985). Pasch et al (2001) have introduced Maldi-ToF as technique to analyze the structure of mimosa, quebracho and pecan nut tannins. The characterization of tannins from different sampling areas was investigated using this technique. Thus, many researchers have used Maldi-ToF to investigate tannins isolated from the roots of Tunisian *zizyphus jujuba* (Abdalla et al. 2014), maritime pine (Navarrete et al. 2010), Norway spruce and Silver fir (Bianchi et al. 2014), *Pinus brutia* (Ucar et al. 2013), African mahogany (Bikoro Bi Athomo et al. 2018) and from grape, green tea leaves and limousin oak heartwood (Ricci et al. 2017). However, to date and according to our knowledge there are no Maldi-ToF data available for *Aucoumea klaineana* Pierre (Okoume) sapwood and heartwood. Okoume is an important economical resource for plywood preparation. It is an Africa tropical hardwood mainly located in Congo and Gabon. Among the 400 million m^3 volume of standing tree potentially exploited in Gabon, Okoume represents 130 million m^3, which corresponds to 31% of the total wood of Gabon forest reserve. Therefore, since the prohibition of exporting logs by the Gabonese government in 2010, the trade of wood locally transformed knows an important increase, and it is the case for wood species like Okoume, African mahogany, etc. Nevertheless, the timber sector mainly focused on the first transformation of wood generated a high content of non-valorized wood wastes accounting approximately for 50 % of the wood transformed.

The aim of this research was to analyze the chemical structure of Okoume's sapwood and

heartwood condensed tannins by Maldi-TOF and FTIR techniques in order to propose a suitable valorization of such a tropical hardwood wastes.

III.D. Experimental

III.D.1. Samples

Sapwood and heartwood of Okoume were collected in the logging concessions of SED (Société Equatoriale de Déroulage) located in Nzamaligue natural forest (Estuary Province of the West part of Gabon). All the samples were collected from one piece of one tree (83 cm x 10 cm) of around 41 years old (Zaou et al. 1998) at 1.3 m from the soil. The wood samples were ground with a Retsch SK1 mill to pass 60 mesh and oven-dried for 24 hours at 105 °C.

III.D.2. Chemical

All the chemicals used in this study were purchased from Fisher Scientific and Sigma Aldrich. Solvents and reactants were used without further purification.

III.D.3. Extraction

The wood meal was three time extracted with 30 ml of acetone/water (7/3: v/v) solution at room temperature during three hours. Supernatant was collected after each extraction by filtration through Whatman paper n° 1. The solvent was then evaporated under reduced pressure and some drops of orthophosphoric acid (d=1.21) were added.

III.D.4. Samples for Maldi-ToF analysis

The tannins were obtained after acetone/water extraction (ratio7/3, v/v), oven-dried at 50°C for 24 hours and dissolved in a water/acetone (1:1, v/v) solution up to 7.5 mg/ml. NaCl solution was added to enhance the ion formation and the mixture was placed on the Maldi target. Then, the sample solutions and the matrix (2, 5-dihydroxy benzoic acid) were mixed in equal amounts and 1.5 µl of the resulting solution was placed on the Maldi target. Red phosphorous (500–3000 Da) was used as reference for spectrum calibration. Finally, after evaporation of the solvent, the Maldi target was introduced into the spectrometer.

III.D.5. Maldi-ToF-MS acquisition

The spectra were recorded on a KRATOS AXIMA Performance mass spectrometer from Shimadzu Biotech (Kratos Analytical Shimadzu Europe Ltd., Manchester, UK). The irradiation source was a pulsed nitrogen laser with a wavelength of 337 nm. The length of one laser pulse was 3 ns. Measurements were carried out using the following conditions: polarity-positive, flight path-

linear, 20 kV acceleration voltages, 100-150 pulses per spectrum. The delayed extraction technique was used applying delay times of 200-800 ns. The software Maldi-MS used for the data treatment.

III.D.6. FTIR spectroscopy

A Perkin Elmer Frontier-ATR (Attenuated total reflection) spectrophotometer equipped with diamond/ZnSe crystal was used to analyze tannins. About 2 mg ground powder was placed on the crystal and the contact was obtained applying a force of about 150 N on the sample. Each spectrum was obtained with 32 scans with the resolution of 4 cm-1 from 4000 to 600 cm^{-1}.

III.E. Results and discussion

Maldi-ToF analysis has been carried out on both Okoume sapwood and heartwood to compare the distribution of sugars and flavonoids structures in both parts of this wood species.

III.E.1. Low molecular weight compounds

III.E.1.a. Non sugar compounds

The Maldi-ToF analysis was conducted under two different molecular weight ranges. The first one is in the 100-200 Da and the second one in the 250-2000 Da range. However, the presence of low molecular weight is therefore hinted (Table 1). These molecules could be aromatic compounds, sugars or some hydrolysable tannins attached or not to Na+. NaCl has been added to the system for peaks detection enhancement. The spreading of molecules between Okoume sapwood and heartwood tannins in the 100-200 Da range pointed out the following results: a weak peak at 128.8 Da was observed in the sapwood (Fig. 1b). This peak could be related to sotolon as this compound was previously found in high content in funegrec (Guerra and Yaylayan 2011). The presence of this compound in Okoume acetone/water extract is supported by the utilization of Okoume resin in perfumery, sotolon and other aromatic compounds (Renimel and Andre 2004) being responsible for its characteristic odor. In addition, the occurrence of weak quantity of resinic compounds such as sotolon in Okoume wood as revealed by the Maldi analysis agreed with the GC-MS results reported by Medzegue et al. (2013) who found other resin volatile compounds like amyrin in Okoume heartwood extracts.

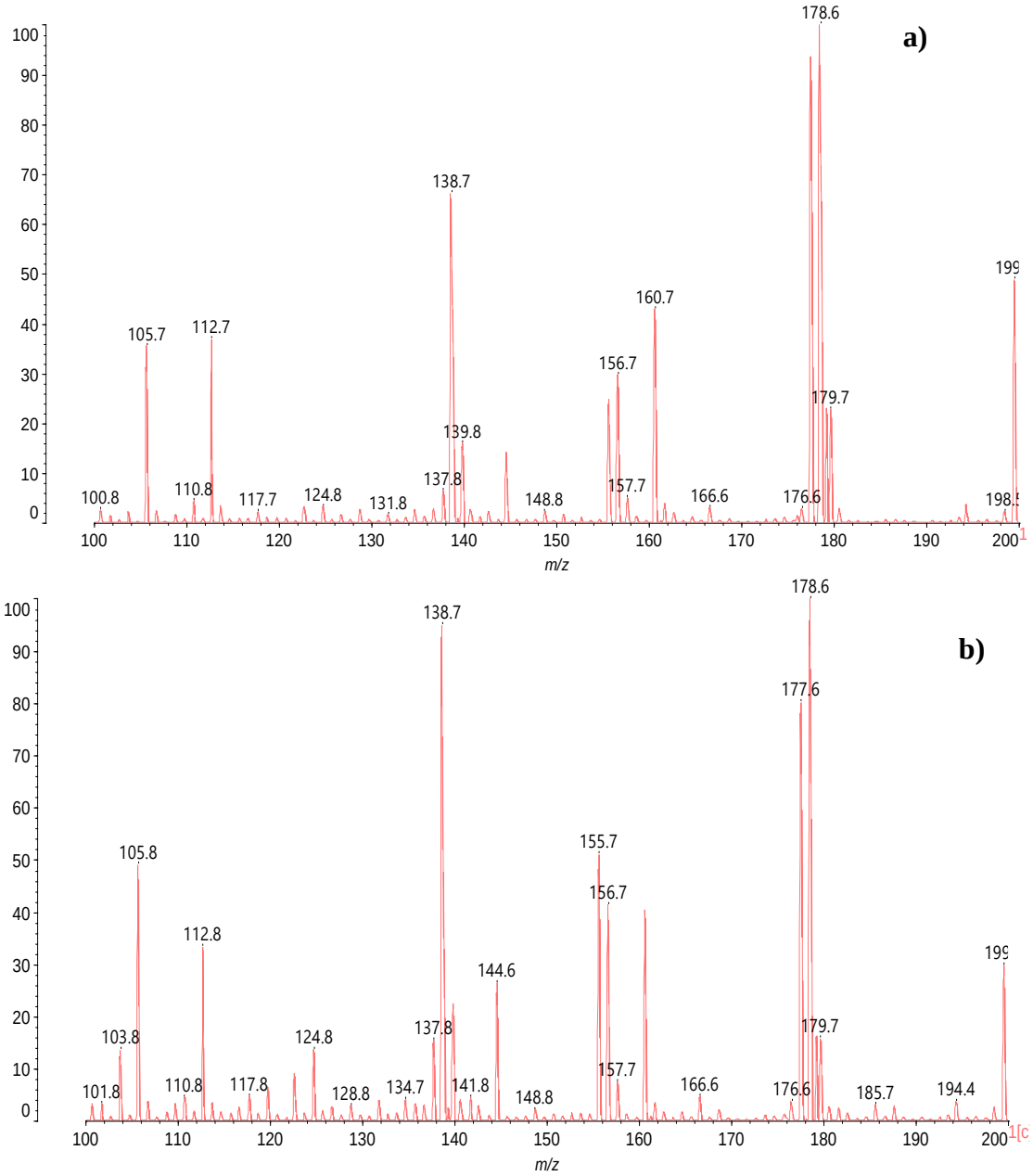


***Figure 1**. Maldi-ToF spectra of heartwood (a) and sapwood (b) in the range 100-200 Da*

The peak at 138.12 Da belonging both to the heartwood and to the sapwood extracts was more strengthened in the sapwood. This peak is attributed to para-hydroxybenzoic acid and was previously observed in other tropical hardwood acetone/water bark extracts such as African mahogany (Bikoro Bi Athomo et al. 2018).

III.E.1.b. Sugar compounds

A small peak at 131.8 Da in the heartwood extract was previously attributed (Bikoro Bi Athomo et al. 2018; Ricci et al. 2016) to a glucose ring fragmentation induced by cleavage in Maldi-ToF analysis. However, the low intensity of 148.8 Da peak assigned to xylose or arabinose

of *m/z* 150 Da supported that these pentosans identified in Okoume's hemicellulose chains (Safou-Tchiama et al. 2016, 2007) were weakly solubilized. Additional sugars were found at 176.6, 178.6 and 179.7 Da peak series indicating the presence of glucose, mannose or galactose of *m/z* 180 Da in the acetone/water extracts as reported for African mahogany's bark (Bikoro Bi Athomo et al. 2018). Despite these dominating glycosidic sugars, a small content of mannitol which has lost water ($-H_2O$) appears at 166.6 Da (Fig. 1). The occurrence of these sugar units confirms the 2960-2925 cm^{-1} FTIR bands assigned to -CH, $-CH_2-$ and $-CH_3-$ stretching vibrations ν(C-H) of carbohydrates and sugars (Grasel et al. 2015). In addition, strong characteristic ν(C-O) of C-OH bonds in secondary alcohols were observed in the 1050-1160 cm^{-1} region (Fig. 7), that emphasized the presence of alcohol type structures in the studied extracts. FTIR spectra of the samples showed 1084.4 and 1075.4 cm^{-1} bands, respectively (Fig. 7). Similar signals, ascribed to C-O deformation in secondary alcohols and aliphatic ethers (Popescu et al. 2007) corroborated that stated above. However, these bands should be more likely assigned to δ(C-O) of glucose and/or β(1→3) vibration in glucans (Galichet et al. 2001; Kacuráková and Mathlouthi 1996). The presence of glucose units to account for one of the major sugars in Okoume's acetone/water extracts is reinforced by the 1030 cm^{-1} (Kacuráková and Mathlouthi 1996) or ν(C-O) of primary alcohols in cellulose (Lv et al. 2015) without excluding the C-C mannan rings vibration possibility (Estevez et al. 2009).

However, the more resolved 1030 cm^{-1} band depicted in the heartwood FTIR spectrum (Fig. 5a) suggests a high glucose and mannose content on that part of Okoume.

Table 1. Maldi-ToF peaks assigned of Okoume's sapwood and heartwood in the range 100-200 Da

Experimental m/z (Da)		Calculated m/z (Da)		Unity Type		Oligomer Type	
112.8	112.7	112	112	Furfural oxydation in β-formylacrylic acid	Furfural oxydation in β-formylacrylic acid	Monomer	Monomer
128.8	128.8	128.8	128.8	Sotolon	Sotolon	Monomer	Monomer
131.8	131.8	131.8	131.8	Glucose fragmentation residue	Glucose fragmentation residue	Monomer	Monomer
138.12	138.12	138.7	138.7	Parahydroxybenzoic	Parahydroxybenzoic	Monomer	Monomer
139.8	139.8	139	139	Levulinic Ac (+ Na)	Levulinic Ac (+ Na)	Monomer	Monomer
144.6	144.6	148	148	Quercitol (-H2O)	Quercitol (-H2O)	Monomer	Monomer
148.8	148.8	150	150	Xylose, arabinose	Xylose, arabinose	Monomer	Monomer
166.6	166.6	165.24	166	Mannitol (-H2O)	Mannitol (-H2O)	Monomer	Monomer
178.6	178.6	180	180	Glucose, galactose, mannose,	Glucose, galactose, mannose,	Monomer Monomer	Monomer Monomer

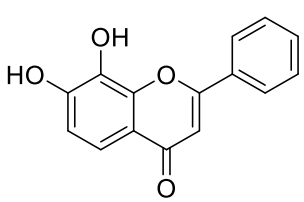
Furthermore, the abundance of mannose in the heartwood is supported by the strongest sharp band at 962.18 cm^{-1} assigned to mannan glycosidic linkages (Kato et al. 1973) as the sapwood ones arising at 971.24 cm^{-1} were less intense (Fig. 7b).

Two tannin oligomer series appeared to be present in this study (Table 2): the one was composed of non-glycosylated flavonoid oligomers and the other was a mixture of flavonoids attached to carbohydrate monomers. The latter could be glucose or another carbohydrate monomer residue, probably mannose or galactose. However, the weak proportion of galactose obtained in Okoume's wood and the small average fraction of mannose stemming from acid or enzymatic hydrolysis (Safou-Tchiama et al. 2016) supported that the dominating glycosyl moiety was glucose (Glu).

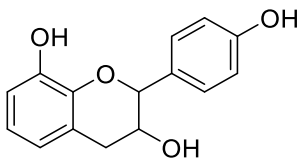
III.E.2. High molecular weight compounds

III.E.2.a. Non-glycosylated condensed tannins

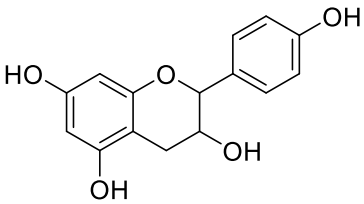
The Maldi-ToF fragmentation patterns of Okoume's heartwood and sapwood tannins showed the presence of several types of non-glycosylated condensed flavonoid oligomers (Fig. 2). Their corresponding structures are listed in Table 2. Three identified typical condensed tannins oligomers namely trihydroxyflavan (B), fisetinidin (C) and gallocatechin (E) of respectives *m/z* 258.4; 274.4 and 305.5 Da were identified (Table 2). No evidence of free catechin or robitinidin (D) of *m/z* 290.3 as well as dihydroxyflavon (A) monomer of calculated *m/z* 254.2 Da was found in the acetone/water extracts. Therefore, Okoume's sapwood and heartwood condensed tannins should be fisetinidin and gallocatechin type flavonoid units which contain very low trihydroxyflavan content.



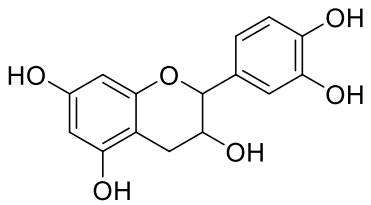
A) Dihydroxyflavone
M=254.28 Da



B) Trihydroxyflavan
M= 258 Da

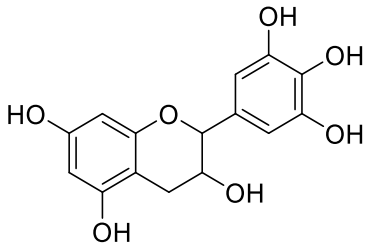


C) Fisetinidin
M= 274.3 Da



D) Catechin (Robitinidin)

M= 290.3 Da

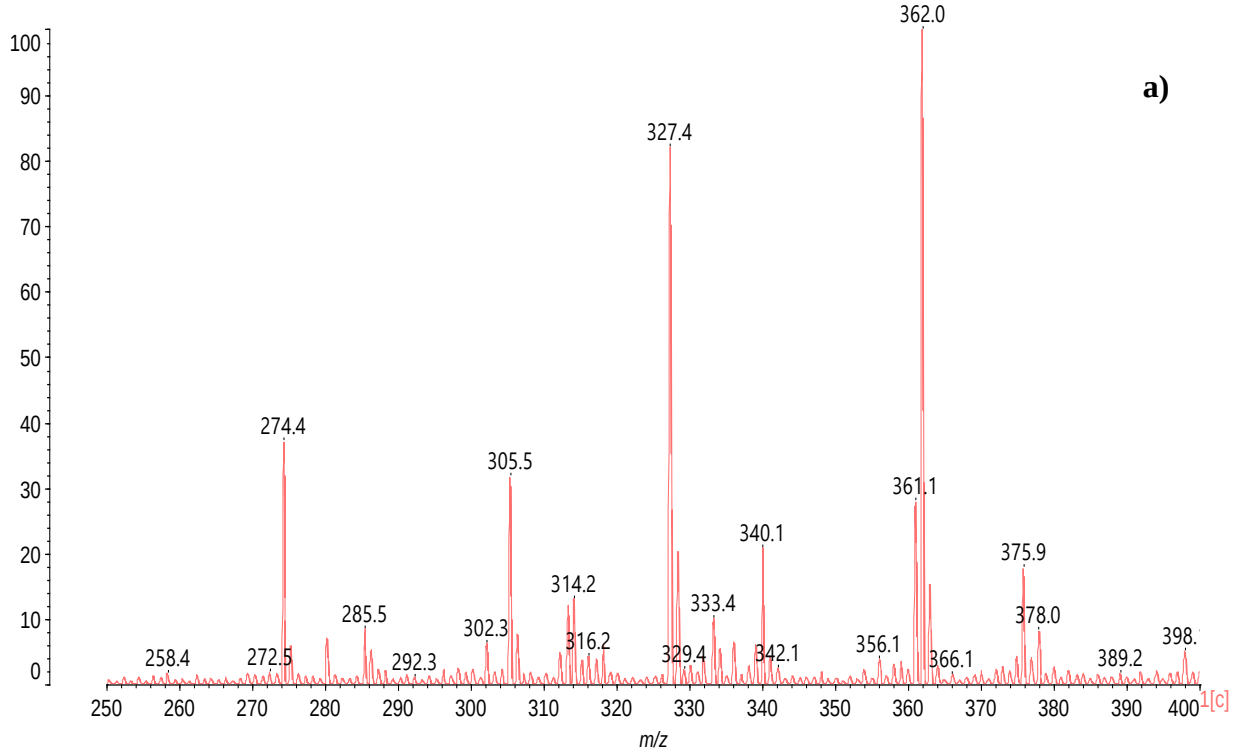


E) Gallocatechin

M=306.3 Da

The distribution of non-glycosylated flavonoid dimer or trimer structures presented in Table 2 for signals arising in the 250-2000 Da range (Figure 2-6) didn't show any remarkable differences between Okoume's sapwood and heartwood flavonoid structures.

The first non-glycosylated condensed tannin dimer observed at 478.8-477.8 Da was assigned to a trihydroxyflavan/dihydroxyflavan which has lost 3*x*H (Fig. 5).



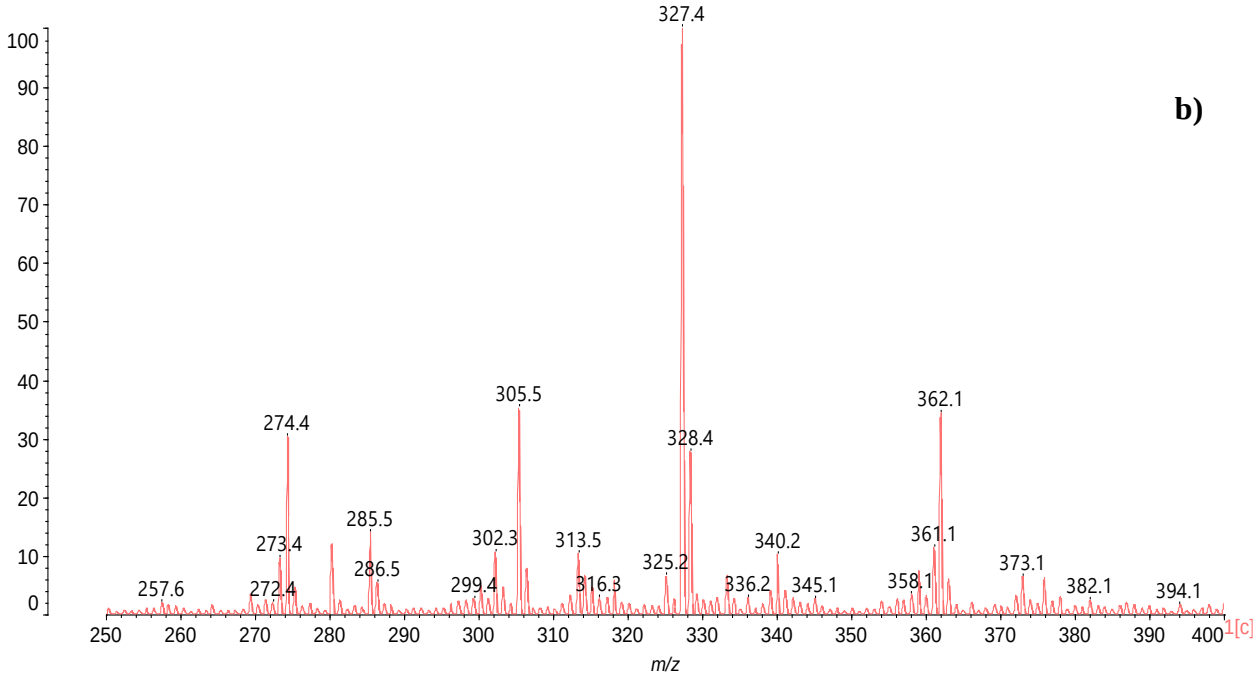
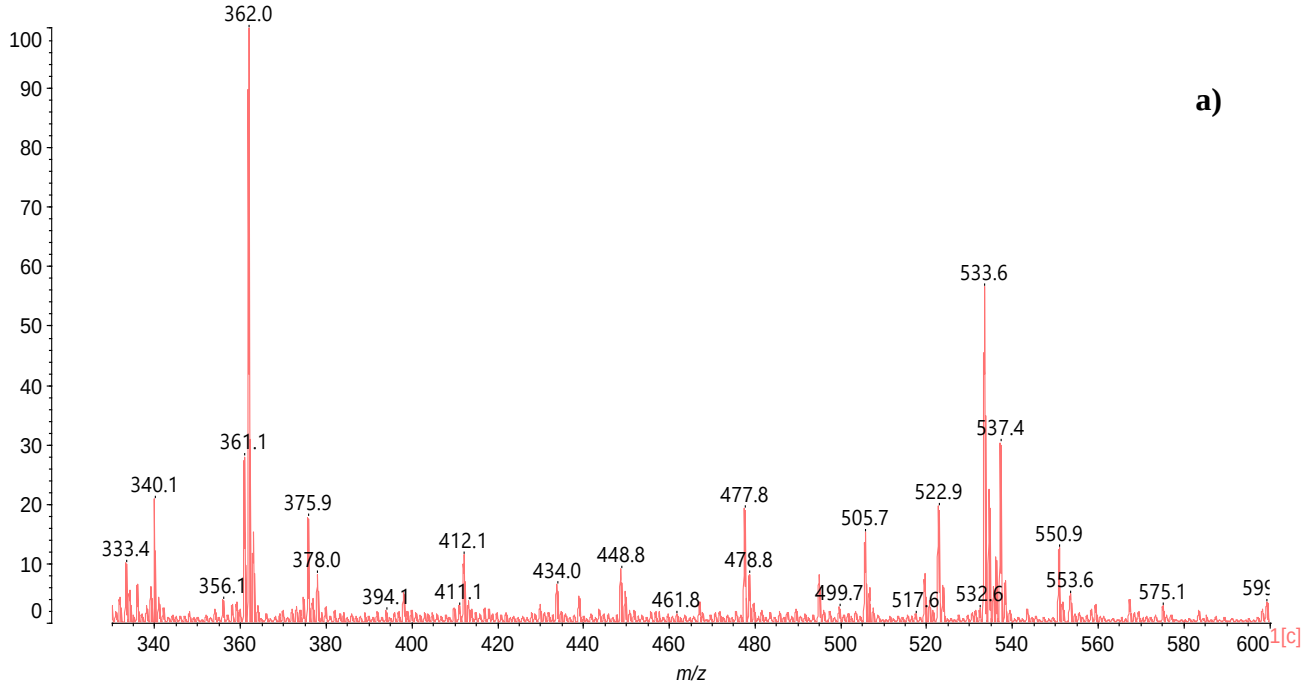


***Figure 2**. Maldi-ToF spectra of heartwood (a) and sapwood (b) in the range 250-400 Da*



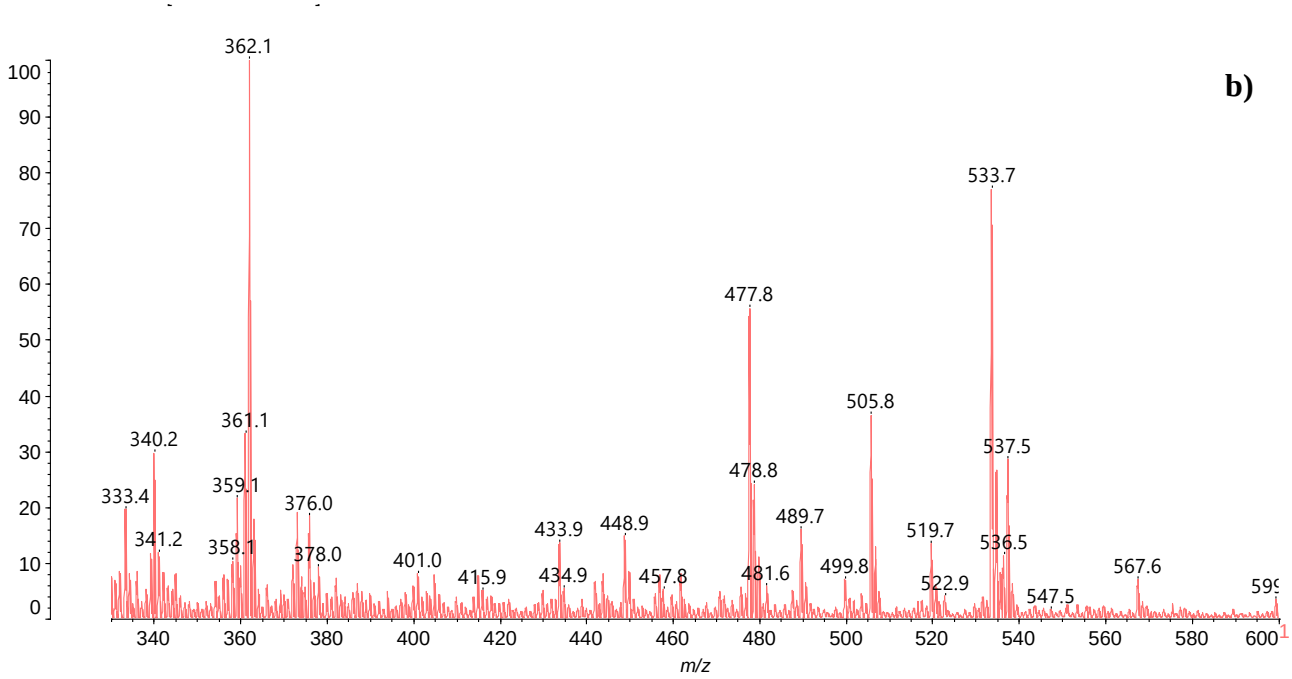


***Figure 3**. Maldi-ToF spectra of heartwood (a) and sapwood (b) in the range 330-600 Da*

The highest relative peak intensity of this compound for the sapwood extracts suggested a high content of that dimer on the sapwood compared to heartwood. The abundance in Okoume sapwood of non-glycosylated condensed tannins was reinforced by the signal strength of the 505.8 Da peak assigned to 2*x*trihydroxyflavan dimer which has lost 3*x*H (Fig. 5). This dominating sapwood trend on non-glycosylated polyphenols was strengthened by the occurrence of the 533.7-533.6 Da peak attributed to a fisetinidin-trihydroxyflavan dimer of *m/z* 532.3 Da. This strong peak could also be assigned to catechin-dihydroxyflavan or robitinidin-dihydroxyflavan dimer as previously reported for African mahogany's bark (Bikoro Bi Athomo et al. 2018). However, Okoume's heartwood should be more abundant in gallocatechin-catechin-Na^+ or gallocatechin-robitinidin-Na^+ such as the non-glycosylated tannin dimers assigned to the strong 617.7-617.4 Da peak (Fig. 5). The first non-glycosylated tannin trimer was found at 833.2-833 Da and assigned to 2*x*fisetinidin-catechin or 2*x*fisetinidin-robitinidin of *m/z* 832.2 Da. The relative intensity of this compound (Fig. 5a) suggests a high content of such a dimer in the heartwood compared to the sapwood.

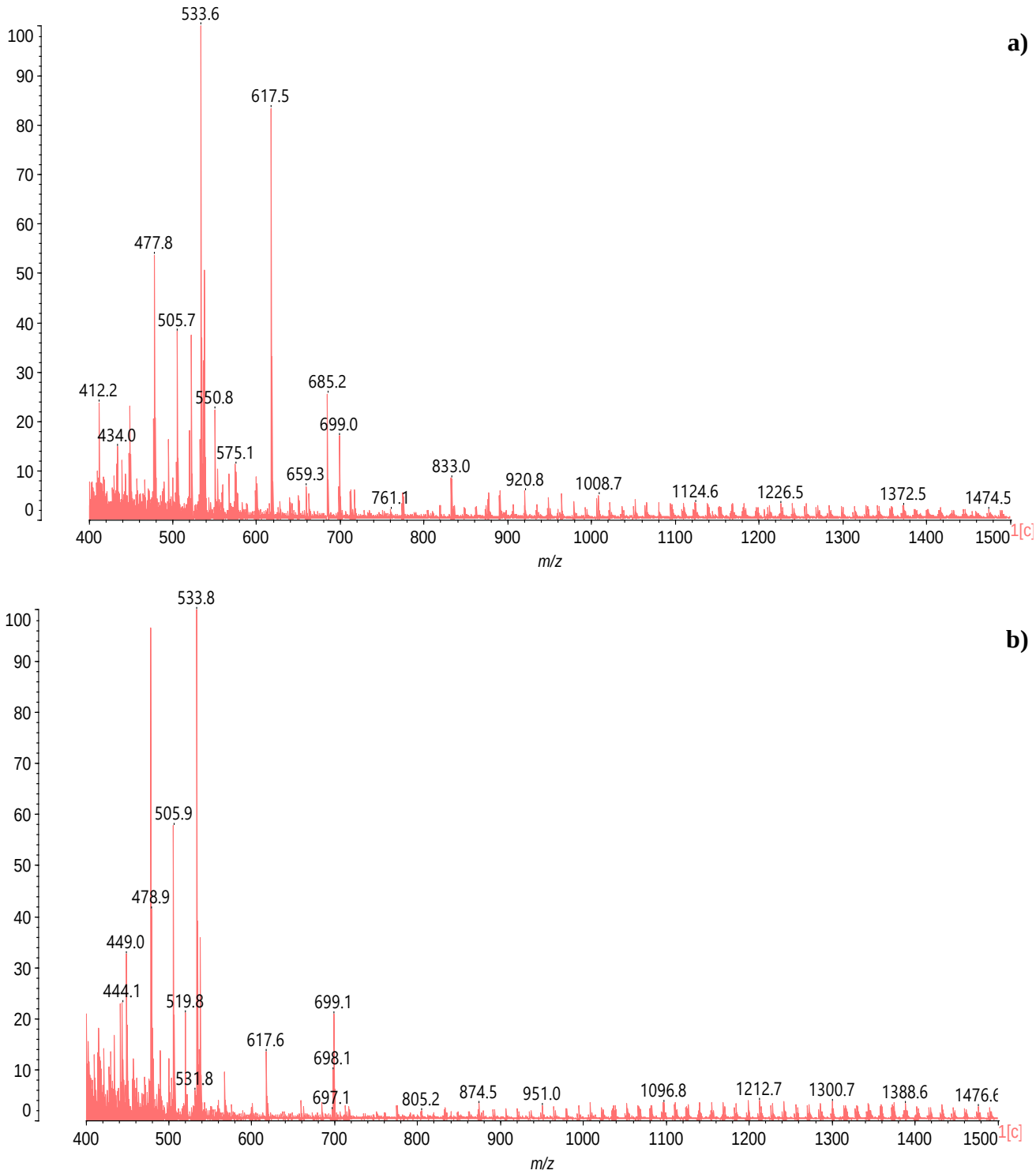


***Figure 4**. Maldi-ToF spectra of heartwood (a) and sapwood (b) in the range 400-1500 Da*

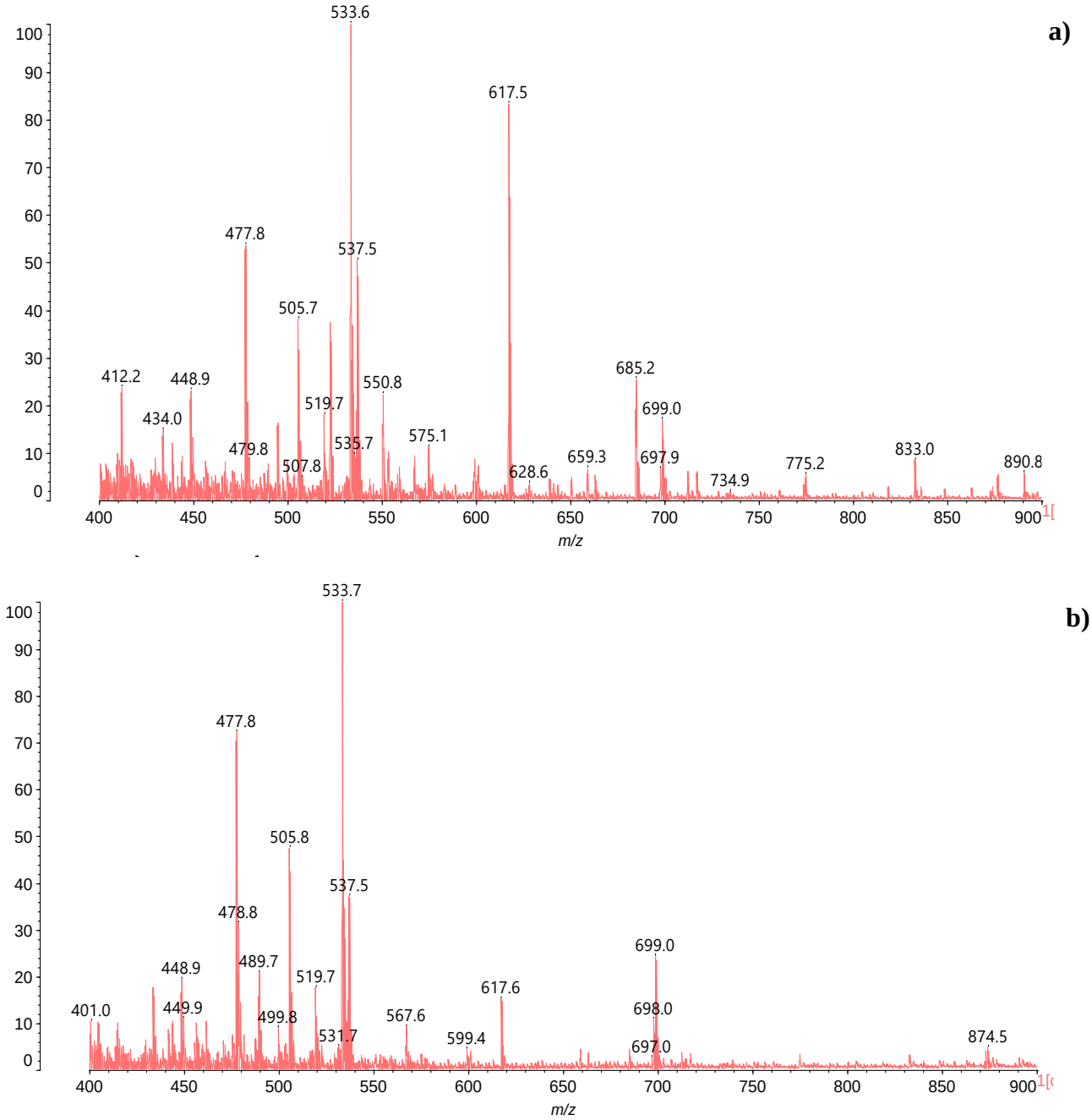


***Figure 5**. Maldi-ToF spectra of heartwood (a) and sapwood (b) in the range 400-900 Da*

Among the non-glycosylated tannins listed in Table 2, remarkable compounds such as fisetinidin, catechin/robitinidin and gallocatechin trimers can be found at 820, 874.5-874.4 and 921.1-920.8 Da peaks respectively (Fig. 6b, Table 2). A non-glycosylated tannin tetramer series was found in Okoume's xylem as listed in Table 2, and all their relative intensities showed their high content in the Sapwood with regard to the heartwood (Fig. 6).

Table 2. Maldi-ToF peaks assigned of Okoume's sapwood and heartwood in the range 250-2000 D

Experimental m/z (Da)		Calculated m/z (Da)		Unity type		Oligomer type	
Sapwood	Heartwood	Sapwood	Heartwood	Sapwood	Heartwood	Sapwood	Heartwood
258.2	258.4	257.6	258	Trihydroxyflavan	Trihydroxyflavan	Monomer	Monomer
274.4	274.4	274	274.4	Fisetinidin	Fisetinidin	Monomer	Monomer
285.5	285.4	286.23	286.23	Kaempferol	Kaempferol	Monomer	Monomer
292.3	-	290.3	290.3	Catechin or Robitinidin	Catechin or Robitinidin	Monomer	Monomer
305.5	305.5	305.5	305.5	Gallocatechin	Gallocatechin	Monomer	Monomer
314.2	313.5	313.2	313.2	Catechin (+ Na) Robitinidin (+ Na)	Catechin (+ Na) Robitinidin (+ Na)	Monomer Monomer	Monomer Monomer
327.4	327.4	329	329	Gallocatechin (+ Na)	Gallocatechin (+ Na)	Monomer	Monomer
340.1	340.2	342	342	Cellobiose (Glu2)	Cellobiose (Glu2)	Dimer	Dimer
362.0	362	362.1	362	(Dihydroxyflavan-3xH)-(p-hydroxybenzoic acid) (Trihydroxyflavan-3xH)-Benzoic acid	(Dihydroxyflavan-3xH)-(p-hydroxybenzoic acid) (Trihydroxyflavan-3xH)-Benzoic acid	Dimer Dimer	Dimer Dimer
375.9	375.9	376	376	Dihydroxyflavon-3-benzoate	Dihydroxyflavon-3-benzoate	Dimer	Dimer
398	-	395.4	395.4	Dihydroxyflavan-3 benzoate	Dihydroxyflavan-3 benzoate	Dimer	Dimer
412	412	411	411	Triihydroxyflavan-3 benzoate	Triihydroxyflavan-3 benzoate	Dimer	Dimer
430.0	430.0	433.9	433.9	Fisetinidin-3-O-Glycoside (Astragalin)	Fisetinidin-3-O-Glycoside (Astragalin)	Dimer	Dimer
448.8	448.8	448.37	448.37	Catechin-3-O-Glycoside (Astragalin)	Catechin-3-O-Glycoside (Astragalin)	Dimer	Dimer
478.8-477.8	478.8-477.8	479.6	479.6	Trihydroxyflavan/Dihydroxyflavan-3xH	Trihydroxyflavan/Dihydroxyflavan-3xH	Dimer	Dimer
499.8	499.7	489.6	498.6	Trihydroxyflavan/Dihydroxyflavan	Trihydroxyflavan/Dihydroxyflavan-	Dimer	Dimer

505.8	505.8	508.2	508.2	2xTrihydroxyflavan-3xH Dihydroxyflavone	2xTrihydroxyflavan-3sH -	Dimer	Dimer
519.7	519.6	514.6	514.6	2xTrihydroxyflavan	2xTrihydroxyflavan	Dimer	Dimer
533.7	533.6	532.3	532.3	Fisetinidin/Trihydroxyflavan Catechin/Dihydroxyflavan Robitinidin/Dihydroxyflavan	Fisetinidin/Trihydroxyflavan Catechin/Dihydroxyflavan Robitinidin/Dihydroxyflavan	Dimer Dimer Dimer	Dimer Dimer Dimer
599.4	599.4	604.4	604.4	2xGallocatechin-3xH	2xGallocatechin-3xH	Dimer	Dimer
617.6	617.4	616.3	616.3	Isoquercetin-Gallate Gallocatechin/catechin (+Na)	Isoquercetin-Gallate Gallocatechin/catechin (+Na)	Dimer Dimer	Dimer Dimer
699	697.9	700.6	700.6	2xFisitinidin-1xGallic Ac Catechin-(Trihydroxyflavan-3(3,4-dihydroxy) benzoic acid)	2xFisitinidin-1xGallic Ac Catechin-(Trihydroxyflavan-3(3,4-dihydroxy) benzoic acid)	Trimer Trimer	Trimer
820.0	820.0	819.0	819.0	3xFisetinidin	3xFisetinidin	Trimer	Trimer
833.2	833.0	832.2	832.2	2xFisitinidin-1xCatechin	2xFisitinidin-1xCatechin	Trimer	Trimer
874.4	874.5	872.7	872.7	3xCatechin or 3xRobitidin 2xfisetinidin-gallocatechin (+Na)	3xCatechin or 3xRobitidin 2xfisetinidin-gallocatechin (+Na)	Trimer Trimer	Trimer Trimer
890.0	890.0	986.6	896.9	1xCatechin-2xGallocatechin	1xCatechin-2xGallocatechin	Trimer	Trimer
921	920.8	916.5 923	916.5 823	3xGallocatechin 2xGallocatechin-1xGallic acid/Glycoside	3xGallocatechin 2xGallocatechin-1xGallic acid/Glycoside	Trimer Tetramer	Trimer Trimer
937	937	946.4	-	3,3’,4’,5,5’,7,8-heptahydroxyflavone-2gallocatechin	-	Trimer	-
951	-	974.4	-	3,3’,4’,5,5’,7,8-	-	Trimer	-

				heptahydroxyflavone-1 gallocatechin (-Na)			
964.8	964.8	-		-	Trihydroxyflavan (+ Na)	-	Trimer
1010	1010	1011	1011	4xDihydroxyflavone	4xDihydroxyflavone	Tetramer	Tetramer
1000	1000	1008.6	1008.6	2',3',4',5,5',6,7-heptahydroxyflavanone (C15H12O9)	-	Monomer	-
1036.7	1036.7	1035 1040	1035 1040	4xDihydroxyflavan protonated 2xGallocatechin-1xFisetinidin-Glu	4xDihydroxyflavan protonated 2xGallocatechin-1xFisetinidin-Glu	Tetramer Tetramer	Tetramer Tetramer
1052.8	1052.8	1052.8	1052.8	1xCatechin-2xGallocatechin-Glu	1xCatechin-2xGallocatechin-Glu	Tetramer	Tetramer
1124.6	1124.6	1126	1126	2xFisetinidin-1xCatechin	2xFisetinidin-1xCatechin	Tetramer	Tetramer
1140.8	1140.8	1139	1139	1xFisitinidin-3xCatechin	1xFisitinidin-3xCatechin	Tetramer	Tetramer
1156.7	1154.5	1155	1155	4xCatechin or 4xRobitinidin	4xCatechin or 4xRobitinidin	Tetramer	Tetramer
1170.7	1168.8	1169	1169	2xGallocatechin-1xCatechin-1xFisitinidin	2xGallocatechin-1xCatechin-1xFisetinidin	Tetramer	Tetramer
		1215.2	1215.2	4xGallocatechin	4xGallocatechin	Tetramer	Tetramer
1212.7	1212.7	1214	1214	1xCatechin-2xGallocatechin-2xGlu	1xCatechin-2xGallocatechin-2xGlu	Pentamer	Pentamer
1242.7	1242.7	1238	1238	Gallocatechin (+ Na)	Gallocatechin (+ Na)	Tetramer	Tetramer
1285.5	1285.5	1282	1282	2xFisetinidin-2xCatechin-1xGlu	2xFisetinidin-2cCatechin-1xGlu	Pentamer	Pentamer
1286.7	1286.7	1286	1286	Trihydroxyflavan	Trihydroxyflavan	Pentamer	Pentamer
1300.7	1300.7	1302.9	1302.9	1xFisitinidin-3xCatechin--1xGlu	3xCatechin-1xFisitinidin-1xGlu	Pentamer	Pentamer
1360	1360	1364	1364	5xFisetinidin	5xFisetinidin	Pentamer	Pentamer
1372.5	1372.5	1376	1376	1xCatechin-2xGallocatechin-3xGlu	1xCatechin-2xGallocatechin-3xGlu	Hexamer	Hexamer
1388.6	1388.6	1387	1387	5xFisetinidin+Na	5xFisetinidin+Na	Pentamer	Pentamer
1402.7	1402.7	1402.7	1402	4xFisetinidin-Catechin (+Na)	4xFisetinidin-Catechin (+Na)	Pentamer	Pentamer
1410	1410	1412	1412	2xFisitinidin-3xCatechin	2xFisitinidin-3xCatechin	Pentamer	Pentamer
1462.7	1462.7	1458	1458	4xCatechin-1xGallocatechin	4xCatechin-	Pentamer	Pentamer

					1xGallocatechin		
1490.6	1490.6	1487	1487	2xGallocatechin-1xFisitinidin	2xGallocatechin-1xFisitinidin	Pentamer	Pentamer
1520	1520	1518.5	1518.5	5x2xGallocatechin	2xGallocatechin	Pentamer	
1540	1540	1538	1538	1xCatechin-2xGallocatechin-4xGlu	1xCatechin-2xGallocatechin-4xGlu	Heptamer	Heptamer
1548.7	1548.7	1549	1549	6xTrihydroxyflavan protonated	6xTrihydroxyflavan protonated	Hexamer	Hexamer
1562.7	1562.7	1566	1566	6xDihydroxyflavone (+Na)	6xDihydroxyflavone (+Na)	Hexamer	Hexamer
1578.7	1578.7	1574	1574	5xDihydroxyflavone-Gallocatechin protonated	5xDihydroxyflavone-Gallocatechin protonated	Hexamer	Hexamer
1650.7	1650.7	1652	1652	5xFiesetinidin-1xCatechin	5xFiesetinidin-1xCatechin	Hexamer	Hexamer
1680	1680	1683	1683	4xFisetinidin-1xCatechin-1xGallocatechin	4xFisetinidin-1xCatechin-1xGallocatechin	Hexamer	Hexamer
1694.8	1694.8	1700	1700	1xCatechin-2xGallocatechin-5xGlu	1xCatechin-2xGallocatechin-5xGlu	Octamer	Octamer
1740	1740	1741.8	1741.8	5xCatechin-1xGallocatechin	5xCatechin-1xGallocatechin	Hexamer	Hexamer
1860	1860	1862	1862	1xCatechin-2xgallocatechin-6xGlu	1xCatechin-2xgallocatechin-6xGlu	Nonamer	Nonamer
1820.7	1820.7	1822	1822	6xGallocatechin	6xGallocatechin	Hexamer	Hexamer
1916.9	1916.9	1914	1914	7xFisetinidin protonated	7xFisetinidin protonated	Heptamer	Heptamer

The first tetramer appearing at 1010 Da peak was ascribed to dihydroxyflavone whereas signal of protonated dihydroxyflavan tetramer was noted at 1036.7 Da. Other tetramers rising at 1142.6, 1140.8, 1156.7-1154.4, 1170.7-1168.8 and 1242.8 Da should result from various combinations of fisetinidin, catechin/robitinidin and gallocatechin as shown in Table 2. The strong signal at 1242.8 Da peak of gallocatechin tetramer underlines the abundance of this oligomer in the sapwood, although the content of its corresponding monomer at 305.5 Da (Fig. 2) would be equivalent for the two wood materials. Furthermore, signals at 1286.7, 1360, 1388.6, 1402.7, 1410, 1462.7 and 1490.6 Da highlights the presence of a non-glycosylated tannin pentamer series (Fig.6a, Table 2) which remains also the strongest for the sapwood. Note that the peak at 1388.6 Da assigned to Na^+ attached to fisetinidin pentamer should contain C_4-C_8 and C_4-C_6 interflavonoid bonds as previously reported for maritime pine by Navarrete et al (2010). In addition, the basic sapwood and heartwood condensed tannin monomers like trihydroxyflavan, fisetinidin and gallocatechin lead to their corresponding pentamers at 1286.7, 1360 and 1520 Da respectively. Condensed tannin series up to six hexamers free from glycosyl units was found in Okoume's xylem at 1548.7, 1562.7, 1578.7, 1680, 1740 and 1820 Da (Fig. 6, Table 2). A protonated trihydroxyflavan hexamer of *m/z* (1543 Da + 5*x*H) would correspond to 1548.7 Da peak while the signal at 1820 Da was assigned to gallocatechin hexamer. The four other signals matched for various monomer condensations as depicted in Table 2. It was worth mentioning that only one heptamer corresponding to seven protonated condensed fisetinidin units of *m/z* (1908 Da + 6*x*H) was found at 1916.9 Da peak (Fig. 6).

The presence of condensed tannins in the non-glycosylated flavonoid series discussed above is supported by FTIR spectra (Fig. 7). Typical C=C-C aromatic and C-O stretching bonds, ν(C=C-C) and ν(C-O) respectively were easily identified. The ν(C=C-C) appeared in the 1606-1445.7 cm^{-1} region and the ν(C-O) was observed at 1368-1158 cm^{-1} and 1042-1028 cm^{-1} as reported by various authors (Falcão and Araújo 2013; Ping et al. 2012). Both the sapwood and heartwood displayed intense signals of ν(C=C-C) which agreed with the presence of condensed tannins in Okoumé's acetone/water extracts. However, the strongest signal strength of 1606-1445.7 cm^{-1} bands (Fig. 7) suggested a high content of condensed tannins in Okoumé's sapwood compared to the heartwood. This finding, which supported the signal strength intensities displayed by Maldi-ToF was confirmed by the dominating anthocyans and pro-anthocyans content in the sapwood (Table 3), thus showing for the first time a significant difference ($p<0.001$) between Okoume's sapwood and heartwood condensed tannins content. However, a convenient discussion regarding the ν(C-O) of condensed tannins was not achieved at this level of our study since the ν(C-O) of condensed tannins overlapped the C-C, C-O of

glycosyl units which raised at 1368-1158 cm^{-1} and 1042-1028 cm^{-1}.

Table 3. Phenolic content of Okoume bark expressed in % of dry bark

Okoume type sample	Total phenolic content (% of dry sample)*	Anthocyans content (% of dry sample)*	Pro-anthocyans content (% of dry sample)*
Sapwood	2.04±0.78b	0.48±0.10e	0.30±0.04h
Heartwood	0.74±0.11c	0.22±0.15f	0.10±0.20i

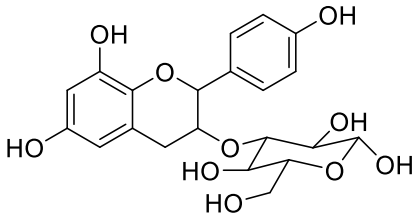
N=4

*: Means±SD

In a column, value with the same letter was not statistically different at α=0.05 level of significance

III.E.2.b. Glycosylated condensed tannins

The second flavonoid series of the extracts was carbohydrates linked to condensed and non-condensed tannin oligomers. Only ten glycosylated tannins were identified in Okoume's xylem extracts as shown by the peak series at 430.0, 448.8, 699-697.7, 921-920.8, 1036.7, 1285.5, 1300.7 Da of Table 2. It is worth mentioning that condensed tannins coupled with carbohydrates linked by ether bridges to the C_3 position of flavonoid units were reported by Pizzi 1980, and more recently by other authors (Abdalla et al. 2014; Drovou et al. 2015). As previously observed for the non-glycosylated tannins, the glycosylated series was the same for the sapwood and heartwood although their relative intensities were strengthened for peaks above 699-697.7 Da (Fig. 5). The first glycosylated tannin detected at 433.9-433 Da was assigned to fisetinidin-3-O-glycosyl.



M=433.9 Da

The strongest signals at 1285.5 and 1300.7 Da peaks (Fig. 6b) were assigned to 3*x*fisetinidin-1*x*catechin and 1*x*fisetinidin-3*x*catechin linked to one glycosyl unit, respectively.

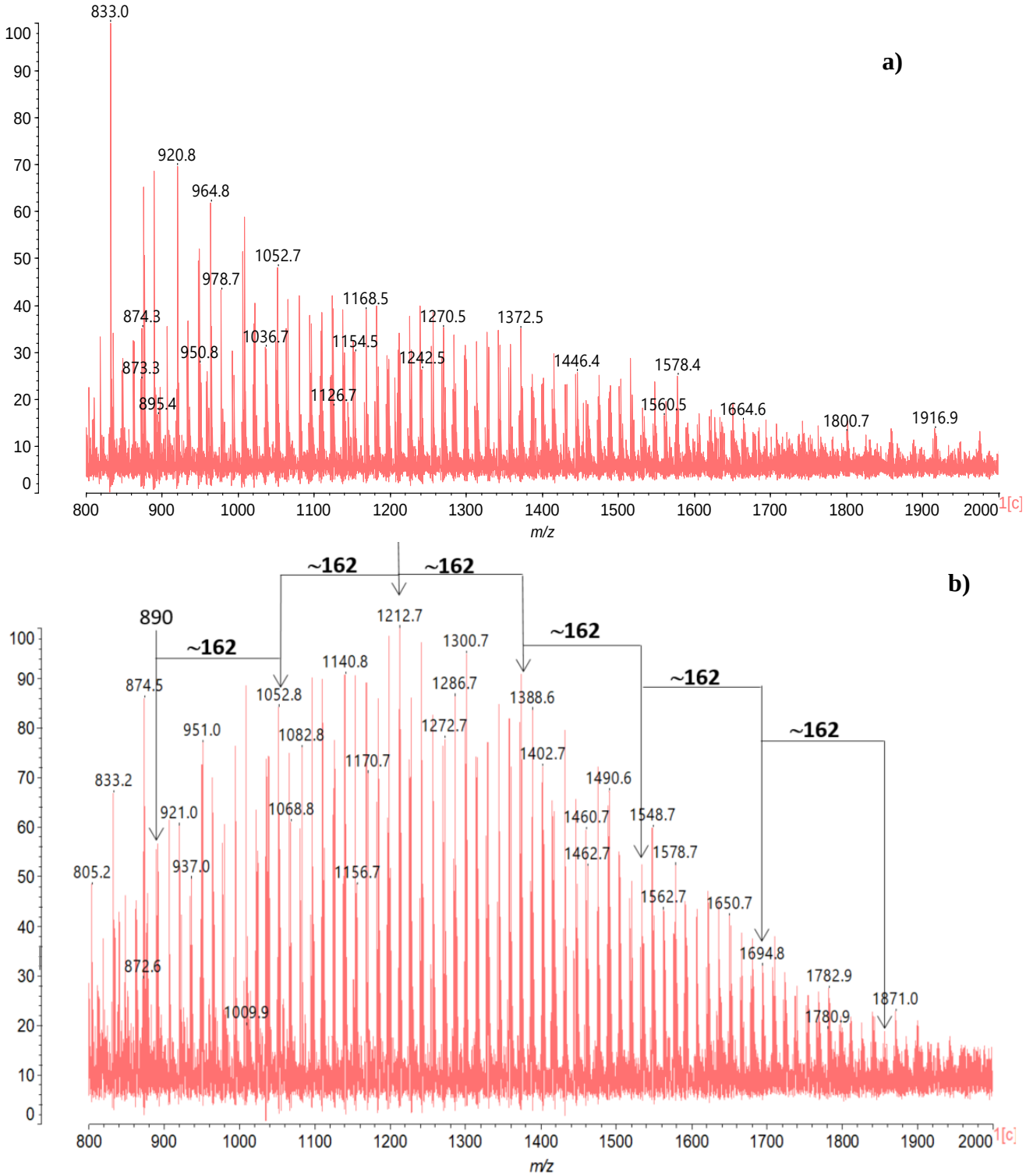


***Figure 6**. Maldi-Tof spectra of heartwood (a) and sapwood (b) in the range 800-2000 Da*

These findings supported the abundance in Okoume's sapwood of glycosylated condensed tannins, and the occurrence of glycosyl units linked to condensed tannins agreed with the presence of 1086-1075 cm^{-1} bands assigned to δ(C-O) in secondary alcohol (Popescu et al. 2007). However, the high glucose content in Okoume's xylem allowed to ascertain that the carbohydrate monomers linked to Okoume's tannins should be strongly dominated by glucose (Glu) as previously claimed.

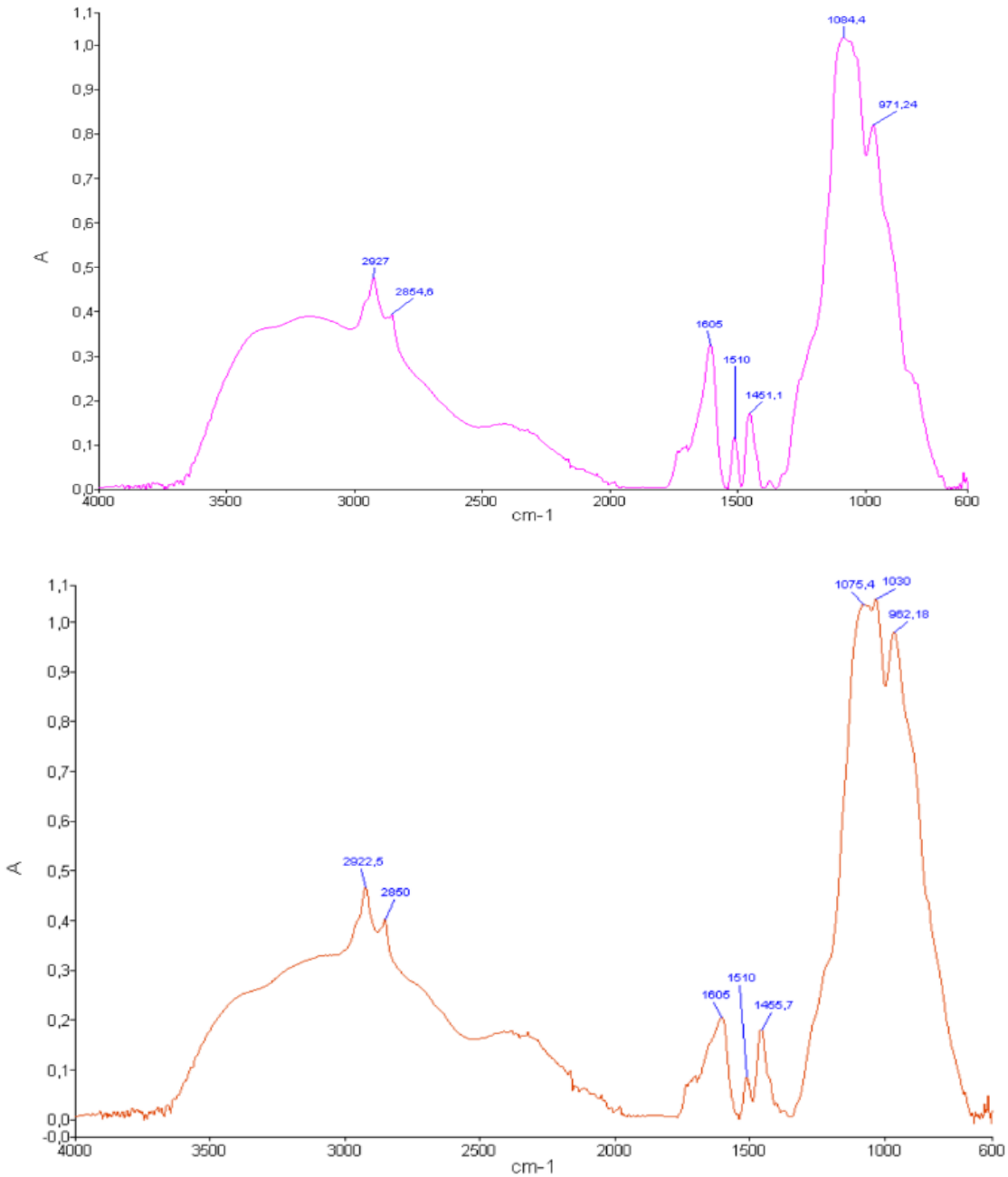
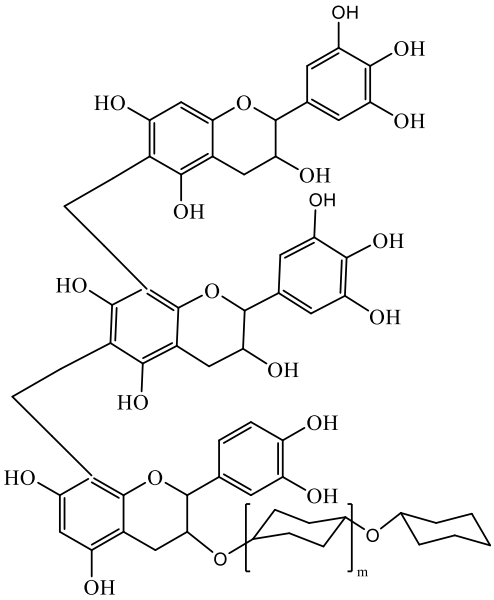


***Figure 7**. FTIR spectra of heartwood (a) and sapwood (b) extractives*

A fine analysis of Figure 6b showed that signals at 890, 1052.8, 1212.7, 1372.5, 1538, 1694..8, 1860 Da correspond to a glycosyl series (+162) starting from the 890 Da peak assigned to 2*x*gallocatechin-1*x*catechin trimer of *m/z* 886.6 leading to an oligomer up to six glycosyl units long at 1860 Da (890 Da + 6*x*162) (Fig. 6). The corresponding glycosylated condensed tannin series should be represented by the structure below:



M= (890 +m*x*162) Da with 1 ≤ m ≤ 6

Nevertheless, only few additional compounds belong to hydrolysable tannins linked to glucose was found in the extracts. The one rising at 921-920.8 Da peak was assigned to 2*x*gallocatechin-1*x*gallic acid glycosyl linked tetramer of *m/z* 923 Da. Although the signal of isoquercetin-gallate (Ricci et al. 2017) raised with high strength at 617.4 Da in the heartwood extracts; other esters depicted at 362, 375.9, 412 and 699-697.9 Da (Table 2) accounting for low content agreed with the weak signal strength at 1730 cm^{-1} of gallic acid moieties ν(C=O) (Falcão and Araújo 2013) in FTIR spectra of Fig. 7.

III.F. Conclusion

The main monomeric constituents in Okoume sapwood and heartwood tannins are fisetinidin and gallocatechin. Structures of condensed tannins from Okoume elucidated by Maldi-ToF and FTIR analysis showed that the condensed tannins were dominated by flavonoid structures weakly esterified and some extent of glycosylated flavonoid moieties. The sapwood and heartwood of Okoume were rich in condensed tannins free from glycosyl units, an oligomer up to seven fisetinidin units was found. Oligomer series of condensed tannins linked to one glycosyl unit and long chain of 2*x*gallocatechin-1*x*catehin linked to six glycosyl residues were found in Okoume's wood. These finding showed the strong potential of Okoume wood wastes to be source of various molecules of high added-value for fine chemistry with biological or food applications. The possibility of an industrial line to produce ecofriendly adhesives in response to the formaldehyde glue based concern would be a promising future for the weak valorized Okoume wood wastes in Central Africa country like Gabon.

Acknowledgements

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Conflict of interest

The authors declare that this work is no in conflicts with any interest.

III.G. References

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Chapitre 3

Valorisation

I. Présentation

Cette partie correspond à la valorisation des déchets de bois d'okoumé.

Le premier chapitre concerne l'élaboration d'une colle biosourcée à base de tanins. Les résultats obtenus dans les articles 1, 2 et 3 nous ont permis d'améliorer un point essentiel, la réduction de la quantité de soude de 5% à 0,25% lors de l'extraction industrielle des tanins. En effet, la quantité élévée de soude limite la performante collante de la résine tanin/hexamine au regard des premières constatations. Ce chapitre met en évidence, la performance de la résine produite en mélangeant, les tanins d'Okoumé à l'hexamine. Ces tanins n'ont subi aucune modification chimique préalable de leur strusture.

Le second chapitre met en évidence les résulats obtenus concernant la mise au point d'un composite bois d'Okoumé- plastique. En effet, nous avons développé un composite par thermocompression sans pour autant passer par une étape d'extrusion, en mélangeant de la sciure de bois d'Okoumé aux broyats de plastique à différentes granulométries. Le composite obtenu a été caractérisé en se basant sur différentes normes d'application : duretée Monnin , Brinell et Shore A0 (B51 125, B51 126 et NF EN ISO 868 respectivement), résistance à la flexion (NF EN 310), test de gonflement (NF EN 317), test de conductivité (ISO 22007-2) et de diffusivité thermique (ISO 22007-2).

II. Development of green adhesives for fibreboard manufacturing, using Okoume bark tannins and hexamine: Characterization by ^{1}H NMR, TMA, TGA and DSC analysis

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II.A. Résumé

Le bois d'Okoumé, largement connu pour avoir une excellente capacité de déroulage, a été utilisé dans notre cas pour l'extraction des tanins afin de formuler, pour la première fois avec ce bois, un adhésif respectueux de l'environnement. Les tanins extraits de l'écorce, puis mélangés à l'hexamine comme durcisseur ont montré des valeurs élevées du module d'élasticité (3681 MPa, 3752 MPa, 3894 MPa, 3971 MPa) selon l'analyse thermomécanique. Une bonne apparence cohésive ≥ 80% et ≥ 60% basée sur la norme NF EN 341-1 et NF EN 314-2 a été obtenue. L'analyse par RMN ^{1}H a été utilisée pour comprendre la réactivité entre les tanins d'Okoumé et l'hexamine, et également pour observer la variabilité de cette réactivité en fonction des extraits des différentes écorces d'Okoumé. Le contrôle de la stabilité thermique a révélé une température de dégradation élevée de l'adhésif.

II.B. Abstracts

Okoume wood, is widely known for having an excellent unwinding capability, this hardwood specie was used for tannins extraction in order to formulate for the first time with that tropical hardwood, an eco-friendly adhesive. The tannins were extracted from the bark, then, mixed to the hexamine as hardener. The system obtained showed high values of elasticity

modulus (3681MPa; 3752MPa; 3894MPa; 3971MPa) according to thermomechanical analysis. A good seemingly cohesive of ≥ 80% and ≥60% based on standard NF EN 314-1 and NF EN 314-2. ^{1}H NMR analysis was used to understand the reactivity occurring between Okoume tannins and hexamine and also to observe tannins reactivity variability with hexamine between tannins extracted from different Okoume barks. Thermal stability control revealed a high thermal degradation of the adhesive system.

Keywords: adhesive, bio-based, hexamine, tannins, Okoume

II.C. Introduction

The main stimulus for today's renewed interest in bio-based adhesives is the acute sensitivity of the general public toward anything concerning the environment and its protection [1]. Governments are still fighting against reducing the use of synthetic resins. Moreover, phenol formaldehyde, resorcinol formaldehyde resins and some amino resins such us urea-formaldehyde and melamine-formaldehyde are the most used today. Globally, there is growing awareness and interest in the use of renewable feedstocks and chemicals as alternative to petrochemicals. It is due to many actors including an emerging industrial biotechnology sector is driving an interest on using differing bioresources.

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 [2]. They offer the advantage over the other two types of not needing any reinforcement with an oil-derived synthetic resin [1]. 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 presently limited by the relatively low stocks supply of these materials [1]. Thus, the aim is to prepare an adhesive based on materials from Okoume, 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.

The used of Okoume as the main raw for particles board is unknown. However, this wood has revealed to have high Stiasny number properties [3]. 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.

II.D. Materials and Method

Samples of *A. klaineana* were collected in the logging concessions of SED (Société Equatoriale de Déroulage) forestry. Four different trees were so collected in three different areas of Gabon. In the north, the south and the west. All the samples were collected from pieces of four trees (83 cm x 10 cm) of around 41 years old [4] at 1.3 m from the soil. Barks samples were dried in an oven for 24 hours at 105 °C, ground in Retsch SK1 mill and screened at 60 mesh.

All the chemicals used in this study were purchased from Fisher Scientific and Sigma Aldrich. Solvents and reactants were used without further purification.

II.D.1. Tannin extraction for adhesive formulation

The tannins were extracted from Okoume bark. The extraction was carried out in water containing 0.25% of NaOH + 0.25 % $NaHSO_3$ + 0.25 % Na_2SO_3 solvent, with a sample-water ratio of 1/5 at 80°C for 3h. The extraction was conducted in several barks grams in order to produce sufficient quantity of tannin for adhesives preparations.

II.D.2. Adhesive properties of tannins

The hardening reaction of a resin system or glue mixes can be evaluated by thermomechanical analysis (TMA), by studding the rigidity of the wood–resin joint as a function of temperature. Thus, the different adhesive system tannins were thermomechanical analyzed under the same conditions. Samples of 38% tannin solution in water, to which 8% hexamine on tannin extract solids content had been added with a pH=10, were prepared. Quadruplet samples of beech (*Fagus sylvatica*) of two beech wood plies, each 0.6 mm thick, bonded with 30 mg of resin, for a total samples dimension of 16*x*5*x*1.1 mm^3. The whole were tested in non-isothermal mode between 25 °C and 250 °C, at a heating rate of 10 °C/min, with a Mettler TMA/SDTA840 apparatus in three point bending, on a span of 14 mm, exercising a force cycle of 0.1/0.5 N. The classical mechanics relation between force and deflection E= [L3/(4bh3)] [ΔF/(Δ f)] allowed the calculation of Young's modulus E for each case tested. The deflection curves that allow MOE determination were obtained in the three-point 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.

II.D.3. Bonging quality test

The Bonding quality test EN 314-1 of 2004

II.D.3.a. Pre-testing process

Immersion of the 100*x*25 mm^2 samples 6h in boiling water, followed by cooling in water at 20 ±3 °C for at least 1h.

II.D.3.b. Determination of bond strength by shear test

Before the water treatment, the lengths of the shear surface are measured and noted down to 0.1 mm. Then, the shear test is carried out on the wet test pieces which can be wiped beforehand. The test pieces are arranged centrally in the clamping devices so that the load can be transmitted from the shear test machine via the test pieces. This, without transverse load. Sliding is only allowed at the start of loading and tightening is carried out on the faces. Thereafter a load is applied at constant speed so that the rupture occurs within 30±10 s.

The breaking load is determined with an accuracy of 1%. The shear strength f_v is calculated according to the formula (1) :

$$f_v = \frac{F}{l_1 x b_1} \qquad (1)$$

With F Breaking load of test piece (N)

l_1 Length of shear surface (mm²)

b_1 Width of shear surface (mm²)

After the shear test, the apparent cohesive failure in the wood is determined

II.D.4. Thermogravimetric analysis (TGA)

The experimental work was carried out on a computerized thermobalance (TGA Q50 instrument) using a furnace which allows a heating rate of 10°C/min. The thermobalance configuration gives a sensitivity of ±0.4lg. It allows to use a small sample mass (10–50 mg) which is needed to ensure isothermal conditions in the samples. In order to establish an inert atmosphere during all experiments, a controlled airflow (fixed at 60mL. min^{-1}, 1 atm) sweeps the measurement cell that is purged for 20 min before starting the heating program. The temperature program was 25 to 600 °C.

II.D.5. Differential scanning calorimetry analysis (DSC)

Measurements were carried out on a DSC (TA Instruments, Q20) equipped with a rapid cooling system. Individual samples of tannins industrial extracted were weighed (~ 7mg) in standard aluminum pans (TA Instruments) and data acquisitions were carried out using the Universal Analysis 2000 program (TA Instruments). A standard heating rate of 10◦C/min was employed from room temperature to 400◦C.

II.D.6. Fourier Transformed Infrared (FTIR) Spectroscopy

FTIR Spectra were recorded on a Perkin Elmer Spectrum One equipped with an ATR-FTIR unit. A few milligrams of resin sample were placed on a crystal (diamond/ZnSe). The spectra were obtained with a resolution of 4 cm^{-1} and 10 co-addition scans in a wavelength range of 650-4000 cm^{-1}. The spectra were collected and analyzed using Spectrum software (Perkin Elmer).

II.D.7. ^{1}H NMR analysis

90 mg of tannin-hexamine resin was dissolved in 0.9 mL of D_2O purchased form ACROS ORGANICS. ^{1}H NMR spectra were recorded at 25°C in a Bruker AVANCE 500 MHz

equipped with a z-gradient double resonance probe. The spectra widths were 5000 and 12,300Hz for the ^{1}H dimension. The number of collected complex points was 1024 for the ^{1}H dimension with a recycle delay of 1.5 s. The number of transients was 64. The $^{1}J_{CH}$ used was 145 Hz. Prior to Fourier transformation. Data processing was preformed using MestReNova software.

II.E. Results and discussion

II.E.1. TMA behavior of tannin-hexamine adhesive

The TMA showed in Fig.1 the Elasticity Modulus (MOE) of the different tannins-hexamine adhesives made with the tannins extracted from Okoume bark. Those tannins were provided from four various Okoume trees located in different areas. The reported values were respectively of 3681MPa; 3752MPa; 3894MPa; 3971MPa for Milole; Nzamiligue 1; Nzamaligue 2 and Mitzic. The adhesive formulation made with tannins extracted from Milole seems to have the lowest value. However, statistical analysis performed with R software showed that there is no significant difference between trees with a p-value=0.61. Furthermore, comparing tree by tree, it appears that there is still no significant difference between Mitzic and Milole with a p-value=0.74; between Nzamaligue and Milole the p-value=0.59 and between Nzamaligue and Mitzic the p-value=0.98. The obtained results were higher than those so obtained by Navarrete et al. [5] on maritime pine bark conducted under the same condition. The elasticity modulus for this specie presented a maximal value of 3744 (MPa). The MOE of the wood joints bonded with different resin gives a good indication of the final strength of the adhesive system tested and the possible final performance of the adhesive system tested. Additionally, these results could be related to the Stiasny number of the species [3].

However, the adhesive starts to polymerize between 50°C and 100°C according to the sample. This temperature is in the same trend with that published by Navarrete et al. [6] and Tondi, [7].

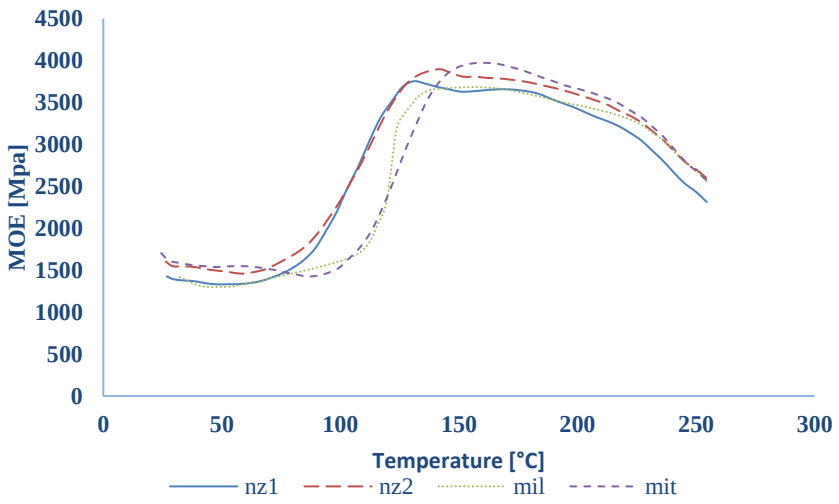

Figure1: Thermomechanical analysis (TMA) of wood panels prepared with tannin-hexamine adhesives: comparison of elasticity modulus (MOE, MPa) obtained for different laboratory-made wood bark extracts area. With Nz=Nzamaligue ; Mil=Milole ; Mit= Mitzic

To understand the reactivity occurring, the FT-IR spectra Observed in Fig. 6 could be appropriated to explain that. In fact, the infrared region between 1800 and 650cm^{-1} is the most significant for tannin extracts and it can be useful to interpret the different reactivity. The C=O stretching are commonly observed in 1800-1650cm^{-1} region. All the powders present some absorbance in this region and the activated tannins show some significant increase, which must be due to the nature of the interaction of the hardener with tannins. In case of hexamine, this interaction is due to the broad stretching of the secondary amines [7]. Regarding aromatic C=C stretching in the region 1650-1400 cm^{-1}. Tannin-hexamine seem to show limited absorption at around 1500 cm^{-1}, as observed by Tondi, [7] who attributed this curve either to delocalization of the π electrons when tannin-hexamine becomes polymerized or to the possibility of signal shifting to around 1450 cm^{-1} which indeed appears broader for every tannin-hardener formulation and it can be assigned to several transitions involving aromatic atoms: C-H bending as well as C–O and C–C stretching are all possible. The broader profiles of this spectral area suggest some similarity between the “activated formulations” [7]. The presence of hydrolysable tannins is therefore hinted. This is therefore observed at the band 1350 cm^{-1} attributed to pyrogallic moieties which is going until disappear when hardener is added to the system. The signal attributed to C-O stretching and C-C bending of aromatics decrease for tannin-hexamine adhesive made with tannins from Milole and seems to resist for Nzamaligue 1 and 2. Furthermore, all the powders present a broader peak in the region close to 1100 cm^{-1}, this might be due to C-H bending of aromatic. Tondi, [7] has attributed this observation to sterical hindrance of the polymerized molecule. Additionally, the absence of peak at 975 cm^{-1} in the system tannin-hexamine guaranty a high cross-linked polymers of the system comparing to FT-

IR peaks of Okoume bark tannins in the low wave number region 1100-600 cm^{-1} (personal data).

II.E.2. Bonding quality test

Table 1 shows the bonding quality test results. The shear strength of Mitzic adhesive studied according to the standard EN 314-1 of 2004 method showed that the system tannin-hexamine of that geographic area would be the least resistance among the investigated adhesives. However, the standard EN 314-2 displayed that all the adhesives respect the required means. The shear strength of Mitzic, Nzamaligue 1 and Nzamaligue 2 adhesives were 0.21±0.05; 0.47±0.09 and 0.49±0.11 N/mm² respectively (Table 1). However, the absence of Mitzic tannins does not allow us to present its results. Regarding the seemingly cohesive mean wood failure, and according to the EN 314-2 standard, the system Milole tannin-hexamine should have an apparent cohesive of ≥80% while Nzama 1 and Nzama 2 should have an apparent cohesive ≥60%. Moreover, the lowest the apparent cohesive wood failure is, better the adhesive is. These results corroborated that previously obtained for Stiasny number [3].

Table 1 : Shear force of Okoume tannin-hexamine adhesives end requirements according to EN341-2 standard

A. klaineana type adhesive samples (condensed tannin-hexamine)	**Shear Force [MPa]**		**Requirements (EN341-2)**	
	Average	Standard deviation	Average shear strength f_v [N/mm²]	Average apparent cohesive failure in the wood [%]
Milole	**	**	**	**
Mitzic	0.21	0.05	$0.2 \leq f_v \leq 0.4$	≥80
Nzamaligue1	0.47	0.09	$0.4 \leq f_v \leq 0.6$	≥60
Nzamaligue2	0.49	0.11	$0.6 \leq f_v \leq 1.0$	≥40
-	-	-	$1.0 \leq f_v$	No Requirement

II.E.3. Thermal stability control of tannin-hexamine

The thermograms of tannin-hexamine obtained under nitrogen depicted in Fig. 2 and represented in Table 2 exhibited three major steps of the tannin-hexamine adhesive degradation process. The first step occurred at 47.83; 48.97; 55.79 and 63.75 in TGA analysis of tannin-hexamine of Milole, Nzamaligue1, Mitzic and Nzamaligue 2 respectively. These first onset temperatures of degradation (T_{d1}) should be associated to preliminary oxidation steps and elimination of volatile fractions such as CO and CO_2 released by the resin (Table 2). Similar temperatures of degradation were observed for tannic acid [8]. The second onset of degradation

(T_{d2}) occurring between 151 and 205°C (Fig. 2) would correspond to the first depolymerization of resins. The third onset temperature of degradation (T_{d3}) assigned to the second depolymerization of the material which would correspond to carbon dioxide and further loss of hydroxyls [9] appeared between 206 and 298°C. The Differential Thermogravimetric analysis (DTG) thermograms of Fig. 3 corresponding to resin derivatives pointed out T_{max}=288.48°C, T_{max}=321.88°C, T_{max}=322.88°C and T_{max}=333.97°C respectively for Nzamaligue 1, Nzamaligue 2, Mitzic and Milole. These temperatures raising in the range 300-800°C should belong to hexamine decomposition that produced mainly ammonia and formaldehyde [10]. But, the occurrence of Okoume tannins which degraded at 250°C cannot be completely ruled out [3].

The TG analysis of the resins at T< 100°C showed a low thermal stability for Nzamaligue 1 and Milole resins who pointed out mass losses of 15% and 12% respectively at the first onset of degradation T_{d1}=48.97°C for Nzamaligue 1 and T_{d1}=47.83°C for Milole. Whereas, Mitzic and Nzamaligue 2 resins begin to degrade at T_{d1}=55.79° and 63.75°C respectively. In addition to their thermal stability, the mass losses of these two resins (7 and 5%, respectively) were also the lowest compared to Milole and Nzamaligue ones (Fig. 2). Moreover, the four resins showed difference on their temperature of degradation at 15% mass loss ($T_{85\%}$) and at 30% mass loss ($T_{70\%}$) which underlined the variability of wood thermal stability as shown in Table 2. The better thermal stability of the resins performed with condensed tannins extracted from Okoume bark was corroborated by their residual rate at the end of decomposition process (T=600°C) comparing to anningre tannin resin as performed by Konai [11]. The residual masses were 47.50 and 31.66% for Nzamaligue 2 and Mitzic respectively, while Nzamaligue 1 and Milole pointed out a rate of 27.08 and 28.05% respectively. The close residual masses and T_{d1}, T_{d1} and T_{d3} of Nzamaligue 1 and Milole resins suggested similarities about their degree of polymerization or interflavonoid bonds (Table 2). Moreover, the residual masses released by the Okoume tannin-hexamine resins at 600°C were in the rough size with that published for Urea Formaldehyde

(UF) by Gonulas [12]. It was remarkable that, with the exception of the sample collected in Nzamaligue 1 natural forest, all the Okoume based tannin-hexamine resins were slightly more stable than UF (T_{max} =310°C) [12].

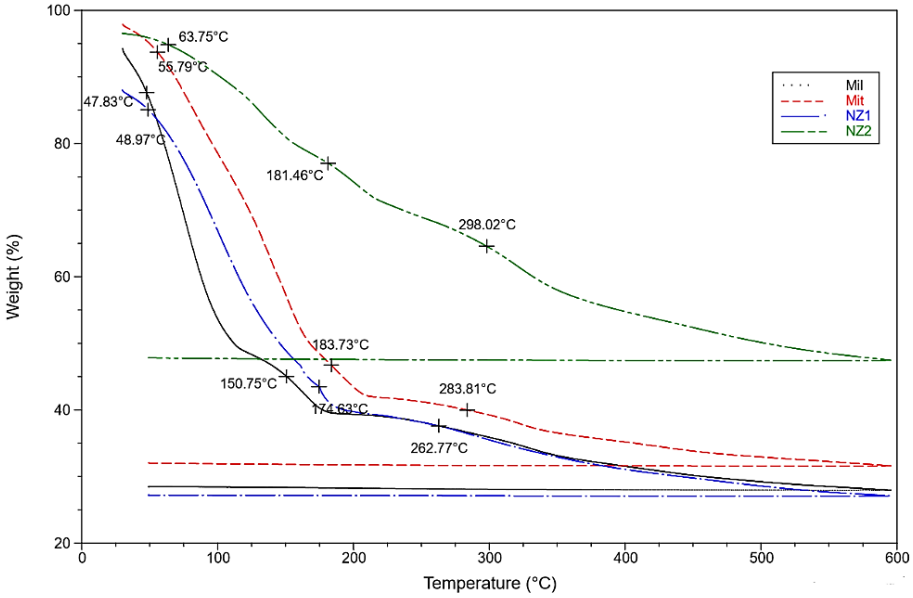

Figure 2: Thermal degradation of Okoume bark tannins-hexamine resin

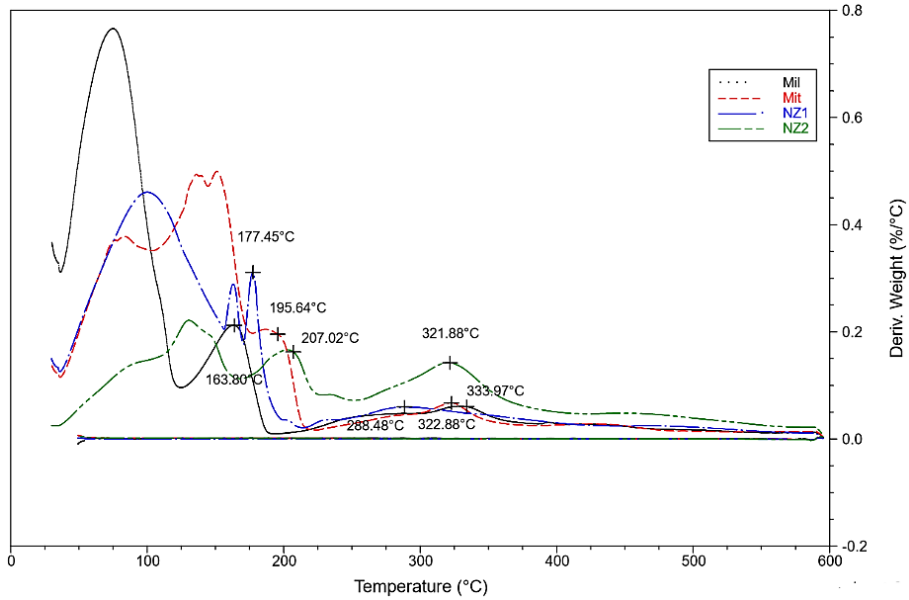

Figure.3: Thermal degradation of Okoume bark tannins-hexamine resin derivatives

II.E.4. Differential scanning calorimetry analysis (DSC)

The four based Okoume tannin resins were analyzed by DSC which is the most widely accepted method for determining the glass transition temperature (T_g) of natural or synthetic polymers [13]. The analysis was carried out at rate of 10°C/min, and the thermograms so obtained were depicted in Fig. 4. All the resins pointed out typical endothermic peaks of elimination reactions [14], the first one showed curve at about 80 and 100°C for Mitzic and Nzamaligue 1. These peaks should belong to water removal as previously observed in case of condensed tannins [15], [16]. That loss of water was also present in strong peaks at 106.08, 111.48, 113.19 and 114.61°C for the Mitzic, Milole, Nzamaligue 1 and Nzamaligue 2 adhesives (Fig. 4) while Nzamaligue 1 resin displayed another endothermic at 178.86°C. However, the

thermal curves of Fig. 4 did not revealed significant thermal differences according to the wood origin. Although Nzamaligue 1 pointed out two additional shoulders at T≈100°C and 178.86°C.

Furthermore, this adhesive highlights a crystallization reaction located at 107.9°C temperature before a glass transition (Tg) at 101.57°C. Mitzic tannin-hexamine has also revealed a crystallization reaction at 102.25°C before a Tg at 79.44°C. However, any evidence of Tg were clearly identified for Nzamaligue 2 and Milole resin respectively. DSC was undertaken on relevant samples to further assess how the sample decomposed (Fig. 4). Therefore, tannin-hexamine adhesive from with Mitzic and Milole extracts was stable up to 216 and 215.04°C, respectively. These two resins were much more stable to oxidation than those made with Nzamaligue 1 and 2 tannins which seemed stable until 196.78 and 191.32°C, respectively. These results indicated a similar behavior between Okoume resins and mimosa tannin-hexamine resin performed by Peña [17]. No evidence of auto-condensation of Okoume resins was observed at pH≤10 indeed, which agreed with that ascertained by Peña [17].

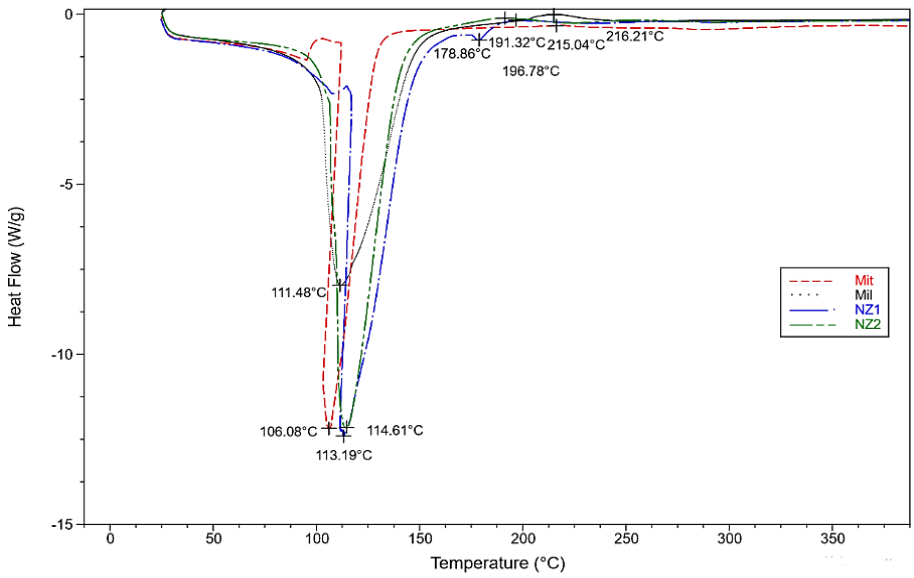

Figure 4: DSC curves of Okoume bark tannins-hexamine resin

II.E.5. Analysis of Okoume tannin-hexamine adhesive composition by ^{1}H-RMN

In order to understand the chemical shift occurred, spectra can be divided in three regions as described by Inaki et al [18]. The high field region (0.0-2.5 ppm) contains mainly signals from the alkyl residues some organic acid and alcohols. Additionally, this field should correspond to H-C, aliphatic protons in extended alkyl chains. The low field region (2.5-5.8 ppm) is dominated by signals from the components of highest concentration: glucose, fructose and sucrose. However, regarding the region between 2 and 3ppm, the occurrence of H-C=, protons attached to carbon atoms adjacent to a carbonyl or aromatic group appears to be mainly

dominated. On the other hand, protons attached to carbon atoms singly bonded to oxygen is therefore hinted into the region 3.3-5ppm as described by Zghari et al [19]. The low field region (5.8-8.5 ppm) contains a number of signals from aromatic protons of phenols constituents and correspond to the most explainable region for condensed tannins characterization. This region is dominated by A_r-H of aromatic protons. However, O-H phenolic appears into the field 4-10 ppm. Internal composition of aliphatic moieties like CH_3 were identified with the resonance signal occurred at 0.8 ppm and confirmed by FTIR analysis, which pointed an absorbance peak occurring between 1300-1400 cm^{-1} (Fig. 5)

^{1}HNMR highlighted the tannins and hexamine reactivity betwixt 3 and 5 ppm with an increasing of integration surface, which could be explained by decomposition of hexamine structure and formation imino-amino methylene bases (Fig. 7).

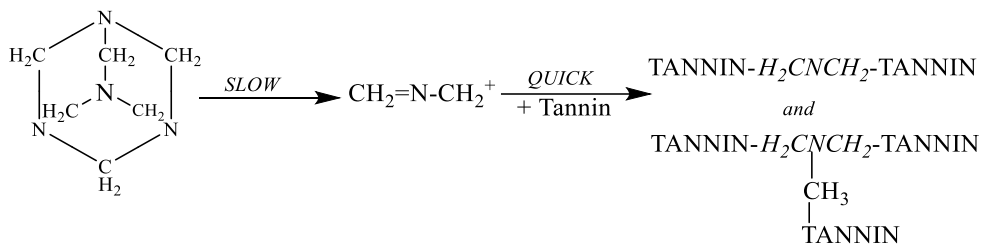

Figure 5: Mechanism of hexamine decomposition to imino-amino methylene bases in presence of fast-reacting species such as tannins and their fast reaction with tannins to form benzylamine brides [20].

One of practical method for estimating the stoichiometry and binding constants of intermolecular complexation is by titration experiment with NMR or UV-Visible spectroscopy [21]. In our case, ^{1}H NMR was used to observe the potential variability of Okoume's bark tannins according to the area of sampling and the reactivity tannin-hexamine occurred. ^{1}H NMR showed that the chemical structure of the four tannins appeared slightly the same. The slight different occurs between 3 and 5 ppm where integration surface shown values of 134.44; 149.59 and 187.9 ppm for Nzamaligue 1; Mitzic and Nzamaligue 2 respectively (Fig. 7). The same observation was made while analyzing the resins 166.73, 204.78, 357.24 ppm. However, resins ^{1}HNMR analyzed were emphasis by the curve between 4 and 5 ppm when overlaying tannins and resins peaks for each sample. One peak at 4.5 ppm in tannin-hexamine curves, belonging to hexamine (Fig. 7). This one is not present in tannins curves. The high reactivity of Okoume tannins was in agreement with the Stiasny Number reported by Engozogho et *al* [3].

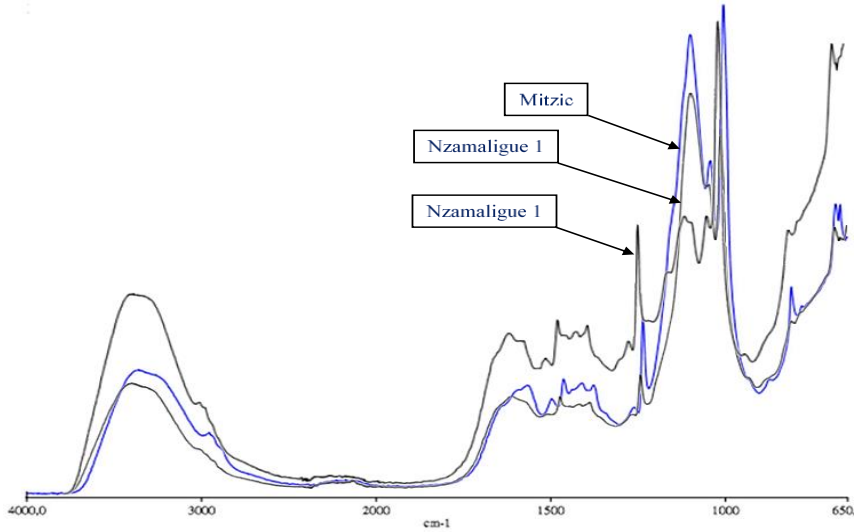

Figure 6: FTIR peaks of Okoume bark tannins-hexamine

II.F. Conclusion

According to the results obtained in this characterization, we noticed that for Okoume bark adhesives from different areas of Gabon presented very high performance for TMA tests. In fact: 3681MPa; 3752MPa; 3894MPa; 3971MPa for Milole; Nzamiligue 1; Nzamaligue 2 and Mitzic respectively.

^{1}H NMR displayed that the chemical structure of the four Okoume tannins came out analogous. The minor disparity occurs between 3 and 5 ppm where integration surface obtained values of 134.44; 149.59 and 187.9 ppm for Nzamaligue 1, Mitzic and Nzamaligue 2 respectively.

The four Okoume tannins adhere to the EN 314-2 standards. In effect, the shear strength of Mitzic, Nzamaligue 1 and Nzamaligue 2 were of 0.21±0.05; 0.47±0.09 and 0.49±0.11 N/mm² with the respective seemingly cohesive of ≥80%; ≥60% and ≥60%.

The residual masses delivered by the Okoume tannin-hexamine resins at 600°C were higher than those typically reported for UF resins.

All the Okoume resins revealed typical endothermic peaks of elimination reactions, the first one appeared at about 80 and 100°C for Mitzic and Nzamaligue 1. These results shown a comparable performance between Okoume resins and mimosa tannin-hexamine resins.

Acknowledgements

We gratefully acknowledge the Société Equatoriale de Déroulage (SED) for their full collaboration in wood material provision.

Funding

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Conflict of interest

The authors declare that this work is no in conflicts with any interest.

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NF EN 314-2. Qualité du collage - Partie 2: Exigences: Juin 1993. B51-1338-2

III. Utilization of *Aucoumea klaineana* Pierre (Okoume) wood wastes in plastic panel composites

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III.A. Résumé

Dans une société où le recyclage devrait être plus important, comme en Afrique, il existe une possibilité de valorisation des déchets pour la production de composite bois-plastique (WPC). Ces produits à base de bois pourraient être utiles pour la construction et la fabrication de meubles.

En règle générale, les WPC sont fabriqués via 2 processus succesifs : l'extrusion et la thermocompression. Cependant, il est possible de les produire directement par thermocompression. Les sciures d'aubier et de bois de cœur d'Okoumé ont été utilisées sans extractions préalable des composés chimiques. Les particules de bois utilisées presentaient une granulométrie de 0,5 mn pour tous les panneaux, tandis que celles des particules de plastique étaient de 1,5 mm pour les panneaux P1, P2, P3, P4 et P5 et de 0,5 mm pour les panneaux P6 et P7.

Les processus d'analyse ont été effectués selon les différentes normes européenne et les résultats obtenus étaient en accord avec les recommandations de ces normes. Les principaux résultats ont montré que, l'ajout de plastique a augmenté la résistance thermique du bois. Ce comportement devrait être utile pour l'industrie du bâtiment. De plus, l'analyse mécanique des panneaux a montré une bonne tenue mécanique au regard des résulats présentés en flexion et en module de flexion.

III.B. Abstract

Objective: In a society where recycling should be more significant, like in Africa, there are great possibilities of waste valorization in the area of wood plastic composite (WPC production. These wood-based products could be useful for building and furniture manufacturing which dominate the use of wood plastic composites. Therefore, African societies are on the trail of new technologies.

Methodology: Typically, WPC are made by 2 successful processes: extrusion and thermocompression. However, it is possible to make them just with thermocompression process. The sapwood and heartwood sawdust were used without further chemical extractions. These innovative panels are interesting, because of the reducing of two mains matters met in Africa societies, especially in Gabonese industry and society. Open-air burned waste woods (85%) and, open-air waste plastics burned. This last one, representing 70% of daily waste in Gabon. In the obtained panels, wood is used as reinforcement at 50%, plastic is therefore used as matrix at 47% and 3% for binder. OWP (Okoume wood plastic) were manufactured with plastic particles sizes of 0.5 mm for panels P5, P6, and 1.5 mm for P1, P2, P3, and P4 panels.

Results: The analysis processes were performed according to various standard recommendations and the results obtained were in agreement with the relevant standards. From the mains results, it was observed that adding plastic helped the wood to better withstand the temperature as described with the thermogravimetric analysis, differential scanning calorimetry and hot disk tests. This behavior should be useful for building industry using. The mechanical analysis behavior of the panels, shown good values of bending strength and modulus elasticity for the 6 panels performed. The small the plastic particle sizes was, the higher the compactness was. This last behavior was appreciated for panels water recovery, which highlighted small yield of water recovery with the main high value of about ≈7%, comparing to the minimum value recommended by the standard (13%).

Keywords: Composite, Okoume, plastic recycling, wood.

III.C. Introduction

Gabon is a country covered with 85% of forests. This national heritage is exploited and about sixty species are mainly exported to Europe and Asia. The timber industry is the second largest resource in this country and *Aucoumea klaineana* Pierre (Okoume) is the most abundant species [1]. Other species such as the African mahogany (*Khaya ivorensis*) are also exploited because of their wood quality. In order to promote the local processing of wood, the Gabonese government has banned raw logs exportation since the year 2010 and any felled trees must undergo at least one transformation before any exportation according to the law N°016/01 bearing on the Forest Code promulgated on December 31, 2001. This measure should increase the industrial activities of timber companies in the country, thus increasing this sector employability. However, according to the final report of the first African wood forum occurred at Libreville June 20, 2018, the high content of wood wastes, which accounts for 50% of the wood transformed in that Central African country, is the major drawback of the dominating first transformation of logs.

Today, the valorization of these wood wastes is very limited. Therefore, one of the promising strategies of wood wastes valorization is their coupling with underutilized plastic wastes which represent 70% on the 600 tons of garbage produced per day at Libreville [2]**.** Despite the abundant literature dealing with the different ways of plastic wastes valorization [3,4], developing countries are hampered by the lack of adequate technologies for reducing this scourge**.** Nevertheless, some authors have investigated strategies for wood wastes valorization in various ways. Esterification of tropical wood sawdust with succinic anhydride showed variability on the micromechanical stability of the resulting composites. The crystalline lattice of succinylated Okoume sawdust pointed out its good stability through the coupling agent [5]. The enzymatic conversion of Okoume polysaccharides in neutral sugars for bioethanol [6], or the recent isolation of its lignin by steam explosion [7], as well as the molecular structure and the ease of depolymerization of Okoume lignin [8] showed the potential that hardwood wastes for biorefinery. In addition, the thermal stability of Okoume tannins exhibited the capability of that hardwood wastes to be used as source of condensed tannins for adhesives [9].

However, the considerable development of the housing market in the hot and humid Congo basin countries must solve the question of maintaining thermal comfort of homes. That requires a better knowledge of the thermal characteristics of the materials which compose the walls.

Therefore, the aim of this study is to produce wood-plastic composites with simple and easily transferable thermo-compression technology from Okoume wood wastes and plastic

sawdust entirely dominated by polyethylene. The obtained panels should be used in the building industry as they constitute a solution for wood and plastic wastes management. This work will be focused on two mains sittings : understand the thermal behavior of the wood, but also of the plastic and the OWP; and figure out the physical-mechanical behavior of the material in order to preview the possible use of composite. The main objective of this article is to assess the potential of this wood-plastic composite manufactured by direct compression.

III.D. Experimental

III.D.1. Samples

The sapwood and the inner heartwood (from rotary cutting logs) of Okoume were collected in the logging concessions of SED (Société Equatoriale de Déroulage). All the samples were collected from four pieces of trees (83 cm x 10 cm) of around 41 years old [10] at 1.3 m from the soil. The wood samples were ground with a Retsch SK1 mill to pass 60 mesh and oven-dried for 24 hours at 105 °C. Various recycled plastic bottles were collected and ground at 0.5mm and 1.5mm of plastic particles sizes diameter with a RETSH ZM200 machine. The peculiarity of this research work is that the matrix thermoplastic used is as set of different polymers taken in proportions well defined. The percentages considered of the matrix obtained represent the shares of polymer waste found in the city of Libreville (capital of Gabon). The bottle were grounded without further cleaning.

III.D.1.a. Panel manufacturing

Six wood-plastic panels of 280*x*280*x*10 mm^3 were made as follows: 3% of MAPP (Maleic anhydrid coupled with the polypropylene) purchased from Sigma Aldrish was added to the mix 47% of plastic powder and 50% of wood powder. The percentages were chosen based on previews works described by sigworth and Wefer [11]. Generally, MAPP is added from 1 up to 5% [12]. We have chosen 3% of MAPP in order to reduce the total cost of the material, since MAPP represents 4 to 20% of the total cost of the material [13]. The whole was mixed with a CAFRAMO machine mixer for 3 minutes at room temperature. The obtained mixture was carried to an industrial press with hot plate heated at 210°C and the press cycle used for shaping the panel is presented below. The six panels made called P1 to P6 (Table 1) were different. Panels P1-P4 were made with plastic particle sizes of 1.5 mm of diameter, whereas the panels P5-P6 were made with plastic particle sizes of 0.5 mm of diameter. The lack of panels of plastic particle sizes of 0.5 mm is mainly due to the difficulty of crushing plastic until 0.5 mm of diameter.

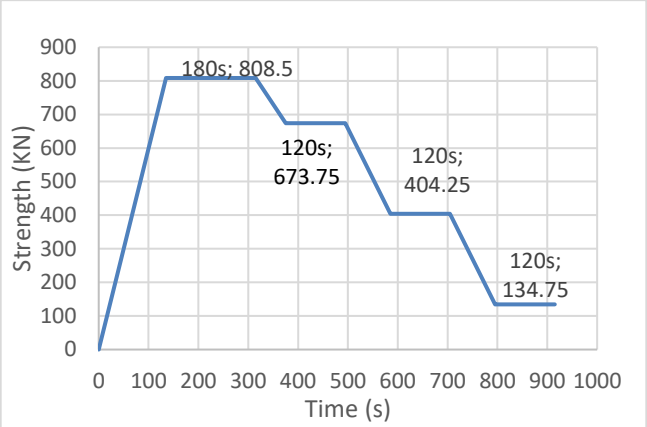

Figure 1: Press cycle for shaping the Okoume wood-plastic panel.

III.D.2. Water recovery

Water recovery consists in determining the composite swelling rate using small samples of 50±1 mm of sides. Water recovery were performed according to standards NF EN317 and NF EN 325. The panels were firstly conditioned at 65±5% of relative moisture content and 20±2°C of temperature until getting a constant mass. The panels were secondly immersed into a constant-temperature bath (20±1°C) and maintained at this temperature during the assay. The calculation of the swelling in thickness of each test piece, expressed in centimeters from its original thickness, use the following formula (1) :

:

$$Gt = \frac{t2-t1}{t1}\ x\ 100 \qquad (1)$$

With G_t swelling, t_1 piece thickness before immersion expressed in millimeters, t_2 piece thickness after immersion expressed in millimeters

III.D.3. Thermal characterization of Okoume wood, plastic and wood-plastic composite

III.D.3.a. Hot Disk

The device used is a Hot Disk with a reference probe 5465 and a radius of 3.189 mm. The samples are 50*x*50*x*10 mm^3. A Kapton wired nickel probe is placed between two samples of the same material. The probe will emit thermal energy for a short or long time and will thus locally heat the material. The probe, which is transmitter and receiver, senses this temperature variation. It will measure the heat flux in an axial and radial way based on ISO 22007-2 standard.

III.D.3.b. Themalgravimetric analysis (TGA)

Thermal decomposition was performed using a TA Instrument (TGA Q50 instrument).

The temperature program was from 25 to 600°C at a heating rate of 10°C/min. the measurement were conducted under nitrogen (40 ml/min). The results were analyzed with TA Instruments Universal Analysis 2000 software.

III.D.3.c. Differential Scanning Calometric (DSC)

The differences in heat exchange between an analysis sample and a reference were carried out on a TA Instruments (DSC Q20 instrument) equipped with a rapid cooling system. Samples of Okoume and plastics were weighed (~ 7mg) in standard aluminum pans (TA Instruments) and data acquisitions were carried out using the Universal Analysis 2000 program (TA Instruments). The measurement was conducted under nitrogen (40 ml/min) with a standard heating rate of 10◦C/min from room temperature to 400°C.

III.D.4. Mechanical characterization of Okoume wood, plastic and wood-plastic composite

III.D.4.a. Hardness

Three different hardness methods were performed on the wood-plastic composite in order to assess the variability of results. The tests were performed on the pieces of 50*x*50*x*thickness mm^3.

Monnin (B51-125): the pieces were first conditioned up to a constant mass (20°C of temperature end 65% of relative humidity). The principle of the method consists of printing with a 30 mm diameter steel cylinder a face of the piece, under a certain load. The cylinder moves at a speed of 10 mm / min up to the load of 300 DaN and proceed to immediate discharge. In order to obtain a visible imprint, a carbon sheet is interposed between the cylinder and the piece.

The results of each piece was expressed as follows (2):

$$t = 15 - \frac{1}{2}\sqrt{900 - a^2} \qquad N = \frac{1}{t} \qquad (2)$$

With a the print width (mm) ; t penetration arrow (mm) ; N Monnin hardness

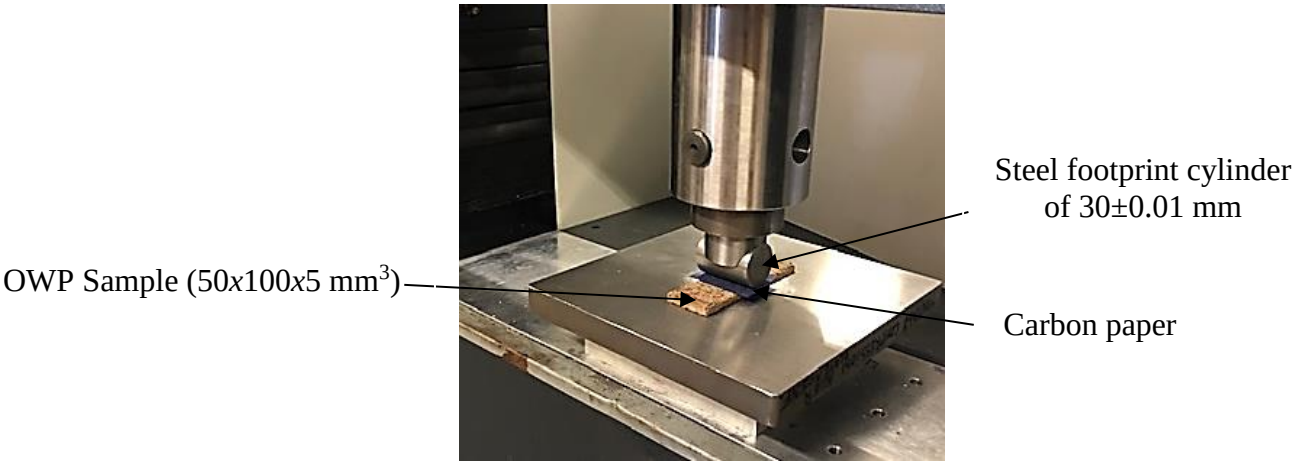

Figure 2: Monnin hardness testing device

Brinell (B51-126 the pieces were first conditioned up to a constant mass (20°C of temperature end 65% of relative humidity). The method consists of the determination of the punching resistance of a panel by applying a loaded to the facing of the test piece with a 10 mm diameter ball. The evaluation of the punching resistance is made by measuring the depth of penetration and the residual depth. A preload of 1% of the test load is applied in order to determine the reference position of the load head; then an increasing force is applied at such a speed that the depth p = 2.5±0.5 mm was reached in 15±5s. Finally, the force corresponding to this depth is maintained for 25±5s and the depth is recorded.

Brinell hardness (HB) is obtained using the formula (3):

$$HB = \frac{3.18\ x\ F}{1000\ x\ P} \tag{3}$$

With F the force determining the retained depth (Newtons); P the depth of penetration of the ball (mm); HB Brinell hardness (dan/mm²).

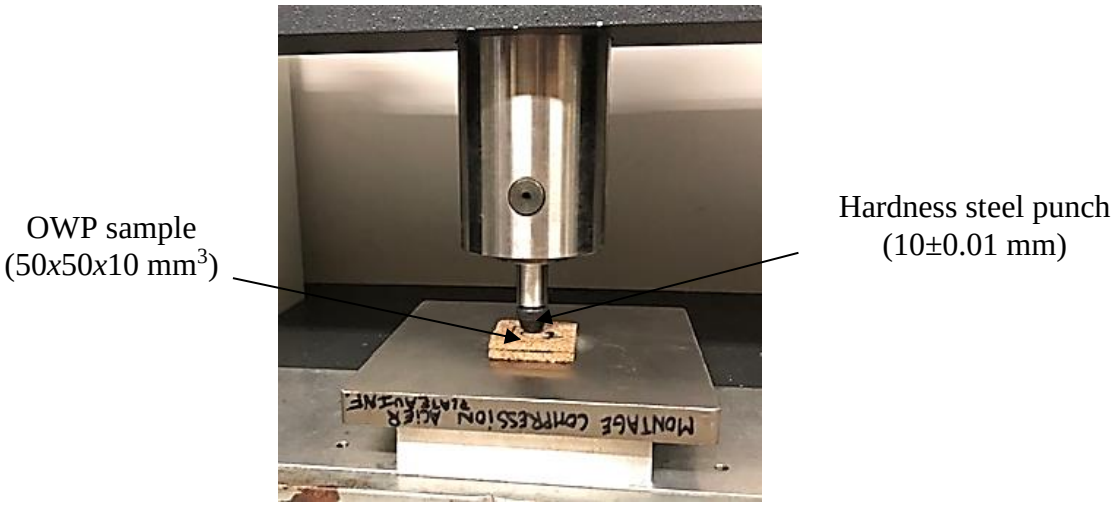

Figure 3: Brinell hardness testing device

Shore AO (NF EN ISO 868) : The durometer must be mounted on the test stand using the mounting device. Then place the hardness reference block on the glass plate. It is necessary to lower the lever, to remain in balance, so to press the lace in the hole of the reference block, until the foot of the instrument touches the reference block completely. During this time, the dial hardness value should be ± 1 compared to the embossed value below the reference block. If the value is not between 100 ± 1, the adjusting nut under the glass plate must be adjusted so that the value will reach 100 ± 1. The lever should be lowered carefully using the force of the given weight (1 kg for Shore A0 hardness). If the durometer touches the test equipment completely, the value can be read on the dial. The reading time for thermoplastic gum is 15 seconds, in vulcanized gums or other kinds of unknown gum it is 3 seconds.

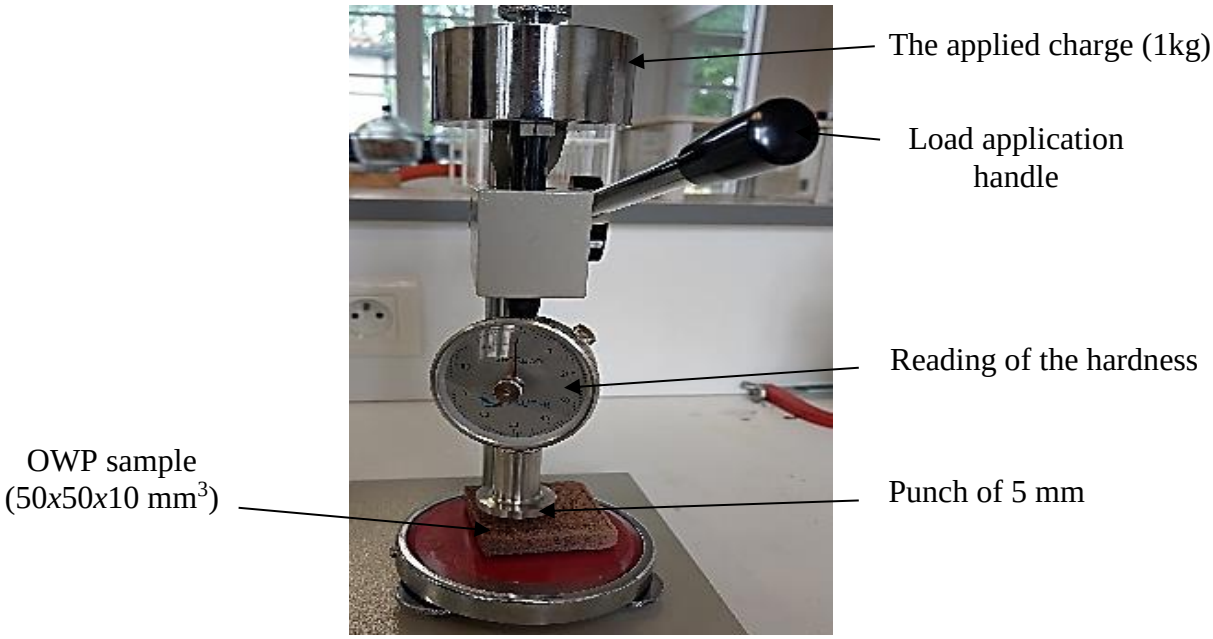

Figure 4: Shore A0 hardness-testing device

III.D.5. Bending strength and modulus elasticity

The determination of 3 points bending strength and modulus of elasticity was performed according to EN 310 relevant standard. The pieces were first conditioned (20°C and 65% of relative humidity) up to a constant mass. A bending load is applied in the middle of a test piece of 150x50xthickness mm^3 of section, according to NF EN 326-1 standard. The elasticity modulus is calculated using the slope of the straight part of the load–deformation curve. During the test, a load at constant speed is applied in the center of the specimen with an adjusted speed , so that, the breaking loaded was reached in 60±30s. The deflection in the middle of the test piece was measured with a LVDT Solartron sensor AX5S.

The results obtained were expressed as follows (4) and (5) :

$$E_m = \frac{t_1^3\ (F_2 - F_2)}{4bt^3\ (a_2 - a_1)} \quad (4)$$

With E_m elasticity modulus in bending (N) ; t_1 distance between centers of support (mm) ; b width of the piece (mm) ;

T thickness of the piece (mm) ; $F_2 - F_1$ Load increasing (N) on the right section of the curve load-deformation, F_1 should be of about 10% and F_2 of about 40% of the tensile strength a_2- a_1 boom increasing halfway down the test piece.

$$f_m = \frac{3F_{max}\ t_1}{2bt^2} \quad (5)$$

With F_{max} tensile strength (N); f_m bending strength (N)

Figure 5: Bending elasticity modulus and strength testing device

In summary, nine parameters will be studied in this work:

Water recovery, thermal conductivity, thermal diffusivity, particle sizes, Shore A0 hardness, 3 points bending strength and modulus of elasticity , Monnin and Brinell hardness.

III.D.6. Multivariate data analysis: Principal Component Analysis (PCA)

The objective of a loading projection is to visualize the variables' position with respect to one another in two-dimensional space and their related correlations. Variables closed to one another and far from the plot origin are positively correlated (such as, particle sizes and water recovery in PC1-PC2 plots), while variable opposite one another on the plot are inversely proportional (such as, thermal conductivity and water recovery in PC1-PC2 plot). The generated Loading plots set from the Table 1 explain the relationships between two variables

by their angle from the center as described by Shin et *al* [14].

III.E. Results and discussion

III.E.1. Thermal properties of Okoume wood

The thermal degradation of the materials obtained was conducted under nitrogen and the relevant results are depicted in Figure 6. Data collected in Fig. 6b showed that the onset temperature of degradation (T_d^i) was 217.5°C for the sapwood and heartwood, this why only one curve of Okoume biomasses is presented. The same trend was observed for the temperature at 5% weigh loss of the wood($T_{5\%}^w$), which did not show difference between the studied wood samples (250.2 °C). Although the temperature of decomposition of cellulose, hemicelluloses and lignin would partially overlap in the range 200-400 °C [15], the mass loss occurring at 250.2 °C should be assigned to hemicellulose chains breakdown [16] which raised their maximum temperature of degradation (T_{max}^1) at the 306.25 °C shoulder. That first derivative thermogravimetric curves (DTG) fingerprint of active pyrolysis of hemicelluloses and amorphous celluloses agreed with that found for white willow and black alder [17], lime wood [15], poplar [18], eucalyptus and other wood species [19]. Moreover, the temperatures at 50% weight loss ($T_{50\%}$) did not vary between Okoume biomasses, $T_{50\%}$was 350°C both for the sapwood and heartwood indeed. That very close temperature of the second maximum DTG curve of wood ($T_{max}^2 = 345.53°C$) should be endorsed to active pyrolysis of Okoume's crystalline cellulose agreed with that reported for beech, oak or sapele hardwood species. The TG curve (Fig. 6b) showed a last stage of Okoume's thermal degradation occurring in the range 350-500°C (Fig. 6b) attributed to active pyrolysis breakdown of lignin [20]. That was ascertained by the third DTG maximum temperature of degradation of Okoume's biomasses (T_{max}^3) raising at 476.82 °C (Fig. 6a) and assigned to thermal decomposition of lignin in other studies [14,15].

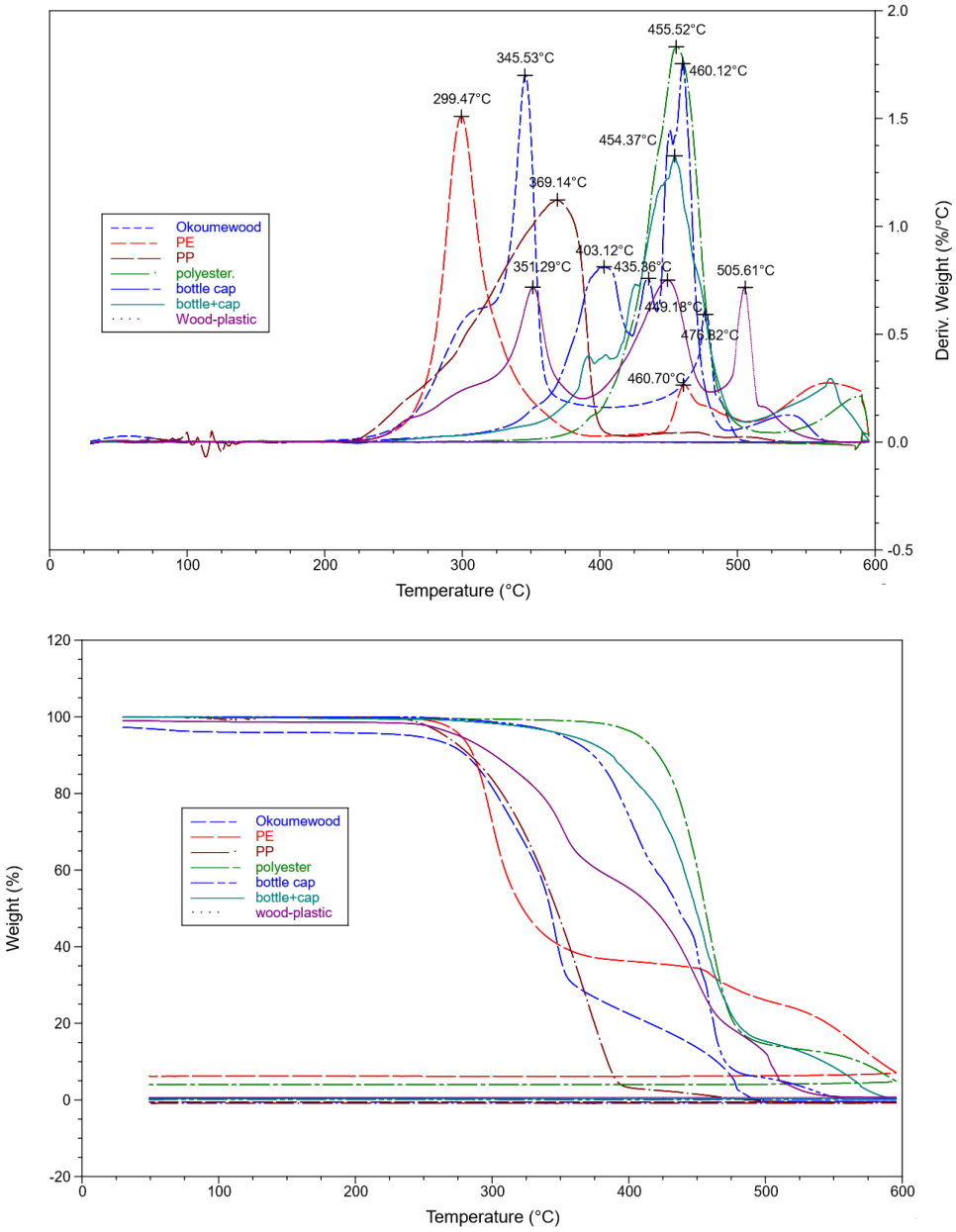

Figure. 2 : DTGA (a) and TGA (b) curves of materials used heated at 10°C/ min under nitrogen. With Okoumewood= blue; polyethylene= red; polypropylene= marron; polyester=green; bottle cap= blue; bottle+cap= aqua and woodplastic= purple.

The lack of differences about the thermal stability of Okoume's sapwood and heartwood agreed with that published for Chinese fir [21]. That would be related to the similar chemical composition of the two biomasses on behalf of their similar, cellulose [22] and lignin [6] content. Conversely, the highest extracts content in Okoume's heartwood [6] which would degrade at low activating energy as found for Chinese fir's heartwood [21] did not reduce the T_d^i of Okoume's heartwood compared to the sapwood one.

Moreover, both the sapwood and heartwood did not release any residual mass at the final temperature of wood degrading process ($T_f = 500°C$). That result was in close agreement

with the weak ash content (0.2-0.47% of dry wood) released by Okoume's sapwood and heartwood ignited at 500 and 600 °C reported by Minkue M'Eny (2000) and Safou Tchiama et al. (2016) works.

The strong thermal stability of Okoume's biomasses discussed above was corroborated by their DSC thermograms, which superimposed completely (Fig. 7), in the figure, sapwood and heartwood did not show any different on their degradation process; this is why only one curve of Okoume biomasses is presented. The first middle point of the glass transition (T_g) of Okoume was observed at 120.98 °C. That temperature should be related to the weakening of the hydrogen bonds of the cellulose-hemicelluloses carbohydrate complex like amorphous phase occurring with the loss of physically bonded water in the range 80-120°C [23]. The two endothermic peaks at 154.16 and 158.33°C should be assigned to active pyrolytic degradation of Okoume's xylan like hemicelluloses for xylans start to decompose at 150°C [24]. The strongest endothermic peak at 176.71°C was ascribed to Okoume's cellulose degradation as similar peak related to cellulose transition phase has been reported for Shisham (*D. sissoo*) wood [23]. Moreover, the DSC curve displayed two exothermic broads centered at 293.84 and 374.97°C, respectively assigned to isolate hemicelluloses and cellulose crystalline phase degradation of Okoume. That agreed with that reported for *Pinus elliotii* and *Eucalyptus grandis* woods by Poletto et al. (2016).

III.E.2. Thermal properties of Okoume plastic composite (OWP)

The literature reveals that the body of plastic bottles is generally composed of poly (hetero) terephthalate (PET). The thermal curves of the cap showed that it would be a mixture of polyethylene (PE) and polypropylene (PP). The thermal stability of the grinded body bottles and caps mixed with Okoume's wood sawdust were controlled by TG/DTG and DSC (Figs. 6; 7). TG data collected in Fig. 6 showed that all the bottle's thermal parameters were accordingly better than that displayed by Okoume's wood. Furthermore, the temperature at 5% and 50% mass loss of the body bottle composed with PET and labelled $T_{5\%}^{PET}$ and $T_{50\%}^{PET}$ respectively, as well as its residual mass at $T_f = 500°C$ were higher than that found for the cap. That result showed that the thermal stability of plastic bottle wastes would be controlled by the bottle body made with PET. In addition, the thermal properties of the entire ground bottle (body + cap) were intermediates between the body and the cap as revealed by the TG curves and data (Fig. 6).

For a convenient understanding of the thermal properties of plastic wastes used in this study, the chemical composition of the bottle was controlled by DSC. That thermal technique revealed the absence of characteristic endothermic peak at 167.61°C assigned to PP in the

plastic bottle. Hence, it should be ascertained that most of the ground plastic material corresponded to PET as its characteristic melting point was noted at 127.8 °C whereas its endothermic peak of degradation appearing at 246.84 °C agreed with that previously described for PET by other works [25].

The thermal behavior of the manufactured OWP was analyzed by TG/DTG and DSC. It was noteworthy that mixing the wood sawdust with plastic increased the thermal stability of the composite compared to the wood alone as shown in Fig. 6. The most important temperature gain was for the mass loss at 50% which increased from 350 °C in the wood particles to 408.33 °C for the OWP. In addition, the thermal stability gain of OWP was also remarked for the residual mass at $T_f = 500\ °C$ which became very close (~15%) to that displayed by the plastic waste (~16.25 °C).

However, the DTG curves of OWP showed a decrease of the rate of degradation (weight %/C) for hemicelluloses and cellulose in particular whereas no change was observed for the hemicelluloses T_{max}^1 shoulder which remained 306.25 °C in OWP (Fig. 6b). The presence of plastic in OWP should have enrobed the wood particles so that the T_{max}^2 assigned to cellulose degradation shifted at 351.29 °C (Fig. 6b), but that thermal stability gain (+5.76 °C) was least than that displayed by Okoumé's lignin for which the T_{max}^3 shifted at 505.61 °C in the OWP compared to the wood (476.82 °C); thus leading to a thermal stability gain of +51.24 °C. That better lignin thermal stability in OWP suggest good interactions between the lignin moieties and the hydrophobic -$(CH_2)_2$- aliphatic chains and the aromatic rings of PET plastic.

That good coating effect of the wood flour in OWP was supported by the DSC curves (Fig. 7) which did not exhibit the endothermic melting temperatures of hemicelluloses and cellulose at 153 and 176.71 °C respectively, observed in the wood free from plastic (Fig. 7). The coating of wood flour with PET increased the Tg of OWP which took place around 170 °C above the 120.98 °C for Okoume's wood (Fig 7). Accordingly, the melting point of hemicellulose chains located in the wood cells coated with plastic chains in OWP should have shifted to the weak endothermic peak at 212.50 °C (Fig. 7). Furthermore, the DSC curves did not show evidence of lignin degradation at T>400 °C (Fig. 7). The thermal stability of OWP's lignin should be related to possible crosslinking between the hydrophobic units of PET and the phenylpropane units of lignin. These intermolecular interactions would support the shifting of T_{max}^3 at 505.63 °C as observed in the DTG curves of OWP (Fig. 6b).

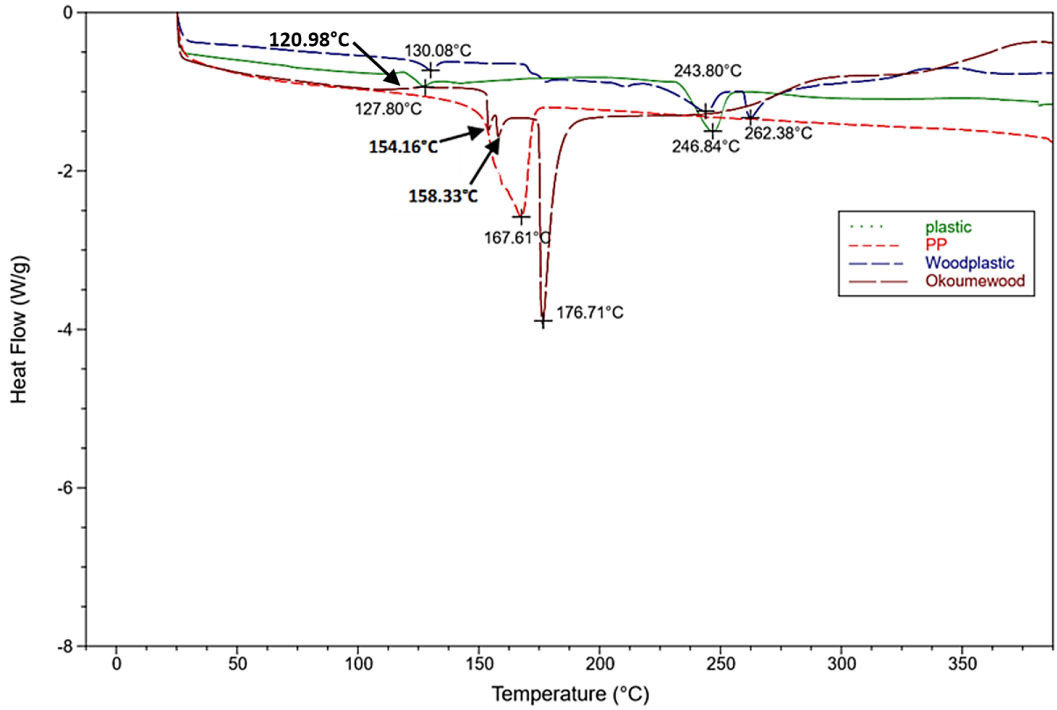

Figure 3: DSC of materials used heated at 10°C/ min under nitrogen with plastic= green; woodplastic= blue; polypropylene= red and Okoumewood=marron.

III.E.3. Thermal stability control of the wood, plastic and wood-plastic composite

The thermal behavior of the panels was studied to appreciate their relevant applications, for an interior or exterior cladding. The thermal conductivity of a material is defined as its capability to carry thermal energy. The results obtained for 50*x*50*x*10mm^3 OWP panels are reported in Table 1. The measurement points were chosen so that the depth probed is between 7 and 8 mm on the 10 mm available. Composites P5 and P6 panels with 0.5 mm of wood sawdust and plastic have significant higher thermal conductivity ($p<0.0001$) than wood-plastic with 1.5 mm of plastic sawdust (Table 1). Additionally, no significant difference were observed between the panels with 1.5 mm plastic particle sizes ($p<0.5$). The difference observed between panels according to thermal conductivity should be related to the small gaps appearing at the surface of 1.5 m plastic particle size panels inside, due to the lack of compactness between particles. These air gaps are surely more abundant with particles of different sizes than with particles of the same size. The same trend was observed by Rebolledo et *al* [26] while working on the effect of density and fiber size on porosity and thermal conductivity of fiberboard mats. Generally, Okoume wood has a thermal conductivity of λ=0.120 W/mK, according to EN 13986 international standard. More generally, hardwood species could have a thermal conductivity up to λ= 0.13 W/mK very closed to PET one (λ=0.13-0.15 W/m. K). However, mixing both the wood flour and the plastic particles slightly reduced the thermal conductivity of the composite final around 0.035 W/mK for P5 and P6 panels and 0.04 W/mK for P1, P2, P3 and P4 panels.

The literature stated that, the thermal conductivity decreases with the wood flour [27]. Furthermore, PCA revealed that the thermal conductivity and diffusivity were entirely correlated to the plastic particle sizes (Fig. 8).

III.E.4. Mechanical analysis

III.E.4.a. Hardness properties

The durometer Shore A0 was used for hardness tests and the results obtained are reported in Table 1. The Shore hardness is mostly made for rubbery and non-hard materials. The details provided by PCA revealed that Shore A0, which is inversely proportional to the particle sizes gives the best results for the P5 end P6 panels. Additionally, ANOVA test highlighted that the difference between P5, P6 and the other panels was significant ($p<0.05$). All the panels of 1.5 mm plastic particle sizes are significantly different at the 4.67% threshold of 0.5 mm panels except P4, which should be different at a lower threshold. Additionaly P5 and P6 have no significant difference at the 0.34% threshold. These results first emphasized that the particles size could directly affect by the hardness of the panels. Nevertheless, all the panels are hard according to the Shore A0 scale as 0 would be soft and 100 would be hard. The results obtained were all higher than that exhibited by white polypropylene block copolymer used for steel pipe coating [28]. Therefore, we are at the limit of the high/maximum hardness values. This hardness test is not the best suited for this type of material, hence, the need to check these results with other types of hardness tests.

Brinell method was used to evaluate the punching resistance of a panel. The results obtained reported in Table 1 showed that Brinell test remains almost the same for panels P5 and P6 of 0.5 mm of particle sizes. However, the Brinell test performed on panels of 1.5 mm revealed a variability between the samples ($p<0.03$). Furthermore, only P2 panel appears to be statistically different regarding the others panels. This could be due to the lack of homogeneity between the wood particles size of 0.5 mm and plastic powder of 1.5 mm. Additionally, the higher Brinell test values obtained for the P1, P3 and P4 panels could be related to the area where the ball hitted. Indeed, if the ball hits on a plastic particle (1.5 mm), the result is higher than that obtained when the ball hits in the gape formed by of the less compacted panel's parts. The Brinell test carried out in this studied underlined the variability of panels of high particles size (1.5 mm). That would result from the created vacuums or the unrobed wood particles, which lowered their Brinell value (3.665±1.567) compared to the better homogenized panels of 0.5 mm particles size (4.897±1.688). These values are in accordance with the relevant hardness rating for Australia Jarrah (4.7), Malaysia Merbau (4.9), North America Maple (4.8), Sweden Ash (4.0), Sweden Beech (3.8), American Cherry, European Red Oak (3.8), North America

Walnut (3.4) and the Sweden White Oak (3.7) trees [29]. However, it can also be assumed that carrying out local and very punctual measurements can disturb the evaluation of the average hardness measurement, hence the performance of Monnin hardness tests

Monnin hardness test was then used to determine the hardness of wood-based panels. The results obtained are reported in Table 1. According to the standard NFB51-125, the smaller the width of the print is, the greater the numeric value of the hardness is. The Monnin hardness making a hardness measurement over the entire width of the test piece is less affected by local hardness differences, hence the fact that it is less well correlated with the particle size or at least less affected by the heterogeneity linked to different particle sizes. Indeed, a more global and less local measurement is done. Unfortunately, it was noteworthy that no significant difference was found between the Monnin hardness values of the panels ($p>0.05$). That result supported again the existence of an important variability regardless of the particle size (slightly more important for panel with 1.5 mm particles size, $p>0.05$). PCA revealed that Monnin hardness is not correlated to the plastic particle sizes. Indeed, the variable associated to the Monnin hardness test in Fig. 8 evolves in PC2, whereas, the plastic particle sizes evolves in PC1. Nevertheless, in comparison with results obtained by CIRAD (2012), where the Monnin hardness test of Okoume wood was 1.6 at 12% humidity, results obtained are encouraging. Adding wood particles increased significantly the final panels' hardness of around ≈3.9 (Table 1). It is the phenomenon of densification, and the addition of plastic particle that increased the hardness of 240% (≈3.9).

III.E.4.b. Characterization of OWP in bending

The bending strength allowed to assess the deformability of OWP under loading. 3-point bending was performed on OWP containing 0.5 mm and 1.5 mm of plastic particle sizes without varying the grain size of wood particles. The results obtained are reported into the Table 1. The first observation made was that, bending strength and elastic modulus values range from 2.80 MPa for P1 panel up to 7.37 MPa for P2 panel, and from 570.13 MPa for P1 panel up to 2463.013 MPa for P2 panel respectively. However, the average score of the OWP performed with 1.5 mm of plastic particle sizes is of 1349.76±802.16 MPa, while for panels performed with 0.5 mm, was of 954.04±130.52MPa. The standard deviation observed, is related to the variability of results obtained during mechanical test. This variability was more present into the panels with 1.5 mm of plastic particle sizes (Table 1). The second observation made was that, panels performed with 1.5 mm of plastic particle sizes appear to have high bending elasticity and strength values (P2, P3 and P4) comparing to 0.5 mm panels (P5 and P6). Notwithstanding, the poor mechanical properties presented by the panel P1 (570.136 MPa), despite the 1.5 mm

of plastic particle sizes used for shaping, that means that, the plastic particle sizes directly influenced the mechanical aspect of the final product with a heterogeneity of values obtained while analysing panels made with 1.5 mm of plastic particles sizes (Table 1). Which is quite logical because of the representative elementary volume not reached and especially more vacuum in the panels at 1.5 mm and on the fact that the panels were very thin, then, risk of having very heterogeneous zones not representative of the overall characteristics of the mixture.

Table 1 present bending strength and bending elasticity modulus properties of composite prepared at 210°C. Compared to pure polyester as describe by Gunduz et *al* [30], the addition of wood fibers could at first sight caused decrease in the bending strength or bending elasticity modulus in some cases such as panels P1 and P5. According to Gunduz et *al*, (2016) pure polyester has modulus of elasticity of 920 MPa. Nonetheless, panels P2, P3, P4 and P6 have elasticity modulus higher than pure polyester (920 MPa). In addition, polyester has been described by TG analysis to be present in high content in plastic particles (Fig. 6). This increasing of MOE was mainly influenced by fiber reinforcement in the polymer. That behavior is due to wood fiber containing bound moisture, which could hydrolyzed the coupling agent. That hydrolyzed product, may develop either co-valent or hydrogen bonds with the hydroxyl-rich wood fiber, improving interfacial adhesion at the fiber-matrix interface [29,30].

According to Febriando et *al* [33], addition of wood flour onto the matrix resulted in linearly decreasing both tensile strength and breaking elongation values and linearly increasing in Young's modulus value. The results obtained during test and reported in Table 1, are, in closed agreement than these published by Febriando et *al* [33] while working on the wood/polymer composite by using Polypropylene as matrix with first hot mixed step at 50 % of wood flour. The same closed agreement was also observed for the published results of Coutinho et *al* [31]; on the propylene-wood fiber composite while looking for the effect of treatment (silane-treated) and mixing conditions on mechanical properties (2961 MPa). Gunduz et *al*. (2016) exhibited a value of 2464 MPa of bending [30] and Delviawan et *al*. (2019) exhibited MOE value of 2337 MPa [34]. According to PC analysis, mechanical properties are correlated to Monnin hardness on PC2 and correlated to the density of the final panels on PC1 (Fig. 8).

Table 1 : the average values of the various tests carried out for the validation of the results obtained

With n=3

	Different tests								
WPC panels samples	Monnin	Brinell	Shore A0	Thermal conductivity (λ=W·m^{-1}·K^{-1})	Thermal diffusivity (α=m²·s-1)	Bending strength (MPa)	Modulus of Elasticity in bending (Mpa)	water recovery (%)	Density
P1(1.5mm)	8.406±2.517	5.224±1.447	89.000±2.000	0.091±0.002	0.044±0.001	2.628±0.661	502.542±232.210	6.823±0.453	1.066±0.023
P2(1.5mm)	3.400±0.917	2.311±0.394	90.000±1.000	0.092±0.001	0.044±0.001	6.832±0.955	2059.104±705.850	7.725±1.300	0.991±0.042
P3(1.5mm)	6.240±1.169	4.364±1.079	92.333±0.577	0.090±0.001	0.044±0.001	0.339±0.311	210.533±128.599	7.682±1.083	1.007±0.068
P4(1.5mm)	4.633±1.534	4.015±0.145	91.333±0.577	0.092±0.002	0.044±0.001	4.248±3.593	784.390±608.889	7.165±0.792	1.050±0.028
P5(1.5mm)	7.524±2.439	3.772±0.888	93.333±2.516	0.090±0.001	0.045±0.001	4.519±1.358	1076.9813±687.762	7.909±1.012	1.020±0.054
P6(0.5mm)	3.758±1.449	5.047±1.025	95.333±1.115	0.105±0.001	0.051±0.001	3.515±0.168	825.826±62.229	3.332±0.419	1.115±0.042
P7(0.5mm)	7.219±4.224	4.381±1.504	95.000±1.000	0.101±0.005	0.049±0.002	4.519±2.167	942.344±269.653	3.724±1.119	1.082±0.071

III.E.5. Water recovery

The swelling tests allowed to study the water recovery of panels in water according to the NF EN 317 standard swelling test used for wood based composites. Furthermore, Standard NF EN 312 of November 2010 for particle boards indicated that a panel working and used in a humid environment must not have a swelling higher than 13%. The average swelling values collected in Table 1 showed that panels of 1.5 mm particles size had a water uptake (7.46±0.77) higher than displayed by the 0.5 mm ones (2.83±1.33). These results emphasized the potential of OWP to be used as panels in tropical area where the weather conditions are humid and warm all the year long. The correlation between the particle size and the swelling was corroborated by PCA which confirmed that, the smaller the size the plastic particles, the less the water recovery phenomena was (Fig. 8). Table 1 showed no significant different between OWP density, this lack of density different is due to the plastic particle sizes density (p=0.48). PCA showed that thermal conductivity was conversely opposite to the water recovery. The larger the size of the plastic particles, the greater the water absorption. The same trend was observed by Kaluzova et *al* [35], while they worked on the production of thermal insulation composite material based on polymers, with an emphasized on the water and the thermal conductivity.

III.E.6. Multivariate data analysis: Principal Component Analysis (PCA)

The data matrix of variables analyzed (i.e, the nine parameters measured of the relevant panels) was subjected to PCA in order to visualize the links between the different parameter and the correlations obtained. The results of PCA analysis presented in Fig. 8 provide information on both studied variables and samples. PC1 and PC2 representing more than 80% of the data matrix information. It was then enough to summarize the data. The horizontal axis of the graph is the first Principal Component (PC1). It describes 62.2% of the data matrix, and indicate high contributions of variable from water recovery, thermal conductivity, thermal diffusivity, particle sizes, density and Shore A0 hardness test variables. The vertical axis is the second Principal Component (PC2), it describes 26.8% of data matrix. This latest summarized the bending parameters, Brinell and Monnin hardness tests. The remaining PCs decreasing in explained variances, and did not explain significant variability in the data (<20%). Specific patterns of correlation between the variables tested can be visualized when compares one another loading between the PCs; see PC1-PC2 plots (Fig. 8).

The samples analyzed are composed of two different particle sizes, which can influence the behavior of the final panels. These differences are important to understand the influence of variables on OWP behavior.

On PC1, it appears two different groups of sample, on the left side, samples with 1.5 mm of plastic particle sizes, and on the right side, samples with 0.5 mm of plastic particle sizes. Considering the 1.5 mm plastic particle sizes, they are associated to physical properties (water recovery, particle size) and are conversely opposed to thermal properties (thermal diffusivity and conductivity). The same trend can be observed for the 0.5 mm plastic particle sizes. Therefore, according the PCA results (Fig. 8), physical and thermal properties are opposed: the larger the particle size, the greater the water recovery (P3-P4 panels), the less conductive the material (better for P5-P6 panels), the less dense it is and the less durable it is [36].

Regarding PC2, mechanical (bending strength and elastic modulus) and hardness tests (Monnin and Brinell) are inversely correlated. However, the heterogeneity of the results is too important and therefore making the analysis very complicated. Moreover, some panels were too thin for certain tests resulting in large dispersions. Finally, the lack of samples did not allow to confirm the variables influence on PC2.

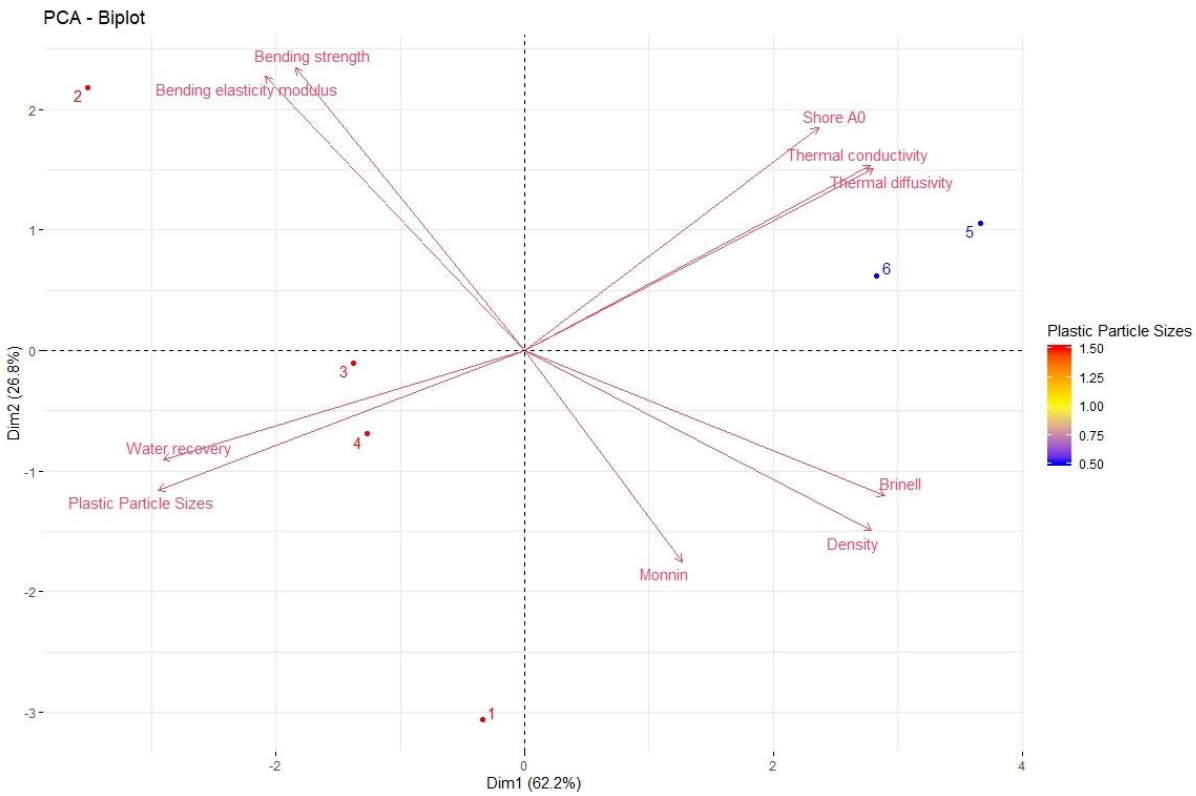

Figure 4: Biplot: Projection of variables and samples onto the first two PCs. Coloration made from the two different sizes of the plastic particle sizes

III.F. Conclusions

A OWP in compression without using hot mix extruder is feasible. The first results on the mechanical resistance and the physical properties of particles panels produced showed that the simple hot press system seemed promising while comparing with composite made by extrusion process published by several authors as discussed above. Additionaly, we have worked with 50 % of wood fibers. A major difference with the extrusion operation is that the plastic state does not change during the implementation. Therefore, the compression force has a very important role.

The thermal analyzes allowed checking the thermal stability of the panels and making sure that the components combination was judicious to control, in particular by governing the wood quantity which is the critical element when the temperature increased. But it was observed that adding plastic helped the wood to better withstand the temperature as seen with the TGA, DSC and hot disk tests. The strong stability of Okoume biomasses corroborated with many biomasses species such as eucalyptus, poplar, white willow, black alder and lime, known to have an excellent quality for wood composite manufacturing. The water recovery indicated good homogeneity of the particles in the panels as well as a small presence of gap area according to particles sizes. Above all, it is particularly interesting to obtain a significant low thermal conductivity and low swelling for the panel's application in humid areas.

Okoume wood was used because it has a low thermal conductivity (0.12 λ/W/m.°C) regarding others hardwood species such as oak, beech, ash pitch pine, thus thermal conductivity is of 0.23 λ/W/m.°C. Hardness tests performed are in accordance with the relevant standard values. Additionaly, OWP has the lowest thermal conductivity (≈0.1 λ/W/m.°C) comparing to composite performed with low medium density polyethylene (≈0.2 λ/W/m.°C) as published by Bourai et al. (2013). They showed the strong potential of Okoume wood wastes to be used as source for wood plastic composites. In the context of our study, the particle sizes (of 0.5 mm and 1.5 mm) seems to have an important effect on the physical properties, but not on the mechanical properties that remain nevertheless very good for composite panels in comparison with this already exists.

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Declaration of interest statement

The authors declare that this work is no in conflicts with any interest

III.G. References

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TROISIEME PARTIE
CONCLUSION ET PERSPECTIVES

I. Conclusion générale

Les travaux présentés dans cette étude ont permis de faire progresser les connaissances sur l'essence d'Okoumé, que ce soit sur la nature chimique de ses extraits, ou sur sa valorisation.

L'étude des extraits d'Okoumé a été réalisée pour atteindre trois objectifs principaux: (i) quantifier la teneur totale en polyphénols selon une méthode colorimétrique ; (ii) Caractériser pour la première fois les extraits de tanins condensés de cette écorce par MALDI-ToF, LC-MS et FTIR; et (iii) Comprendre le comportement thermique des tanins condensés d'Okoumé.

Les résulats obtenus à l'issue de la première analyse du taux d'extractibles de l'Okoumé et de leur caractérisation ont montré que ce bois était riche en composés aussi bien polaires qu'apolaires. De plus, la variabilité du taux d'extractibles dans les différentes parties du bois est apparue élevée. La spectroscopie MALDI-ToF a révélé la présence de composés distincts de type tanins condensés contenant un taux notable de tanins hydrolysables portant des fragments galloyle, et également la présence d'oligomères de tanins condensés glycosylés. Les tanins d'Okoumé sont dominés par deux grandes familles : prodelphidine/profisénidine. En revanche, la catechine ne fait pas partir des unités principales des tanins d'Okoumé.

Pour l'écorce, la structure moléculaire des tanins condensés obtenus par macération dans un mélange acétone/eau a mis en évidence la présence de fisétinidine, gallocatéchine et trihydroxyflavane comme principaux monomères. Les chaînes d'oligomères les plus élevées détectées dans ces extraits étaient des tétramères. Une série d'oligomères formés par un trimère flavonoïde lié à jusqu'à dix unités de sucre a également été identifiée pour la première fois.

Dans le cas de l'aubier et du bois de cœur, nous avons également constaté que les principaux constituants monomères sont la fisétinidine et la gallocatéchine. Les structures des tanins condensés d'Okoumé détectés étaient majoritairement composées par des flavonoïdes faiblement estérifiés, une grande partie non glycosylées, et une faible proportion de fragments flavonoïdes glycosylés. Un oligomère possédant jusqu'à sept unités fisétinidine non glycosylées a été trouvé pour la première fois. Des oligomères de tanins condensés liés à une unité glycosylique et à une longue chaîne de 2*x*gallocatéchine-1*x*catehine liée à six résidus glycosyliques ont été également détectés pour la première fois.

Ces résultats ont montré le fort potentiel des déchets de bois d'Okoumé comme source de diverses molécules à haute valeur ajoutée pour la chimie fine à usage biologique ou alimentaire.

A l'issue des résulats originaux présentés dans cette thèse, une première voie de valorisation a été proposée par la mise au point d'un adhesif tanins-hexamine sans modification

préalable de la structrure chimique du tanin. En effet, la mesure de l'indice de Stiasny et l'analyse thermique ont révélé le potentiel des déchets de bois d'Okoumé comme source de tanins condensés possédant de fortes propriétés adhésives.

L'inconvenient rencontré pour la formulation d'une colle à base de tanins a été la présence élévée de composés inorganiques provenant de la méthode d'extraction. Ces sels étaient clairement observés lors de l'analyse thermique des tanins. Cette problématique a été résolue en diminuant la quantité de soude dans la solution d'extraction.

Les tanins issus des trois parties du bois d'Okoumé, à savoir l'écorce, l'aubier et le bois de cœur ont montré une réactivité très élévée avec le formaldéhyde jusqu'à atteindre une valeur de l'indice de Stiasny maximale de 93%.

Le controle de la stabilité thermique des tanins condensés d'Okoumé réalisé sous azote a montré en ATG deux phases majeures de degradation. Le premier niveau se situe entre 100 et 120°C. Il est associé au départ d'eau résiduelle ou aux molécules de faibles masses molaires détectées entre 100 et 200 Da. Le deuxième se situe entre 240 et 260°C et correspond à la dégradation des tanins. Ces résulats montrent ainsi que les tanins d'Okoumé se dégradent à des températures très élévées.

Les formulations collantes préparées à partir de tanins issus d'écorce de bois d'Okoumé ont montré de bonnes propritété mécaniques. Les valeurs du module d'élasticité déterminées par TMA étaient de 3681 MPa, 3752 MPa, 3894 MPa, 3971 MPa pour Milolé, Nzamaligue 1, Nzamaligue 2 et Metzic respectivement. De plus, selon la norme NF EN 314-2, tous les adhesifs ont respecté la moyenne requise d'utilisation au regard des résulats de la résistance au cisaillement (0,21 ± 0,05; 0,47 ± 0,09 et 0,49 ± 0,11 N/mm²) et de la cohésion apparente (≥80%; ≥60% et ≥60% respectivement). Par ailleurs, l'analyse ^{1}H RMN a confirmé la bonne réactivité des tanins vis-a-vis de l'hexamine. Enfin, le système tanins d'Okoumé-hexamine se dégrade à hautes températures (>288°C).

Il a ainsi été démontré la pertinence des tanins d'Okoumé comme substrats pour la fabrication d'adhésifs respectueux de l'environnement. Ils pourraient s'intégrer dans une ligne de production industrielle pour remplacer des colles de type urée-formaldéhyde et pourraient constituer un avenir prometteur pour les déchets de bois d'Okoumé faiblement valorisés dans un pays d'Afrique centrale comme le Gabon.

Dans le cadre des recherches de voies de valorisation, nous avons également étudié la faisabilité d'un composite bois-plastique (WPC) en thermocompression directe, sans extrusion préalable.

Ces premiers résulats ont montré que les tailles des particules de plastique avaient un impact direct sur les propriétés des panneaux (0,5 mm préférable à 1,5 mm), ainsi que la force de compression et la température des plateaux chauffant. Malgré un faible échantillonnage et une forte variabilité, les observations visuelles, la résistance mécanique et les propriétés physiques des panneaux produits par un système simple de presse à chaud se sont révélées prometteuses. Tout les résultats obtenus sont basés sur les protocoles de différentes normes internationales.

Les propriétés mécaniques des panneaux se sont révélées sensibles à la taille des particules de plastique (0,5 ou 1,5 mm). Avec celles de 1,5 mm, une forte variabilité a été observée, attribuée à un problème d'homogénéité. Par exemple le module d'élasticité en flexion variait entre 210±128 et 2059±705 MPa. Avec les particules de 0,5 mm de diamètre, ces valeurs variaient de 825±62 à 942±269 MPa. Par contre, la conductivité thermique s'est révélée peu sensible à la taille des particules de plastique, avec par ailleurs une bonne homogénéité des valeurs : entre 0,090±0,001 et 0.092±0.002 $W.m^{-1}.K^{-1}$ pour les particules en 1,5 mm et entre 0,101±0,005 et 0,105±0,001 $W.m^{-1}.K^{-1}$ pour les particules en 0,5 mm. Enfin, les tests de dureté effectués sont conformes aux valeurs standards.

Les analyses thermiques ont permis de vérifier la stabilité des panneaux et de s'assurer que la combinaison des composants était adaptée afin de contrôler la quantité de bois qui est l'élément critique lorsque la température augmente. Il a été observé par ATG, DSC et au hot disk, que l'ajout de plastique permet au bois de mieux résister à la chaleur. Le faible gonflement à l'eau observé (compris entre 6,8±0,4 et 7,9±1,0 % pour les particules de 1,5 mm et de 3,5±0,75 pour celles de 0,5 mm), indique une bonne compacité des particules dans les panneaux ainsi qu'une faible présence d'espace entre-elles, en particulier avec les particules de plastique de garnulométrie 0,5 mm.

II. Perpectives

L'ensemble des résulats obtenus a ouvert de nouvelles voies de valorisation possibles des déchets de bois d'Okoumé, à savoir une meilleure connaisance des extraits, la formulation de colles bio-sourcées et la fabrication de composite bois-plastique. Plusieurs pistes et optimisations restent à explorer.

La valorisation de la fisetinidine, du trihydroflavane dans le secteur phytochimique pourrait être considérée. De plus, il serait intéressant de poursuivre les analyses d'autres extraits de l'écorce, l'aubier et du bois de cœur, peu considérés dans ces travaux, à savoir les acides gras. Une meilleure connaissance de leurs activités chimiques pouraient également apporter de nouvelles opportunités de valorisation.

Le développement des adhésifs bio-sourcés pourrait comporter deux phases. La première correspondrait à l'amélioration des formulations, notamment par la modification chimique des tanins d'Okoumé pour les rendre plus réactifs. La seconde serait la montée en échelle, du pilote vers l'industrie.

La fabrication des composites s'est révélée prometteuse. Des travaux complémentaires restent nécessaires pour la validation et l'optimisation des procédés. Il serait ainsi judicieux d'utiliser des poudres de bois et de plastiques de granulométrie plus faible, de manière à améliorer l'homogénéité. Des travaux pourraient également être conduits sur la transformation par extrusion pour produire des granulés plus homogènes et faciles à employer par des filières liées à la plasturgie.

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Annexe 1 : Liste des publications

Durant la thèse :

Premier auteur :

Parus

Engozogho Anris, S.P., Bikoro Bi Athomo, A., Vidal, M., Denaud, L., Safou-Tchiama, R., Charrier, B. 2019. Extraction and characterization of Aucoumea klaineana Pierre (okoume) extractives. Journal of Renewable Materials. 7(6),519-522.

Engozogho Anris, S.P., Bikoro Bi Athomo, A., Safou-Tchiama, R., Santiago-Medina, F.J., Cabaret, T., Pizzi, A., Charrier, B. Molecular structure and thermal stability of Aucoumea klaineana Pierre (Okoume) condensed tannins: An attempt to African wood wastes valorization. Scientific Reports, 10(1). 1-14.

Soumis

Engozogho Anris, S.P., Bikoro Bi Athomo, A., Safou-Tchiama, R., Santiago-Medina, F., Pizzi, A., Charrier, B. Maldi-ToF analysis and FTIR characterization of *Aucoumea Klaineana* Pierre (Okoume) sapwood and heartwood condensed tannins from Gabon natural forest (submitted to Journal of wood science and technology, reference number : WSAT-D-19-00269

Engozogho Anris, S.P., Bikoro Bi Athomo, A., Safou-Tchiama, R., Vidal, M., Leroyer, L., Charrier, B. Development of green adhesives for fibreboard manufacturing, using Okoume bark tannins and hexamine: Characterization by 1H NMR, TMA, TGA, and DSC analysis

Engozogho Anris, S.P., Bikoro Bi Athomo, A., Eyma, F., Safou-Tchiama, R., Rubini, M., Boucard, M., Charrier, B. Utilization of *Aucoumea klaineana* Pierre (Okoume) wood wastes in plastic panel composites

Co-Auteur :

Bikoro Bi Athomo A, **Engozogho Anris SP**, Safou-Tchiama R, Santiago-Medina FJ, Cabaret T, Pizzi A., Charrier, B. 2018. Chemical composition of African mahogany (K. ivorensis A. Chev) extractive and tannin structures of the bark by MALDI-TOF, Industrial Crops and Products. 113, 167-178.

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African tropical heartwoods toward Pycnoporus sanguineus and Antrodia sp.: lignin structure and cellulose morphology control. Journal of the Indian Academy of Wood Science, 15(2), 162-171.

Safou Tchiama R, Bikoro Bi Athomo A, **Engozogho Anris PS**, Akagah AG, De jeso B. 2019. Characterization of some African tropical heartwood lignins by ^{1}D 1^{3}C and ^{1}H-NMR: molecular structure and hydroxyl groups' distribution. Journal of the Indian Academy of Wood Science, 16(1), 73-86.

Annexe 2 : Communications (Poster)

- PHDTALENT, 18 octobre 2019, Paris, France
- Conférence internationale WOODCHEM, 20-22 novembre 2019, Nancy, France
- Journée Nationale du Composite (JNC), 1-3 juillet 2019, Bordeaux, France
- Journée du Cosmétique, 18 juin 2019, Poitiers, France
- Ecole thématique PLURIBOIS, 3-7 juin 2019, Fréjus, France
- Conférence internationale ICOME MATERIALS & ENERGY, 30 avril-4 mai 2018, Saint Sebastien, Espagne
- GDR science du bois, 20-22 novembre 2018, Cluny, France
- GDR science du bois, 21-23 novembre 2017, Nantes, France
- Ecole thématique PLURIBOIS, 12-16 juin 2017, Mont de Marsan (**Participation à l'organisation)**

Annexe 3 : Encadrement de stages

- **Mathieu Boucard** (Faculté de Sciences & Sciences de l'ingénieur, Université de Bretagne Sud), Eco-Conception polymeres & Composites, stagee de 3 mois de fin d'étude niveau licence (2015)

  Sujet : Développer un nouveau composite à base de bois tropicaux et de plastiques recyclés

- **Martin Cegarra** (IUT de Mont de Marsan), stage licence Pro métiers du bois

  Sujet : Bureau d'étude, conception de charpente

- **Robin Charrier** (IUT de Mont de Marsan), stage licence Por métiers du bois

  Sujet : Fabrication de menuiseries mixtes bois-aluminium

- **Louise Watcher** (IUT de Mont de Marsan), DUT Science et Génie des Matériaux

  Sujet : Construction bois : de l'idée au concret

- **Léon Depaire**. Dimensionnement et conception d'un broyeur à serment. Stage DUT Science et Génie des Matériaux. 35 pp.

- **Adrien Boumedine et Valerie Mbouefet Mbami** (IUT de Mont de Marsan), Projet tuteuré Licence Pro métiers du bois

  Sujet : Analyse et optimisation du timisage des copaux dans la couche interne des panneaux de particules EGGER

- **Serge Fota** (IUT de Mont de Marsan), Porjt tuteuré Licence Pro métiers du bois

  Sujet : Développement de composites plastiques recyclé-sciure de bois

Annexe 4 : Formation doctorale

- **1ère année de thèse :**

-Introduction à la recherche scientifique (10h)

-Fête de la science (10h)

-Formation à la recherche documentaire et à la publication scientifique (12h)

-Formation à l'éthique de la recherche et à l'intégrité scientifique (4h)

- **2ième année de thèse :**

-Anglais certification Cambridge (10h)

-Doctoriales transfrontalières UPPA-UP/EHU (40h)

-Enseignement (34h) (cours et TP sutructure de la matière, Projet tutoré et suivi des étudiants en stage et alternance. Niveau DUT)

-Formation en matériau et sensation

-Formation en public speaking

-Formation en neuroscience et management

-Enseignement mode emploi (14h)

-Valorisation de la recherche (5h30)

- -Formation éthique et intégrité scientifique

-Séminaire de sensibilisation à l'entrepreneuriat

-Formation préparation aux entretiens d'embauche

- **3ième année de thèse :**

-Insertion professionnelle : Séminaire Bac +8 (12h)
-Ma thèse en 180 secondes (40h) **2ième prix**
-Formation en neuroscience et management
-Enseignement (56h) (TP mise au point des matériaux bio-sourcés, suivi des étudiants en stage et alternance : Niveau DUT et Licence Pro)

Annexe 5 : Analyse RMN ^{1}H de l'adhesif

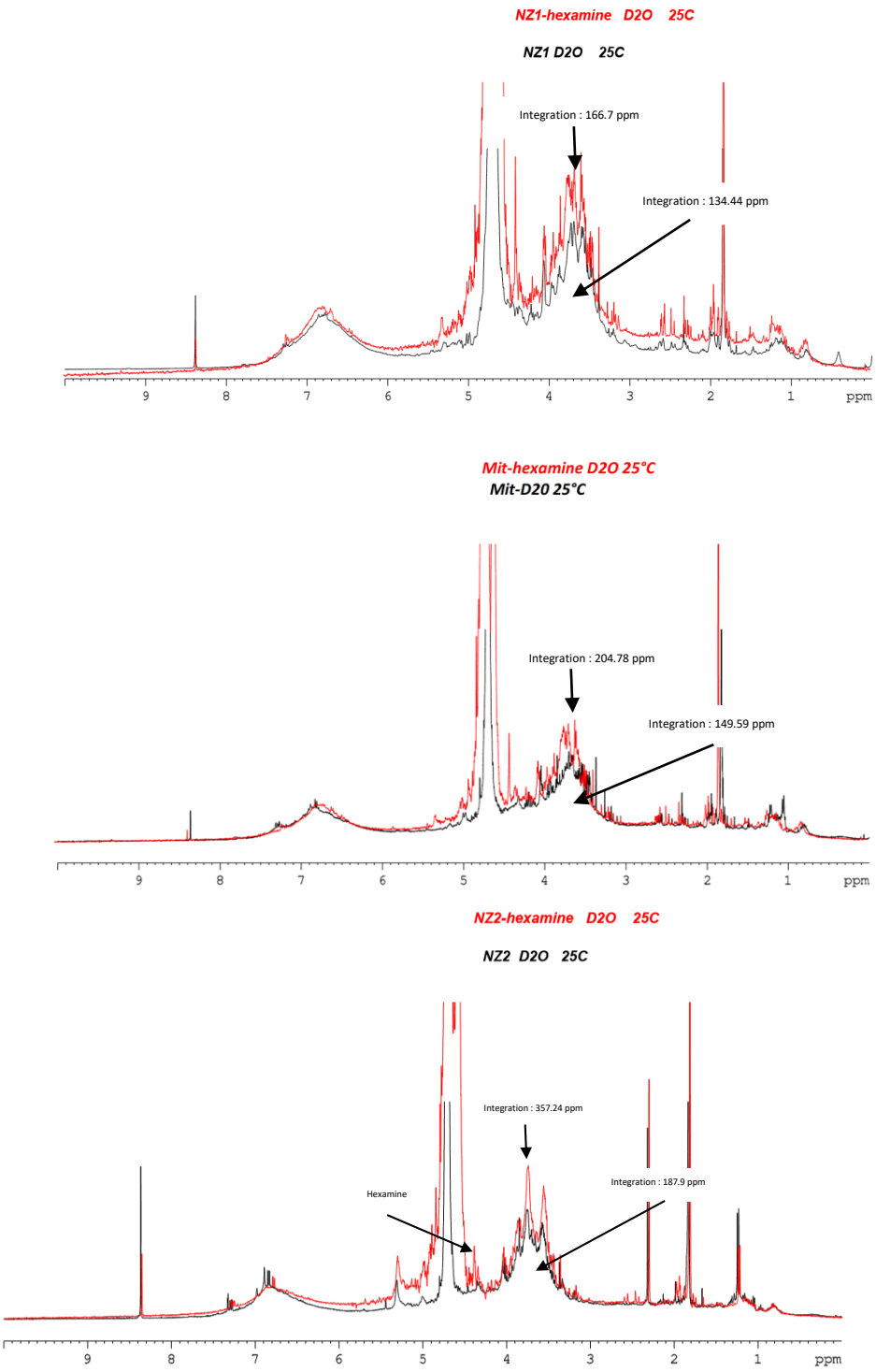
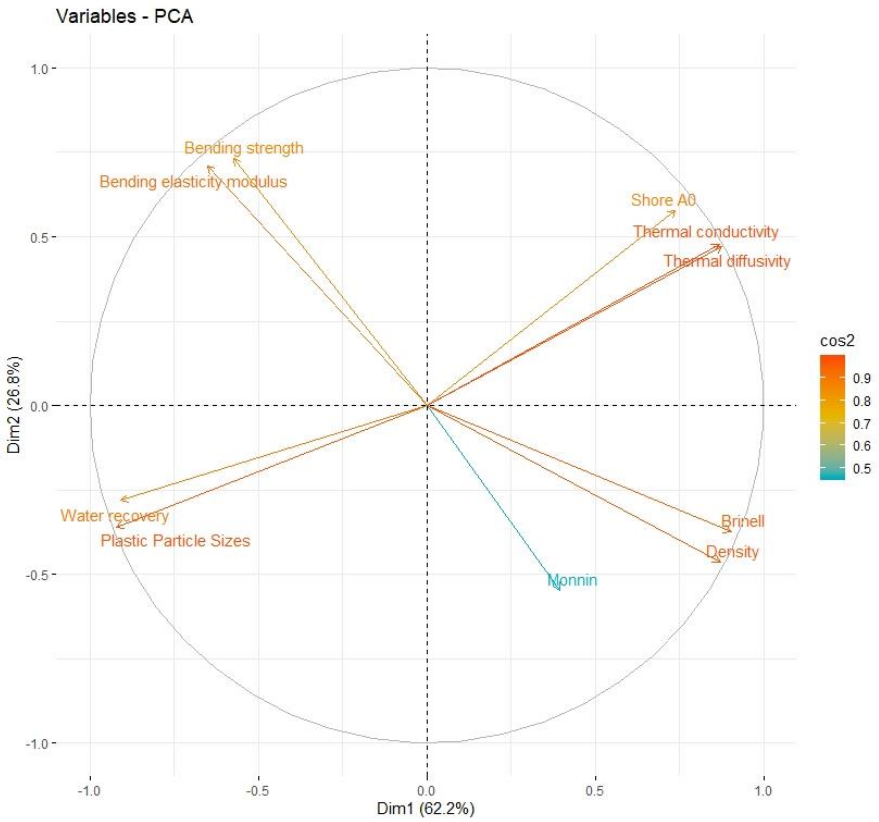
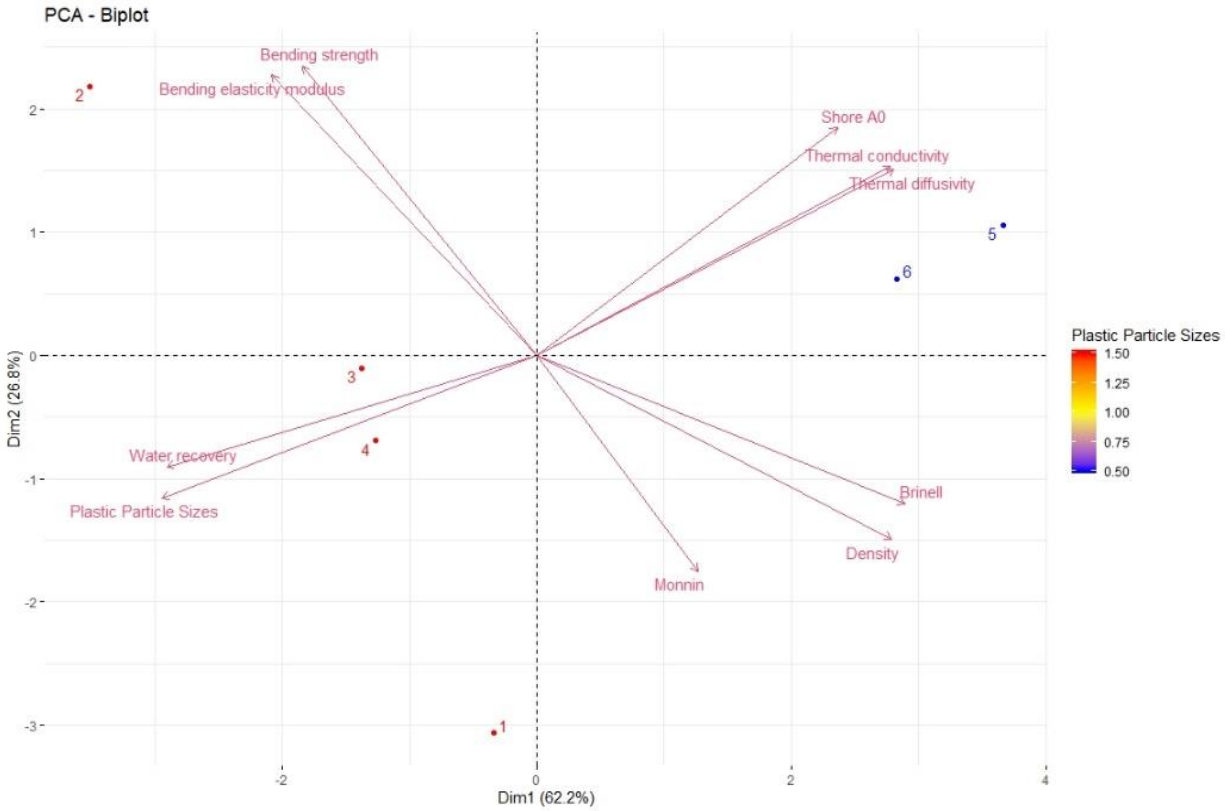
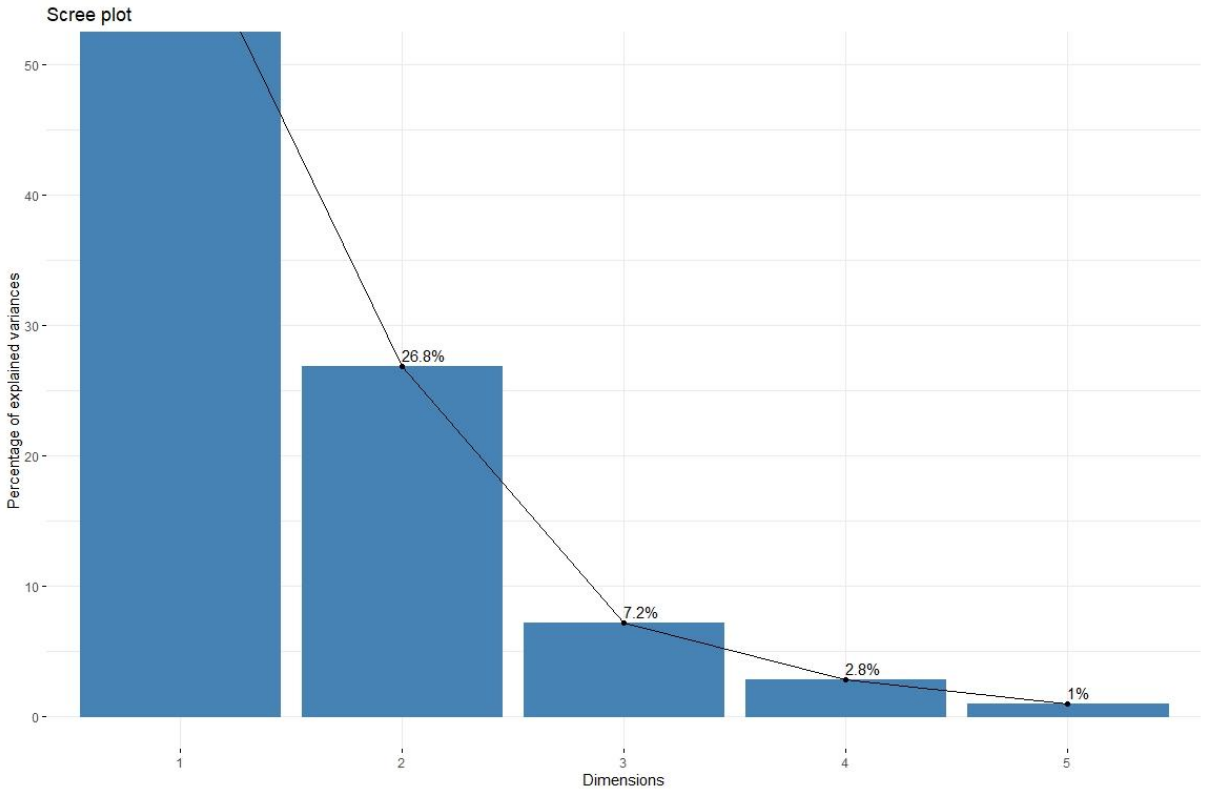


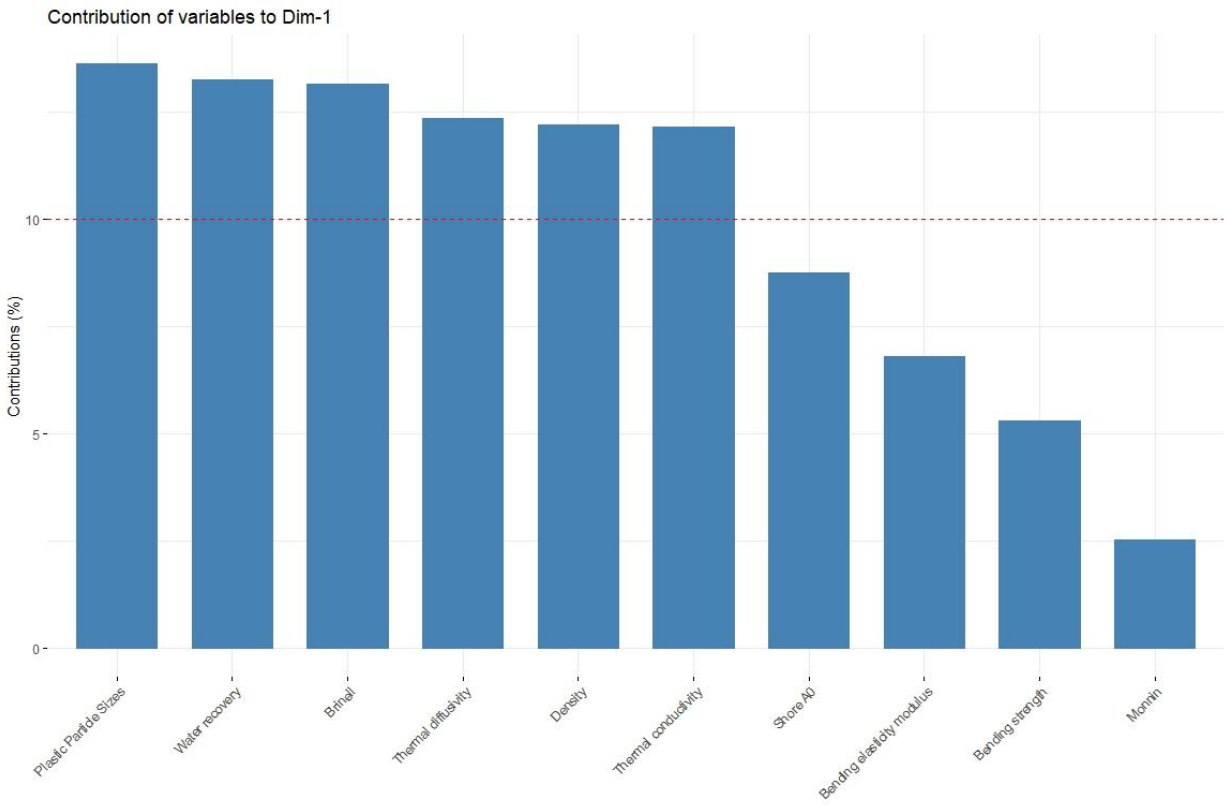
Figure 7: ^{1}H NMR of tannin-hexamine resin

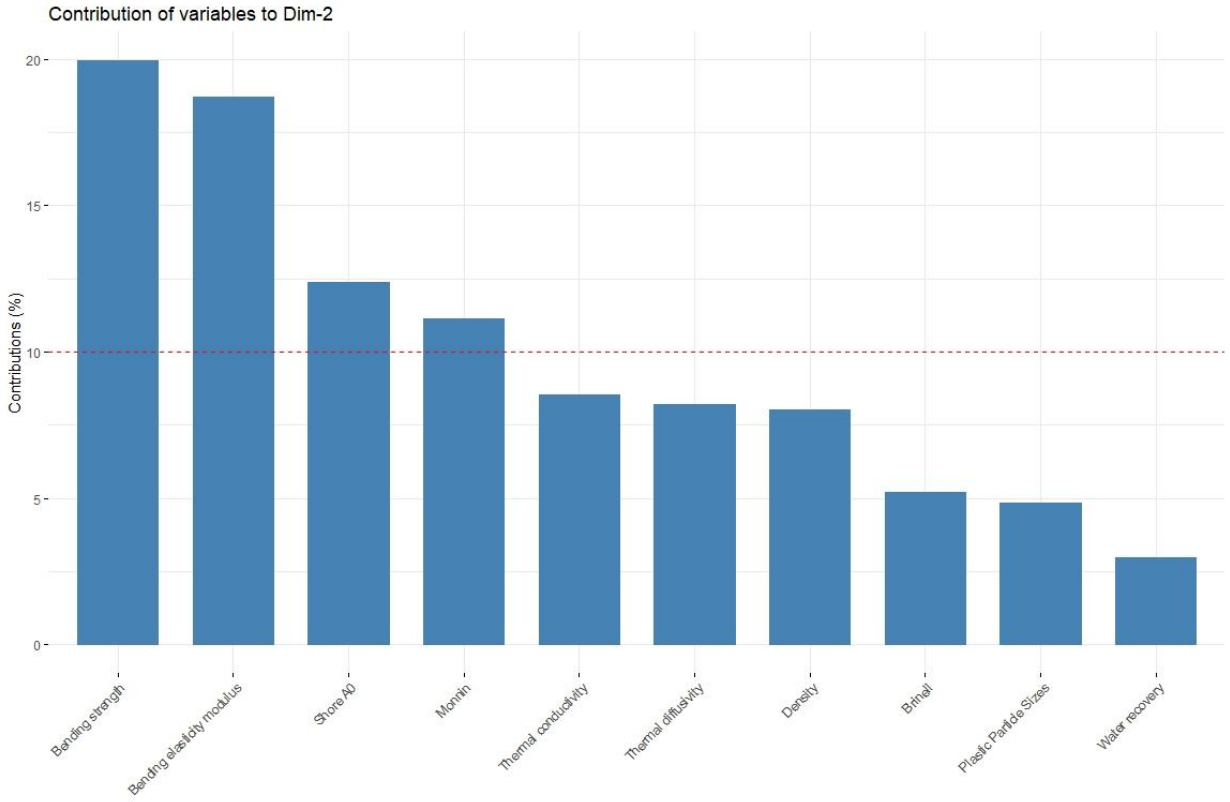
Annexe 6 : Analyse en composante principale du composite

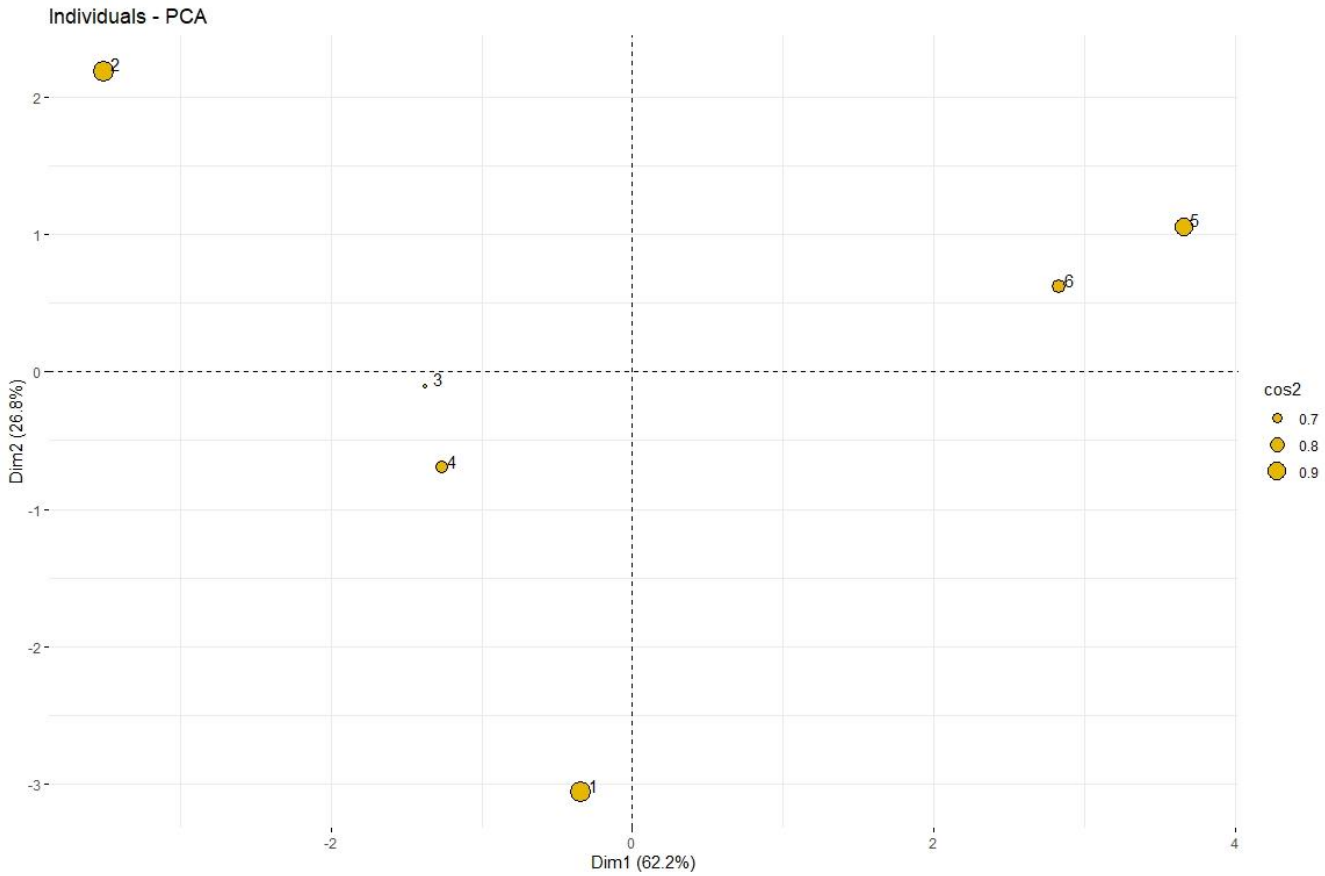





Scree plot
Percentage of explained variances
50
40
30
20
10
0
26.8%
7.2%
2.8%
1%
1
2
3
4
5
Dimensions


Contribution of variables to Dim-1
Contributions (%)
10
5
0
Plastic Particle Sizes
Water recovery
Brinell
Thermal diffusivity
Density
Thermal conductivity
Shore A0
Bending elasticity modulus
Bending strength
Mornin


Contribution of variables to Dim-2
Contributions (%)
20
15
10
5
0
Bending strength
Bending elasticity modulus
Shore A0
Monnin
Thermal conductivity
Thermal diffusivity
Density
Brinell
Plastic Particle Sizes
Water recovery


Individuals - PCA
Dim2 (26.8%)
Dim1 (62.2%)
2
1
0
-1
-2
-3
-2
0
2
4
1
2
3
4
5
6
cos2
0.7
0.8
0.9

Résumé

Ce travail de thèse s'est organisé autour de la valorisation des produits connexes issus de la première et de la deuxième transformation des bois du Bassin du Congo en général, et de d'*Aucoumea klaineana* Pierre (Okoumé) du Gabon en particulier.

En effet, 85% des produits connexes issus de cette industrie, sont incinérés à ciel ouvert, pour raison d'absence ou de faibles voies de valorisations possibles.

Notre objectif premier est de présenter des voies de valorisations capables de réduire considérablement l'incinération de ces produits connexes. Pour répondre à cette première problématique, nous avons procédé, par une analyse chimique des trois parties du bois (écorce, aubier, cœur), pour mettre en évidence les différentes molécules et leurs potentiels domaines de valorisation. Les molécules principalement visées étant les polyphénols. Nous avons par la suite porté un accent particulier sur les tanins condensés extraits de l'Okoumé, que nous avons caractérisés thermiquement et chimiquement par différentes spectroscopies. Afin d'observer leur pouvoir collant, notamment leur capacité de ces tanins a être utilisé comme substrat pour la mise en point d'un adhésif bio-sourcé pour l'industrie du contreplaqué et du panneau aggloméré.

La deuxième voie de valorisation est la mise au point d'une composite bois-plastique par thermocompression sans passé par une étape d'extrusion préalable. Ce composite pourra trouver son utilisation dans la construction et l'aménagement interne.

Mots clés : Adhésif, composite, Okoumé, polyphénols, tanins

Abstract

This PhD, was organized around the promotion of related products from the first and second processing of wood from the Congo Basin in general and Aucoumea Klaineana Pierre (Okoume) from Gabon in particular.

Indeed, 85% of related products from this industry are incinerated open air, the reason being the absence or the weak ways of possible valorizations.

Our primary objective is to present ways of recovery capable to reduce considerably the incineration of this waste. Therefore and to answer this first issue, we proceeded first, by a chemical analysis of the three parts of the wood (bark, sapwood, heart), aiming to highlight the different molecules and their potential areas of "development''. The molecules mainly targeted are polyphenols. We then focused in particular on the condensed tannins extracted from

Okoume, which we characterized thermally and chemically with different spectroscopy. To observe their stickiness, including their ability to be used as a substrate for the development of a bio-sourced adhesives for the plywood and panel industry.

The second recovery method highlighted is the development of a wood-plastic composite by thermocompression without having gone through a prior extrusion step. This composite can find its use in construction and internal design.

Key words : Adhesive, composite, Okoume, polyphenol, tannins