Heartwood formation process in teak (Tectona grandis L. f): fate of non-structural carbohydrates and characterization of forsythoside B

Bobelé Florence NIAMKE, Augustin Amissa ADIMA, Kati-Coulibaly SERAPHIN, Nadine AMUSANT and Christian JAY-ALLEMAND

Laboratoire de Procédés Industriels de Synthèse de l’Environnement et de l’Énergie Nouvelle (LAPISEN), Unité Chimie des Eaux et des Substances Naturelles, Institut National Polytechnique Houphouët Boigny, B.P. 1313 Yamoussoukro, Côte d’Ivoire.

Laboratoire de Nutrition et Pharmacologie de l’UFR Biosciences 22 BP 582 Abidjan 22, Côte d’Ivoire.

Unité Mixte de Recherche (UMR) ECOFOG, 2091 Route de Baduel - BP 792 - 97337 Cayenne cedex. Guyane, France.

Laboratoire de Biochimie et de Physiologie Végétale, Université de Montpellier II, UMR 1208 IATE (UM, INRA, SupAgro) CC024, Place Eugène Bataillon, 34095 Montpellier Cedex 05 France.

Corresponding author; E-mails: bobele.niamke@inphb.ci / bobeleflo@yahoo.fr

ABSTRACT

Heartwood formation is an important process in perennial plants as trees. Non-structural carbohydrates (NSC) storage in wood is an important feature of heartwood formation properties before and after wood drying. To better understand how NSC are stored and transformed in teak wood, their radial distribution was studied before and after chemical hydrolysis by using a spectrophotometric method coupled to enzymatic assays. In sapwood, NSC (starch, glucose, fructose and sucrose) and condensed NSC (mainly phenolic glucosides) were strongly accumulated respectively 100.9 ± 5.9 and 30.2 ± 5.5 μmol.g.dw⁻¹. They decreased abruptly (5-10 folds lower) from sapwood to heartwood. However, if the proportion of condensed glucose was 3 times higher than that of non-condensed glucose in the sapwood, this ratio increases 20 folds in the heartwood indicating that glucosylation process could occur. The forsythoside B, a trisaccharide of caffeic acid present in sapwood, was likely hydrolyzed during heartwood formation. Our results show that high proportions of starch and soluble NSC (glucose, fructose and sucrose; 80%) and condensed NCS (essentially glucose after chemical hydrolysis of the extract; 20%) were mobilized in the sapwood and used in the transition zone leading to their abrupt depletion in the heartwood where condensed NSC dominated (75-90%). The unmetabolized glucose was likely stored mainly by etherification. Significant correlations between glucose contents (before and after hydrolysis) and natural durability (−0.43 ≤ R ≤ −0.67) were found. These results indicate that high levels of NSC mobilization in the stem could be a relative efficient strategy of teak for enhancing its heartwood natural durability.

© 2018 International Formulae Group. All rights reserved.

Keywords: Teak, sapwood, non-structural carbohydrate, hydrolysis, natural durability.

INTRODUCTION

Teak (Tectona grandis L. f.) is indigenous to India, Myanmar, Thailand and Laos. It was introduced in the tropics as exotic species and is one of the most valuable tropical timbers on the international market (Bhat and Ma, 2004). Teak has long been known to possess highly durable wood, due to its high natural durability due to high levels of non-structural carbohydrates (NSC). These carbohydrates are stored in the sapwood and used in the transition zone leading to their abrupt depletion in the heartwood. The unmetabolized glucose is likely stored mainly by etherification. Significant correlations between glucose contents (before and after hydrolysis) and natural durability were found. These results indicate that high levels of NSC mobilization in the stem could be a relative efficient strategy of teak for enhancing its heartwood natural durability.
largely to the presence of extractives in the heartwood, e.g. anthraquinones and naphthoquinones (Niamké et al., 2011). In contrast to heartwood, teak sapwood is less durable mainly due to the presence of reserves material and its low content of extractives (Niamké et al., 2011). This difference of natural durability between sapwood and heartwood has been suggested to be strongly related to the storage of NSC which are responsible for the biosynthesis of toxic secondary substances (Hamada et al., 2017). In addition, it is shown that high content of sucrose and other sugars in wood promotes its rapid colonization by fungi (Okahisa et al., 2006; Ja’a’faru and Onilude, 2014), indicating that sugars status of a tissue could be an indicator for its susceptibility to pathogens, especially to fungi attacks.

The differences in natural durability between sapwood and heartwood are related to heartwood formation. Heartwood formation is an important process in perennial plants as trees. It is the ultimate process leading to the death of living sapwood tissues due to internal phenomenon depending on the cycle of tree life involving both water and mechanical gradients (Berthier et al., 2001; Kokutse et al., 2009). During heartwood formation, several widespread reactions (oxidation, hydrolysis, methylation and glycosylation) occurred and lead to the improvement of wood properties such as natural durability and colour. Glycosylation (the conjugation of a sugar moiety) lead to modifications of chemical structures and is catalysed by a family of enzymes called glycosyltransferases. It plays many roles in plant: (1) it contributes to the high chemical diversity among plant secondary metabolites, (2) it is implied in the control of the compartmentalization of metabolites, stabilization, enhancement of water solubility and deactivation/detoxification of natural products, leading to the regulation of metabolic homeostasis, detoxification of xenobiotics and the biosynthesis, storage and transport properties of secondary metabolites (Yonekura-Sakakibara, 2009).

Reserve materials are present in the wood as lipids and non-structural carbohydrates. Storage of non-structural carbohydrates (NSC), principally sugars and starch (Hoch et al., 2003) is very important for woody species. Indeed, these reserves were used in long-lived organisms for their perennity by fighting against biotic and abiotic stress, including pests, disturbance, and drought (Dietze et al., 2014). NSC play many functional roles such as nutrient transport, carbon providing for energy metabolism and osmoregulation, and the biosynthesis of toxic extractives for defense mechanisms (Kampe and Magel, 2013), they are highly relevant in the context of tree resilience to many global change factors. However, it remains unclear for how these reserves are stored in the wood.

Therefore, we are interested by the mobilization of NSC and their possible relationship to natural durability in teak. In teak, previous studies have shown that NSC (starch, glucose, fructose and sucrose) and fats were highly accumulated in sapwood (Nobuchi et al., 2005). Moreover, Windeissen et al. (2003) found that hydrolyzed methanol extracts of all parts of Panama teak wood contained mono and disaccharides and sugar derivatives. They suggested that a part of teak extractives occurred in condensed forms or as glycosides with more than two sugar components.

In the present work, we attempted to better understand the potentialities of perennial plant to mobilize and use their NSC in the wood to strengthen their defence system and improve their natural durability. For that, the radial distribution of NSC was examined before and after chemical hydrolysis of wood samples. Soluble NSC and starch, and condensed NSC were quantified through the analysis of major soluble NSC in both sapwood and heartwood of trees from Malaysian plantation-growth teak. Their natural durability was already determined (Niamké et al. 2011). The content of NSC was determined by spectrophotometric method after enzymatic assays and the major phenolic compounds were analyzed by HPLC.
MATERIALS AND METHODS

Materials
Six trees of teak from Malaysia, ranging in age from 5.5 to 10 years were used and sampled as previously described by Niamké et al. (2010). All the wood slices were conditioned in a climatic room (humidity: 65% ± 5%, temperature 20 °C ± 1 °C) until their use. From one radius, four sections of wood (25 x 25 x 10 mm, R,T,L) were taken successively from sapwood to the pith into sapwood (SW), outer heartwood (OHW), middle heartwood (MHW), inner heartwood (IHW). The wood samples were ground to 0.5 mm of size of the particles in a Retsh ZM 200 mill (Oise, France).

Non-Structural Carbohydrates extraction and quantification
Non-structural carbohydrates (NSC-starch, glucose, fructose and sucrose) were extracted and measured enzymatically by UV methods (spectrophotometer Bio-Tek Instruments, Colmar, France) according to Niamké et al. (2010). Glucose and fructose were determined directly, and sucrose (on the extract) and starch (on the wood residue) were determined after enzymatic hydrolysis. The content of soluble non-structural carbohydrates is expressed as µmole per gram (g) dry weight (dw) and the content of starch as µmole equivalent glucose per gram (g) dry weight (dw). The coefficient of variation of the extraction and measurement procedure determined from three independent replicates of the same powder never exceeded 8%.

Acid hydrolysis
Acetonic extract (750 µL) was mixed with 750 µL of 2 N hydrochloric acid; nitrogen was added and the mixture was conserved at 4 °C during 2 h. After neutralisation until pH 2, the extract was dried under nitrogen. The dried extract was dissolved in an aqueous methanolic solution (50%). The hydro-methanolic extract was filtered and the final solution was analyzed for non-structural carbohydrates as described above. The basic hydrolysis of chlorogenic acid (cafeoyl-quinic acid) was used as control.

Isolation, purification and identification of the phenolic compound H1
Grounded dry matter (12.8 g) of sapwood were extracted with 140 mL of acetone/water (80:20, v/v) at 4 °C and pH = 2 (see extraction procedure of polyphenols above). Acetone was evaporated to dryness (rotator evaporator Buchi R-215 coupled to a vacuum controller V 850, Marolles en Brie, France) and the remaining aqueous solution was lyophilized (Bioblock Christ Alpha 1-2, Paris, France). The dry residue (0.41 g) was dissolved in 10 mL of pure methanol. The solution was fractionated using a preparative HPLC (Agilent Technologies 1200 series, Paris, France) coupled to a fraction collector (Agilent Technologies 1200 series, Paris, France) with a reverse 5 µm C18 column (SA AB250SP1), 250 x 10 mm (Cluzeau, Paris, France). Fraction F containing H1 was collected at 9.7 min. The semi-preparative conditions were used as follow: mobile phase: solvent A = water/acetic acid (99:1, v/v), solvent B = methanol/acetonitrile (1:1 v/v); elution gradient: 0-13 min, 30% solvent B, 14-15 min , 30-100% B, 16-25 min, 100% B, 26-28 min, 30% B. The flow rate is fixed at 2.5 mL.min⁻¹, a maximum pressure at 250 bars and the detection at 254 nm. The injection volume was 500 µl of purified methanolic
extract of sapwood. The lyophilisation of the fraction F (m = 6.1 mg) yielded 0.05% of dry wood powder.

To confirm the chemical structure of H1, the fraction F was analyzed using a MS-ESI analytical technique. MS experiments were conducted on a QStar Elite (Applied Biosystems SCIEX) Q-TOF apparatus (Paris, France). The sample (6.1 mg) was dissolved in 300 µl and diluted in a solution of methanol with 3 mM of ammonium acetate. All mass spectrometry data were acquired with a positive ionization mode. The MS conditions were used as follow: the tension of the electrospray (ISV) was 5500 V; the tension of the orifice (OR) was 30 V and the pressure of the atomization gas (air) was 16 psi. The mass of the compound was verified twice according to the theoretical mass of [M+NH4]+ which is 774.28.

NMR experiments were performed on a spectrometer (Bruker Avance DRX-500, Wissembourg, France) operating at 500 MHz for 1H using a 5 mm triple resonance TXI cryoprobe tube and 125 MHz for 13C. One-dimensional (1D) 1H and 13C, two dimensional (2D) 1H COSY and NOESY and 2D (1H,13C) HSQC and HMBC spectra of samples dissolved in methanol-D were recorded at 27 °C. Chemical shifts (δ) are given in ppm and coupling constant J values are given in Hz. Spectra signals were referenced using 1H H and 13C signals of internal tetramethylsulfoxide (TMS; 0.0 ppm).

Statistical analysis

The XLSTAT software package (Paris, France) was used for statistical analysis. Significant differences of non-structural carbohydrate and phenolic contents were determined within the radial distribution between sapwood and outer heartwood, between outer heartwood and middle heartwood and between middle heartwood and inner heartwood using the non-parametric Mann Whitney U test. Correlations between NSC content (before and after chemical hydrolysis) and natural durability were determined using Spearman correlation’s test. Values were considered to be statistically significant when p < 0.05.

RESULTS

Characterization of non-structural carbohydrates (NSC) in teak wood

Table 1 shows the content of non-structural carbohydrates before and after chemical hydrolysis. Two types of NSC were identified in teak wood: Soluble NSC and starch or non-condensed NSC obtained before chemical hydrolysis on a hand and on another hand condensed NSC. Non-condensed NSC are accumulated in sapwood (SW; 100.9 ± 5 µmoles.g.dw⁻¹). They represent 77.7% of total NSC (before and after chemical hydrolysis) of sapwood from where they depleted to inner heartwood (0.6 µmoles.g.dw⁻¹; Table 1).

In Heartwood, at least 60 times low amount of non-condensed NSC were found, when we compare to sapwood. Condensed NSC were quantified after chemical (acid and basic) hydrolysis. Only glucose and fructose were detected in teak sapwood. Glucose was the most important condensed-NSC found. Fructose which was mainly hydrolyzed from raffinose and stachyose was only detected in extracts from basic hydrolysis of sapwood. In sapwood, condensed (conjugated) glucose from basic hydrolysis (21 µmoles.g⁻¹ dw) was two-fold higher than condensed glucose from acid hydrolysis (Figure 1; 9 µmoles.g⁻¹ dw). While in heartwood, the opposite trend was observed. Condensed glucose from acid hydrolysis was higher than that of basic hydrolysis, indicating that condensed glucose is mostly etherified.

Correlations between NSC and natural durability

Interesting Spearman correlations (0.43 ≤ R ≤ 0.67) were found between NSC (soluble NSC and starch - non-condensed and condensed forms) and decay resistance (natural durability against fungi) of teak (Table 2). The highest correlation was observed with condensed glucose (R = 0.67). It is follow by that of non-condensed glucose (R = 0.46) and starch (R = 0.43). No significant correlation was found between
decay resistance and the content of fructose and sucrose.

**Identification of the compound H1**

H1 UV spectra in methanol showed three bands of absorption: band I = 240 nm, band II = 290 and band III 329 nm. These absorption bands are typical of a benzoyl system (band II), such as the dihydroxyphenylethanol moiety, and the cinnamoyl system (band III), such as caffeoyl moiety both being present in verbascoside and forsythoside B, as previously reported (Delazar et al., 2005; Funes et al., 2010). A 100% base peak [M+] for H1 was observed at m/z 757 with additional fragment ions at m/z 625 [M+], 471 [M+] and 325 [M+] corresponding to successive loss of the apiose, rhamnose and a glucosyl moiety respectively.

The $^1$H and $^{13}$C-NMR spectra of H1 exhibited signals arising from a caffeoyl and 3,4-dihydroxy phenethyl moieties (Table 3). $^{13}$C-NMR spectrum displayed 34 signals of which 17 were assigned to the aglycone and the acyl moieties, including two aromatic rings and the remaining 17 signals corresponded to one pentose and two hexose sugar residues. The 2D COSY spectrum shows cross-peaks for the glucose moiety between H-1 (δ 4.37) and H-2 (δ 3.38), H-2 and H-3 (δ 3.80), H-3 and H-4 (δ 4.94), indicates that this latter position was the acylation site of (E)-caffeic acid on the trisaccharide core. High resonances observed for the signals H-3 glucose (δ 3.80) and CH2-6 glucose (δ 3.73-3.49) (Figure 2), indicate that these positions were the glycosylation sites. Finally, the 2D $^{13}$C-$^1$H allows us to assign unambiguously the $^{13}$C resonance of the three sugars units and the cross-peaks observed between H-3 glucose and C-1 rhamnose and, between CH2-6 glucose and C-1 apiose permitted us to determine the sugar sequence. These NMR data are consistent for a compound with the chemical structure with β-D-glucopyranosyl, 2-(3,4-dihydroxyphenyl) O-D-apio-ß-furanosyl-(1→6)-O-[6-deoxy-α-L-rhamnopyranosyl-(1→3)]-4-{[(2E)-3-(3,4-dihydroxyphenyl)-2-propenoate] corresponding to the forsythoside B (C$_{34}$H$_{44}$O$_{19}$) and prove that glycosylated compounds were present in teak wood.

**Table 1:** Non-structural carbohydrates (NSC) content and their percentages before and after chemical hydrolyse in the sapwood, the outer heartwood, the middle heartwood and the inner heartwood of teak (**Tectona grandis**) from Malaysia.

| Content of NSC                     | Sapwood          | Outer heartwood | Middle heartwood | Inner heartwood |
|-----------------------------------|------------------|-----------------|------------------|-----------------|
| Before chemical hydrolyse (µmoles.g⁻¹ dw) | 100.9 (5.9)$^a$ | 1.7 (0.4)$^b$  | 1.2 (0.1)$^b$   | 0.6 (0.1)$^c$  |
| After chemical hydrolyse (µmoles.g⁻¹ dw) | 30.2 (5.5)$^a$  | 5.6 (0.3)$^b$  | 4.2 (0.2)$^b$   | 4.5 (0.3)$^b$  |
| Before chemical hydrolyse (%)     | 77.7             | 24.5            | 16.2             | 14.2            |
| After chemical hydrolyse (%)      | 22.3             | 75.5            | 83.8             | 85.8            |

NSC: Non-structural carbohydrates; N = 24. Standard deviations are in brackets; different letters within a line indicated the level of significance.
**Figure 1:** Radial distribution of conjugated non-structural carbohydrates: glucose from acid hydrolysis (GA), glucose from basic hydrolysis (GB) in sapwood (SW) and heartwood (HW) which is divided into outer heartwood (OHW), middle heartwood (MHW) and inner heartwood (IHW). Values are the mean of 6 independent analyses. Standard deviations are displayed as bars. Letters a, b, c and d indicate statistically significant values at $\rho < 0.05$.

**Table 2:** Spearman correlation’s between non-structural carbohydrates and Conjugated glucose and natural durability against Antrodia sp. in the heartwood of teak from Malaysia. N= 72. Numbers which are in bold are statistically significant.

| Non-structural carbohydrates | Coefficients of correlation (R) |
|-----------------------------|---------------------------------|
| Condensed glucose           | -0.67                           |
| Non-condensed glucose       | -0.46                           |
| Starch                      | -0.43                           |
| Fructose                    | 0.24                            |
| Sucrose                     | 0.03                            |
Table 3: $^1$H (500 MHz, coupling constant J in Hz in parentheses) and $^{13}$C (125 MHz) data of Forsythoside B in CD$_3$OD.

| Carbon number | Chemical shift $\delta$ in ppm | Carbon number | Chemical shift $\delta$ in ppm |
|---------------|--------------------------------|---------------|--------------------------------|
| 3,5-dihydroxy-phenethyl | Glucose moiety | | |
| 1 | - | 131.4 | 1″ | 4.37; d (7.6) | 104.3 |
| | 2 | 6.70; d (2.1) | 117.1 | 2″ | 3.38; dd (9.2; 7.9) | 76.2 |
| | 3 | - | 146.4 | 3″ | 3.80; m | 81.6 |
| | 4 | - | 144.7 | 4″ | 4.94; t (9.5) | 70.9 |
| | 5 | 6.68; d (7.9) | 116.3 | 5″ | 3.70; m | 74.6 |
| | 6 | 6.57; dd (7.9; 2.1) | 121.3 | 6″ | 3.73; m | 68.5 |
| 7 | 2.80; m | 36.6 | Rhamnose moiety | 1″ | 5.18; d (1.5) | 103.0 |
| 8 | 3.73; m | 72.4 | 2″ | 3.92; m | 72.3 |
| | 4.00; m | | | | |
| Caffeoyl moiety | | | |
| | 7.07; d (1.8) | 115.2 | 3″ | 3.55; m | 72.1 |
| | - | 146.9 | 4″ | 3.28; m | 73.8 |
| | - | 149.9 | 5″ | 3.57; m | 70.4 |
| | 6.78; d (8.2) | 116.5 | 6″ | 1.08; d (6.1) | 18.4 |
| | 6.96; dd (8.2; 1.8) | 123.2 | Apiose moiety | 1″ | 4.91; d (2.4) | 111.1 |
| | 7.59; d (16.0) | 148.0 | 2″ | 3.87; m | 78.2 |
| | - | 168.1 | 3″ | - | 80.7 |
| | | 4″ | 3.92; m | 75.1 |
| | | 3.74; m | 65.7 |
| | | 5″ | 3.55; s |

Assignment was confirmed from 2D $^1$H - $^1$H COSY, $^1$H - $^1$H NOESY, $^1$H – $^{13}$C HMQC and $^1$H – $^{13}$C HMBC spectra analyses.

Figure 2: Essential NOE correlations, $^2$J and $^3$J distances observed for NOESY-NMR and HMBC-NMR spectra respectively for the Forsythoside B.
DISCUSSION

Non-structural carbohydrates are involved in many metabolic processes and their mobilization in wood of high durable species may provide relevant information for wood properties (Safou-Tchiam et al., 2017). In the present study, soluble NSC and starch (non-condensed NSC) were quantified from ethanolic extracts and condensed NSC were quantified from acetic extracts. It was checked that NSC contents in ethanolic and acetic extracts show similar results (data not shown).

In teak sapwood, NSC were monomeric and polymeric forms mainly as starch, glucose, fructose and sucrose. Starch is the main NSC in teak as previously shown in other temperate and tropical species (Barbaroux et al., 2003). These results are in good accordance with histochemical results found on Thailand teak by Nobuchi et al. (2005). Sucrose content was found to be the lowest among all studied NSC that is a surprising result by comparison to temperate species such as Robinia pseudoacacia and Fagus sylvester (Label et al., 2000; Barzot et al., 2013; Kampe and Magel, 2013) which accumulated high amount of sucrose in sapwood. This may be explained either by species and differences of climatic conditions. Indeed, Kampe and Magel (2013) reported the important role of sucrose and its derivatives in the resistance to frost during winter and autumn. However, we could not exclude that hydrolysis process could take place during the wood drying procedure. Even though these quantitative differences between soluble NSC, the content of all NSC (soluble and starch) decreased abruptly from the sapwood to the heartwood indicating that they are probably involved in heartwood formation. They provided carbon skeletons for metabolic activities.

In teak, during heartwood formation process, high content of reserve materials (NSC) of the sapwood were cleared up while high amounts of toxic quinones were synthesized and enhanced the natural durability property of heartwood (Niamké et al., 2011). Indeed, the sapwood was non-durable (class 5) in opposition to the outer heartwood which was highly durable (class 1, according to the current standard EN 350-1 (AFNOR, 2016; Kokutse et al., 2006). The high accumulation of NSC associated with the absence or low content of toxic quinone in sapwood may explain its lower natural durability in comparison to heartwood (Niamké et al., 2011). These quinones were mainly tectoquinone, 2-(hydroxymethyl)anthraquinone and 4',5'-dihydroxy-episocatalponol (Niamké et al., 2014). They were shown to have high correlations with decay resistance (natural durability) in teak heartwood and may play a key role in teak decay resistance (Niamké et al., 2014).

Despite the key role of toxic quinone in teak natural durability, the mobilization of non-structural carbohydrates (NSC) throughout the stem of teak could likely influence the natural durability property. Indeed, this mobilization occurred in two forms: non-condensed (soluble NSC and starch) and condensed NSC. Two processes may be involved in the storage of NSC and their transformation in teak wood. Firstly, non-condensed NSC (starch, glucose, fructose and sucrose) were accumulated and available in sapwood. As previously shown in temperate species such as Robinia pseudoacacia, Acer pseudoplatanus and Fagus sylvatica, this part of NSC represented an important fraction of storage NSC (Label et al., 2000). In teak sapwood, these NSC represented 80% of total mobilized NSC (Table 4). According to the woody species, these carbohydrates can be totally or partially used for metabolic activities such as respiration and the transformation of sapwood into heartwood. Secondly, monosaccharides (glucose, rhamnose) or disaccharides as rutinose can be bounded to aglycone parts such as flavonoids and quinones (Verma et al., 2005) and phenolic acid (forsythoside B), through the activities of glycosyltransferases. Forsythoside B, identified for the first time in teak sapwood, is a glycosylated caffeic acid derivative bounded with three molecules of sugar moieties (Delazar et al., 2005) and is
only found in the sapwood of teak (Niamké et al., 2011). This finding confirms that glycosylation of sugar moieties occurred in teak and represents 20% of total NSC in sapwood and 75 – 90% in heartwood.

These hypotheses are supported by new advances concerning carbon allocation from NSC in the trees (Hartmann and Trumbore, 2016). Indeed, Richardson et al. (2013) after using radiocarbon 14C in the wood of Acer rubrum, observed that carbon allocation depended on the dynamics of NSC which can stored during decade before their use for metabolic functions. Trumbore et al. (2015) found that in Californian oaks, the carbon bounded in soluble NSC (such as glucose, fructose) were younger than that of insoluble pools. This indicates that NSC stored as insoluble structures in stemwood but rapidly exchange into soluble pools for metabolic functions when needed.

Glycosides could be degraded by glycosidases during the death of cells. The conjugated phenolic (forsythoside B) identified in sapwood was probably hydrolysed during heartwood formation releasing phenolic aglycones and monosaccharides. The same process was found for hydrojuglone glucoside in walnut (Babula et al., 2009). Indeed, hydrojuglone glucoside, a molecule of hydrojuglone bounded to glucose (Strugstad et al., 2012), was found to be the major phenolic in the sapwood of walnut (Babula et al., 2009). The transformation of sapwood into heartwood lead to the hydrolysis of glucosides as observed in walnut. Therefore, comparable storage process of phenolic precursors for the formation of heartwood substances could be observed in both walnut and teak. However, in the transition zone of teak and black locust, hydrolysis of these glycosides could be associated with the high utilisation of both non-condensed NSC and condensed glucose. In some cases, deglycosylation process of phenolics can occur without enzymatic catalysers but also under chemical conditions (pH, O₂, metals…) which are suitable for oxidation (Witkowski and Wałejko, 2002).

Concomitantly, secondary metabolites such as phenolics are synthesized from NSC and other precursors present in sapwood via shikimate and mevalonate pathways, then accumulated in heartwood (Nobuchi et al., 2005; Niamké et al., 2011). After their formation, other processes such as glycosylation (conjugated sugar corresponded to 75-90% of soluble and condensed NSC in heartwood) mainly by etherification occurred in teak. As shown in conifers (Betula platyphylla and Syringa vulgaris), glycosylation in teak could be consider as the capability of this species to store overproduced products during the growing season (Marjamaa et al., 2003). In teak sapwood, glycosylation could be a way to store more reserves and to reduce the availability of NSC in heartwood. These reactions could induce quantitative variations in phenolic content in heartwood with a reliable impact on its natural durability as defence potentialities. During natural drying process, we could not exclude that degradative reactions of biochemical compounds (oxidation, deglycosylation, polymerization) by fungal, bacterial and termite attacks may occur. For industry purposes, the biochemical status of air-dried wood with reference to natural durability brings relevant information. More investigations including controlled wood samples will be useful to make the link between wood properties and the physiological process of heartwood formation.

ACKNOWLEDGEMENTS
The authors are grateful to the Montpellier section of the “Association Française des Femmes Diplômées des Universités” (AFFDU), France for providing one month of funding to Niamké Bobélé Florence during her Ph.D. We thank Dr Herbette Gaëtan in spectropole Marseille for MNR analyses and Dr Thévenon Marie-France in CIRAD Montpellier UR40, for her advices about processes in durable species.

COMPETING INTEREST
There is no competing interest.
AUTHORS’ CONTRIBUTIONS

CJ-A coordinates all scientific activities; NA supervises the technical activities and the writing of the manuscript; SK-C and AAA contributed by a critical reading of the manuscript and finally, BFN conceived and conducted all manipulations, analyses and this manuscript.

REFERENCES

AFNOR. 2016. NF EN 350: Durability of wood and wood-based products - Testing and classification of the durability to biological agents of wood and wood-based materials.

Babula P, Adam V, Havel L, Kizek R. 2009. Noteworthy Secondary Metabolites Naphthoquinones – their Occurrence, Pharmacological Properties and Analysis. Curr. Pharm. Anal., 5(1): 47–68. DOI: https://doi.org/10.2174/157341209787314936

Barboux C, Breda N, Dufrene E. 2003. Distribution of above-ground and below-ground carbohydrate reserves in adult trees of two contrasting broad-leaved species (Quercus petraea and Fagus sylvatica). New Phytol., 157: 605-615. DOI: 10.1046/j.1469-8137.2003.00681.x.

Barzot S, Barthes L, Fresneau C. 2013. Distribution of non-structural nitrogen compounds in mature oak trees at four key phenological stages. 2013. Trees, 27(4). DOI 10.1007/s00468-013-0853-5

Berthier S, Kokutse AD, Stokes A, Fourcaud T. 2001. Irregular heartwood formation in maritime Pine (Pinus pinaster Ait) consequences for biomechanical and hydraulic Tree Functioning. Ann. Bot., 87: 19-25. DOI: https://doi.org/10.1006/anbo.2000.1287

Bhat KM, Ma HO. 2004. Teak growers unite. ITTO Tropical Forest Update 14/1: 3-5.

Delazar A, Gibbons S, Kumarasamy Y, Nahar L, Shoeb M, Sarker SD. 2005. Antioxidant phenylethanoid glycosides from the rhizomes of Eremosthasys glabra (Lamiaceae). Biochem. Syst. Ecol., 33: 87-90. DOI: 10.1016/j.bse.2004.05.011

Dietze, MC, Sala, A, Carbone, MS, Czimczik, CI, Manton, J, Richardson, AD, Vargas, R. 2014. Nonstructural carbon in woody plants. Annu. Rev. Plant Biol., 65: 667-687. DOI: https://doi.org/10.1146/annurev-arplant-050213-040054

Funes L, Laporta O, Cerdan-Calero M, Micol V. 2010. Effects of verbascoside, a phenylpropanoid glycoside from Lemon verbena, on phospholipid model membranes. Chem. Phys. Lipids, 163: 190-199. DOI: 10.1016/j.chemphyslip.2009.11.004

Hamada J, Petriassins A, Mothe F, Ruelle J, Pétrissans M, Gérardin P. 2017. Intraspecific variation of European oak wood thermal stability according to radial position. Wood Sci. Technol. DOI : 10.1007/s00226-017-0910-0

Hartmann H, Trumbore S. 2016. Understanding the roles of nontrustural carbohydrates in forest trees - from what we can measure to what we want to know. New Phytol., 211: 386-403. DOI: 10.1111/nph.13955

Hoch, G, Richter, A., & Körner, C. 2003. Non-structural carbon compounds in temperate forest trees. Plant Cell Environ., 26: 1067-1081. DOI: 10.1046/j.0016-8025.2003.01032.x

Ja’afaru MI, Onilude AA. 2014. Induction characteristics and optimization of production conditions of Xylanase by selected fungal species on lignocellulosic substrates. Int. J. Biol. Chem. Sci., 8(1): 324-339. DOI: http://dx.doi.org/10.4314/ijbcs.v8i1.28

Kampe A, Magel E. 2013. New Insights into
Heartwood and Heartwood Formation. Cellular Aspects of Wood Formation. In *Plant Cell Monographs* Fromm J (ed), Vol. 20, pp. 71-95.

Kokutse AD, Stokes A, Bailleres H, Kokou K, Baudasse C. 2006. Decay resistance of Togolese teak (*Tectona grandis* L.f) heartwood and relationship with colour. *Trees, 20*: 219-223. DOI: http://dx.doi.org/10.1007/s00468-005-0028-0

Kokutse AD, Stokes A, Adjonou K, Kokou K. 2009. Relationship between ecological indicators and teak wood characteristics in Tchorogo plantation (Togo). *Int. J. Biol. Chem. Sci.*, 3(3): 483-491, 2009. DOI: http://dx.doi.org/10.4314/ijbcs.v3i3.45352

Label P, Beritognolo I, Burtin P, Couée I, Breton C, Charpentier J-P, Jay-Allemand C. 2000. Cambial activity and xylem differentiation in walnut (*Juglans* sp.). 2000. Cell and molecular biology of wood formation. Chapter 16, pp 209 – 221. SN - 1-85996-123-1.

Marjamaa K, Lehtonen M, Lundell T, Toikka M, Saranpaa P, Fagerstedt K. 2003. Developmental lignification and seasonal variation in –glucosidase and peroxidase activities in xylem of Scots pine, Norway spruce and silver birch. *Tree Physiol.*, 23: 977-986.

Niamké FB, Amusant N, Kadio AA, Thevenon MF, Nourissier S, Adima AA, Jay-Allemand C, Chaix G. 2014. Rapid prediction of phenolic compounds as chemical markers for the natural durability of teak (*Tectona grandis* Linn f.) heartwood by near infrared spectroscopy. *J. Near Infrared Spec.*, 22: 35-43. DOI: 10.1255/jnirs.1091

Niamké BF, Amusant N, Charpentier J-P, Chaix G, Baissac Y, Boutahar N, Adima AA, Kati-Coulibaly S, Jay-Allemand C. 2011. Relationships between biochemical attributes (non-structural carbohydrates and phenolics) and natural durability against fungi in dry teak wood (*Tectona grandis* L.). *Ann. For. Sci.*, 68(1): 201-211. DOI: http://dx.doi.org/10.1007/s13595-011-0021-2

Niamké BF, Amusant N, Kokutse AD, Chaix G, Charpentier J-P, Adima AA, Kati-Coulibaly S, Jay-Allemand C. 2010. Radial distribution of non-structural carbohydrates in Malaysian teak. *Int. J. Biol. Chem. Sci.*, 4(3): 710-720. DOI: http://dx.doi.org/10.4314/ijbcs.v4i3.60498

Nobuchi T, Okada N, Nishida M, Siripatanadilok S, Veenin T, Tobing TL, Sahri MH. 2005. Some characteristics of wood formation in Teak (*Tectona grandis*) with special reference to water conditions. in Bhat K M, Nair KKN, Bhat KV, Muralidharan EM, Sharma JK, (eds) *Quality timber products of teak from sustainable forest management*. India Pp 495-499.

Okahisa Y, Yoshihura T, Imamura Y. 2006. Seasonal and height-dependant fluctuation of starch and free glucose contents in moso bamboo (*Phyllostachys pubescens*) and its relation to attack by termites and decay fungi. *J. Wood Sci.*, 52: 445-451. DOI: 10.1007/s10086-005-0776-x

Richardson AD, Carbone MS, Keenan TF, Czimczik CI, Hollinger DY, Murakami P, Schaberg PG, Xu X. 2013. Seasonal dynamics and age of stemwood nonstructural carbohydrates in temperate forest trees. *New Phytol.*, 197: 850-861. DOI: 10.1111/nph.12042

Safou-Tchiam R, Andzi Barhé T, Soulounganga P, Ngwa Obame S, Mabicka Iwangou SB, Bikoro Bi Athomo B, Engozogho Anris PS, Jeso BD, Akagah AG. 2017. Chemical reactivity and supramolecular...
susceptibility of hardwood celluloses towards succinic anhydride. Int. J. Biol. Chem. Sci., 11(6): 3110-3131. DOI: https://dx.doi.org/10.4314/ijbcs.v11i6.44

Strugstad PM, Despotovski S. 2012. A summary of extraction, synthesis, properties and potential uses of juglone: a literature review. JEM, 13(3): 1-16. http://jem.forrex.org/index.php/jem/article/viewFile/119/473

Trumbore S, Czimczik CI, Sierre CA, Muhr J, Xu X. 2015. Non-structural carbon dynamics and allocation relate to growth rate and leaf habit in California oaks. Tree Physiol., 35: 1206-1222. DOI: https://doi.org/10.1093/treephys/tpv097

Verma SC, Singh NP, Sinha AK. 2005. Determination and locational variations in the quantity of hydroxyanthraquinones and their glycosides in rhizomes of *Rheum emodi* using high-performance liquid chromatography. J. Chromatogr. A, 1097: 59-65. DOI: 10.1016/j.chroma.2005.08.018

Windeisen E, Klassen A, Wegener G. 2003. On the chemical characterization of plantation teakwood from Panama. Holz Roh-Werkst., 61: 416-418. DOI: http://dx.doi.org/10.1007/s00107-003-0406-5

Witkowski S, Walejko P. 2002. Studies in the acid catalysed glycosylation of α-tocopherol. Z Naturforsch, 57b: 571-578.

Yonekura-Sakakibara K. 2009. Functional genomics of family 1 glycosyltransferases in *Arabidopsis*. Plant Biotechnol, 26: 267-274. DOI: 10.1039/B817077K.