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Lucero Chavez Salgado

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Université  
de Toulouse

# THÈSE

En vue de l'obtention du

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Mme LUCERO CHAVEZ SALGADO

le vendredi 9 juillet 2021

**Titre :**

Study of the valorization of teak (*Tectona grandis*) by-product from a sawmill by a continuous process under subcritical water

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**Directeur(s) de Thèse :**

MME VIRGINIE VANDENBOSSCHE

**Rapporteurs :**

M. CHAKER EL KALAMOUNI, UNIVERSITE DE LA REUNION

M. EDUARDO BARZANA GARCIA, UNIVERSITE NATIONALE AUTONOME DE MEXICO

**Membre(s) du jury :**

M. DOMINIQUE DE CARO, LABORATOIRE DE CHIMIE DE COORDINATION, Président

M. GERARD VILAREM, TOULOUSE INP, Invité(e)

MME CHRISTINE RAYNAUD, TOULOUSE INP, Invité(e)

MME VIRGINIE VANDENBOSSCHE, TOULOUSE INP, Membre



When you set out on your journey to Ithaca,  
pray that the road is long,  
full of adventure, full of knowledge.  
The Lestrygonians and the Cyclops,  
the angry Poseidon – do not fear them:  
You will never find such as these on your path,  
if your thoughts remain lofty, if a fine  
emotion touches your spirit and your body.  
The Lestrygonians and the Cyclops,  
the fierce Poseidon you will never encounter,  
if you do not carry them within your soul,  
if your soul does not set them up before you.

...

Ithaca has given you the beautiful voyage.  
Without her you would have never set out on the road.  
She has nothing more to give you.

**Ἰθάκη**

Κωνσταντίνος Καβάφης



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## Résumé

*Tectona grandis* L.f. (teck) est un bois tropical apprécié sur le marché du bois en raison de ses performances exceptionnelles et de sa valeur économique. Parmi ses principales qualités figurent la stabilité dimensionnelle et la résistance à la pourriture, toutes deux attribuées à ses matières extractibles. Ils contiennent des composés biologiquement actifs, principalement des quinones et des anthraquinones qui confèrent une résistance naturelle aux termites et aux champignons, ainsi qu'une activité antioxydante et pharmacologique. La demande mondiale de teck devrait continuer à augmenter, et avec elle les résidus à traiter. Le teck ayant des caractéristiques excellentes, les sous-produits des usines de transformation du bois pourraient constituer une bioressource potentielle de composés chimiques. La technologie d'extrusion bivis a été étudiée comme technique d'extraction solide/liquide de la sciure de teck. L'utilisation de l'eau comme solvant a été proposée, non seulement parce qu'il s'agit d'un solvant respectueux de l'environnement, mais aussi parce que les conditions de pression et de température appropriées peuvent être atteintes à l'intérieur de l'extrudeur, conduisant l'eau à des conditions sous-critiques. Différentes conditions de température et de rapport liquide/solide ont été évaluées selon un plan expérimental défini. Des métabolites secondaires avec un contenu phénolique total élevé ont été obtenus. La valorisation de l'extrudat comme matériau pour la préparation de biocomposites a également été étudiée.

### Mots clés :

Teck, extraction solide/liquide, eau subcritique, métabolites secondaires, biocomposite

## **Abstract**

*Tectona grandis* L.f. (teak tree) is a tropical hardwood exceptionally appreciated in the timber market due to its outstanding performance and economic value. Among the main qualities are dimensional stability and decay resistance, both ascribed to its extractives. They contain biologically active compounds, mainly quinones and anthraquinones that confer a natural resistance against termites and fungi, as well as antioxidant and pharmacological activity. The increase in teak global demand is likely to continue, and along with this the residues to deal with. Since teak has amazing features, the byproducts from wood processing plants could be a potential biosource of chemical compounds. The twin-screw technology as a solid/liquid extraction technique of teak sawdust has been studied. The use of water as a solvent has been proposed, not only because it is a green solvent, but the appropriate pressure and temperature conditions can be reached inside the extruder leading water to subcritical conditions. Different conditions of temperature and liquid to solid ratio have been evaluated according to a defined experimental design. There have been obtained secondary metabolites with a high total phenolic content. The valorization of the extrudate as a raw material for the preparation of biocomposites has been as well studied.

### **Keywords:**

Teak, solid/liquid extraction, subcritical water, secondary metabolites, biocomposite

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## **General introduction**



This thesis was conducted under the direction of Gérard Vilarem until he departed from the laboratory (LCA) in August 2020, the direction of the thesis was then continued by Virginie Vandenbossche.

*Tectona grandis* L.f. (teak tree) is one of the most valuable timber due to its remarkable performance in the timber industry and its economic importance. Teak occurs naturally in India, Myanmar, Thailand, and Laos People's Democratic Republic. Nevertheless, Myanmar is the only country that commercializes high-quality teak from natural forests on the international market. The other countries with native forests have logging bans or log export restrictions. Consequently, the increasing global demand, mainly by India and China, is supplied by planted forests.

The significant value of teak wood and the ease of plantation establishment have made it one of the most important tree species for planting in the tropics, also outside its natural distribution area (Keogh, 1996). In this context, Latin America emerges as a promising region where teak has been translocated and the planted area has considerably increased. In Mexico, in particular, forestry plantations represent a good long-term business opportunity, and their promotion is strategic for the country.

Mexico has 138 million hectares of forest vegetation, equivalent to 70.4 % of its territory, of which 47 % are forests and jungles. Additionally, it has around 7 million hectares of temperate and tropical climate suitable for developing commercial forest plantations (CFP), of which 80 % are located in tropical and subtropical regions of the country whose soil types and climates are favorable to achieve rapid growth of plantations, and the advantage of the availability of labor (CONAFOR, 2020).

According to the National Forestry Commission (CONAFOR), Mexico has 270 000 ha of commercial forest plantations and the main timber species planted are *Cedrela odorata*, *Eucalyptus spp.*, *Tectona grandis*, *Gmelina arborea* *Pinus spp.*, *Swietenia macrophylla*, *Prosopis spp.*, and *Tabebuia rosea*.

*Tectona grandis* is the third more planted species with 25, 324 ha mainly in the southeast of the country due to the favorable environmental conditions in this area. Teak cultivation includes the states of Campeche (8,803 ha), Tabasco (7,268 ha), Chiapas (2,941 ha), Veracruz (2,300 ha), and Nayarit (1,955 ha) (CONAFOR, 2015).

The potential for the production of raw materials represents an alternative for the economic development of rural areas. Furthermore, through the CFP of timber species of high economic value or of rapid growth, it is possible to reduce their importation. In this sense, the Mexican government, through the National Forest

Program, promotes the expansion of CFP to contribute to the balance of trade. The objective is to encourage the development of plantations in regions with high potential to create supply areas and reinforce the link between the producers and the companies.

One of the major Mexican teak plantations is Agropecuaria Santa Genoveva S.A.P.I. de C.V., located in Campeche. This company manages important volumes of timber for both domestic and international markets. The main products are logs and wooden boards. Due to the large quantities of material processed, the disposal of byproducts (chips and sawdust) is a challenge to overcome.

The conventional uses of woody residues are as biofuel feedstock, as a substrate for processing compost that can be commercialized for gardening or agricultural activities, and as soil conditioning. However, considering that *Tectona grandis* is not a conventional but a remarkable tree, its byproducts could be a raw material rather than a waste. Since teak has outstanding features attributed to its extractives, the byproducts from a wood processing plant could be a potential biosource of chemical compounds.

Nowadays, there are many techniques for the extraction of bioactive compounds from natural sources. However, the conventional ones (soxhlet, maceration, percolation, etc.) present decisive disadvantages when processing large quantities of material. Capacity is a crucial factor on an industrial scale, for this reason, it is imperative to have a technology that allows managing large volumes of material. The twin-screw extrusion technology seems to be an adequate solution by virtue of its capacity, flexibility, ability to handle wider varieties of raw materials in a continuous way, and good control of process parameters. Regarding the solvent, the use of water is proposed, not only because it is a green solvent, but also because suitable pressure and temperature conditions can be achieved inside the extruder leading water to subcritical conditions. The properties of water under subcritical conditions change considerably, in particular, the dielectric constant and, accordingly, its solvent properties, which become similar to those of organic solvents such as ethanol or methanol.

The objectives of this work were to study the potential of teak sawdust as a source of phytochemical compounds and to propose an approach for its integral valorization. For these aims, two options are explored, the recovery of extractives by twin-screw technology using water as a solvent, and the reuse of the extrudate as a raw material for the preparation of biocomposites.

To develop this study, the first chapter is dedicated to the literature review that presents this exceptional tree and describes the extractives and secondary metabolites that confer it its highly appreciated natural

resistance. It also discusses the advantages of the extraction method chosen in this work, twin-screw extruder, with respect to the conventional ones, as well as the convenience of the use of water.

The second chapter describes the origin of teak sawdust and details its characterization. The analysis include fiber distribution (cellulose, hemicellulose, and lignin by Van Soest and Wine method) and the characterization of its extracts. Extractions are carried out both with a conventional method (using a Soxhlet apparatus) and a pressurized extraction (accelerated solvent extraction, ASE). The extractives are analyzed by high-performance liquid chromatography and gas chromatography in order to identify and quantify the secondary metabolites extracted. The antioxidant activity (by DPPH assay) and the total phenolic content (by Folin-Ciocalteu method) are also quantified in the extracts. These results allow us to determine the potential of teak sawdust and to have reference values for the evaluation of the novel extraction method proposed in this work.

The third chapter presents the study of the solid/liquid extraction with twin-screw technology. The first approach is performed using an extruder BC21, where different conditions of temperature and liquid to solid ratio are studied according to a defined experimental design. The extracts obtained are analyzed to evaluate the feasibility of this extraction method. The extraction yield, the identification and quantification of the extracted compounds, and the reducing capacity of the extracts are the main responses evaluated. A scale-up study is then conducted using an extruder BC45. This last extrusion is mainly focused on the recovery of the extrudate for a subsequent valorization.

The fourth chapter explores the valorization of the teak extrudate as a filler for composites. The preparation of the biocomposites is done using poly(lactic acid) (PLA) as a polymeric matrix and no additives or coupling agents are used. The choice of this polymer responds to the environmental concerns about the biodegradability of new materials. PLA exhibits not only a biodegradable character, but also has good mechanical properties (compared with other biopolymers) and is easy to process. The blending of wood fibers with the PLA is ensured by a twin-screw extruder. The mechanical test specimens are then prepared using an injection molding machine. The mechanical features, such as tensile and flexural modulus and strength of the biocomposites are evaluate to assess the reinforcement provided by teak fibers to the biopolymer.

Finally, the fifth chapter presents in detail the procedures followed and materials used in this study.



## **Chapter 1**

### ***Tectona grandis* (teak) byproducts: a source of valuable bioactives compounds**





## **Chapter 1. *Tectona grandis* (teak) byproducts: a source of valuable bioactives compounds**

*Tectona grandis* L.f. (teak tree) is an exceptional tropical hardwood appreciated in the timber market due to its outstanding performance and economic value. The characteristics of teak wood make it suitable for a broad array of applications. Among the main qualities are dimensional stability and decay resistance, both ascribed to its extractives. They contain biologically active compounds, mainly quinones and anthraquinones that confer a natural resistance against termites and fungi, as well as antioxidant and pharmacological activity.

Teak tree occurs naturally in Asia, but it has been successfully translocated to other regions, such as Latin America, thanks to its wide tolerance to climatic conditions. It is an interesting species to be planted due to its sustainability, high quality, and economic value in the market. In addition, the fast growth rate of teak tree makes the return of investment shorter than other tropical species (CONAFOR, 2020). Consequently, there is international attention and effort to promote teak commercial forests. In line with this trend, Mexico is also investing in teak plantations. Mexico has around 7 million hectares with suitable conditions for developing commercial forest plantations (CFP), and competitive advantages to increase its extension. The potential of the soil, the climate, and the geography with adequate conditions for the CFP confirm these advantages. Among the main cultivated timber species in CFP, teak tree is the third most important. The most extensive plantations are located in the southeast of the country, with the state of Campeche having the largest area. In Campeche, the wood processing enterprise Agropecuaria Santa Genoveva S.A.P.I. de C.V. manages one of the major teak plantations, and as expected the amount of wood wastes is considerable. Nonetheless, this is not a domestic concern, the increase in teak global demand is likely to continue, and along with this the byproducts (like shavings, chips, and sawdust) to deal with.

This first chapter presents the literature review and essential definitions for better comprehension and contextualization of this study. It includes basic concepts of the tree's structural components, teak tree generalities with an emphasis on its extractives and secondary metabolites. After, it gives a brief summary of extractive methods used to obtain the phytochemical components highlighting the twin-screw technology as an innovative method that could be used for solid/ liquid extractions. Lastly, subcritical water is presented as a suitable solvent for the extraction process in alignment with environmental concerns.

## 1.1 Plant biomass with multiple interests: Trees

Biomass refers to the mass of living organisms, including plants, animals, and microorganisms; from a biochemical perspective it includes cellulose, lignin, sugars, fats, and proteins. Biomass comprises both the above and belowground tissues of plants, for example, leaves, twigs, branches, roots, etc. (Houghton, 2008).

Among the plant biomass, forests are of interest as a source of wood for timber, pulp and phytochemical compounds with and precious pharmacological value. Moreover, they are the major source of biomass for energy. Forest are the most important biomass carbon producers and the ones that store the most standing biomass carbon (Gonçalves et al., 2018).

### 1.1.1 Tree external structure

A tree is a woody perennial plant, with a single main stem or in case of coppice several stems, and has a more or less definitive crow.

The whole tree comprises all parts or organs of a tree, ranging from the leaves, flowers, fruits and buds to the branches, stem roots and fine roots, see Figure 1. The distinction of the above-ground (aerial part) and below-ground (underground) part of a tree is important in terms of biomass estimation. The above-ground part of a tree can be partitioned into stem, lateral parts or branches and foliage.

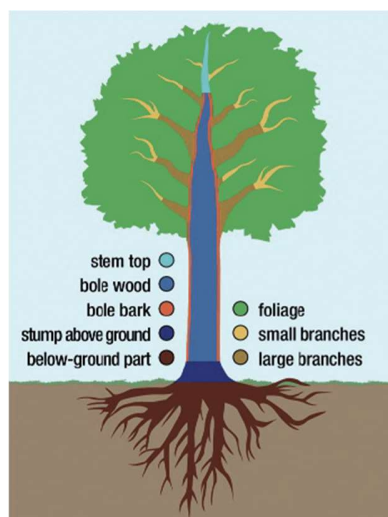


Figure 1. Parts of a tree (Gschwantner et al., 2009)

The crown consists of the living branches and their foliage. The foliage comprises all temporary parts such as leaves and needles, reproductive parts and buds. For most of higher vascular plants, the leaves are the principal photosynthetic. They are attached by a continuous vascular system to the rest of the plant so that free exchange of nutrients and water can be carried to various parts of the plant.

The stem of a tree is the above-ground part of the main shoot with apical dominance (main axis or body of a plant). It can be further divided into stump, bole, and stem top. The distinction between them depends of diameter threshold (economically relevant). The stump is the part of the stem, which would remain after a tree was cut under normal felling practices. The bole is the part of the stem between stump and the stem top. The stem top is the topmost of the stem. The main stem bears, as a result of branching, the secondary or laterals parts of a tree.

Stems of woody plants support the crown; store water, carbohydrates, and minerals; conduct water and minerals upward from the roots; and transport foods and hormones from points where they are synthesized to those where they are used in growth or stored for future use.

A mature tree stem typically consists of a tapering column of wood (xylem) composed of a series of layers or annual increments, added one above the other, like a series of overlapping cones, and enclosed in a covering of bark. At the apex of the stem and each of its branches is a terminal growing point where increase in length occurs. Between the bark and wood of the stem, branches, and major roots is the vascular cambium, a thin, sheathing lateral meristem.

Young xylem or sapwood conducts sap, strengthens the stem, and to some extent serves as a storage reservoir for food. The living parenchyma cells in the sapwood, which are very important because they store foods, consist of horizontally oriented ray cells and, in many species of woody plants, of vertically oriented axial parenchyma cells as well. On the average only about 10 % of the sapwood cells are alive. As the xylem ages all the living cells die and the tissue often becomes darker, producing a central cylinder of dark-colored dead tissue, called heartwood, which continues to provide mechanical support but is no longer involved in physiological processes (Pallardy, 2008), see Figure 2.

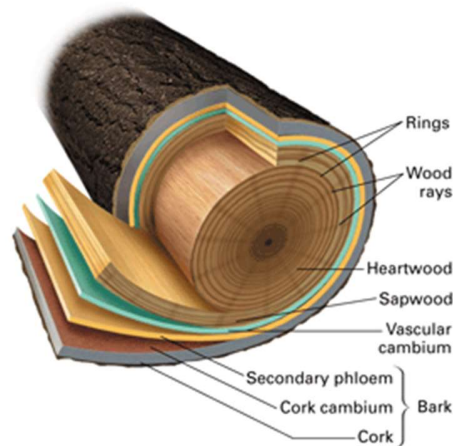


Figure 2. Tree cross section

A protective layer of bark normally covers all woody parts of trees. In plant science and forestry, the bark comprises the tissues outside the cambium. In terms of wood-processing only the xylem is relevant. Therefore, all tissues outside the xylem should be assigned to the bark from a wood-manufacturing point of view (Gschwantner et al., 2009).

Finally, roots provide structural anchorage to keep trees from toppling over. They also have a massive system for harvesting the enormous quantities of water and the mineral resources of the soil required by the tree and serve as storage depots, especially in seasonal climates (Encyclopedia Britannica, 2019).

### 1.1.2 Tree internal structure

Vascular plants, also known as higher plants or tracheophytes, are defined as those that possess specialized conducting tissues called xylem and phloem. In addition to vascular tissue, both tree parts aerial and ground are composed of dermal tissue and ground tissue, see Figure 3. Furthermore, each organ can be comprised of various other tissues distinguished by the specific characteristics of their cells (Carrillo-López and Yahia, 2019).

Dermal tissue system protects the internal tissues against pathogen attacks, mechanical injuries, temperature, stress, penetration of chemicals and loss of moisture. This system comprises different elements such as, epidermis, cuticular membrane (cuticle), stomata, and lenticels. The outermost layer of cells surrounding the roots and stems of the primary body (including the leaves, flowers, fruits, and seeds) is called the

epidermis. The cuticle is a waxy layer that covers the outside part of the epidermis decreasing water loss by transpiration. Stomata (pores) are interspersed throughout the epidermal cells of the leaves (and to some extent on the stems). They regulate the exchange of gases and water vapor into and out of the plant body.

Immediately adjacent is a cylinder of ground tissue; in the stem the outer region is called the cortex and the inner region the pith. The roots of woody dicotyledonous and conifers develop only a cortex (the pith is absent), the innermost layer of which comprises thick-walled wall cells called endodermal cells.

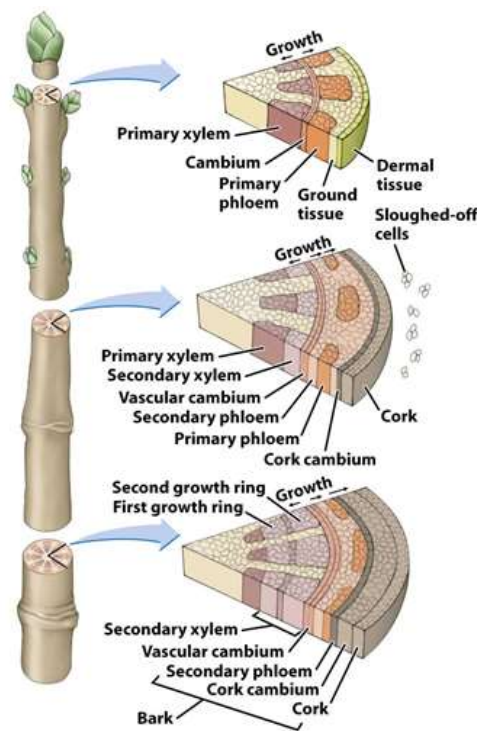


Figure 3. Tree internal structure (Michael L. Cain et al., 2006)

The vascular system is a continuous assemblage of conducting and supporting tissues that extends throughout the plant body, xylem and phloem. The xylem tissue transports water and dissolved minerals from the roots to the stem and leaves; and provides mechanical strength to plant parts. Xylem tissue consists mainly of dead cells that lack end walls between adjacent cells. The side walls are thick and reinforced with lignin, which makes them stiff and water proof. On the other hand, phloem tissue transports products of photosynthesis (oxygen and carbohydrates) to other parts of the plant for growth or storage. This type of tissue consists of living cells that are separated by end walls with pores, see Figure 4.

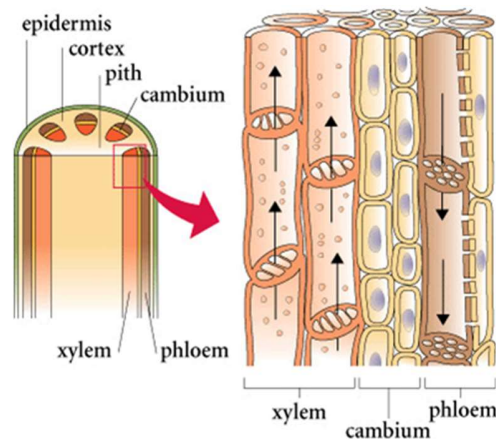


Figure 4. Vascular system (xylem and phloem tissues) (OER, 2018)

In the stems and roots, the vascular tissues are arranged concentrically, in the order of a series of cylinders. Each column, or cylinder, of primary vascular tissue develops the primary xylem toward the inner aspect of the column and the primary phloem toward the outer aspect. The multiple vascular cylinders are arranged throughout the cortex, either in an uninterrupted ring between the cortex and pith or separated from each other by ground tissues (Encyclopedia Britannica, 2019).

### 1.1.3 Forestry residues valorization

The ordinary uses of woody wastes are as biofuel feedstock or disposal in landfills. By contrast, there have recently emerged innovative alternatives for valorizing them. For instance, as a raw material for activated carbon production (Adegoke and Bello, 2015); in construction materials like clay matrix bricks (Barbieri et al., 2013); as a source for rearing edible insects (Varelas and Langton, 2017); in biocomposites manufacturing (Väisänen et al., 2016); and biomass-derived fuel, bio-ethanol, and bio-diesel production (Lu et al., 2017).

Most of the commercial activated carbon has as a precursor coal, agricultural by-products or lignocellulosic materials, which have a remarkable carbon content, few inorganic compounds and a notable mechanical strength, desired characteristics for a natural active carbon precursor (Cansado et al., 2018). Teak sawdust has been used for high activated carbon production. It is a promising precursor due to its high carbon content (50 %).

Activated carbon is an efficient and non-expensive adsorbent of gaseous and aqueous phase pollutants such as pesticides and phenolic compounds (Mohanty et al., 2005; Patil et al., 2011). Cansado et al. (Cansado et al., 2018) studied the high porous carbon production from teak sawdust activated by carbon dioxide (physical activation) and its application in the removal of pesticides (4-chloro-2-methyl-phenoxyacetic acid) in liquid phase. They got values of removal capacity similar to commercial ones, concluding that a feasible adsorption can be reached with adsorbents produced from sawdust wastes.

On the other hand, Mohanty et al. (Mohanty et al., 2005) investigated chemically activated carbon prepared from teak sawdust. The activation was carried out with zinc chloride and it was tested on phenol. They obtained encouraging results. The activated carbon showed a high surface area (about 585 m<sup>2</sup>/g) and an interesting adsorption capability (2.82 mg/g at pH 3.5).

Ismadji et al. (Ismadji et al., 2005) prepared activated carbon from vacuum pyrolysis char of teak sawdust. This method developed mainly microporous structure. The results obtained suggest that this material is an adequate precursor for activated carbon. The surface area and pore volume were 1150 m<sup>2</sup>/g and 0.43 cm<sup>3</sup>/g, respectively.

The bark is also considered a viable adsorbent, as it contains a high amount of tannin. It is assumed that the polyhydroxyl groups of tannin are active species in the adsorption process (Ali et al., 2012). The ability of pine bark in adsorption process has been studied with good results, for instance in phenols (Vázquez et al., 2007) and polycyclic aromatic hydrocarbon removal (Li et al., 2010) and as activated carbon precursor for removing organochlorine pesticides (Sousa et al., 2011). Regarding teak bark, it has been tested in powder form for the adsorption of methylene blue in batch process (varying initial concentration of adsorbate, agitation time and speed, pH, and particle size). Teak bark was used without treatment but wash with distilled water and sun-dried, after it was grounded. The adsorbent demonstrated an outstanding adsorption capacity reaching 333.3 mg/g (Patil et al., 2011).

Additionally, woody biomass wastes are a sustainable option for building material production, such as ceramic bricks. The residues could be incorporated in the bricks at the beginning of the process in replacement of clays in an amount lower than 10 % wt. (Barbieri et al., 2013).

Another possibility to recycle woody residues is the preparation of composites. The aforementioned advantages and their low abrasiveness make them suitable as fillers in wood-polymer composites (WPC) (Petchwattana and Covavisaruch, 2014). Composites are mainly manufactured with a plastic matrix and

wood, additives may be used. Among the most used wood species are pine, maple, and oak. In the particular case of teak, Patel et al. (Patel and Rawat, 2017) evaluated WPC with different charges of teak wood flour, they found that the composite consisting of 10 % of wood mixed with 15 % of gum rosin had excellent impact strength, high abrasive wear resistance, and high ductility.

Furthermore, taking into account that extractives play an essential role in natural resistance of wood, they can be valorize as potential preservatives to protect others tree species with low deterioration resistance (Brocco et al., 2017). Wood extracts have been used in commercial products for the treatment against rot, decay, and insect damage, for instance, Cedarshield® that contains natural cedar wood oil. Moreover, the allelopathic potential of teak is an opportunity to explore as a source of natural herbicidal (Lacret et al., 2012)

However, it is important to take into account the limitations of using natural compounds in wood protection products, for instance, the incompatibility of results from laboratory studies with field trials, the narrow range of the activity of some compounds, the legislation and registration of new compounds or formulations, and the risk to human health and environment due to their intrinsic biological activity (Singh and Singh, 2012)

The utilization of forest and timber industry residues displays many advantages for instance low cost, eco-friendly disposal and vast availability (they are among the most abundant renewable sources) (Adegoke and Bello, 2015; Ghaedi et al., 2014). Furthermore, residues from woodworking industry (wood shavings and sawdust), as well as forest activities are considered some of the most important biomass (Barbieri et al., 2013).



## 1.2 A tree with exceptional attributes: *Tectona grandis* L.f.

### 1.2.1 *Tectona grandis* L.f. generalities

*Tectona grandis* L. f. is a deciduous tropical tree of the Lamiaceae family. According to the Integrated Taxonomic Information System (ITIS) its taxonomic hierarchy is as follow, see Table 1 (ITIS, 2019).

Table 1. *Tectona grandis* L.f. taxonomy (Taxonomic Serial No.: 32247)

Kingdom:	Plantae
Subkingdom:	Viridiplantae
Infra kingdom:	Streptophyta
Superdivision:	Embryophyta
Division:	Tracheophyta
Subdivision:	Spermatophytina
Class:	Magnoliopsida
Superorder:	Asteranae
Order:	Lamiales
Family:	Lamiaceae
Genus:	<i>Tectona</i>
Species:	<i>Tectona grandis</i> L. f.

There are three species of the genus *Tectona*, namely *Tectona philippinensis* Benth. and Hooker f., *Tectona hamiltoniana* Wall., and *Tectona grandis* Linn. f.

*Tectona philippinensis* (Philippine teak) is endemic of the Philippines, occurring in Luzon Island and Iling Island. Philippine teak is a small to medium sized tree (up to 15 m) with thin and flaky bark and bright purple, bluish to lilac blooms (Caringal et al., 2015). Philippine teak wood and leaves have medicinal value

(Briz, 2017; Ragasa et al., 2008). In addition, this tree may have potential as a genetic resource for future teak breeding programs aimed at improving supplies of this highly popular wood (IUCN, 2008).

*Tectona hamiltoniana* (Dahat teak) is confined to the dry zone of central Myanmar like Magway and Mandalay. It naturally occurs in areas where annual rainfall is between 400 and 800 mm, it is therefore drought tolerant. This species is a medium sized deciduous tree (approx. 8 m in height). Dahat teak wood shows favorable properties, such as high stability and density, and very high natural resistance to insects (Minn et al., 2015). Furthermore, its bark is used for medical purposes. Nevertheless, it is economically not as important as *Tectona grandis*.

*Tectona grandis* (teak tree), the species studied here, is native to India, Myanmar, Thailand and Laos People's Democratic Republic (Kaosa-ard, 1981). Teak tree is a large, deciduous tree that can reach a height from 20 m to 35 m, and a diameter at breast height (DBH) from 29 cm to 54 cm in 50 years (Palanisamy et al., 2009), see Figure 5. Its stem is straight with rough and fissured bark of brown color. It is usually cylindrical but becoming fluted and slightly buttressed at base when mature. The roots are thick and often no deeper than 30 cm because they are sensitive to oxygen deficiency (ITTO, 2019) however they extend laterally up to 15 m from the stem (Orwa et al., 2009).



Figure 5. *Tectona grandis* tree (Agropecuaria Santa Genoveva S.A.P.I. de C.V. plantation, Campeche Mexico)

Its crown is open with many small branches (Nidavani, 2014). Leaves are opposite, broadly elliptic, obovate, from 11 cm to 85 cm long and 6 cm to 50 cm broad, glabrescent above and stellate-pubescent below, at the base rounded to acute, in apex obtuse to acute, and for petiole stout from 5 cm to 6 cm long

(ITTO, 2019; Palanisamy et al., 2009). Teak flowers are small (from 6 mm to 8 mm in size) and white or rarely purplish pink color. They occur in large inflorescences or panicles (from 20 cm to 90 cm) which initiate from terminal buds of stem and branch shoots. Fruit is a drupe, globose, from 5 mm to 20 mm in size, enclosed by an accrescent calyx with thick shaggy exocarp of matted hairs, epicarp inflated, spongy, and staellate pubescent, endocarp stony, 4-celled, seeds 1-4, oblong and exalbuminous (Palanisamy et al., 2009).

*Tectona grandis* is a highly recognized hardwood tree in the wood industry due to its economic value and outstanding characteristics, such as strength, lightness, dimensional stability, ease of work, pleasing aesthetic appearance, waterproof and decay resistance (Kokutse et al., 2006). Teak heartwood can be used in considerable applications both interior and exterior purposes. For instance, furniture, veneer, carving, boat decks, railway ties, shipbuilding, and construction industry (Nidavani, 2014).

Furthermore, teak exhibits non-corrosive properties, termite and fungi resistance, as well as antioxidant and pharmacological activities (Moya et al., 2014; Nidavani, 2014; Vyas et al., 2018). These qualities are ascribed to the secondary metabolites present in their extractives (Gupta and Singh, 2004; Lukmandaru, 2012a; Niamké et al., 2011). Due to the above mentioned we are interested in the extraction and identification of teak compounds.

### **1.2.1.1 Distribution around the world**

#### **1.2.1.1.1 Geographical requirements**

*Tectona grandis* plantations are well grown in tropical and sub-tropical zones. It has a natural distribution range from the longitude of 73° E in India to 104° 30' in Thailand. The northern boundary limit of teak is about 25° 30' N lat. in Myanmar; and its southern boundary limit lies from 9°N lat. in India through 15°-16°N lat. in Myanmar to 16° 30' lat. in Thailand (Kaosa-ard, 1981). The altitude can vary from zero up to 1,200 masl.

Teak plantations requires well-drained alluvial soils, quite moist and tropical climate. The best conditions for growing teak are soils derived from rocks of volcanic origin (pH between 6.5 and 7.5), a temperature between 40 °C and 13 °C, intensity of full day-light between 75 % and 94 %, and an annual rainfall from less than 900 mm to 3,500 mm (Kaosa-ard, 1981; Palanisamy et al., 2009).

The extensive climatic conditions for teak growth make it very tolerant. Consequently, it was successfully translocated outside of its native occurrence to several countries in Africa and Latin America during the past century where planted forest seems to be a good investment by the private sector (Hansen et al., 2017).

### 1.2.1.1.2 Natural forests

Natural teak forests are located in the Indian Peninsular, Myanmar, Thailand and Laos People's Democratic Republic (Kaosa-ard, 1981), see Figure 6. It is estimated that this kind of forests cover approximately 29 million ha along this four countries. Actually, teak wood from natural forest commercialized on market comes exclusively from Myanmar, where teak plantations covers 16 million ha (Kollert and Kleine, 2017).

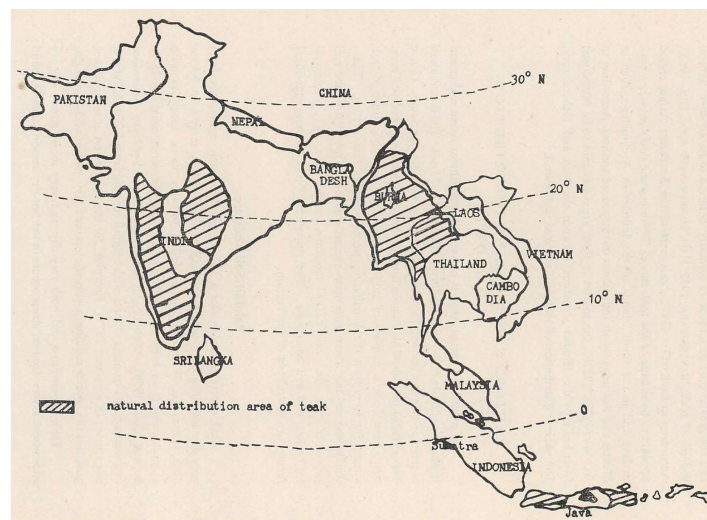


Figure 6. Natural distribution of *Tectona grandis* (Kaosa-ard, 1981)

The quality of teakwood from virgin forests, where rotation time may exceed 100 years, is generally accepted to be distinctly superior to that from plantations. Teakwood from natural sites is appreciated worldwide in particular for its exceptional decay resistance (Windeisen et al., 2003). Unfortunately, native teak forests are declining, especially old-growth and high-quality reservoirs. Among the main causes are overexploitation, deforestation, agricultural expansion, grazing, and conversion to other land uses. As a result, the natural populations are endangered.

It is important to highlight that teak natural forests represents only the 0.7 % of the total forests in the world, in other words, it is just a minor share of the global trade. Nevertheless, teak timber represents a relevant luxurious heartwood resource, which is emerging with an increasing growth rate, and this trend is likely to

continue. That and the fact that teak is easily adaptable to the climatic conditions and it needs short rotations could explain the investment that many countries in tropical Asia, Africa and Latin America are doing in teak plantations (Camino and Morales, 2013; Kollert and Walotek, 2015). The private sector manages 88 % in Central America and 99 % in South America (Kollert and Cherubini, 2012).

### **1.2.1.1.3 Planted forests**

It is assumed that *Tectona grandis* was exported for the first time from its endemic occurrence to Java, Indonesia, between the fourteenth and sixteenth centuries. In Africa, the introduction of teak in trial gardens was attributed to German colonial administration in nineteenth century. It is supposed that the African landraces come from India, Myanmar or Thailand (Verhaegen et al., 2010). In Latin America the first pure teak plantation was established in 1913 in Trinidad and Tobago with seeds coming from Tenasserim, Myanmar. After that, in 1926 another introduction was carry out in Panama, this time with seed from Sri Lanka. It was until 1928 when a definitive planting program in Trinidad started (Keogh, 1982).

Teak tree grows outside its native range in planted forests in around 70 tropical countries. According to various assessments, they cover between 4.35 and 6.89 million ha (1.1 % of the world's plantations), of which more than 80 % grow in Asia, 10 % in Africa, and 6 % in tropical America. Nevertheless, these figures may underestimates the real area since there are not available data in all countries with teak plantations (Kollert and Kleine, 2017).

Currently, this species reaches 74 % from planted area of tropical hardwoods representing the most grown species in this category among others, such as mahogany (*Swietenia macrophylla*), red cedar (*Cedrela odorata*) and rosewood (*Dalbergia sissoo*). It is expected that the production of planted forests continues increasing and taking into account the decrease of teak supply from natural ones, timber from plantations are a promising source for long-term market (Camino and Morales, 2013). Figure 7 illustrates a teak plantation in Mexico.



Figure 7. *Tectona grandis* plantation in Campeche, México (Agropecuaria Santa Genoveva S.A.P.I. de C.V.)

### 1.2.2 *Tectona grandis* L.f. extractives

Heartwood extractives are non-structural components produced by a living tree in response to wounds, physiological stress, or as waste products. Extractives are highly variable and consist of a wide range of chemical compounds (Mankowski et al., 2016).

The content of extractives depends on environmental and genetic factors, radial position, and tree age (Lukmandaru and Takahashi, 2009; Niamké et al., 2014). Regarding radial position, some works indicate that the concentration of extractives is higher in heartwood than in sapwood. Normally, it increases radially towards the outer heartwood (where it is more concentrated). Nonetheless, the sapwood and the transition zone between them contain likewise some chemicals (Thulasidas and Bhat, 2007). For instance, in Malaysian teak were identified tectoquinone (between 0.3 wt. % and 0.5 wt. %) and 2-(hydroxymethyl) anthraquinone in heartwood, the first one was also detected in sapwood but in a significantly smaller amount and the second one was not present. Other compounds found were anthraquinone-2-carboxylic acid, 1,4-naphthoquinone (0.02 wt. % dw.) and lapachol. The concentration of these compounds were higher in heartwood than in sapwood, being tectoquinone and a hydroxycinnamic acid the major compounds in sapwood. The radial pattern showed that the main teak extractives were synthesized in the aging sapwood (transition zone), and the outer heartwood concentrates the maximal amount of extractives. The differences recognized throughout the radial position was attributed to the possible transformation of these compounds during heartwood formation and maturation process as occurs in others species (Niamké et al., 2011).

In addition, many authors have found a direct relationship between wood durability and extractive content. For instance, Kokutse et al. (Kokutse et al., 2006) affirm that the inner teak heartwood is less resistance to pathogen attack than the intermediate or outer heartwood. This reduction in durability may be due to a decrease in the amount of extractives present in the heartwood nearer the pith, or an ageing of these extractives. Concerning the age of tree, some researches (Haupt et al., 2003; Thulasidas and Bhat, 2007) confirm the general perception of a positive correlation between the age of the tree and its amount of total extractives.

Table 2 summarizes some extractives from teak heartwood according to the solvent utilized and the results of their biocide tests. These figures confirm that teak extractives have an attractive termicidal and fungicide potential. This bioactivity might not be attributed only to the toxicity but to the synergic effect of toxic and antioxidant properties (Hassan et al., 2017). On the other hand, it is well established that hot water extract has low amounts of compounds and they are not associated to wood natural resistance in comparison with those extracted with ethanol or another solvents (Brocco et al., 2017).

Regarding teak bark extracts, they have a complex composition that contains a considerable amount of tannins and phenols, as well as sterols, anthraquinones, triterpenic, hemi-terpenic and naphthalene compounds in their extracts. Baptista et al. (Baptista et al., 2013) focused their investigation on teak bark (from 50- 60 year-old in East Timor) characterization. They measured the extractives content obtained from Soxhlet extractions using dichloromethane, ethanol and water as solvents. The bark was ground in three different sizes fine (<0.180 mm), medium (0.250–0.450 mm) and coarse (>2 mm). Mean total extractives were 10.7 % of total dry weight assigned as follows 84 % to polar extractives removed with ethanol and water, and 16 % to waxes and others non-polar compounds removed with dichloromethane. They observed as well the effect of particle size in the difference between the fractions; it was recovered 30 % more extractives in fine fraction than the coarse one.

The natural durability is defined as the ability of a timber species to prevent attack caused by wood-destroying agents without any physical or chemical modifications (Sundararaj et al., 2015). Teakwood is very resistant to wood-destroying fungi without additional preservation even under high wood moisture conditions or when in contact with the soil (Windeisen et al., 2003). This resistance is attributed mainly to the quantity and type of secondary metabolites present in their extractives, such as anthraquinones and tectoquinones, that act as defensive compounds to environmental stresses (Brocco et al., 2017; Kirker et al., 2013; Kokutse et al., 2006). It is worth to observe that the durability depends as well of some factors including hardness, density, location of origin and stand management practices (Mankowski et al., 2016).

It is also remarkable that teak extractives not only evidence weather and pathogens tolerance but therapeutic and pharmacological activity (Nidavani, 2014; Ramesh and Mahalakshmi, 2014; Vyas et al., 2018). To cite some examples: antibacterial (Khan et al., 2010; Krishna and Nair, 2010; Lanka and Parimala, 2017; Neamatallah et al., 2005), antioxidant, analgesic, antipyretic (Sultana et al., 2015), anti-inflammatory, anthelmintic, hypoglycemic, diuretic, gastroprotective, haemolytic anaemia (Diallo et al., 2008), antiviral (chikungunya) (Sangeetha et al., 2017), bronchitis, antiplasmodial (Kopa et al., 2014), cytotoxic, hair growth activity, among others. Beyond these properties, teak extractives could have an allelopathic potential playing a role in the control of weeds and pest control (Macías et al., 2010). Hence, *Tectona grandis* extractives could be a relevant source of bioactive ingredients.



Table 2. Summary of extractives in *Tectona grandis* wood and their bioactivity potential

<i>Tectona grandis</i> extract (origin)	Extraction method	Extractive content/ identified compound	Bioassay	Results	Reference
Methanolic (heartwood)	successive extraction (6 h, refluxed 3 times)		Brine shrimp lethality test Anti-termic test ( <i>Reticulitermes speratus</i> Kolbe termites, 10 days)	LC <sub>50</sub> (ppm) >200 Weight loss (mg): 21.32	(Lukmandaru, 2012a)
n-hexane (heartwood)	successive extraction (6 h, refluxed 3 times)		Brine shrimp lethality test Anti-termic test ( <i>Reticulitermes speratus</i> Kolbe termites, 10 days)	LC <sub>50</sub> (ppm): 6.71 Weight loss (mg): 8.37	(Lukmandaru, 2012a)
n-hexane/unsaponifiable fraction (heartwood)	successive extraction (6 h, refluxed 3 times) after saponified with 1.3 % ethanolic KOH	Squalene Tectoquinone	Brine shrimp lethality test Anti-termic test ( <i>Reticulitermes speratus</i> Kolbe termites, 10 days)	LC <sub>50</sub> (ppm): 6.77 Weight loss (mg): 8.34	(Lukmandaru, 2012a)
Ethyl acetate (heartwood)	successive extraction (6 h, refluxed 3 times)		Brine shrimp lethality test Anti-termic test ( <i>Reticulitermes speratus</i> Kolbe termites, 10 days)	LC <sub>50</sub> (ppm): 6.60 Weight loss (mg): 5.39	(Lukmandaru, 2012a)

<i>Tectona grandis</i> extract (origin)	Extraction method	Extractive content/ identified compound	Bioassay	Results	Reference
Acetone: water (80: 20 v/ v, Malaysia)	Sonication (two times)	Tectoquinone (7.9 mg equivalent 5- methoxyflavone/ g dw in outer heartwood, 6.0 mg equivalent 5-methoxyflavone/ g dw in inner heartwood)  2-(hydroxymethyl)anthraquinone (3.0 mg equivalent 5-methoxyflavone/ g dw in heartwood)  Anthraquinone-2-carboxylic acid (0.54 mg equivalent 5-methoxyflavone/ g dw in heartwood)  1,4-naphthoquinone (0.13 mg equivalent 5- methoxyflavone/ dw in heartwood)  Lapachol (0.26 mg equivalent 5- methoxyflavone/ g dw in heartwood)	Antifungal test ( <i>Antrodia</i> sp, 16 weeks)	Weight loss (%): 0.1-14.5	(Niamké et al., 2011)

<i>Tectona grandis</i> extract (origin)	Extraction method	Extractive content/ identified compound	Bioassay	Results	Reference	
Acetone: water (80: 20 v/ v, heartwood)	Sonication (two times, 45 min)	Tectoquinone (0.48 mg/ g dw to 17.72 mg/ g dw)			(Niamké et al., 2014)	
		2-(hydroxymethyl)anthraquinone (0.07 mg/ g dw to 11.81 mg/ g dw)				
		Anthraquinone-2-carboxylic acid (0.03 mg/ g dw to 3.23 mg/ g dw)				
		1,4-naphthoquinone (0.0 mg/ g dw to 0.34 mg/ g dw)				
		4',5'-dihydroxyepiisocatalponol (0.0 mg/ g dw to 9.67 mg/ g dw)				
		Total phenolic (1.96 mg/ g dw to 43.58 mg/ g dw)				
Acetone: water (80: 20 v/ v, Malaysia heartwood)		4',5'-dihydroxyepiisocatalponol (0.0 mg/ g dw to 9.67 mg/ g dw)	Antifungal test (14 weeks) <i>T. versicolor</i>	Minimum inhibitory concentration: 0.22 mM IC <sub>50</sub> : 0.11mM	(Niamké et al., 2012)	
Ethanol: benzene (1: 2, wet site home garden, heartwood)	Soxhlet (8 h)	Total extractives (12.44 %)	Antifungal test (8 weeks)	Weight loss (%):	(Thulasidas and Bhat, 2007)	
		Tectoquinone (0.23 %)	<i>P. palustris</i>	43.30		
		1,4-naphthoquinone (0.62 %)	<i>G. rabeum</i>	7.05		
			<i>P. sanguineus</i>	1.86		
			<i>T. hirsuta</i>	2.76		
		<i>T. versicolor</i>	1.94			

<i>Tectona grandis</i> extract (origin)	Extraction method	Extractive content/ identified compound	Bioassay	Results	Reference
Ethanol: benzene (1: 2, dry site home garden, heartwood)	Soxhlet (8 h)	Total extractives (15.98 %) Tectoquinone (0.34 %) 1,4-naphthoquinone (1.26 %)	Antifungal test (8 weeks) <i>P. palustris</i> <i>G. rabeum</i> <i>P. sanguineus</i> <i>T. hirsuta</i> <i>T. versicolor</i>	Weight loss (%): 18.41 4.28 1.70 3.02 1.73	(Thulasidas and Bhat, 2007)
Ethanol: benzene (1: 2, plantation, heartwood, Kerala, India)	Soxhlet (8 h)	Total extractives (13.31 %) Tectoquinone (0.32 %) 1,4-naphthoquinone (0.97 %)	Antifungal test (8 weeks) <i>P. palustris</i> <i>G. rabeum</i> <i>P. sanguineus</i> <i>T. hirsuta</i> <i>T. versicolor</i>	Weight loss (%): 26.88 2.34 2.06 2.16 1.65	(Thulasidas and Bhat, 2007)
Aqueous (hot water, heartwood, Brazil)	Maceration (autoclave, two times, 2.5 h)	Total extractives (6.56 %)	Antifungal test (12 weeks, Teak wood substrate) <i>Postia placenta</i> <i>Gloeophyllum trabeum</i> <i>Neolentinus lepideus</i> <i>Trametes versicolor</i>	Weight loss (%): 11.93 21.12 2.39 10.82	(Brocco et al., 2017)
			Antifungal test (12 weeks, <i>Pinus</i> sp substrate) <i>Postia placenta</i> <i>Gloeophyllum trabeum</i> <i>Neolentinus lepideus</i> <i>Trametes versicolor</i>	Weight loss (%): 55.82 43.49 37.50 11.43	

<i>Tectona grandis</i> extract (origin)	Extraction method	Extractive content/ identified compound	Bioassay	Results	Reference				
Ethanolic (absolute ethanol, heartwood, Brazil)	Maceration (two times, 24 h)	Total extractives (9.17 %)	Antifungal test (12 weeks, Teak wood substrate)	Weight loss (%):	(Brocco et al., 2017)				
			<i>Postia placenta</i>	6.65					
			<i>Gloeophyllum trabeum</i>	7.93					
			<i>Neolentinus lepideus</i>	2.51					
			<i>Trametes versicolor</i>	12.10					
			Antifungal test (12 weeks, Pinus sp substrate)	Weight loss (%):					
			<i>Postia placenta</i>	55.82					
			<i>Gloeophyllum trabeum</i>	43.49					
			<i>Neolentinus lepideus</i>	37.50					
			<i>Trametes versicolor</i>	11.43					
			Ethanol: water (1: 1, heartwood, Brazil)				Antifungal test (12 weeks, Teak wood substrate)	Weight loss (%):	(Brocco et al., 2017)
							<i>Postia placenta</i>	5.32	
							<i>Gloeophyllum trabeum</i>	5.51	
							<i>Neolentinus lepideus</i>	3.20	
<i>Trametes versicolor</i>	10.48								
Antifungal test (12 weeks, Pinus sp substrate)	Weight loss (%):								
<i>Postia placenta</i>	41.25								
<i>Gloeophyllum trabeum</i>	57.43								
<i>Neolentinus lepideus</i>	20.98								
<i>Trametes versicolor</i>	10.92								

<i>Tectona grandis</i> extract (origin)	Extraction method	Extractive content/ identified compound	Bioassay	Results	Reference
Ethanol: toluene (2:1, heartwood, Pittsburg, USA)	Soxhlet (6 h)	Squalene (28.24 % of sample)	Anti-termic test	LC <sub>50</sub> (mg/ ml):	(Hassan et al., 2017)
		2-methyl-9,10-anthracenedione (24.03 % of sample)	<i>Reticulitermes flavipes</i>	4.43	
		1-methyl-3,4-dihydroisoquinoline (5.22 % of sample)	<i>Heterotermes indicola</i>	3.21	
Ethanol: toluene (2:1, heartwood, Myanmar, India)	Soxhlet (6 h)	Total extractives (5.51 %)			(Mankowski et al., 2016)
		3-(1-hydroxyethyl)-2-methyl-1-benzofuran-5,6-dicarbonitrile (3.53 % of sample)			
		1-(1,1-dimethylethyl)-4-phenoxy-benzene (3.06 % of sample)			
		2-methyl-9,10-anthracenedione (24.03 % of sample)			
		1-methyl-3,4-dihydroisoquinoline (28.24 % of sample) Squalene (28.24 % of sample)			
Dichlorometane (bark, East Timor)	Soxtec (1.5 h)	1.6 % of total dw			(Baptista et al., 2013)
Ethanol (bark, East Timor)	Soxtec (1.5 h)	2.9 % of total dw 0.9 % of ethanolic soluble phenolic compounds			(Baptista et al., 2013)

<b><i>Tectona grandis</i> extract (origin)</b>	<b>Extraction method</b>	<b>Extractive content/ identified compound</b>	<b>Bioassay</b>	<b>Results</b>	<b>Reference</b>
Water (bark, East Timor)	Soxtec (1.5 h)	6.1 % of total dw 0.7 % of water soluble phenolic compounds			(Baptista et al., 2013)
NaOH (Costa Rica)	ASTM D-1109-84 standard	Total extractives (23.83 %)			(Moya et al., 2017)
Aqueous (hot water, Costa Rica)	ASTM D-1110-84 standard	Total extractives (7.22 %)			(Moya et al., 2017)
Aqueous (cool water, Costa Rica)	ASTM D-1110-84 standard	Total extractives (8.51 %)			(Moya et al., 2017)
Dichloromethane (Costa Rica)	ASTMD-1108-96 standard	Total extractives (13.01 %)			(Moya et al., 2017)
Ethanol: toluene	ASTM D-1107-96 standard	Total extractives (1.18 %)			(Moya et al., 2017)

dw: dry weight

### 1.2.3 *Tectona grandis* L.f. secondary metabolites

Secondary metabolites are chemical compounds produced by the plant cell through metabolic pathways derived from the primary metabolic pathways. They do not constitute primary functions like cell growth, development, or reproduction, but in the interaction of the cell with its environment. Secondary metabolites are also known as extractives in the context of forest products (Celedon and Bohlmann, 2018). They are present practically in every part of teak tree. Extractives contains quinones, triterpenoids, apocarotenoids, steroids, terpenes, lignans, fatty esters and phenolic compounds (Lukmandaru, 2012a).

Table 3 displays the secondary metabolites identified in *Tectona grandis* leaves, roots and bark. (Kopa et al., 2014; Lacret et al., 2012; Lanka and Parimala, 2017; Macías et al., 2010, 2004; Nayeem and Karvekar, 2010; Sangeetha et al., 2017; Vyas et al., 2018). This work is focused on teak heartwood that is why a more specific and detailed information of secondary metabolites in this tree part is classified in Table 4. The predominant compounds are naphthoquinones and anthraquinones (Vyas et al., 2018).

In relation to innate tolerance to wood destroying organisms, even that it is attributed to secondary metabolites there is not a consensus on the main components concerned. On one hand, some authors attribute it to compounds such as tectoquinone, lapachol, and deoxylapachol. For instance, Lukmandaru (Lukmandaru, 2012a) showed that tectoquinone has a strong termicidal activity and Haupt et al. (Haupt et al., 2003) identified tectoquinone as a bioactive compound for the inhibition of fungus. In accordance with them, Niamké (Niamké et al., 2011) found a strong correlation between decay resistance and 2-hydroxymethyl anthraquinone and tectoquinone. According to Thulasidas and Bhat (Thulasidas and Bhat, 2007) 2-methyl anthraquinone and 1,4-naphthoquinone are the principal components that influence the durability and dimensional stability of teak.

On the other hand, studies conducted by Windeisen et al. (Windeisen et al., 2003) suggest tectoquinone and 2-hydroxymethylanthraquinone possess any effective potential with regard to durability. They suggest that other compounds such as squalene, may contribute to durability in the form of a hydrophobic barrier or as precursor to toxic triterpene compounds. In line with this, tests carried out by Haupt et al. (Haupt et al., 2003) showed that deoxylapachol has no fungicidal effect. If deoxylapachol is considered as a precursor of tectoquinone, the different composition of extractives of non-durable teakwood can probably be attributed to a lower biosynthetic efficiency concerning the conversion of deoxylapachol into tectoquinone.



Nonetheless, it is well established that secondary metabolites have a demonstrated bioactivity and there is an increasing demand of phytochemical constituents by industries such as pharmaceutical, chemical, cosmetic and food. Since the qualitative and quantitative studies of bioactive compounds from plants mostly rely of the selection of proper extraction method and type of solvent, their development is as well growing (Golam Rasul, 2018). The next section discuss briefly some techniques commonly used in phytochemical constituent extraction and the performance of solvents.

Table 3. Summary of secondary metabolites present in *Tectona grandis* tree

Leaves	Roots	Bark
Acetovanillone		
E-isofuraldehyde		
3-hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)propan-1-one		
Evofolin A		
Gallic acid		
Ellagic acid		
<b>Naphthoquinones</b>		
Hydroxysesamone	Lapachol	
	$\alpha$ -Lapachone	
	$\beta$ -Lapachone	
	Dehydro- $\alpha$ -lapachone	
	Tectol	
	Tecomaquinone-I	
<b>Anthraquinones</b>		
3-acetoxy-8-hydroxy-2-methylanthraquinone (grandiquinone A)	1-hydroxy-3-methylanthracene-9,10-dione (pachybasin)	
5,8-dihydroxy-2-methylanthraquinone	Munjistin	
3-hydroxy-2-methylanthraquinone	2-methylquinizarin	
quinizarine	1-hydroxy-2-methyl anthraquinone	
Tectograndone	Obtusifolin	
Anthraceutone		
Naphthotectone		
Tectoleafquinone		

Leaves	Roots	Bark
<b>Flavonoid</b>		
Rutin	$\beta$ -Sitosterol	
Quercitin		
<b>Monoterpenes</b>		
(6RS)-(E)-2,6-dimethyl-2,7-octadiene-1,6-diol		
<b>Diterpenes</b>		
Abeograndinoic acid		Abeograndinoic acid
phytol		Phytol
7,11,15-trimethyl-3-methylene-hexadecan-1,2-diol		7,11,15-trimethyl-3-methylene-hexadecan-1,2-diol
rhinocerotinoic acid		Rhinocerotinoic acid
2-oxokovalenic acid		2-oxokovalenic acid
19-hydroxyferruginol		19-hydroxyferruginol
Tectograndinol		Solidagonal acid
Solidagonal acid		
<b>Triterpenes</b>		
Ursolic acid	Squalene	5-hydroxy-1,4-naphthalenedione lup-20(29)-en-3 $\beta$ -ol
Betulinic acid	Betulinic acid	Betulin
Corosolic acid		Betulinic acid
Oleanolic acid		Betulinaldehyde stigmast-5-en-3-O- $\beta$ -D-Glucopyranoside
Maslinic acid		
methyl-2 $\alpha$ ,3 $\alpha$ -dihydroxyurs-12-en-28-oate		
Euscaphic acid		

Leaves	Roots	Bark
<b>Sesquiterpenes</b>		
1 $\beta$ -6 $\alpha$ -dihydroxy-4(15)-eudesmene		1 $\beta$ -6 $\alpha$ -dihydroxy-4(15)-eudesmene
7-epieudesm-4(15)-ene-1 $\alpha$ ,6 $\alpha$ -diol		7-epieudesm-4(15)-ene-1 $\alpha$ ,6 $\alpha$ -diol
<b>Lignans</b>		
Syringaresinol		
Medioresinol		
1-hydroxypinoresinol		
Lariciresinol		
Balaphonin		
Zhebeiresinol		
<b>Norlignans</b>		
Tectonoelin A ((7Z)-9'nor-3',4,4'-trihydroxy-3-methoxylign-7-ene-9,7'-lactone)		
Tectonoelin B ((7Z)-9'nor-3',4,4'-trihydroxy-3,5-dimethoxylign-7-ene-9,7'-lactone)		
<b>Apocarotenoids</b>		
3 $\beta$ -hydroxy-7,8-dihydro- $\beta$ -ionone		
9(S)-4-oxo-7,8-dihydro- $\beta$ -ionol		
Tectoionol A ((3R,5R,6S,9R)-6,9-epoxiionane-3,5-diol)		
Tectoionol B (2-hydroxy-7,8-dihydro- $\beta$ -ionol)		
3 $\beta$ -hydroxy-7,8-dihydro- $\beta$ -ionol		
<b>Sterols</b>		
sitosterol 3-O- $\beta$ -D-glucopyranoside		
<b>Fatty acids</b>		
Benzene-1-carboxylic acid-2-hexadeconate		

Leaves	Roots	Bark
<b>Fatty esters</b>		
		7'-hydroxy-n-octacosanoyl n-decanoate
		20'-hydroxyeicosanyl linolenate
		18'-hydroxy-n-hexacosanyl-n-decanoate
<b>Steroids</b>		
		$\beta$ -sitosterol- $\beta$ -D-[4'-linolenyl-6'-(tridecan-4'''-one-1'''-oxy)] glucuranopyranoside
		Stigmast-5-en-3-O- $\beta$ -d-glucopyranoside
<b>Acyclic alkanes</b>		
		n-docosane

Table 4. Summary of secondary metabolites present in *Tectona grandis* heartwood

<b>Heartwood</b>					
<b>Chemical compound</b>		<b>Molecular</b>	<b>CAS</b>	<b>Extraction solvent</b>	<b>References</b>
<b>Name</b>	<b>Common name</b>	<b>formula</b>			
<b>Naphthoquinones</b>					
4-hydroxy-3-(3-methylbut-2-enyl) naphthalene-1,2-dione	Lapachol 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthoquinone	C <sub>15</sub> H <sub>14</sub> O <sub>3</sub>	84-79-7	Ethanol: benzene Ethyl acetate n-hexane Petrol Chloroform Acetone: water Petroleum ether Acetone (dichloromethane-soluble fraction) Chloroform Chloroform: methanol Water: acetone Methanol	(Lukmandaru, 2012b; Lukmandaru and Takahashi, 2009) (Lukmandaru, 2015a) (Lukmandaru, 2015a) (Khan and Mlungwana, 1999; Singh et al., 1989) (Gupta and Singh, 2004) (Niamké et al., 2011) (Windeisen et al., 2003) (Windeisen et al., 2003) (Gupta and Singh, 2004) (Sumthong et al., 2008) (Carrieri et al., 2014) (Carrieri et al., 2014; Lukmandaru, 2015a)

Name	Common name	Molecular formula	CAS	Extraction solvent	References
2-(3-methylbut-2-enyl) naphthalene-1,4-dione	Deoxylapachol	C <sub>15</sub> H <sub>14</sub> O <sub>2</sub>	3568-90-9	Petroleum ether	(Windeisen et al., 2003)
				Acetone	(Windeisen et al., 2003)
				(dichloromethane-soluble fraction)	
				Ethanol: benzene	(Lukmandaru, 2012b; Lukmandaru and Takahashi, 2009)
				Ethyl acetate	(Lukmandaru, 2015a)
				n-hexane	(Lukmandaru, 2015a)
				Chloroform	(Gupta and Singh, 2004)
Chloroform: methanol	(Sumthong et al., 2008)				
Water: acetone	(Carrieri et al., 2014)				
Methanol	(Carrieri et al., 2014; Lukmandaru, 2015a)				
Isodeoxylapachol		C <sub>15</sub> H <sub>14</sub> O <sub>2</sub>		Ethanol: benzene	(Lukmandaru, 2012b; Lukmandaru and Takahashi, 2009)
				Ethyl acetate	(Lukmandaru, 2015a)
				n-hexane	(Lukmandaru, 2015a)
				Methanol	(Lukmandaru, 2015a)
				Acetone	(Windeisen et al., 2003)
				(dichloromethane-soluble fraction)	
Chloroform: methanol	(Sumthong et al., 2008)				
2-[(1E)-3-hydroxy-3-methylbut-1-enyl] naphthoquinone	3'-OH-deoxyisolapachol			Chloroform: methanol	(Sumthong et al., 2008)
4,5-dihydroxy-3-(3-methylbut-2-enyl) naphthalene-1,2-dione	5-hydroxylapachol	C <sub>15</sub> H <sub>14</sub> O <sub>4</sub>	221350-50-1	Petrol	(Khan and Mlungwana, 1999)

Name	Common name	Molecular formula	CAS	Extraction solvent	References
1,4-naphthalenedione	1,4-naphtoquinone	C <sub>10</sub> H <sub>6</sub> O <sub>2</sub>	130-15-4	Ethanol: benzene Acetone: water	(Thulasidas and Bhat, 2007) (Niamké et al., 2014, 2011)
Dehydro- $\alpha$ isodunnione		C <sub>15</sub> H <sub>12</sub> O <sub>3</sub>		Chloroform	(Gupta and Singh, 2004)
<b>Naphthoquinone derivatives</b>					
2,2-dimethylbenzo[g]chromene-5,10-quinone	Xyloidone Dehydro- $\alpha$ -lapachone	C <sub>15</sub> H <sub>12</sub> O <sub>3</sub>	15297-92-4	Chloroform Petrol	(Gupta and Singh, 2004) (Khan and Mlungwana, 1999; Singh et al., 1989)
Dehydro- $\beta$ -lapachone					(Neha and Sangeeta, 2013)
5-(6-hydroxy-2,2-dimethylbenzo[h]chromen-5-yl)-2,2-dimethylbenzo[h]chromen-6-ol	Tectol	C <sub>30</sub> H <sub>26</sub> O <sub>4</sub>	24449-39-6	Ethanol: benzene Chloroform Chloroform: methanol Methanol	(Lukmandaru, 2012b; Lukmandaru and Takahashi, 2009) (Gupta and Singh, 2004) (Sumthong et al., 2008) (Windeisen et al., 2003)
2,2-dimethyl-2H-benzo[h]chromen-6-ol	Hemitectol			Chloroform: methanol	(Sumthong et al., 2008)
Tecomaquinone-I		C <sub>30</sub> H <sub>24</sub> O <sub>4</sub>	89355-02-2	Chloroform Petrol	(Gupta and Singh, 2004) (Singh et al., 1989)
(5E)-5-(2,2-dimethyl-6-oxobenzo[h]chromen-5-ylidene)-2,2-dimethylbenzo[h]chromen-6-one	Dehydrotectol	C <sub>30</sub> H <sub>24</sub> O <sub>4</sub>	20213-28-9		(Neha and Sangeeta, 2013)
4',5'-dihydroxy-epiisocatalponol				Acetone: water	(Niamké et al., 2014, 2012)

Name	Common name	Molecular formula	CAS	Extraction solvent	References
<b>Anthraquinones</b>					
2-methyl-9,10-anthracenedione	Tectoquinone 2-methylanthraquinone	C <sub>15</sub> H <sub>10</sub> O <sub>2</sub>	84-54-8	Ethanol: benzene	(Lukmandaru, 2012b; Lukmandaru and Takahashi, 2009; Thulasidas and Bhat, 2007)
				n-hexane	(Lukmandaru, 2012a, 2015a)
				Ethyl acetate	(Lukmandaru, 2015a)
				Methanol	(Lukmandaru, 2015a)
				Acetone: water	(Niamké et al., 2014, 2011)
				Ethanol: toluene	(Hassan et al., 2017; Mankowski et al., 2016)
				Petroleum ether	(Windeisen et al., 2003)
				Acetone (dichloromethane-soluble fraction)	(Windeisen et al., 2003)
				Chloroform	(Gupta and Singh, 2004)
				Petrol	(Singh et al., 1989)
Chloroform: methanol	(Sumthong et al., 2008)				
9,10-dihydro-9,10-dioxo-2-anthracene carboxylic acid	Anthraquinone carboxylic acid	-2- C <sub>15</sub> H <sub>8</sub> O <sub>4</sub>	117-78-2	Acetone: water	(Niamké et al., 2014, 2011)
2-hydroxymethyl anthracene-9,10-dione	2-hydroxymethyl-anthraquinone  Anthraquinone-2-methanol	C <sub>15</sub> H <sub>10</sub> O <sub>3</sub>	17241-59-7	Petroleum ether	(Windeisen et al., 2003)
				Acetone (dichloromethane-soluble fraction)	(Windeisen et al., 2003)
				Chloroform: methanol	(Sumthong et al., 2008)
				Acetone: water	(Niamké et al., 2014, 2011)



Name	Common name	Molecular formula	CAS	Extraction solvent	References
2-(1,1-dimethylethyl)-9,10-anthracenedione	2-tert-butylanthraquinone	C <sub>18</sub> H <sub>16</sub> O <sub>2</sub>	84-47-9	Acetone (dichloromethane-soluble fraction)	(Windeisen et al., 2003)
9,10-dimethoxy-2-methylanthracene-1,4-dione	9,10-dimethoxy-2-methyl-1,4-anthraquinone	C <sub>17</sub> H <sub>14</sub> O <sub>4</sub>		Petrol	(Singh et al., 1989)
2,8-dihydroxy-1-methoxy-3-methylanthracene-9,10-dione	obtusifolin	C <sub>16</sub> H <sub>12</sub> O <sub>5</sub>	477-85-0		(Neha and Sangeeta, 2013)
1-hydroxy-3-methylanthracene-9,10-dione	pachybasin	C <sub>15</sub> H <sub>10</sub> O <sub>3</sub>	2549-78-2		(Neha and Sangeeta, 2013)
1,4-dihydroxy-2-methylanthraquinone	2-methylquinizarin	C <sub>15</sub> H <sub>10</sub> O <sub>4</sub>	2589-39-1	Petrol	(Khan and Mlungwana, 1999)
<b>Triterpenes</b>					
2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene	Squalene	C <sub>30</sub> H <sub>50</sub>	111-02-4	Ethanol: benzene Ethanol: toluene Ethyl acetate n-hexane Methanol Petroleum ether Petrol	(Lukmandaru, 2012b) (Hassan et al., 2017; Mankowski et al., 2016) (Lukmandaru, 2015a) (Lukmandaru, 2012a, 2015a) (Lukmandaru, 2015a) (Windeisen et al., 2003) (Khan and Mlungwana, 1999)
<b>Fatty acids</b>					
n-hexadecanoic acid	Palmitic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	57-10-3	Ethanol: benzene Acetone	(Lukmandaru, 2012b; Lukmandaru and Takahashi, 2009) (Windeisen et al., 2003)

<b>Name</b>	<b>Common name</b>	<b>Molecular formula</b>	<b>CAS</b>	<b>Extraction solvent</b>	<b>References</b>
<b>Sterols</b>					
22,23-dihydrostigmasterol	$\beta$ -sitosterol	C <sub>29</sub> H <sub>50</sub> O	83-46-5	Petroleum ether	(Lukmandaru, 2012a) (Windeisen et al., 2003)
<b>Quinolines</b>					
1-methyl-3,4-dihydroisoquinoline	3,4-dihydro-1-methylisoquinoline	C <sub>10</sub> H <sub>11</sub> N	2412-58-0	Ethanol: toluene	(Hassan et al., 2017; Mankowski et al., 2016)
<b>Aromatic ether</b>					
1-(1,1-dimethylethyl)-4-phenoxy-benzene	1-(tert-butyl)-4-phenoxybenzene	C <sub>16</sub> H <sub>18</sub> O	5331-28-2	Ethanol: toluene	(Mankowski et al., 2016)

#### 1.2.4 *Tectona grandis* L. f. structural composition

Along with the analysis of chemical composition and bioactivity potential of teak extractives, the structural composition is relevant in its characterization. There are many studies referring to structural carbohydrates of teak wood, the Table 5 summaries some research made on this subject. In addition, Table 6 shows the monosaccharides analysis of teak wood.

Regarding *Tectona grandis* volatiles liberated from the breakdown of hemicelluloses, cellulose and lignin fractions, Balogun et al. (Balogun et al., 2014) carried out assays of teak chips from a timber processing plant in Nigeria. They found 55 compounds from pyrolytic conversion process analyzed by CG-MS, among the most abundant were carbon dioxide (10.4 %), acetic acid (6.4 %), furan +2-butanone (5.7 %), and levoglucosan (5.2 %), and trans-coniferyl alcohol (4.6 %). Products of hemicelluloses decomposition (that occurs below 538 K) were acetic acid and 2-furaldehyde (furfural). From decomposition of cellulose (598 K- 630 K) arises levoglucosan. The major phenolic compounds identified derived from lignin fraction were isoeugenol (2.7 %), acetoguaiacone (2.1 %), 4-vinylguaiacol (2.0 %), methyl guaiacol (1.6 %), guaiacol (1.4 %), and vanillin (1.2 %)<sup>1</sup>.

On the other hand Lourenço et al. (Lourenço et al., 2015) identified 127 compounds from teak pyrolysis (Py-CG-MS/ FID). They reported the follow major lignin-derived compounds for heartwood were 4-vinylsyringol (6.9 %), trans-coniferyl alcohol (4.4 %), coniferaldehyde (2.0 %), 4-vinylguaiacol (1.6 %), 4-methylguaiacol (1.6 %), and vanillin (1.5 %). From polysaccharides, the main compounds produced was levoglucosan (14.4 %), 2-hydroxymethyl-5-hydroxy-2,3-dihydro-4H-pyran-4-one (3.6 %), and furfural (1.2 %)<sup>1</sup>. In accordance with their results, they suggest that the high lignin content in teakwood, particularly the enrichment of lignin monomeric unit guaiacyl may contribute to the wood strength as well as high durability.

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<sup>1</sup> Results expressed as percentage of total chromatogram area

Table 5. *Tectona grandis* structural composition analysis

<i>Tectona grandis</i> origin (age)	Lignin (wt. %)	Cellulose (wt. %)	Hemicelluloses (wt. %)	Ash (wt. %)	Reference
Bhumo, Myanmar	35.4 (trunk)	36.9 (trunk)	26.0 (trunk)		(Gašparík et al., 2019)
East Timor (70 year-old)	37.3 (heartwood, extractive-free) <sup>2</sup> : 20.3 G-units, 16.4 S-units, 0.7 H-units 35.4 (sapwood, extractive-free): 20.4 G-units, 14.4 S-units, 0.6 H-units 28.0 (bark, extractive-free): 14.9 G-units, 11.8 S-units, 1.3 H-units				(Lourenço et al., 2015)
East Timor (50- 70 year-old)	32.2 (heartwood) 32.4 (sapwood)				(Miranda et al., 2011)

<sup>2</sup> G-units: Guaiacyl units, S-units: Syringyl units, H-units: p-hydroxyphenyl units

East Timor (50-60 year-old)	20 (bark, total lignin) 16 (bark, Klason lignin)			18.5 <sup>3</sup>	(Baptista et al., 2013)
Java, Indonesia (40 year-old, long rotation; 10 year-old, short rotation)	32.19 (long rotation) 35.53 (short rotation)	49.18 (long rotation) 48.80 (short rotation)	19.35 (long rotation) 18.70 (long rotation)		(Rizanti et al., 2018)
Randublatung, Java	30.33 (sapwood, extractive-free) 30.71-30.02 (heartwood, extractive-free)	49.28 (sapwood, extractive-free) 44.84-47.01 (heartwood, extractive-free)	29.67 (sapwood, extractive-free) 22.96-27.04 (heartwood, extractive-free)	1.5	(Lukmandaru, 2015b)
Costa Rica (6-18 year-old)	21.9 (trunk)	54.4 (trunk)		2.81	(Moya et al., 2017)
Panama (30 year-old)	33.3-34.3 (heartwood) 33.9 (sapwood) 38.3 (transition)				(Windeisen et al., 2003)
Iloring, Nigeria (40-45 year-old)	36.5 (tree lumber, dry-ash-free)			0.7	(Balogun et al., 2014)

<sup>3</sup> Ash elemental composition: calcium 93 %, potassium 4.8 %, and magnesium 1.9 %

Table 6. *Tectona grandis* monosaccharide composition

<b>Monosaccharide</b>	<b>Heartwood (wt. % of wood)</b> (Miranda et al., 2011)	<b>Sapwood (wt. % of wood)</b> (Miranda et al., 2011)	<b>Bark (wt. %)</b> (Baptista et al., 2013)
Glucose	44.6	43.7	61.7
Xylose	8.3	7.8	20.4
Mannose	3.2	3.1	5.6
Arabinose	0.6	0.6	4.2
Galactose	0.6	0.5	3.1
Rhamnose	0.2	0.5	5.0

### 1.3 Extraction methods

Extraction, as the term is used pharmaceutically, involves the separation of medicinally active compounds of plant or animal tissues from the inactive or inert components (desired and undesired) by using selective solvents in standard extraction procedures (Golam Rasul, 2018).

The extraction of natural products progresses through the following stages: the solvent penetrates into the solid matrix; the solute dissolves into the solvent; the solute is diffused out of the solid matrix; and the extracted solutes are collected (Zhang et al., 2018).

There are many extraction techniques to obtain compounds from plants. Conventional methods (mastered since long time ago) utilize organic solvents or water at atmospheric pressure while new ones (developed more recently) apply pressure and elevated temperatures. Among the conventional methods, we can cite maceration, infusion, percolation, decoction, Soxhlet, and hydrodistillation.

Maceration involved soaking plant materials (coarse or powdered) in a closed vessel with a solvent and it is allowed to stand at room temperature for a long time (from several hours to days) with frequent agitation. The process intend to soften and break the plant's cell wall to release the soluble phytochemicals. The process takes place only by molecular diffusion. After the extraction time, the mixture is pressed or strained off by filtration.

Infusion and decoction have the same principle as maceration; in both techniques, the sample is soaked in cold or boiled water. However, the maceration period for infusion is shorter and the sample is boiled in a specified volume of water and for decoction in a defined time. Decoction is only suitable for extracting heat-stable compounds, hard plants materials (e.g. roots and barks) and usually resulted in more oil-soluble compounds compared to maceration and infusion (Azwanida, 2015)

The percolation is common in the preparation of tinctures. In this method the dried sample is packed in the percolator (a narrow, cone-shaped vessel open at both ends), then the solvent is added and it is macerated for several hours. After that, the saturated solvent is replaced by fresh solvent. The process is repeated until a drop of the extractant from the percolator when evaporated does not leave a residue.

A classic Soxhlet extractor operates as follows: the sample is placed in a thimble holder, which is then gradually filled with condensed fresh extractant from a distillation flask. When the liquid reaches the overflow level, a siphon aspirates the extract from the thimble holder and unloads it back into the distillation flask, thus carrying the extracted analytes into the bulk liquid. This operation is repeated until extraction is complete (Luque de Castro and Priego-Capote, 2012).

In hydrodistillation method, plant materials are packed in a still compartment then water is added in sufficient amount and brought to a boil. Alternatively, direct steam may also be injected into the plant sample. The vapor mixture of water and oil is condensed by indirect cooling with water. Condensed mixture flows from condenser to a separator, where oil and bioactive compounds are separated automatically from the water (Hasbay and Galanakis, 2018)

Pressurized liquid extraction (PLE) also known as accelerated solvent extraction (ASE) performs extractions at elevated solvent temperatures and pressures to achieve higher extraction efficacy. After loading a sample into the extraction cell, the cell is filled with solvent, heated and pressurized, and held at the pressure and temperature for a predetermined time. The clean solvent is pumped into the sample cell, and the sample cell is purged with nitrogen gas. This extract is collected into a collection vial (Richter and Raynie, 2012). High pressure keeps solvents in a liquid state above their boiling point resulting in a high solubility and high diffusion rate of lipid solutes in the solvent, and a high penetration of the solvent in the matrix. PLE decreased the consumption of extraction time and solvent and had better repeatability compared to other methods (Zhang et al., 2018).

However, the most popular extraction methods have the difficulty of automation and the commercial-scale development for a competitive use (Luque de Castro and Priego-Capote, 2012). This drawback could be overcome by twin-screw technology, which enables the continuous process of large quantity of raw materials, among them lignocellulosic biomass. This technology performs the following functions in a continuous process: thermomechanical defibration, chemical impregnation (intense solid-liquid mixing), micro/ macro mixing, solid-liquid separation.

### **Twin-screw extrusion technology**

Twin-screw extrusion is a process based on the action of two screws that spin into a tight barrel, which is equipped with temperature control. This action produces high shearing forces between the raw material, the screws and the barrel that lead to locally temperature and pressure rise along the extruder. This technology is very attractive because it can combine mechanical, thermal and chemical actions in a single step and in continuous mode (Vandenbossche et al., 2019). Typical operations in extruders include transporting, compressing, pumping, mixing, cooking, heating, cooling, melting, blending, reacting, degassing, filling and shaping of materials.

Twin-screw extruders are comprised of two parallel rotative shafts in a bilobed barrel. In these shafts, specific intermeshing and identical screws are assembled. They can be classified as counter rotating or corotating depending on the direction of rotation. The barrel can be divided in different modules such



as open modules to introduce either liquid or solid material, closed and thermo-regulated modules or filtration modules equipped with a filtering mesh.

Typical elements are conveying, mixing (mainly kneading discs), and reverse screws; they produce different effects on the raw material. Conveying screws are used for the transportation of material throughout the profile and between mixing zones. They are self-cleaning and have a good dissociation capacity. Contrary to reverse screws, conveying elements are designed to impart low mechanical energy (low shear force).

The mixing elements increase the distributive and dispersion mixing performance. They improve the micro mixing as a result of the interpenetration between them, furthermore when they turn the heat transfer and the product uniformity is enhanced (Uitterhaegen and Evon, 2017). They are characterized by the number of discs used, and by the staggering angle between two sequential discs. Comparing to conveying screws, kneading elements generate high shear-rate regions, with high fluxes through these regions. The intensity is controlled by the thickness of the discs (the number of discs per length). Narrow discs promote distributive mixing and impose less shear intensity. Broader discs increase the amount of elongational flow and thus dispersive mixing. The staggering angle between the discs also dictates the conveying capacity of the mixing elements and they can be configured as forward, backward transporting or neutral. Increasing the staggering angle of the kneading block increases cross-sectional mixing, but decreases axial mixing and conveying capacity. For distributive mixing in these elements the influence of the staggering angle is more important than that of the width of the discs. To maintain a constant flow rate it is necessary to increase the pressure to push the fluids through kneading blocks (Sarhangi Fard and Anderson, 2013).

The reverse screws are utilized to build pressure and apply high shearing forces on the treated material. They create a dynamic plug in solid/ liquid process. The use of restrictive elements allows to increase the residence time in the extruder that range from 10 s to around 5 min, most process operating from 30 s to 2 min (Vandenbossche et al., 2019).

The type, arrangement and length of screw elements is called the screw profile (or screw configuration), see Figure 8. The arrangement of the profile depends on the unitary operation and the mechanical treatment to be imposed to the material along the screw profile.



Figure 8. Twin-screw profile, from left to right reverse, conveying and kneading elements

Among the advantages of twin-screw technology, we can list the following:

- Continuous production
- Modular system
- Good control of process parameters
- Thermo-mechanical versatility
- Good mixing and heat transfer capabilities
- High solid load capability
- Good handling of large amounts of raw material
- No waste production
- Large-scale availability

The typical processing lines of extrusion are plastic, chemical, food, feed, pulp and paper, as well as compounding processes since they provide short residence times and extensive mixing. Nevertheless, this thermo-mechanical technology has been expanded to a full range of purposes since it is very flexible and allows both bio-source and synthetic raw material. The raw material may be solids (powders, granulates, flours), liquids, slurries, and possibly gases. It is suitable to process biomass in multiple ways, such as pretreatment, extraction, and reactive extrusion.

Regarding the use of twin-screw extruder in biomass pretreatment, there are studies that evaluate and prove its effectiveness in the deconstruction and enzymatic hydrolysis of lignocellulosic biomass such as blue agave bagasse, oil palm empty fruit bunch, barley straw (Vandenbossche et al., 2014) and sweet corn co-products (Fong Lopez et al., 2019; Vandenbossche et al., 2015). Furthermore, extraction processes have been conducted with this technology, such as mechanical pressing to extract essential oil from rapeseed, sunflower seeds, coriander seeds, jatropha seeds, camellia seeds, and pongamia pinnata seeds (Uitterhaegen and Evon, 2017). It can be used as well for extraction of xylans from straw and bran (Jacquemin et al., 2015), and of hydroxycinnamic acids from hemp by-products as an efficient solid/liquid extraction (Candy et al., 2017).

Taking into account the above-mentioned works the twin-screw extrusion can be an interesting method for extractions. In solid/ liquid extractions of biomass using an extruder, the mechanical work that the screw profile exerts on the material strongly modifies the cell wall, it causes its degradation and promotes the solvent diffusion (Evon et al., 2018). In this regard, Celhay et al. (Celhay et al., 2014) developed an extraction process of wood by-products in order to recover hydrophilic polyphenols and antiradical compounds. This work turned out to be a good option for residues valorization applicable to the paper industry.

The excellent performance and flexibility of this process make it a promising way to maximize the value of biomass components by extraction. That is why twin-screw technology is suggested as extraction method of bioactive compounds of teak sawdust.

## 1.4 Solvents in extraction process

Along with the appropriate extraction method, the selection of the suitable solvent is essential for secondary metabolites extraction. The choice of the solvent will determine the type of compounds extracted from the samples, see Table 7 (Pandey and Tripathi, 2014). Alcohols (ethanol and methanol) are universal solvents in solvent extraction for phytochemical investigation (Zhang et al., 2018). However, selectivity, solubility, cost and safety should be considered in selection of solvents. Based on the law of similarity and intermiscibility (like dissolves like), solvents with a polarity value near to the polarity of the solute are likely to perform better and vice versa.

Table 7. Solvents used for active components extraction

<b>Water</b>	<b>Ethanol</b>	<b>Methanol</b>	<b>Chloroform</b>	<b>Ether</b>	<b>Acetone</b>
Tannins	Tannins	Anthocyanins	Flovonoids	Alkaloids	Phenols
Anthocyanins	Polyphenols	Terpenoids	Terpenoids	Terpenoids	Flavonols
Terpenoids	Polyacetylenes	Saponins		Fatty acids	Flavonoids
Saponins	Flavonols	Tannins			
Terpenoids	Terpenoids	Xanthoxyllines			
Polypeptides	Sterols	Terpenoids			
Lectins	Alkaloids	Flavones			
Starches		Polyphenols			

Conventional extraction solvents are generally volatile organic compounds obtained from non-renewable resources, mainly petroleum-based, and suspected to be harmful to both human health and the environment (Chemat et al., 2019). As an alternative, a new class of solvents emerged in the market, less hazardous, the so-called green solvents. They comes from renewable and organic/ inorganic (ionic liquids) sources, and may even be of fossil origin, providing they satisfy the criteria.

Green solvents, or environmentally friendly solvents, are a class of solvents focused on minimizing the environmental impact resulting from the use of solvents of fossil origin in the chemical industry. For a solvent to be considered green, it should meet most of the fundamental principles of green chemistry. These principles summarize the rational and methodological use of chemicals, with preference for the use of renewable feedstock or innocuous substances that will not remain in the environment. The use of auxiliary substances (such as solvents, separation agents, etc.) and the formation of hazardous products should preferably be avoided (de Jesus and Filho, 2020).

Water is the green solvent par excellence. It is not only inexpensive and environmentally benign, but also non-toxic and non-flammable, providing opportunities for clean processing and pollution prevention (Chemat et al., 2019). It is also convenient for extracting bioactives compounds from different sources maintaining its biological activity. The downside is that the properties of water at atmospheric pressure are not the suitable for obtaining high extraction yields. However, this can be improved by modifying the physical-chemical properties of the water by bringing it to subcritical conditions. The use of subcritical water for extractions is an alternative technique that deals with the inconveniences of other ones, such as large amount of waste of organic solvents, long time extraction and low efficiency in impregnation.

### Subcritical water extraction

Subcritical water (SCW) is defined as liquid water under pressure at temperatures above its boiling point (100 °C and 0.1 MPa) and below its critical point 374 °C and 22.1 MPa (Möller et al., 2011), see Figure 9.

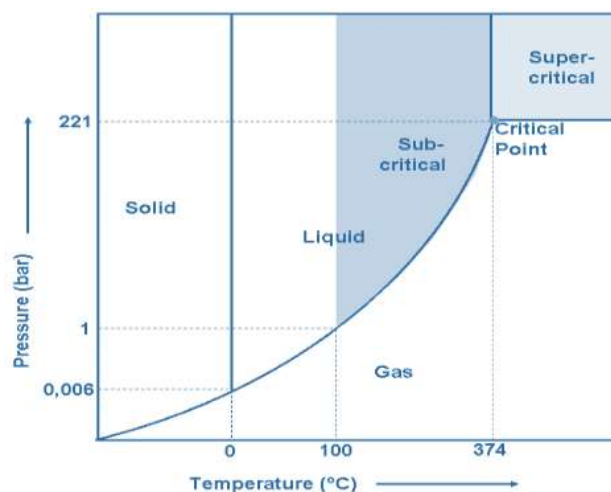


Figure 9. Phase diagram of water

The temperature and pressure have an important effect on water properties such as, density, dielectric constant, viscosity, ion product ( $K_w$ ), and diffusion coefficient (Akizuki et al., 2014). The viscosity and surface tension decrease with increasing the temperature. Thus, subcritical water is a more diffusive solvent than water at room temperature. The change in major physical and chemical properties of water in subcritical conditions decisively influences its properties as a solvent (Möller et al., 2011).

The solubility depends of the polarities of both the compound to be extracted and the SCW. There are complex molecular interactions that cause different solubility trend over a broad temperature range. For

most compounds a larger solubility is beyond 150 °C. On the other hand, the effect of the pressure is negligible on the solubilities of hydrophobic organic compounds in SCW.

The solvent properties of water has a high dependency of dielectric constant. The dielectric constant decrease with increasing temperature, see Figure 10. At room temperature and atmospheric pressure, the dielectric constant for water is around 78.4. It is very high due to its extensive hydrogen bonding structure (Zhang et al., 2020). Water is a polar solvent at these conditions; therefore, non-polar compounds are poorly miscible with it. Contrary, when temperature and pressure increase the dielectric constant decrease significantly, it becomes similar to that of organic solvents. Hence, hydrophobic organic compounds solubilize in water at subcritical conditions. The dielectric constant of water at temperatures of 280 °C, 300 °C, and 420 °C at 25 MPa are similar to ethanol, acetone and hexane at 20 °C and 0.1 MPa, 24.6, 21.01, and 2.02 respectively (Dominguez, 2017; Moldoveanu and David, 2013).

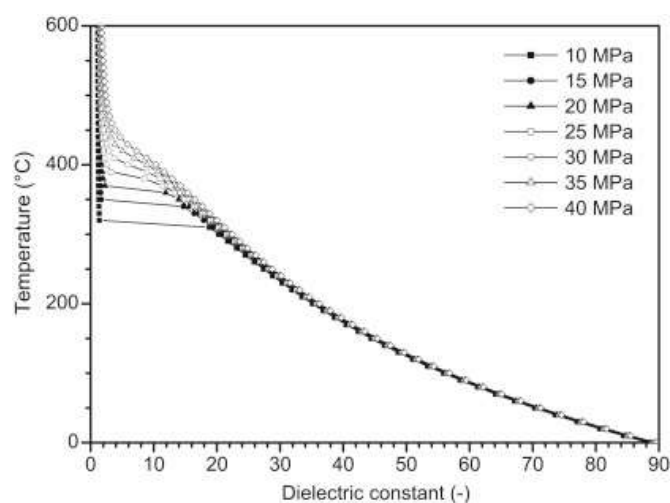


Figure 10. Dielectric constant of water (Dominguez, 2017)

The extraction process using SCW is influenced not only by the physicochemical properties of water but the properties of the compounds to be extracted, structure of the matrix, interaction between them (solvent, matrix and compounds), ratio of solvent and matrix, temperature and pressure of the process, and time (Zhang et al., 2020).

Temperature and pressure are variables that possess a direct (activation energy reaction equilibria) and indirect reaction impact (solvent properties) on the extraction process. Temperature can be consider the key reaction variable because it can be directly controlled and has the highest impact. An increasing process temperature increases the thermal energy and thus allows increase the reaction rates and change the reaction mechanism. Whereas most physical properties of SCW are only weakly dependent of pressure (Möller et al., 2011).

Subcritical water causes mass transfer through diffusion and convection process. The energy supplied with SCW can interrupt the interaction between adhesive (solute-matrix) and cohesive (solute-solute) by reducing the activation energy required for the desorption process, while the elevated pressure can assist in the extraction by forcing water to penetrate into the matrix (pores), where it is impossible to achieve it under the normal pressure (Zhang et al., 2020)

The subcritical water technology can be successfully applied to different purposes, for instance: extraction, hydrolysis, and gasification processes. It allows extract polysaccharides, proteins, antioxidants and polyphenols. It is a suitable technique to extract therapeutic compounds from plants and other materials without inducing chemical degradation up to 200 °C. In general, SCW has higher extraction yields than conventional methods (Carr et al., 2011).

It is worth to mention that the benefits of using subcritical water for extractions compared with conventional solvents are very remarkable. Water is ubiquitous, inexpensive, non-toxic, non-flammable, clean, environmental friendly (green solvent), has low disposal costs, and hazardous solvent free in the final extract. In this regard, SCW is appropriated and attractive for use as a solvent for pharmaceutical, cosmetic and food processing industry. Hence, taking into account the advantages listed above, this work proposes the use of subcritical water as a solvent in the extraction of secondary metabolites.

## Conclusions of Chapter 1

Whereas the international demand for teak timber continues to grow, natural teak forests, mainly old-growth high-quality stands, are declining. Hence, forest plantations represent the source of supply to the international market. Teak is one of the few emerging valuable hardwood species that has been planted in about 70 tropical countries and for most of them, it represents a good business opportunity attracting large investments. Mexico is one of the countries where teak plantations have been promoting and several timber-processing companies have benefited from government programs to increase teak wood production.

The characteristics that make teak wood so prized and demanded in the construction industry, luxury market, and furniture manufacturing are, among others, its strength, dimensional stability, and natural durability. It is well accepted that its natural decay resistance is due to secondary metabolites present in their extractives. Teak extractives not only evidence weather resistance, termicidal and fungicide potential, but therapeutic and pharmacological activity, for instance, antibacterial, antioxidant, analgesic, antipyretic, anti-inflammatory, anthelmintic, hypoglycemic, diuretic, and gastroprotective. The content of extractives depends on environmental and genetic factors, radial position, and tree age. Normally, it increases radially towards the outer heartwood (where it is more concentrated). Concerning age, there is a positive correlation between the age of the tree and its amount of total extractives. In general, the largest amount of extractives are obtained with polar solvents like methanol and ethanol. There have been found values up to 9.17 % of extractives content with ethanol and 15.98 % with ethanol: benzene; for methanolic extractives, the yields reach values from 2.31 % to 5.5 %.

The predominant secondary metabolites that have been identified in teakwood extractives are quinone derivatives such as naphthoquinones: lapachol, deoxylapachol, isodeoxylapachol, 5-hydroxylapachol, 1,4-naphthoquinone, tectol, and hemitectol; anthraquinones: tectoquinone, anthraquinone-2-carboxylic acid, 2-hydroxymethyl-anthraquinone; and triterpenes: squalene and betulinic acid. The phytoconstituents of teak wood have an interesting potential to explore.

There are many extraction techniques to obtain compounds from plants. However, the most popular methods have the difficulty of automation and the commercial-scale development for competitive use. This drawback could be overcome by twin-screw technology, which enables the continuous processing of a large quantity of raw materials. The effectiveness of the twin-screw technology in the treatment of lignocellulosic biomass has been studied with promising results, as well as an alternative method to solid/liquid extractions.



Along with the appropriate extraction technique, the selection of the solvent is very important. The use of water as a solvent is presented as an excellent alternative due to its ecological character. Moreover, it is inexpensive, non-toxic, non-flammable, and the extraction with water delivers a final extract appropriate for the pharmaceutical, food, and cosmetic industry since it is hazardous solvent-free. The solvent properties of water in subcritical conditions (mainly dielectric constant and polarity) become similar to organic solvents (ethanol or methanol), this improves the solubility of phenolic compounds in aqueous extractions. The yields achieved using SCW are comparable to techniques that use organic solvents. Moreover, it has been demonstrated that the pressure and temperature conditions reached in the mechanical stress zones in twin-screw extruders are favorable for achieving subcritical water conditions.



## **Chapter 2**

### **Characterization of teak sawdust and chemical analysis of extractives from conventional extractions**



## **Chapter 2. Characterization of teak sawdust and chemical analysis of extractives from conventional extractions**

The first part of this chapter covers the description of the raw material, namely teak sawdust (TS), which was obtained from a wood processing enterprise. Two lots were received for the study. The first lot (1-lot TS) of less than one kilogram was used for preliminary characterization. The second lot (2-lot TS) of 500 Kg was received later to perform the extractions using twin-screw technology. The second part presents the results of the characterization of TS and its extracts. The tests included fiber determination (cellulose, hemicelluloses, and lignin content), extractives content (using different extraction methods), and extractives characterization (total polyphenols content, identification and quantification of secondary metabolites, and antioxidant activity). Details of experimental procedures are described in the material and methods section. Finally, a brief overview of the potential uses of the secondary metabolites identified in teak extractives is discussed.

### **2.1 Origin and description of teak sawdust**

*Tectona grandis* sawdust was obtained as waste from 14 to 16-year-old debarked teak logs (heartwood and sapwood) from Agropecuaria Santa Genoveva S.A.P.I de C.V. sawmill, which handles large volumes of wood and, consequently, generates a considerable amount of waste that must be treated. The plantation is located in Campeche, Mexico, see Figure 11. The predominant climate in this region is warm sub-humid with a rainfall abundant to very abundant during the summer. The total annual precipitation varies between 1200 mm and 2000 mm, and the average annual temperature fluctuates from 23.9° C to 27.6° C. The main soil types are leptosol, phaeozem, and vertisol (classification according to world reference base for soil resources) (INEGI, 2018). These geographic and climatic characteristics are suitable for the planting of tropical species.



Figure 11. Campeche, Mexico, *Tectona grandis* sawdust provenance (INEGI 2018)

The first lot of teak sawdust (1-lot TS), received in 2017, was used to make the preliminary tests and to have an approach to its potential. The second one (2-lot TS), received in 2019, was also characterized and utilized for twin-screw extrusion. It was air-dried at the exit of the wood processing enterprise before shipment to preserve it from degradation due to oven drying. Figure 12 illustrates both lots as received.



Figure 12. Raw teak sawdust 1-lot TS (left) and 2-lot-TS (right)

Figure 13 and Figure 14 show the particle size distribution of teak sawdust estimated by sieving. For both lots, almost 99 % of the material pass through the 1-mm mesh, and more than 96 % is retained by the 32- $\mu\text{m}$  mesh. The majority fraction is between 250- $\mu\text{m}$  and 500- $\mu\text{m}$  sieve. The particle size distribution is comparable in 1-lot TS and 2-lot TS.

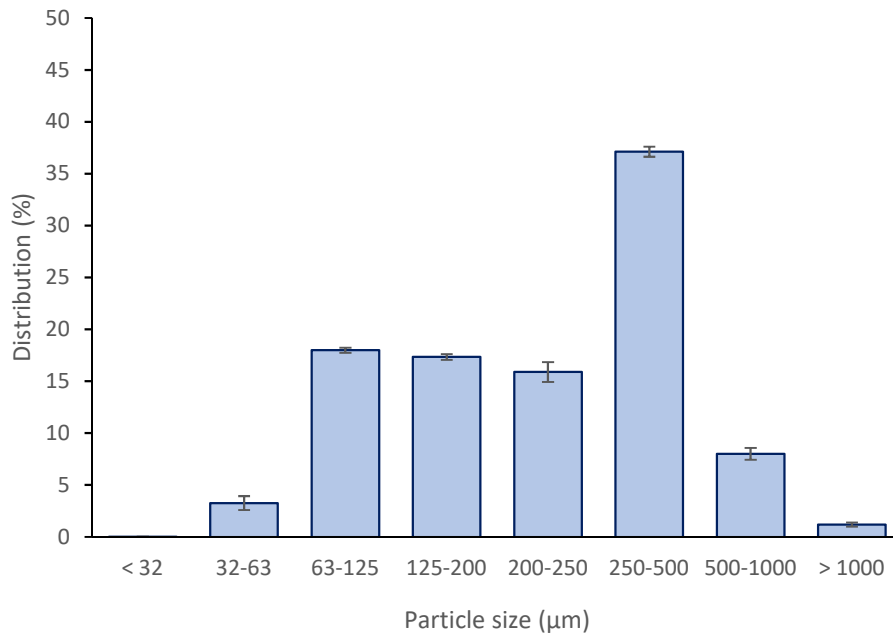


Figure 13. Particle size distribution of teak sawdust from 1-lot TS

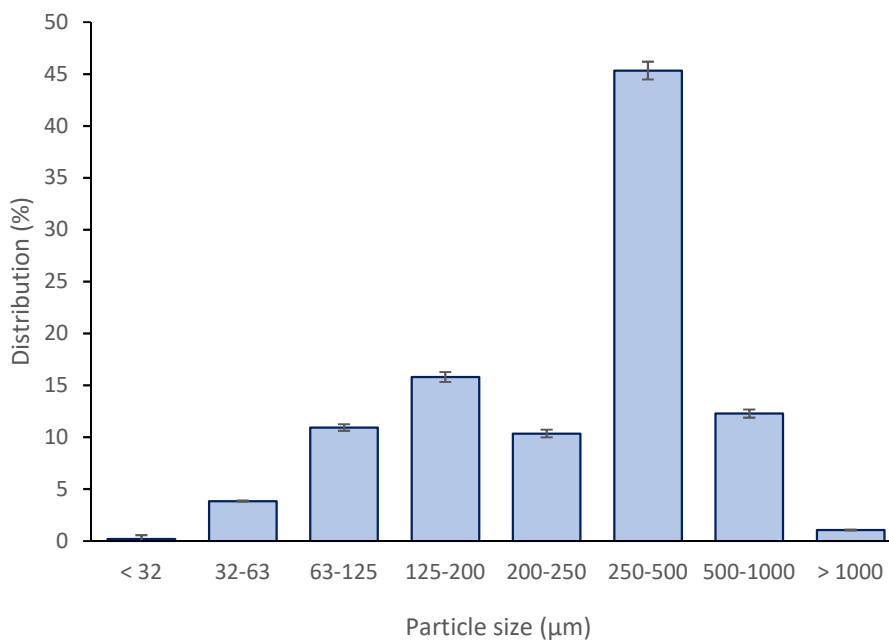


Figure 14. Particle size distribution of teak sawdust from 2-lot TS

## 2.2 Lignocellulosic fiber analysis

The teak sawdust was dried at 103 °C overnight before the fiber analysis. The estimation of cellulose, hemicelluloses, and lignin was carried out by Van Soest and Wine method (Soest and Wine, 1967) with slight modifications. In this technique, the quantification of each type of fiber is done by gravimetric

differences of sequential washings. From each wash, a fraction of the fiber is separated. The neutral detergent fiber (NDF) fraction is the organic matter not solubilized in a neutral detergent solution. It includes hemicelluloses, cellulose, lignin, and minerals. The acid detergent fiber (ADF) fraction is the organic matter not solubilized in an acid detergent solution. It includes cellulose, lignin, and minerals. Subsequently, the lignin is separated by oxidation in the presence of a buffer. Finally, the mineral content is determined by calcination. The characterization of teak sawdust is presented in Table 8.

Table 8. Characterization of teak sawdust from Campeche, Mexico

	1-lot teak sawdust	2-lot teak sawdust
Dry matter as received (%)	65.49 (0.22) <sup>1</sup>	80.41 (4.04) <sup>1</sup>
Dry matter after drying (%)	91.15 (0.19) <sup>1,3</sup>	85.65 (0.002) <sup>1,3</sup>
<b>Fiber analysis (%)</b>		
Cellulose	51.7 (1.61) <sup>2,6</sup>	52.8 (1.70) <sup>1,6</sup>
Hemicelluloses	11.0 (0.98) <sup>2,6</sup>	11.2 (0.40) <sup>1,6</sup>
Lignin	28.7 (2.48) <sup>2,6</sup>	27.3 (0.40) <sup>1,6</sup>
Minerals	1.73 (0.25) <sup>1,4</sup>	2.3 (0.08) <sup>1,4</sup>
Protein		1.12 (0.01) <sup>2,5</sup>

1. Average of triplicated. Standard deviation in parentheses. Results expressed on dry weight basis.

2. Average of duplicated. Standard deviation in parentheses. Results expressed on dry weight basis.

3. Oven drying at 50 °C.

4. Minerals determination at 550°C during 8 h.

5. Kjeldahl method (N-factor: 6.25).

6. Van Soest and Wine method

According to Table 8, the lignocellulosic content of both lots are practically the same, which means that the structure of teak sawdust is quite homogeneous despite coming from different harvests. They are also congruent with those reported by other researchers. For instance, Windeisen et al. (Windeisen et al., 2003) published values from 33.3 % (in heartwood) to 33.9 % (in sapwood) of lignin, and from 51.5 % (in transition zone) to 54.5 % (in sapwood) of polysaccharides for 30-year-old teak trees from a plantation in Panama. Moya et al. (Moya et al., 2017) studied 6 to 18-year-old teak trees from Costa Rica and reported the composition of 54.4 % of cellulose, 21.9 % of lignin, 2.81 % of minerals, and 0.20 % of nitrogen (equivalent to 1 % of protein). On the other hand, for 50 to 70-year-old teak trees from northeast East Timor, the lignin values reported are 32.2 % in heartwood and 32.4 % in sapwood (Miranda et al., 2011).

Comparing the lignin content of plantations within the same age range, this study shows that teak sawdust of 14 to 16-year-old trees from Campeche presents a higher lignin content (27 % to 29 %) than 6 to 18-year-old trees from Costa Rica (22 %). It is well accepted that lignin contributes to the natural durability of teakwood. It provides cell wall resistance, increases hydrophobicity, and acts as a protective



barrier against microbial degradation. Additionally, lignin content and its compositional type are related to the wood stiffness and mechanical resistance (Lourenço et al., 2015).

For both 1-lot TS and 2-lot TS, the sum of the fibrous and mineral content is about 93 %, this implies that there could be up to 7 % of extractives to be recovered from the sawdust. Nevertheless, it includes, in addition to secondary metabolites (e.g. polyphenolic compounds) gums, sugar, waxes, coloring matter, and starch. The interest of this work is in the secondary metabolites and their potential. Therefore, the following analysis is the quantification of total extractives and their characterization in order to identify the compounds extracted.

### 2.3 Extractives content

The extractives contain secondary metabolites that confer a selective advantage to the tree. It has been well established that the quantity of extractives is an indicator of natural durability (decay, insects, and weather resistance) and dimensional stability (Kollert and Kleine, 2017).

The quantification of extractives was done by two methods, using a Soxhlet apparatus and with an accelerated solvent extraction (ASE) system. The extractions were carried out using solvents with different polarities. The variances in polarity make it possible to extract different families of compounds according to the affinity to the solvent. Table 9 displays some important properties of the solvents used to take into account (Reichardt, 2003).

Table 9. Properties of solvents

<b>Solvent</b>	<b>Molecular formula</b>	<b>Molecular weight (g/mol)</b>	<b>Boiling point (° C)</b>	<b>Relative polarity</b>
Cyclohexane	C <sub>6</sub> H <sub>12</sub>	84.16	80.7	0.006
Acetone	C <sub>3</sub> H <sub>6</sub> O	58.08	56.2	0.355
Ethanol	C <sub>2</sub> H <sub>6</sub> O	46.0	78.5	0.654
Methanol	CH <sub>4</sub> O	32.04	64.6	0.762
Water	H <sub>2</sub> O	18.02	100.0	1.000

### 2.3.1 Extractives content of Soxhlet extraction

Soxhlet extraction is an exhaustive technique with which high yields can be obtained. It is commonly applied for extracting bioactive compounds from natural sources. In a conventional Soxhlet extraction procedure, the sample material is placed in a thimble-holder, and it is gradually filled with condensate fresh solvent from a distillation flask. When the liquid reaches the overflow level, a siphon aspirates the solution of the thimble-holder and it falls back into the distillation flask carrying the extracted analytes into the bulk liquid. This operation is repeated until complete extraction is achieved (Luque de Castro and García-Ayuso, 1998).

The most important benefits of conventional Soxhlet extraction include the following: the sample is repeatedly brought into contact with fresh portions of extractant, which facilitates displacement of the transfer equilibrium; no filtration is required after extraction, so sample throughput can be increased by performing parallel simultaneous extractions; the procedure is very simple; the performance is seemingly unaffected by matrix effects.

By contrast, the drawbacks of Soxhlet extraction as compared with other techniques for solid sample preparation are the long time required for extraction; a large amount of extractant used, which is not only expensive to dispose of, but also the source of additional environmental problems; the potential thermal decomposition of thermolabile target species by the effect of samples usually being extracted at the solvent boiling point over long periods; the absence of agitation; and the difficulty of automating. However, this technique is still widely used (Luque de Castro and Priego-Capote, 2012).

For the quantification of extractives in teak sawdust, a Soxhlet device was used. TS was dried at 50° C overnight before performing the extractions. In order to follow the evolution of the recovery of extracts for 1-lot TS, the extractives content was determined after seven hours of extraction. Afterward, the process was continued until the solvent in the siphon was colorless. It was an exhaustive extraction with the purpose of obtaining the maximum amount of extractives. After that, the organic solvent was evaporated under vacuum using a rotary evaporator to determine the yield. Details are presented in the chapter on materials and methods. Table 10 and Table 11 present the results of Soxhlet extractions from 1-lot TS and 2-lot-TS respectively.

Table 10. Extractives content of Soxhlet extractions from 1-lot TS

<b>Extractives from 1-lot TS (g/100 g dry teak sawdust)<sup>1</sup></b>						
<b>Solvent</b>	<b>Time (h)<sup>2</sup></b>	<b>Extractives (%)<sup>3</sup></b>	<b>Time (h)<sup>4</sup></b>	<b>Extractives (%)<sup>5</sup></b>	<b>Total time (h)</b>	<b>Total extractives (%)</b>
Cyclohexane	7	2.45 (0.30)	7	0.27 (0.001)	14	2.72
Acetone	7	3.07 (0.40)	7	0.25 (0.06)	14	3.32
Ethanol	7	5.42 (0.59)	7	0.46 (0.01)	14	5.88
Methanol	8	5.24 (0.33)	7	0.45 (0.001)	15	5.68

1. Average of duplicated. Standard deviation in parentheses. Results expressed on dry weight basis. Sample weight: 8 g approx. Solvent volume: 150 mL approx.

2. Duration of the first extraction series

3. Extractives obtained after the first extraction series

4. Additional extraction duration until colorless solvent in siphon is obtained

5. Extractives from the second extraction series

Table 11. Extractives content of Soxhlet extractions from 2-lot TS

<b>Extractives from 2-lot TS (g/100 g dry teak sawdust)<sup>1</sup></b>		
<b>Solvent</b>	<b>Time (h)</b>	<b>Extractives (%)</b>
Cyclohexane	7	2.34 (0.24)
Acetone	7	3.05 (0.07)
Ethanol	7	4.74 (0.23)
Methanol	7	4.74 (0.27)

1. Average of duplicated. Standard deviation in parentheses. Results expressed on dry weight basis. Sample weight: 13-14 g approx. Solvent volume: 200 mL approx.

For the extractions of 1-lot TS (see Table 10), 90 % of the total yield is already recovered in the first part of the extraction (after 7 h) when using cyclohexane, and 92 % for all other solvents. In other words, the additional time practically does not increase the amount of extractives, no more than 0.5 g of extractives per 100 g of dry teak sawdust is obtained in the second part of the extraction. Consequently, the time for the extractions of 2-lot TS is set to 7 h.

Overall, the differences between the two lots are no major, especially considering that, the amount of extracts depends on several factors and the samples come from separated productions. Not surprisingly, for both lots, the extracted compounds with methanol and ethanol are the most abundant; the yield is even the same for ethanolic and methanolic extracts of each lot. Commonly, the amount extracted decreased as the polarity of the solvent decreased, which means that the extract is richer in high polar compounds.

In general, the published results also show this trend, the largest amount of extractives are obtained with high polarity solvents. The methanolic yield quantified in this work is somewhat higher than those reported in the literature. For instance, the extractive content was 2.31 % and 4.0 % for inner sapwood and outer heartwood, respectively for 8-year-old teakwood; and 2.14 % and 3.69 % for inner sapwood and outer heartwood, respectively for 22-year-old teakwood, both samples from a plantation in Indonesia (Lukmandaru, 2013). In another work, the methanolic yield was 3.41 % for a 20-year-old teak heartwood from Indonesia, 4.72 % for a 16-year-old teak heartwood from Myanmar, and 3.16% for a 15-year-old teak heartwood from Thailand, all the samples from plantations (Da Costa et al., 1958). To cite another reference, methanolic extractives of teakwood from India were from 2.3 % to 5.5 % for 10-year-old teak; and from 2.0 % to 4.4 % for 30-year-old teak (Narayanamurti et al., 1962).

Methanol has been widely used as a solvent for the extraction of phytochemicals due to its efficiency. Several authors have reported that methanolic extracts contain the highest amount of secondary metabolites from teakwood, such as lapachol and deoxylapachol (Carrieri et al., 2014; Lukmandaru, 2015a); isodeoxylapachol and tectoquinone for sapwood region (Lukmandaru, 2015a). Additionally, methanol soluble extracts are attributed with fungicidal and termicidal properties.

Regarding the ethanolic extractives content, they are comparable to the results published. For instance, it was observed an extract yield of 3.9 % and 6.3 % in ethanol: water (8:2) fraction of 29-year-old teak heartwood from Panama, and 100-year-old teak heartwood from Myanmar, respectively (Haupt et al., 2003). In other work, for 20-year-old teak heartwood from a plantation in Brazil, the yield for absolute ethanol extraction was 9.17 % (Brocco et al., 2017).

The yield of acetone extracts is also similar to those observed in other works. For instance, Rizanti et al. (Rizanti et al., 2018) found an acetone extract content, of a sequential extraction, of 0.3 % and 1.1 % for 10-year-old and 40-year-old teak trees, respectively from Java, Indonesia. On the other hand, Haupt et al. (Haupt et al., 2003) obtained an extract yield of 3.7 % and 6.5 % in acetone: water (9:1) fraction of 29-year-old teak heartwood from Panama and 100-year-old teak heartwood from Myanmar, respectively. Moreover, they found a high antifungal activity in acetone: water extract.

On the other hand, the content of lipophilic compounds (extracted with cyclohexane) is higher than that observed with hexane, from 0.32 % to 1.89 % for 8 to 22-year-old teakwood from Indonesia (Lukmandaru, 2013) or toluene: ethanol, 1.18 % in for 9-year-old teakwood from Costa Rica (Moya et al., 2017). However, these extractives are only moderately correlated with the natural termite resistance (Lukmandaru and Takahashi, 2008).

The high extractives content by Soxhlet method suggests that the teak sawdust from Campeche, Mexico could be a good source of extractives.

### **2.3.2 Extractives content of accelerated solvent extraction (ASE)**

Accelerated solvent extraction is an automated extraction technique that uses elevated temperatures and pressures to achieve extractions of organic compounds in very short periods of time. The elevated temperature and pressure improve the efficiency of the extraction process. Increasing the temperature facilitates the capacity of solvents to solubilize analytes; it also leads to faster diffusion rates. This means that analytes move more quickly from the boundary layer near the surface of the matrix from which they are extracted to the bulk solvent. Higher temperatures also mean lower solvent viscosities, meaning that the solvent can penetrate the pores of the matrix more easily. Finally, increased temperature makes it easier to disrupt solute-matrix interactions (dipole attractions, van der Waals forces, hydrogen bonding, etc.) and remove analytes from the matrix. Likewise, if sufficient pressure is exerted on the solvent during the extractions, temperatures above the boiling point can be used. Operating at elevated pressures also helps the overall extraction process to happen more quickly. The pressurized solvent is forced into the pores of the sample matrix, coming into more intimate contact with analytes in those areas (TFS, 2011).

The second method of extract quantification was using an ASE system. The teak sawdust was dried at 50° C overnight before performing the extractions. During a run of ASE extraction, the cell is filled with solvent and then heated and pressurized to maintain the solvent in a liquid state. After the run, the extract is rinsed from the cell into a collection vessel (a bottle or vial). Afterward, the solvent is evaporated under vacuum using a rotary evaporator to determine the yield. Details are presented in the chapter on materials and methods. Table 12 presents the results of ASE extractions from 1-lot TS and 2-lot TS.

Table 12. Extractives content of ASE extractions from 1-lot TS and 2-lot TS

Solvent	ASE extractives from 1-lot TS	ASE extractives from 2-lot TS
	(g/100 g dry teak sawdust) <sup>1</sup>	(g/100 g dry teak sawdust) <sup>1</sup>
	Extractives (%)	Extractives (%)
Cyclohexane <sup>2</sup>	1.10 (0.25)	1.88 (0.03)
Acetone	2.40 (0.16)	2.45 (0.13)
Ethanol	4.80 (0.27)	4.66 (0.17)
Methanol	4.78 (0.32)	3.48 (0.06)
Water	2.59 (0.76)	3.14 (0.16)

1. ASE (Dionex 350 Thermo Scientific). Average of triplicated. Standard deviation in parentheses. Results expressed on dry weight basis. Sample weight: 1.8 g approx. (1-lot TS)/ 2 g approx. (2-lot TS). Static time: 7 min. Number of cycles: 3. Maximum pressure: 117 bar. Total extraction time: 32 min. 2. ASE static time: 5 min

Similar to the results obtained with the soxhlet apparatus, the highest yields are obtained with methanol and ethanol. Although the yield is higher using a Soxhlet apparatus, in the case of the ethanol and methanol solvents of 1-lot TS, a pressurized extraction achieves up to 90 % of the extractives obtained with the classical extraction.

The results for the extractives in the present work are similar to those described by other studies. For example, Rodríguez Anda et al. (Rodríguez Anda et al., 2019) reported values from 0.83 % to 1.54 % of extractives in methanol: water (8:2 v/v) and from 2.84 % to 6.07 % of extractives in acetone: water (9:1 v/v) of ASE extraction of 9-15-year-old teak trees from a plantation located also in Campeche, Mexico.

Concerning the aqueous extracts, our data are comparable to those reported in the literature for the aqueous fraction of sequential extractions using a Soxhlet apparatus. It was observed a yield of 2.5 % for both 10-year-old and 40-year-old teak trees from Indonesia (Rizanti et al., 2018), and from 1.5 % to 2.9 % for teak heartwood from East Timor (Miranda et al., 2011).

## 2.4 Total phenolic content (TPC) analysis

Phenolic compounds include naphthoquinones, phenolic acids (benzoic and cinnamic acid derivatives), coumarins, flavonoids, stilbenes, hydrolyzable, and condensed tannins, and lignans. These compounds are among the most widely occurring secondary metabolites in the plant kingdom, acting mainly as phytoalexins, attractants for pollinators, contributors to plant pigmentation, antioxidants, and protective agents against UV light, among others (Blainski et al., 2013).

The TPC in the extracts was achieved by colorimetry using the Folin-Ciocalteu (F-C) method and gallic acid as standard, details are displayed in the chapter on materials and methods. It is important to mention that the Folin–Ciocalteu assay actually measures sample reducing capacity, which does not necessarily reflect the total phenolic content. F-C reagent is nonspecific to phenolic compounds as it can be reduced by many non-phenolic compounds (such as proteins). Phenolic compounds react with F-C reagent only under basic conditions (adjusted by a sodium carbonate solution). Dissociation of a phenolic proton leads to a phenolate anion, which is capable of reducing F-C reagent. This supports the notion that the reaction occurs through electron transfer mechanism. The blue compounds formed between phenolate and F-C reagent are independent of the structure of phenolic compounds, therefore ruling out the possibility of coordination complexes formed between the metal center and the phenolic compounds. (Huang et al., 2005). Despite its lack of specificity the total phenolic content determined by F-C is convenient, simple, and reproducible. The results of TPC are presented in Table 13 and Table 14.

Table 13. Total phenolic content of Soxhlet extracts from 2-lot TS

<b>Solvent</b>	<b>Polyphenols (mg GAE/g teak)<sup>1</sup></b>	<b>Polyphenols (mg GAE/g extract)<sup>1</sup></b>
Cyclohexane <sup>2,3</sup>	10.3 (0.6)	439.8 (25.9)
Acetone <sup>2,3</sup>	18.1 (1.5)	594.4 (47.8)
Ethanol <sup>2</sup>	32.8 (1.9)	693.4 (40.9)
Methanol <sup>2</sup>	35.4 (1.5)	568.0 (24.5)

1. Folin-Ciocalteu method. TPC expressed as mg of gallic acid equivalent (GAE) per gram of dry weight. Average of triplicated. Standard deviation in parentheses. BMG-Labtech Spectrostar-Nano, 96-well microplate. Absorbance recorded at 700 nm. Temperature: 45°C. Incubation time: 45 min.

2. Soxhlet extraction. Average of duplicated. Standard deviation in parentheses. Results expressed on dry weight basis. Sample weight: 13-14 g approx. Solvent volume: 200 mL approx. Total extraction time: 7 h.

3. For TPC quantification the solvent was evaporated and the extract re-dissolved into ethanol.

Table 14. Total phenolic content of ASE extracts from 2-lot TS

<b>Solvent</b>	<b>Polyphenols (mg GAE/g teak)<sup>1</sup></b>	<b>Polyphenols (mg GAE/g extract)<sup>1</sup></b>
Cyclohexane <sup>2,3</sup>	11.8 (0.9)	627.3 (47.1)
Acetone <sup>2,3</sup>	11.4 (0.7)	467.3 (28.2)
Ethanol <sup>2</sup>	23.4 (1.4)	505.6 (29.8)
Methanol <sup>2</sup>	28.4 (0.5)	811.0 (14.4)

1. Folin-Ciocalteu method. TPC expressed as mg of gallic acid equivalent (GAE) per gram of dry weight. Average of triplicated. Standard deviation in parentheses.

2. ASE (Dionex 350 Thermo Scientific). Sample weight: 1.8 g approx. Static time: 7 min. Number of cycles: 3. Maximum pressure: 117 bar. Total extraction time: 32 min.

3. For TPC quantification the solvent was evaporated and the extract re-dissolved into ethanol.

The concentration in polyphenols does not change considerably between the two extraction techniques, being, in any case, higher when using a Soxhlet apparatus. This suggests on the one hand that the reduction capacity of phenolic compounds extracted by Soxhlet device remains stable despite the long extraction time (7 h) with respect to ASE (32 min). On the other hand, the ASE method is efficient enough to allow a polyphenol content almost as high as the Soxhlet method.

For both cases, the polyphenol content tends to increase as the polarity of the solvent increases. As expected, the TPC is higher in methanol and ethanol extracts. The highest value obtained is 35.4 mg GAE/g dry teak for the methanol extract (the most polar solvent used) while the lowest is 10.3 mg GAE/g dry teak for the cyclohexane extract (the most nonpolar solvent used) both extractions using a Soxhlet apparatus. Observing the amount of polyphenols with respect to the extract, in general, it represents more than 50 % of the extract content for all samples. For instance, 81 % of the methanolic extractives by ASE corresponds to compounds capable of reducing the F-C reagent, which are presumably phenolic compounds.

The results of the present study are in accordance with those reported for other wood with similar durability, in some cases, they are even higher. For teak bark, it has been reported a TPC in ethanolic extract of 0.9 mg GAE/mL (Baptista et al., 2013). For *Maclura tinctoria* (L.) D. Don ex Steud. a Brazilian tree whose resistance rates equivalent to teak, there have been found TPC values of 38.4 mg GAE/g wood and 45.3 mg GAE/g wood for methanol: water extract (4:1 v/v) and acetone: water extract (7:3 v/v), respectively (Lamounier et al., 2012).

Other references may include tropical species with highly decay resistance such as moabi (*Baillonella toxisperma* Pierre); bilinga (*Nauclea diderrichii* (De wild, Merr); okan (*Cylicodiscus gabunensis* Harms); and padouk (*Pterocarpus soyauxii* Taub) with a TPC in ethanolic extracts of 51 mg GAE/g



extract, 239 mg GAE/g extract, 559 mg GAE/g extract, and 172 mg GAE/g extract, respectively (Mounguengui et al., 2016).

On the other hand, for oak (*Quercus robur*), which is also classified as durable timber, there have been observed values from 5.0 mg GAE/g dry matter to 13.4 mg GAE/g dry matter in ethanolic extracts from oak bark (Dedrie et al., 2015). In addition, for methanolic extracts of oak wood there have been reported figures from 0.101 mg GAE/g wood to 0.028 mg GAE/g wood (Alañón et al., 2011).

## **2.5 Secondary metabolites identification and quantification**

Teakwood from natural plantations belongs to durability class I (highly resistance timbers) as assessed by the international standard classification (ASTM, 1981). Fast-growing conditions, as the plantation in Mexico where the samples come from, generally do not significantly compromise timber strength. However, wood from fast-growth teak trees is apparently less durable than teakwood from natural forests, probably due to the juvenile wood and the lower extractive content. The durability in the heartwood from fast-growth plantations is influenced by the height of the tree, age, radial position, soil, and growing conditions (Moya et al., 2014).

Durability is largely determined by the polyphenolic compounds contained in the cell wall and other anthraquinone and naphthoquinone derivatives, which offer various degrees of decay resistance to teak timber. It is accepted that the high durability of teakwood is due to the high extractive content. Recently, it has been reported that individual chemical compounds, even if present in small quantities, provide durability to teak heartwood more than the total extractives content (Kollert and Kleine, 2017).

The identification and quantification of phenolic compounds were performed by high-performance liquid chromatography (HPLC-DAD Dionex Ultimate 3000, Thermo Scientific). Compounds were identified by the comparison of the retention time and UV spectrum recorded between 200 nm and 400 nm with those obtained by standards. The quantification of phenolic compounds was measured at 254 nm. The secondary metabolites identified in Soxhlet extracts from 2-lot TS are presented in Table 15.

Table 15. Secondary metabolites identified in Soxhlet extracts from 2-lot TS

Solvent	2-hydroxymethyl anthraquinone <sup>1</sup>		Anthraquinone-2-carboxylic acid <sup>1</sup>		Lapachol <sup>1</sup>		2-methyl anthraquinone <sup>1</sup>		Squalene <sup>2</sup>		Total	
	(mg/g extract)	(mg/g teak)	(mg/g extract)	(mg/g teak)	(mg/g extract)	(mg/g teak)	(mg/g extract)	(mg/g teak)	(mg/g extract)	(mg/g teak)	(mg/g extract)	(mg/g teak)
Cyclohexane <sup>3,4</sup>	0.24	< 0.01	nd	nd	0.49	< 0.01	1.61	< 0.01	5.96	0.08	8.30	0.08
Acetone <sup>3,5</sup>	4.20	0.13	3.92	0.12	1.27	0.04	6.50	0.20	0.92	0.03	16.81	0.52
Ethanol <sup>3,5</sup>	3.02	0.14	4.12	0.19	2.20	0.10	4.41	0.20	0.50	0.03	14.25	0.64
Methanol <sup>3,5</sup>	1.70	0.10	1.88	0.11	1.05	0.06	2.93	0.18			7.56	0.46

nd: not detected

1. HPLC-DAD Dionex Ultimate 3000 (Thermo Scientific). Column Luna 3u PFP(2)100Å, 150 x 3.0 mm (Phenomenex). Quantification measured at 254 nm. Results expressed on dry weight basis.

2. CG-FID Varian 3900. Results expressed on dry weight basis.

3. Soxhlet extraction. Sample weight: 13-14 g approx. Solvent volume: 200 mL approx. Total time: 7 h.

4. For HPLC quantification the solvent was evaporated and the extract re-dissolved into methanol.

5. For CG quantification the solvent was evaporated and the extract re-dissolved into cyclohexane.

According to data from Table 15, there is no decisive difference in the quantity of secondary metabolites obtained (mg/g teak) for ethanol, acetone, or methanol extracts. However, it is slightly higher in ethanolic extract followed by acetone extract. On the other hand, for the cyclohexane extract, only squalene is identified in amounts greater than 0.01 mg/g teak. It is worth noting that the total amount of secondary metabolites presented in Table 15 corresponds to the sum of those identified and quantified (by comparisons with the authentic ones). Nevertheless, not all peaks of the chromatograms have been assigned to a compound. Consequently, the extract may contain other unidentified polyphenols. For instance, a well-defined peak stands out in the acetone and methanol extracts at 34 min that has not been identified. Figure 15 displays the chromatograms of each extract. In addition, 1,4-naphthoquinone has been also searched for but the UV-vis spectrum in the extracts does not fully correspond to the authentic one.

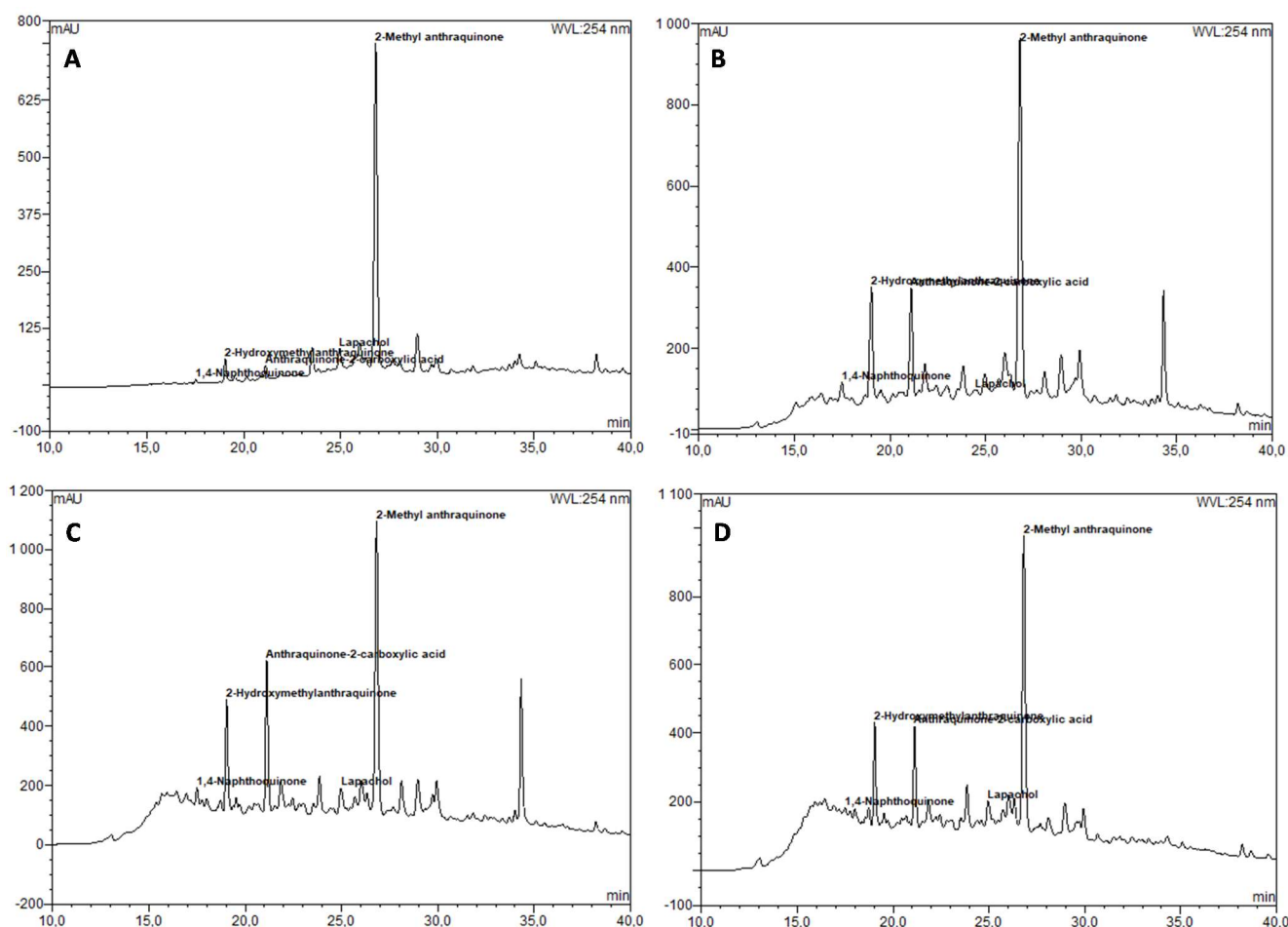


Figure 15. Chromatograms of extracts from teak sawdust extraction using a Soxhlet apparatus. A) Cyclohexane extract. B) Acetone extract. C) Methanolic extract. D) Ethanolic extract

The major compound in ethanol, methanol, and acetone extracts is 2-methyl anthraquinone (2-MA, also known as tectoquinone). It is even achieved in the same quantity in all three solvents (0.2 mg/g dry teak). In the acetone extract; however, it represents a higher proportion (6.5 mg/g extract). Tectoquinone is one of the most identified and studied secondary metabolites in teakwood (in both sapwood and heartwood region) and it is presumed that it is the main compound that influences the durability and dimensional stability of teak (Haupt et al., 2003; Thulasidas and Bhat, 2007).

Tectoquinone can be extracted with a wide variety of solvents. For instance, for acetone: water (80:20 v/v) extracts of 5-37-year-old teak trees from Sabah, Malaysia have been reported values of 0.48 mg/g dw to 17.72 mg/g dw (Niamké et al., 2014). On the other hand, in ethanol: benzene (1:2 v/v) extracts of 35-year-old teak trees there have been quantified from 2.3 mg/g dw to 3.4 mg/g dw of tectoquinone (Thulasidas and Bhat, 2007). For methanolic extractives, there have been found values from 0.07 mg/g dw in inner sapwood to 1.01 mg/g dw in outer heartwood for 8 to 22-year-old teak trees from Java, Indonesia (Lukmandaru, 2015a). In our study, the contribution of extractives from each part of the trunk

cannot be differentiated since the sawdust comes from a cross-section of the trunk and it contains both heartwood and softwood. Overall, tectoquinone appears to a lesser extent in our extracts.

Concerning anthraquinone-2-carboxylic acid (AQ-2-CA) and 2-hydroxymethyl anthraquinone (2-HM-AQ), they are the second most abundant polyphenols, and the higher concentration is found in ethanol extract (0.19 mg/g dry teak and 0.14 mg/g dry teak and for AQ-2-CA and 2-HM-AQ, respectively). The values observed in this work are in the same range as those reported in the literature. For instance, from 0.07 mg/g dw to 11.81 mg/g dw and from 0.03 mg/g dw to 3.23 mg/g dw of AQ-2CA and 2-HM-AQ, respectively in acetone: water (80:20 v/v) extracts of 5-37-year-old teak trees from Sabah, Malaysia (Niamké et al., 2014). Furthermore, Windeisen et al. (Windeisen et al., 2003) also identified 2-HM-AQ in petroleum ether and acetone extracts. It is interesting to note that the identification of AQ-2-CA has not been as published as the other secondary metabolites.

Regarding lapachol (4-hydroxy-3-(3-methylbut-2-enyl) naphthalene-1,2-dione), it is extracted mostly with ethanol. This naphthoquinone has been frequently identified in teakwood extracts. For instance, for southeast Asia teakwood, there have been reported values from 0.06 mg/g to 2.61 mg/g using as solvents methanol, ethyl acetate, water: ethanol, and water: acetone (Carrieri et al., 2014). For methanolic extractives, there have been found values from 0.0069 mg/g dw in outer sapwood to 0.41 mg/g dw outer heartwood for 8 to 22-year-old teak trees from Java (Lukmandaru, 2015a). As we can see, our results are comparable to those found by other researchers.

The analyses of the extractives by means GC prove squalene to be the main compound in cyclohexane, as expected due to its hydrophobic nature. This non-polar lipid has been identified in teak extracts with solvents as petroleum ether (Windeisen et al., 2003), petrol (Khan and Mlungwana, 1999), n-hexane, ethyl acetate, and methanol (Lukmandaru, 2015a). For instance, for methanolic extractives, the content of squalene was from 0.02 mg/g dw in outer sapwood to 1.43 mg/g dw in outer heartwood for 8 to 22-year-old teak trees from Java (Lukmandaru, 2015a).

Overall, the results of this study are congruent with those published by other authors. It is worth mentioning that there is variability in respect to the quantity of secondary metabolites that can be extracted from teakwood because there are many factors affecting the composition of the extracts.

The secondary metabolites identified in ASE extracts from 2-lot TS are presented in Table 16 and Figure 16 shows the corresponding chromatograms.

Table 16. Secondary metabolites identified in ASE extracts from 2-lot TS

Solvent	2-hydroxymethyl anthraquinone <sup>1</sup>		Anthroquinone-2-carboxylic acid <sup>1</sup>		Lapachol <sup>1</sup>		2-methyl anthraquinone <sup>1</sup>		Squalene <sup>2</sup>		Total	
	(mg/g extract)	(mg/g teak)	(mg/g extract)	(mg/g teak)	(mg/g extract)	(mg/g teak)	(mg/g extract)	(mg/g teak)	(mg/g extract)	(mg/g teak)	(mg/g extract)	(mg/g teak)
Cyclohexane <sup>3,4</sup>	1.41	0.02	nd	nd	nd	nd	5.72	0.06	5.96	0.11	13.09	0.19
Acetone <sup>3,5</sup>	2.58	0.10	1.60	0.06	1.02	0.04	4.04	0.16	1.46	0.03	10.70	0.39
Ethanol <sup>3,5</sup>	2.44	0.10	2.96	0.12	1.19	0.05	3.42	0.14	0.23	0.01	10.24	0.43
Methanol <sup>3,5</sup>	2.69	0.09	3.71	0.13	1.48	0.05	3.80	0.13	0.26	0.01	11.94	0.41
Water <sup>3,5</sup>	1.27	0.01	3.69	0.02	0.59	< 0.01	0.36	< 0.01	nd	nd	5.91	0.03

nd: not detected

1. HPLC-DAD Dionex Ultimate 3000 (Thermo Scientific). Column Luna 3u PFP(2)100Å, 150 x 3.0 mm (Phenomenex). Quantification measured at 254 nm. Results expressed on dry weight basis.

2. CG-FID Varian 3900. Results expressed on dry weight basis.

3. ASE (Dionex 350 Thermo Scientific). Sample weight: 2 g approx. Static time: 7 min. Number of cycles: 3. Maximum pressure: 117 bar. Total extraction time: 32 min.

4. For HPLC quantification the solvent was evaporated and the extract re-dissolved into methanol.

5. For CG quantification the solvent was evaporated and the extract re-dissolved into cyclohexane.

For the samples performed by ASE, the solvents acetone, ethanol, and methanol do not determine the quantity of secondary metabolites identified, which means that the same quantity of compounds is obtained in all these extracts. There have been reached values up to 0.10 mg/g dry teak, 0.13 mg/g dry teak, 0.05 mg/g dry teak, and 0.16 mg/g dry teak for 2-HM-AQ, AQ-2-CA, lapachol, and 2-MA, respectively. Regarding squalene, it is found in low amounts and mainly in cyclohexane extract (0.11 mg/g dry teak).

Considering the analogous analysis for extractions using a Soxhlet device, the quantity measured for ASE is slightly lower. In line with these results, the total phenolic content is also lower in ASE extracts than in Soxhlet extracts (see Table 13 and Table 14).

Comparing our results with those published by Rodríguez et al. (Rodríguez Anda et al., 2019) who studied teak extractives from the same region as we did (Campeche, Mexico) and using also an ASE system, their data exceeds ours. The tectoquinone content was 265.54 mg/g and 322.12 mg/g for acetone: water (9:1 v/v) extracts of 9-year-old and 15-year-old teak trees, respectively.

Regarding aqueous extractives, there have been identified mainly AQ-2-CA and 2-HM-AQ but in amounts lower than 0.02 mg/g dry teak. Since in this work we are interested in water as a solvent, longer extractions are carried out using an ASE device and with another technique to deepen this analysis. The results are discussed in a later section.

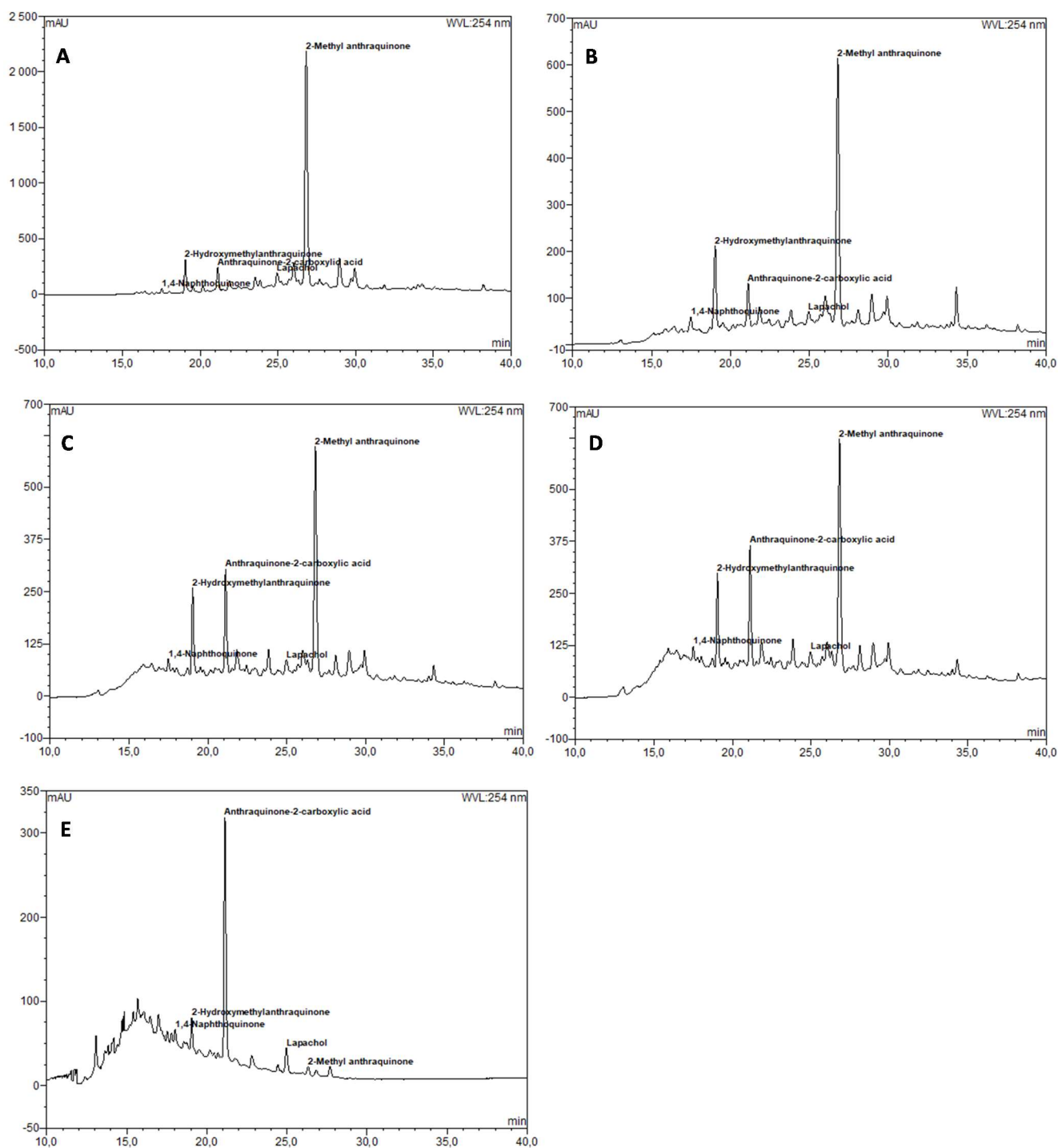


Figure 16. Chromatograms of extracts from teak sawdust extraction using an ASE system. A) Cyclohexane extract. B) Acetone extract. C) Methanolic extract. D) Ethanol extract. E) Aqueous extract

## 2.6 Antioxidant activity assay

Antioxidants protect living cells against the damaging effects of reactive oxygen species like singlet oxygen, and other free radicals such as superoxide, hydroxyl, peroxy, and peroxyxynitrite (Rana et al., 2014). They combat the free radicals by intervening at any one of the three major steps of the free radical mediated oxidative process (initiation, propagation, and termination) (Kedare and Singh, 2011).

It has been found that *Tectona grandis* bark is effective against free radical mediated diseases. Moreover, ethanolic extract of bark showed potent in vitro antioxidant potential and marked hyperglycemic activity (Rana et al., 2014).

The method of free radical scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) provides an approach for evaluating the antioxidant potential of certain compounds. The assay is based on the measurement of the scavenging capacity of antioxidants towards it. The odd electron of nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine (Kedare and Singh, 2011). Table 17 displays the results of DPPH antioxidant assay of extracts from 2-lot TS, details are presented in the chapter on materials and methods.

Table 17. Antioxidant activity of Soxhlet and ASE extracts from 2-lot TS

Solvent	Extraction method	Antioxidant
		activity IC <sub>50</sub> (mg/L) <sup>1</sup>
Acetone	Soxhlet <sup>2</sup>	238.16
	ASE <sup>3</sup>	146.50
Ethanol	Soxhlet <sup>2</sup>	108.52
	ASE <sup>3</sup>	145.61
Methanol	Soxhlet <sup>2</sup>	161.54
	ASE <sup>3</sup>	196.29
Water	ASE <sup>3</sup>	662.50

1. DPPH assay. Antioxidant activity expressed as the concentration of the extractive that inhibits 50% of radicals (CI<sub>50</sub>). BMG-Labtech Spectrostar-Nano, 96-well microplate. Incubation time: 40 min. Absorbance recorded at 516 nm. Temperature: room temp.

2. Soxhlet extraction. Sample weight: 8 g approx. Solvent volume: 150 mL approx. Total extraction time: 7 h.

3. ASE (Dionex 350 Thermo Scientific). Sample weight: 1.8 g approx. Static time: 7 min. Number of cycles: 3. Maximum pressure: 117 bar. Total extraction time: 32 min.

The Soxhlet ethanolic extract shows the best antioxidant activity. In agreement with this result, the quantity of the identified secondary metabolites and the value of TPC are the highest in ethanolic extracts

(for both extraction methods). The hydrogen donating activity of ethanol and acetone extractives from ASE is practically the same.

Other researchers also performed DPPH assay in methanolic extractives obtaining analogous results. For instance, for teak bark extracts at a concentration of 100 mg/L and 250 mg/L the scavenging percentages of the initial DPPH concentration were 19 % and 27.3 %, respectively, and for teakwood extracts at the same concentrations, the scavenging percentages were 47.5 % and 60 %, respectively (Krishna and Nair, 2010). On the other hand, if the antioxidant activity is compared with other species of the same family or with similar durabilities, such as *Gmelina arborea* Roxb. (also known as white teak) whose  $IC_{50}$  was 46.2 mg/L for the methanolic extract of stem bark (Patil et al., 2009); or *Maclura tinctoria* (L.) D. Don ex Steud. with  $IC_{50}$  values of 18.7 mg/L and 20.9 mg/L for wood and bark methanolic extracts, respectively (Lamounier et al., 2012), it is observed that teak extracts exhibit a lower capacity.

Not surprisingly, the lowest DPPH radical scavenging activity is attributed to the aqueous extract, even at the maximum concentration evaluated, 50 % inhibition is not reached, and therefore, the concentration at which 25 % of radicals are inhibited is reported.

It is assumed that there is a positive correlation between the content of phenolic compounds and antioxidant activity. Nevertheless, in our study TPC values do not directly correlate with antioxidant activity for all solvents. This suggests that the antioxidant capacity of the extracts could be the result of a synergic effect of other factors, even the presence of other kinds of secondary metabolites. Neither is a clear correspondence between the extraction method and the free radical-scavenging activity ascribable to all solvents. Nevertheless, in general, the ethanolic extract exhibits the best antioxidant capacity and TPC values.



## 2.7 Water extractives

Considering that in this work there is a particular interest in using water as a solvent, more extractions are performed of a longer duration than the one presented in Table 12 for a more in-depth study. The first extraction is carried out using a Fibertec 1020 (Foss, France) device with a cooling system at atmospheric pressure. The second one is through an ASE system. The extractives content is presented in Table 18.

Table 18. Water-soluble extractives content of 2-lot TS

<b>Water-soluble extractives content for 2-lot TS (g/100 g teak sawdust)</b>		
<b>Method</b>	<b>Time (h)</b>	<b>Extractives (%)</b>
Fibertec <sup>1</sup>	2	4.02 (0.02)
ASE <sup>2</sup>	2	3.79 (0.02)

1. Fibertec 1020 extractor (Foss) Average of triplicated. Standard deviation in parentheses. Results expressed on dry weight basis. Sample weight: 1.3 g approx. Solvent volume: 100 mL approx. Temperature: boiling temp.

2. ASE Dionex 350 Thermo Scientific. Average of triplicated. Standard deviation in parentheses. Results expressed on dry weight basis. Sample weight: 2.0 g approx. Temperature: 100°C.

As expected, as the extraction time increases, the extract content increases, however, no more than 1 g/100 g is gained with respect to the ASE extraction for 32 min (3.14 %). This time the extraction by ASE is performed in successive runs every 30 min up to 2 h. Figure 17 illustrates the vials with the extracts of each run. It can be observed that the color intensity decreases significantly in each fraction until the solvent in the last vial is very clear. It could be said that it is an exhaustive extraction where it is expected to recover practically all the extractives. The wood color depends on the chemical composition of its components (cellulose, hemicelluloses, lignin, and extractives) and their interaction with light. The color is determined more by the distinctiveness of the individual wood components than their amount, and the best example of this is cellulose. Cellulose is white in color and its content in wood is the highest, but its color is often overlaid by more distinctive extractives and lignin, whose content in wood is lower (Gašparík et al., 2019). The color could correlate with the amount of extractives. In addition, the class of compounds influences colorimetric properties (Shahid-ul-Islam et al., 2018).

The quantification of the different secondary metabolites identified in each fraction is presented in a later section in order to study the distribution of the compounds over time.



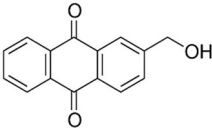
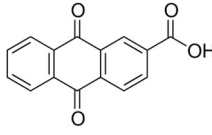
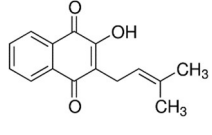
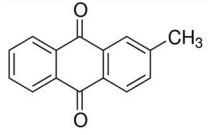
Figure 17. Aqueous extraction of teak sawdust using an ASE system, from left to right at 30 min, 60 min, 90 min, and 120 min

A noteworthy point is that the extraction method does not influence the total amount of extracts. This is surprising because the pressurized method would be expected to favor the extraction of compounds. Probably the extraction time is long enough to allow, in the case of Fibertec extraction, all water-soluble compounds to be extracted. In general, the hot water extractives content is lower than that reported by other authors. There have been published data of 7.22 % and 8.51 % for hot water and cool water extraction, respectively for 6 to 18-year-old teakwood from Costa Rica (Moya et al., 2017). On the other hand, for 50 to 60-year-old teak bark from East Timor, an aqueous fraction with 6.1 % of extractives has been achieved (Baptista et al., 2013). The extraction carried out by Baptista et al. was using a Soxtec device, which is comparable to the Fibertec, for a total time of 1.5 h. Another work observed an aqueous extractive content of 6.56 % for 20-year-old teak heartwood from a plantation in Brazil (Brocco et al., 2017).

### 2.7.1 Secondary metabolites

The secondary metabolites identified in ASE and Fibertec extracts (extraction performed for 2 h) from 2-lot TS are shown in Table 19.

Table 19. Secondary metabolites identified in water-soluble extracts from 2-lot TS

Secondary metabolites in water-soluble extracts for 2-lot TS referred to the raw material					
Method	2-hydroxymethyl anthraquinone (mg/g teak) <sup>1</sup>	Anthroquinone-2-carboxylic acid (mg/g teak) <sup>1</sup>	Lapachol (mg/g teak) <sup>1</sup>	2-methyl anthraquinone (mg/g teak) <sup>1</sup>	Total (mg/g teak)
					
Fibertec <sup>2</sup>	0.04 (7E-4)	0.08 (8E-4)	0.04 (7E-4)	0.01 (2E-4)	0.14
ASE <sup>3</sup>	0.06 (1E-3)	0.08 (8E-3)	0.06 (2E-3)	0.03 (9E-3)	0.23
Secondary metabolites in water-soluble extracts for 2-lot TS referred to the extract					
Method	2-hydroxymethyl anthraquinone (mg/g extract) <sup>1</sup>	Anthroquinone-2-carboxylic acid (mg/g extract) <sup>1</sup>	Lapachol (mg/g extract) <sup>1</sup>	2-methyl anthraquinone (mg/g extract) <sup>1</sup>	Total (mg/g extract)
Fibertec <sup>2</sup>	1.04 (2E-2)	2.07 (2E-2)	1.00 (2E-2)	0.30 (6E-3)	3.41
ASE <sup>3</sup>	1.60 (9E-2)	2.13 (1E-1)	1.63 (9E-3)	0.77 (2E-2)	6.13

1. HPLC-DAD Dionex Ultimate 3000 (Thermo Scientific). Column Luna 3u PFP(2)100Å, 150 x 3.0 mm (Phenomenex). Average of duplicated. Standard deviation in parentheses. Results expressed on dry weight basis.

2. Fibertec 1020 extractor (Foss). Sample weight: 1.3 g approx. Solvent volume: 100 mL approx. Temperature: boiling temp. Total time: 2 h.

3. ASE Dionex 350 Thermo Scientific. Sample weight: 2.0 g approx. Temperature: 100°C. Total time: 2 h.

In general, the extracts obtained using an ASE device show a higher content of each identified compound except for AQ-2-CA whose content is the same in both extracts. In the case of 2-HM-AQ and lapachol, the ASE system allows 65 % more recovery than the Fibertec system. Furthermore, comparing these results with those presented in Table 14 (ASE extraction for 32 min) the increase of the extraction time leads coherently to a rise in the amount of secondary metabolites for both methods.

Figure 18 displays the distribution of the secondary metabolites identified in each successive fraction of the aqueous extraction using an ASE device. After 30 minutes of extraction, 100 % of AQ-2-CA and 97 % of lapachol have been already recovered. The 2-methyl anthraquinone and 2-HM-AQ show a more progressive increase over time.

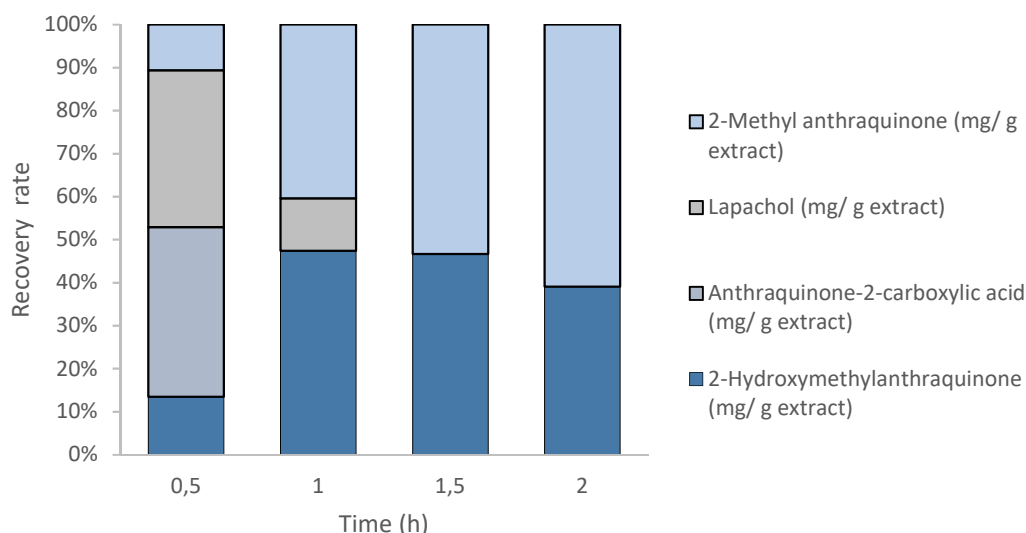


Figure 18. Distribution of the recovery of phenolic compounds over time extracted using an ASE system

### 2.7.2 Antioxidant activity assay

The antioxidant activity of ASE and Fibertec extracts is presented in Table 20. The DPPH radical scavenging potential is significantly improved with respect to the value found for the ASE aqueous extract for 32 min ( $IC_{50}$ : 662.5 mg/L) being the Fibertec extract the one that exhibits the best antioxidant potential. This latter is even better than the values of ethanol, methanol, and acetone extracts presented in Table 17 using ASE and Soxhlet apparatus.

Although increasing the extraction time enhances only 20 % of the amount of extracts, the DPPH assay suggests that the secondary metabolites extracted after 2 h exhibit better antioxidant properties.

Table 20. Antioxidant activity of water-soluble extracts from 2-lot TS

Method	Antioxidant activity
	$IC_{50}$ (mg/L) <sup>1</sup>
Fibertec <sup>2</sup>	122.50
ASE <sup>3</sup>	172.96

1. DPPH assay. Antioxidant activity expressed as the concentration of the extractive that inhibits 50% of radicals ( $CI_{50}$ ). BMG-Labtech Spectrostar-Nano, 96-well microplate. Incubation time: 40 min. Absorbance recorded at 516 nm. Temperature: room temp.

2. Fibertec 1020 extractor (Foss). Temperature: boiling temp. Total extraction time: 2 h.

3. ASE Dionex 350 Thermo Scientific. Temperature: 100°C. Total extraction time: 2 h.

### 2.7.3 Total phenolic content (TPC) analysis

According to Table 21, the ASE extract is slightly better in terms of reduction capacity. Both aqueous extracts have a phenolic content almost twice as high as that found in extracts obtained with other solvents (methanol, ethanol, acetone, and cyclohexane) by Soxhlet or ASE (32 min) device, see Table 13.

The values obtained in this study are higher compared with others works. In a study carried out by Qiu et al. (Qiu et al., 2019) for 18-year-old teakwood from China, the TPC of hot water extracts were 13.5 mg GAE/g in heartwood and 9.3 mg GAE/g in sapwood. Another study has been reported phenolic content of 0.7 mg GAE/mL for an aqueous extract of teak bark (Baptista et al., 2013).

Table 21. Total phenolic content of water-soluble extracts from 2-lot TS

<b>Method</b>	<b>Polyphenols (mg GAE/g teak)<sup>1</sup></b>
Fibertec <sup>2</sup>	41.2 (0.2)
ASE <sup>3</sup>	55.3 (2.0)

1. Folin-Ciocalteu method. TPC expressed as mg of gallic acid equivalent (GAE) per gram of dry weight. Average of triplicated. Standard deviation in parentheses. BMG-Labtech Spectrostar-Nano, 96-well microplate. Absorbance recorded at 700 nm. Temperature: 45°C. Incubation time: 45 min.

2. Fibertec 1020 extractor (Foss). Temperature: boiling temp. Total extraction time: 2 h.

3. ASE Dionex 350 Thermo Scientific. Temperature: 100°C. Total extraction time: 2 h.

## 2.8 Potential uses of secondary metabolites identified in teak sawdust extracts

As confirmed by the results presented in this chapter, teak extracts contain attractive secondary metabolites. These could be recovered and valorized in a variety of uses. Among the molecules that could be exploited in the pharmaceutical, cosmetic or food industry are squalene, lapachol, 2-methylanthraquinone, and anthraquinone -2-carboxylic acid.

Squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene) is a triterpene that acts as intermediate in the biosynthesis of phytosterol or cholesterol in plants, animals and humans. Liver oil of sharks from deep sea represents the richest natural source of squalene. There are important reasons limiting the use of this source, such as the presence of persistent organic pollutants in the sea, which can still be found in the purified squalene; the regulations against the overfishing of sharks; and the increasing trend among the consumers towards the use of plant-based products. Squalene has multiple applications due to its unique properties. It presents remarkable antioxidant and antibiotic activities derived from its chemical structure of six double bonds. The use of squalene has been cited in formulations of nutraceuticals and cosmetics products (e.g. lipsticks, makeup, and hair products). It offers a wide range of benefits such as emollient, hydrating, high spreadability, light consistency, non-greasy texture, rapid transdermal absorption, and reduction of skin damage by UV radiation; moreover, it has been used in healing eczema and in the protection against aging and wrinkles. It is also used in the preparation of stable emulsion as an adjuvant for vaccine delivery stimulating the immune response and increasing the patient's response to the vaccine. Furthermore, there are studies about the potential of squalene in cancer therapy (Popa et al., 2014). For the above mentioned, it exists a great interest in finding new natural, mainly vegetable, sources for squalene. The most relevant plant sources of squalene are oils extracted from amaranth, olive, palm, rice, wheat germ, grape seed, peanut, and soybean (Micera et al., 2020). Most vegetal oils are obtained by mechanical pressing or extraction with solvents like hexane. Recently the biosynthesis of squalene from microbial sources has been also studied as an alternative, using for instance yeast, bacteria, algae, and microalgae.

Concerning the squalene market, its size is projected to reach USD 184 million by 2025 from USD 129 million in 2020, at a CAGR of 7.3 %. The growth in the cosmetics and pharmaceutical industry, along with the increasing research in the oncology segment, are the major factors driving the growth of the squalene market (MarketsandMarkets, 2021).

Another interesting phytoconstituent is lapachol (4-hydroxy-3-(3-methylbut-2-enyl) naphthalene-1,2-dione), it is a natural naphthoquinone widely distributed in plants belonging to the family Bignoniaceae, such as *Tabebuia avellanedae* (lapachol tree, Brazilian taheebo), *Catalpa ovata* G.Don. *Tecomella undulata* (Sm.) Seem., but it has also been found in other families from the order Lamiales (Christensen,

2015), such as *Tectona grandis* L.f. Lapachol is known for its anti-inflammatory, analgesic (Costa et al., 2011), antibiotic (Rocha et al., 2013), antimalarial (Zofou et al., 2013), anticancer (Kandioller et al., 2013), antibacterial and antifungal properties (Ravelo et al., 2003). There is a growing interest in the study of the pharmacological activity of lapachol and its derivatives in the treatments of tumors and the cytotoxic effects against different cancer types (Lu et al., 2013; Niehues et al., 2012). This compound displays not only antiproliferative activity but also antimetastatic effects both in vitro and in vivo tests (Epifano et al., 2014). Additionally, it is used as a coloring matter to dye textile fibers (Har Bhajan Singh and Bharati, 2014)

Regarding 1, 4-naphtoquinone (naphthalene-1,4-dione), it is a naturally occurring compound, especially in several families of higher plants, such as Plumbaginaceae, Juglandaceae, Boraginaceae, Dioncophyllaceae, Verbenaceae, Scrophulariaceae, Avicenniaceae, Balsaminaceae, Bignoniaceae, Gentianaceae, and Droseraceae, also it is present in algae, fungi, some animals, and products of metabolism of some bacteria. It shows antimalarial, antibacterial, antifungal, and anticancer activities (Checker et al., 2018). Besides, 1, 4-naphtoquinone has been pointed out as a potential pharmacophore for inhibition of both monoamine oxidase and DNA topoisomerase activities, this latter associated with tumor activity (Coelho-Cerqueira et al., 2014).

With respect to one of the most studied quinones in teak extracts, namely 2-methylanthraquinone (2-methyl-9,10-anthracenedione), it and its derivatives are important as intermediates for manufacturing various kinds of vat dyes and brilliant blue (turquoise blue) disperse dyes (Hattori, 2000).

Concerning anthraquinone-2-carboxylic acid (9,10-dihydro-9,10-dioxo-2-anthracene carboxylic acid), it is a polyphenol found in fruits, vegetables, herbs, and widely identified in *Tabebuia avellanedae* (Brazilian taheebo). It has proved anti-inflammatory and antinociceptive activity in vivo, hence it has a potential in the development of functional foods (Park et al., 2016). In addition, some studies suggest that AQ-2-CA has a strong and long-acting anti-passive cutaneous anaphylaxis activity. Its therapeutic use could be also considered in the treatment of asthma (Cheng et al., 1999).

The above studies indicate that the secondary metabolites that can be found in teak wood have quite attractive properties and a real potential to be used in cosmetic or nutraceutical products, in the dyeing industry, or for medical and pharmaceutical purposes.

## Conclusion of Chapter 2

The teak sawdust was obtained from Agropecuaria Santa Genoveva, a wood processing enterprise located in Mexico. Two lots were received and characterized, the first one for a preliminary approach and the second one for a larger-scale study. In general, both lots have the same characteristics. The predominant particle size (about 85 %) is between 63  $\mu\text{m}$  and 500  $\mu\text{m}$ . The lignocellulosic content is 52 % cellulose, 11 % hemicelluloses, and 28 % lignin, plus 2 % mineral matter.

Regarding the extractives content for 2-lot-TS, using a Soxhlet apparatus for 7 h of extraction, there have been reached values of 2.3 %, 3.1 %, 4.7 %, and 4.7 % for cyclohexane, acetone, ethanol, and methanol extracts, respectively. On the other hand, for extractions using an ASE system for 32 min, there have been obtained yields of 1.9 %, 2.5 %, 4.7 %, 3.5 %, and 3.1 % for cyclohexane, acetone, ethanol, methanol, and water extracts, respectively. These results are in agreement with those reported by other studies, and even higher when compared to extracts from teak trees of the same age range. It means that despite being a by-product, teak sawdust could be a potential source of extractives.

The TPC and the antioxidant activity of the extracts have also been determined. For the phenolic content, the highest value has been found in the methanolic and ethanolic extracts of both extraction types (Soxhlet and ASE), which is practically the same (about 30 mg GAE/g dry teak). The difference is in the phenolic content with respect to the extract, where the best result has been obtained with methanol and using an ASE system (811 mg GAE/g extract). For the antioxidant activity, the ethanolic extract using a Soxhlet apparatus has shown the best  $\text{IC}_{50}$  (108 mg/L).

There is practically no difference in the amount of secondary metabolites quantified in acetone, methanol, and ethanol extracts for both extraction methods (Soxhlet and ASE), being slightly higher in ethanolic Soxhlet extract with values of 14 mg/100 g teak, 19 mg/100 g teak, 10 mg/100 g teak, 20 mg/100 g teak for 2-hydroxymethyl anthraquinone, anthraquinone-2-carboxylic acid, lapachol, 2-methyl anthraquinone, respectively. The major compound in all extracts is 2-MA. Regarding the squalene, it has been found up to 11 mg/100 g teak in the cyclohexane ASE extract. In general, these values are comparable to those found by other authors.

In the case of aqueous extraction, up to 4 % of extractives content has been achieved (for 120 min of extraction), and 3 % for 30 min of extraction using an ASE device. In contrast to extracts of other solvents, where mostly 2-MA has been identified, when using water as a solvent the major compound extracted is AQ-2-CA, 2 mg/100 g teak and 8 mg/100 g teak for 30 min and 120 min, respectively. Regarding the DPPH radical scavenging potential, it is improved from an  $\text{IC}_{50}$  of 662.5 mg/L to 84 mg/L by increasing the ASE extraction time. Furthermore, the TPC has been attained values of 55 mg GAE/g



teak and 1564 mg GAE/ g extract, these figures are higher than those found in the literature for teakwood and even in other similar species.

The characterization results demonstrate that teak sawdust has an interesting content of extractives (obtained with solvents of a wide range of polarities), a high TPC, and secondary metabolites that could be recovered. Therefore, teak sawdust, which is considered a woody waste, has the potential to be a source of extractives. The next chapter studies the possibility of a liquid/ solid extraction from a larger scale perspective using a twin-screw extruder, and water at subcritical conditions as a solvent.



## **Chapter 3**

### **Study of the aqueous solid/liquid extraction of teak sawdust using a twin-screw extruder**



## **Chapter 3. Study of the aqueous solid/liquid extraction of teak sawdust using a twin-screw extruder**

Once previous studies have confirmed the potential of teak sawdust as a source of extractives, since it has a high extractives yield with compounds of interest and its extracts exhibit an attractive reducing capacity measured as total phenolic compounds, the next step is the recovery of the compounds with a commercial scale perspective. In this regard, twin-screw technology is evaluated as an extraction method since it offers the possibility of processing large quantities of raw material continuously.

This chapter presents the study of the solid/liquid extraction of teak sawdust. The first approach was performed using an extruder BC21 (Cleextral). Different conditions of temperature and liquid to solid ratio were evaluated according to a defined experimental design. The extracts obtained were then analyzed to determine the extractives content, identify and quantify the extracted compounds, and estimate the total polyphenol content. Then a scale-up study of the twin-screw technology was carried out with an extruder BC45 (Cleextral). This last extrusion was mainly focused on the recovery of the extrudate for a subsequent valorization.

In line with the trend of extracting bioactive compounds with reduced environmental impact, the solvent used in all extraction was water. It has been demonstrated that the pressure and temperature conditions reached in the mechanical stress zones in twin-screw extruders are favorable for achieving subcritical water conditions (Evon et al., 2018). Under these conditions, the solvent properties (mainly dielectric constant and polarity) of water become similar to organic solvents (ethanol or methanol) (Zhang et al., 2020), which could improve the low solubility of phenolic compounds in aqueous extractions.

### **3.1 Study of the solid/liquid extraction of teak sawdust using a twin-screw extruder BC21 (Cleextral)**

The first part of the study focused on defining the barrel configuration as well as the extruder screw profile that would allow the necessary process steps to be carried out. The extruder used for this approach was a BC21 (Cleextral, France). It is a co-rotating and intermeshing twin-screw, which consists of two parallel screw shafts inside a closed barrel of seven modules of 100 mm in length. Once the extruder configuration was completed, the limits of the experimental range that would allow the steady-state process were searched. The impact of temperature (T) and solid to liquid ratio (L/S) were studied using an experimental design. Additional tests were then performed to complete the experimental design and refine the interpretation of the results.

### 3.1.1 Study of the solid/liquid extraction of teak sawdust using a twin-screw extruder BC21: principle of the procedure

The barrel configuration pursues to define three zones: feeding and conveying zone, shearing and mixing zone, and liquid/solid separation zone, see Figure 19.

The first one is the feeding and conveying zone (modules 1 and 2). In module 1, the teak sawdust is fed and its transport along the barrel starts. In module 2, a first water inlet is injected.

In the second zone (modules 3, 4, and 5) the mixture is subjected to a strong shearing. The mechanical force exerted by the mixing elements (bilobe paddle screw, BB) in modules 3 and 5 promotes an intimate interaction between the solid matter and the solvent. In these areas of intense mechanical stress, favorable conditions are expected to be reached to bring the water to subcritical conditions. This zone corresponds, then, to the solid/liquid extraction. Another water supply is entered in module 5.

In the third zone (modules 6 and 7) the liquid/solid separation is carried out through the press of the mixture by the reversed double-thread screw (CF2C) placed in module 7. The extrudate is conveyed to the outlet of module 7 and the liquid is filtered in module 6.

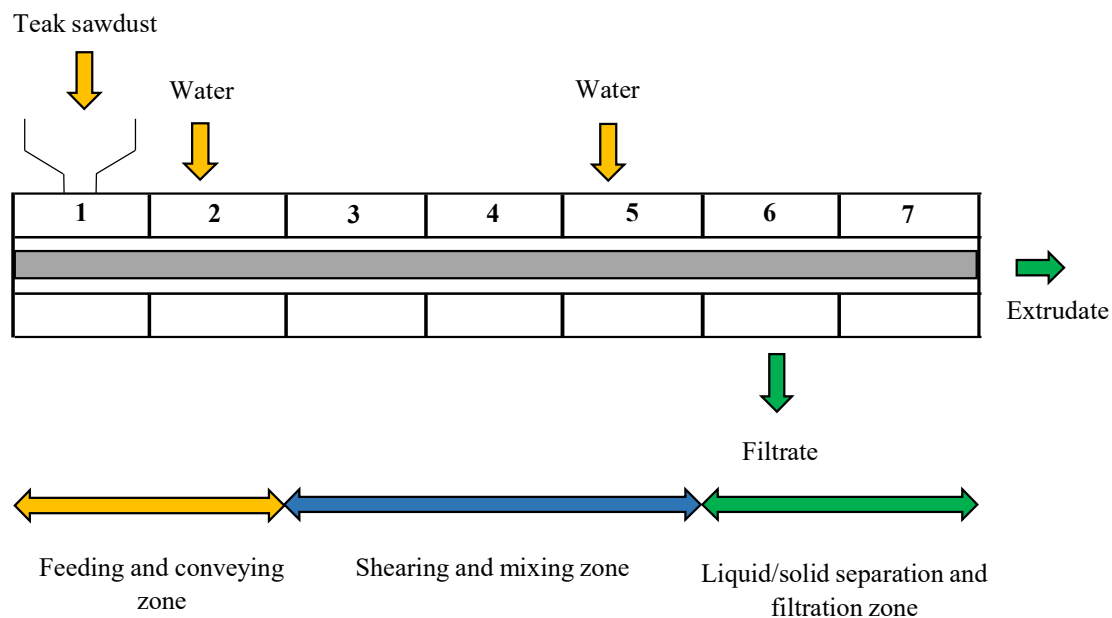


Figure 19. Schematic of extruder barrel zones

In the configuration used in trials, modules 2, 3, 4, 5, and 7 are thermo-regulated. They are heated by thermal induction and cooled by water recirculation. The process parameters (temperature, screw speed,

and teak sawdust flow) are controlled by a control panel. The common steps of the different screw profiles tested are as follows:

The teak sawdust (TS) is fed at module 1 by a twin-screw-volumetric feeder (KCL-KT20 K-Tron Soder). The sawdust is used as received from the sawmill, without prior conditioning. The screw elements T2F placed at the beginning of the configuration allow the feeding of the raw material and then it is transported to module 2. This module is not heated.

In module 2, the first injection of water is made using a piston pump. The water flow rate is kept constant at liquid to solid ratio (L/S) equal to 1 Kg of total liquid/Kg of dry TS in this module. The mixture is transported by the conveying double-thread C2F screws to module 3.

In module 3, the bilobe-paddle screws BB ensure a shear mixing of the sawdust with the liquid. This type of element generates high shear-rate regions, with high fluxes through these regions. The staggering angle between the discs dictates the conveying capacity of the BB elements and they can be configured as forward or backward transporting, or neutral. Increasing the staggering angle causes leakage flow between the discs to increase, and thus the backflow, and consequently the residence time distribution. In the profile here proposed, the BB elements are arranged at 90°. For BB screws with 90° staggering angle, the forward transport capacity of the disc is zero. By contrast, at 90° there is a better distributive mixing because of the larger leakage flows. To maintain a constant flow rate, more pressure is needed to push the fluids through these elements (Sarhangi Fard and Anderson, 2013). Consequently, there are placed conveying screws C2F upstream (in module 2 and at the beginning of module 3), the conveying elements build up the required pressure.

In module 4, the transport of the mixture continues by C2F screw elements to module 5. From module 3 to module 5, the intimate contact between the raw material and the solvent facilitates the extraction process.

In module 5, a second water inlet is injected using a piston pump. The flow rate is varied in such a way that this module has an L/S ratio from 3.25 Kg/h to 4.25 Kg/h according to the experimental design detailed in a later section.

Module 6 was equipped with a filter made up of four hemispherical dishes with conical holes of 1 mm of inner diameter and 2 mm of outer diameter. The filter ensures the flow and collection of the filtrate. The filtrate has then centrifuged to remove the fine particles (sediment) due to the solid breakdown. This module is not heated.

In module 7, the reversed double-thread screw CF2C gives a good distributive mixing and transports the mixing upstream instead of downstream, this makes possible the mixture compression and promotes a zone of high mechanical pressure resulting in the formation of a dynamic plug. Lastly, C2F elements convey the extrudate (solid phase) to the exit of the equipment.

### 3.1.2 Preliminary trials: screw profile selection and configuration

Preliminary tests were carried out before defining the one that was appropriate to the material size and the feedstock flowrates. The profiles are shown in Figure 20 to Figure 22. The first two (A and B) had the inconvenience that some of the raw material was returned and overflowing by the feeding module (module 1). Finally, profile C was the one that allowed a continuous flow of both extrudate and filtrate without clogging.



Module	1		2		3		4		5		6			7		
Screw type	T2F	T2F	C2F	C2F	C2F	BB	C2F	C2F	C2F	BB	C2F	C2F	C2F	C2F	CF2C	C2F
Length (mm)	50	50	50	50	50	50	50	50	50	50	25	25	50	25	50	25
Pitch (mm) or angle	50	50	33	33	25	90°	33	33	25	90°	25	25	16	16	-33	33

Figure 20. Configuration and screw profile A (preliminary test) of twin-screw extruder BC21 (Clextal) for teak sawdust extraction. T2F: trapezoidal double-thread screw, C2F: conveying double-thread screw, BB: bilobe paddle screw, CF2C: reversed double-thread screw

Module	1		2		3		4		5		6			7				
Screw type	T2F	T2F	C2F	C2F	C2F	BB	C2F	C2F	C2F	C2F	BB	C2F	C2F	C2F	C2F	CF2C	C2F	C2F
Length (mm)	50	50	50	50	50	25	50	50	50	25	50	25	25	50	25	25	25	25
Pitch (mm) or angle	50	50	33	33	25	90°	33	33	25	16	90°	25	25	16	16	-25	33	33

Figure 21. Configuration and screw profile B (preliminary test) of twin-screw extruder BC21 (Clextal) for teak sawdust extraction. T2F: trapezoidal double-thread screw, C2F: conveying double-thread screw, BB: bilobe paddle screw, CF2C: reversed double-thread screw





Module	1		2		3		4		5		6			7			
Screw type	T2F	T2F	C2F	C2F	C2F	BB	C2F	C2F	C2F	C2F	BB	C2F	C2F	C2F	C2F	CF2C	C2F
Length (mm)	50	50	50	50	50	25	50	50	50	25	50	25	25	50	25	50	25
Pitch (mm) or angle	50	50	33	33	25	90°	33	33	25	16	90°	25	25	16	16	-33	33

Figure 22. Configuration and screw profile C (preliminary test) of twin-screw extruder BC21 (Clextral) for teak sawdust extraction. T2F: trapezoidal double-thread screw, C2F: conveying double-thread screw, BB: bilobe paddle screw, CF2C: reversed double-thread screw

Modules 1 and 2 in all three configurations have the same screw profile. In module 1, the feeding of the material is achieved with the screws type trapezoidal double-thread screw (T2F50). Afterward, in module 2 the conveying of the sawdust is made thanks to conveying double-thread screws (C2F33 and C2F25).

In configuration A, in the second part of module 3, there are restrictive elements namely bilobe mixing disks (BB) kneading type, the arrangement consists of ten pairs of BB elements at 90° making a total length of 50 mm. Qualitatively, mixing disks generate high and low-shear regions, near the tip and the screw root, respectively. Mixing effects are produced by backward or reverse and tangential flows (Yerramilli and Karwe, 2004). Contrary to conveying elements in which the direction of the flow is towards the exit, in the BB disks arranged at 90° the axial flow is neutral. This type of elements creates a dynamic plug that corresponds to an accumulation of material constantly renewed by the downstream thrust of the material conveyed by the screws. If the plug is too consistent and the pressure of the conveying screws is not sufficient, a blockage is created thus the material and liquid accumulate along the screw go upstream. To reduce stress and dynamic plug, the number of disks in configuration B is reduced to a total length of 25 mm.

After the BB elements, the profile with screws type C2F33 and C2F25 (in modules 4 and 5) is kept the same in configurations A and B. The decrease of the length of the mixing elements is compensated in configuration B by adding a conveying element (C2F16) before the second stack of mixing elements (BB at 90°) in module 5. The conveying elements in module 6 (C2F25, C2F25, C2F16) are also the same for A and B.

In module 7, a reversed double-thread screw (CF2C) is used. This element creates a strong stress zone. The pitch of this type of screw is reversed and slotted on the external thread, parallel to the screw axis. The material is thus pushed to the opposite direction by the reverse pitch, creating a strong dynamic plug and material passes through the screw element through the slots. It is placed after the filtration

module and allows the compression of the mixture and flush the filtrate out by the filter. This element is a CF2C-25 of 25 mm length and a CF2C-33 of 50 mm length in configurations A and B, respectively.

This screw element not only allows the compression and consequently the solid/liquid separation, but also has a very important mechanical effect on the raw material. Figure 23 illustrates the teak sawdust as received and Figure 24 after extrusion (extrudate). It is observed that the CF2C-33 (50 mm) screw causes a higher fiber shredding than CF2C-25 (25 mm) screw. This mechanical work on the material is very important because it allows greater exposure of the fibers with the solvent, which would favor the extraction process.

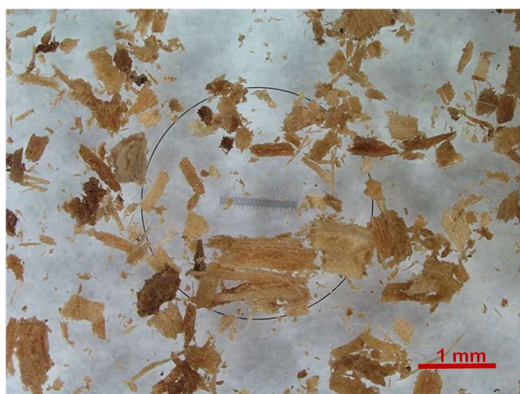


Figure 23. Teak sawdust as received

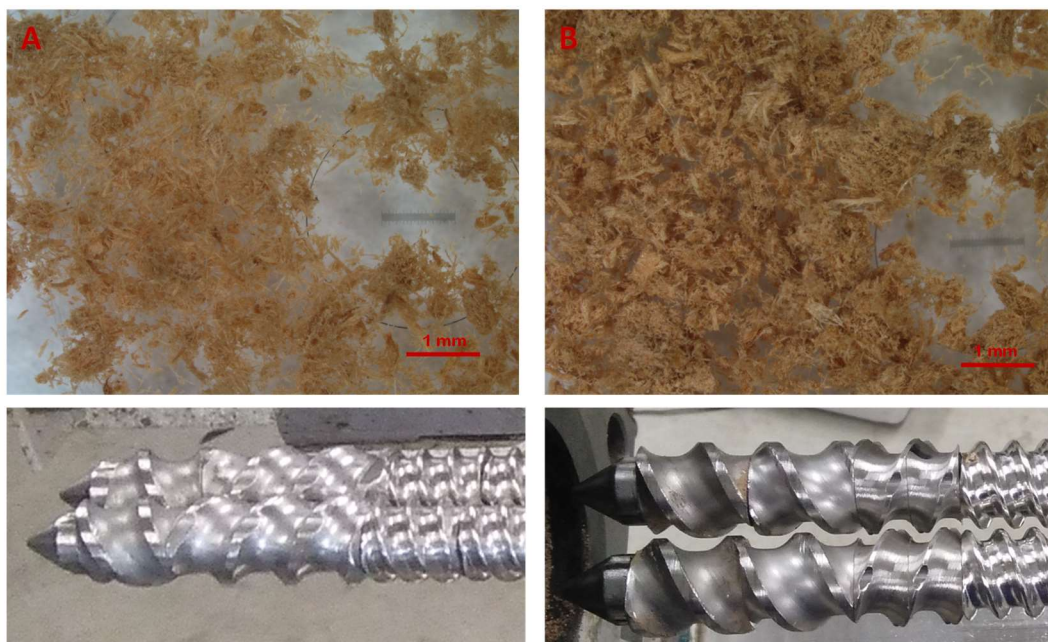


Figure 24. Mechanical effect of CF2F screw on teak sawdust in the extraction process by means of twin-screw extruder BC21 (Cletral). A: teak extrudate using a CF2F-33 (50 mm) screw element; B: teak extrudate using a CF2F -25 (25 mm) screw element

Configuration C is the same as configuration B except for reversed double-thread screw in module 7 (where CF2F-33 (50 mm) screw is used to promote the material defibration). This last screw profile allowed the recovery of the extrudate and filtrate in a continuous and stable way without refluxing the material. The solid/liquid extraction study is therefore performed using this configuration. The following section describes the operating conditions in more detail.

### 3.1.3 Solid/liquid extraction of teak sawdust using a twin-screw extruder BC21: definition of the experimental design

Once the profile has been defined, the aqueous extraction study is performed following a Doelhart experimental design where the effects of the extraction temperature and the L/S ratio are evaluated. The configuration, screw profile, and process temperatures of twin-screw extruder BC21 (Clextal) for teak sawdust extraction are shown in Figure 25.

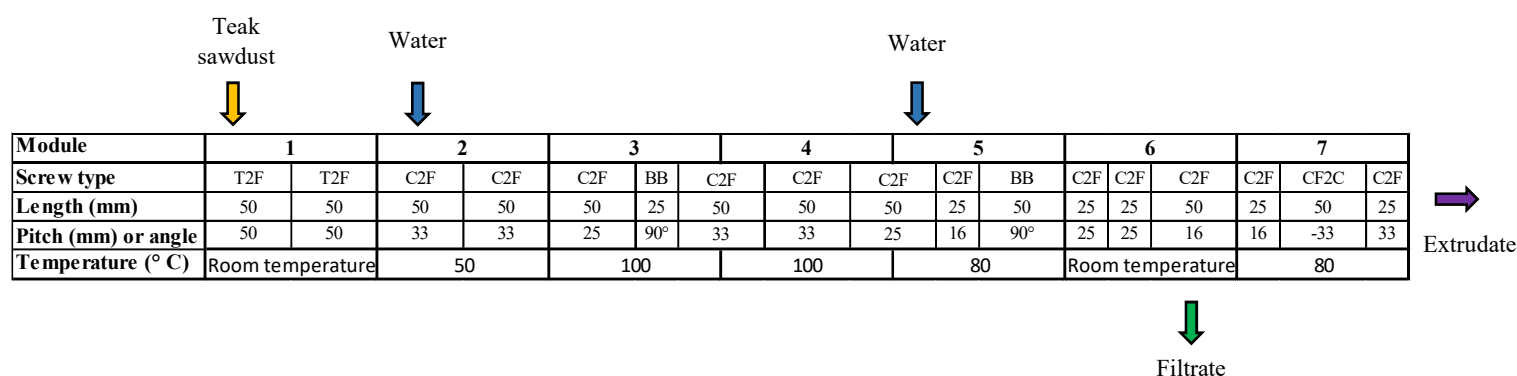
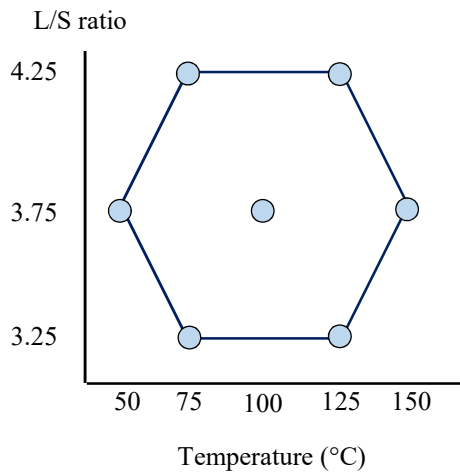


Figure 25. Configuration and screw profile of twin-screw extruder BC21 (Clextal) for teak sawdust extraction. T2F: trapezoidal double-thread screw, C2F: conveying double-thread screw, BB: bilobe paddle screw, CF2C: reversed double-thread screw

For the experimental design, the extraction temperature is adjusted in a range between 50°C and 150°C in modules 3 and 4 (both at the same T), according to the values presented in Table 22. The temperature in module 2 is set at 50 °C, and in modules 5 and 7 at 80°C (to avoid water evaporation). Modules 1 (teak feeding module) and 6 (filter module) are not heated. Concerning the L/S ratio, it is modulated between 3.25 Kg of total liquid/ Kg of dry TS and 4.25 Kg of total liquid/ Kg of dry TS in module 5 (second water injection), according to the values presented in Table 22. The solid/liquid ratio in module 2 (first water injection) is fixed at 1 Kg of total liquid/Kg of dry TS for all the trials. The rotation speed of the screws is fixed at 250 rpm.

Table 22. Real and coded values of temperature and L/S ratio of the experimental design for the solid/liquid extraction of teak sawdust using a twin-screw extruder BC21 (Cletral)



Experiment	Coded values		Real value	
	X <sub>T</sub>	X <sub>L/S</sub>	X <sub>T</sub>	X <sub>L/S</sub>
1	0	0	100	3.75
2	1	0	150	3.75
3	0.5	0.866	125	4.25
4	-0.5	0.866	75	4.25
5	-1	0	50	3.75
6	-0.5	-0.866	75	3.25
7	0.5	-0.866	125	3.25

The experimental design allows estimating the effects of the chosen variables (T, L/S) in the evaluated domain by calculating the coefficients of a quadratic polynomial model, which relates the responses to the studied variables.

$$Y = a_0 + a_T x_T + a_L \frac{x_L}{S} + a_T \frac{L}{S} x_T \frac{x_L}{S} + a_{TT} x_T^2 + a_{LL} \frac{x_L^2}{S^2}$$

Where:

Y: response

a: coefficient

x<sub>T</sub>: temperature

x<sub>L/S</sub>: L/S ratio

For each point of the experimental design, the twin-screw extraction is performed 15min before sampling to stabilize the operating conditions. The raw filtrate and the extrudate are collected for 15min. Each extraction is performed once, except for the central point (T= 100° C, L/S= 3.75), which is made in triplicate to estimate the standard deviation.

### 3.1.4 Responses of the solid/liquid extraction of teak sawdust using a twin-screw extruder BC21

The thermo-mechanical extraction (as defined in sections 3.1.1 and 3.1.3) implemented with an extruder BC21 (Figure 26 A, C and E) leads from teak sawdust (Figure 26 B) to a filtrate loaded with fine particles (Figure 26 F and G) and a solid extrudate (Figure 26 F and D).

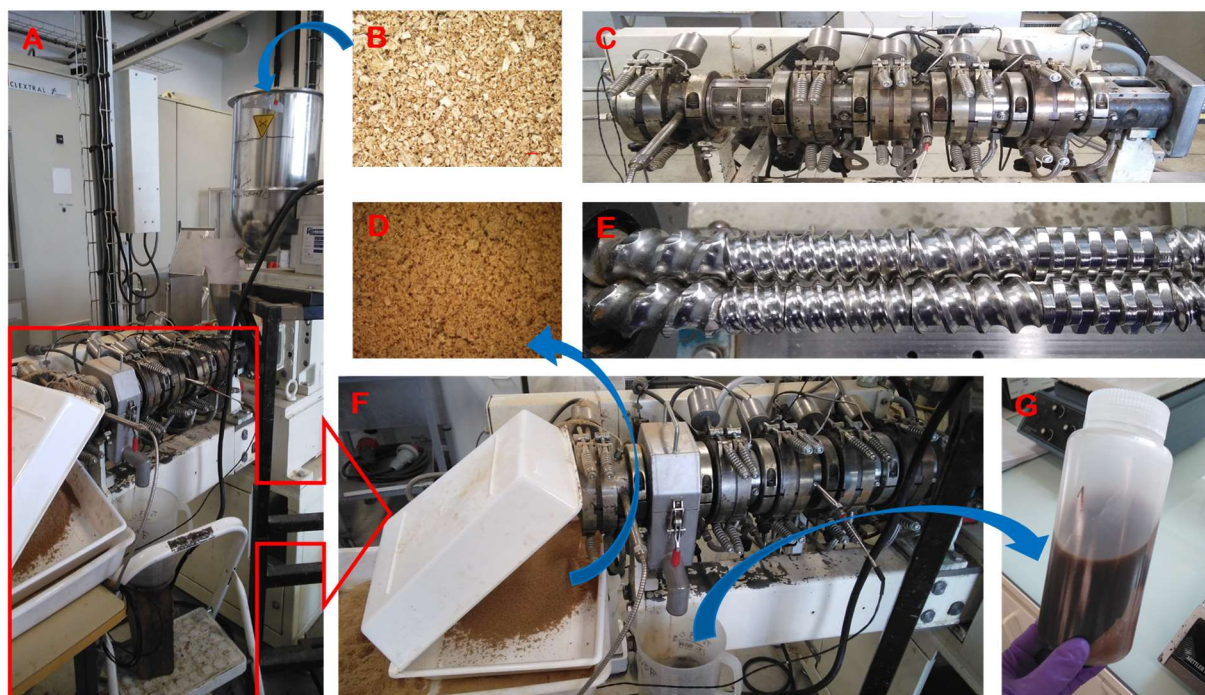


Figure 26. A: Twin-screw extruder BC21 (Clextal, France); B: Teak sawdust as received; C: extruder modules; D: extrudate; E: screw profile, from left to right: reversed double-thread screw CF2C -33, conveying double-thread screw C2F16, C2F16, C2F25, C2F25, bilobe paddle screw BB 90°; F: Outputs (extrudate and filtrate); G: Filtrate after centrifugation (clarified filtrate and sediments)

Figure 27 illustrates the solid/liquid extraction of teak sawdust. There are three inputs to the process, one solid and two liquid. The solid input corresponds to teak sawdust (TS), which is fed to the extruder without pre-drying, therefore it has an associated moisture, which is taken into account for the calculations. The liquid inputs correspond to two water injections named in the order of introduction as 1W and 2W. At the extruder outlet, two streams are recovered a liquid one called raw filtrate (RF) and a solid one called raw extrudate (RE). The raw extrudate is dried and its moisture content is estimated. On the other hand, the raw filtrate is centrifuged to separate the clarified filtrate (CF), solid-free liquid, and the sediment (S) the solid particles entrained by the filter. Further analyses of the CF are carried out (extractives yield, compounds identification, and total phenolic content determination). Both outputs are quantified for mass balance purposes.

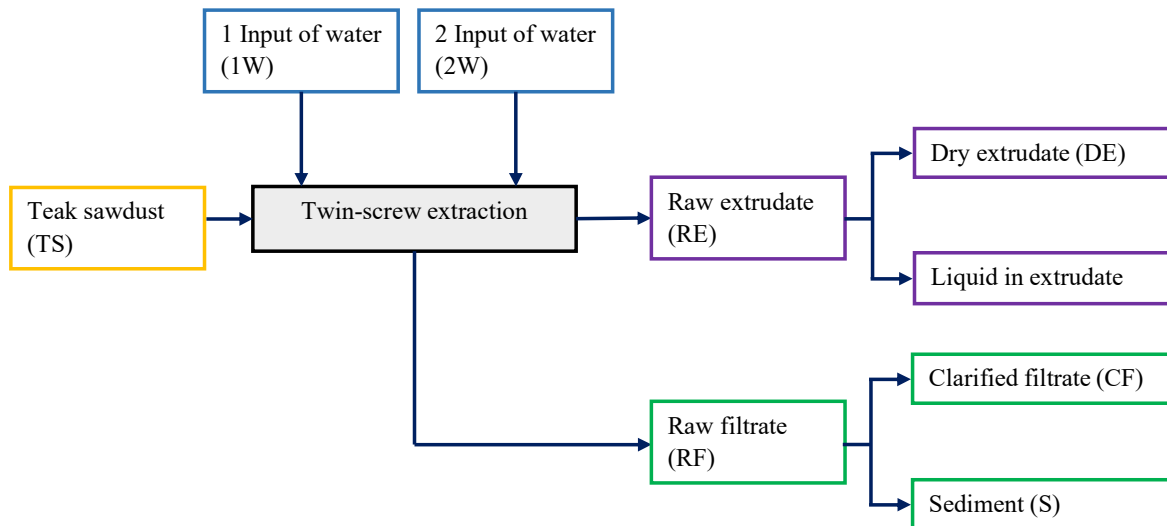


Figure 27. Schematic of solid/liquid extraction of teak sawdust using a twin-screw extruder

To observe the behavior of the material throughout the extruder barrel and to visualize the zones of material accumulation, a dead stop was conducted by suddenly stopping the extruder after reaching stationary conditions and material samples were taken from various zones of the barrel. The experimental point where sampling was carried out was at  $T= 100^{\circ} \text{C}$  and  $L/S= 3.25$  (the measured values were average values over the sampling zone).

It is found that the dry matter decreases in each zone including a water contribution and increases strongly after filtration. The photos confirm the absence of empty zones. The extruder is globally well filled with the presence of more compacted zones at the level of the stress zones, see Figure 28.

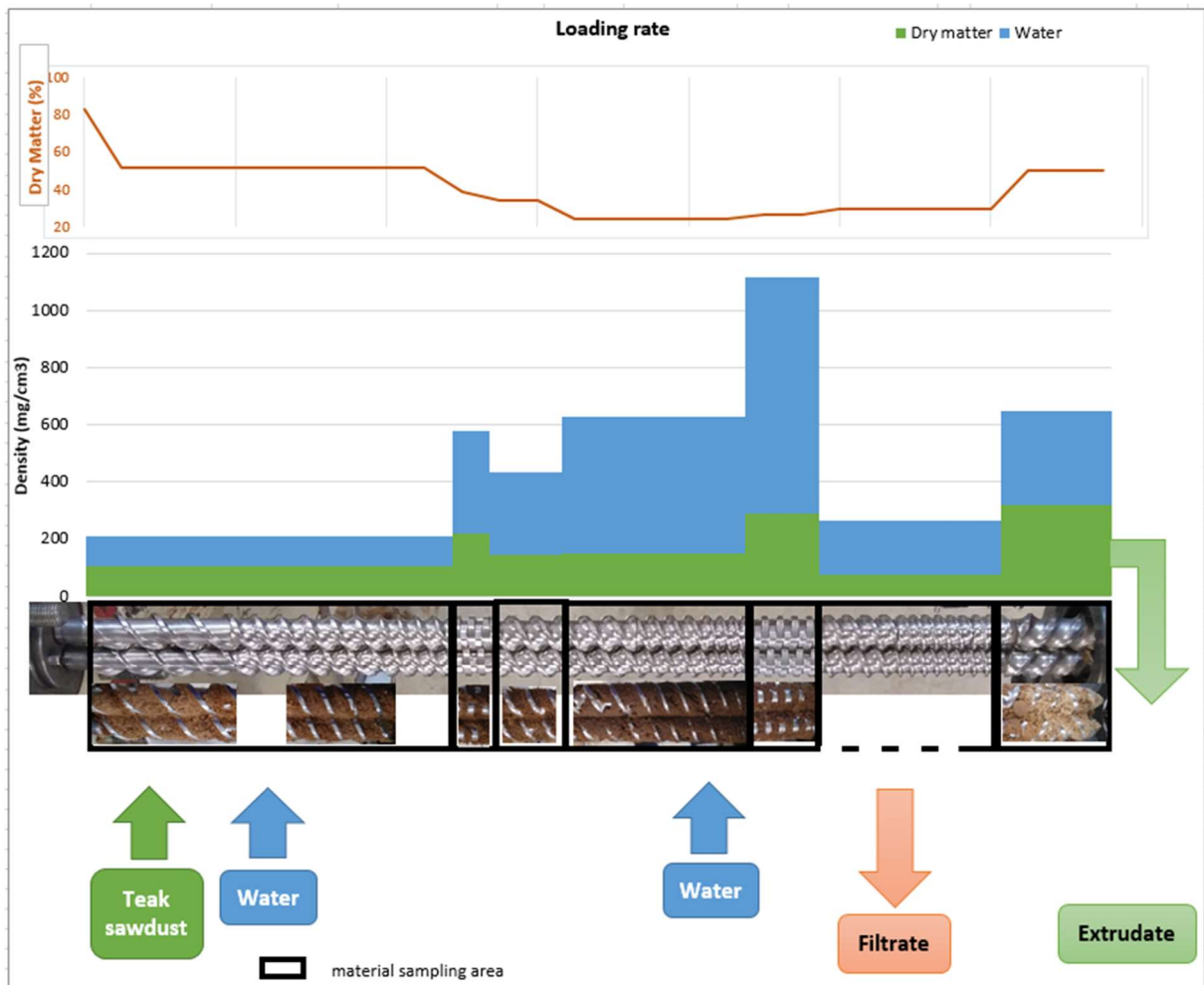


Figure 28. Diagram of the distribution of solid and liquid material along the screw profile after a dead stop and progressive opening of the extruder BC21

Table 23 presents the parameters and the process flow rates (inputs and outputs) of the experimental design and Table 24 shows the mass balance.

The following notation is used for the input flow rates:

$Q_{TS}$ : flow rate of teak sawdust (Kg/h)

$DM_{TS}$ : dry matter of teak sawdust (82.36 %)

$Q_{DTS}$ : flow rate of dry teak sawdust (Kg/h)  $Q_{DTS} = Q_{TS} * DM_{TS}$

$Q_{1W}$ : flow rate of first water input (Kg/h)

$Q_{2W}$ : flow rate of second water input (Kg/h)

The liquid/solid ratio (L/S) in module 5 is calculated by the equation:

$$\frac{L}{S} = \frac{Q_{1W} + Q_{2W} + Q_{TS}(1 - DM_{TS})}{Q_{TS} * DM_{TS}}$$

The following notation is used for the output flow rates:

$Q_{RE}$ : flowrate of raw extrudate (Kg/h)

$DM_{RE}$ : dry matter of raw extrudate

$Q_{DE}$ : flowrate of dry extrudate (Kg/h)  $Q_{DE} = Q_{RE} * DM_{RE}$

$Q_{RF}$ : flowrate of raw filtrate (Kg/h)

$Q_{DS}$ : flowrate of dry sediment (in the raw filtrate) (Kg/h)

$Q_{CF}$ : flowrate of clarified filtrate (Kg/h)  $Q_{CF} = Q_{RF} - Q_{DS}$

The total mass balance error is calculated by the equation:

$$\text{Total mass balance error} = \frac{\text{Total input} - \text{Total output}}{\text{Total input}} * 100$$

$$\text{Total mass balance error} = \frac{(Q_{1W} + Q_{2W} + Q_{TS}) - (Q_{RE} + Q_{RF})}{(Q_{1W} + Q_{2W} + Q_{TS})} * 100$$

The dry solid mass balance error is calculated by the equation:

$$\text{Total dry solid mass balance error} = \frac{Q_{DTS} - (Q_{DE} + Q_{DS})}{Q_{DTS}} * 100$$



Table 23. Variables and process flows for the study of the influence of temperature and L/S ratio on the solid/liquid extraction of teak sawdust using a twin-screw extruder BC21 (Cleextral)

Exp. point	Inputs				Outputs							
	Process variables		Teak sawdust		Water		Extrudate			Filtrate		
	Temp. (° C)	Ratio (L/S) <sup>1</sup>	Q <sub>RTS</sub> (Kg/h)	Q <sub>DTS</sub> (Kg/h)	Q <sub>1w</sub> (Kg/h)	Q <sub>2w</sub> (Kg/h)	Q <sub>RE</sub> (Kg/h)	DM <sub>RE</sub> (%)	Q <sub>DE</sub> (Kg/h)	Q <sub>RF</sub> (Kg/h)	Q <sub>CF</sub> (Kg/h)	Q <sub>DS</sub> (Kg/h)
1	100	3.75	2.05	1.69	1.41	4.57	1.73	80.9	1.40	4.81	4.62	0.19
1	100	3.75	2.24	1.85	1.41	4.77	2.19	72.7	1.59	3.94	3.75	0.19
1	100	3.75	2.24	1.85	1.41	4.77	1.97	80.7	1.59	4.86	4.64	0.22
2	150	3.75	2.16	1.78	1.41	4.77	2.05	76.3	1.56	4.70	4.51	0.19
3	125	4.25	2.16	1.78	1.41	5.63	1.97	76.7	1.51	5.64	5.43	0.21
4	75	4.25	2.16	1.78	1.41	5.63	2.13	72.5	1.55	4.72	4.54	0.18
5	50	3.75	2.24	1.85	1.41	4.77	2.30	72.2	1.66	5.02	4.84	0.18
6	75	3.25	2.36	1.94	1.41	4.77	2.12	72.3	1.53	5.76	5.45	0.31
7	125	3.25	2.24	1.85	1.41	3.91	2.20	74.0	1.63	3.75	3.56	0.19

1. in module 5

Table 24. Mass balance of the solid/liquid extraction of teak sawdust using a twin-screw extruder BC21 (Clextral)

Process variables			Mass balance					
Exp. point	Temp. (° C)	Ratio (L/S)	Total input (Kg/h)	Total output (Kg/h)	Total balance error (%)	Total dry solid input (Kg/h)	Total dry solid output (Kg/h)	Total dry solid balance error (%)
1	100	3.75	8.03	6.54	18.5	1.69	1.59	5.98
1	100	3.75	8.42	6.13	27.2	1.85	1.79	3.33
1	100	3.75	8.42	6.84	18.8	1.85	1.82	1.70
2	150	3.75	8.34	6.75	19.1	1.78	1.76	1.37
3	125	4.25	9.20	7.62	17.2	1.78	1.72	3.20
4	75	4.25	9.20	6.85	25.5	1.78	1.73	3.05
5	50	3.75	8.42	7.33	13.0	1.85	1.84	0.16
6	75	3.25	8.54	7.88	7.7	1.94	1.84	5.11
7	125	3.25	7.56	5.95	21.3	1.85	1.82	1.46

The mass balance is the practical application of the law of conservation matter. It considers the input, output, and distribution of a substance between streams in a process. In this case, the extractions are performed in a steady-state, there is no mass accumulation in the system. Therefore, the mass entering the system is equal to the mass leaving the system.

In the total mass balance (see Table 24), the difference between the total input and the total output with respect to the total input is due to small variations in flow rates that may occur at the pumps and solids feeder hopper, as well as unquantified material in the output streams. During the extraction process, part of the liquid is lost by evaporation at the level of the filter and the extruder exit. There is also a loss of solid matter due to water vapor entrainment. According to the mass balance applied to the dry solid matter, it is found that the maximum difference is 6 %.

The evaluated responses of the variation of temperature and L/S ratio of the experimental points are the following (see Table 25):

- Dry matter in the extrudate ( $DM_{RE}$ )

The dry matter in the extrudate gives information on the efficiency of the pressing performed by the reversed double-thread screw in module 7. The higher the value, the more efficiently the material has been pressed and the more liquid has been evacuated by the filter.

- Dry extrudate yield ( $Y_{DE}$ )

$$Y_{DE} = \frac{Q_{RE} * DM_{RE}}{Q_{TS} * DM_{TS}} * 100$$

The dry extrudate yield is an indicator of the amount of dry matter extruded in relation to the amount of dry matter fed. This value decreases according to the material extracted by filtration in the form of sediments (solid particles entrained by the filter) and compounds solubilized.

- Filtrate recovery ( $R_F$ )

$$R_F = \frac{Q_{RF} * (1 - DM_{RF})}{Q_{1W} + Q_{2W} + Q_{TS}(1 - DM_{TS})} * 100$$

The filtrate recovery indicates the amount of liquid recovered by the filter in relation to the total amount of liquid fed to the extruder.

- Dry sediment yield ( $Y_{DS}$ )

$$Y_{DS} = \frac{Q_{DS}}{Q_{TS} * DM_{TS}} * 100$$

The dry sediment yield indicates the amount of dry matter entrained by the filter in relation to the amount of dry matter fed.

- Extractives yield ( $Y_E$ )

$$Y_E = \frac{Q_{CF} * DM_{CF}}{Q_{TS} * DM_{TS}} * 100$$

The extractives yield indicates the amount of solubilized compounds recovered through the filter.

- Secondary metabolites identification

The clarified filtrate of each extraction was analyzed by HPLC-DAD (Dionex Ultimate 3000, Thermo Scientific) in order to identify and quantify the secondary metabolites extracted. The compounds were identified by the comparison of the retention time and UV spectrum recorded between 200 nm and 400 nm with those obtained by standards. The quantification was conducted at 254 nm.

- Total phenolic content (TPC)

Another property evaluated in the clarified filtrates is their reducing capacity associated with the total phenolic content (TPC). It is determined by the Folin-Ciocalteu colorimetric assay and results are expressed as gallic acid equivalent (GAE).

Table 25. Responses of the solid/liquid extraction of teak sawdust using a twin-screw extruder BC21 (Clextral)

Process variables			Extrudate		Filtrate	Extractives analysis			
Exp. point	Temp. (° C)	Ratio (L/S)	Dry extrudate	Dry matter in the extrudate	Filtrate recovery R <sub>F</sub> (%)	Dry sediment yield	Extractives yield Y <sub>E</sub> (%)	Total phenolic content	Anthraquinone-2-carboxylic acid (mg/100 g teak) <sup>2</sup>
			Y <sub>DE</sub> (%)	DM <sub>RE</sub> (%)		Y <sub>DS</sub> (%)		(g GAE/100 g teak) <sup>1</sup>	
1	100	3.75	83.0	80.9	72.9	11.1	1.34	0.58	2.68
1	100	3.75	86.2	72.7	57.0	10.4	1.36	0.57	2.07
1	100	3.75	86.2	80.7	70.6	12.1	1.33	0.59	2.67
2	150	3.75	87.8	76.3	68.7	10.8	1.41	0.67	3.17
3	125	4.25	85.1	76.7	73.2	11.7	1.48	0.58	2.14
4	75	4.25	87.0	72.5	61.1	10.0	1.29	0.32	2.18
5	50	3.75	89.9	72.2	73.6	9.9	1.02	0.52	2.03
6	75	3.25	78.8	72.3	82.7	16.1	1.27	0.63	2.57
7	125	3.25	88.3	74.0	62.3	10.3	1.41	0.44	2.68
Standard deviation <sup>3</sup>			1.9	4.7	8.6	0.9	0.01	0.01	0.35

1. Folin-Ciocalteu method. TPC expressed as g of gallic acid equivalent (GAE) per 100 gram of dry weight. Average of triplicated. BMG-Labtech Spectrostar-Nano, 96-well microplate. Absorbance recorded at 700 nm. Temperature: 45°C. Incubation time: 45 min.

2. HPLC-DAD Dionex Ultimate 3000 (Thermo Scientific). Column Luna 3u PFP(2)100Å, 150 x 3.0 mm (Phenomenex). UV spectrum recorded between 200 nm and 400 nm. Quantification at 254 nm. Results expressed on dry weight basis

3. Standard deviation calculated from the triplicated of the point L/S= 3.75, T= 100° C

### 3.1.4.1 Responses of the solid/liquid extraction of teak sawdust using a twin-screw extruder

#### BC21: analysis of experimental

The experimental design is analyzed from two angles, on the one hand, the aspect of mass balance and filtration efficiency, and on the other hand, the extraction of extracts with chemical properties of interest. To facilitate the visualization of the whole range of influence of the variables, the representation of the results through response surfaces is presented in Figure 29 and Figure 32.

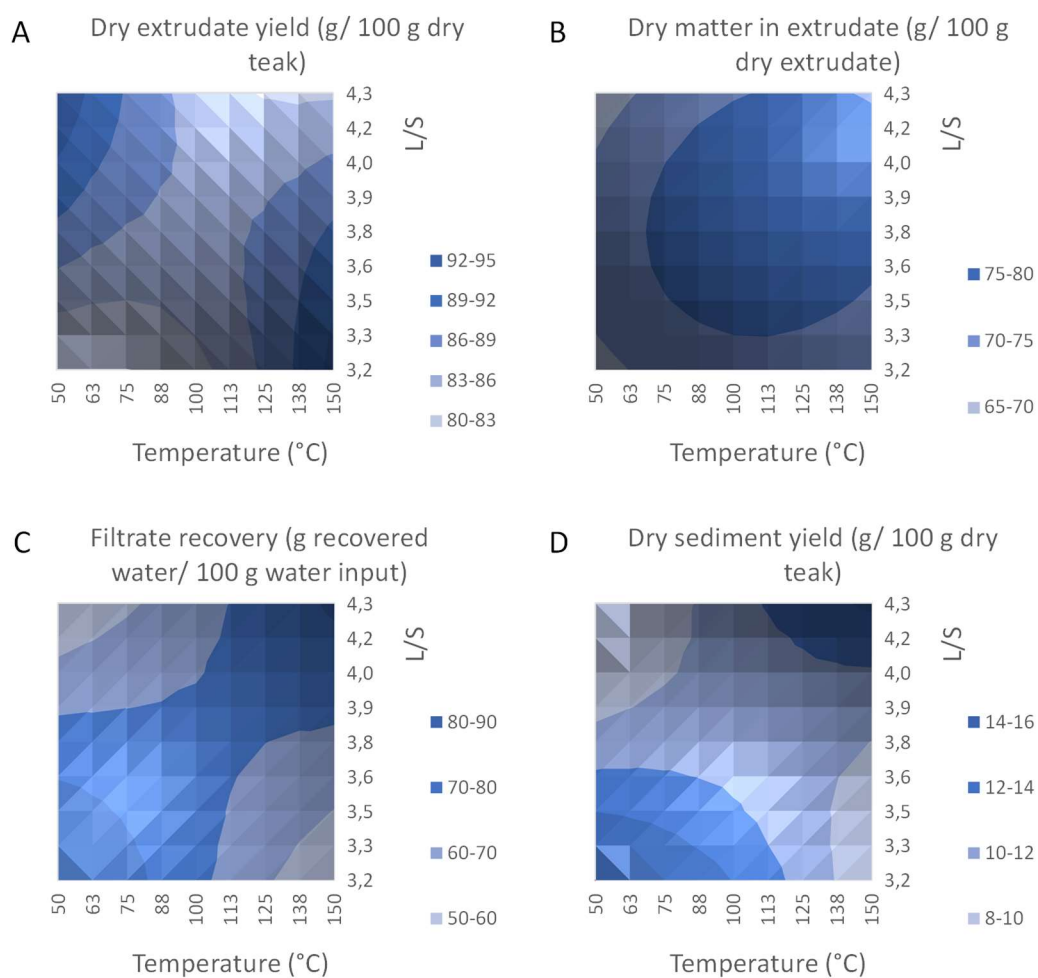


Figure 29. Response surfaces of experimental design of teak sawdust extraction using a BC21 extruder. Effects of extraction efficiency on A: dry extrudate yield (g/100 g dry teak); B: dry matter in extrudate (g/100 g dry extrudate); C: filtrate recovery (g recovered water/100 g dry matter); D: dry sediment yield (g/100 g dry teak)

The efficiency of the pressing, which is carried out by the counter-pressure exerted by the reverse screw conveying the mixture upstream and the sequence of direct pitch screws conveying the material

downstream, could be evaluated by the amount of dry matter in the extrudate ( $DM_{RE}$ ) and the filtrate recovery ( $R_F$ ).

According to the response surface, the high dry extrudate yield (80 % - 90 %,  $SD \pm 1.9$  %), the content of dry matter in the extrudate (70 %-80 %,  $SD \pm 4.7$  %), as well as the elevated filtrate recovery (60 %-80 %,  $SD \pm 8.6$  %) demonstrate a good solid/liquid separation. The predominant range of  $Y_{DE}$  is found in the central values of the experimental design. The highest values of  $DM_{RE}$  are found in the intermediate conditions of T and L/S ratio and decrease radially. Regarding the  $R_F$ , the results vary over a considerably wide range, the high standard deviation does not allow a conclusive discussion, however, the prevalent percentages are those around 70 %.

The mechanical work exerted inside the extruder mainly in zones that generate a strong shear causes the breaking and defibration of the material. Figure 30 shows extrudates obtained with different operational conditions, as well as the sediment. Comparing the extrudates with the raw sawdust (see Figure 23), it is observed that the material has been shredded. On the other hand, when comparing the sediment with the raw sawdust, defibration is not observed, even the particle size distribution (measured by means of laser diffraction, see Figure 31) is similar with values of  $D[4;3]$ : 403  $\mu\text{m}$  and  $D_v(50)$ : 346  $\mu\text{m}$  for raw sawdust, and  $D[4;3]$ : 401  $\mu\text{m}$  and  $D_v(50)$ : 343  $\mu\text{m}$  for the sediment. This means that the greatest mechanical work is carried out after the filter, by the action of the CF2C screw element.

Concerning the particle size of the extrudates, several attempts were made to determine it by sieving and laser diffraction, however, no congruent results were obtained. The material is difficult to handle due to its very small size (like flour) and hygroscopicity. The fibers separated after mechanical treatment tend to re-agglomerate after drying to form clumps that are difficult to separate during particle size measurements.

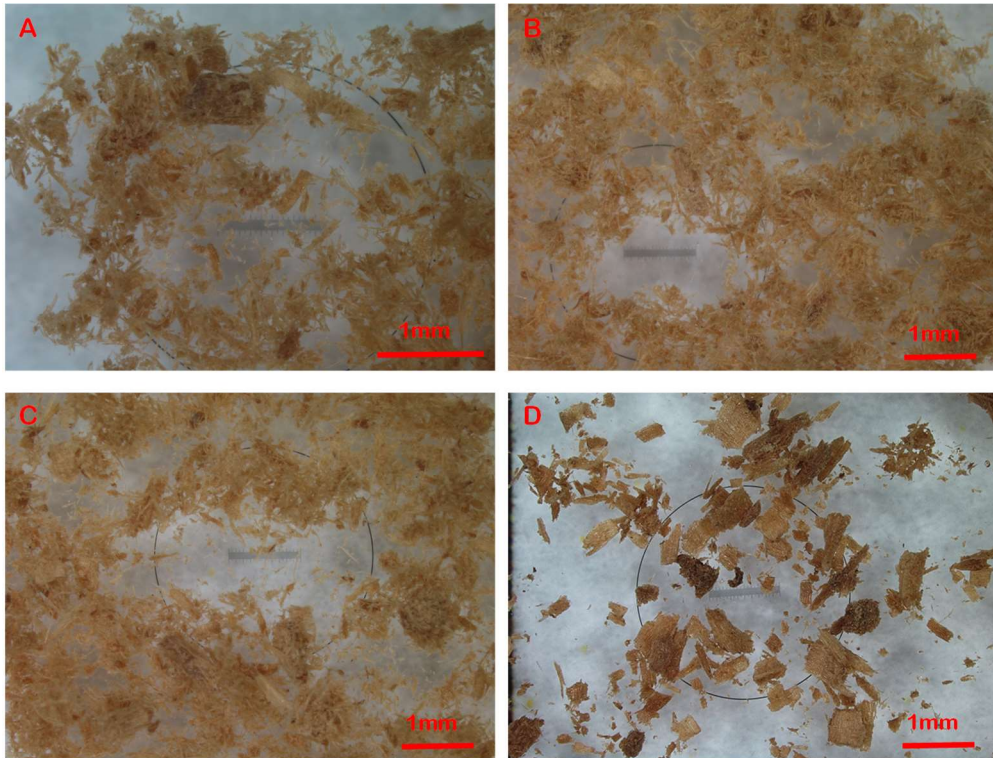


Figure 30. Magnified photographs of teak extrudates from extraction process by means of twin-screw extruder BC21 (Clextal). A: teak extrudate at T=75 °C and L/S=3.75; B: teak extrudate at T=100 °C and L/S=3.75; C: teak extrudate at T=125 °C and L/S=3.25; D: sediment from extraction at T=100 °C and L/S=3.75

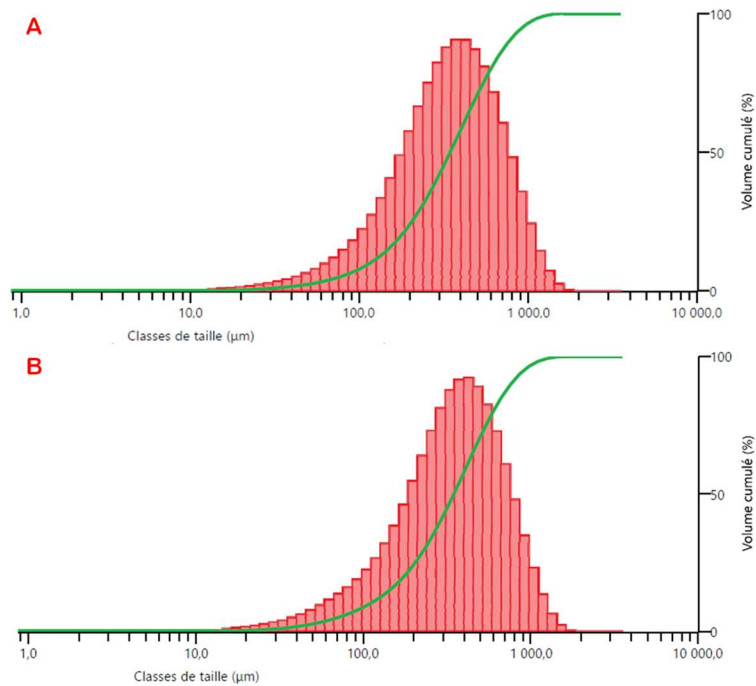


Figure 31. Particle size distribution measure by means of laser diffraction (Mastersizer 3000, Malvern). A: Raw teak sawdust B: Sediment from extraction at T=100 °C and L/S=3.75



Concerning the extractives content, it is observed that the change of the process conditions does not have a significant impact on this value, the majority values are between 1.3 g/100 g dry teak and 1.5 g/100 g dry teak. Contrasting these results with those of the previous study, it is observed that in a very short contact time between the solvent and substrate, about 1min (Celhay et al., 2014), the extruder allows to extract up to 37.5 % of the potential quantified by a Fibertec device for 2h (4 g/100 g dry teak, see Table 11 in Chapter 2).

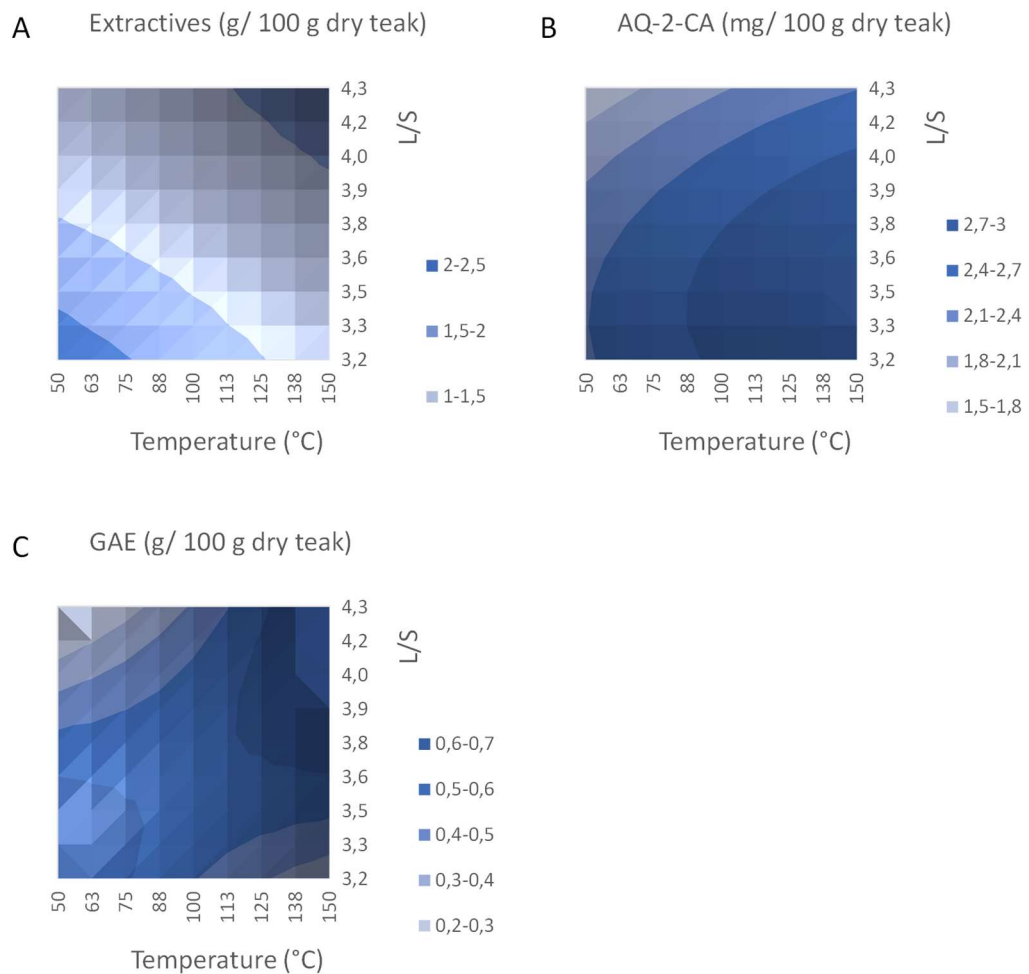


Figure 32. Response surfaces of experimental design of teak sawdust extraction using a BC21 extruder. Effects of extraction efficiency on A: extractives content (g/100 g dry teak); B: anthraquinone-2-carboxylic acid (g/100 g dry teak); C: Gallic acid equivalent (g/100 g dry teak)

Two additional points were performed ( $T= 100^{\circ} \text{C}$ ,  $L/S= 3.25$ ; and  $T= 100^{\circ} \text{C}$ ,  $L/S= 3.45$ ) in order to see more clearly the effect of L/S keeping the temperature constant. The results are presented in Figure 25 to Figure 27

Table 26. Variables and process flows for the study of the influence of temperature and L/S ratio on the solid/liquid extraction of teak sawdust using a twin-screw extruder BC21 (Cleextral). Additional points

Process variables		Inputs				Outputs					
		Teak sawdust		Water		Extrudate			Filtrate		
Temp. (° C)	Ratio (L/S)	Q <sub>RTS</sub> (Kg/h)	Q <sub>DTS</sub> (Kg/h)	Q <sub>1w</sub> (Kg/h)	Q <sub>2w</sub> (Kg/h)	Q <sub>RE</sub> (Kg/h)	DM <sub>RE</sub> (%)	Q <sub>DE</sub> (Kg/h)	Q <sub>RF</sub> (Kg/h)	Q <sub>CF</sub> (Kg/h)	Q <sub>DS</sub> (Kg/h)
100	3.25	2.05	1.69	1.38	3.91	1.72	80.7	1.39	3.95	3.80	0.15
100	4.25	2.05	1.69	1.38	5.63	1.79	79.7	1.43	5.66	5.48	0.18

Table 27. Mass balance of the solid/liquid extraction of teak sawdust using a twin-screw extruder BC21 (Cleextral). Additional points

Process variables		Mass balance					
Temp. (° C)	Ratio (L/S)	Total input (Kg/h)	Total output (Kg/h)	Total balance error (%)	Total dry solid input (Kg/h)	Total dry solid output (Kg/h)	Total dry solid balance error (%)
100	3.25	7.34	5.67	22.75	1.69	1.54	8.88
100	4.25	9.06	7.45	17.78	1.69	1.61	4.78

Table 28. Responses of the solid/liquid extraction of teak sawdust using a twin-screw extruder BC21 (Clextral). Additional points

Process variables		Extrudate		Filtrate	Extractives analysis			
Temp. (° C)	Ratio (L/S)	Dry	Dry matter	Filtrate	Dry	Extractives	Total phenolic	Anthraquinone-2-
		extrudate	in the	recovery	sediment			
		Y <sub>DE</sub> (%)	DM <sub>RE</sub> (%)	R <sub>F</sub> (%)	Y <sub>DS</sub> (%)	Y <sub>E</sub> (%)	(g GAE/100 g teak) <sup>1</sup>	(mg/100 g teak) <sup>2</sup>
100	3.25	82.1	80.7	67.3	9.1	1.33	0.45	2.41
100	4.25	84.7	79.7	74.3	10.5	1.34	0.63	2.71

1. Folin-Ciocalteu method. TPC expressed as g of gallic acid equivalent (GAE) per 100 gram of dry weight. Average of triplicated. BMG-Labtech Spectrostar-Nano, 96-well microplate. Absorbance recorded at 700 nm. Temperature: 45°C. Incubation time: 45 min.

2. HPLC-DAD Dionex Ultimate 3000 (Thermo Scientific). Column Luna 3u PFP(2)100Å, 150 x 3.0 mm (Phenomenex). UV spectrum recorded between 200 nm and 400 nm. Quantification at 254 nm. Results expressed on dry weight basis

3. Standard deviation calculated from the triplicated of the point L/S= 3.75, T= 100° C

Considering the  $DM_{RE}$ , for the three L/S ratio evaluated the maximum values are reached at 100 °C. Taking the results at 75° C it can be observed that the increase of L/S from 3.25 to 4.25 has no effect on the  $DM_{RE}$ , while at 125° C the increase of L/S increases the  $DM_{RE}$ . Regarding the filtrate recovery, overall at 100° C and 125 °C the percentage of  $R_F$  increases when the L/S increases, on the other hand, at a fixed L/S of 3.25 and 3.75 the filtrate recovery decreases when the temperature increases. Concerning the dry extrudate yield, at L/S ratio of 3.75 and 4.25, the lowest percentage is observed at 100° C. On the other hand, at 3.25 the  $Y_{DE}$  increases when the temperature increases.

Regarding the analysis of the clarified filtrate, the compounds searched with the original standard as a reference were 2-hydroxymethyl anthraquinone, anthraquinone-2-carboxylic acid (AQ-2-CA), lapachol, and 2-methyl anthraquinone. However, only AQ-2-CA and lapachol have been identified and the latter in amounts less than 1 mg/100g dry teak. Therefore, AQ-2-CA is the compound on which the effect of the process variables has been evaluated.

By way of example, Figure 33 shows chromatograms of the extracts. In these chromatograms, which are representative of all conditions studied, it is observed that the peak corresponding to AQ-2-CA is the one found in the highest proportion, therefore it can be affirmed that it is the compound extracted in greater quantity. At the L/S value of 3.75 the higher the temperature, the greater the amount of this phenolic compound is extracted. Comparing these values with that determined using an ASE device and water as a solvent for 32 min (2 mg AQ-2-CA/100 g dry teak), it can be seen that extractions using an extruder allow the recovery of a slightly higher amount of anthraquinone-2-carboxylic acid (from 1.3 mg/ 100 g teak to 3 mg/ 100 g teak) in the whole range of conditions studied. On the other hand, the result of the extraction ASE performed for 2h (8 mg/100 g teak, see Table 12 in Chapter 2) indicates that potentially higher recovery values could be achieved. The extraction time is a very important variable to take into account in extractions.

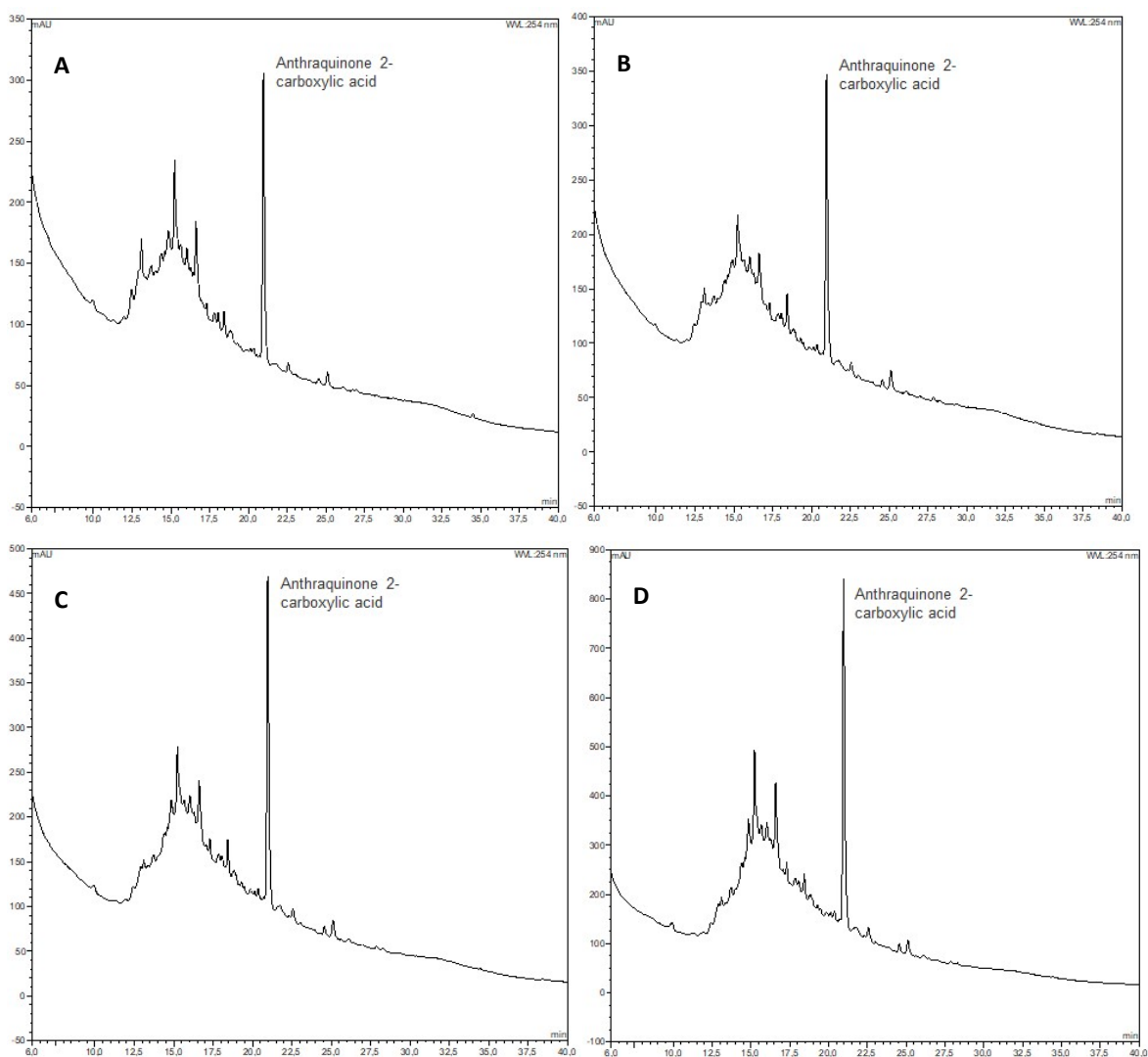


Figure 33. Chromatograms of the aqueous extracts from teak sawdust extraction using a twin-screw BC21 (Cletral). A) L/S: 3.75 and T: 50° C. B) L/S: 3.75 and T: 100° C. C) L/S: 3.5 and T: 150° C. D) L/S: 3.25 and T: 100° C (HPLC-DAD Dionex Ultimate 3000 (Thermo Scientific). Column Luna 3u PFP(2)100Å, 150 x 3.0 mm (Phenomenex)

Considering the TPC in the clarified filtrates, phenolic contents ranges from 0.3g GAE/100 g teak to 0.67 g GAE/100 g teak. Considering the test at L/S= 3.75 it is observed that with increasing temperature the TPC also increases, the opposite case at L/S= 3.25 where with increasing temperature the TPC decreases. On the other hand, at T= 100° C, the increase in the L/S ratio favors the polyphenols content. Comparing the reducing capacity of the extracts obtained by extrusion with that of the extract by Fibertec device (4.1 g GAE/100 g teak). It is shown that using twin-screw technology, it is possible to achieve up to 15 % of the TPC highest value quantified for teak sawdust aqueous extraction.

The values obtained in this study are comparable even higher to extracts of other types of woody by-products also using an extruder as an extraction method. There have been reported figures from 0.11g GAE/100g dry matter to 0.21g GAE/100g dry matter for maritime pine knots; 0.09g GAE/100g dry

matter to 0.16g GAE/100g dry matter for aspen knots; 0.26g GAE/100g dry matter to 0.97 g GAE/100g dry matter for aspen bark, for different conditions (temperature between 50°C to 150°C and a wide range of L/S ratio) performed with a extruder BC45 (Clextral) (Celhay et al., 2014).

### 3.1.4.1 Antioxidant activity assays

The free radical scavenging activity of the extracts is studied using the DPPH antioxidant assay, which represents the ability of a complex mixture of compounds to quench the free radical DPPH. Figure 34 and Figure 35 illustrate the effects of temperature and L/S ratio on the antioxidant activity, respectively. The displayed range of concentrations corresponds to the range following a linear response, which in some cases is not very wide. In general, DPPH has a relatively small linear reaction range of only from 2 to 3-fold (Prior et al., 2005). Various antioxidant mechanisms are involved in DPPH radical scavenging assay, and they are not the same for every single compound present in the extract (Chen et al., 2013). In general, the antioxidant activity of the teak extracts is not very high.

The antioxidant potential is usually expressed using the  $IC_{50}$  value, defined as the concentration of antioxidants that causes a 50 % decrease in the DPPH radical absorbance. However, 50 % inhibition is not achieved in all cases or it is not within a range of concentrations that allows a reliable determination. This first part describes the overall effect of the variables evaluated and the calculation of DPPH inhibition concentration is covered below.

It is known that in the estimation of the antioxidant activity of biological samples the chromogens (in this case DPPH radical) have an important limitation because interferences at specific wavelengths may occur (Arnao, 2000), for instance, colored antioxidant compounds from plant-derived materials have spectra that overlap DPPH (Locatelli et al., 2009). The aqueous extracts are of a dark brown color (see Figure 26.G) and even when diluted, the intensity of the color is still strong, which is linked to the presence of colored molecules bearing polyphenolic and/or anthraquinonic structures. According to the studies dealing with this problem, following the decrease in absorbance at 516nm resulting from the increase in concentration, the absorbance value becomes therefore modified by the presence of colored extracts, even when working with minimal sample volumes. The interferences could also arise due to the apparition of secondary reaction products between the chromogen and the samples being analyzed. Therefore, it is advisable to choose a wavelength far from the visible region to avoid interferences. However, in the case of DPPH the problem is serious since its maximum absorbance range is between 515 and 520 nm. Thus, at wavelengths near the visible region, the antioxidant activity measured is underestimated due to sample interferences (Arnao, 2000). Moreover, the assay is not a competitive reaction because DPPH is both a radical probe and oxidant. DPPH color can be lost via either radical reaction or reduction as well as unrelated reactions, and steric accessibility is a major determinant of the reaction (Prior et al., 2005). To avoid an inadequate determination that could lead to an underestimation of the antioxidant capacity due to the interference caused by the presence of colored compounds, the absorbance contribution of the color was subtracted. Therefore, it could explain why the linearity of the curve is not observed for the totality of extracts.

When comparing different temperatures while keeping a fixed L/S ratio, see Figure 34, in general, the antioxidant capacity decreases with increasing temperature. For instance, for an L/S ratio of 3.25, if we take the concentration for a 50 % radical inhibition, we have 1.36 g/L, 1.51 g/L, and 2.03 g/L for 75°C, 100 °C, and 125 °C, respectively. The same effect is presented for an L/S of 3.75 with values of 2.06 g/L, 2.44 g/L, and 3.13 g/L for 50°C, 100 °C, and 150 °C, respectively. In the case of the highest L/S (4.25), it is also found that at the highest temperature the radical scavenging is the lowest (3.36 g/L at 125°C). This behavior could be attributed to the fact that the extracted compounds with antioxidant activity are thermosensitive, therefore, the increase in temperature decreases the activity of the extract by the decomposition of antioxidant compounds. It should also be considered that the extraction process using an extruder is different from the conventional ones. In the extruder, the mechanical work exerted on the material causes defibration and breakage of the cell wall of the material, which directly affects the interaction of the solvent with the material. The more intimate contact of the solvent with the substrate may favor the extraction of other compounds that do not necessarily exhibit an antioxidant capacity, resulting in a dilution effect of the extract activity. This is observed in the extract yields, whose highest values are obtained at the highest temperatures, for example, for T=150°C and L/S=3.75; and T=125°C and L/S=4.25, the extractives content are 1.41 % and 1.48 %, respectively.

Concerning the effect of the liquid to solid ratio with respect to temperature, see Figure 35, two kinds of behavior could be observed depending on the concentration range. In all cases, the best antioxidant activity at low concentrations is achieved with an L/S ratio of 3.25. Nevertheless, it is also at the lowest L/S that the change in concentration has the least influence on the DPPH radical inhibition. For instance, when extracting at 75 °C (see A) the slope of the curve at L/S 4.25 is steeper than that at 3.25 by more than 50 %, and consequently, the observed difference between the two curves in the percentage inhibition at low concentrations decreases with increasing concentration up to 3 g/L experimentally the same response would be obtained. This effect is most evident with the experiment at 125°C and L/S=3.25 (see Figure 35. C), where the least influence of extract concentration on the antioxidant capacity is observed.

This indicates that the ratio L/S has a strong influence on the variability of the extracted compounds. The accessibility of the antioxidant compounds to the solvent as well as their solubility herein is favored with a high ratio, giving a higher “priority” to antioxidant compounds, while a lower ratio sounds to favored extraction of non-antioxidant. These two extraction mechanisms may be co-existing depending on their solubility and diffusion in the solvent, therefore offering extracts of variable antioxidant capacity.



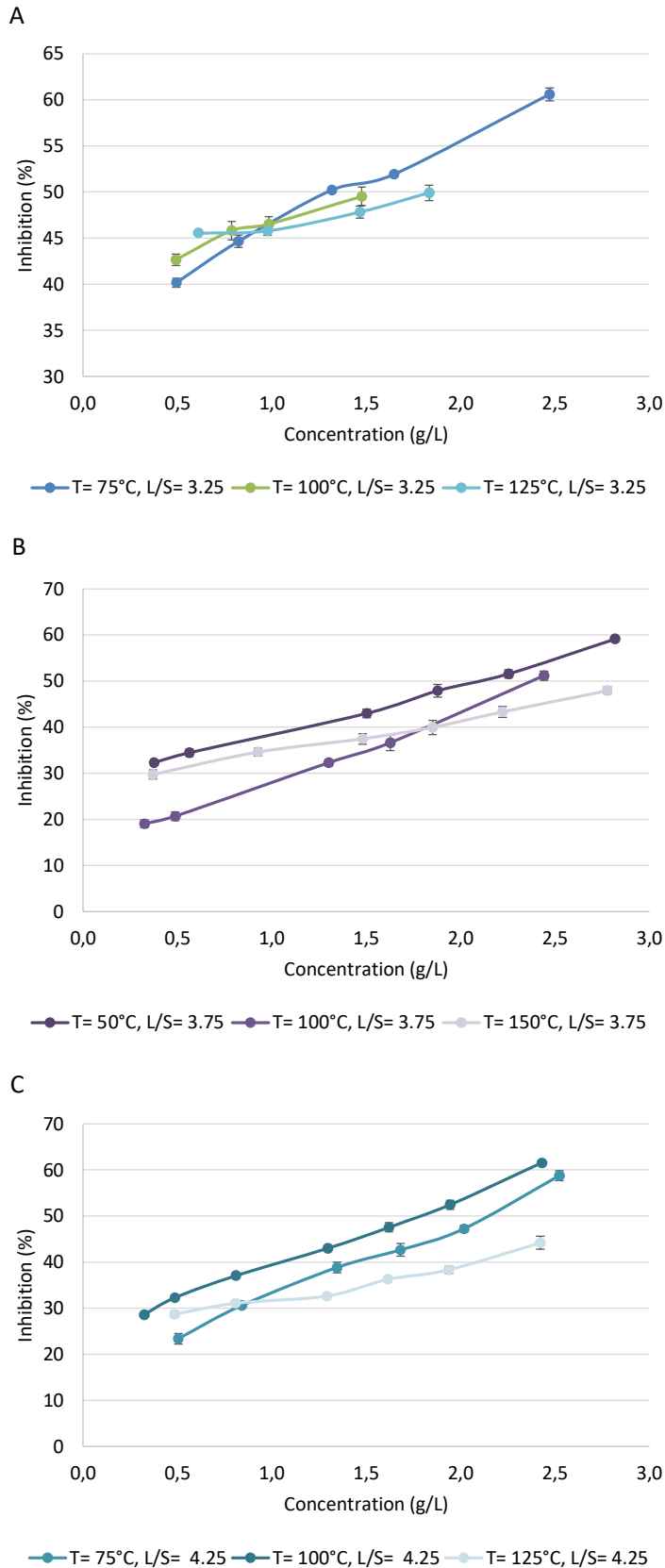


Figure 34. Effect of temperature in antioxidant activity. (A) L/S= 3.25, (B) L/S= 3.75, (C) L/S= 4.25 (DPPH radical scavenging assay, BMG-Labtech Spectrostar-Nano, 96-well microplate. Incubation time: 40 min at room temperature. Absorbance recorded at 516 nm)

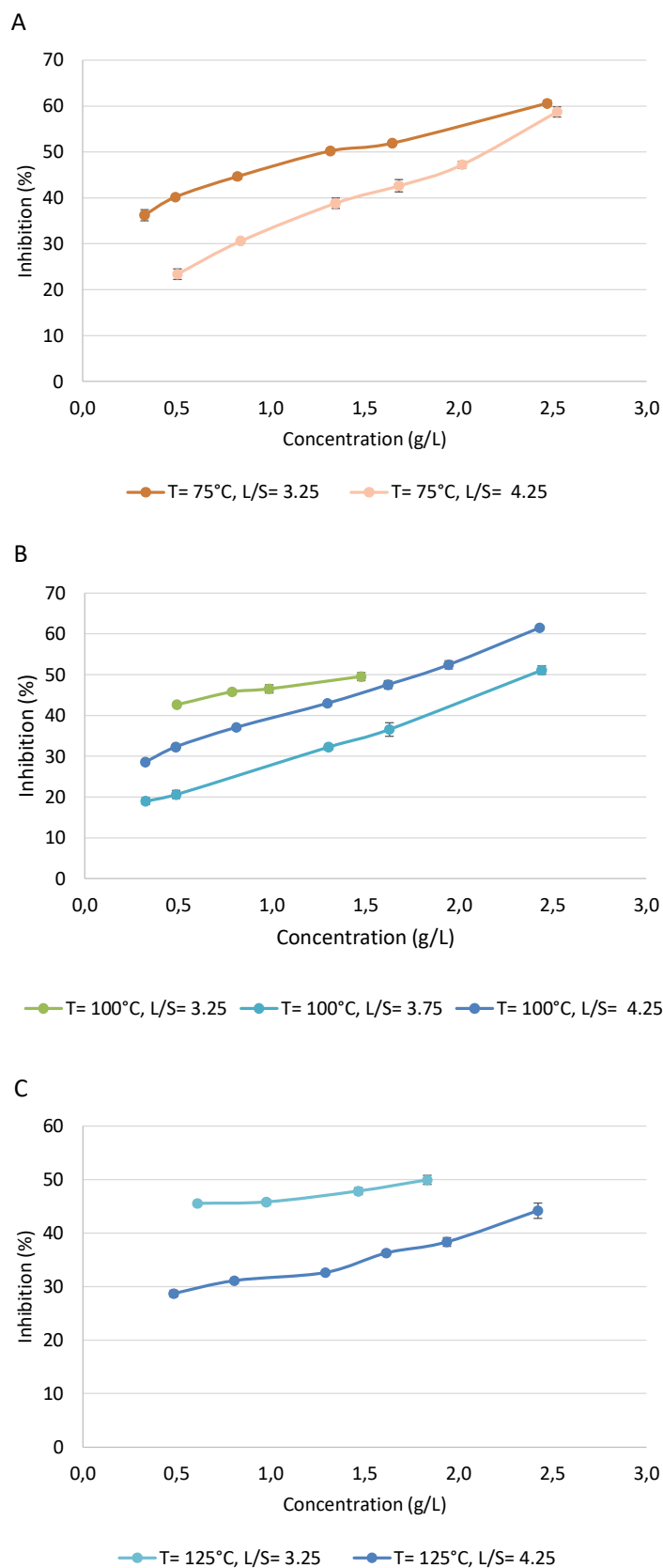


Figure 35. Effect of L/S ratio in antioxidant activity. (A) T= 75°C, (B) T= 100°C, (C) T= 125°C (DPPH radical scavenging assay, BMG-Labtech Spectrostar-Nano, 96-well microplate. Incubation time: 40 min at room temperature Absorbance recorded at 516 nm)

Although the determination of an IC value is not an exhaustive or discriminant assay for the measure of antioxidant activity, it is useful and interesting when comparing different extraction conditions. Considering that 50 % inhibition of DPPH radicals is not obtained for the whole extracts within the ranges of concentrations evaluated and to avoid the discrepancy between the observed values and those estimated through calculation models. The extracts were diluted in such a way that the response was linear for all extracts and a reliable value could be given for this assay. The concentration of the extract that scavenges 25 % of DPPH free radical (IC<sub>25</sub>) was then determined. Table 29 presents the results of the antioxidant capacity and summarizes other related responses.

From these results, it is noted that as the liquid to solid ratio increases, the radical scavenging activity decreases. When comparing, for instance, the values at a constant temperature of 75°C, the extract obtained at L/S of 4.25 needs to have twice the concentration than the one at L/S of 3.25 to reach the same percentage of inhibition. The same occurs at 125°C where IC<sub>25</sub> values of 0.539 g/L and 0.198 g/L are found for an L/S of 4.25 and 3.25, respectively. It also stands out that the effect of temperature is more evident at a low L/S ratio, in general, the increase in temperature favors the antioxidant capacity at concentrations lower than 0.5 g/L. For an L/S= 3.25, a difference of 50°C represents a 70 % difference between the concentrations giving the same IC<sub>25</sub>, while at L/S of 4.25 the concentrations only vary by less than 10 %.

Table 29. Antioxidant activity (IC<sub>25</sub>), total phenolic content (TPC), and secondary metabolites identified in aqueous extracts of teak sawdust using a twin-screw extruder BC21 (Clextal)

Experimental point	T (°C)	L/S	IC <sub>25</sub> (g/L) <sup>1</sup>	TPC	AQ-2-CA	Lapachol
				(mg GAE/g dry extract) <sup>2</sup>	(mg/g dry extract) <sup>3</sup>	(mg/g dry extract) <sup>3</sup>
1	100	3,75	0,409	429,76	1,89	0,26
2	150	3,75	nd	474,54	2,25	0,22
3	125	4,25	0,539	395,40	1,44	0,10
4	75	4,25	0,571	249,49	1,71	0,14
5	50	3,75	0,328	351,37	1,37	nd
6	75	3,25	0,285	318,24	1,43	0,09
7	125	3,25	0,198	312,21	1,98	0,18

1. DPPH assay. Antioxidant activity expressed as the concentration of the extract that inhibits 25% of radicals (IC<sub>25</sub>). BMG-Labtech Spectrostar-Nano, 96-well microplate. Incubation time: 40 min. Absorbance recorded at 516 nm. Temperature: room temperature

2. Folin-Ciocalteu method. TPC expressed as grams of gallic acid equivalent (GAE) per gram of dry extract. BMG-Labtech Spectrostar-Nano, 96-well microplate. Absorbance recorded at 700 nm. Temperature: 45°C. Incubation time: 45 min.

3. HPLC-DAD Dionex Ultimate 3000 (Thermo Scientific). Column Luna 3u PFP(2)100Å, 150 x 3.0 mm (Phenomenex). Quantification measured at 254 nm.

nd: not determined

The antioxidant capacity can be related to the total phenolic content considering that the DPPH radical scavenging is mainly attributed to the polyphenols that can donate an electron or hydrogen radical. The results from the experimental design confirm this positive correlation when extracting at lower temperatures, the higher the TPC, the better the antioxidant activity. On the other hand, at higher temperatures, this correlation is not observed. For instance, when comparing the L/S ratios at 75°C, it is observed that the extracts obtained at L/S=3.25 exhibit higher TPC (318.24 g GAE/g dry extract) than that at L/S= 4.25 (249.49 g GAE/g dry extract), as well as the lowest concentration that inhibits the 25 % of DPPH radicals (0.285 g/L and 0.571 g/L for L/S=3.25 and L/S=4.25, respectively).

According to the data, in general, for the same L/S, the increase in temperature leads to more interesting TPC values. Nevertheless, these greater TPC values do not necessarily exhibit interesting antioxidant activities. By increasing the temperature, the extraction yield is increased by the enrichment of non-antioxidant compounds that become accessible through a heated solvent. The non-antioxidant compounds contribute to diluting the active compounds, and therefore lowering the overall antioxidant activity of the extract.

The polyphenols identified in the extracts are AQ-2-CA and lapachol. There is a direct correspondence between the amount of AQ-2-CA and the antioxidant activity only at 100°C and 125 °C. For instance, at T=125 °C, the AQ-2-CA content is 1.98 mg/g dry extract and 1.44 mg/g dry extract for an L/S ratio of 3.25 and 4.25, respectively, and the IC<sub>25</sub> of 0.198 g/L and 0.539 g/L, for an L/S ratio of 3.25 and 4.25, respectively. Regarding the effect of the temperature, in general, at low L/S ratios, increasing temperature favors the extraction of AQ-2-CA. For example, at L/S=3.75 the values are 1.37 mg/ g dry extract, 1.89 mg/ g dry extract, and 2.25 mg/ g dry extract for 50 °C, 100 °C, and 150 °C, respectively.

In the same way that AQ-2-CA, the lapachol content in the extract obtained at 125°C shows a direct correlation with antioxidant capacity. However, there is no clear trend regarding the influence of temperature and the amount of solvent used.

The antioxidant activity of the standards of both phenolic compounds was also determined, AQ-2-CA shows a value of 7.5 % inhibition of DPPH radicals with a concentration of 0.16 g/L. For lapachol; an inhibition of DPPH radicals 25 % is reached with a concentration of 0.081 g/L. Lapachol could be then attributed a greater contribution to the antioxidant capacity of the extracts than anthraquinone. However, this compound is found in a lower proportion than AQ-2-CA. The extracts have a complex and heterogeneous chemical composition and the interaction mechanisms with the organic nitrogen radical DPPH are as well complex and synergistic effects could be involved. Therefore, it is difficult to attribute the low antioxidant activity of the extracts to the weak presence of lapachol. Its antioxidant capacity has been found to be around 566 mg/L (IC<sub>50</sub>), which remains low to impute the antioxidant to its presence.

On the other hand, considering the results of the literature, the values of this study are lower. For instance, in an evaluation of the scavenging activity of the polysaccharides (isolated from water-soluble fraction) of stem bark of *Tectona grandis* from Assam, India, it was found a 62.93 % of inhibition at the concentration of 1.0 g/L (Rana et al., 2014). The authors attributed the radical scavenging to the polysaccharides, but they did not measure the polyphenols content in this study. This difference could easily be explained by the difference of vegetal material because stem bark contains polyphenolic compounds in high concentration, while sawdust is mainly constituted of inner parts of the tree without bark.

In terms of content of TPC, the pilot extracts obtained by water extraction under mild subcritical conditions offer values between 249 mg GAE/g extract and 351 mg GAE/g extract. With such a concentration of polyphenols, the antioxidant activity should have been better.

By firstly comparing TPC in water ASE, the variation of extraction time under subcritical conditions from 30 min to 120 min allows to double the level of extracted polyphenols from 2.31 up to 5.53 mg GAE/g teak, showing the factor time is really efficient, and partly explaining why the polyphenols are “poorly” extracted by means of extrusion. The gain in antioxidant capacity ( $IC_{25}$ ) is around 10 times higher in 120 minutes extraction than in 30 minutes. Previous studies on wood extraction by extrusion have shown that the residence time of the matter, at a subcritical water pressure, is around 1 minute (Celhay et al., 2014). The contact time between the solvent and teak is, therefore, an important factor to access to polyphenolic compounds with great antioxidant capacity.

Comparing the antioxidant potential of the extracts obtained by means of the twin-screw technology with that of the extract using an ASE system and water as a solvent for 30 min whose  $IC_{50}$  is 0.662 g/L, the results of extraction using an extruder present a lower DPPH radical scavenging activity for all the conditions studied. Moreover, the volume of water related to volume of teak is quite low in the extrusion experiments between 3.25 and 4.25, increases to 16 in ASE experiments, and rise up to 100 in Fibertec experiments. Regarding the consequent antioxidant capacities, the active compounds are better extracted with a large ratio L/S, and the resulting extract offers a strong antioxidant activity, linked to a large content of polyphenols.

Table 30. Total phenolic content (TPC) and antioxidant activity of Soxhlet and ASE extracts from 2-lot TS

Solvent	Extraction method	TPC	TPC	Antioxidant
		(mg GAE/g teak) <sup>1</sup>	(mg GAE/g extract) <sup>1</sup>	activity IC <sub>50</sub> (mg/L) <sup>2</sup>
Acetone	Soxhlet <sup>3</sup>	18.1 (1.5)	594.4 (47.8)	238.16
	ASE <sup>4</sup>	11.4 (0.7)	467.3 (28.2)	146.50
Ethanol	Soxhlet <sup>3</sup>	32.8 (1.9)	693.4 (40.9)	108.52
	ASE <sup>4</sup>	23.4 (1.4)	505.6 (29.8)	145.61
Methanol	Soxhlet <sup>3</sup>	35.4 (1.5)	568.0 (24.5)	161.54
	ASE <sup>4</sup>	28.4 (0.5)	811.0 (14.4)	196.29
Water	ASE <sup>4</sup>	23.1 (0.3)		662.5
	ASE <sup>5</sup>	55.3 (2.0)		172.96
	Fibertec <sup>6</sup>	41.2 (0.2)		122.5

1. Folin-Ciocalteu method. TPC expressed as mg of gallic acid equivalent (GAE) per gram of dry weight. Average of triplicated. Standard deviation in parentheses. BMG-Labtech Spectrostar-Nano, 96-well microplate. Absorbance recorded at 700 nm. Temperature: 45°C. Incubation time: 45 min.

2. DPPH assay. Antioxidant activity expressed as the concentration of the extractive that inhibits 50% of radicals (CI<sub>50</sub>). BMG-Labtech Spectrostar-Nano, 96-well microplate. Incubation time: 40 min. Absorbance recorded at 516 nm at room temperature

3. Soxhlet extraction. Sample weight: 8 g approx. Solvent volume: 150 mL approx. Total extraction time: 7 h.

4. ASE (Dionex 350 Thermo Scientific). Sample weight: 1.8 g approx. Static time: 7 min. Number of cycles: 3. Maximum pressure: 117 bar. Total extraction time: 32 min.

5. ASE (Dionex 350 Thermo Scientific). Sample weight: 1.8 g approx. Static time: 7 min. Number of cycles: 3. Maximum pressure: 117 bar. Total extraction time: 120 min.

6. Fibertec 1020 extractor (Foss). Temperature: boiling temp. Total extraction time: 2 h.

Water as an extraction solvent for teak is very efficient with a large ratio L/S, and with long contact times, to obtain antioxidant extracts. This means the polarity of water is too high to access such antiradical compounds, even if the pressure is slightly increased, it appears to not be enough to maintain water under subcritical conditions (giving to water a decreased polarity).

The importance of polarity of the solvent to access the antioxidant compounds is shown Table 30. Ethanol and methanol showed very interesting extraction capacities with polyphenols yield of 32.8 and 35.4 mg GAE/g teak wood by Soxhlet extraction.

To conclude on that study, recovery antioxidant from teak sawdust by means of twin-screw extraction technology with water as solvent presents the advantage of being a method of refining lignocellulosic material in a continuous process. The mechanical stresses applied to the teak sawdust makes it possible to obtain extracts with interesting polyphenol contents. However, the antioxidant activities of these polyphenols are rather low.

It may be worthwhile to modify the extraction conditions to use a less polar solvent to access the antioxidant fraction and to undertake a complementary radical-scavenging method of evaluation of the twin-screw water extracts.

However, twin-screw extruder fractionation provides refined lignocellulosic fibers with antioxidant compounds that have not been extracted from the matrix. On the other hand, many other compounds that react as polyphenols have been removed from the matrix, resulting in particularly refined fibers. Their use as basic fibers for the design of new materials would be perfectly appropriate. Indeed, the antioxidant compounds of wood from secondary metabolism allow the tree to defend itself against external aggression and stresses (mechanical load, humidity, extreme temperatures, UV), it would be natural that these activities could be reinvested in building materials subjected to the same climatic stresses. This study therefore could offer a valorization of sawmill by-products in generating refined fibers with high antioxidant activities.

### 3.2 Scale-up and production of teak fibers on twin-screw extruder B45 (Cleextral) for a valorization of sawdust in the field of composite materials

After having studied the extraction of extractives from teak sawdust on the extruder BC21, the validation of the scale-up (with a scale-up factor of 2) was conducted on the extruder BC45. This process was carried out to increase the quantity of extrudate produced and to study its valorization in the field of composite materials.

#### 3.2.1 Configuration and screw profile of extruder BC45 (Cleextral)

The scale-up was performed using a co-rotating and intermeshing twin-screw extruder BC45 (Cleextral, France), see Figure 36.

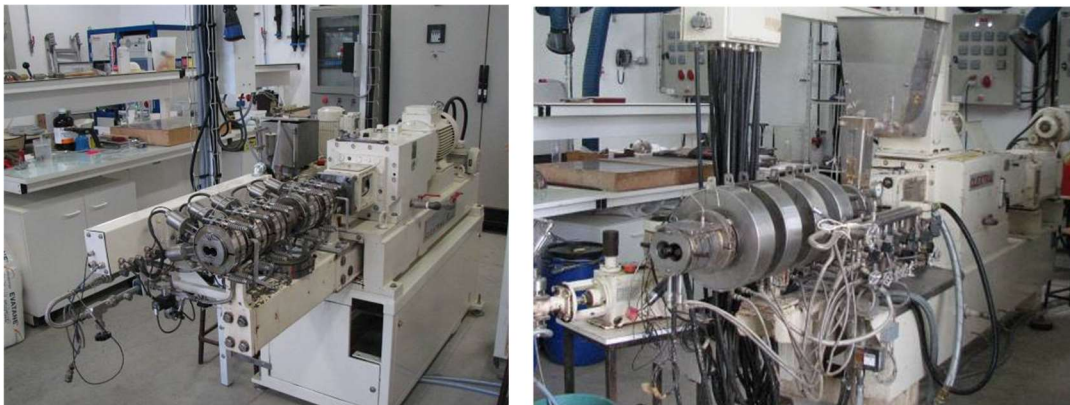


Figure 36. Twin-screw extruder BC21 (left) and BC45 (right)

The extruder consists of a closed barrel with seven modules of 200 mm in length. Modules 2, 3, 4, 5, and 7 are heated by thermal induction and cooled by water recirculation. Module 6 is equipped with a filter made up of six hemispherical dishes with conical holes of 1 mm of inner diameter and 2 mm of outer diameter. A control panel monitored the process parameters (temperature, screw speed, and teak sawdust flow rate). For comparison purposes, the technical data of the two extruders is given in Table 31.



Table 31. Comparison of the technical characteristics of the BC21 and BC45 extruder

<b>Extruder</b>	<b>BC45</b>	<b>BC21</b>
Barrel and screw		
Number of modules	7	7
Unit module length (cm)	20	10
Useful length of barrel (m)	1.4	0.7
Number of thermos-regulated modules	5	5
Heating mode	Induction (5kW)	Electric
Cooling mode	Water cooling (500 to 1000L/h, 4 to 6 bar)	Water cooling (2m <sup>3</sup> /h, 1.8 bar)
Shaft spacing (cm)	45	21
Maximum diameter of screw elements (mm)	55	25
Interpenetration of screw elements (mm)	10	6
Maximum screw rotation speed (rpm)	600	682
Motor		
Motor type	GS 1607	AC
Power (kW)	44	8.5
Maximum rotation speed (rpm)	600	682
Theoretical efficiency (cos φ)	0.95	0.90
Peripheral devices		
Solid supply	Volumetric screw hopper Clextral 40 (Clextral, France), (Volume: 40L, max screw speed: 227rpm, power: 0.75kW)	Volumetric screw hopper KCL-KT20 (K-Tron, USA), (Volume: 40L, max screw speed: 87 hz, power: 0.5kW)
Position	Module 1	Module 1

The configuration, screw profile, and process temperature are adapted from the point T= 100° C, L/S= 3.25 carried out in BC21. The profile configuration is defined in order to promote the size reduction of the raw material, the intimate contact between the material and the solvent by shear forces that allow

cell wall breakage and thus solid/liquid extraction; and liquid/solid separation by pressing and filtration. Figure 37 presents the configuration, screw profile, and process temperature and Figure 38 shows the screw elements used in the profile.

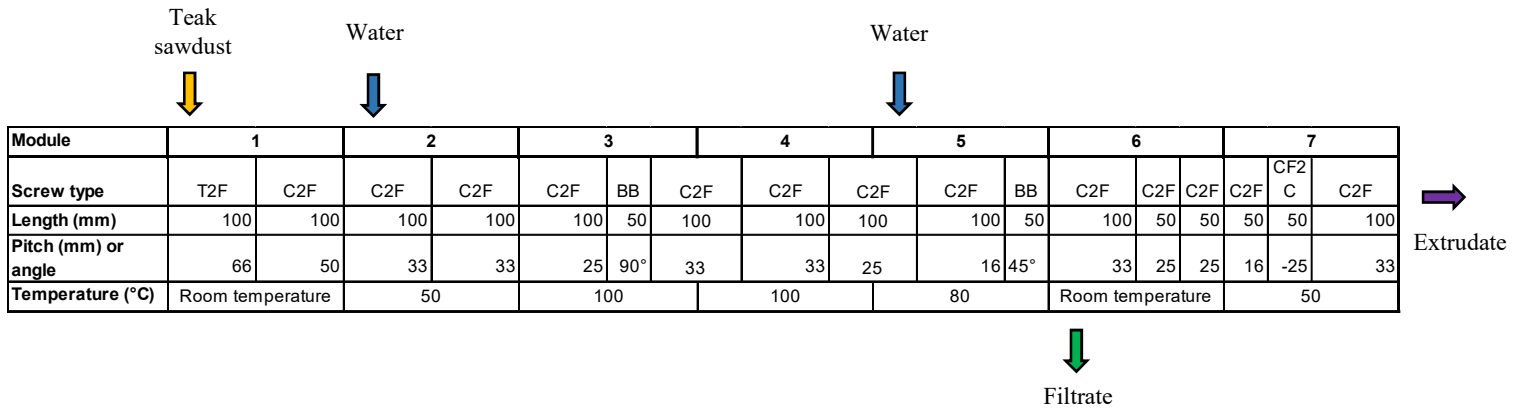


Figure 37. Configuration and screw profile of twin-screw extruder BC45 (Cletral) for teak sawdust extraction. T2F: trapezoidal double-thread screw, C2F: conveying double-thread screw, BB: bilobe paddle screw, CF2C: reversed double-thread screw

The first screws of the profile, trapezoidal double-thread screw and high pitch (T2F66) (Figure 38 B) are conveying screws; they facilitate the introduction of the raw material into the barrel. Then, the conveying double-thread screw, direct and decreasing pitch (C2F50, C2F33, C2F33, C2F25) (Figure 38 C, D, and E) ensure the efficient transport of the material as well as compression and initial contact with water (extraction solvent), which is injected in module 2. These screws carry out, in a lesser proportion, mixing and shearing operations because of their intermeshing character.

The following elements are the bilobed mixing discs (BB arranged at 90° and 50 mm length) (Figure 38 F), they are restrictive elements that ensure a high shear effect and therefore defibration and mixing of the solid material with the liquid. The stagger angle plays an important part. The elements with a stagger angle of 90° are flow-neutral and lack any axial conveying capability (Bauer et al., 2021).

Later, there is a sequence of double threaded, direct, and decreasing pitch screws (C2F33, C2F33, C2F25, and C2F16) (Figure 38 D, E, G). They allow the contact between the material and water, transport, and compression of the mixture. A second water input is made in module 5. The water is brought into contact with the mixture in C2F screws and the mixture is transported to the kneading elements. Unlike the first arrangement, the BB screws are arranged at 45°, this staggered angle has a higher pressure build-up capability and better mixing capability. After, a filter is placed in module 6 (Figure 38 K) to recover the filtrate. The separation of the filtrate is possible by the counter effect

generated by the opposite flow elements. A sequence of direct conveying screws (C2F33, C2F25, C2F25, and C2F16) (Figure 38 D, H, I) continue the transport of the mixture through the barrel and build up pressure to counteract the effect of the downstream flow of the reversed pitch element (CF2C-25) (Figure 38 J). The maximum pressure occurs at the intersection of the forward- and reverse-conveying screw sections. The absolute value of the pressure rise in the forward-conveying screw section is equal to the absolute value of the pressure loss in the reverse-conveying screw section. The combination of forward-conveying (pressure-gaining) and reverse-conveying (pressure-losing) screw elements constitutes a complete mixing section, however, reverse pitch screws could present points of stagnancy, the particles are held up during irregular time periods, before further axial transport (van Zuilichem et al., 1999). The last element is a forward-conveying screw, which allows the exit of the extrudate

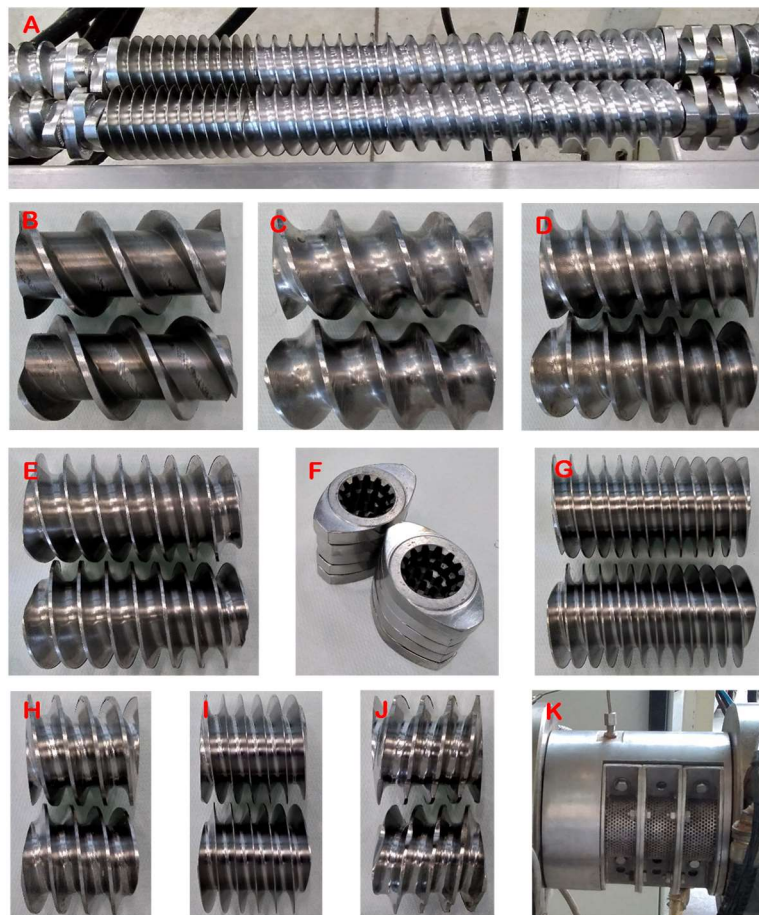


Figure 38. A: Screw profile section of twin-screw extruder BC45 (Cleextral, France), from left to right: BB 45° (50 mm), C2F16 (100 mm), C2F25 (100 mm), C2F33 (100 mm), C2F33 (100 mm), BB 90° (50 mm); B: T2F66 (100 mm); C: C2F50 (100 mm); D: C2F33 (100 mm); E: C2F25 (100 mm); F: BB (50 mm); G: C2F16 (100 mm); H: C2F25 (50 mm); I: C2F16 (50 mm); J: CF2C-25 (50 mm); K: Filter

The screw profile proposed allows the extraction process in steady state. The formation of a dynamic plug responsible for the pressing of the raw material is ensured by the reversed double-thread screw. The presence of the filter upstream of the reversed screw allows the recovery of the filtrate without clogging the filtration grid.

The extraction process is performed at a teak sawdust flowrate of 10.14 kg of fresh matter/h. TS is fed at module 1 by a twin-screw-volumetric feeder (LWF-D5 S K-Tron Soder), it is used as received from the sawmill without prior conditioning. Two water inputs are connected to the extruder. The first one is injected in module 2 at a flow rate of 7.23 Kg/h. The second water injection is made in module 5 at a flow rate of 21.20 Kg/h.

At the exit of the extruder, a filtrate and an extrudate are recovered. The filtrate (obtained in module 6) contains fine fibrous solid particles designated as sediment (see Figure 39 A). It is centrifuged with a centrifuge (Rousselet) (see Figure 39 B) at 1000 rpm through a bag with a porosity of 5  $\mu\text{m}$ , to separate the clarified filtrate and the sediment. The extrudate is recovered in module 7 and used after for the manufacture of biocomposites (see Figure 40). The solid/liquid extraction schema is the same as the one presented for extruder BC21 (see Figure 27) and the responses evaluated in the previous extractions are as well discussed in this extraction.



Figure 39. A: filtrate obtained by twin-screw extrusion of teak sawdust using an extruder BC45 (Clextral, France). B: centrifuge (Rousselet)



Figure 40. Extrudate obtained by twin-screw extrusion of teak sawdust using an extruder BC45 (Clextal, France)

### 3.2.2 Responses of the solid/liquid extraction of teak sawdust using a twin-screw extruder BC45 (Clextal)

The total mass balance of the solid/liquid extraction is presented in Table 32 and the process flows rates (i.e. inputs and outputs) in Table 33. According to the dry solid matter figures, the maximum difference is 2.49 %. The same responses were evaluated as for previous extractions. From the point of view of filtration efficiency: dry extrudate yield ( $Y_{DE}$ ), dry matter in the extrudate ( $DM_{RE}$ ), filtrate recovery ( $R_F$ ), and dry sediment yield ( $Y_{DS}$ ); and of filtrate properties: extractives yield ( $Y_E$ ), TPC (g GAE/100g teak) and AQ-2-CA content.

Regarding the solid/ liquid separation, the  $R_F$  value of 83.4 % demonstrate a high filtration efficiency of the extraction by means of extruder BC45, better than that carried out with the extruder BC21 ( $R_F= 67.3$  %). It would be expected that a high filtrate recovery would be also expressed in a high dry matter in the extrudate, which is not observed, the  $DM_{RE}$  achieved is 61.8 %, but can be explained by the high dry sediment yield (38.8 %). This means that, although a lot of liquid is recovered through the filter, it also allows a large amount of sediment to pass through. Consequently, the dry extrudate yield is also low (58.7 %). In contrast, for the extruder BC21 the elevated  $Y_{DE}$  (82.1 %) and low  $Y_{DS}$  (9.1 %) prove a good extrudate recovery.

The extraction by means of an extruder BC45 also exerts an important mechanical work on the material, causing the sawdust to separate into thin fibers, see Figure 41.

Table 32. Mass balance of the solid/liquid extraction of teak sawdust using a twin-screw extruder BC45 (Clextral)

Process variables		Mass balance					
Temp. (° C)	Ratio (L/S)	Total input (Kg/h)	Total output (Kg/h)	Total balance error (%)	Total dry solid input (Kg/h)	Total dry solid output (Kg/h)	Total dry solid balance error (%)
100	3.31	38.57	36.22	6.09	8.95	8.73	2.49

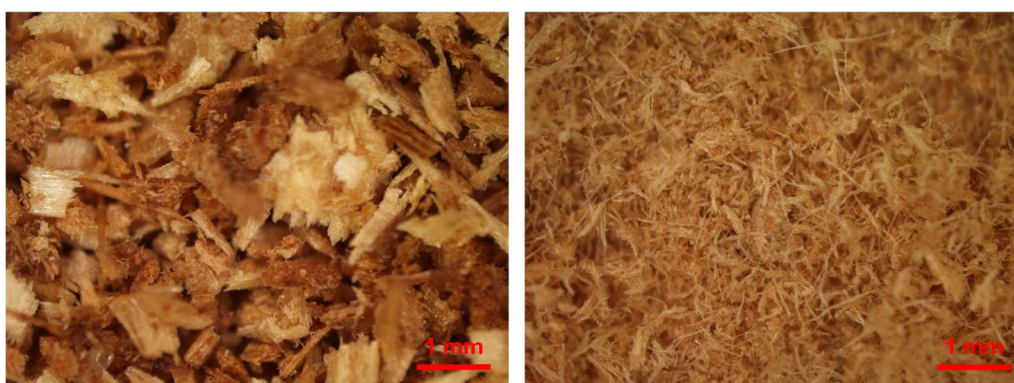


Figure 41. Teak sawdust as received (left) and after extrusion using a extruder BC45 (right)

On the side of characteristics of the filtrate, larger scale extraction yields a higher content of extractives (1.54 %) and the filtrate has a better reducing capacity, practically double amount of polyphenols is obtained (0.84 g GAE/ 100g teak) and congruently the antioxidant activity is improved by 50 %. On the other hand, the recovery of AQ-2-CA is higher with the extruder BC21, as discussed in the previous section this compound is not associated with high antioxidant activity, however, its extraction is important because it could be valorized in the pharmaceutical or food industry.

After having studied the possibility of obtaining extracts with interesting properties from teak sawdust by an innovative process using water as the only extraction solvent, the study focuses on the reuse of the extrudate, which is constituted by lignocellulosic fibers. Its valorization is studied in Chapter 4.

Table 33. Variables and process flows of the solid/liquid extraction of teak sawdust using a twin-screw extruder BC45 (Clextal)

Extruder	Inputs						Outputs					
	Process variables		Teak sawdust		Water		Extrudate			Filtrate		
	Temp. (° C)	Ratio (L/S)	Q <sub>RTS</sub> (Kg/h)	Q <sub>DTS</sub> (Kg/h)	Q <sub>1w</sub> (Kg/h)	Q <sub>2w</sub> (Kg/h)	Q <sub>RE</sub> (Kg/h)	DM <sub>RE</sub> (%)	Q <sub>DE</sub> (Kg/h)	Q <sub>RF</sub> (Kg/h)	Q <sub>CF</sub> (Kg/h)	Q <sub>DS</sub> (Kg/h)
BC45	100	3.31	10.14	8.95	7.23	21.2	8.51	61.78	5.26	27.71	24.71	3.47
BC21	100	3.25	2.05	1.69	1.38	3.91	1.72	80.70	1.39	3.95	3.80	0.153

Table 34. Responses of the solid/liquid extraction of teak sawdust using a twin-screw extruder BC45 (Clextal)

Process variables		Extrudate		Filtrate		Extractives analysis				
Temp. (° C)	Ratio (L/S)	Dry extrudate yield Y <sub>DE</sub> (%)	Dry matter in the extrudate DM <sub>RE</sub> (%)	Filtrate recovery R <sub>F</sub> (%)	Dry sediment yield Y <sub>DS</sub> (%)	Extractives yield Y <sub>E</sub> (%)	Total phenolic content (g GAE/100 g teak) <sup>1</sup>	Anthraquinone-2-carboxylic acid (mg/100 g teak) <sup>2</sup>	Antioxidant activity, IC <sub>50</sub> (g/L) <sup>3</sup>	
BC45	100	3.31	58.7	61.8	83.4	38.8	1.54	0.84	1.83	0.76
BC21	100	3.25	82.2	80.7	67.3	9.1	1.33	0.45	2.41	1.51

1. Folin-Ciocalteu method. TPC expressed as g of gallic acid equivalent (GAE) per 100 gram of dry weight. BMG-Labtech Spectrostar-Nano, 96-well microplate. Absorbance recorded at 700 nm. Temperature: 45°C. Incubation time: 45 min.

2. HPLC-DAD Dionex Ultimate 3000 (Thermo Scientific). Column Luna 3u PFP(2)100Å, 150 x 3.0 mm (Phenomenex). UV spectrum recorded between 200 nm and 400 nm. Quantification at 254 nm. Results expressed on dry weight basis.

DPPH assay. Antioxidant activity expressed as the concentration of the extractive that inhibits 50% of radicals (CI<sub>50</sub>). BMG-Labtech Spectrostar-Nano, 96-well microplate. Incubation time: 40 min. Absorbance recorded at 516 nm. Temperature: room temp.

### Conclusions of Chapter 3

The evaluation of the twin-screw technology as a method of solid/liquid extraction for teak sawdust was first performed with an extruder BC21 and then a scale-up of the process was carried up with an extruder BC45. The barrel configuration, screw profile, and operating conditions allowed the extraction process in a steady state on both extruders. Three zones were well defined and achieved (feeding, extraction, and liquid/solid separation). The screw profile allowed the formation of a dynamic plug responsible for the pressing of the raw material and the recovery of the filtrate was done without clogging the filtration grid. Teak sawdust did not require any pretreatment and showed good processability in the extruder. In addition, the extruder was overall well filled.

For all extrusions, intense mechanical work on the sawdust was observed, which was attributed to the stress generated in the area of the reversed double-thread screw. This resulted in a highly shredded extrudate.

The effect of temperature and L/S ratio were evaluated over a range of 50 °C-125 °C and 3.25-4.25, respectively, for the extrusions using the extruder BC21. In general, high filtrate recovery values have been obtained (between 60 % and 80 %) as well as an elevated dry extrudate yield (80 % - 90 %), proving a good liquid to solid separation. The sediment yield did not vary more than 1 % among all conditions evaluated, averaging 11 %. No conclusive correlation was observed between the effect of the variation of the evaluated parameters and the filtration efficiency.

Concerning the properties of the filtrates, the extractives yield has been found to be between 1.0 % and 1.5 %. Not surprisingly, it was enhanced by the increase of both temperature and L/S ratio. These values represent between 32 % and 47 % of the extractives obtained with an ASE system and water as a solvent. However, the extraction time is considerably different for both kinds of methods, about 1 min for the extruder and 32 min for the ASE, as well as the L/S ratio, whose value is 17 for the ASE. The extraction time and the liquid to solid ratio are decisive factors in the extraction of compounds.

The phenolic content estimated as a GAE showed that the use of twin-screw extraction technology with water as a solvent makes it possible to obtain extracts with interesting polyphenol content (from 249 mg GAE/g extract to 475 mg GAE/g extract). However, the antioxidant activities of these polyphenols were rather low (IC<sub>25</sub> from 198 mg/L to 571 mg/L). The polyphenols identified in the extracts were AQ-2-CA and lapachol. There have been obtained figures from 1.37 mg/ g dry extract to 2.25 mg / g dry extract for the anthraquinone, and from 0.1 mg/ g dry extract to 0.45 mg / g dry extract for the lapachol. The average AQ-2-CA content in the extracts obtained with the extruder represented more than 80 % of the



content reached in aqueous extractions performed with an ASE system and Fibertec with an extraction time of 2h.

The scale up of the extraction by means of an extruder BC45 made it possible to obtain a high filtrate recovery (83 %), even higher than with the extruder BC21 at the same temperature and L/S ratio conditions. However, the dry sediment yield was also very high (39 %), which means that although a lot of liquid was recovered by the filter, a high amount of extrudate material was also entrained. Consequently, the dry extrudate yield was low (58 %). On the filtrate quality side, an extractives yield of 1.54 % has been obtained and both phenolic content (543 mg GAE/ g dry extract) and antioxidant activity ( $IC_{50}=0.76$  g/L) were better in the extract from the extruder BC45. The same secondary metabolites were identified as in the extracts obtained with the extruder BC21. There have been reached values of 1.18 mg AQ-2-CA/ g dry extract and 0.21 mg lapachol/ g dry extract. In general, the scaling was well achieved. In addition to a filtrate with high phenolic content, a refined lignocellulosic material was obtained, well defibrated and with extractives still retained.

The extrudate could be evaluated as reinforcement in polymeric matrices. It could not only contribute to the improvement of the mechanical properties but also provide environmental resistance qualities due to the remaining extractives it contains.



## **Chapter 4**

### **Valorization of teak sawdust extrudate in the preparation of biocomposites**



## **Chapter 4. Valorization of teak sawdust extrudate in the preparation of biocomposites**

This chapter studies the use of teak extrudate as a reinforcement for biocomposites. The first part of the chapter deals briefly with biocomposites, in particular PLA-based composites. This is followed by the description of composites manufacturing. The teak fibers were compounded, without any treatment other than drying, with poly(lactic acid) (PLA) using a twin-screw extruder and the mechanical test specimens were manufactured using an injection molding machine. The biocomposites were prepared with different teak fiber loadings (10 wt.%, 20 wt.%, and 30wt.%). The discussion of their mechanical performance (tensile and flexural properties) is then presented.

### **4.1 Biocomposites**

Composites are structural materials made by combining two or more constituent materials with significantly different properties. Fiber-reinforced polymers are composites in which fibers are embedded in a polymeric matrix. The materials used to form conventional composites are often synthetic, for example, glass and carbon fibers and petroleum-based polymers. While these materials show significant strength and do not corrode, their constituents and their manufacturing processes require the use of nonrenewable materials and fossil fuels. Natural fibers are more beneficial reinforcement than glass fiber composites from an ecological point of view (Vigneshwaran et al., 2020). Although natural fibers are not as strong as synthetic fibers, extensive studies have been shown that they have the potential of reinforcing polymer matrix composites (Venkatarajan and Athijayamani, 2021). Biocomposites are natural fiber-reinforced biopolymers. They are being developed to provide an environmentally responsible alternative to conventional materials (Christian, 2020).

Once teak sawdust extractives have been characterized and the potential of twin-screw extruder as an extraction method with water as a solvent has been studied. The possibility of valorizing the extrudate is explored. In this way, an integral valorization of teak sawdust is proposed. Natural fibers are used as a replacement for glass fiber in composites attributed to their advantages such as low density and environmental aspects in terms of low energy consumption and renewability. Despite its heterogeneity, limited processing temperatures as well as being hydrophilic and thus inherently incompatible with hydrophobic thermoplastic matrices, the attention to the use of natural fibers for several applications has increased due to the global ecological concerns (Pappu et al., 2019). The benefits of biocomposites include that the materials are derived from renewable resources and in many cases can completely biodegrade when subjected to an appropriate environment (Christian, 2020).

When the fibers used in the production of composites are woody, they are called wood polymer composites (WPC). Among the most used wood species are pine, maple, and oak (Patel and Rawat, 2017). The advantages offered by timber residues such as low cost, environmental-friendly, availability, and low abrasiveness make them suitable as fillers in the WPC preparation (Petchwattana and Covavisaruch, 2014).

The attractive features of natural fiber over the traditional counterparts include relatively high specific strength, free formability, low self-weight, substantial resistance to corrosion and fatigue, and biodegradability (Gurunathan et al., 2015). In contrast, there are several drawbacks to the use of natural fibers, such as hydrophilicity, the hydroxyl (OH) groups located on the backbone of the cellulose structure readily bond with water, which limits the hydrogen bonding sites available for bonding with a matrix. The difference in polarity between the highly polar natural fibers and nonpolar polymer matrices leads to poor interfacial bonding (Christian, 2020). The adhesion between the fiber and the matrix plays a significant role in the final mechanical properties of the biocomposites since the stress transfer between matrix and fiber determines the reinforcement efficiency (Gurunathan et al., 2015). The compatibility and wettability between natural fibers and polymers can be improved by adding coupling and dispersing agents, or treatments to reduce the difference between hydrophilic/hydrophobic characters of the fiber and the matrix. The treatment not merely enhances interfacial adhesion, but also provides better fiber dispersion (Ibrahim et al., 2021). Another way to improve the affinity between the matrix and the fibers is the use of less hydrophobic polymeric matrices. Poly(lactic acid) (PLA) has been found to result in better mechanical performances. However, due to the reduced hydrophobicity of the PLA, moisture is able to diffuse into the material resulting in dimensional changes of the reinforcing fibers (Joffre et al., 2017).

Regarding the polymeric matrix, the biocomposites are based on biodegradable polymers. Biopolymers are polymers that are produced by or derived from living organisms, such as plants and microbes, rather than from petroleum, the traditional source of polymers. The primary sources of biopolymers are renewable (Reddy et al., 2012). Poly (lactic acid) (PLA) has achieved great commercial interest in the polymer industry among the biopolymers due to its attractive mechanical properties (compared to other biopolymers), potential biodegradability, and high industrial production capacity (Goutianos et al., 2019; Sudamrao Getme and Patel, 2020).

Poly(lactic acid) is a crystallizable thermoplastic polymer. It belongs to synthetic linear aliphatic polyesters produced from natural resources (such as corn and sugar beets). PLA is synthesized either through ring-opening polymerization of lactide or polycondensation of lactic acid (2-hydroxy propionic acid) (Gurunathan et al., 2015), see Figure 42. Lactic acid is mainly derived from plant or animal sources such as starch, cellulose, kitchen waste, and fish waste. As a monomer, lactic acid has two types of

optical isomers: left-handed and right-handed. Thus, PLA has three polymer configurations: left-turning poly-L-lactide (PLLA), right-turning poly-D-lactide (PDLA), and copolymer of D- and L-blocks P(DL)LA. The homopolymers PDLA and PLLA have a higher melting point and faster crystallization rate than conventional PLA (Jin et al., 2019). The uniformity of the different structure isomer can be mainly influenced by the extraction process to which it was subjected, the component of isomers itself, the processing involved, and the molecular weight of PLA. It might also be affected by the thermal processing of polymer which contributed to some properties changes such as crystallization capability (Lazim et al., 2021). The properties of PLA are tabulated in Table 35.

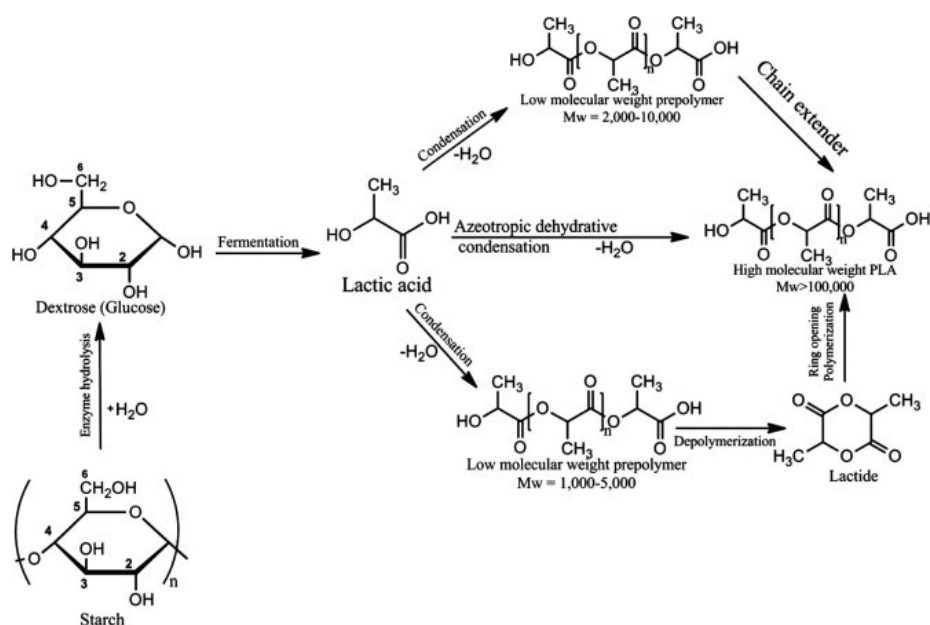
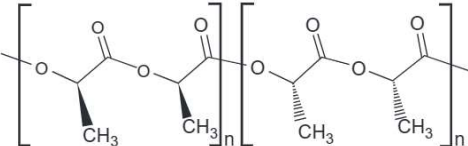


Figure 42. Synthesis pathway of poly(lactic acid) (Gurunathan et al., 2015)

Table 35. Poly(lactic acid) (PLA) properties (Lazim et al., 2021)

PLA polymers	Structure	Elastic modulus (GPa)	Tensile strength (MPa)	Elongation at break (%)	T <sub>m</sub> (°C)	T <sub>g</sub> (°C)
Poly(D-lactide)		8.6	0.88	30	220-230	65-72
Poly(L-lactide)		2.7-4.14	15.5-150	3.0-10	170-190	55-65

Poly(D,L-lactide)		1.5-1.9	0.04-0.05	5-10	170-190	50-60
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Tm: melting point

Tg: glass transition temperature

PLA presents good stiffness, high strength, and low elongation at break (Dong et al., 2014). It is easy to process, biodegradable, and has good mechanical properties. PLA can be processed by conventional processing methods, such as injection molding and extrusion. In comparison to petroleum-based polymers, such as polyethylene, polypropylene, polystyrene, and polyethylene terephthalate, the mechanical properties of PLA are appealing, particularly its Young's modulus, making it an excellent substitute for petroleum-based polymers in short-time packaging (Kaseem, 2019). PLA can be found commercially in various molecular weights in a wide range of applications such as textiles, pharmaceutical products, and also biomedical devices. On the other hand, as a pure raw material, it is brittle, has a poor impact strength, and low heat distortion temperature. Consequently, it is commonly mixed with fillers. Wood fibers have shown to be an excellent option for reinforcing PLA (Trinh et al., 2021). WPC materials comprising PLA have been evaluated for a range of applications including furniture elements, plastic components, and films (Luedtke et al., 2019).

The composite performance is not only a combination of the properties of the different phases but also governed by the stress transfer between them (Joffre et al., 2017). Furthermore, fiber-PLA matrix adhesion and interfacial properties of molded or multilayered composites can also be influenced by other composite properties, for example, the fiber dispersion or particle size (Luedtke et al., 2019).

## 4.2 Preparation of biocomposites

For the manufacture of biocomposites, NatureWorks® Injection grade PLA Ingeo™ biopolymer 3251D was selected as a matrix polymer. Two series of biocomposites were prepared at three different teak fiber loadings, the first one as a preliminary test and the second one for validation. For the preparation of the first series (1-BC), all the extrudates obtained from extractions using the extruder BC21 (Clextral, France) were recovered and mixed. The fibers were then derived from extrusions under different conditions, however, no significant differences were observed in the texture of the fibers. The second series (2-BC) was manufactured with the extrudate from the extraction using the extruder BC45 (Clextral, France) under controlled fiber production conditions.

The teak extrudate was dried at 70°C overnight before blending using a ventilated oven to a moisture content of 3 %- 4 %. This drying operation is important because natural fibers can readily absorb a



substantial amount of moisture from the surrounding environment (Zhang et al., 2014). When moisture reaches the fiber, the hydrogen bonds break and hydroxyl groups form new hydrogen bonds with water molecules. This not only may affect the internal structure and the mechanical properties of the natural fiber reinforcement but may also alter the fiber surface chemical composition, which is more exposed to the environment. The reduction of mechanical properties of natural fiber composites is typically attributed to the inherent interaction of natural fibers with water, which may lead to damage at the interface. Therefore, several reports conclude that natural fibers need to be dried before matrix blending (Fuentes et al., 2016). Figure 43 illustrates the general sequential process of biocomposites preparation.

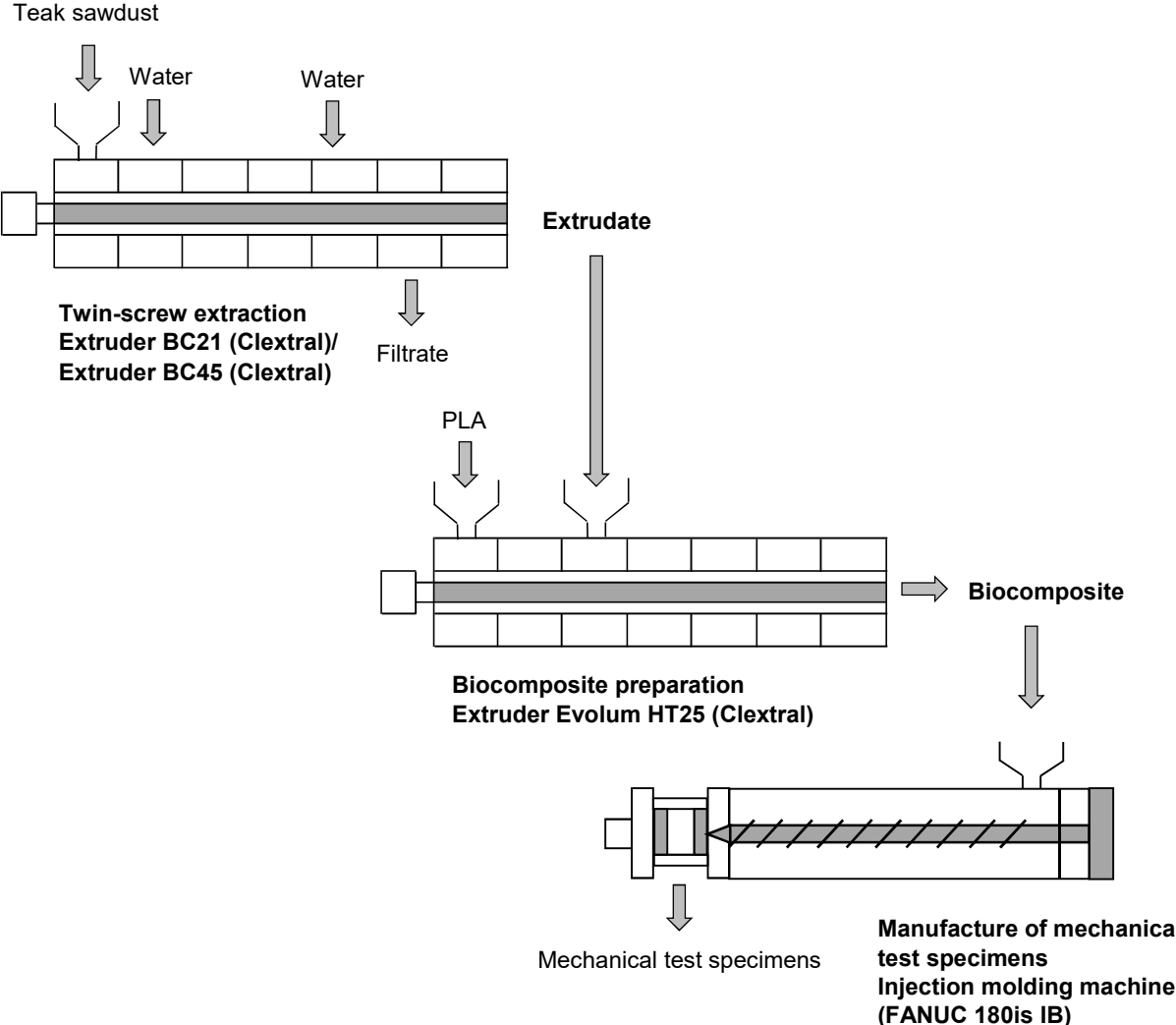


Figure 43. Biocomposite manufacturing from teak sawdust extrudate

The PLA biopolymer and the dried extrudate were compounded using twin-screw extruder Evolum HT25 (Clextral, France), see Figure 44. Several studies have shown that the compounding of natural fiber-based composites can be successfully achieved in a single step using twin-screw extrusion since

they provide a good dispersion of fiber and matrix (Bouafif et al., 2009; Gamon et al., 2013; Huerta-Cardoso et al., 2020).



Figure 44. Twin-screw extruder Evolum HT25 (Clextrel)

Biocomposites were prepared at three different loads of teak extrudate designed according to the fiber loading as PLA/10 wt.% teak extrudate, PLA/20 wt.% teak extrudate, and PLA/30 wt.% teak extrudate. Table 36 displays the sample codes and compositions of prepared biocomposites.

Table 36. Sample codes and compositions of biocomposites

Sample code	Polymer/ teak extrudate content (wt.%)	Teak fiber origin
Neat PLA	100/0	
<b>1-BC series</b>		
PLA/10 wt.% TE	90/10	Extrusion with extruder BC21
PLA/20 wt.% TE	80/20	Extrusion with extruder BC21
PLA/30 wt.% TE	70/30	Extrusion with extruder BC21
<b>2-BC series</b>		
PLA/10 wt.% TE	90/10	Extrusion with extruder BC45
PLA/20 wt.% TE	80/20	Extrusion with extruder BC45
PLA/30 wt.% TE	70/30	Extrusion with extruder BC45

The configuration and screw profile of the twin-screw extruder Evolum HT25 (Clextrel) are presented in Figure 45. The extruder barrel, with a length of 1 m and an L/D of 40, consisted of 11 successive modules, the last module being the one corresponding to the extrusion die.

There are two zones in the configuration:

- Zone of PLA melting. The PLA is fed by a volumetric feeder in module 1 (not heated). The conveying screws placed in modules 1-4 allow the transport of the biopolymer and the bilobe screw element followed by a reversed double-thread screw the homogenization.
- Zone of blending the PLA in the molten state and the teak fibers. The feeding of the latter is ensured by a lateral screw feeder. The mixing is performed in three zones by bilobe paddle screws in modules 6, 7, and 8. The adequate dispersion of the fibers in the polymeric matrix is ensured by the reversed double-thread screw placed immediately after the stacking of BL22 elements in module 7. The mixture is then degassed in module 9 and extruded through a 2-hole die in module 11. For the composites at 10 wt.% and 20 wt%, the rod formed at the exit of the barrel is continuously cooled in water and then passed through an automatic granulator cutter. For the composite at 30 wt.%, it is recovered directly at the extruder outlet and cooled in the open air, after that, it is granulated using a granulator. The difference in the type of cut was because the speed of the rod exit and the speed of the automatic cutter do not match. Figure 46 illustrates the biocomposites.

For neat PLA, PLA/10 wt.% TE, and PLA/20 wt.% TE, the temperatures of the extruder barrel are 80 °C for module 2 and 180 °C for modules 3-11; and the screw speed is maintained at 200 rpm. For PLA/30 wt.% TE, the temperatures are 80 °C for module 2 and 190 °C for modules 3-11; and the screw speed is maintained at 250 rpm. For all runs, a total feed flow rate of 10Kg/h is fixed.

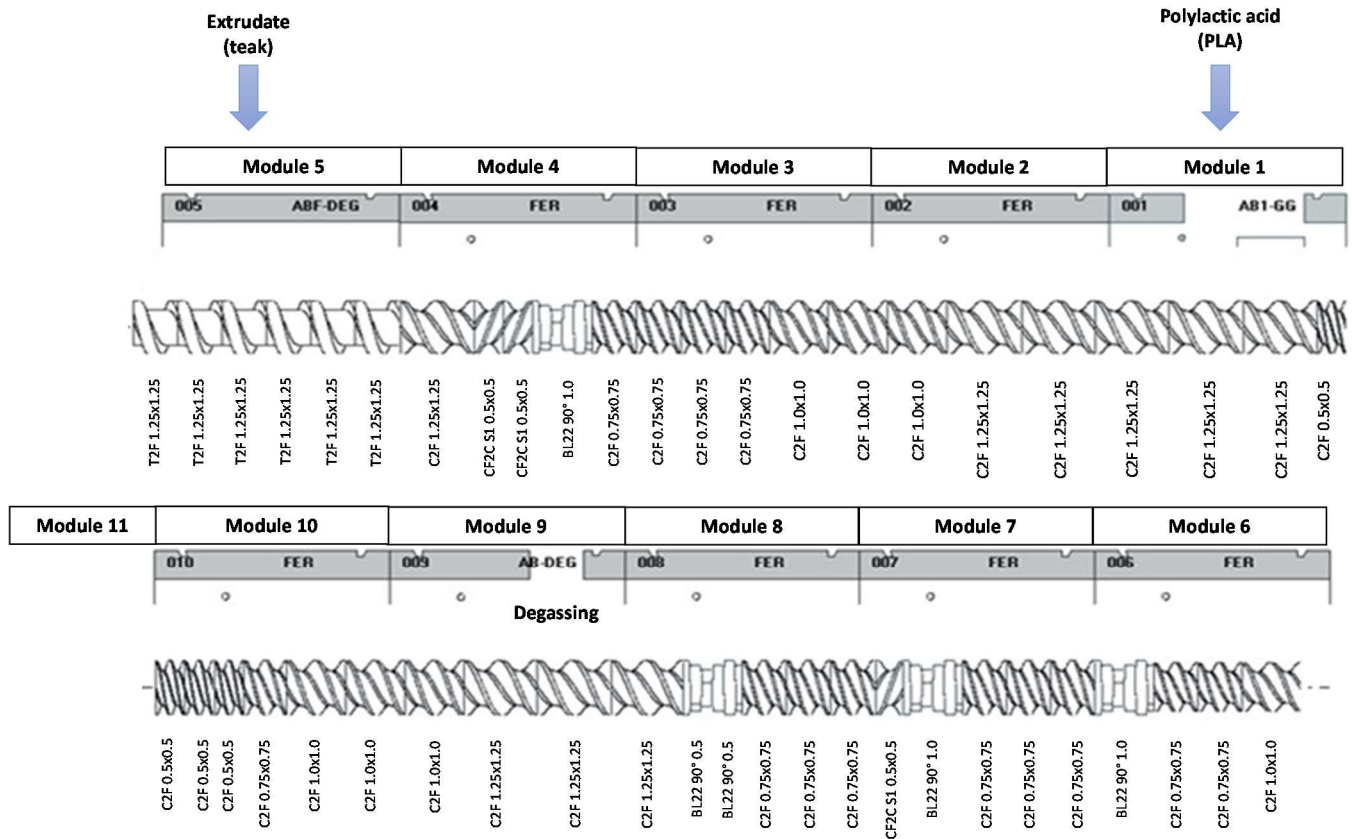


Figure 45. Configuration and screw profile of twin-screw extruder Evolum HT25 (Clextral, France) for biocomposite manufacturing. T2F: trapezoidal double-thread screw, C2F: conveying double-thread screw, BL22: bilobe paddle screw, CF2C: reversed double-thread screw



Figure 46. Biocomposites: PLA/20 wt.% TE (left) and PLA/30 wt.% TE (right)

After processing, the biocomposites are injected into an injection-molding machine (FANUC Series 180is-IB) to make the specimens for mechanical tests. Injection molding is a key polymer processing technology whereby parts with complex geometry are easily attained by injecting plastic materials molten by heat into a mold, and then cooling and solidifying them. The temperature profile along the plasticizing screw was 20 °C (feeding)/ 160 °C/ 170 °C/ 170 °C/ 180°C (nozzle).

As a reminder, an injection molding machine consists of a plasticizing unit, a clamping unit (in which the mold is fixed), and a control unit. The operating principle is as follows: In the plasticizing unit, the screw is placed in a cylindrical, conductively heated barrel. When it rotates, its function is first to melt the polymer pellets, mix them and convey the material to the inlet of the closing unit. Then it acts as a piston to inject the molten material into a mold. The latter, generally regulated at a temperature close to room temperature, will allow the polymer to set as quickly as possible (Pham, 2013) Figure 47 illustrates an injection molding machine.

The process of injection molding is divided into 5 major steps: dosing, injection, compaction, cooling, ejection.

Step 1: the dosing phase. The polymer pellets fall from a hopper into a screw and barrel assembly. They are then progressively melted by the combined actions of the clamps heating the barrel, the shearing of the material, caused by the rotation of the screw and the friction of the pellets (between them and against the walls of the barrel and the screw). The molten material is then conveyed in front of the screw (via a non-return valve), in the space created by the progressive backward movement of the latter during its rotation.

Step 2: the injection phase. Thanks to a translation movement of the screw, the molten polymer is transferred from the tank to a mold via channels. This translational movement is generated by a cylinder, through the non-return valve which, in this phase, prevents the material from turning back.

Stage 3: the compaction phase. The mold is composed of two parts, one fixed and one mobile. During compaction, the mold is kept under pressure to remain closed, while the material is compacted by the screw. During this phase, the molten polymer continues to be injected to compensate for the shrinkage of the material as it cools.

Step 4: the cooling phase. When the polymer is fully set at the gate(s), there is no need to apply holding pressure, and the part continues to cool until it is fully solidified.

Step 5: the ejection phase. The mold opens, and the formed part is ejected from the mold

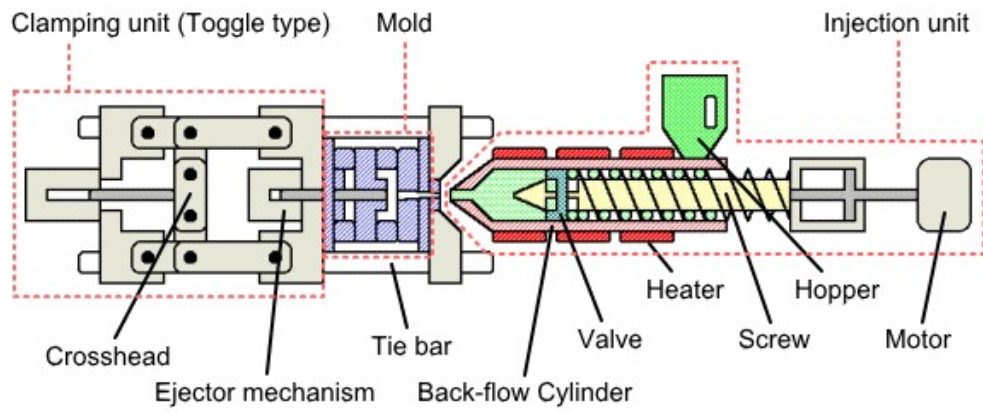


Figure 47. Injection molding machine (Polyplastics Co., 2021)

For the study, two types of materials are produced: neat PLA specimens (used as reference material) and PLA/ teak extrudate specimens.

### 4.3 Mechanical tests

Fiber-reinforced composites express a mechanical behavior significantly different from that of conventional materials, such as metals, owing to their nature (anisotropic and heterogeneous). The mechanical performance of composites is essential to determine the material properties for subsequent use in product design, quality control, application performance requirements, and production process. It involves the determination of mechanical parameters such as strength and stiffness. The most common standardized mechanical tests of polymer composites include tensile, flexural, impact, bending, and compression tests (Saba et al., 2019).

For the three different wood fiber loadings of both series, the visual appearance of the biocomposites seems to be relatively uniform, mainly in the samples with lower fiber content, however, some very small wood fiber chips can be seen mostly in the 1-BC composites series. The smooth finish is as well observed in all composites. The color of the specimens becomes more intense with increasing fiber content due to the higher content of lignin.

Figure 48 and Figure 49 show magnified photographs of the series 1-BC and 2-BC, respectively. The magnified images confirm the first visual evaluation, the lower the fiber load, the better the incorporation of the fiber and the PLA, on the other hand, at the maximum content evaluated, the more evident the polymer on the surface.

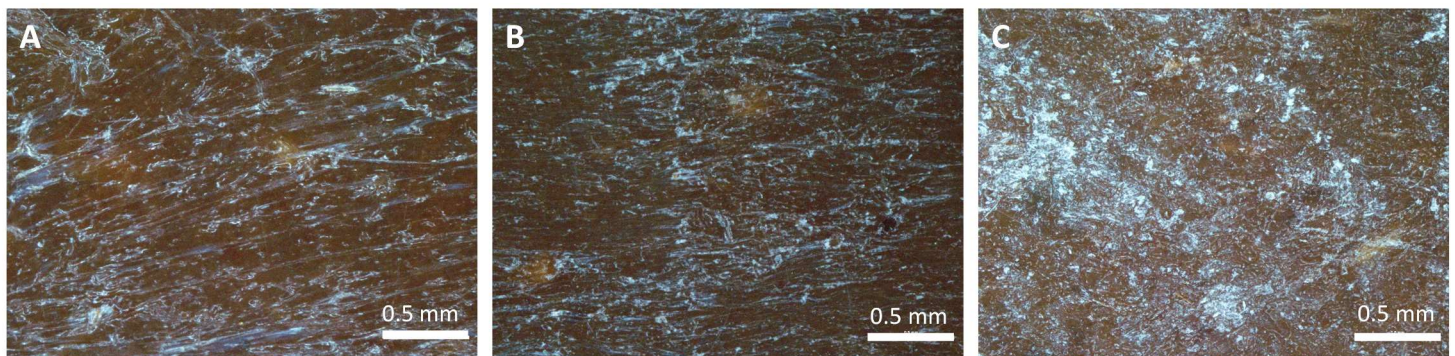


Figure 48. Magnified photographs of biocomposites (PLA/ teak extrudate) 1-BC samples. A: PLA/10 wt.% TE; B: PLA/20 wt.% TE; C: PLA/30 wt.% TE

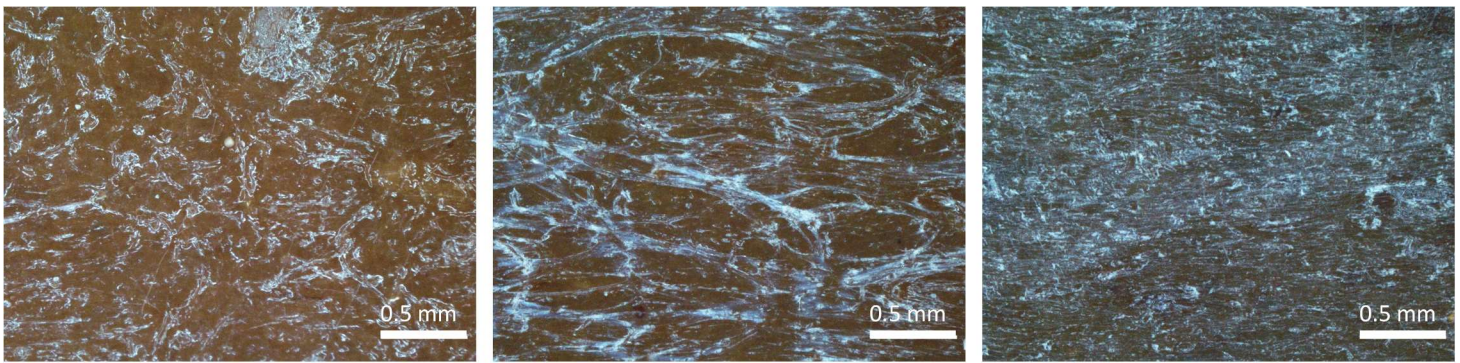


Figure 49. Magnified photographs of biocomposites (PLA/ teak extrudate) 2-BC samples. A: PLA/10 wt.% TE; B: PLA/20 wt.% TE; C: PLA/30 wt.% TE

The Figure 50.A illustrates the extrudate coming from the extrusions carried out with the extruder BC21. It is observed, in addition to wood fibers small pieces of whole sawdust, Figure 50.B shows the zoom of this piece of wood and it is more clearly seen that although the size is very small, the wood is not defibrated. These pieces of sawdust remain in the mechanical specimens (see Figure 50.C and Figure 50.D), this means that the thermo-mechanical process of composite manufacturing (blending using an extruder) does not defibrate completely the wood. On the other hand, the extrudate from the extrusions performed with the extruder BC45 is more defibrated (see Figure 51.A), small pieces of complete wood are also observed, but, in general, the material is more unstructured, clusters of fibers can also be seen. Figure 51.B shows the zoom of this cluster where it can be noted that it is a skein-like cluster. Macro and microscopic observations of the mechanical specimens do not exhibit these clusters (see Figure 51.C and Figure 51.D), suggesting that during the blending of the polymer and the extrudate, the mechanical and thermal work exerted on the mixture allows the agglomerates to break up.

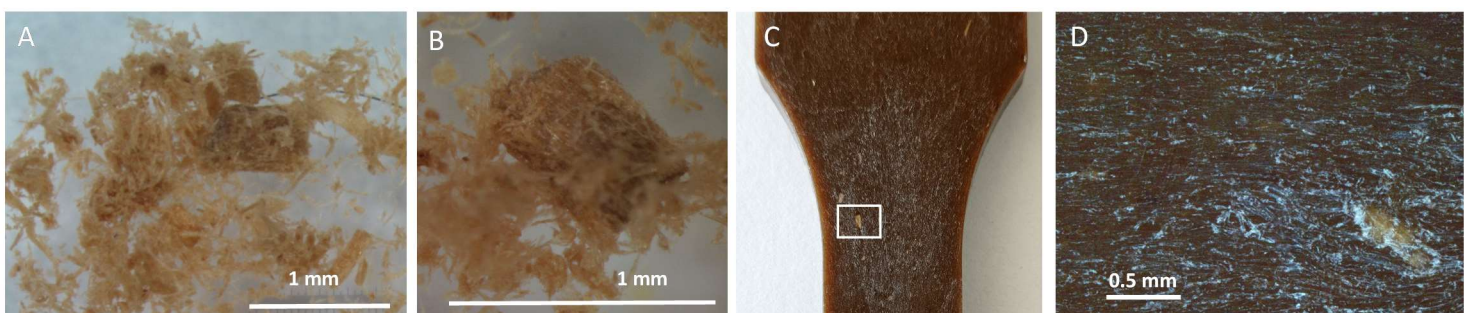


Figure 50. A: magnified photo of extrudate from extrusion using the extruder BC21; B: magnification of extrudate from extrusion using the extruder BC21; C: mechanical test specimen (PLA/20 wt.% teak extrudate) from 1-BC series; D: magnification of mechanical test specimen (PLA/20 wt.% teak extrudate) from 1-BC series



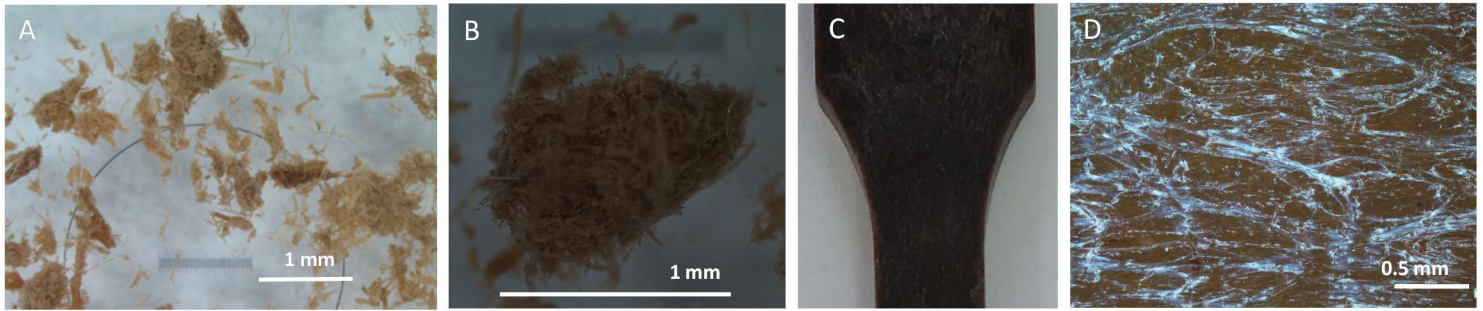


Figure 51. A: magnified photo of extrudate from extrusion using the extruder BC45; B: magnification of extrudate from extrusion using the extruder BC45; C: mechanical test specimen (PLA/20 wt.% TE) from 2-BC series; D: magnification of mechanical test specimen (PLA/20 wt.% TE) from 2-BC series

The biocomposites prepared from PLA and teak extrudate were subjected to tensile and flexural tests. The flexural test under three-point bending was determined by using a universal testing machine (Tinius Olsen H5KT) in accordance with French Standard NF EN ISO 14125. The specimens for the flexural test were rectangular bars with dimensions of 80 mm in length, 10 mm in width, and 4 mm in thickness. The tensile test was conducted according to French Standard NF EN ISO 527 by using the same machine. The specimens for the tensile test were dumbbell-shaped (Type 1A) with dimensions of 170 mm in overall length, 10 mm in width at narrow portion, and 4 mm in thickness. Figure 52 illustrates the mechanical specimens.

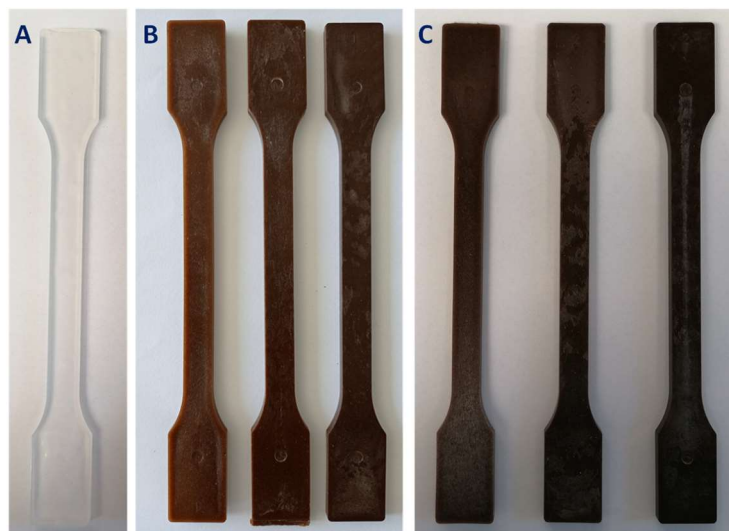


Figure 52. Tensile test specimens of biocomposites (PLA/ teak extrudate) prepared by injection molding. A: neat PLA (3251D). B: 1-BC series, from left to right: PLA/10 wt.% TE, PLA/20 wt.% TE, and PLA/30 wt.% TE; C: 2-BC series, from left to right: PLA/10 wt.% TE, PLA/20 wt.% TE, and PLA/30 wt.% TE

From the results of mechanical tests, the stress-strain curves are plotted and the tensile modulus and tensile strength are determined. Stress is defined as the ratio of the applied force to the cross-sectional area of the material it is applied to.

$$\sigma = \frac{F}{A}$$

Where:

$\sigma$ : stress

F: load (force)

A: cross-sectional area

Strain is defined as the ratio of the change in dimension to the initial dimension of the material. The type of strain applied to the test specimens was normal. Normal strain (or longitudinal strain) concerns itself with the change in only one dimension.

$$\varepsilon = \frac{L - L_0}{L_0}$$

Where:

$L_0$ : initial gauge length

L: final length

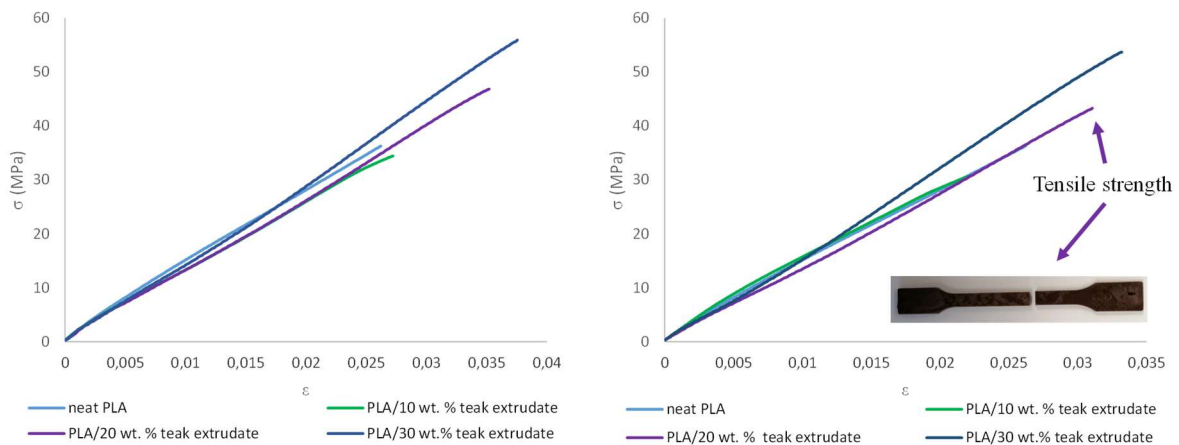


Figure 53. Stress-strain curve of neat PLA and biocomposites prepared at different compositions. PLA/teak sawdust extrudate 1-BC (left) and PLA/teak sawdust extrudate 2-BC (right)

Figure 53 displays the stress-strain curves of biocomposites at different fiber loading. It is observed that the biocomposites show the same brittle character as neat PLA. They do not demonstrate any plastic deformation but fail in the elastic region. In other words, the test pieces undergo a strain proportional

(linearly) to the tensile force during the entire mechanical test. There is no permanent change in the original shape, and hence no ductility. Figure 53 also shows a photograph of the tensile failure mode of the fractured sample specimen (PLA/20 wt.% teak extrudate) in the tensile testing. All samples of both series of biocomposites (1-BC and 2-BC) at the three different loadings showed the same failure mode.

The mechanical properties of neat PLA and biocomposites (tensile modulus, tensile strength at break, elongation, and flexural strength) are graphically presented in Figure 54.

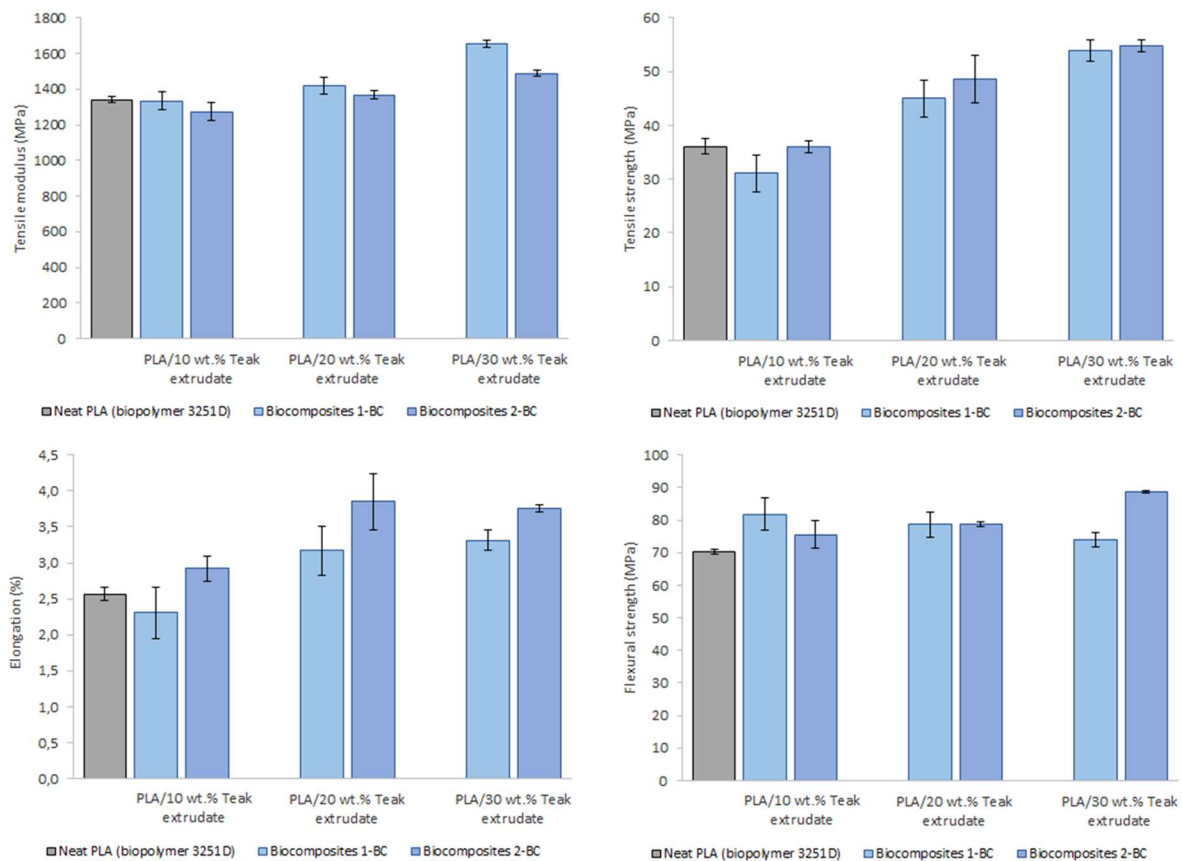


Figure 54. Mechanical tests of biocomposites prepared with teak extrudate (1-BC and 2-BC) and PLA (3251D) at different compositions (PLA/teak sawdust extrudate). A) Tensile modulus (MPa); B) Tensile strength at break (MPa); C) Elongation (%); D) Flexural strength (MPa)

The tensile modulus describes the stiffness or resistance to elastic deformation of a material under load. It is obtained by the slope of the stress-strain curve within the elastic limits of the material. The effect of the addition of the teak fiber to the PLA on the tensile modulus is observed at loads higher than 10 wt.%. It is improved from 1341 MPa for neat PLA to 1419 MPa and 1652 MPa for composites with 20 wt.% and 30 wt.%, respectively of 1-BC series; and to 1367 MPa and 1489 MPa for composites with 20 wt.% and 30 wt.%, respectively of 2-BC series. In accordance with this, the tensile strength of neat PLA (36 MPa) is enhanced up to 55 MPa for composites with 30 wt.% of both series, which means 50%.

Tensile strength is considered as the failure criteria for brittle material and it is used for designing components or structures.

The elongation at break, also known as fracture strain, is the ratio between changed length and initial length after breakage of the test specimen. It expresses the capability of the material to resist changes of shape without crack formation (Djafari Petroudy, 2017). As well as the above traction properties, the elongation is favored with higher teak fiber loadings, however, in this case, the difference between the results obtained at 20% and 30% do not vary more than 5% between them. The highest percentage of elongation is reached with the composite at 20 wt.% TE (2-BC) being of 3.9%, compared to neat PLA (2.6%), it represents a gain in the elasticity of 50%. When comparing the two series, it can be seen that for all compositions, the 2-BC series always shows higher values. The low elongation at break is characteristic of brittle materials such as PLA. The natural fibers, on the other hand, present higher values of elongation, hence this property is better in biocomposites reinforced with natural fibers.

On the other hand, for flexural strength, the loading effect was evident since 10 wt.% of the wood fiber. An opposite trend can be observed between the two series. For the 1-BC series, the flexural strength decreases as the amount of extrudate in the composite increases. The maximum value reached is 82 MPa for PLA/10 wt.% TE composite. For the 2-BC series, this property is favored at higher wood fiber loadings. The performance was increased from 70 MPa for neat PLA up to 89 MPa for PLA/30 wt.% TE. This could be attributed to the fact that the extrudate of the second series is more defibrated, which would leave more lignin exposed. Lignin has polar and non-polar sides due to its hyperbranched structure (Ilmiati et al., 2018). The hydrophobic side of lignin may have favored the adhesion between the polymer and the fiber. This better compatibility between the composite components could then be reflected in increased flexural strength.

It is noticeable that even that no additives were used in the synthesis of the teak biocomposites the mechanical features of neat PLA are improved and the results are comparable with other works of wood reinforced composites (Haque et al., 2020; Petchwattana et al., 2017). For instance, WPC with teak wood flour and unsaturated polyester have been achieved tensile strength from 14 MPa to 21 MPa and flexural strength from 5.9 MPa to 6.8 MPa for loads of teak from 5% to 20% (Patel and Rawat, 2017).

## Conclusions of Chapter 4

The extrudate from teak sawdust extractions was valorized as a reinforcement of a biodegradable polymeric matrix (PLA). Two series of biocomposites were prepared, the first (1-BC) with the extrudates obtained from the BC21 extruder. Although the process conditions were different, the extractions were performed with the same screw profile and the same appearance and texture were observed in all extrudates. The second series (2-BC) was manufactured with the extrudate from the extraction using the extruder BC45. Biocomposites were prepared at three different fiber loading 10 wt.%, 20 wt.%, and 30 wt.% of teak extrudate. Those with higher fiber content, probably as a consequence of the increased lignin content and remaining extractives, had a more intense color.

In general, extrudates of both extrusions were well shredded with a flour-like appearance, and only a few very small pieces of whole wood and clusters of fibers were present. These small pieces of sawdust remained in the mechanical specimens. The thermo-mechanical work of the twin-screw extruder acted on the mixture to incorporate the PLA and the fibers, however, it no longer contributed to the destructuring of the material. On the other hand, the process conditions did allow the fiber clusters to separate.

The proposed process for composites preparation by means of a twin-screw extruder (Evolum HT25, Clextral) to blend the teak fibers with PLA, followed by an injection molding machine (FANUC Series 180is-IB) to mold the specimens for the mechanical tests allowed obtaining biocomposites with a smooth and homogeneous overall appearance.

Regarding the mechanical tests. The biocomposites of both series and of all PLA/teak extrudate compositions did not demonstrate any plastic deformation but failed in the elastic region in the same way as neat PLA. In terms of tensile properties, the addition of 10 wt.% of teak extrudate had no hardening effect on the biopolymer in any of the series. Nevertheless, at higher loads, the tensile strength increased as the fiber content increased. It has been improved from 36 MPa for neat PLA to 54 MPa for composites with 30 wt.% TE of both series. The tensile modulus (of 1341.3 MPa for neat PLA) has been as well enhanced by up to 10% in the 1-BC series and 20% in 2-BC. Concerning the elongation of the materials, the highest percentage has been reached with the composite at 20 wt.% TE (2-BC) being of 3.9%, compared to neat PLA (2.6%), it means a gain in the elasticity of 50%. None of the series showed an improvement in elongation when the fiber content was increased from 20% TE to 30% TE.

On the other hand, for flexural strength, the loading effect was evident since 10 wt.% of the wood fiber. For series 1-BC, increasing the load slightly decreased the flexural strength values, with a maximum value of 82 MPa for PLA/10 wt.% TE, whereas series 2-BC showed an enhanced from 70 MPa for neat

PLA up to 89 MPA for PLA/30 wt.% TE. In general, the composite of the 2-BC series displayed slightly better mechanical properties.

It would also be important to evaluate other properties such as moisture absorption, thermogravimetric analysis, accelerating aging, scanning electron microscopy, among others to complete the characterization and to deepening the interfacial interactions between fiber and matrix. The proposal to use teak extrudate as a natural fiber in the reinforcement of PLA completes the sawdust valorization approach of this study.

## **Chapter 5**

### **Materials and methods**





## **Chapter 5. Materials and methods**

### **5.1 Conditioning of raw material**

Teak sawdust was obtained as waste from 14 to 16-year-old debarked teak logs (heartwood and sapwood) from Agropecuaria Santa Genoveva sawmill located in Campeche, Mexico. Two lots were received, the first one (1-lot TS) of less than one kilogram for the preliminary characterization, the second lot (2-lot TS) of 500 Kg for the extractions using twin-screw technology.

#### **5.1.1 Teak sawdust drying**

Teak sawdust was air-dried before shipment to preserve it from degradation due to oven drying (75 %-80 % of dry matter).

For the fiber analysis (cellulose, hemicelluloses, and lignin content), the teak sawdust was oven-dried at 103°C overnight.

For the quantification of total extractives by a Soxhlet apparatus, ASE system, and Fibertec device, teak sawdust was oven-dried at 50° C overnight before performing the extractions.

For extractions using a twin-screw technology, the teak sawdust was used as received without pre-drying.

For the preparation of biocomposites, the teak extrudate was dried at 70°C overnight before blending with PLA. The composite (PLA/teak extrudate) was dried at 90°C before injection molding for mechanical test specimens manufacturing. The material was spread on thin canvas covering grids arranged on three trays in the oven. Drying was made by hot air ventilation.

### **5.2 Particle size distribution**

The particle size distribution was determined using a Retsch AS 200 automated vibratory shaker machine. The material is thrown upwards by the vibrations on the sieve bottom and subsequently falls back down onto the sieve mesh fabric due to gravitation forces. Thereby, the sample material is subjected to a three-dimensional movement. Hence, the sample is spread uniformly across the entire surface of the sieve bottom, whereas the particles are subjected to an acceleration in vertical direction. In this process, they perform free rotations and are compared with the mesh size when falling back down statistically

orientated. In the shaker machine, an electromagnetic drive sets a spring–mass system in motion and transfers the oscillations to the sieve stack. The mesh size used were 1000 µm, 500 µm, 250 µm, 200 µm, 125 µm, 63 µm, and 32 µm. For the test, the sample mass was about 70 g. The sieving was carried out for 40 minutes at 70 % of the amplitude of the apparatus. The results were displayed graphically as histograms (percentage of the material retained on the sieves of different sizes). Each sample was taken in triplicate.

### 5.3 Dry matter content

The dry matter content (DM) was calculated by drying the material in the oven at 105°C for 12 hours. The sample was cooled in a desiccator before weighing. The measurements were made in triplicate and the results were expressed as a percentage (g of dry teak sawdust/ 100 g of raw teak sawdust).

$$DM(\%) = \frac{m_{dry\ sample}}{m_{sample}} * 100$$

Where:

$m_{sample}$ : initial sample mass (g)

$m_{dry\ sample}$ : dry sample mass (g)

### 5.4 Mineral matter content

The mineral matter (MM) content was evaluated after sample calcination at 550°C for 3 hours using a Nabertherm oven (Lilienthal, Germany). The sample was cooled in a desiccator before weighing. This analysis was performed in triplicated and the results were expressed as a percentage on dry weight basis (g of MM/100 g of dry teak sawdust).

$$MM(\%) = \frac{m_{ac}}{m_{sample}} * 100$$

Where:

$m_{ac}$ : sample mass after calcination (g)

$m_{sample}$ : initial sample mass (g)

### 5.5 Lignocellulosic fiber analysis

The quantification of each type of fiber (i.e. cellulose, hemicelluloses, and lignin) was carried out by Van Soest and Wine method (Soest and Wine, 1967) using a Fibertec 1020 device (Foss, France) with a cooling system, see Figure 55. This method is based on gravimetric differences of sequential washings

(attacks). The neutral detergent fiber (NDF) fraction is the organic matter not solubilized in a neutral detergent solution consisting of sodium laurylsulphate. NDF includes hemicelluloses, cellulose, lignin, and minerals. The acid detergent fiber (ADF) fraction is the organic matter not solubilized in an acid detergent solution consisting of acetyltrimethylammonium bromide in sulfuric acid. ADF includes cellulose, lignin, and minerals. Subsequently, the lignin is separated by oxidation using potassium permanganate in the presence of a buffer, see Figure 56.

The reagent solutions used are the following:

- NDF solution: sodium lauryl sulfate, sodium ethylene diamine tetra-acetate, disodium phosphate, sodium borate decahydrate, ethyl cellulose.
- ADF solution: hexadecyltrimethyl ammonium bromide at 2 % in 1N sulfuric acid.
- Potassium permanganate reagent: 2 volumes of saturated potassium permanganate solution to 1 volume of buffer solution.
- Buffer solution: iron nitrate, silver nitrate aqueous solution, glacial acetic acid, potassium acetate, tertiary butyl alcohol.
- Demineralizing solution: Oxalic acid dihydrate, ethanol (95 %), hydrochloric acid (12 N), distilled water.

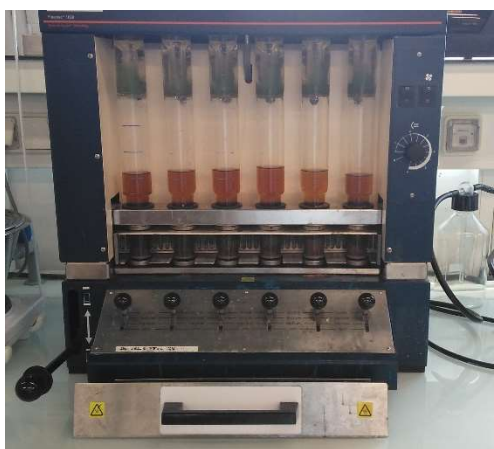


Figure 55. Fibertec 1020 device (Foss, France)

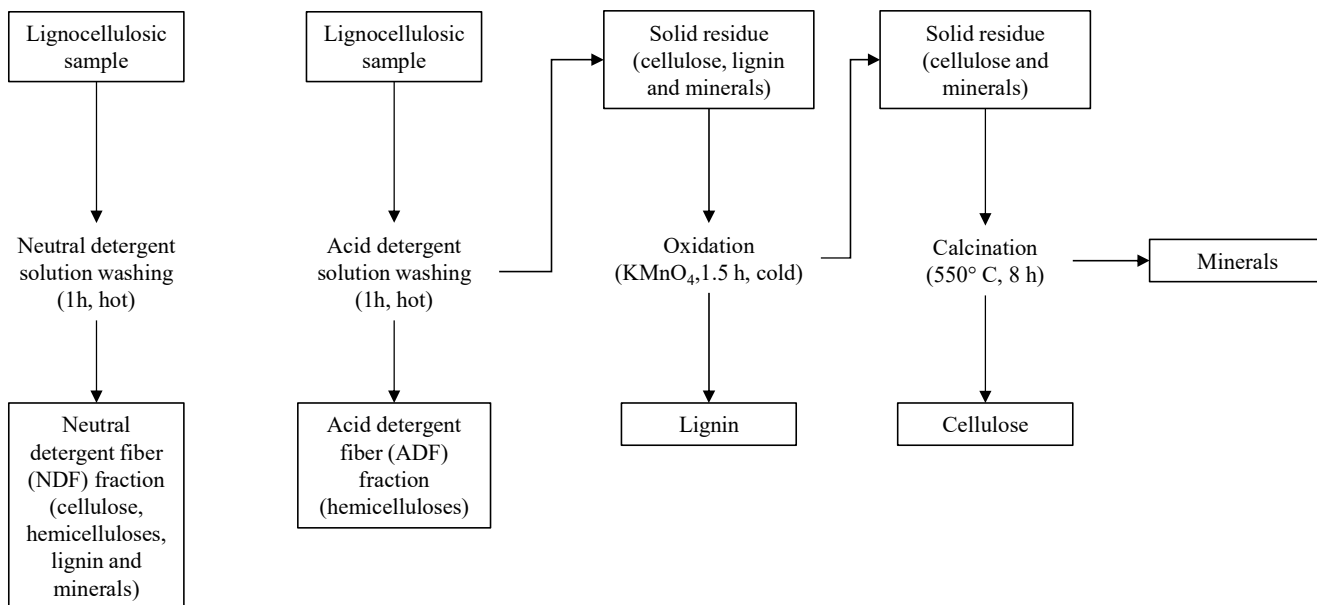


Figure 56. ADF-NDF method scheme

To perform the attacks, 1 g of sample was placed in a sintered glass filter crucible. The NDF attack was made by adding 100 mL of the NDF solution to the sample. After one hour of reaction at boiling temperature, the solution was removed by suction and the solid residue was rinsed with boiling water until the foam disappeared. The sample was then removed from the system and left to dry in an oven at 103° C for 12 hours. Afterward, it was cooled in a desiccator and then weighed. Finally, the sample was calcined at 550° C for 8 hours. Subsequently, it was cooled in a desiccator and then weighed.

For the ADF attack, the same procedure was followed but using the ADF solution. The potassium permanganate attack was carried out on the samples that had undergone the ADF attack using Fibertec 1020 cold extraction unit (Foss, France) by the addition of 25 mL of a mixture of saturated potassium permanganate solution and a buffer solution (2 : 1, v/v). After 1 h 30 min of contact and regular stirring, the reagents were removed by suction and the residue was rinsed first with a demineralizing solution until white fibers were obtained, then by ethanol 80 % and ethanol 95° rinses. The sample was then removed from the system and left to dry in an oven at 105° C for 12 hours. Afterward, it was cooled in a desiccator and then weighed. The sample was then calcined at 550° C for 8 hours. Subsequently, it was cooled in a desiccator and then weighed. The analysis was made at least in duplicate for each sample and the results were expressed as a percentage on dry weight basis (g/ 100 g of dry teak sawdust).

By gravimetric differences the content of each fiber was calculated as follows:

Cellulose (C) content:

$$C (\%) = \frac{(m_{KMnO_4} - m_c) * (1 - MM)}{m_{sample} * DM} * 100$$

Lignin (L) content:

$$L (\%) = \frac{m_{ADF} - m_{MnKO_4}}{m_{sample} * DM} * 100$$

Hemicelluloses (HC) content:

$$HC (\%) = \frac{(m_{NDF} - m_c) * (1 - MM)}{m_{sample} * DM} * 100 - L - C$$

Where:

$m_{sample}$ : sample mass (g)

$m_{NDF}$ : mass of sintered glass filter crucible after NDF attack (g)

$m_{ADF}$ : mass of sintered glass filter crucible after ADF attack (g)

$m_{KMnO_4}$ : mass of sintered glass filter crucible after  $KMnO_4$  attack (g)

$m_c$ : mass of sintered glass filter crucible (g)

MM: mineral matter content (%)

DM: dry matter content (%)

## 5.6 Protein content

The protein content was estimated by Kjeldahl method assisted by a Tecator Kjeltex 2020 digestion system and Kjeltex 8400 analyzer (Foss, France). The Kjeldahl method involves the determination of the total nitrogen in the products (i.e. it does not directly measure protein). The percentage of protein present is calculated from the nitrogen content using a factor. The correct factor would depend on the amino acid composition and the concentration of the different proteins present in the product as well as the non-protein nitrogen content (Evers and Hughes, 2002).

The analysis procedure is as follows the sample is digested at 400° C for 2 hours with concentrated sulfuric acid (98.5 %) catalyzed by hydrated copper sulfate. The nitrogen in the sample is thus converted to nonvolatile ammonium sulfate. After cooling and dilution of the digest, the ammonium sulfate is converted to volatile ammonia gas by heating with sodium hydroxide. The ammonia is steam-distilled

into an excess of boric acid solution (4 %), where it is trapped by forming ammonium borate. The amount of borate formed is determined by titration using hydrochloric acid and methyl red, and bromocresol as indicators. The protein content is then calculated by multiplying the equivalent nitrogen content given by the Kjeldahl procedure by the protein factor. In this work, we used the classical factor (6.25) which considers 16 % of nitrogen content in proteins. The results reported were the average of duplicated and expressed in dry weight basis.

$$\text{Protein content (\%)} = \frac{(V_{\text{sample}} - V_{\text{blank}}) * N_{\text{HCl}} * 14.007}{m_{\text{sample}} * DM} * 6.25 (\%)$$

Where:

$V_{\text{sample}}$ : Volume of hydrochloric acid used in the titration of the sample (L)

$V_{\text{blank}}$ : Volume of hydrochloric acid used in the titration of the blank (L)

$N_{\text{HCl}}$ : Normality of HCl (mol/L)

$m_{\text{sample}}$ : sample mass (g)

DM: dry matter content (%)

## 5.7 Extractives content (yield)

The extractives content (extractives yield) is the ratio of the dry matter of an extract to the dry matter of the raw material from which it was obtained. The results were the average of duplicated or triplicated and expressed as a percentage on dry weight basis (g of extractive/100 g of dry teak sawdust). The extractives content was determined by three methods, namely Soxhlet, ASE, and Fibertec extractions.

$$\text{Extractive content (\%)} = \frac{m_{de}}{m_{ds}} * 100$$

Where:

$m_{de}$ : dry extract (g)

$m_{ds}$ : dry sample mass (g)

### 5.7.1 Soxhlet extraction

Soxhlet extraction is one of the most popular techniques for extraction of analytes from solid materials. Since its discovery in 1879, the standard Soxhlet technique has been routinely applied in almost every analytical laboratory. Up to this day, Soxhlet extraction technique remains a standard technique to which the performance of modern extraction techniques is compared (Zygler et al., 2012). The inconvenience is that the long time and temperature could degrade the compounds if they are not thermally stable.

The sample material was packed in a handmade-thimble of filter paper with a small amount of cotton placed above to contain the sawdust into the thimble. The thimble-holder is gradually filled with condensate fresh solvent from a distillation flask. When the liquid reaches the overflow level, a siphon aspirates the solution of the thimble-holder and it falls back into the distillation flask carrying the extracted analytes into the bulk liquid. This operation is repeated until complete extraction is achieved.

The conditions of the extractions performed in a Soxhlet apparatus are presented in Table 37. Solvents were obtained from VWR International.

Table 37. Conditions of Soxhlet extractions

	<b>1-lot TS</b>	<b>2-lot TS</b>
Sample weight (g)	8	13-14
Solvent volume (mL)	150	200
Extraction time (h)	7-15	7
Solvents	cyclohexane, acetone, ethanol, methanol, water	cyclohexane, acetone, ethanol, methanol, water

After the extraction, the organic solvent was evaporated under vacuum using a rotary evaporator. Crude extracts were stored in desiccators for final drying and weighed to determine extractive content. The results were the average of duplicated or triplicated and expressed as a percentage on dry weight basis (g of extractive/100 g of dry teak sawdust).

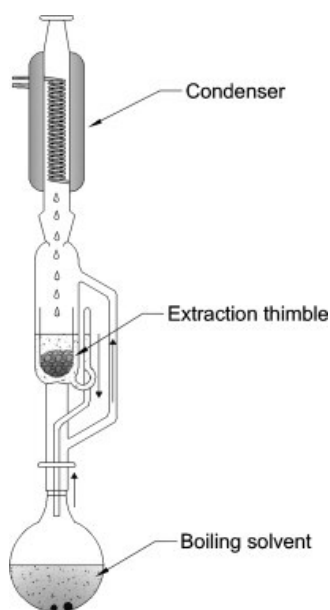


Figure 57. Soxhlet device

### 5.7.2 Accelerated Solvent Extraction

Accelerated solvent extraction (ASE) was carried out using an ASE Dionex 350 Thermo Scientific system. Figure 58 shows a schematic of the extraction process. The teak sawdust is loaded into a sample cell and the end caps (each containing a frit) are tightened by hand onto the cells. The filled sample cells are loaded onto a cell tray and collection vessels (bottles or vials) are loaded onto a collection tray. A robotic arm transfers each cell separately into the oven for extraction. The oven is maintained at the selected operating temperature throughout the extractions. The cell design allows the operation of the extractions at elevated pressures to maintain the solvents as liquids at temperatures above their boiling points. The temperature and pressure are controlled independently for each cell regardless of the solvent used, the moisture or mineral content of the sample, or any characteristic of the matrix that might affect the actual extraction temperature. Once the cell is in place in the oven, the pump immediately begins to deliver the solvent of choice to the sample cell. Once the solvent has made its way through the sample cell and reaches the collection vessel, the static valve closes to allow pressurization of the cell. Since the solvent expands as it heats, the pressure in the cell will increase when the static valve closes. When the pressure reaches 200 psi above the set point, the static valve rapidly opens to relieve the pressure and then closes again. The pump also delivers fresh solvent to the cell in an effort to return the pressure to the set point value. This addition of fresh solvent during ASE is analogous to fresh solvent dripping down from the condenser onto the extraction thimble during Soxhlet extraction.

During the heat-up time, the cell contents are heated by the oven to the selected operating temperature. After the heat-up time, the extraction enters a static period. After the static time, fresh solvent is pumped



through the cell to remove extracted analytes while the sample and solvent are still hot. The number of static cycles is the number of times the sample will be in the static mode. The rinse volume is divided by the number of static cycles, so that fresh solvent is present at the beginning of each static cycle. Following the final solvent rinse, solvent is purged out of the cell (using nitrogen at 150 psi) for a predetermined period of time. The extracts are delivered to the collection vessels through a filter.

The variables and operating conditions of ASE are presented in Table 38. Each extraction was performed in triplicate and the results were expressed as a percentage on dry weight basis (g of extractive/100 g of dry teak sawdust).

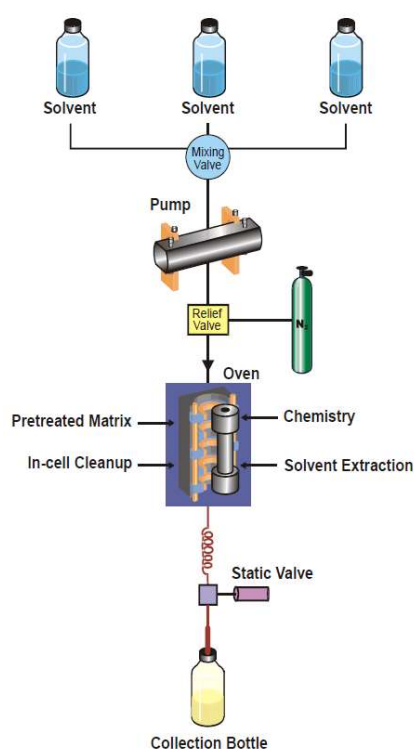


Figure 58. Schematic of the accelerated solvent extraction (ASE) process

Table 38. Variables and operating conditions of ASE

Sample weight (g)	1.8-2
Extraction temperature (°C)	100
Heat-up time (min)	5
Static time (min)	5-7
Maximum pressure (bar)	200
Rinse volume (% of the cell volume)	150
Cell volume (mL)	10
Number of cycles	3
Purge time (s)	120
Total extraction time (min)	32
Solvents	cyclohexane, acetone, ethanol, methanol, water

### 5.7.3 Fibertec extraction

Water extraction was performed with a Fibertec 1020 extractor (Foss). An amount of about 1.3 g of teak sawdust was placed in a sintered glass filter crucible (Foss). The glass crucible was placed in the hot extraction unit equipped with a cooling circuit to prevent vapor loss. Then, a volume of 100 mL of deionized water was poured from the top of the extractor to the glass crucible. The system was heated to the boiling temperature of the water and held for 2 h. After the time had elapsed, the extraction water was recovered by suction using a vacuum tube and stored at -24°C for further assays. The results were the average of triplicates and expressed as a percentage on dry weight basis (g of extractive/ 100 g of dry teak sawdust).

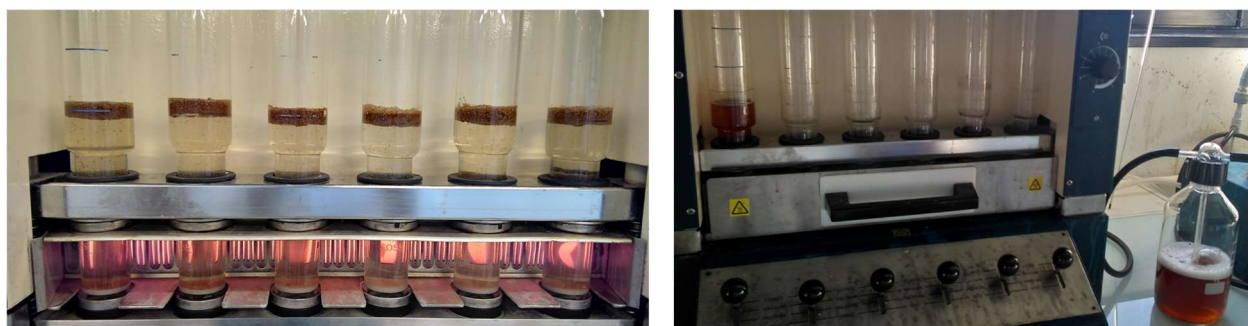


Figure 59. Hot water extraction with a Fibertec 1020 (Foss) system (left) and extract recovery (right)

## 5.8 Total phenolic content

The amount of total phenolic content (TPC) was determined using the Folin-Ciocalteu (F-C) method (Singleton and Rossi, 1965) with gallic acid as standard, see Figure 60. It involves oxidation in alkaline solution of phenols by the yellow molybdotungstophosphoric heteropolyanion reagent and colorimetric measurement of the resultant molybdotungstophosphate blue, see Figure 61 (Ford et al., 2019). These blue pigments have a maximum absorption depending on the qualitative and/or quantitative composition of phenolic mixtures and on the pH of solutions, usually obtained by adding sodium carbonate (Cicco et al., 2009). This method lacks sensitivity and its accuracy is uncertain due to interference from other substrates (e.g. soluble sugars and proteins) in the plant matrix that can react with the F-C reagents, causing artificially high values. Therefore, the F-C reaction is not sensitive to phenols but a measurement of anything in the matrix that is able to reduce, and therefore be oxidized itself. There is an assumption that the majority of reducing power in a plant matrix is due to phenolics and therefore the F-C (Ford et al., 2019). However, the total phenolic content determined by F-C is convenient, simple, and reproducible.

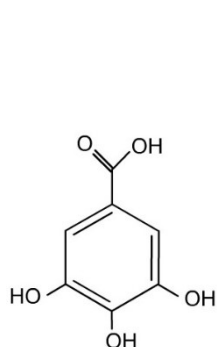


Figure 60. Chemical structure of gallic acid

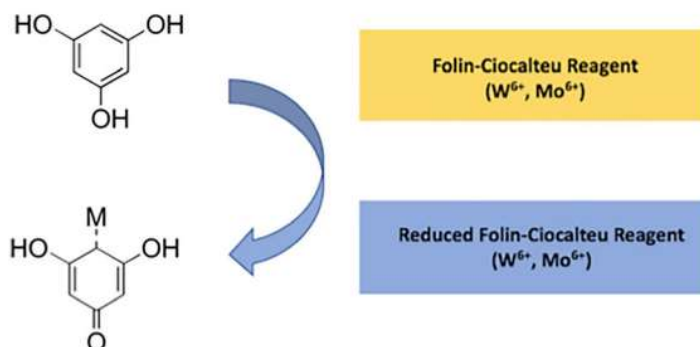


Figure 61. Reduction of the Folin-Ciocalteu reagent by the oxidation of phenols in a sample

The assay was performed by the sequential addition of 20  $\mu\text{L}$  of the sample, 10  $\mu\text{L}$  of Folin-Ciocalteu reagent, and 170  $\mu\text{L}$  of sodium carbonate solution (2.36 % w/w) in a 96-well microplate. The calibration curve was prepared with solutions of gallic acid at different concentrations ranging from 0 to 100 mg/L. The absorbance was measured at 700 nm with a microplate reader (Spectrostar-Nano, BMG Labtech) after 45 min of incubation at 45°C. Gallic acid monohydrate (GA,  $\geq 99\%$ ), Folin-Ciocalteu reagent, and sodium carbonate were purchased from Sigma Aldrich.

Using the Beer–Lambert law the absorbance of the blue wavelength was calibrated using gallic acid as standard. The phenolic content is calculated as equivalents of the standard.

$$TPC = \frac{C_{GAE} * V_{extract} * d}{m_{sample} * DM}$$

Where:

$C_{GAE}$ : Polyphenol concentration (mg of gallic acid equivalent/ mL) in the reaction mixture

$V_{extract}$ : Volume of extract (mL)

$m_{sample}$ : sample mass (g)

d: dilution factors of extract

DM: dry matter content

All measurements were performed in quadruplicate. The results were expressed as mg of gallic acid equivalent per gram of dry extract (mg GAE/ g extract).

## 5.9 Secondary metabolites identification and quantification

### High-performance liquid chromatography-diode-array UV/VIS detector (HPLC-DAD)

The identification and quantification of phenolic compounds were performed by high-performance liquid chromatography with a diode-array detector (HPLC-DAD) Dionex Ultimate 3000 (Thermo Scientific), a column Luna 3u PFP(2)100Å, 150 x 3.0 mm (Phenomenex), and a guard column with the same characteristics. Compounds were identified by the comparison of the retention time and UV spectrum recorded between 200 nm and 400 nm with those obtained by standards. The quantification of phenolic compounds was measured at 254 nm.

HPLC conditions were as follows, mobile phase: solvent A: water; solvent B: acetonitrile; solvent C: methanol, the three eluents with 0.1% v/ v of formic acid. Elution gradient: 0-6 min: 80-75% A, 5-10% B; 6-10 min: 75- 50% A, 10-35% B; 10- 25 min: 50- 30% A, 35- 60% B; 25-27 min: 30- 25% A, 60- 70% B; 27- 30 min: 25- 20% A, 70- 75% B; 30- 35 min: 20-15% A, 75- 80% B; 35- 50 min: 15-1 0% A, 80- 85% B. Flow rate: 0.3 mL/ min. The quantification of main phenolic compounds was measured at 254 nm. Before injection, the extract was filtered through a 0.2 µm PTFE syringe filter. The results were expressed as mg of the phenolic compound per gram of dry extract (mg/ g extract).

The authentic standards: 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthoquinone (Lapachol, 98%), anthraquinone-2-carboxylic acid (98%), and 2-methylantraquinone (tectoquinone, ≥95%) were purchased from Sigma Aldrich. Solvents acetonitrile and methanol (HPLC grade) were obtained from VWR International; ultra-pure water was used (18.2 MΩ cm, Purelab classic apparatus, Elga).

## 5.10 Antioxidant activity assay

The method of free radical scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) provides an approach for evaluating the antioxidant potential of certain compounds. It was developed by Blois in 1958 with the viewpoint to determine the antioxidant activity by using a stable free radical DPPH. The assay is based on the measurement of the scavenging capacity of antioxidants towards it. The odd electron of nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine, see Figure 62. DPPH is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole so that the molecules do not dimerise, like most other free radicals. The delocalization also gives rise to the deep violet color, with an absorption in ethanol solution at around 520 nm (Kleinrichert and Alappat, 2019), see Figure 63. On mixing DPPH solution with a substance that can donate an electron or hydrogen radical, it becomes a stable, diamagnetic molecule, it gives rise to the reduced form with the loss of violet color. The resulting decolorization is stoichiometric with respect to the number of electrons taken up. The diphenylpicrylhydrazine can be oxidized only with difficulty, and then irreversibly. Representing the DPPH radical by  $Z\cdot$  and the donor molecule by AH, the primary reaction is as follows:

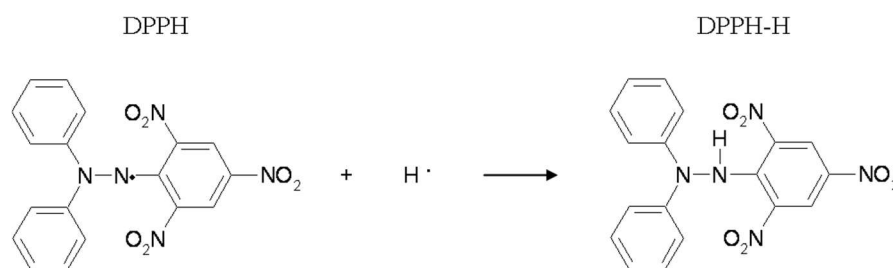
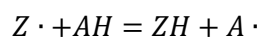


Figure 62. 1,1-diphenyl-2-picrylhydrazyl (free radical, violet color) 1,1-diphenyl-2-picrylhydrazine (non-radical)

Where ZH is the reduced form and  $A\cdot$  is free radical produced in the first step. The latter radical will then undergo further reactions which control the overall stoichiometry. The reaction is therefore intended to provide the link with the reactions taking place in an oxidizing system, the DPPH molecule  $Z\cdot$  is thus intended to represent the free radicals formed in the system whose activity is to be suppressed by the substance AH (Kedare and Singh, 2011).

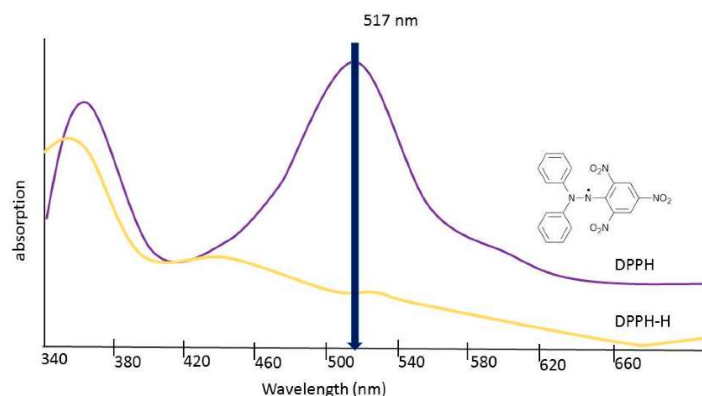


Figure 63. 1,1-diphenyl-2-picrylhydrazyl (DPPH) adsorption spectra

It is a rapid, simple, inexpensive, and widely used method to measure the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity.

The assay was carried out by the sequential addition of 150  $\mu\text{L}$  of the extract diluted in ethanol and 150  $\mu\text{L}$  of DPPH ethanolic solution (0.238 mM) in a 96-well microplate. For each extract, seven concentrations were tested and each dilution was conducted in six-fold. The blank was made by mixing the DPPH solution with ethanol. The absorbance was then recorded at 516 nm after 40 min of incubation in darkness at room temperature. The equipment utilized was BMG-Labtech Spectrostar-Nano and the DPPH was purchased from Sigma Aldrich. The antioxidant activity is determined from the response curve plotted from the following function:

$$f(MS_i) = 1 - \frac{A}{A_0}$$

Where:

$MS_i$ : concentration of each extract dilution

A: final absorbance of the reaction mixture

$A_0$ : initial absorbance of DPPH

The antiradical activity of the extracts obtained using soxhlet, ASE, and Fibertec devices was evaluated in relation to the value of the 50 % DPPH radical inhibitory concentration ( $IC_{50}$ ). It means the minimum concentration of extract necessary to reduce half of the DPPH molecules present in the reaction medium. Thus, the lower the IC value, the higher the antiradical activity of an extract. For the aqueous extracts from extractions performed with a twin-screw extruder, the evaluation included the subtraction of the contribution of color absorbance when determining it at each dilution without DPPH. The  $IC_{25}$  was reported.

## 5.11 Extractions using a twin-screw extruder

Twin-screw extruders consist of two intermeshing, co-rotating screws mounted on splined shafts in a closed barrel. The barrel can be divided into different types of modules: open modules to introduce materials (liquid or solid), closed and thermo-regulated modules or filtration modules equipped with a filter. The different screw elements fitted on the shaft can have different shapes, conformation, and actions. The successive arrangement of the screw elements depends on the unit operation and the mechanical treatment imposed on the biomass through the screw profile. Table 39 displays the characteristics of the screw elements used in this study (Vandenbossche et al., 2019).

Table 39. Characteristics of common screw elements

Denomination	Mixing	Shear	Transport	Remarks
<b>Direct pitch screw elements</b>				
Trapezoidal double-thread T2S	+	+	+++	Non self-cleaning Improve the input in the feeding zone
Conjugated double-thread C2F	+	+	++	Self-cleaning
<b>Mixing elements</b>				
Bilobe mixing disk BB	+++	++	Depend on angle	High mixing capacity Transport capacity depends on the angle between elements Residence time increase
<b>Reverse screw elements</b>				
Reversed double-thread CF2C	++++	++++	---	High counter pressure Residence time increase Dynamic plug formation for liquid/solid extraction

### 5.11.1 Twin-screw extruder Clextral BC21

The modularity of twin-screw extruders enables the use of numerous types of elements (conveying screw, back-flowing screw, mixers, kneaders, etc.) which can be combined with countless possibilities. The design of the screw profile will affect flow properties within the extruder and local parameters like filling ratio, shear rate, temperature and residence time (Domenech et al., 2013).

Twin-screw extruder BC21 (Clextral, France) consisted of a closed barrel with seven modules of 100 mm in length. The configuration, screw profile, and process temperature are shown in Figure 64.



Module	1		2		3		4		5			6		7			
Screw type	T2F	T2F	C2F	C2F	C2F	BB	C2F	C2F	C2F	C2F	BB	C2F	C2F	C2F	C2F	CF2C	C2F
Length (mm)	50	50	50	50	50	25	50	50	50	25	50	25	25	50	25	50	25
Pitch (mm) or angle	50	50	33	33	25	90°	33	33	25	16	90°	25	25	16	16	-33	33

Figure 64. Configuration and screw profile of twin-screw extruder BC21 (Clextral, France) for teak sawdust extraction. T2F: trapezoidal double-thread screw, C2F: conveying double-thread screw, BB: bilobe paddle screw, CF2C: reversed double-thread screw

In the configuration used in trials, modules 2, 3, 4, 5, and 7 were thermo-regulated. They were heated by thermal induction and cooled by water recirculation. The teak sawdust (TS) was fed in module 1 by a twin-screw-volumetric feeder (KCL-KT20 K-Tron Soder). The TS flow was adjusted to a value of approximately 2.2 kg of fresh matter/h, it was used as received from the sawmill without prior conditioning. The solvent (water) was input through two injections in modules 2 and 5. The water flow rate was kept constant at liquid to solid (L/S) ratio equal to 1 Kg of total liquid/ Kg of dry TS at module 2. In module 5, the water flow rate was varied in such a way that this module had an L/S ratio from 3.25 Kg/h to 4.25 Kg/h in accordance with the experimental design defined. Module 6 was equipped with a filter made up of four hemispherical dishes with conical holes of 1 mm of inner diameter and 2 mm of outer diameter. The filter allowed the recovery of the filtrate. The extrudate was recovered at the exit of the extruder in module 7. After extrusion, the extrudate was dried for biocomposite manufacturing. Likewise, the filtrate was clarified through a centrifugation process and then using filter paper because the particle size of the sediment was so small that its content in the filtrate after centrifugation was very high. Further analyses were then carried out (compounds identification and total phenolic content determination). Both outputs were quantified for mass balance purposes.



The process parameters (temperature, screw speed, and teak sawdust flow rate) were controlled by a control panel.

### 5.11.2 Twin-screw extruder Clextral BC45

Twin-screw extruder BC45 (Clextral, France) consisted of a closed barrel with seven modules of 200 mm in length. Module 1 was open at the top for a solid feed using twin-screw-volumetric feeder (LWF-D5 S K-Tron Soder). Module 6 was open at the bottom for filtrate recovery. All closed modules (2, 3, 4, 5, and 7) were thermo-regulated by an induction heating system and the cooling system was controlled by solenoid valves. The configuration, screw profile, and process temperature are shown in Figure 65.

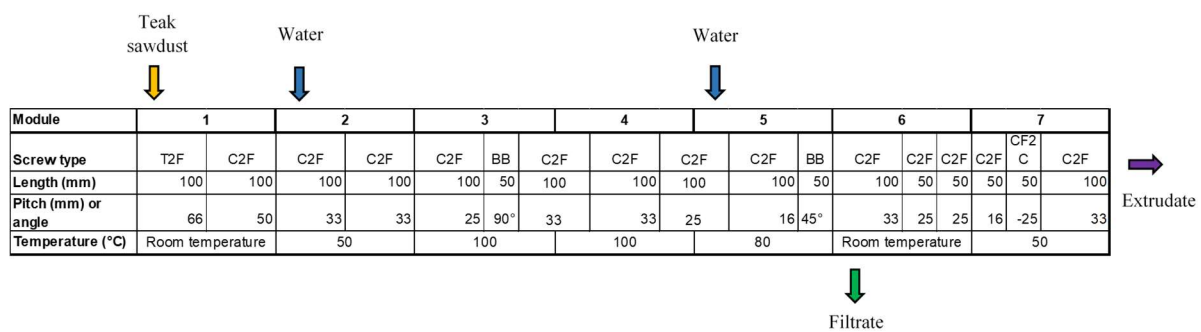


Figure 65. Configuration and screw profile of twin-screw extruder BC45 (Clextral, France) for teak sawdust extraction. T2F: trapezoidal double-thread screw, C2F: conveying double-thread screw, BB: bilobe paddle screw, CF2C: reversed double-thread screw

The first screws of the profile, trapezoidal double-thread screw and high pitch (T2F66) are conveying screws; they facilitate the introduction of the raw material into the barrel. Then, the conveying double-thread screw, direct and decreasing pitch (C2F50, C2F33, C2F25) ensure the efficient transport of the material as well as compression and initial contact with water (extraction solvent), which is injected in module 2. These screws carry out, in a lesser proportion, mixing and shearing operations because of their intermeshing character.

The following elements are the bilobed mixing discs (BB arranged at 90°), they are restrictive elements that ensure a high shear effect and therefore defibration and mixing of the solid material with the liquid. The stagger angle plays an important part. The elements with a stagger angle of 90° are flow-neutral and lack any axial conveying capability (Bauer et al., 2021).

Later, there is a sequence of double threaded, direct and decreasing pitch screws (C2F33, C2F25, and C2F16). They allow the contact between the material and water, transport and compression of the

mixture. A second water input is made in module 5. BB elements are placed after the water feeding. Unlike the first arrangement, the BB screws with 45° stagger angle have a higher pressure build-up capability and better mixing capability

## 5.12 Biocomposites

### 5.12.1 Preparation of biocomposites

Poly(lactic acid) was the thermoplastic polymer used for biocomposites manufacturing. Injection grade PLA Ingeo™ biopolymer 3251D was purchase from NatureWorks®. The physical and mechanical properties of PLA are shown in Table 40 and the processing temperature profile recommended is presented in Table 41.

Table 40. Physical and mechanical properties of PLA

<b>Physical properties</b>	<b>Ingeo 3251D</b>
Specific Gravity	1.24
MFR, g/10 min (210°C, 2.16kg)	80
MFR, g/10 min (190°C, 2.16kg)	35
Relative Viscosity	2.5
Crystalline Melt Temperature (°C)	155-170
Glass Transition Temperature (°C)	55-60
Clarity	Transparent
<b>Mechanical Properties</b>	
Tensile Yield Strength, psi (MPa)	9,000 (62)
Tensile Elongation, %	3.5
Notched Izod Impact, ft-lb/in (J/m)	0.3 (16)
Flexural Strength (MPa)	15,700 (108)
Notched Izod Impact, ft-lb/in (J/m)	0.3 (16)
Heat Distortion Temperate (°C)	55

Table 41. Processing temperature profile recommended for PLA extrusion

<b>Processing temperature profile</b>	
Melt Temp.	188-210°C
Feed Throat	20°C
Feed Temp.	166-177°C
Compression Section	182-193°C
Metering Section	188-205°C
Nozzle	188-205°C
Mold	25°C
Screw Speed	100-200 rpm
Back Pressure	50-100 psi
Mold Shrinkage	0.004 in/in. +/-0.001

The teak extrudate was dried at 70°C overnight before blending with PLA. The biocomposites were prepared at three different loads of teak extrudate (10 wt.%, 20 wt.%, and 30 wt.%) using a twin-screw extruder Evolum HT25 (Clextral, France). The configuration and screw profile were the same for all compositions, they are shown in Figure 66. The operating temperatures of the extruder were 50 °C in the feeding zone, 180 °C/190°C in the mixing zone, and 180°C/190°C at the die exit, see Table 42. The process conditions measured at the extrusion process are presented in Table 43.

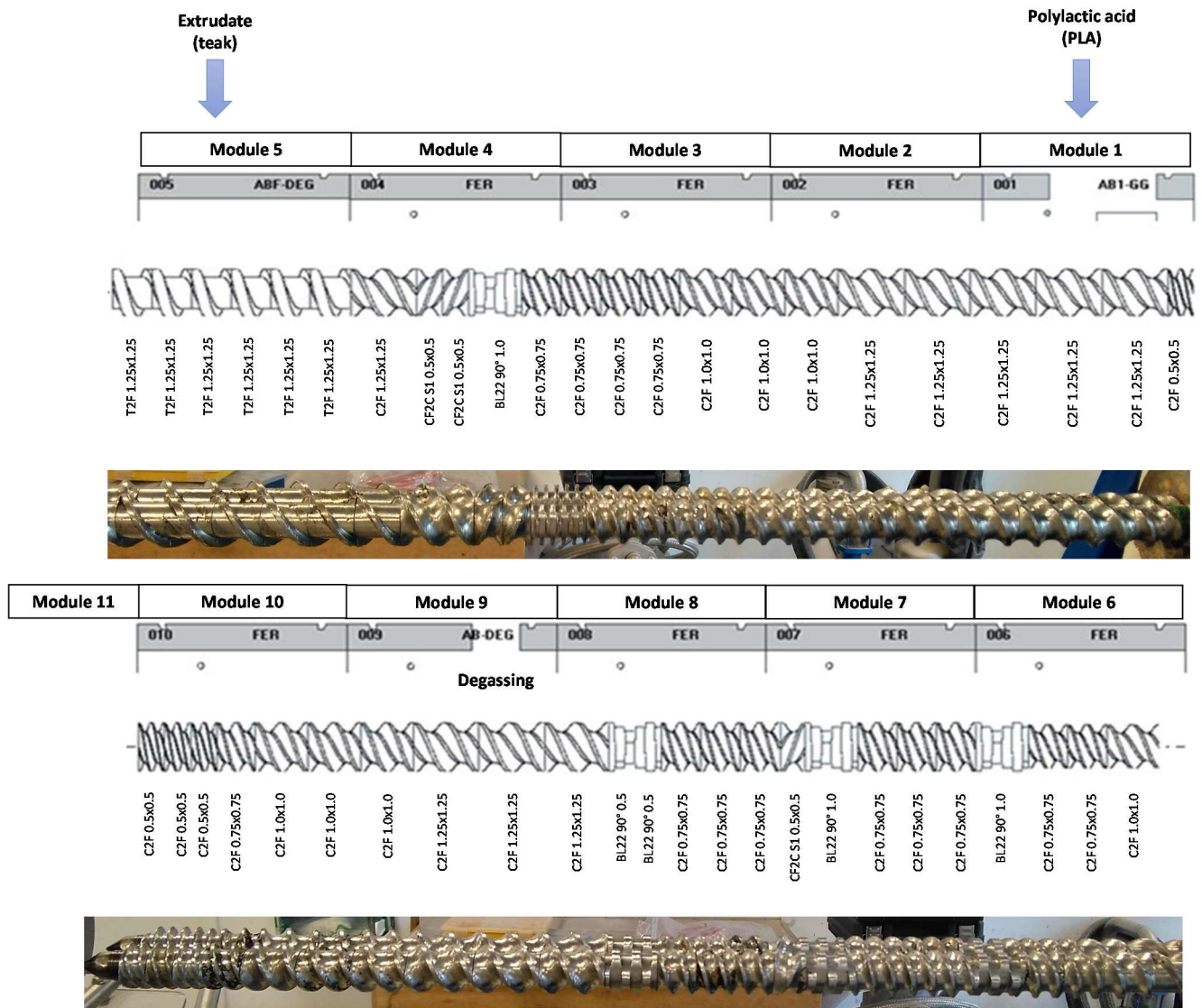


Figure 66. Configuration and screw profile of twin-screw extruder Evolum HT25 (Clextral, France) for biocomposite manufacturing. T2F: trapezoidal double-thread screw, C2F: conveying double-thread screw, BL22: bilobe paddle screw, CF2C: reversed double-thread screw

Table 42. Process temperature of each module of extruder Evolum HT25 (Clextal) in biocomposite manufacturing at different load of teak extrudate

Module	Temperature set point (°C)											
	1	2	3	4	5	6	7	8	9	10	11	
PLA neat	-	80	180	180	180	180	180	180	180	180	180	180
PLA/10 wt.% teak extrudate	-	80	180	180	180	180	180	180	180	180	180	180
PLA/20 wt.% teak extrudate	-	80	180	180	180	180	180	180	180	180	180	180
PLA/30 wt.% teak extrudate	-	80	180	180	180	180	180	180	180	190	190	190

Table 43. Process conditions of biocomposite manufacturing using an extruder Evolum HT25 (Clextal)

	PLA neat	PLA/10 wt.% teak extrudate	PLA/20 wt.% teak extrudate	PLA/30 wt.% teak extrudate
Motor current (A)	32.8±1.0	33.7±0.8	37.6±0.8	36.4±0.9
Screw speed (rpm)	199±2	199±2	200±2	247±1
Stop pressure (bar)	3.1±1.0	4.9±0.4	8.7±0.4	11.7±1.0

The extruded composites at 10 wt.% and 20 wt% were granulated using an automatic cutter installed at the outlet of the extruder after a tray water cooling system, see Figure 67. The extruded compound at 30 wt.% was recovered directly at the extruder outlet and cooled in the open air, after that, it was granulated using a granulator, see Figure 68.

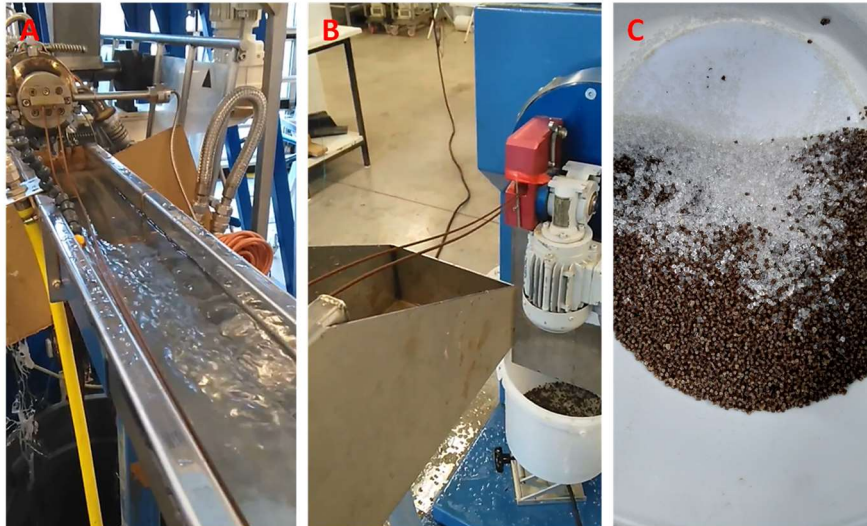


Figure 67. Production of biocomposites PLA/10 wt.% teak extrudate and PLA/20 wt.% teak extrudate. A: cooling system installed at the extruder outlet; B: automatic cutter placed after cooling system; C: neat PLA pellets (above) and PLA/10 wt.% teak extrudate pellets (below)

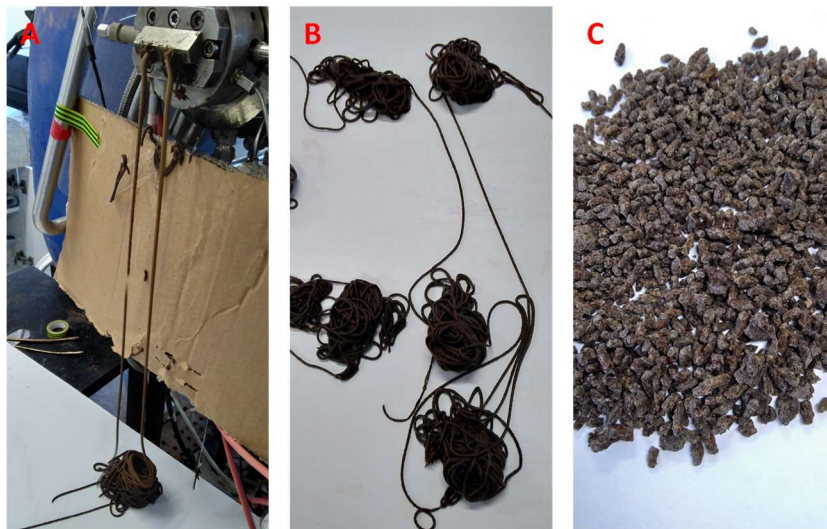


Figure 68. Production of biocomposites PLA/30 wt.% teak extrudate. A: biocomposite at extruder outlet; B: air-drying of biocomposites; C: PLA/30 wt.% teak extrudate pellets

Once the biocomposites were prepared in pellet form, they were injected into an injection-molding machine to make the specimens for mechanical tests, see Figure 69. The biocomposites (PLA/teak extrudate) were dried at 90°C before injection molding. The operating temperatures were 20 °C in the feeding zone, 160 °C in the mixing zone, 170 °C in the reacting zone, and 180 °C at the exit (nozzle). Figure 70 illustrates the process of preparing the mechanical specimens for testing.



Figure 69. Injection-molding machine (FANUC Series 180is-IB) (left) and clamping unit with mold for flexural test specimens (right)

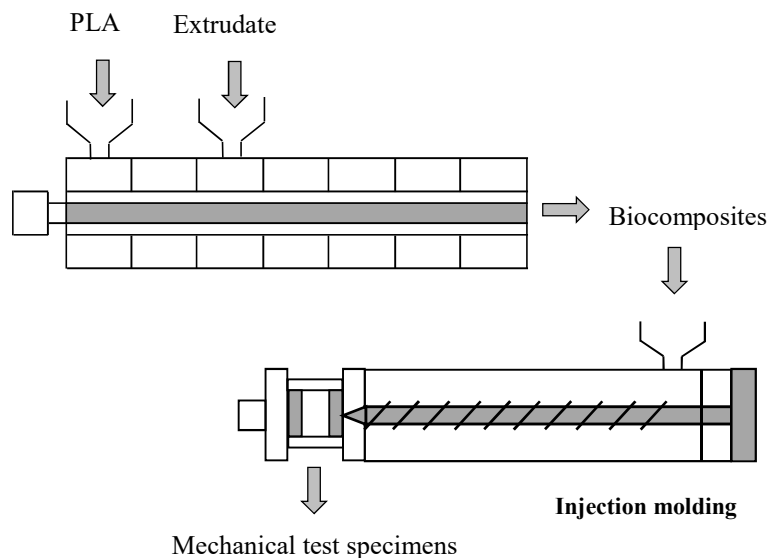


Figure 70. Schematic of the manufacture of mechanical test specimens

### 5.12.2 Mechanical tests

The flexural test under three-point bending was determined by using a universal testing machine (Tinius Olsen H5KT) in accordance with French Standard NF EN ISO 14125. The specimens for the flexural test were rectangular bars with dimensions of 80 mm in length, 10 mm in width, and 4 mm in thickness. The test was performed at a crosshead speed of 2 mm/min. The tensile test was conducted according to French Standard NF EN ISO 527 by using the same machine. The specimens for the tensile test were dumbbell-shaped (Type 1A), see Figure 71; the dimensions are presented in Table 44. The test speed was 1 mm/min. The flexural and tensile test are illustrate in Figure 72.

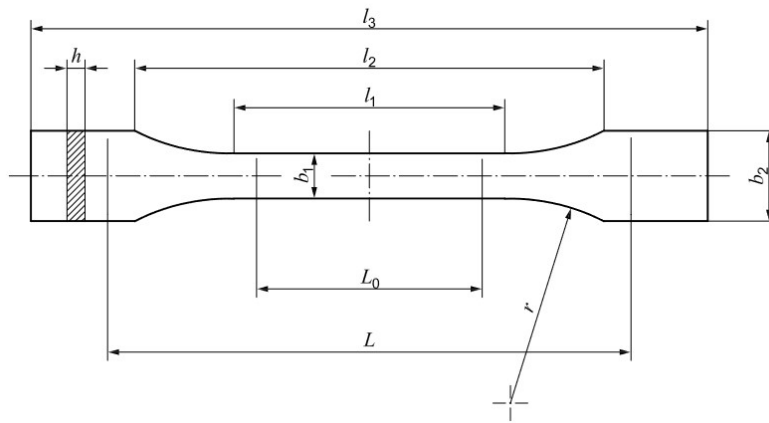


Figure 71. Specimen type 1A for tensile tests

Table 44. Dimensions of specimen type 1A for tensile tests

$l_3$	Overall length (mm)	170
$l_1$	Length of narrow parallel-sided portion (mm)	$80 \pm 2$
$r$	Radius (mm)	$24 \pm 1$
$l_2$	Distance between broad parallel-sided portions (mm)	$109.3 \pm 3.2$
$b_2$	Width at ends (mm)	$20 \pm 0.2$
$b_1$	Width at narrow portions (mm)	$10.0 \pm 0.2$
$h$	Preferred thickness (mm)	$4.0 \pm 0.2$
$L_0$	Gauge length (mm)	$75.0 \pm 0.5$
$L$	Initial distance between grips (mm)	$115 \pm 1$





Figure 72. Flexural test (left) and tensile test (right) with a universal testing machine (Tinius Olsen H5KT)

The mechanical properties of natural fiber composites are known to be influenced by a number of factors including fiber length, the volume fraction of fibers, fiber aspect ratio, their orientation and interfacial adhesion between the fiber-matrix (Huerta-Cardoso et al., 2020).

Tensile strength is one of the important properties for describing the mechanical performance of the material. Tensile strength is the maximum stress that a material can withstand while being stretched or pulled before failing or breaking.

$$TS = \frac{F}{A}$$

Where:

TS: Tensile strength

F: maximum load (force)

A: cross-sectional area

Tensile modulus is the ratio of stress ( $\sigma$ ) to strain ( $\epsilon$ ) at any point along the elastic portion of a stress-strain (load/deformation) curve. It was determined graphically.

In the test of tensile strength, the specimen is pulled from both ends. As the pulling progresses, the specimen bar elongates at a uniform rate that is proportionate to the rate at which the load or pulling force increases. The elongation is the ratio between increased length and initial length.

$$\text{Elongation (\%)} = \frac{L - L_0}{L_0} * 100$$

Where:

$L_0$ : initial gauge length

L: final length

Flexural testing is used to determine the flex or bending properties of a material. It is defined as the maximum stress at the outermost fiber on either the compression or tension side of the specimen.

$$\sigma = \frac{3FL}{2bd^2}$$

Where

F: applied load

L: length of the support span

b: width of test specimen

d: thickness of test specimen

## **General conclusion**



Teak tree cultivation has been growing, mainly in tropical countries, because it is easy to grow, represents a good commercial investment, and is highly valued in the timber market. Global demand for teak is likely to continue to increase, and along with it the by-products (such as chips, shavings, and sawdust) that must be dealt with.

The common disposal of wood industry residues does not offer proposals for their utilization in value-added applications. In addition, it faces the challenge of responding to current environmental concerns, such as the reduction of negative environmental impacts, and the minimization of material and energy resources.

It is in this context that this work proposes a different perspective on the management of wood by-products, in particular teak sawdust. Taking into account the properties attributed to teak wood and its extractives, it was considered as a raw material from which value-added products could be obtained. The potential of teak sawdust as a source of phytochemical compounds and its subsequent use for an integral valorization approach were the aims of this study. First, the recovery of the extractives by means of a twin-screw extruder and water as solvent was evaluated, and then the use of the sawdust after extraction (called extrudate) as a reinforcing material in the preparation of biocomposites was explored.

Initially, a comprehensive review of the literature was conducted. It is well established that teak extractives contain biologically active compounds, mainly quinones and anthraquinones that confer natural resistance against termites and fungi, as well as antioxidant and pharmacological activity. The predominant secondary metabolites that have been identified in teakwood extractives are lapachol, deoxylapachol, 1,4-naphthoquinone, tectoquinone, tectol, anthraquinone-2-carboxylic acid, 2-hydroxymethyl-anthraquinone, and squalene. The content of extractives depends on many factors such as environmental and genetic aspects, radial position, and tree age; in addition, they are more concentrated in the heartwood. In general, the largest amount of extractives is obtained with polar solvents like methanol and ethanol. This allowed us to have a first approach to the material and to know the amount of total extracts that could be achieved, as well as to identify the potentially extractable compounds.

Furthermore, the principle of operation of the twin-screw technology and its use in the treatment of plant material was reviewed. The advantages it offers and its proven use in the processing of lignocellulosic material in a continuous way confirmed it as a solid/liquid extraction method for teak sawdust. Furthermore, it has been demonstrated that the pressure and temperature conditions reached in the mechanical stress zones in twin-screw extruders are favorable for achieving subcritical water conditions.

Teak sawdust was obtained as waste from 14 to 16-year-old debarked teak logs (heartwood and sapwood) from Agropecuaria Santa Genoveva S.A.P.I de C.V. sawmill (Campeche, Mexico). It was received without any treatment other than sun drying. The teak sawdust was characterized (from its physical appearance to the chemical composition of its extracts) to evaluate its potential. In general, the sawdust presented a homogeneous appearance, whose particle size has been found to be in the range of 250  $\mu\text{m}$ - 500  $\mu\text{m}$ . The fiber distribution analysis resulted in a composition of 52.8 % cellulose, 11.2 % hemicelluloses, and 27.3 % lignin.

For the determination of the extractives content, two methods were selected, a conventional and exhaustive method that expends the matter and recovers the maximum amount of compounds (extraction with a Soxhlet apparatus) and a method that pressurizes the solid-liquid system and to which a better efficiency is attributed (ASE). For both extractions, solvents with different polarities were evaluated. The maximum content of extractives, as expected, has been obtained with methanol and ethanol. The yield was 4.74 % for both solvents (ethanol and methanol) when extracted using a soxhlet apparatus. The ASE system achieved up to 4.66 % yield using ethanol and 3.48 % using methanol.

The quality of the extracts was also evaluated in terms of their total phenolic content, antioxidant capacity, and secondary metabolites identified. The TPC, congruently with the yield of extracts, was higher in the methanol and ethanol extracts, where values of 693 mg GAE/g extract and 568 mg GAE/g extract, respectively, for soxhlet extractions have been reached, and up to 811 mg GAE/ g extract for the methanol extract of the ASE extraction. The antioxidant capacity can be related to the total phenolic content considering that the DPPH radical scavenging is mainly attributed to the polyphenols that can donate an electron or hydrogen radical. The antioxidant activity of the extracts obtained was also evaluated. The highest capacity measured as the percentage of DPPH free radical inhibition has been found in the Soxhlet ethanolic extract (108.52 mg/L).

The highest amount of secondary metabolites has been identified in ethanolic Soxhlet extract with values of 14 mg/100 g teak, 19 mg/100 g teak, 10 mg/100 g teak, 20 mg/100 g teak for 2-hydroxymethyl anthraquinone, anthraquinone-2-carboxylic acid, lapachol, 2-methyl anthraquinone, respectively.

Teak sawdust showed a high yield of extractives (obtained with solvents of a wide range of polarities), an elevated TPC, and interesting secondary metabolites, demonstrating its potential as a source of extractives. The recovery of extractives in a continuous process with the possibility of scaling up and handling large volumes of material was then evaluated.

The study of the twin-screw technology as a method of solid/liquid extraction for teak sawdust was first performed with an extruder BC21 and then a scale-up of the process (with a factor of 2) was carried up

with an extruder BC45. Teak sawdust did not require any pretreatment, this represented advantages such as reduction of process time, costs, and materials. Moreover, it showed good processability in the extruder. The extraction of sawdust by means of an extruder led to two products: a filtrate rich in polyphenolic content and a well defibrated extrudate with extractives still retained.

In general, high filtrate recovery values have been obtained (between 60 % and 80 %) as well as an elevated dry extrudate yield (80 % - 90 %), evidencing a good liquid to solid separation when using an extruder BC21. The figures for the extruder BC45 have been 83 % for filtrate recovery and 58 % for dry extrudate yield, in this case, the recovery of the extrudate lower.

Extractive yields between 1.0 % and 1.5 % have been obtained for extractions using an extruder. These values represent only one-third of the extractives that could be obtained with other methods such as ASE or soxhlet. Nevertheless, it must be considered that the residence time of the sawdust in the extruder is very short, which directly impacts the efficiency of the extraction. Regarding the phenolic content in the extracts, overall, high values have been obtained (from 249 mg GAE/g extract to 475 mg GAE/g extract). The polyphenols identified in the extracts were AQ-2-CA and lapachol, this latter to a lesser extent. An average of 25 mg of AQ-2-CA could be recovered per kilogram of teak.

Overall, the scaling was well achieved, obtaining a filtrate with better properties in terms of phenolic content (543 mg GAE/ g dry extract) and antioxidant potential ( $IC_{50} = 0.76$  g/L) than the extrusion performed using an extruder BC21.

The extraction method using the twin-screw technology does not offer an exhaustive extraction of the material but rather continuous and fast processing, capable of handling important volumes of material, which conventional methods used mainly on a laboratory scale could not do. Moreover, the liquid to solid ratio is low, which means less solvent consumption as well as lower costs related to solvent evaporation after processing.

The extrudate from teak sawdust extractions was valorized as a reinforcement of a biodegradable polymeric matrix (PLA). Biocomposites were prepared with the mixture of extrudates from the extruder BC21 for a first series, and with the extrudate from the extruder BC45 for a second series. Both series were prepared at three different fiber loading 10 wt.%, 20 wt.%, and 30 wt.% of teak extrudate. The manufacturing by means of a twin-screw extruder to blend the teak fibers with PLA, followed by an injection molding machine to mold the specimens for the mechanical tests allowed obtaining biocomposites with a smooth and homogeneous overall appearance.

Overall, the mechanical features of the composites prepared with both extrudate sources were similar, with slightly better performance of the second series. The effects of fiber loading on the tensile properties of the composites have been observed at contents higher than 10 %. The tensile modulus of neat PLA has been enhanced up to 20 % and the tensile strength up to 50 % for the composites with 30 wt.% TE. On the other hand, for flexural strength, the loading effect has been evident since 10 wt.% of the wood fiber, with a maximum value of 89 MPA for PLA/30 wt.% TE

This study provides a basis for future valorization studies with a novel extraction method not only for teak sawdust but also for other types of wood and forest residues. Work remains to be done to optimize the process and to purify and evaluate the extracts so that they can be used for subsequent applications. Other points for further research could be:

- The aqueous extracts obtained by means of extruders had a high phenolic content. However, it was not observed a consistent antioxidant activity, evaluated as the ability of the reacting mixture to scavenge DPPH free radicals. It would be interesting to evaluate the antioxidant activity with another assay better adapted to the complex chemical composition of extracts
- The compound identified in greater quantity in the extracts was AQ-2-CA, considering that the extraction was performed in mild subcritical conditions, it would be expected to find also other compounds extractable with less polar solvents such as methanol and ethanol (as 2-methyl anthraquinone). Another screw profile could be undertaken with more restrictive elements that expand the favorable zones to bring water to subcritical conditions.
- A new screw profile of the extruder BC45 that decreases the amount of sediment and therefore increases the dry extrudate yield. In addition, other operating conditions aimed at increasing the content of extracts can be explored, even further scaling could be tested to allow the handling of larger quantities of raw materials could be tested.
- Complement the characterization of biocomposites, for example with moisture absorption evaluation. One of the problems of composites prepared with natural fibers is that they absorb water from the environment and this can compromise their mechanical properties, another aspect to consider regarding moisture is that it can promote the proliferation of fungi in the fibers; this would have a direct impact on the adhesion of the fibers and the polymeric matrix. The teak extrudate still contains extracts that are attributed with fungicidal and decay resistance properties; these qualities could be expected to be maintained and benefit the biocomposite in a particular way with features that other natural fibers would not provide.



- Another way to deepen the assessment of biocomposites could be to take SEM micrographs and to carry out thermal analysis to discuss the interfacial bonding.
- It is well established that teak extracts exhibit fungicidal properties. It would be interesting to study the fungicidal activity of extracts obtained by extrusion.



## Résumé en français

### **Etude de la valorisation du coproduit de scierie de Teck (*Tectona grandis*) par un procédé d'extraction en continu sous eau subcritique**

La direction de cette thèse a été assurée par Gérard Vilarem jusqu'à son départ du laboratoire en août 2020, la direction de la thèse a ensuite été reprise par Virginie Vandebossche.

Le *Tectona grandis* L.f. (teck) est un bois dur tropical bien apprécié sur le marché du bois en raison de ses performances exceptionnelles et de sa valeur économique. Le teck est naturellement présent en Inde, au Myanmar, en Thaïlande et en République démocratique populaire lao (Kaosa-ard, 1981). Les caractéristiques du bois de teck font qu'il convient à un large éventail d'applications. Parmi ses principales qualités figurent la stabilité dimensionnelle et la résistance à la pourriture, toutes deux attribuées à ses matières extractibles. Ceux-ci contiennent des composés biologiquement actifs, principalement des quinones et des anthraquinones qui confèrent une résistance naturelle aux termites et aux champignons, ainsi qu'une activité antioxydante et pharmacologique.

La valeur importante du bois de teck et la facilité d'établissement des plantations en ont fait l'une des espèces d'arbres les plus importantes pour la plantation sous les tropiques, même en dehors de son aire de distribution naturelle (Keogh, 1996). L'Amérique latine apparaît comme une région prometteuse où le teck a été transplanté et où la superficie plantée a considérablement augmenté. Au Mexique en particulier, les plantations forestières représentent une bonne opportunité commerciale à long terme. Selon la Commission nationale des forêts (CONAFOR), le Mexique compte 270 000 ha de plantations forestières commerciales et le *Tectona grandis* est la troisième espèce plantée. L'une des principales plantations de teck du Mexique est l'Agropecuaria Santa Genoveva S.A.P.I. de C.V., située à Campeche. Cette entreprise gère d'importants volumes de bois destinés aux marchés nationaux et internationaux. En raison des grandes quantités de matériaux traités, l'élimination des coproduits est un défi à relever. Néanmoins, il ne s'agit pas d'une préoccupation nationale, l'augmentation de la

demande mondiale de teck va probablement se poursuivre, et avec elle les résidus (copeaux et sciure) à traiter.

Les utilisations ordinaires des déchets ligneux sont les suivantes : matière première pour les biocarburants, substrat pour la transformation en compost qui peut être commercialisé pour les activités de jardinage ou agricoles, et pour l'amendement des sols. Néanmoins, compte tenu du fait que le *Tectona grandis* n'est pas un arbre conventionnel mais un arbre remarquable renfermant des composés d'intérêt, ses coproduits pourraient être source de matière première plutôt que d'être considérés comme déchets. Parmi les principaux métabolites secondaires identifiés dans les matières extractibles du teck figurent le lapachol, le désoxylapachol, la 1,4-naphtoquinone, la 2-hydroxyméthyl-anthraquinone, le squalène et la tectoquinone.

Il existe aujourd'hui de nombreuses techniques pour l'extraction de composés bioactifs à partir de sources naturelles. Cependant, les techniques conventionnelles présentent des inconvénients limitants lorsqu'il s'agit de traiter de grandes quantités de matière. La capacité est un facteur crucial à l'échelle industrielle, c'est pourquoi il est impératif de disposer d'une technologie permettant de gérer de grands volumes de matière. La technologie d'extrusion bi-vis semble être une solution adéquate en raison de sa capacité, de sa flexibilité, de son aptitude à traiter une grande variété de matières premières en continue et avec un bon contrôle des paramètres du processus. En ce qui concerne le solvant, l'utilisation de l'eau est proposée, non seulement parce qu'il s'agit d'un solvant vert, mais aussi parce que les conditions de pression et de température appropriées peuvent être atteintes à l'intérieur de l'extrudeuse en amenant l'eau à des conditions sous-critiques. Les propriétés de l'eau dans des conditions sous-critiques changent considérablement, en particulier la constante diélectrique et par conséquent ses propriétés de solvant qui deviennent similaires aux solvants organiques tels que l'éthanol ou le méthanol.

Les objectifs de ce travail étaient d'étudier le potentiel de la sciure de teck comme source de composés phytochimiques et de proposer une approche pour sa valorisation intégrale. Pour ces objectifs, deux options sont explorées, la récupération des extractibles par la technologie bi-vis utilisant l'eau comme solvant, et la réutilisation de l'extrudat comme matière première pour la préparation de biocomposites.

Pour développer cette étude, le premier chapitre est consacré à la revue de la littérature qui présente cet arbre exceptionnel et décrit les extractibles et métabolites secondaires qui lui confèrent sa résistance naturelle très appréciée. Il aborde également les avantages de la méthode d'extraction choisie dans ce travail, l'extrudeuse à bi-vis, par rapport aux méthodes conventionnelles, ainsi que la commodité de l'utilisation de l'eau.

Le deuxième chapitre décrit l'origine de la sciure de teck et détaille sa caractérisation. L'analyse comprend la distribution des fibres (cellulose, hémicelluloses et lignine par la méthode de Van Soest et Wine) et la caractérisation de ses extraits. Les extractions sont effectuées à la fois avec une méthode conventionnelle (en utilisant un appareil Soxhlet) et une extraction pressurisée (ASE). Les produits extraits sont analysés par chromatographie liquide haute performance (HPLC) et par chromatographie en phase gazeuse (CPG) afin d'identifier et de quantifier les métabolites secondaires extraits. L'activité antioxydante (par test DPPH) et le contenu phénolique total (par méthode Folin-Ciocalteu) sont également quantifiés dans les extraits. Ces résultats nous permettent de déterminer le potentiel de la sciure de teck et d'avoir des valeurs de référence pour l'évaluation de la nouvelle méthode d'extraction proposée dans ce travail.

Le troisième chapitre présente l'étude de l'extraction solide/liquide avec la technologie bi-vis. La première approche est réalisée à l'aide d'une extrudeuse BC21 (Cletral, France), où différentes conditions de température et de ratio liquide/solide sont étudiées selon un plan d'expérience défini. Les extraits obtenus sont analysés pour évaluer la faisabilité de cette méthode d'extraction. Le rendement d'extraction, l'identification et la quantification des composés extraits, et le pouvoir réducteur des extraits sont les principales réponses évaluées. Une étude de mise à l'échelle est ensuite réalisée à l'aide d'une extrudeuse BC45 (Cletral, France). Cette dernière extrusion est principalement axée sur la récupération de l'extrudat pour une valorisation ultérieure.

Le quatrième chapitre explore la valorisation de l'extrudat de teck comme charge pour les composites. La préparation des biocomposites se fait en utilisant du poly(acide lactique) (PLA) comme matrice polymérique et aucun additif ou agent de couplage n'est utilisé. Le choix de ce polymère répond aux préoccupations environnementales concernant la biodégradabilité des nouveaux matériaux. Le PLA présente non seulement un caractère biodégradable, mais aussi de bonnes propriétés mécaniques (par rapport à d'autres biopolymères) et est facile à mettre en œuvre. Le mélange des fibres de bois avec le PLA est assuré par une extrudeuse bi-vis. Les

éprouvettes d'essai mécanique sont ensuite préparés à l'aide d'une machine d'injection. Les caractéristiques mécaniques, telles que le module de traction et de flexion, sont évaluées pour déterminer le renforcement apporté par les fibres de teck au biopolymère.

## **Chapitre 1. Les coproduits de *Tectona grandis* (teck) : une source de composés bioactifs précieux**

Les plantations de teck sont bien développées dans les zones tropicales et subtropicales. Son aire de répartition naturelle s'étend de la longitude 73° E en Inde à 104° 30' en Thaïlande. La limite septentrionale du teck se situe à environ 25° 30' de latitude nord au Myanmar ; et sa limite méridionale se situe entre 9° de latitude nord en Inde, 15°-16° de latitude nord au Myanmar et 16° 30' de latitude en Thaïlande (Kaosa-ard, 1981). L'altitude peut varier de zéro à 1 200 mètres. Les conditions climatiques étendues pour la croissance du teck le rendent très tolérant. Par conséquent, il a été transplanté avec succès en dehors de son lieu d'origine.

Les forêts naturelles de teck ne représentent que 0,7 % de l'ensemble des forêts du monde, c'est-à-dire qu'elles ne constituent qu'une part mineure du commerce mondial. Néanmoins, le bois de teck représente une ressource de bois de cœur luxueuse et pertinente, qui émerge avec un taux de croissance grandissant, et cette tendance devrait se poursuivre. Cela et le fait que le teck s'adapte facilement aux conditions climatiques et qu'il nécessite des rotations courtes pourraient expliquer les investissements que de nombreux pays d'Asie tropicale, d'Afrique et d'Amérique latine réalisent dans les plantations de teck (Camino et Morales, 2013 ; Kollert et Walotek, 2015). Le secteur privé en gère 88 % en Amérique Centrale et 99 % en Amérique du Sud (Kollert et Cherubini, 2012).

Le *Tectona grandis* est un arbre à bois dur très reconnu dans l'industrie du bois en raison de sa valeur économique et de ses caractéristiques exceptionnelles, telles que la résistance, la légèreté, la stabilité dimensionnelle, la facilité de travail, l'aspect esthétique agréable, l'imperméabilité et la résistance à la pourriture (Kokutse et al., 2006). Le duramen du teck peut être utilisé dans de nombreuses applications, tant à l'intérieur qu'à l'extérieur. Par exemple, les meubles, le placage, la sculpture, les ponts de bateaux, les traverses de chemin de fer, la construction navale et l'industrie de la construction (Nidavani, 2014).

En outre, le teck présente des propriétés non corrosives, une résistance aux termites et aux champignons, ainsi que des activités antioxydantes, thérapeutiques et pharmacologiques (Moya et al., 2014 ; Nidavani, 2014 ; Vyas et al., 2018). Ces qualités sont attribuées aux métabolites secondaires présents dans leurs extractibles et à l'effet synergique des propriétés toxiques et antioxydantes (Gupta et Singh, 2004 ; Lukmandaru, 2012 ; Niamké et al., 2011).

Les matières extractibles du duramen sont des composants non structurels produits par un arbre vivant en réponse à des blessures, à un stress physiologique ou comme déchets. Les extractibles sont très variables et se composent d'un large éventail de composés chimiques (Mankowski et al., 2016). De nombreux auteurs ont trouvé une relation directe entre la durabilité du bois et la teneur en matières extractibles (Haupt et al., 2003 ; Kokutse et al., 2006 ; Thulasidas et Bhat, 2007). La teneur en extractibles dépend de facteurs environnementaux et génétiques, de la position radiale et de l'âge de l'arbre (Lukmandaru et Takahashi, 2009 ; Niamké et al., 2014). En ce qui concerne la position radiale, certains travaux indiquent que la concentration en matières extractibles est plus élevée dans le duramen que dans l'aubier. Normalement, elle augmente radialement vers le duramen externe (où elle est plus concentrée). Néanmoins, l'aubier et la zone de transition entre les deux contiennent également certains produits chimiques actifs (Thulasidas et Bhat, 2007).

La durabilité naturelle est définie comme la capacité d'une espèce de bois à prévenir les attaques causées par les agents destructeurs du bois sans aucune modification physique ou chimique (Sundararaj et al., 2015). Le teck est très résistant aux champignons destructeurs du bois sans protection supplémentaire, même dans des conditions de forte humidité du bois ou en contact avec le sol (Windeisen et al., 2003). Cette résistance est attribuée principalement à la quantité et au type de métabolites secondaires présents dans ses extractibles, tels que les anthraquinones et les tectoquinones, qui agissent comme des composés protecteurs face aux stress environnementaux (Brocco et al., 2017 ; Kirker et al., 2013 ; Kokutse et al., 2006). Il convient de noter que la durabilité dépend également de certains facteurs, notamment la dureté, la densité, le lieu d'origine et les pratiques de gestion des plantations (Mankowski et al., 2016).

Il existe de nombreux métabolites secondaires identifiés dans le bois de teck, à savoir les naphtoquinones : lapachol, désoxylapachol, isodéoxylapachol, 5-hydroxylapachol, déhydro-alpha-lapachone (xyloidone), déhydro-bêta-lapachone, 3'-OH-désoxyisolapachol (2-[(1E)-3-hydroxy-3-méthylbut-1-ényl]naphtoquinone), 1,4-naphtoquinone, tectol, hémitectol (2,2-

diméthyl-2H-benzo[h]chromène-6-ol), tecomaquinone-I, déhydrotectol, 4'-5'-dihydroxy-épiisocatalponol, déhydro-alpha-isodunnione. Anthraquinones : tectoquinone, acide 2-anthraquinone carboxylique, 2-hydroxyméthyl-anthraquinone, 9,10-diméthoxy-2-méthyl-1,4-anthraquinone, 2,8-dihydroxy-1-méthoxy-3-méthylanthracène-9,10-dione (obtusifoline), 5-hydroxy-2-méthyl anthraquinone, 1-hydroxy-5-méthoxy-2-méthylanthraquinone, 1,5-dihydroxy-2-méthylanthraquinone, 2-acétoxyméthyl-anthraquinone, anthraquinone-2-carbaldéhyde, 3-hydroxy-2-méthylanthraquinone, 1-hydroxy-3-méthylanthracène-9,10-dione (pachybasine), 2-méthylquinizarine. Triterpènes : squalène, acide bétulinique, polyisoprène. Acides gras : acide palmitique. Stérols : bêta-sitostérol.

Il est bien établi que les métabolites secondaires ont une bioactivité démontrée et il y a une demande croissante de constituants phytochimiques dans l'industrie, en particulier dans les domaines de la pharmacie, la chimie, les cosmétiques et l'alimentation. Comme les études qualitatives et quantitatives des composés bioactifs des plantes reposent principalement sur la sélection d'une méthode d'extraction et d'un type de solvant appropriés, leur développement est également en pleine croissance (Golam Rasul, 2018).

L'extraction des produits naturels progresse à travers les étapes suivantes : le solvant pénètre dans la matrice solide ; le soluté se dissout dans le solvant ; le soluté est diffusé hors de la matrice solide ; et les solutés extraits sont collectés (Zhang et al., 2018).

Il existe de nombreuses techniques d'extraction pour obtenir des composés à partir des plantes. Les méthodes conventionnelles utilisent des solvants organiques ou de l'eau à la pression atmosphérique tandis que les nouvelles méthodes appliquent une pression et des températures élevées. Parmi les méthodes conventionnelles, on peut citer la macération, l'infusion, la percolation, la décoction, le Soxhlet et l'hydrodistillation.

Cependant, les méthodes d'extraction les plus populaires présentent des difficultés d'automatisation et de développement à l'échelle commerciale pour une utilisation compétitive (Luque de Castro et Priego-Capote, 2012). Cet inconvénient pourrait être surmonté par la technologie bi-vis, qui permet de traiter en continu de grandes quantités de matières premières, dont la biomasse lignocellulosique. Cette technologie remplit les fonctions suivantes dans un processus continu : défibrage thermomécanique, imprégnation chimique (mélange solide-liquide intense), micro/macro mélange, séparation solide-liquide.



L'extrusion bi-vis est un procédé basé sur l'action de deux vis qui tournent dans un fourreau fermé, équipé d'un contrôle de température. Cette action produit des forces de cisaillement élevées entre la matière première, les vis et le fourreau, ce qui entraîne une augmentation locale de la température et de la pression le long de l'extrudeuse. Cette technologie est très attractive car elle permet de combiner des actions mécaniques, thermiques et chimiques en une seule étape et en mode continu (Vandenbossche et al., 2019).

Parmi les avantages de la technologie bi-vis, nous pouvons citer les suivants :

- Production en continu
- Système modulaire
- Bon contrôle des paramètres du processus
- Polyvalence thermo-mécanique
- Bonnes capacités de mélange et de transfert de chaleur
- Capacité de charge solide élevée
- Bonne manipulation de grandes quantités de matières premières
- Pas de production de déchets
- Disponibilité à grande échelle

En ce qui concerne l'utilisation de l'extrudeuse bi-vis dans le prétraitement de la biomasse, il existe des études qui évaluent et prouvent son efficacité dans la déconstruction et l'hydrolyse enzymatique de la biomasse lignocellulosique, comme la bagasse d'agave bleu, pinzote de palmier à huile (régime après dégrappage des fruits), la paille d'orge (Vandenbossche et al., 2014) et les coproduits de maïs doux (Fong Lopez et al., 2019 ; Vandenbossche et al., 2015). En outre, le processus d'extraction a été mené avec cette technologie, comme le pressage mécanique pour extraire l'huile essentielle de graines de colza, de graines de tournesol, de graines de coriandre, de graines de jatropha, de graines de camélia et de graines de pongamia pinnata (Uitterhaegen et Evon, 2017). Elle peut également être utilisée pour l'extraction de xylanes à partir de la paille et du son de blé (Jacquemin et al., 2015), et d'acides hydroxycinnamiques à partir de coproduits du chanvre pour une extraction solide/liquide efficace (Candy et al., 2017). Les avantages qu'offre la technologie bi-vis et son utilisation éprouvée dans le traitement de la matière lignocellulosique de manière continue l'ont confirmé comme méthode d'extraction solide/liquide pour la sciure de teck. En outre, il a été démontré qu'il est possible d'atteindre des conditions de pression et de température favorables à

l'obtention de conditions d'eau sous-critique, dans les zones de contraintes mécaniques des extrudeuses bi-vis.

Parallèlement à la méthode d'extraction, la sélection du solvant adéquat est essentielle pour l'extraction des métabolites secondaires. Les solvants d'extraction conventionnels sont généralement des composés organiques volatils obtenus à partir de ressources non renouvelables, principalement à base de pétrole, et soupçonnés d'être nocifs pour la santé humaine et l'environnement (Chemat et al., 2019).

L'eau est le solvant vert par excellence. Elle est non seulement peu coûteuse et inoffensive pour l'environnement, mais aussi non toxique et ininflammable, offrant des possibilités de traitement propre et de prévention de la pollution (Chemat et al., 2019). Il permet également d'extraire des composés bioactifs de différentes sources en maintenant leur activité biologique. L'inconvénient est que les propriétés de l'eau à la pression atmosphérique ne permettent pas d'obtenir des rendements d'extraction élevés. Toutefois, il est possible d'améliorer cette situation en modifiant les propriétés physico-chimiques de l'eau en la portant à des conditions sous-critiques. L'utilisation de l'eau sous-critique pour les extractions est une technique alternative qui résout les inconvénients des autres techniques, tels que la grande quantité de déchets de solvants organiques, la longue durée d'extraction et la faible efficacité de l'imprégnation.

## **Chapitre 2. Caractérisation de la sciure de teck et analyse chimique des extractibles issus des extractions conventionnelles**

La sciure de teck a été obtenue comme déchet de tronc de teck écorcés de 14 à 16 ans (duramen et aubier) provenant de la scierie Agropecuaria Santa Genoveva S.A.P.I de C.V. (Campeche, Mexique). Elle a été reçue sans autre traitement que le séchage au soleil. Concernant la caractérisation initiale, la taille prédominante des particules (environ 85 %) est comprise entre 63  $\mu\text{m}$  et 500  $\mu\text{m}$ . Le contenu lignocellulosique est de 52,8 % de cellulose, 11,2 % d'hémicelluloses et 27,3 % de lignine, plus 2 % de matière minérale.

La quantification des extractibles a été faite par deux méthodes, en utilisant un appareil Soxhlet et en utilisant un système d'extraction accélérée par solvant (ASE). La teneur en extractibles a

atteint, respectivement, des valeurs de 2,3 %, 3,1 %, 4,7 % et 4,7 % pour les extraits de cyclohexane, d'acétone, d'éthanol et de méthanol, obtenus par Soxhlet pendant 7 h d'extraction. D'autre part, pour les extractions utilisant un système ASE pendant 32 min, on a obtenu, respectivement des rendements de 1,9 %, 2,5 %, 4,7 %, 3,5% et 3,1 % pour les extraits de cyclohexane, acétone, éthanol, méthanol et eau.

Des extractions aqueuses supplémentaires d'une durée plus longue (2 h) sont réalisées pour une étude plus approfondie. Une extraction par ASE donnant 3,8 % d'extractibles et une autre utilisant un appareil Fibertec 1020 (Foss, France) à pression atmosphérique donnant 4 % d'extractibles. Comme prévu, plus le temps d'extraction augmente, plus la teneur en extraits augmente. La méthode d'extraction n'influence pas la quantité totale d'extraits, même si on s'attendait à ce que la méthode pressurisée favorise l'extraction des composés. Il est probable que le temps d'extraction soit suffisamment long pour permettre, dans le cas de l'extraction Fibertec, l'extraction de tous les composés hydrosolubles.

En général, les résultats publiés montrent également cette tendance, la plus grande quantité d'extractibles est obtenue avec des solvants à haute polarité. Le rendement méthanolique quantifié dans ce travail est un peu plus élevé que ceux rapportés dans la littérature. Par exemple, le contenu extractif était de 2,31 % et 4,0 % pour l'aubier interne et le duramen externe, respectivement pour du bois de teck de 8 ans ; et de 2,14 % et 3,69 % pour l'aubier interne et le duramen externe, respectivement pour du bois de teck de 22 ans, tous deux provenant d'une plantation en Indonésie (Lukmandaru, 2013).

Concernant les extraits aqueux issus de l'extraction ASE, nos données sont comparables à celles rapportées dans la littérature pour la fraction aqueuse d'extractions séquentielles utilisant un appareil Soxhlet. Il a ainsi été observé un rendement de 2,5 % pour des tecks de 10 et 40 ans d'Indonésie (Rizanti et al., 2018), et de 1,5 % à 2,9 % pour du bois de cœur de teck du Timor oriental (Miranda et al., 2011).

Le contenu phénolique total (TPC) a été obtenu par colorimétrie en utilisant la méthode Folin-Ciocalteu (F-C) et l'acide gallique (GA) comme standard. Comme prévu, les valeurs les plus élevées ont été trouvées dans les extraits méthanoliques et éthanoliques des deux types d'extraction, atteignant 32,8 mg GAE/g de teck sec et 35,4 mg GAE/g de teck sec pour les extraits éthanoliques et méthanoliques Soxhlet, respectivement ; et 23,4 mg GAE/g de teck sec

et 28,4 mg GAE/g de teck sec pour les extraits éthanoliques et méthanoliques ASE, respectivement. En observant la quantité de polyphénols par rapport à l'extrait, en général, elle représente plus de 50 % de la teneur de l'extrait pour tous les échantillons, par exemple, 693 mg GAE/ g d'extrait et 568 mg GAE/ g d'extrait, pour les extraits Soxhlet éthanolique et méthanolique, respectivement. Il existe des composés capables de réduire le réactif F-C, qui sont vraisemblablement des composés phénoliques.

L'activité de piégeage des radicaux libres des extraits a été étudiée à l'aide du test antioxydant DPPH (1,1-diphényl-2-picrylhydrazyl). Elle est exprimée à l'aide de la valeur  $IC_{50}$ , définie comme la concentration d'antioxydants qui provoque une diminution de 50 % de l'absorbance du radical DPPH. L'extrait éthanolique Soxhlet présente la meilleure activité antioxydante. En accord avec ce résultat, la quantité de métabolites secondaires identifiés et la valeur du TPC sont les plus élevées dans les extraits éthanoliques (pour les deux méthodes d'extraction). L'extrait éthanolique utilisant un appareil Soxhlet a montré la meilleure  $IC_{50}$  (108 mg/L).

L'identification et la quantification des composés phénoliques ont été réalisées par HPLC-DAD (Dionex Ultimated 3000, Thermo Scientific). La quantité de métabolites secondaires quantifiée dans les extraits d'acétone, de méthanol et d'éthanol pour les deux méthodes d'extraction (Soxhlet et ASE) a été pratiquement la même, étant légèrement supérieure dans l'extrait éthanolique Soxhlet. On a obtenu des valeurs de 14 mg/100 g de teck, 19 mg/100 g de teck, 10 mg/100 g de teck, 20 mg/100 g de teck pour la 2-hydroxyméthyl anthraquinone, l'acide anthraquinone-2-carboxylique, le lapachol, la 2-méthyl anthraquinone, respectivement. En ce qui concerne le squalène, il a été trouvé jusqu'à 11 mg/100 g de teck dans l'extrait cyclohexane ASE. En général, ces valeurs sont comparables à celles trouvées par d'autres auteurs.

Contrairement aux extraits d'autres solvants, où la 2-MA a été principalement identifiée, en utilisant l'eau comme solvant, le principal composé extrait est l'AQ-2-CA, 2 mg/100 g de teck et 8 mg/100 g de teck pour 30 min et 120 min, respectivement, pour les extractions ASE. En ce qui concerne le potentiel de piégeage du radical DPPH, il est amélioré d'un  $IC_{50}$  de 662,5 mg/L à 173 mg/L en augmentant le temps d'extraction de l'ASE. En outre, le TPC a atteint des valeurs de 55 mg GAE/g de teck et de 1564 mg GAE/ g d'extrait, ces chiffres étant supérieurs à ceux trouvés dans la littérature pour le teck et même pour d'autres espèces similaires.

Dans l'ensemble, les résultats de la caractérisation démontrent que la sciure de teck a une teneur intéressante en matières extractibles, une capacité réductrice élevée mesurée en composés phénoliques totaux, et des métabolites secondaires qui pourraient être récupérés.

### **Chapitre 3. Étude de l'extraction solide/liquide aqueuse de la sciure de teck à l'aide d'une extrudeuse bi-vis**

Après les essais initiaux de la sciure de teck, l'extraction solide/liquide a été étudiée dans une perspective d'échelle commerciale en utilisant la technologie bi-vis. La première approche a été réalisée à l'aide d'une extrudeuse bi-vis BC21 (Clextral, France) constituée de deux arbres parallèles co-rotatifs, supportant des vis co-pénétrantes de forme adaptée au traitement, en rotation à l'intérieur d'un fourreau fermé de sept modules de 100 mm de long. Différentes conditions de température et de ratio liquide/solide ont été évaluées selon un plan d'expérience défini en utilisant cette extrudeuse.

La configuration du fourreau a permis de définir trois zones : zone d'alimentation et de transport (modules 1 et 2), zone de cisaillement et de mélange (modules 3, 4 et 5) et zone de séparation liquide/solide (modules 6 et 7).

Dans la configuration utilisée lors des essais, les modules 2, 3, 4, 5 et 7 sont thermorégulés. Ils sont chauffés par induction thermique et refroidis par recirculation d'eau. La sciure de teck (TS) a été alimentée dans le module 1 par un doseur volumétrique (KCL-KT20 K-Tron Soder). Le débit de TS a été ajusté à une valeur d'environ 2,2 kg /h, elle a été utilisée telle que reçue de la scierie sans conditionnement préalable. Le solvant (eau) a été introduit par deux injections dans les modules 2 et 5. Le débit d'eau a été maintenu constant à un ratio liquide/solide (L/S) égal à 1 Kg de liquide total/ Kg de TS sec au module 2. Dans le module 5, le débit d'eau a été modifié de telle sorte que ce module avait un ratio L/S de 3,25 à 4,25. Le module 6 était équipé d'un filtre composé de quatre plats hémisphériques avec des trous coniques de 1 mm de diamètre intérieur et 2 mm de diamètre extérieur. Le filtre permettait la récupération du filtrat. L'extrudat était récupéré à la sortie de l'extrudeuse en fin de module 7. Après extrusion, l'extrudat a été séché en vue de la fabrication du biocomposite. Le filtrat a été clarifié par une centrifugation et des analyses complémentaires ont ensuite été réalisées, telles que l'identification des composés, le contenu phénolique total et l'activité antioxydante.

Le profil des vis était le suivant : T2F50 (50 mm), T2F50 (50 mm), C2F33 (50 mm), C2F33 (50 mm), C2F25 (50 mm), Mal2 (90°, 25 mm), C2F33 (50 mm), C2F33 (50 mm), C2F25(50 mm), C2F16 (25 mm), Mal2 (90°, 50 mm), C2F25 (25 mm), C2F25 (25 mm), C2F16 (50 mm), C2F16 (25 mm), CF2C-33 (50 mm), C2F33 (25 mm), (T2F : double-filet trapézoïdal, C2F : double-filet conjugué, Mal2 : disques malaxeurs bilobes, CF2C : contre-filets en double-filet conjugué).

Le plan d'expérience était de type Doelhart, où les effets de la température d'extraction et du ratio L/S ont été évalués. Il comprenait sept points expérimentaux : (1) T= 100° C, L/S= 3,75 ; (2) T= 150° C, L/S= 3,75 ; (3) T= 125° C, L/S= 4,25 ; (4) T= 75° C, L/S= 4,25 ; (5) T= 50° C, L/S= 3,75 ; (6) T= 75° C, L/S= 3,25 ; (7) T= 125° C, L/S= 3,25. Deux points supplémentaires du plan d'expérience ont été réalisés (T= 100° C, L/S= 3,25 ; et T= 100° C, L/S= 3,45) afin de voir plus clairement l'effet de L/S en maintenant la température constante.

Les réponses évaluées du point de vue de l'efficacité de la filtration étaient : le rendement en extrudat sec ( $Y_{DE}$ ), la matière sèche dans l'extrudat ( $DM_{RE}$ ), la récupération du filtrat ( $R_F$ ) et le rendement en sédiments secs ( $Y_{DS}$ ) ; et du point de vue des propriétés du filtrat : le rendement en matières extractibles ( $Y_E$ ), le TPC (g GAE/100g de teck) et la teneur en métabolites secondaires.

L'efficacité du passage, qui est assuré par la contre-pression exercée par le contre-filet transportant le mélange en amont et la séquence de vis à pas direct transportant la matière en aval, a pu être évaluée par la quantité de  $DM_{RE}$  et la récupération du filtrat  $R_F$ . Le rendement élevé de l'extrudat sec (80 % - 90 %), la teneur en matière sèche dans l'extrudat (70 %-80 %), ainsi que la récupération élevée du filtrat (60 %-80 %) démontrent une bonne séparation solide/liquide. Le rendement en sédiments n'a pas varié de plus de 1 % parmi toutes les conditions évaluées, avec une moyenne de 11 %. La gamme prédominante de  $Y_{DE}$  se trouve dans les valeurs centrales du plan d'expérience. Les valeurs les plus élevées de  $DM_{RE}$  se trouvent dans les conditions intermédiaires de T et de ratio L/S et diminuent radialement. En ce qui concerne le  $R_F$ , les résultats varient sur une plage considérablement large, l'écart type élevé ne permet pas une discussion concluante, cependant, les pourcentages prédominants sont ceux autour de 70 %.

Le travail mécanique exercé à l'intérieur de l'extrudeuse principalement dans les zones qui génèrent un fort cisaillement provoque la rupture et le défibrage du matériau. L'extrudat est très déchiqueté avec un aspect de farine et des fibres individualisées visibles en microscopie. Le travail mécanique le plus important s'effectue après le filtre, par l'action de l'élément de vis CF2C.

En ce qui concerne la teneur en matières extractibles, on observe que le changement des conditions du procédé n'a pas d'impact significatif sur cette valeur, les valeurs majoritaires se situant entre 1,3 g/100 g de teck sec et 1,5 g/100 g de teck sec. Ces valeurs représentent entre 32 % et 47 % des matières extractibles obtenues avec un système ASE et l'eau comme solvant. Cependant, le temps d'extraction est considérablement différent pour les deux types de méthodes, environ 1 min pour l'extrudeuse et 32 min pour l'ASE, ainsi que le ratio L/S, dont la valeur est de 17 pour l'ASE. Le temps d'extraction et le ratio liquide/solide sont des facteurs décisifs pour l'extraction des composés.

En ce qui concerne l'analyse du filtrat clarifié, les composés recherchés avec le standard original comme référence étaient la 2-hydroxyméthyl anthraquinone, l'acide anthraquinone-2-carboxylique (AQ-2-CA), le lapachol et la 2-méthyl anthraquinone. Les polyphénols identifiés dans les extraits étaient l'AQ-2-CA et le lapachol. On a obtenu des chiffres allant de 1,37 mg/g d'extrait sec à 2,25 mg /g d'extrait sec pour l'anthraquinone, et de 0,1 mg/ g d'extrait sec à 0,45 mg / g d'extrait sec pour le lapachol. La teneur moyenne en AQ-2-CA dans les extraits obtenus avec l'extrudeuse représentait plus de 80 % de la teneur atteinte dans les extractions aqueuses réalisées avec un système ASE et Fibertec avec un temps d'extraction de 2h.

Le contenu phénolique estimé en GAE a montré que l'utilisation de la technologie d'extraction bi-vis avec de l'eau comme solvant permet d'obtenir des extraits ayant une teneur intéressante en polyphénols (de 249 mg GAE/g d'extrait à 475 mg GAE/g d'extrait). Cependant, les activités antioxydantes de ces polyphénols étaient plutôt faibles (IC<sub>25</sub> de 198 mg/L à 571 mg/L).

En comparant le potentiel antioxydant des extraits obtenus à l'aide de la technologie bi-vis avec celui de l'extrait utilisant un système ASE et de l'eau comme solvant pendant 30 min dont la CI<sub>50</sub> est de 0,662 g/L, les résultats de l'extraction à l'aide d'une extrudeuse présentent une activité de piégeage des radicaux DPPH inférieure pour toutes les conditions étudiées. De plus, le volume d'eau par ratio au volume de teck est assez faible dans les expériences d'extrusion entre

3,25 et 4,25, augmente jusqu'à 16 dans les expériences ASE, et monte jusqu'à 100 dans les expériences Fibertec. En ce qui concerne les capacités antioxydantes résultantes, les composés actifs sont mieux extraits avec un grand ratio L/S, et l'extrait résultant offre une forte activité antioxydante, liée à une grande teneur en polyphénols.

La récupération d'antioxydants à partir de sciure de teck au moyen de la technologie d'extraction bi-vis avec de l'eau comme solvant présente l'avantage d'être une méthode de raffinage de la matière lignocellulosique dans un processus continu. Les contraintes mécaniques appliquées à la sciure de teck permettent d'obtenir des extraits présentant des teneurs intéressantes en polyphénols. Cependant, les activités antioxydantes de ces polyphénols sont plutôt faibles.

La mise à l'échelle a été réalisée à l'aide d'une extrudeuse bi-vis BC45 (Clextal, France) co-rotatives et co-pénétrantes. L'extrudeuse se compose d'un fourreau fermé avec sept modules de 200 mm de long. Les modules 2, 3, 4, 5 et 7 sont chauffés par induction thermique et refroidis par recirculation d'eau. Le module 6 est équipé d'un filtre composé de six plats hémisphériques avec des trous coniques de 1 mm de diamètre intérieur et de 2 mm de diamètre extérieur.

La configuration, le profil et la température du procédé sont adaptés du point expérimental  $T=100^{\circ}\text{C}$ ,  $L/S=3,25$  réalisé avec l'extrudeuse BC21. La configuration du profil est définie afin de favoriser la réduction de la taille de la matière première, le contact intime entre la matière et le solvant par des forces de cisaillement qui permettent la rupture de la paroi cellulaire et donc l'extraction solide/liquide, et la séparation liquide/solide par pressage et filtration.

Le profil des vis était le suivant : T2F66 (100 mm), T2F50 (100 mm), C2F33 (100 mm), C2F33 (100 mm), C2F25 (100 mm), Mal2 (90°, 50 mm), C2F33 (100 mm), C2F33 (100 mm), C2F25 (100 mm), C2F16, Mal2 (45°, 50 mm), C2F33 (100 mm), C2F25 (50 mm), C2F25 (50 mm), C2F16 (50 mm), CF2C-25 (50 mm), C2F33 (100 mm), (T2F : double-filet trapézoïdal, C2F : double-filet conjugué, Mal2 : disques malaxeurs bilobes, CF2C : contre-filets en double-filet conjugué).

La mise à l'échelle de l'extraction au moyen d'une extrudeuse BC45 a permis d'obtenir une récupération élevée du filtrat (83 %), encore plus élevée qu'avec l'extrudeuse BC21 aux mêmes conditions de température et de ratio L/S. Cependant, le rendement en sédiments secs était également très élevé (39 %), ce qui signifie que, bien qu'une grande quantité de liquide ait été



récupérée par le filtre, une grande quantité de matière d'extrudat a également été entraînée. Par conséquent, le rendement en extrudat sec était faible (58 %). Du côté de la qualité du filtrat, un rendement en extractibles de 1,54 % a été obtenu et tant le contenu phénolique (543 mg GAE/g d'extrait sec) que l'activité antioxydante ( $IC_{50}=0,76$  g/L) étaient légèrement meilleurs dans l'extrait de l'extrudeuse BC45. Les mêmes métabolites secondaires ont été identifiés que dans les extraits obtenus avec l'extrudeuse BC21. Des valeurs de 1,18 mg d'AQ-2-CA/ g d'extrait sec et de 0,21 mg de lapachol/ g d'extrait sec ont été atteintes. En général, le scale up a été bien réalisé. En plus d'un filtrat à haut contenu phénolique, on a obtenu un matériau lignocellulosique raffiné, bien défibré et avec des extractibles encore conservés.

Le fractionnement par extrudeuse bi-vis fournit des fibres lignocellulosiques raffinées avec des composés antioxydants encore présents dans la matrice. Leur utilisation comme fibres de base pour la conception de nouveaux matériaux serait parfaitement appropriée. En effet, les composés antioxydants du bois issus du métabolisme secondaire permettant à l'arbre de se défendre contre les agressions et les stress extérieurs (charge mécanique, humidité, températures extrêmes, UV), il serait naturel que ces activités puissent être réinvesties dans les matériaux de construction soumis aux mêmes contraintes climatiques. Cette étude pourrait donc offrir une valorisation des coproduits de scierie en générant des fibres raffinées à haute activité antioxydante.

#### **Chapitre 4. Valorisation de l'extrudat de sciure de teck et utilisation potentielle des métabolites secondaires identifiés dans les extraits**

Les biocomposites sont des biopolymères renforcés par des fibres naturelles. Ils sont développés pour offrir une alternative écologique aux matériaux conventionnels. Les fibres naturelles sont utilisées en remplacement de la fibre de verre dans les composites en raison de leurs avantages tels que leur faible densité et leurs aspects environnementaux en termes de faible consommation d'énergie et de caractère renouvelable. Malgré son hétérogénéité, ses températures de traitement limitées ainsi que son caractère hydrophile et donc intrinsèquement incompatible avec les matrices thermoplastiques hydrophobes, l'attention portée à l'utilisation des fibres naturelles pour plusieurs applications a augmenté en raison des préoccupations écologiques mondiales (Pappu et al., 2019).

Lorsque les fibres utilisées dans la production de composites sont ligneuses, elles sont appelées composites bois-polymère. Parmi les essences de bois les plus utilisées figurent le pin, l'érable et le chêne (Patel et Rawat, 2017). Les avantages offerts par les résidus de bois tels que le faible coût, le respect de l'environnement, la disponibilité et la faible abrasivité les rendent appropriés comme charges dans la préparation des biocomposites (Petchwattana et Covavisaruch, 2014).

En ce qui concerne la matrice polymère, les biocomposites sont fabriqués à partir de polymères biodégradables. Les biopolymères sont des polymères produits par ou dérivés d'organismes vivants, tels que les plantes et les microorganismes, plutôt que du pétrole, la source traditionnelle des polymères. Parmi les biopolymères, le poly(acide lactique) (PLA) a suscité un grand intérêt commercial dans l'industrie des polymères en raison de ses propriétés mécaniques intéressantes (par rapport à d'autres biopolymères), de sa biodégradabilité potentielle et de sa capacité de production industrielle élevée (Goutianos et al., 2019 ; Sudamrao Getme et Patel, 2020). Le poly(acide lactique) est un polymère thermoplastique cristallisable. Il appartient aux polyesters aliphatiques linéaires synthétiques produits à partir de ressources naturelles (comme le maïs et la betterave à sucre). Le PLA est synthétisé soit par polymérisation par ouverture de cycle du lactide, soit par polycondensation de l'acide lactique (acide 2-hydroxy propionique) (Gurunathan et al, 2015). Le PLA présente une bonne rigidité, une résistance élevée et un faible allongement à la rupture (Dong et al., 2014). De plus, il est facile à mettre en œuvre et biodégradable. Compte tenu de ces propriétés, le PLA a été choisi comme matrice polymérique.

L'extrudat de teck a été séché à 70°C pendant une nuit avant d'être mélangé au biopolymère PLA pour la fabrication des biocomposites. Deux séries de biocomposites ont été préparées à trois charges différentes en extrudat de teck (TE), la première comme test préliminaire et la seconde pour validation. Pour la préparation de la première série (1-BC), tous les extrudats obtenus à partir des extractions à l'aide de l'extrudeuse BC21 (Clextal, France) ont été récupérés et mélangés. Les fibres ont ensuite été obtenues par extrusion dans des conditions différentes, mais aucune différence significative n'a été observée dans la texture des fibres. La deuxième série (2-BC) a été fabriquée avec l'extrudat issu de l'extraction à l'aide de l'extrudeuse BC45 (Clextal, France) dans des conditions de production de fibres contrôlées.

Le PLA et l'extrudat séché ont été extrudés à l'aide d'une extrudeuse bi-vis Evolum 25 (Clextal, France) à une température comprise entre 180 et 190°C. Les deux séries de biocomposites ont

été préparées avec trois charges différentes d'extrudat de teck (10 %, 20 % et 30 %). Ensuite, les biocomposites ont été injectés dans une machine de moulage par injection afin de fabriquer les éprouvettes destinées aux tests mécaniques.

Pour les trois différentes charges de fibres de bois des deux séries, l'aspect visuel des biocomposites semble être relativement uniforme, principalement dans les échantillons à faible teneur en fibres, cependant, quelques très petits copeaux de fibres de bois peuvent être vus principalement dans la série de composites 1-BC. La finition lisse est également observée dans tous les composites. La couleur des spécimens devient plus intense avec l'augmentation de la teneur en fibres en raison de la teneur plus élevée en lignine.

Les biocomposites ont été soumis à des essais de traction et de flexion. L'essai de flexion sous trois points de flexion a été déterminé à l'aide d'une machine d'essai universelle (Tinius Olsen H5KT) conformément à la norme française NF EN ISO 14125. L'essai de traction a été réalisé selon la norme française NF EN ISO 527 en utilisant la même machine. Les éprouvettes pour l'essai de traction étaient en forme d'haltère (Type 1A).

Les biocomposites des deux séries et de toutes les compositions de PLA/extrudat de teck n'ont pas montré de déformation plastique mais ont cédé dans la région élastique de la même manière que le PLA pur. En termes de propriétés de traction, l'ajout de 10 % d'extrudat de teck n'a eu aucun effet durcissant sur le biopolymère dans aucune des séries. Néanmoins, à des charges plus élevées, la résistance à la traction a augmenté avec la teneur en fibres. Elle a été améliorée, elle est de 36 MPa pour le PLA pur et de 54 MPa pour les composites avec 30 % de TE des deux séries. Le module de traction (de 1341.3 MPa pour le PLA pur) a également été amélioré jusqu'à 10 % dans la série 1-BC et 20 % dans 2-BC. En ce qui concerne l'élongation des matériaux, le pourcentage le plus élevé a été atteint avec le composite à 20 % de TE (2-BC), soit 3,9 %, comparé au PLA pur (2,6 %), ce qui signifie un gain d'élasticité de 50 %. Aucune des séries n'a montré une amélioration de l'élongation lorsque la teneur en fibres a été augmentée de 20 % TE à 30 % TE.

En revanche, pour la résistance à la flexion, l'effet de la charge était évident à partir de 10 % de fibres de bois. Pour la série 1-BC, l'augmentation de la charge de fibre a légèrement diminué les valeurs de résistance à la flexion, avec une valeur maximale de 82 MPa pour PLA/10 % de TE, alors que la série 2-BC a montré une augmentation de 70 MPa pour le PLA pur jusqu'à 89

MPA pour PLA/30 % de TE. En général, le composite de la série 2-BC a présenté des propriétés mécaniques légèrement meilleures.

La proposition d'utiliser l'extrudat de teck comme fibre naturelle dans le renforcement du PLA complète l'approche de valorisation de la sciure de bois de cette étude.

## **Conclusion et perspectives**

La sciure de teck a montré un rendement élevé en matières extractibles (obtenues avec des solvants d'une large gamme de polarités), un TPC élevé, et des métabolites secondaires intéressants, démontrant son potentiel en tant que source de matières extractibles. La récupération des matières extractibles dans un processus continu avec la possibilité de passer à l'échelle supérieure et de traiter de grands volumes de matière a ensuite été évaluée.

L'étude de la technologie bi-vis comme méthode d'extraction solide/liquide de la sciure de teck a d'abord été réalisée avec une extrudeuse BC21, puis une mise à l'échelle du procédé (avec un facteur de 2) a été effectuée avec une extrudeuse BC45. La sciure de teck ne nécessite aucun prétraitement, ce qui présente des avantages tels que la réduction du temps de traitement, des coûts et des matériaux. De plus, elle a montré une bonne aptitude au traitement dans l'extrudeuse. L'extraction de la sciure au moyen d'une extrudeuse a donné lieu à deux produits : un filtrat riche en contenu polyphénolique et un extrudat bien défibré avec des extractibles encore retenus. L'extrudat issu de l'extraction des sciures de teck a été valorisé comme renfort d'une matrice polymérique biodégradable (PLA). La fabrication à l'aide d'une extrudeuse bi-vis pour mélanger les fibres de teck avec le PLA, puis d'une presse à injecter pour mouler les éprouvettes pour les tests mécaniques a permis d'obtenir des biocomposites avec un aspect général lisse et homogène.

La méthode d'extraction utilisant la technologie bi-vis n'offre pas une extraction exhaustive de la matière mais plutôt un traitement continu et rapide, capable de traiter d'importants volumes de matière, ce que les méthodes conventionnelles utilisées principalement à l'échelle du laboratoire ne pouvaient pas faire. De plus, le ratio liquide/solide est faible, ce qui permet de réduire la consommation de solvant ainsi que les coûts liés à l'évaporation du solvant après le traitement.

Cette étude fournit une base pour de futures études de valorisation avec une nouvelle méthode d'extraction non seulement pour la sciure de teck mais aussi pour d'autres types de bois et de résidus forestiers. Des travaux restent à faire pour optimiser le procédé et pour purifier et évaluer les extraits afin qu'ils puissent être utilisés pour des applications ultérieures. D'autres points pourraient faire l'objet de recherches supplémentaires :

- Les extraits aqueux obtenus au moyen de la technologie d'extrusion bi-vis ont un contenu phénolique élevé. Cependant, il n'a pas été observé une activité antioxydante cohérente, évaluée comme la capacité du mélange réactif à piéger les radicaux libres DPPH. Il serait intéressant d'évaluer l'activité antioxydante avec un autre test mieux adapté à la composition chimique complexe des extraits.

- Le composé identifié en plus grande quantité dans les extraits était l'AQ-2-CA, considérant que l'extraction a été effectuée dans des conditions sous-critiques douces, on s'attendrait à trouver aussi d'autres composés extractibles avec des solvants moins polaires comme le méthanol et l'éthanol (comme la 2-méthyl anthraquinone). Un autre profil de vis pourrait être entrepris avec des éléments plus restrictifs qui élargissent les zones favorables pour amener l'eau à des conditions sous-critiques.

- Un nouveau profil de vis de l'extrudeuse BC45 qui diminue la quantité de sédiments et augmente donc le rendement de l'extrudat sec. En outre, d'autres conditions de fonctionnement visant à augmenter la teneur en extraits peuvent être explorées, voire même un changement d'échelle pour permettre la manipulation de plus grandes quantités de matières premières peut être testé.

- Compléter la caractérisation des biocomposites, par exemple par une évaluation de l'absorption d'humidité. Un des problèmes des composites préparés avec des fibres naturelles est qu'elles absorbent l'eau de l'environnement et cela peut compromettre leurs propriétés mécaniques, un autre aspect à considérer concernant l'humidité est qu'elle peut favoriser la prolifération de champignons dans les fibres ; cela aurait un impact direct sur l'adhésion des fibres et de la matrice polymérique. L'extrudat de teck contient encore des extraits auxquels on attribue des propriétés fongicides et de résistance à la pourriture ; on peut s'attendre à ce que

ces qualités soient maintenues et profitent au biocomposite d'une manière particulière avec des caractéristiques que d'autres fibres naturelles ne fourniraient pas.

- Une autre façon d'approfondir l'évaluation des biocomposites pourrait être de prendre des micrographies SEM et de réaliser une analyse thermique pour discuter de la liaison interfaciale.

- Il est bien établi que les extraits de teck présentent des propriétés fongicides. Il serait intéressant d'étudier l'activité fongicide des extraits obtenus par extrusion.

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