COMPARISON OF STARCH CONTENT AND TRANSCRIPTOMES OF FELLED OIL PALM TRUNKS AT 0 AND 15 DAYS OF STORAGE

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by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Page

LIST OF UNITS AND SYMBOLS

~	Approximately
±	Plus minus
#	Number
°C	Degree celsius
A_{260}	Absorbance at 260 nm
A_{280}	Absorbance at 280 nm
cm	Centimetre
MΩ	Megaohms
Mg	Milligram
mL	Millilitre
MPa	Megapascal
ng	Nanogram
nm	Nanometre
pM	Picomolar
µL	Microlitre
µm	Micrometre
–	Negative
g	Gram
L	Litre
β	Beta
α	Alpha
%	Percentage
mM	Millimolar
mm	Millimetre

ng/μL	Nanogram per microlitre
v/v	Volume per volume
TPM	Transcripts per million
w/v	Weight per volume

LIST OF ABBREVIATIONS

β-ME	β-Mercaptoethanol
ADP	Adenosine diphosphate
AGPase	Adenosine diphosphate glucose pyrophosphorylase
AMY	Amylase
ATP	Adenosine triphosphate
BC	Bar code
BE	Branching enzyme
bp	Base pair
C/N	Carbon to nitrogen
cDNA	Complementary deoxyribonucleic acid
DEG	Differentially expressed gene
DEPC	Diethylpyrocarbonate
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
DRS	Direct ribonucleic acid sequencing
dsDNA	Double stranded deoxyribonucleic acid
EFB	Empty fruit bunch
FC	Fold change
FDR	False discovery rate
G-6-PDH	Glucose-6-phosphate dehydrogenase
GBSS	Granule-bound starch synthase
GDP	Gross domestic production
GPase	Glycogen phosphorylase

HK	Hexokinase
HPLC	High-performance liquid chromatography
HS	High sensitivity
INV	Invertase
ISA	Isoamylase-type debranching enzyme
JIS	Japan industrial standards
MDF	Medium density fibreboard
MF	Mesocarp fibres
MGAM	Maltase-glucoamylase
MOPS	3-(N-morpholino)propanesulfonic acid
NADP+	Nicotinamide adenine dinucleotide phosphate (oxidized form)
nt	Nucleotide
OPF	Oil palm fibre
OPT	Oil palm trunk
PCA	Principle coordinate analysis
PCR	Polymerase chain reaction
pH	Potential of hydrogen
PKS	Palm kernel shell
PR protein	Pathogenesis-related protein
RID	Refractive index detector
RNA	Ribonucleic acid
RNA-Seq	Ribonucleic acid sequencing
RNase	Ribonuclease
ROS	Reactive oxygen species
RPM	Revolutions per minute

rRNA	Ribosomal ribonucleic acid
RT	Reverse transcription
Seq	Sequencing
SAG	Senescence-associated gene
SMDS	Single molecule deoxyribonucleic acid sequencing
SS	Starch synthase
TF	Transcription factors
TMV	Tobacco mosaic virus
USM	Universiti Sains Malaysia
UV-VIS	Ultraviolet-visible spectroscopy

LIST OF APPENDICES

PERBANDINGAN KANDUNGAN KANJI DAN TRANSKRIPTOM BATANG POKOK KELAPA SAWIT YANG TELAH DITEBANG SELEPAS SIMPANAN 0 DAN 15 HARI

ABSTRAK

Pokok kelapa sawit ditebang untuk proses penanaman semula selepas mencapai produktiviti maksimum pengeluaran minyak sawit. Sisa biojisim berskala besar seperti batang kelapa sawit (OPT) dijana semasa proses penanaman semula. Sisa biojisim OPT telah dilaporkan mengandungi kandungan kanji dan gula yang tinggi (glukosa, sukrosa dan fruktosa) dan berpotensi untuk digunakan dalam pelbagai kegunaan industri (cth., dalam produk nilai tambah). Walau bagaimanapun, OPT yang mengandungi kanji dan gula yang rendah, tanpa kegunaan dan praktikaliti juga telah pun dikenal pasti dalam kalangan OPT berkanji tinggi di ladang kelapa sawit. Dalam kajian ini, OPT berkanji tinggi dan OPT berkanji rendah pada mulanya ditebang dan terus disimpan selama 15 hari. Tempoh penyimpanan selama 15 hari digunakan kerana ia merupakan tempoh yang mencukupi untuk mengangkut OPT dari ladang dan menghantarnya kepada syarikat industri untuk aplikasi selanjutnya. Khususnya, bahagian atas dan tengah OPT diperiksa pada hari pokok ditebang (hari peyimpanan sifar) dan 15 hari kemudian (hari penyimpanan ke-15). Dalam tempoh penyimpanan, kandungan kanji dan gula dikenal pasti, serta transkriptom OPT yang ditebang telah dijujuk untuk membezakan faktor-faktor yang menyebabkan perbezaan antara kandungan kanji kedua-dua OPT berkanji tinggi dan rendah. Sepanjang tempoh penyimpanan, kandungan kanji berkurangan manakala kandungan gula meningkat. Data penjujukan tinggi yang dijana menggunakan teknologi penjujukan Ion Torrent

menunjukkan bahawa beberapa gen yang terlibat dalam metabolisme kanji dan sukrosa seperti β-fruktofuranosidase, β-amilase and α-amilase telah dikenal pasti dalam semua OPT. Gen-gen ini juga menunjukkan ekspresi yang lebih tinggi pada hari penyimpanan yang kemudian (hari ke-15 penyimpanan) berbanding dengan hari pertama penyimpanan (hari ke-0 penyimpanan). Tambahan pula, OPT berkanji rendah menunjukkan corak protein berkaitan patogenesis (PR) yang sangat jelas. Penemuan kajian ini menunjukkan tindak balas molekul OPT terhadap proses penebangan dan hubungan rapatnya dengan kandungan kanji. Sisa biojisim OPT dengan kandungan kanji yang tinggi adalah penting untuk aplikasi industri, iaitu sebagai bahan berasaskan bio dan produksi bioetanol.

COMPARISON OF STARCH CONTENT AND TRANSCRIPTOMES OF FELLED OIL PALM TRUNKS AT 0 AND 15 DAYS OF STORAGE

ABSTRACT

The oil palm trees are logged for replantation after they reach their maximum productivity of palm oil production. Massive biomass waste such as the oil palm trunk (OPT) is generated during the replanting process. The OPT biomass residues have been reported to contain a high amount of starch and sugar (glucose, sucrose, and fructose) that have high potential for various industrial uses (e.g., in value-added products). However, low starch OPTs with no use and practicality have also been identified in the oil palm plantations. In this study, high-starch and low-starch OPT were firstly felled and further stored for 15 days. A storage period of 15 days was used as 15 days is a sufficient period to transport the OPTs from the plantation and to deliver to industrial companies for further applications. More specifically, the top and middle parts of the trunks containing sugar and starch, on the day the trees were felled (day 0 of storage) and 15 days later (day 15 of storage) were examined. Within the storage period, the starch and sugar contents were identified and the transcriptomes of the felled OPT were sequenced to distinguish the high and low-starch OPT. During the 15 days of storage, the starch content slightly decreased while the sugar content slightly increased in all the OPTs. High sequencing data generated using the Ion Torrent sequencing technology on the high-starch and low-starch OPT showed that a few genes involved in the starch and sucrose metabolism such as β-fructofuranosidase, β-amylase and α-amylase were identified in all the OPTs. These genes also showed higher expression on the later days of storage (15th day of storage) compared to the first day of storage (0 day of storage). Furthermore, the low-starch OPT showed high expressions of the pathogenesis-related (PR) proteins. The findings revealed an insight on the molecular response to the felling of the OPT and its close relationship with the starch content. Thus, OPT biomass waste with a high starch content is important for further industrial applications, namely as feedstock for the biomaterials and bioethanol production.

CHAPTER 1

INTRODUCTION

1.1 Background study

The oil palm tree or *Elaeis guineensis* Jacq., is an economically important plant originated from and cultivated in the West Africa. In the 1870s, oil palm was first introduced in Malaysia as an ornamental plant (Corley *et al.*, 2015). The oil palm is distinct from any other plant due to its unique ability of producing a high amount of yield within a considerably small area of land use (3.8 tonnes of oil per hectare) compared to other plants (USDA, 2023). Malaysia is one of the world's leading producers and exporters of palm oil, after Indonesia. The palm oil supply from these two countries makes up 85% of the palm oil supplied globally. The oil palm industry plays a pivotal role in the Malaysia's agriculture and economy. The applications of oil palm are to produce vegetable oil, soaps, pharmaceutical products, biodiesel, charcoal, pulp and paper, animal feeds, bio-composite, and fertilizers (USDA, 2023).

Every 20 to 25 years, the oil palm trees need to be replanted to maintain the productivity of the palm oil (Corley *et al.*, 2015). The constant supply of palm oil is crucial to meet the demand of approximately 240 million tonnes of oil 2050 (Corley, 2009). Therefore, to clear the plantation field for the next replanting, the old oil palms will be removed either by the push-felled method or the pulverization method. These practices were implemented as an alternative to the conventional open burning of the massive OPT biomass waste that was banned in Malaysia (Noor, 2003).

Although these methods are regarded as more environmentally friendly, it poses some threats. Felled OPTs left in the plantation take at least two years to decompose which eventually becomes a breeding site for beetles such as *Oryctes rhinoceros* (Gnanasegaram *et al.*, 2014). The population of rats also increases shortly after a few months of the replanting process (Hamid *et al.*, 2013). Additionally, replanting contributes to the basal stem rot disease caused by *Ganoderma boninense* (Virdiana *et al.*, 2010) and vascular wilt from *Fusarium oxysporum* (Corley *et al.*, 2015). The OPT is composed of a high C/N (carbon to nitrogen) ratio (Loh *et al.*, 2013) which leads to a prolonged decomposition process (Lin *et al.*, 2019). Thus, the unregulated disposal of OPT in the plantation may alter and affect the soil microbial community as well as the plant growth performance (Uke *et al.*, 2021).

Therefore, studies on the application of the OPT have been conducted extensively to utilize the OPT comprehensively. OPT is composed of 70% sap which is fermentable sugar that will increase after certain duration of storage (Kosugi *et al.*, 2010; Yamada *et al.*, 2010). The OPT sap has the potential to be commercialized for bioethanol production (Bukhari *et al.*, 2015; Kosugi *et al.*, 2010), bioplastic production (Lokesh *et al.*, 2012; Murugan *et al.*, 2016), and nutritional syrups (Sulaiman *et al.*, 2020).

Additionally, the monocotyledonous trunk of an oil palm is rich with parenchyma cells containing starch (Tomimura, 1992). OPT starch has diverse applications such as to produce bioethanol (Eom *et al.*, 2015; Prawitwong *et al.*, 2012), as wood-based panels (Selamat *et al.*, 2014; Sulaiman *et al.*, 2018) and as potential animal feed (Azhary *et al.*, 2018; Widiawati *et al.*, 2024).

Previous studies have identified starch degradation and its enzyme activity of felled OPT for over 30 days. The decrease in starch content of OPT is often associated with the increase in sugar content (Abdul Hamid *et al.*, 2015; Yamada *et al.*, 2010). It has been reported that starch is degraded due to the enzymatic activities of amylase and invertase. Additionally, it was also reported that the content of OPT starch may vary (i.e., high or low levels), but the causative factors for low OPT starch is still unknown (Abdul Hamid *et al.*, 2015). The potential of starch in OPT has made it a focus for biotechnological applications, attracting the interest of industries for a large-scale commercialization. Renewable and environmentally friendly biomass is still the primary feedstock for various applications.

1.2 Problem statement

Replanting of oil palm trees produces massive OPT biomass waste. Furthermore, these residues are left in the plantation to degrade which further contributes to the attraction of undesirable pests and diseases. Thus, in order to make use of this agricultural waste, there is a need to understand the properties of the OPT comprehensively. Among the important OPT components are starch and sugar, both of which have valuable industrial applications. However, after the felling of the oil palm trees, the OPTs should be stored properly for a limited period (i.e., a few weeks) and utilised before the starch begins to degrade into sugar.

Currently, there is minimal knowledge on the types of the OPT (i.e., high-starch and low-starch OPT) and starch and sugar contents. Although the range of the starch available in the OPTs were known, the possible factors affecting its content is not yet studied. Therefore, the condition of the OPT after the felling process within 15 days as well as its relationship with starch needs to be studied to optimize its applications in the industries. OPTs for industrial applications, should be processed almost immediately within the storage period to ensure the starch content is still high and not degraded to sugars. The storage period includes leaving the OPTs in the plantation after felling, transporting the OPTs out from the plantation to its target location, and to prepare the OPTs for their subsequent downstream applications. Additionally, it is equally important that early precautionary measures are adopted to maintain plantation conditions that promotes the production of OPT that is high in starch, thereby adding value to the biomass waste generated during the replanting process. A well-managed plantation will lead to a high palm oil yield and the production of biomass waste that can be stored for a certain time, is useful to produce value-added industrial products.

1.3 Research objectives

The main purpose of this research was to study the starch and sugar content of the different types of OPT conditions (i.e., high-starch and low-starch) on the 0 day and 15th day of storage. The specific objectives of this research are as follows:

1. To determine the starch content of the OPT during the 15-storage days
2. To determine the sugar content of the OPT during the 15-storage days
3. To identify the differentially expressed genes involved in the starch degradation mechanism during the 15-storage days

CHAPTER 2

LITERATURE REVIEW

2.1 Origin and economic importance of *Elaeis guineensis* Jacq. (Oil Palm)

Elaeis guineensis, also known as oil palm, is renowned for its production of palm oil. Oil palm originated from the West Africa. It was initially introduced to Malaysia by the British around 1870s for ornamental purposes. In 1917, the oil palms were cultivated for commercialization in Tennamaran Estate located in Selangor. (Malaysian Palm Oil Board, 2021).

Elaeis is from the Palmae*,* palm family under the Spadiciflorae order. Oil palm is classified as a monoecious plant since it consists of both male and female flowers on the same plant. Approximately 20 to 25 kilograms of fresh fruit bunches with 1000 to 3000 fruitlets are produced by an oil palm (Corley *et al.*, 2015). Its fruitlet is shaped spherically or elongated. The fruitlet is dark purple or close to black when young but as they ripen, they turn orange red (Malaysian Palm Oil Board, 2021). The oil palms start bearing fruits after 2.5 years of planting in the field and will constantly be productive for the next 25 to 30 years (Malaysian Palm Oil Board, 2021). Furthermore, the oil palm produces approximately three times more oil than coconut, seven times more oil than rapeseed, and almost 10 times more oil than soybean (Nair, 2010).

Oil palm is an economically important tropical crop for its variety of applications such as the production of vegetable oil, soaps, pharmaceutical products, charcoal, pulp, paper, and biofertilizers. Palm oil is unique because it needs only a small land area to produce a high amount of oil yield. In contrast to any other plant oil (Murphy *et al.*, 2021). Indonesia and Malaysia are among the top countries that supply palm oil globally, which makes up 85 % of the global oil production (Agriculture, 2020). Other countries that also produce palm oil are Thailand, Columbia, Nigeria, Brazil, and India.

In 2020, Malaysia successfully exported 16.2 million metric tons of palm oil and products that are palm-based. The revenue obtained was approximately 73.3 billion ringgit. The palm oil industry contributed greatly to Malaysia's total Gross Domestic Products (GDP) which was roughly around 36.9 billion ringgit (Hirschmann, 2022). In 2024, Malaysia's palm oil production is expected to increase to 19 million tonnes (Li, 2024). Around 5.9 million hectares of land was used to grow the oil palm trees in 2020 (Hirschmann, 2023). With effective agricultural practices, one hectare of oil palm trees generates about five to seven tons of oil annually (Nair, 2010). Figure 2.1 shows oil palm trees in an oil palm plantation located in Kahang, Johor, Malaysia.

Figure 2.1: Oil palm plantation located in Kahang, Johor, Malaysia

2.2 Replanting of oil palm trees

Despite the abundance of palm oil produced, the aging oil palms need to be replanted around the critical age of between 20 to 25 years to ensure the productivity of palm oil production (Corley *et al.*, 2015). The constant supply and productivity of palm oil is pertinent to meet the demand of approximately 240 millions of tons in 2050 (Corley, 2009). Generally, to replant the younger oil palm seedlings, the old trees in the oil palm plantations are usually cleared off. There are various methods of replanting the oil palm seedlings.

2.2.1 Management of felled oil palm trunks (OPT)

In the initial stages of replanting, the 'clean-clearing technique' was implemented by stacking and burning the felled OPTs. However, this particular method caused major environmental impacts that was later, banned in Malaysia. Although newer and more environmental friendly techniques were introduced after, this 'clean-clearing technique' was completely banned and made illegal by the Government of Malaysia in 2003 (Noor, 2003).

Thus, to clear the plantation fields for the next replanting of oil palms, a method known as chip and windrow, whereby old oil palms will be pushed, felled, chipped, and windrowed into the plantations was implemented as seen in Figures 2.2 and 2.3. The practice implemented is an alternative to the conventional open burning of the massive OPT biomass waste that was banned in Malaysia (Noor, 2003). One OPT windrow is made in between two rows of planted oil palms and then left to decompose in the windrows (Ooi *et al.*, 2005).

Similarly, under-planting is another method that has been implemented by replanting oil palm seedlings under the old palms that are eventually poisoned.

Additionally, pulverization technique using the EnviroMulcher™, Beaver™ and MountainGoat™ technology further pulverizes completely the old OPT into smaller pieces. The pulverized chips were thin and evenly spread to the entire plantation with the root's masses dug during the replanting process. New oil palm seedlings will be replanted almost immediately after the pulverization process (Ooi *et al.*, 2005).

Figure 2.2: Replanting of an oil palm plantation. Plantation section that has been cleared of old oil palm trees, which will be replaced by young oil palm trees.

Figure 2.3: Chipped and windrowed felled OPT.

2.2.2 Challenges arising due to the replantation process

During the replanting process, the oil palm industry produces an enormous amount of lignocellulosic biomass including the OPT, oil palm fronds (OPF), empty fruit bunches (EFB), mesocarp fibers (MF), and palm kernel shell (PKS). Despite this practice being regarded as more environmentally friendly, it poses some serious threats such as the attraction of pests and diseases. Using the chip and windrow method and pulverization technique, the old OPT takes at least two years and 13 months to decompose, respectively (Ooi *et al.*, 2005) which eventually becomes a breeding site for beetles such as *Oryctes rhinoceros* (Gnanasegaram *et al.*, 2014). The immature beetles thrive well in the dead and decomposing OPT whereas the mature beetles use it for breeding and mating purposes (Young, 1986; Zelazny, 1975).

Additionally, the population of wood rats (*Rattus tiomanicus)*, classified as one of the major pests in oil palm plantation (Buckle *et al.*, 1997) also increases shortly after a few months of the replanting process (Hamid *et al.*, 2013). The undisturbed frond and trunk piles are favourable for the rats to nest and breed (Hamid *et al.*, 2013). The effect of the multiplying rats after the replanting process is that they will consume the immature oil palm seedlings' fronds and feed on the succulent growing tissue that directly affects the growth efficiency of the oil palm seedlings (Chung, 2012).

The chipping and windrowing method of the felled old OPT is one of the sources of the basal stem rot disease caused by *Ganoderma boninense* (Virdiana *et al.*, 2010). This disease mainly affects the roots and basal stem of an oil palm (Siddiqui *et al.*, 2021). *G. boninense* is able to spread through the debris left from the previously planted and infected oil palm trees, after the new plants are replanted during the replanting process (Turner, 2008).

In addition, OPT is classified as one of the lignocellulosic biomasses that generally has a high C/N ratio (Lin *et al.*, 2019) . The high C/N ratio in the soil causes the decomposing process to be lengthen (Akratos *et al.*, 2017) and further reduces the biodegradation rate (Lin *et al.*, 2019). Therefore, the uncontrolled disposal of OPT back into the soil may alter and affect the surrounding plant growth and soil microflora as well as causing the soil to be nutrient deficient (Uke *et al.*, 2021; Zhang *et al.*, 2017).

2.3 Composition of an OPT

Generally, oil palm is categorized as a non-woody tree as it is monocotyledonous. It lacks cambium, secondary growth, growth rings and other components that are present in a woody plant. The length of an OPT can be in a range of seven to 13 m. The diameter on the other hand is approximately between 45 cm and 65 cm (Shafawati *et al.*, 2013). OPT is composed of four different parts, i.e., the cortex with bark, outer, middle, and inner regions (Figure 2.4). Contrary to the woody plants, OPT is composed of mainly two components which are the parenchyma and vascular bundle with a ratio of 70:30 (Bakar *et al.*, 2013). The parenchyma cells store starch as food storage, whereas the vascular bundle acts as the transportation system of water as well as nutrients throughout the palm tree (Mhd Ramle *et al.*, 2012; Tomimura, 1992).

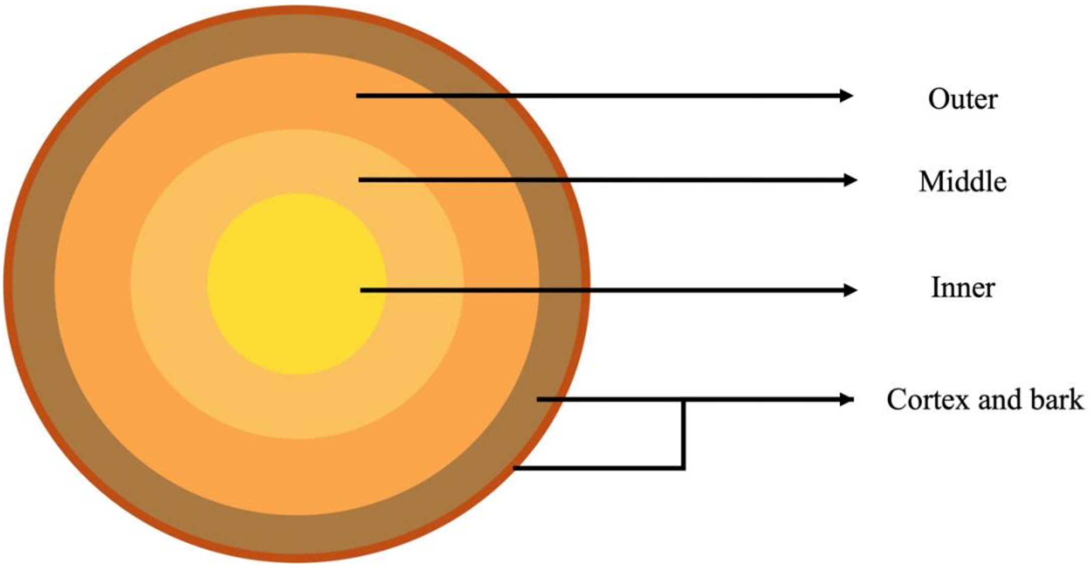

Figure 2.4: The cross section of an OPT showing the various parts having different compositions.

The outer layer of the OPT comprises of thin layers of parenchyma as well as vascular bundles. This layer of the OPT has high content of vascular bundle compared to parenchyma, which contributes to the mechanical support of the OPT. Additionally, these vascular bundles develop secondary cell walls with an increment in its length, beginning from the inner part of the OPT towards the outer parts of the OPT as seen in Figure 2.5 (Rosli *et al.*, 2016).

The middle layer of the OPT is composed of parenchymatous tissues with vascular bundles that are scattered, meanwhile the inner layer of the OPT has less vascular bundles which are randomly scattered. 80% of the OPT is made up of both the middle and inner layers of the OPT (Mhd Ramle *et al.*, 2012). Additionally, the moisture content also differs among the different sections of the OPT. The innermost layer of the OPT contains the highest moisture, followed by the middle and outer layers. This is mainly due to the presence of the parenchymatous tissues as it hold more moisture compared to the vascular bundle (Yamada *et al.*, 2010). Parenchyma cells are naturally spongy, soft, and absorbent compared to the vascular bundles that are fibrous, dense and less absorbent (Mhd Ramle *et al.*, 2012; Tomimura, 1992).

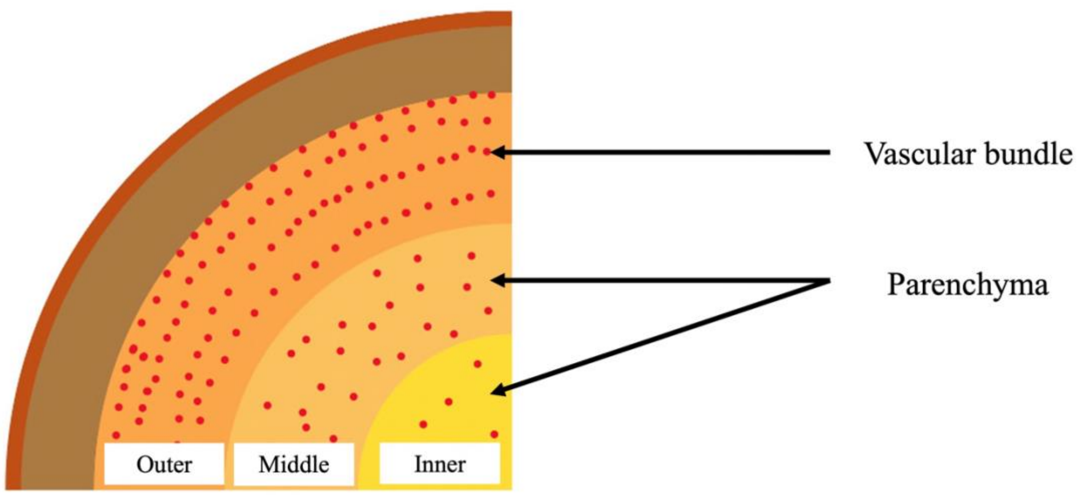

Figure 2.5: Distribution of vascular bundle and parenchyma in various parts of OPT.

Generally, lignocellulosic biomass is composed of polysaccharides, cellulose, hemicellulose, and lignin (Álvarez *et al.*, 2016). Additionally, the amount also differs from the composition in the OPT (Akmar *et al.*, 2001). The OPT consists of complex carbohydrate in forms of lignin, cellulose, hemicellulose, starch, and inorganics (ash) (Okahisa *et al.*, 2018; Saka *et al.*, 2008).

Cellulose is composed of a 8000 to 12000 β-1,4 linked D-glucose units in a linear polymeric chain (Kelly-Yong *et al.*, 2007). The glucose units are linked together to form a ribbon-like structure that is further stabilized with the presence of hydrogen bonds within the chains (Alberts, 2003). The function of cellulose is to provide mechanical strength, support plant growth, differentiation of cells, transportation of water, and defense mechanisms (Rongpipi *et al.*, 2019). It is used in various applications such as paper (Sahin *et al.*, 2008), textile (Stanković *et al.*, 2008), and bioethanol production (Chen *et al.*, 2010).

Hemicellulose on the other hand, is composed of different structural types which are further classified into four groupings such as xylans, mannans, mixed linkage β-glucans, and xyloglucans (Ebringerová *et al.*, 2005). Xylans are present mostly in the hemicellulose class of the hardwood species and herbaceous plants (Saha, 2003). Mannans mainly represented by glucomannans and galactomannans are the primary hemicelluloses in the secondary cell-walls of conifers (Timell, 1967). Mixed-linkage hemicellulose is particularly in Poales and some pteridophytes (Buckeridge *et al.*, 2004; Sørensen *et al.*, 2008). Xyloglucans is commonly present in the primary cell-walls of all the higher plants and they are strongly affixed via hydrogen bond to the cellulose fibrils (Pauly *et al.*, 1999). Hemicellulose functions to further strengthen the cell wall by interacting with cellulose and lignin (Scheller *et al.*, 2010).

The OPT is also composed of lignin, an amorphous biomacromolecule with phenyl propane units linked with different chemical bonds namely the C-C bonds and C-O-C ether bonds (Yang *et al.*, 2022). Lignin provides plants their structural integrity by playing its role of a glue (Zakzeski *et al.*, 2010).

The other important and rich component of an OPT is its starch (Abdul Hamid *et al.*, 2015). Starch is commonly stored in the parenchyma cells embedded within the vascular bundles and fibrous strands in the trunk of the OPT during its photosynthesis process (Tomimura, 1992). Among all the parts of the oil palm tree, the OPT contains the most starch content even though the amount of starch is dependent on the parts of the trunk, the age as well as the condition (Hashim *et al.*, 2011). Some OPT have been reported to contain a lot of starch meanwhile some have a low amount of starch (Abdul Hamid *et al.*, 2015). The apical of the OPT contains the highest amount of starch, followed by the middle and bottom part of the OPT (Abdul Hamid *et al.*, 2015). Additionally, in terms of the diameter of the OPT, the innermost core has the most starch followed by the outer section of the OPT (Yamada *et al.*, 2010).

Additionally, since OPT is slightly different from the general woody biomass, its trunk is composed of 70 % moisture content (Yamada *et al.*, 2010). Thus, the 70 % is mainly in the form of various free sugars, with glucose being dominant followed by sucrose and fructose (Kosugi *et al.*, 2010; Yamada *et al.*, 2010). Amino acids (i.e. aspartic acid, serine, glutamic acid and alanine) (Kosugi *et al.*, 2010), organic acids (i.e. acetic acid, maleic acid and malic acid) and mineral (i.e. calcium, magnesium and sodium) (Komonkiat *et al.*, 2013).

2.4 OPT and its applications

The outer part of an OPT is appropriate for plywood production considering the middle section of the OPT is mainly composed of the soft parenchyma tissues (Mokhtar *et al.*, 2011). It is also used to produce medium-density fiberboard (MDF) (Ibrahim *et al.*, 2013; Ibrahim *et al.*, 2014). Additionally, OPT is also a potential starting material to produce bio pellet (Wistara *et al.*, 2021). Malaysia and Indonesia have been utilizing the OPT for the production of bio pellet that can be further utilized to generate biofuels (Chew *et al.*, 2023). The pretreated OPT biomass may also be a potential raw material for ethanol fermentation (Afedzi *et al.*, 2023).

2.4.1 Starch

Carbohydrates in an OPT are stored in the form of starch in the parenchyma cells (Abe *et al.*, 2013). In general, starch is composed of the two glucose polymers amylose and amylopectin, structuring an intricate semi-crystalline structures and granules located in plastids (Apriyanto *et al.*, 2022). As oil palm trees age, instead of increasing in width size, the parenchyma cells composed of starch granules, will reduce and further be replaced by mature vascular bundles due to the characteristic of a monocotyledonous plants being absent in vascular cambium (Mhd Ramle *et al.*, 2012). Generally, the granule of the OPT starch has an average size of 14.6 µm with a shape of ovoid to elliptical with its main portion of a truncated end. Additionally, some starch granules were observed to be in a bell shape (Apriyanto *et al.*, 2022; Mohd. Noor *et al.*, 1999).

Thus, starch in OPT is classified as the most important polysaccharide and a major carbohydrate reserve (Tomimura, 1992). Starch as carbon storage is more sustainable because starch reduces the disturbances in cells' osmotic balance (Yu *et al.*, 2022). Thus, high starch OPT potentially contributes as value added product due to its many potential applications.

2.4.1(a) Potential of OPT for bioethanol production

Generally, starch from biomass waste has been observed to be used as a source for the production of bioethanol, such as the potato starch residue stream (Hashem *et al.*, 2010), cassava peel (Aruwajoye *et al.*, 2020), bamboo (Kuttiraja *et al.*, 2013), and rice straw (Wi *et al.*, 2013). Starch of the OPT is a possible bioresource to produce ethanol and biochemicals (Eom *et al.*, 2015; Wardani *et al.*, 2021). This is because of the high starch content that will eventually be converted into fermentable sugars in its sap (Kosugi *et al.*, 2010; Yamada *et al.*, 2010). High solid-state simultaneous saccharification and fermentation using the parenchyma and vascular bundle resulted in the maximized utilization of starch from the OPT (Prawitwong *et al.*, 2012). Additionally, the hydrolyzed OPT starch medium made from the starch extraction of amylase and glucoamylase, was able to be fermented by several lactic acid bacteria into lactic acid (Meidiawati *et al.*, 2024).

2.4.1(b) Potential of OPT for wood-based panel

The wood-based panel industry is now leaning towards green adhesives in producing its particleboards. The OPT starch mixed with urea formaldehyde is a potential green adhesive to produce particleboards because its application reduces the reliance of the urea formaldehyde, which is a type of petroleum-based adhesives (Selamat *et al.*, 2014; Sulaiman *et al.*, 2018). Certain minimum requirements such as the modulus of rupture, modulus of elasticity and internal bond strength established by the Japanese Industrial Standards (JIS) were fulfilled using oil palm starch as an adhesive (Sulaiman *et al.*, 2018).

2.4.1(c) Potential of OPT as animal feed

The top part of the OPT containing higher starch content can be ensilaged in the presence of bacterial inoculum such as *Lactobacillus acidophilus* in order to increase its nutritional value. Results showed an increase in the crude protein content, and higher digestibility (Widiawati *et al.*, 2024). Furthermore, fermented OPT using *Phanerochaete chrysosporium* resulted in the highest digestibility as nutrients potential animal feeding stock (Azhary *et al.*, 2018). Therefore, OPT is a potential source as an animal feed material.

2.4.2 OPT's fermentable sugar and its applications

After the felling of the oil palm trees, the starch present in the OPT will eventually be converted into simple sugars such as glucose and fructose by the starch-degrading and sucrose metabolizing enzymes (Yamada *et al.*, 2010). The enzyme activities of amylases and invertases increases after the felling process which was one of the contributing factors in the reduction of the starch content (Abdul Hamid *et al.*, 2015). The sap of the OPT comprises of a considerable amount of fermentable sugar that will increase within a period of storage (Kosugi *et al.*, 2010; Yamada *et al.*, 2010).

2.4.2(a) OPT sap for bioethanol production

The OPT sap has been shown to contain three major sugars, namely glucose, sucrose, and fructose. Bioethanol can be produced using the OPT sap as a carbon feedstock for *Saccharomyces cerevisiae* (Bukhari *et al.*, 2015; Shahirah *et al.*, 2015). *Kluyveromyces marxianus* was also reported to utilize OPT sap to produce bioethanol at a higher productivity compared to *S.* cerevisiae. The supplemented nutrients such as magnesium sulphate and β-alanine promotes higher production of bioethanol (Bukhari *et al.*, 2015). Additionally, the produced ethanol using the OPT sap was similar to the reference fermentation of yeast extract peptone dextrose implying that OPT sap is a good carbon feedstock for bacteria to produce bioethanol (Kosugi *et al.*, 2010).

2.4.2(b) OPT sap for bioplastic production

OPT sap has the potential to be utilized by various bacteria as a medium feedstock to produce biodegradable plastics. *Bacillus megaterium*, a bacteria that was isolated from the termite gut, was able to produce polyhydroxyalkanoate (PHA) when young or old OPT sap was used as its carbon feedstock (Lokesh *et al.*, 2012). Additionally, a combination of sugars from the OPT sap and crude palm kernel oil when used as the carbon source for *Cupriavidus necator* strain Re2058/pCB113, was able to produce copolymers that has properties which are similar to the synthetic plastics (Murugan *et al.*, 2016). Interestingly, microorganisms that are tolerant towards high salinity such as *Halomonas* sp. SK5 successfully utilized mixture of OPT sap and seawater to synthesize homopolymers and copolymers (Rathi *et al.*, 2013). Therefore, OPT sap is a good potential of carbon feedstock to produce bioplastics. OPT sap comprises of 70% of the OPT weight thus increasing the available sugar content within the OPT after a certain period of storage (Yamada *et al.*, 2010).

2.4.2(c) OPT sap as commercialized syrup

There are different types of commercialized syrup such as honey, maple, and grape syrups. A study has shown that OPT sap possesses similar physicochemical properties as other commercialized syrups and has the potential to be a substitute of commercial syrups such as maple and glucose, offering additional nutritional benefits (Sulaiman *et al.*, 2020). Additionally, the OPT syrup was reported to be made into toffee for confectionary purposes with overall best performance compared to other toffees. The recrystallisation of the OPT sap toffee is minimal since the OPT sap after 60 days of storage is composed of mainly glucose, the reducing sugar (Sulaiman *et al.*, 2019)

2.4.2(d) OPT sap with reactive oxygen (ROS) potential

The two free radical scavenging activities identified in the OPT sap were the Vitamin C Equivalent Antioxidant Capacity and Trolox Equivalent Antioxidant Capacity, that are both metrics used to evaluate the sample's antioxidant activity (Sulaiman *et al.*, 2020). Additionally, the OPT sap has reactive oxygen species (ROS) scavenging capacity whereby the middle part showed highest scavenging activity against radicals of alkoxyl and hydroxyl. The shorter storage period of the OPT resulted in the sap with higher ROS activity compared to the sap from OPT incubated for a longer storage period (Arai *et al.*, 2022).

2.5 Pathogenesis-related (PR) proteins

Plants are sessile organisms. They do not possess the ability to move from threats such as adverse environmental conditions or any pathogenic attacks. Therefore, they have developed a unique self-defense mechanism as a response to these conditions. They respond to these stressful conditions on various levels namely the cellular, molecular, and genetic levels. Generally, the plant's immunity is active upon the microbe-associated molecular patterns, pathogen-associated molecular patterns, or damage-associated molecular patterns, which contributes to the pattern-triggered immunity (Jones *et al.*, 2006). Its immunity is also affected by the effector-triggered immunity (Win *et al.*, 2012). These will initiate the local signaling events such as fluxes of ion, ROS, and protein kinases. These initiations will subsequently result in the production of phenolic compounds, phytohormones, phytoalexins as well as the PR proteins (Boccardo *et al.*, 2019).

PR proteins were firstly discovered with the Tobacco Mosaic Virus infection towards the tobacco plants (Harrison *et al.*, 1999). PR proteins are composed of diverse molecules that are activated by phytopathogens, and defense related signaling molecules. The expression of PR proteins is important as an indicator of the plants immune response responding to abiotic and biotic stresses namely the drought, cold, salinity, wounding, heavy metals, and pathogenic attacks (Ali *et al.*, 2018).

Enzymes associated with pathogen defense such as glucanase, chitinase, peroxidase and ribonucleases, are also proteins synthesized from the interaction between the plants and pathogens. Healthy plants may produce this enzyme in minimal quantities, however, the production increases significantly in presence of pathogens (Dos Santos *et al.*, 2023). PR proteins further enhances the immunity of a plant by breaking down the fungal cell wall, making membranes more permeable, suppressing transcription, and deactivating ribosomes (Anroop *et al.*, 2022).

Additionally, PR proteins are classified into different families according to their structural and functional properties. Presently, there are 19 families of PR proteins that has been studied (Dos Santos *et al.*, 2023). These defense related proteins triggered from actions of signaling compounds (i.e., salicylic acid, jasmonic acid, and ethylene), or during stress (i.e., senescence, wounding, or cold stress). Thus, PR proteins are produced not only as defense response, they may be induced from the various stress inflicted on the plants (Loon *et al.*, 2006). The expression of the PR proteins is pertinent in identifying its effect in resisting or combating phytopathogenic attacks. They can be further studied to be incorporated into certain crop plants, thereby producing crop varieties with disease resistance (Islam *et al.*, 2023).

2.6 Gene expression of plants undergoing senescence

Plant senescence is a complex and regulated process that indicates the end of a plant's life cycle. It is a genetically controlled process identified by the aging of leaves or the remobilization of nutrients. It is very important to understand the changes of gene expression during a plant's senescence to enhance the crop yield and quality by improving management practices. The senescence-associated genes (SAGSs), transcription factors (TFs), and regulatory networks are some of the genetic and molecular mechanisms involved in senescence.

Numerous SAGs were identified with high expression as plants' age. It has been reported that more than 5,800 SAGs were expressed in many plant species, including *Arabidopsis thaliana, Oryza sativa* and *Solanum lycopersicum*. Additionally, SAGs play a role during the chlorophyll degradation, nutrient recycling and programmed cell death (Guo *et al.*, 2021).

Transcription factors are also important to regulate the expression of genes when plants undergo senescence. The TFs (i.e., Myeloblastosis and WRKY) been been shown to involve in senescence process (Zhang *et al.*, 2021). The TFs highly impacted the expression of SAGs, which further affects the regulation of time and progress of leaf senescence. This implies the relationship between the TF-mediated regulatory networks and the plant aging process (Koyama, 2014).

The expression of plant hormones also regulates the senescence of plants. Hormones such as ethylene, jasmonic acid, salicyclic acid, and absicis acid promotes senescence. Opposite of cytokinin and gibberellic acid which prolong senescence. The relationship between these plant hormones are crucial to identify the timing and the progress of senescence (Guo *et al.*, 2021).

The abiotic stress such as photoperiod, temperature and water stress contribute to the gene expressions during senescence. For example, when plants are exposed to drought environment, leaf aging will be exacerbated due to the change in hormonal balances and stress-related gene expression will be triggered. (Zhang *et al.*, 2021).

Thus, during plant senescence, the changes in the gene expression are regulated by a network of regulatory mechanisms that involves the SAGs, TFs, signal of hormones as well as the effect from environmental interactions. With this knowledge, innovative strategies to manipulate the senescence process is important to further improve the productivity and sustainability of crops.