

**DETERMINATION OF
2-PYRONE-4, 6-DICARBOXYLIC ACID PRECURSORS
FROM OIL PALM TREE USING DIFFERENT
EXTRACTION PROCESS**

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Universiti Sains Malaysia

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EXTRACTION PROCESS**

By

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ABBREVIATIONS

CH ₃ OH	Methanol
CD ₃ OD	deuterated methanol
H ₂ O	Water
H ₃ PO ₄	Phosphorus acid
NaOH	Sodium hydroxide
NMR	Nuclear Magnetic Resonance
HPLC	High Performance Liquid Chromatography
RP-HPLC	Reverse-phase High Performance Liquid Chromatography
NP-HPLC	Normal-phase High Performance Liquid Chromatography
UV/VIS	Ultraviolet-visible Spectroscopy
PDC	2-pyrone-4,6-dicarboxylic acid
EFB	Empty fruit bunch

**MENENTUKAN UNIT ASAS FENOL UNTUK 2-PIRON-4,6-
DIKARBOKSILIK ASID DARIPADA POKOK KELAPA SAWIT
DENGAN MENGGUNAKAN PROSES PENGEKSTRAKAN
YANG BERBEZA**

Abstrak

Kajian telah dilakukan untuk menentukan unit asas fenol bagi 2-piron-4,6-dikarboksilik asid (PDC) yang terdapat di dalam pelbagai hasil tanaman kelapa sawit di Malaysia. Hasil tanaman kelapa sawit yang terlibat ialah *Dura x Pisifera (Dura x URT)*, *Dura x Tenera*, *Deli dura x AVROS*, *Dumpy x Yangambi x AVROS*, *Deli dura x yangambi*, *Deli dura x Pisifera x H&C*. Setiap sample dibahagikan kepada 11 bahagian iaitu kulit, batang (bahagian dalam), pelepah (pelepah kecil dan pelepah besar), daun, buah (isi buah, albumen dan kulit keras), tandan buah kelapa sawit kosong (fiber tandan buah kosong, tangkai tandan buah dan duri tandan buah). Pengekstrakan subkritikal air dan pengekstrakan subkritikal air beralkali telah dipilih untuk mengkaji kondisi optimum bagi menghasilkan unit asas fenol bagi 2-piron-4,6-dikarboksilik asid (PDC). Kondisi optimum bagi kedua-dua pengekstrakan ini telah ditentukan dengan menggunakan batang kelapa sawit (bahagian dalam). Tiga pembolehubah telah digunakan bagi pengekstrakan subkritikal air iaitu suhu, masa dan isipadu air. Manakala bagi pengekstrakan subkritikal air beralkali empat pembolehubah digunakan iaitu kepekatan, suhu, masa dan isipadu larutan sodium hidroksida (NaOH). Keputusan menunjukkan kondisi optimum bagi pengekstrakan subkritikal air ialah 200°C, 40-60 min dan 100 ml air. Manakala bagi pengekstrakan subkritikal air beralkali menunjukkan kehadiran yang maksimum unit asas fenol bagi

PDC pada 0.1M, 180°C, 20 min dan 100 ml larutan NaOH. Berdasarkan keputusan, *Dura x Pisifera (Dura x URT)* menunjukkan jumlah unit asas fenol bagi PDC yang tinggi iaitu dengan jumlah hasil sebanyak 8.40%. Sementara batang (bahagian dalam) dan kulit keras mengandungi kadar hasil unit-unit asas fenol yang tinggi berbanding bahagian-bahagian yang lain. Keputusan yang ditunjukkan daripada kaedah kromatografi (HPLC) dan disahkan melalui NMR menunjukkan *p*-hidroksibenzoik asid ialah unit utama fenol bagi PDC di dalam sisa kelapa sawit.

DETERMINATION OF 2-PYRONE-4,6-DICARBOXYLIC ACID PRECURSORS FROM OIL PALM TREE USING DIFFERENT EXTRACTION PROCESS

Abstract

The precursors of 2-pyrone-4,6-dicarboxylic acid (PDC) were investigated from selected oil palm cultivars in Malaysia. The studied cultivars include *Dura x Pisifera (Dura x URT)*, *Dura x Tenera*, *Deli dura x AVROS*, *Dumpy x Yangambi x AVROS*, *Deli dura x yangambi*, *Deli dura x Pisifera x H&C*. All samples were divided into 11 parts; outer bark, trunk (inner part), frond (rachis and petiole), leaves, fruits (flesh, albumen and kernel shell) and empty fruit bunch (EFB fiber, midrib spine leaflets and stalk of fruit bunch). Subcritical water extraction and alkaline subcritical water extraction were chosen to investigate the optimum conditions to produce PDC precursors. Oil palm trunk powder (inner part) was used in both extractions to find the optimum condition of PDC precursors. Three parameters were used for subcritical water extraction that includes temperatures, times and volumes of water. For alkaline subcritical water extraction four parameters were used which were concentrations, temperatures, times and volume of sodium hydroxide (NaOH) solutions. The results indicated that, optimum condition for subcritical water extraction was at 200°C, 40-60 min and 100 ml. While for alkaline subcritical water extraction showed the best presence of PDC precursors at 0.1M, 180°C, 20 min and 100 ml of NaOH solvent. From the results *Dura x Pisifera (Dura x URT)* showed high total yield of PDC precursors of 8.40%. While trunk (inner part) and kernel shell consisted high yield of total PDC precursors. Results obtained from high performance liquid chromatography (HPLC) and confirmed by nuclear magnetic

resonance (NMR) showed that the p -hydroxybenzoic acid was the main precursor of PDC in the oil palm biomass.

1.0 INTRODUCTION

1.1 General Background

The botanical name of the oil palm is *Elaeis guineensis*, was originally planted in West Africa where it has been used from ancient times as a source of edible oil. This monocotyledonous plant of the palm family (*Arecaceae*) can be recognized on the basis of their fruit characteristics (Jouannic et al., 2005). In year 2006, the palm oil production in Malaysia increased to 16.5 million metric tons. The amount of biomass produced by an oil palm tree in the year 2008 is on average of 231.5 kg dry weight/year. The oil palm empty fruit bunches and oil palm trunk contributed about 15.8 and 8.2 million tonnes (Khalil et al., 2009). Oil palm tree has positioned Malaysia as the leading country in the oil palm industry from upstream to downstream process (Chew et al., 2008). The rapid expansion of palm oil production in Malaysia has obviously brought about an increase in the number of mills that are in operation in the palm oil industry (Chew et al., 2008).

Palm oil industry in Malaysia also produces huge quantity of biomass including oil palm trunks, oil palm frond, empty fruit bunches (EFB), kernel, shell and fibres in the production of palm. Oil palm biomass had varied in its morphology, so it would be necessary to deal with many compounds in an attempt to convert biomass to useful products (Goldstein, 1978). Oil palm solid waste can be considered as a sustainable and also renewable lignocellulosic material (Chew et al., 2008).

The oil palm cultivation started in Malaysia and Indonesia in the 1910s (Heartly, 1988). The oil palm industry in Malaysia is based on Deli dura population. Three major cultivars of oil palm are dura, pisifera and tenera which can be indentified from fruit palm. The history and development of oil palm variety could be obtained from Kushairi and Rajanaidu (2000). Tenera palm was developed in

industry by crossing the female dura with the male pisifera (Basiron, 2000). Since then various types of hybrids have been obtained through several breeding research programmes conducted by agencies like FELDA, MPOB and SIME DARBY (Norziha, et al., 2008).

Various research had been done and focused on the utilization of empty fruit bunch on the production of pulp for paper making, board-making industry, oil palm fiber mattresses, agriculture mats and organic fertilizer (Astimar et al., 2002; Tanaka et al., 2002; Ridzuan et al., 2002; Mohamad et al., 2002). Research work also undergoing in the converting OPF and OPT for the manufacture of commercially viable composite panel product (Nordin et al., 2004). Therefore besides all findings and developments, these renewable materials can be alternatively used for producing valuable chemical and lignocellulosic products. The oil palm biomass has great potential to be reproduced into high value-added and useful income-generating products.

Precursor for making 2-pyrone-4, 6-dicarboxylic acid (PDC) is synthesized from phenolic compound in lignin monomers (Otsuka et al., 2005). Lignin is generally distributed as a matrix component with hemicelluloses in the spaces of inters cellulose micro fibrils in primary and secondary walls and in the middle lamellae (Higuchi, 1985). 2-pyrone-4,6-dicarboxylic acid (PDC) is a substance with a structure containing a pyran ring with two carboxylic acid groups. The conversion of these two carboxylic groups into derivatives would make it a potential raw material for various novel, bio-based polymers. These ring fission reactions resulting in 2-pyrone-4,6-dicarboxylic acid are mostly known in bacteria like *Pseudomonas* and *Micrococcus* compared to plants (Wilkes and Glasl, 2001). In fact, Michinobu et al. (2008) succeeded in preparing polyesters from 2-pyrone-4,6dicarboxylic acid, a

chemically stable metabolic intermediate of lignin for the first time utilized (Michinobu et al., 2008). Shigehara et al. (2001) also succeeded in producing PDC polyamide as a novel polymer chemicals via the 2-pyrone-4,6-dicarbonyl dichloride derivative (Otsuka et al., 2005).

Subcritical water extraction method was known as a green technology process. From previous research, it was found that this eco-friendly treatment can selectively produce phenolic compounds in a very short time compared to conventional methods. Subcritical water extraction is a simple, non-flammable, practical and cost-effective method that has been recently used to extract phenolic compound (Pourali et al., 2010). In this research, PDC precursors were determined by using high performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR). HPLC offers selectivity, resolution, speed and sensitivity far superior compared to classical techniques such as paper chromatography. Reversed-phase HPLC with UV/VIS detector provides powerful and consistency analysis for phenolic compounds in plant extracts. While NMR was examine the structure of chemical compound that was found in oil palm extract (Tasioula-Margari and Okogeri, 2001).

In this study oil palm biomasses was chosen as a raw material for identification and quantification of 2-pyrone-4,6-dicarboxylic acid precursors. The aim of this study was to optimize a simple method for the determination of PDC precursors and consequently to identify the possibility of PDC precursors in oil palm biomasses that can make a significant contribution for future research on the production of PDC-based materials.

1.2 Hypothesis

Nowadays, there are huge amounts of oil palm biomass left unexploited in oil palm plantation. However, these biomasses had a potential to develop and commercialize in our industry to become useful finding. Oil palm biomasses are expected to be one of the raw materials that can produce 2-pyrone-4,6-dicarboxylic acid precursors. In this research, study on identification and quantification of precursors 2-pyrone-4,6-dicarboxylic acid (PDC) from oil palm tree was developed by varying extraction procedures.

1.3 Objectives

The objectives of the present work are

1. To determine the optimum condition of subcritical water treatment and alkaline subcritical water treatment (extraction of precursor of PDC) of oil palm biomasses.
2. To compare between every parts of oil palm which had a highest yield of precursor of PDC.
3. To compare between cultivars of oil palm which had a highest yield of PDC precursors.
4. To study the chemical structure of the highest yield of PDC precursors.

2.0 LITERATURE REVIEW

2.1 Oil palm (*Elaeisguineensis*)

The oil palm is a monocotyledon plant belonging to the genus *Elaeis* of the palm family (*Arecaceae*). It is a perennial tree crop and the highest oil producing plant, with a long life cycle and no natural vegetative reproduction (Nakkaew et al., 2008). *Elaeis* is derived from the Greek word *elaion*, meaning oil, while the specific name *guineensis* shows Jacquin attributed its origin to the Guinea coast. *Elaeis guineensis* Jacq. is grouped with *Cocos* (coconut) and other genera under the tribe *Coccoineae* (Corley & Thinker, 2003). From time to time other specific names have been attached to supposed species of *Elaeis*, but none has shown any signs of permanency other than *E. melanococca*, now named *E. oleifera*, and *E. madagascariensis*, which legality used. The Index Kewensis lists fourteen names but the majority of them have disappeared from the literature. Many of them either refers to quite different palms or is synonymous with *E. guineensis*. The genus *Elaeis* comprises the two species, namely *E. guineensis* and *E. oleifera*. *E. guineensis* originates from West Africa and the commercial planting material is mainly of this species. *E. oleifera* is a stumpy plant of South American origin and its oil is characterized by high oleic acid content (Hartley, 1988).

Elaeis guineensis originating from West Africa was first introduced to Brazil and other tropical countries in the 15th Century by the Portuguese. It was first brought to Malaysia from tropical Africa in 1870 through the Singapore Botanical Gardens for ornamental purpose (Mohd Zin et al., 1991). Oil palm is a species that contribute to the economic importance as it yielding an average of 3.7 tonnes of oil per hectare per year in Malaysia (Kee, 1957). Its products like palm oil, palm kernel, palm kernel oil, empty fruit bunch (EFB) and oil palm trunk are important sources

for many developing country as a foreign exchange earnings. America Latin countries had established their oil palm plantation and production to achieve domestic oil requirements while Africa countries like Ivory Coast and Cameroons are expanding palm oil production to increase their export earnings (Kee, 1957). This happen because of the need by plantation companies to diversify their interests and dependence on other natural sources and it has become a significant sector of the economy of many developing countries (Nakkaew et al.,2008; Hartley, 1988; Kee, 1957).



Figure 1: Oil palm plantation

(Picture of Felda Kekayaan, Kluang, Johor)

The palm tree as shown in Figure 1 reaches maturity in three years and has economic life of about 35 years. The crop is unique in that it produces two types of oil. Each part of oil palm tree had their valuable value. The fleshy mesocarp produces palm oil, which is used mainly for its edible properties and the kernel produces palm kernel oil, which has wide application in the oleo chemical industry. The two oils have different composition and this is important because the two oils

are often confused by nutritionist. Palm oil consist palmitic acid, oleic acid and most common fatty acid while kernel oil mainly contain lauric acid (Chew and Bhatia, 2008).

Oil palm fruit grows in large bunches weighing 10-20 kg and each size kindly as a small plum. The fruits consist of a hard kernel (seed) inside a shell (endocarp) which is surrounded by fleshy mesocarp. Empty fruit bunch (EFB) of oil palm can be described as a cellulosic residue was used as a raw material for cellulose, lignin and hemicelluloses production (Hartley, 1988).

Oil palm fronds are left rotting between the rows of palm tree and it important for soil conservation, erosion control and nutrient recycling. Besides, oil palm trunk and frond are hugely used in pulp, paper and wood based industry (Chew and Bhatia, 2008).

As we know, palm oil availability is the main sources of oil palm tree therefore, beside that there also generated a huge quantity of oil palm biomasses (Figure 2). These residues had a huge potential to be a major source of energy for mankind and is expected to act as substantial role in the future global energy balance. The physical and chemical properties of different oil palm biomasses significantly give them a high value of commercialize. For example, nowadays oil palm solid waste are cheap and abandoned materials produced during palm oil milling process and these materials can be considered as a sustainable and also renewable lignocellulosic material (Chew and Bhatia, 2008; Luangkiattikhun et al., 2007).The demand of this biomass had increases by year because of new finding in their uses (Alriol et al., 2008).

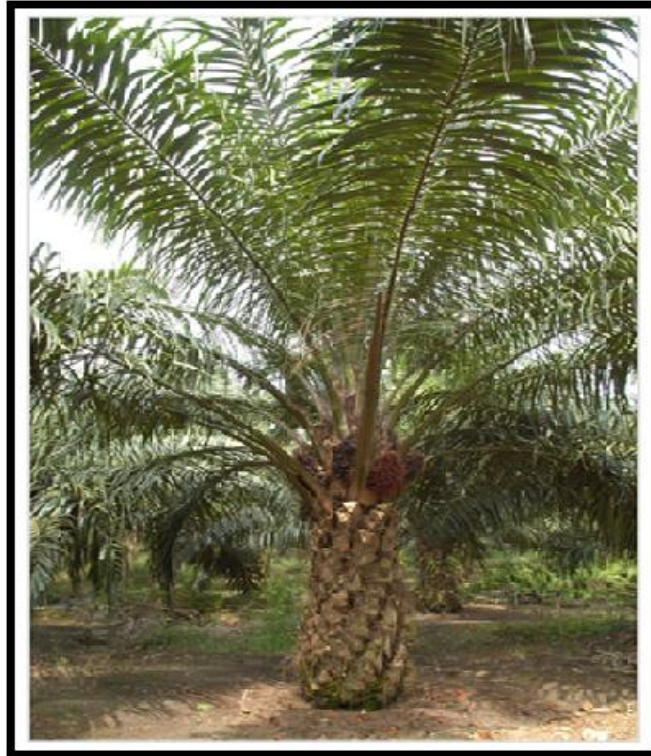


Figure 2: Oil palm tree
(Picture taken from Ladang Pelam, Kulim, Kedah)

2.2 Plant biomass

Biomass is a lignocellulosic materials and it also refers to encompass all plant and animal matter. Lignocellulosic biomass principally made up from lignin, hemicelluloses and cellulose. This kind of biomass consists of fibrous materials from organic sources, woody, agriculture wastes and organic industry wastes. There are various renewable sources of energy, but plant biomass stands out as one of the most valuable and promising (Lim, 2006).

Oil palm biomass comes in abundantly and for the industries this can be advantage if the excess of waste can be used to produce other useful applications. Malaysia as a largest producer of oil palm can generate the largest amount of oil palm biomass as a by-products. MPOB report in June 2006 stated that the Malaysian palm oil industries are producing about 2.85 million tonnes of solid wastes including

fibre, shell and empty fruit bunches. Residues from oil palm production come in two parts. The first part is when harvest and production of oil time, which is fronds, empty fruit bunches (EFB), fibres and kernel shell are produces and second part when replanting in 25th year. During daily harvesting of the fresh fruits bunches for oil production, the fronds are also available (Koh et al, 2006; Lim, 2006).

There are many advantages and benefits using biomass as an energy source and raw material in new development. It is clean sources of energy and if utilisation of them were doing in correct and proper way it will less harmful to environment as shown in Table 1. Beside during the converting of biomass to useable forms, may be useful of chemical by-products can also be produced. On the other hand, the use of plant biomass contribute essentially no net CO₂ emission to the atmosphere and due from that it will not contribute towards global warming (Koh et al, 2006; Lim, 2006). Agriculture wastes also have relatively low calorific values due to the high moisture content and low in bulk density. Somehow moisture content and oil content determine the energy content of the wastes (Ani, 2006). The use of biomass energy not only provides economic, environmental and social benefits but also contributing to national energy security and towards political stability. Since there are a number of advantages on oil palm biomass, it is proposed to develop the technologies, standards and improvement in their utilization.

Table 1: Availability of palm oil mill residues and their energy potential

Year	Total Planted area (Mha)	Amount of mesocarp fibres (MT)	Surplus mesocarp fibres available (MT)	Energy from mesocarp fibres (PJ)	Amount of shell (MT)	Surplus shell available (MT)	Energy from shell (PJ)	Amount of EFB (MT)	Surplus of EFB (MT)	Energy from EFB
1997	2.819	4.74	1.19	22.36	7.16	1.79	36.62	8.6	2.15	38.5
1998	3.078	5.17	1.29	24.23	7.82	1.96	40.1	9.39	2.35	42.08
1999	3.138	5.27	1.32	24.8	7.97	1.99	40.71	9.57	2.39	42.8
2000	3.498	5.88	1.47	27.62	8.88	2.22	45.42	10.67	2.67	47.81
2005	3.798	6.38	1.6	30.06	9.65	2.41	49.31	11.58	2.9	51.93
2015	4.098	6.88	1.72	32.31	10.41	2.6	53.2	12.5	3.13	56.05
2020	4.398	7.39	1.85	34.75	11.17	2.79	57.08	13.41	3.35	59.99

(Source: Ani, 2006)

2.2.1 Oil palm trunk

Oil palm trunk also known as a stem of the palm. It is an erect and fairly uniform column which up to around 12 until 15 years of age. It also covered by persistent leaf bases. Scars are found to occupy a large point of the stem surface when the leaf bases slough off. It varies in diameters from the thickness of a reed to a sturdy pillar-like structure as seen in the date-palm, Palmyra palm or Talipot. Narrow cortex from the wide central cylinder have different outer region of living tissues (Kee, 1957).

At the top of the stem there is one bud that known as a growing point. This growing point is a place where oil palm lives and grows, if the growing point dies the tree dies as well. In many species of oil palm, trunk is covered with a dense network of stiff fibres, often compacted together at the free ends into spines. This fibrous material, which is so valuable for cordage, consists of the fibrous tissue of the leafstalk, which in these cases persists after the decay of the softer portions. It is very characteristic of some palms to produce from the base of the stem a series of

adventitious roots which gradually thrust themselves into the soil and serve to steady the tree and prevent its overthrow by the wind (Kee, 1957).

There are many small and large vascular bundles with a few bundles scattered in the central region at the edge of the cylinder as shown in Figure 3. Single strands of phloem tissues and xylem elements surrounded by parenchyma are consisting in the vascular bundles. These tissues which consists a lot of vascular bundles and parenchyma cells can be separated easily by mechanical crush. Besides, due to the high contents of xylan and starch in addition to cellulose and lignin, trunk has a high potential for food and cattle feed production. Oil palm trunk can be utilized for building materials like a raw material for wood-based panels such as cement board, fibreboard and strand board (Tomimura, 1992).



Figure 3: Oil palm trunk

(Picture taken at Felda Kelating Jernih, Serting Hilir, Negeri Sembilan)

2.2.2 Oil palm leaves

The oil palm leaves are pinnate, and the length of leaf varies in meters but 7 until 8 meters are common and normal. A mature palm had 30 until 40 leaves which are pinnate and the leaflets are arranged in two ranks on either side of rachis as shown in Figure 4. Phyllotaxis is refers to the arrangement of leaves with regard to the axis of the palm. The first open leaf stage was develop about 20 until 24 months and elongation is most rapid in the 5 until 6 months. There are maybe 150 until 250 leaflets per leaf. Two sets of spirals of leaves, eight running one way and thirteen the other can be seen in mature palms. The palm is left handed if the spiral of eight ascends the palm in a clockwise direction and if it in contra direction (anti-clockwise) the palm is right handed. The petiole broadens considerably towards the junction with the stem is used to connect the leaf to the stem. A bud which located in the axil of each leaf (where the leaf meets the stem) may develop into male or female inflorescences (Kee, 1957).



Figure 4: Oil palm leaves
(Picture taken from Felda Kekayaan, Kluang, Johor)

2.2.3 Oil palm frond

Oil palm frond is one of the greatest abundant agricultural by-products in Malaysia. This biomass is collected during pruning and replanting activities. Frond was reported had the longest fiber with an average length of 1.59mm among various fibrous components of the oil palm tree such as trunk, mesocarp and fruit bunch (Wan Rosli et al., 2003). Oil palm frond can be divided into petiole and leaflets (rachis). It belongs to the category of fibrous crop residues which include by products such as rice straw (Kee, 1957; Heartly, 1988)

Oil palm frond as shown in Figure 5 has potential for use as a raw material for pulp and paper industry or wood-based industry (Heartly, 1988). Meanwhile, it also can use as a roughage source or as a component in compound feed for ruminant. Recently, oil palm fronds are left rotting between the rows of palm tree. This is causes to the long-term benefit of nutrient recycling and for soil conservation and erosion control (Kee, 1957).



Figure 5: Oil palm frond
(Picture taken from Ladang Pelam, Kulim, Kedah)

2.2.4 Oil palm fruits

The oil palm fruit consists of a thin external waxy skin or exocarp, the fleshy and oily mesocarp, a hard stony endocarp or shell and an endosperm or kernel. The seed was constituted by the endocarp and kernel. The fruit bunch was developed from female inflorescence and become ripe after about 5 until 6 months. The fruits do not all become ripe at the same time but may be occur over fifteen days though sometimes much shorter than this (Chew and Bhatia, 2008).

The colour appearance of the fruits varies when ripening. The common one is deep violet to black at the apex and colourless at the base before ripening. Besides that, there have fruit which change from orange to red after ripening and it is believed to be associated with carotene content. The uncommon type is green before ripening and after ripening it change to a light reddish orange colour. This is because these kinds of fruit do not contain carotene in their mesocarp (Kee, 1957).

The most important about fruit its shell thickness because it can refers to their cultivar. Dura have intermediate thickness of shells around 2 until 8 millimetres and low to medium mesocarp content of 35 until 55%. While Tenera which is hybrid of shell-less Pisifera and the common thick shelled Dura have very thick shells and a thin outer fleshy layer of pericarp, it's about 2 until 8 millimetres. Third variety is without shell (Pisifera). Commercially a hybrid between the thicker shelled Dura and shell less Pisifera is mostly used at the present time. This crop is unique because it produces two types of oil. The pericarp produces a bright orange oil which is used mainly for its edible properties and the other from the kernel within the shell produce thicker and colourless palm kernel oil which has wide application in the oleochemical industry (Kee, 1957).



Midrib spine
leaflets

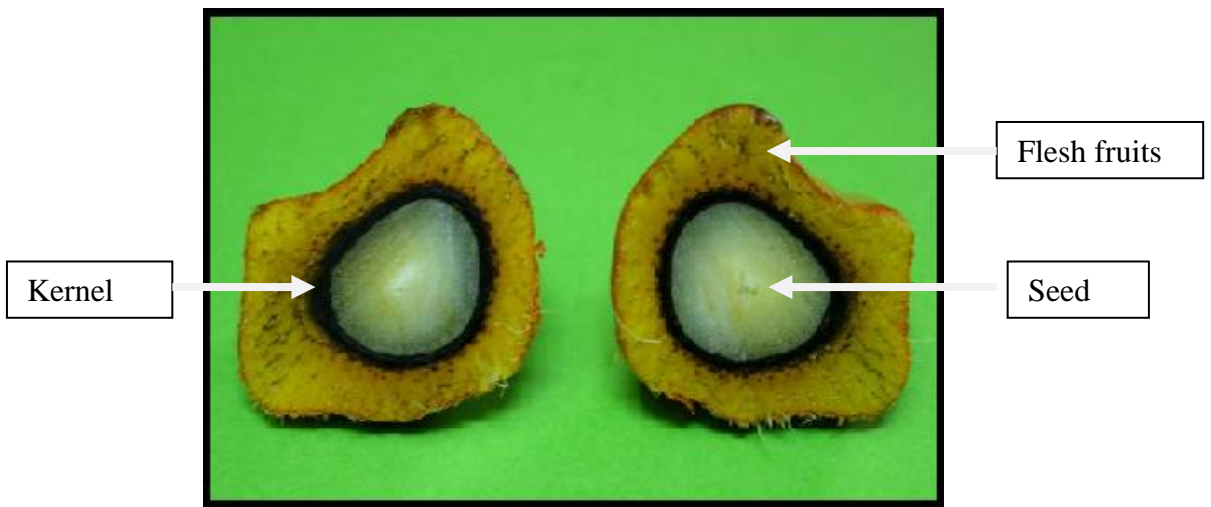


Figure 6: Picture of oil palm fruit
(Picture of sample from Felda Kekayaan, Kluang, Johor)

The oil palm seed is the nut that remains after the oily mesocarp is removed and it consist of a hard shell and kernel as shown in Figure 6. Most cases, it has one kernel but sometime it can contain two or even three. Oil palm seed has length around 2 to 4 cm. The shell has three germ pores and fibres passing longitudinally

through and adhering to it. The kernel which inside the shell consists of layers of hard oil endosperm, greyish white in colour surrounded by a dark brown testa covered with a network of fibres. Additionally, seed obtained from Dura x Pisifera crosses have been used almost exclusively for new planting nowadays (Kee, 1957).

2.3 Oil palm breeding

The aim for oil palm breeding is to produce the maximum quantity of palm oil and kernels per hectare. At first, oil palm breeding focused only to the production of palm oil since *tenera* which can increase the quantity of oil is four to eight times of the kernels. Therefore, oil palm breeding also can be describes as a process of breeding from a pair of individuals. Both parents have male and female flowers, example *dura* has taken on the role of mother palm and the *pisifera* take the role of father. The qualities of the yield performance of the hybrid may lead to the parents to be chosen for continued breeding into further generations (Hartley, 1988). Improvement of this breeding process will require widening of the genetic variability in each of these populations either by inter-crossing or by intro-gressing this material (Wastie and Earp, 1972).

There are some factors contributed to breeding process and one of the reasons is yield factor. Oil palm yield can be determined by a number of yield components after separation such as bunch weight, number of bunches percentage fruit per bunch, percentage oil per mesocarp and percentage kernel. Besides, breeding also happened because of disease resistance. This is very important because some plant disease can be preventing by crossing the cultivar and it will lead to produce high quality of palm. Unfortunately, a few technical problems would occur for breeding against diseases resistance. The reaction of these breeding is different depend on the

environment which can induce differences in the expression of the respective characters or how different in genetic make up can indicative the measurement (Wastie and Earp, 1972).

There are serious breeding works took places such as trials of *Deli* crosses with *Deli* x import *dura* and *tenera* and with crosses within African material. Results from that trial showed that the *Deli* palms gave the highest weight per bunch, with the *Deli* import *dura* and the *Deli tenera* intermediate between *Deli* and imports. While *Elaeis guineensis* oil palm crosses between trees of different geographical origins prove that they had an annual palm oil yield higher than intra-origin ones (Pushparajah and Soon, 1981). Crossing the *dura* and *pisifera* to give the thin-shelled *tenera* fruit type improved partitioning of dry matter within the fruit, giving a 30% increase in oil yield at the expense of shell, without changing total dry matter production. After a lot of research that was revealed that yield of *Deli dura* oil palms after four generations of selection was 60% greater than that of the unselected base population (Hartley, 1988).

Improvement of breeding in oil palm still has to consider the efficiency of the selection method and the rate at which this is achieved depends on available genetic variability. Some research can be continued to investigate the possibility of breeding for such characters as a lower melting point or higher unsaturation. Knowledge of the genetics of the various characteristics of the oil palm which determine the yield of oil is very important for the efficiency of a selection cultivar (Wastie and Earp, 1972).

2.4 Lignin

Lignin as shown in Figure 7 is a major component of the plant cell wall along with cellulose and hemicelluloses. It is known to one of the most abundant aromatic compounds in nature and widely distributed phenolic polymers found in higher plants. Lignin is nontoxic, complex and natural phenolic polymer that has made it exclusively important in many industrial applications. Variety of functional groups and over 10 different types of linkage made the structure of lignin complex and it depends strongly on the original sources and extraction method used (Alriols et al., 2009; Bhat et al., 2009). The dehydrogenative polymerizations of three main mono lignols (p-coumaryls, coniferyls and sinapyls alcohols) are contributed to the complex polymers of lignin. Angiosperms lignin is produced mainly from coniferyl and sinapyl alcohols with a small amount of p-coumaryl alcohols. While gymnosperm lignin is formed from coniferyl alcohols and together with small amount of p-coumaryl alcohols (Del Rio et al., 2007).

Lignin that derived the oxidation products are members of vanillyl, syringyl, p-hydroxyl, and cinnamyl families. 3-methoxy (OCH₃) group and a 4-hydroxyl (OH) group are main group that characterized vanillyls while syringyl had characterized by two 3,5-methoxy groups and a 1-hydroxy group. Each family of vanillyl, syringyl and p-hydroxyl had consists of side chain which are aldehyde, ketone and carboxylic acid. The cinnamyl family has consisted two phenols with trans-propenoic acids. The vanillyl group are found in all four vascular plant types which are woody and non-woody angiosperms and gymnosperms. While for syringyl groups are found in angiosperms only and cinnamyl compounds are detected primarily in non-woody sources of angiosperms and gymnosperms. The p-hydroxyl group are released from

non-woody tissues but sometime can be found in woody tissues in small amount presence (Degryse, 1999).

Many researcher formerly associated with other field to turn their concern to the biosynthesis of these phenolic polymers because of the interest in the biosynthesis of plant constituents has been stimulated by the considerable advances in enzymological technique and the increasing availability of radioisotopes (Harborne, 1964). Plant that derived lignin had many health beneficial such as antibacterial, antiparasitic, antitumoral, antiviral, and immunopotentiating activities. Lignin that isolated from any plants also has antioxidant properties and free radical scavenger; they may improve the effectiveness of chemotherapy, decrease side effects of chemotherapy and radiotherapy, and prevent some types of cancer (Tachakittirungrod et al., 2006). Complicated three-dimensional of lignin in which the monolignols synthesized in plant are randomly polymerized made it difficult to utilization and an advanced system has not been established. Meanwhile, some chemical treatment like aqueous alkaline oxidation can decompose high molecular lignin to low molecular compounds and the various low molecular compounds were produced. However many of these compounds are burned or discarded and just some of these compounds are utilized (Otsuka et al., 2006).

2.4.1 Phenolic compounds

Phenolic compounds or polyphenols are the natural compounds in the plant. It comprises of groups like phenolic acids, flavonoids and tannins (Neo et al., 2010). Natural polyphenols can range from simple molecules (phenolic acids, phenylpropanoids, flavonoids) to highly polymerised compounds (lignins, melanins, tannins), with flavonoids representing the most common and widely distributed subgroup. Phenolic compound rarely occur in the free state in living plant tissue; they are practically always present in conjugated form (Swain, 1977). Phenolic is a class of chemical compounds consisting of a hydroxyl group (-OH) bonded directly to an aromatic hydrocarbon group. Phenolic compounds are composed of one or more aromatic benzene rings with one or more hydroxyl groups (C-OH) (Micheal, 2008). The simplest of the class is phenol (C_6H_5OH) as shown in Figure 8 and as a solid it is a white crystalline compound.

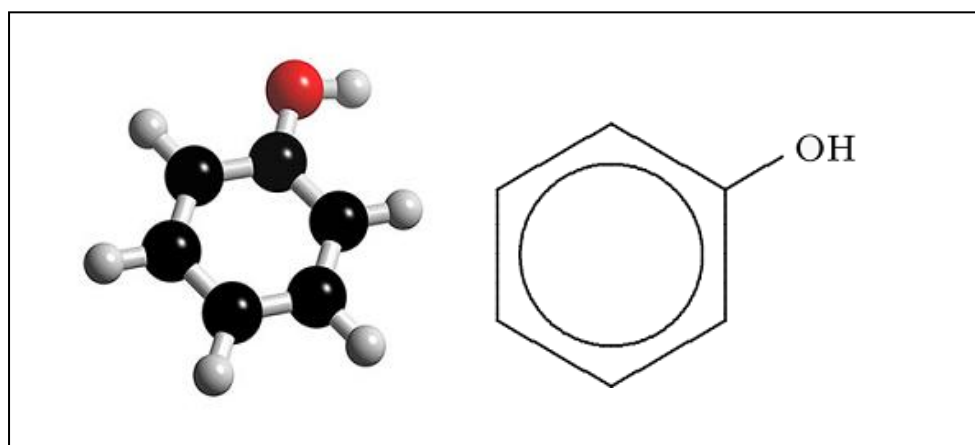


Figure 8: Structure of phenol

(Source: Harborne, 1964)

Some of the phenolic compounds connected with cell walls, while others of them exist without any chemical bonds between the plant cell vacuoles (Pourali et al., 2010). Hydroxy derivatives of aromatic carboxylic acids which arise from either

benzoic acid or cinnamic acid group can be named as phenolic acids. The derivatives of benzoic acid are gallic, protocatechuic, p-Hydroxybenzoic and vanillic acids. While the derivatives of cinnamic acid are caffeic, p-coumaric and ferulic acids (Neo et al., 2010). There is a wide variety of phenolic structures in plant matrix, due to that the identification of phenolic compound can be a complex and difficult task. These compounds can also link with five different sugar moieties or get bonded with one another to form dimers and trimers (Quirantes-Piné et al., 2009).

Phenolic compounds can be identified, isolated and analyzed based on the most part of the polarity of the hydroxyl group attached to an aromatic ring. Some detection and separation of phenolic compounds were referred to their physical properties. Phenolic compounds have been differentiated by solubility properties which occur in plants from the similarly coloured and oil-soluble carotenoid pigments. Phenolic compounds are usually soluble in polar solvent except they are totally esterified, etherified or glycosylated. Many of them dissolve in solution of sodium hydroxide and sodium carbonate but only a few dissolve in bicarbonate. While phenolic compounds which has a few hydroxyl groups are soluble in ether, benzene, chloroform and ethyl acetate (Harborne, 1964). Therefore, there are many factors that should be considerate before choosing a method for isolation of natural phenol. Some of them are the properties of the compounds and chemical composition of the biological source. The phenols can be isolated by extraction using acetone, ethanol, methanol or water. This can be depending on the number of hydroxyl group and sugar in the molecule (Harborne, 1964).

Phenolic compounds had very important roles that contribute to several uses. Plants and their derived products had present phenolic compounds that are widespread group of antioxidant (Kahoun et al., 2008). It enables to protect human

tissues from damage because of oxygen or free radicals. Besides, it can reduce the risk of some diseases and offer beneficial effects to the prevention of cancer, inhibiting tumour initiation, promotion and progression, cardiovascular disease, Alzheimer's disease and diabetes (Pourali et al., 2010). These groups of substances also produce to some characteristics such as colour, flavor, nutritional, organoleptic and commercial properties of fruits and their derived products (Fang et al., 2005). Meanwhile, they also have fungicidal properties and effectively protect the tree against microbiological attack. They also can contribute to the natural colouring of wood (Sjöström, 1981).

Quantitative analyses of phenolic compounds can be done in many ways. According to Swain and Hillis (1959), oxidation with Folin-Ciocalteu reagent which consists sodium phosphomolybdate and sodium tungstate is the most trusted method for determine phenolics (Harborne, 1964). Both paper chromatography and gas chromatography method for separation and identification of phenolics has been developed. Recently for the analysis of phenolic compounds are doing by the most popular and reliable technique which is reversed-phase high performance liquid chromatography (HPLC) (Tasioula-Margari et al., 2001).

2.4.2 Plant phenolics

Phenolic compounds are widely distributed in the plant kingdom. Plant tissues may contain up to several grams per kilogram and the main phenolics which are found are glycosides or esters and it is seldom that free phenols are present in more than trace amounts. Some of natural plant rich in these compound; p-hydroxybenzoic acid, vanillic, syringic, protocatechuic, salicylic, gentisic and o-pyrocatechuic (2,3-dihydroxybenzoic) acids on alkaline hydrolysis of aqueous or

ethanolic extracts. Sometime hydroquinone occurs free in a few amounts although it is always come with huge quantity of its glucoside.

Previous study proves that gallic acid is derived from a non-aromatic precursor in higher plant is indicated that glucose was found to be a far better precursor of gallic acid. β -oxidation of ferulic acid and sinapic acid can produce vanillic and syringic acid. In addition, they also could be formed by the hydroxylation and methylation of acids like *p*-hydroxybenzoic or protocatechuic acid. Phenolic cinnamic acids like *p*-coumaric acid, caffeic acid and ferulic acids play an important role in the biosynthesis of lignin, flavonoids and related compounds. These acids which are widely distributed in plants do not usually occur in the free state but rather as esters (Harborne, 1964).

Phenolic acids that occur in plant and fruit have abilities in many ways such as in biological effects including antioxidant activities, antitumor, anti mutagenic and antibacterial activities. Ferulic acid had a good potential in medical technology to protect against DNA damage, cancer and other human disorders. Beside, ferulic acid in the form of sodium ferulate also has been shown for treatment of cardiocascular and cerebrovascular diseases in China. It has been approved as a food additive in Japan because of curcumin, the major yellow pigment in turmeric and mustard (Chung et al., 2010). *p*-hydroxybenzoic acid is slightly soluble in water and chloroform but more soluble in polar organic like alcohol and acetone. This compound had been used in cosmetic industry as a preservatives and very popular antioxidant because of its low toxicity. Gallic acid is commonly used in the pharmaceutical industry because of the anti-fungal, anti-viral and antioxidant properties. While vanillic acid can be used as a flavoring agent and as an intermediate in the production of vanillin from ferulic acid. Both caffeic acid and

protocatechuic acid has important role in human health because of anti-cancer properties (Harborne, 1964). These chemical structures can be seen in Figure 9.

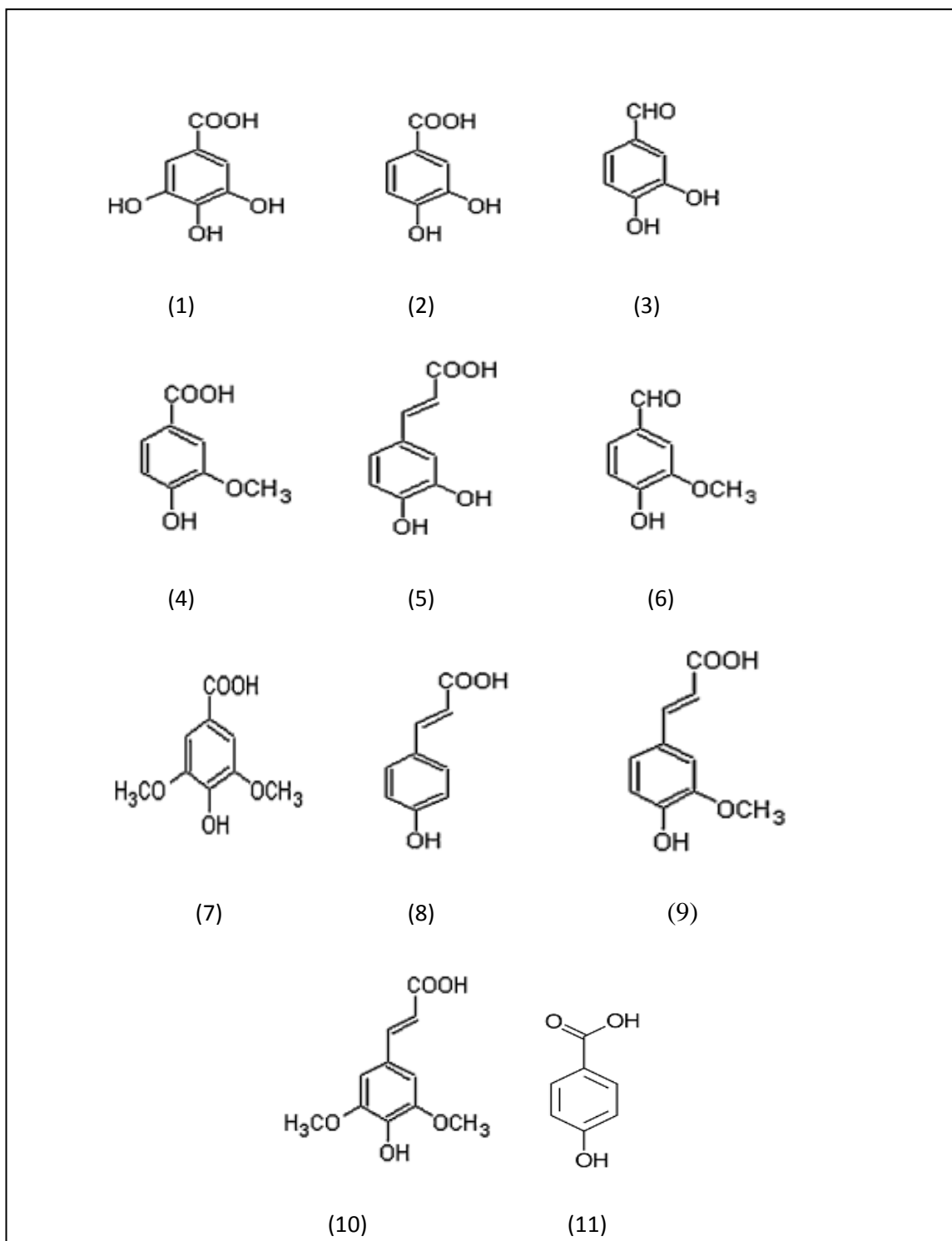


Figure 9: Structure of phenolic compounds (1) gallic acid (2) protocatechuic acid (3) protocatechuicaldehyde (4) vanillic acid (5) caffeic acid (6) vanillin (7) syringic acid (8) p-coumaric acid (9) ferulic acid (10) sinapic acid (11) p-hydroxybenzoic acid

(Source: Kahoun et al., 2008)