ANALYSIS OF SAP SUGAR AND STARCH CONTENT OF FELLED OIL PALM TRUNKS AT DIFFERENT STORAGE TIME

by

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Ethanol and lactic acid production using sap squeezed from old oil palm trunks felled for replanting

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Old oil palm trunks that had been felled for replanting were found to contain large quantities of high glucose content sap. Notably, the sap in the inner part of the trunk accounted for more than 80% of the whole trunk weight. The glucose concentration of the sap from the inner part was 85.2 g/L and decreased towards the outer part. Other sugars found in relatively low concentrations were sucrose, fructose, galactose, xylose, and rhamnose. In addition, oil palm sap was found to be rich in various kinds of amino acids, organic acids, minerals and vitamins. Based on these findings, we fermented the sap to produce ethanol using the sake brewing yeast strain, *Saccharomyces cerevisiae* Kyokai no.7. Ethanol was produced from the sap without the addition of nutrients, at a comparable rate and yield to the reference fermentation on YPD medium with glucose as a carbon source. Likewise, we produced lactic acid, a promising material for bio-plastics, poly-lactate, from the sap using the homolactic acid bacterium *Lactobacillus lactis* ATCC19435. We confirmed that sugars contained in the sap were readily converted to lactic acid with almost the same efficiency as the reference fermentation on MSR medium with glucose as a substrate. These results indicate that oil palm trunks felled for replanting are a significant resource for the production of fuel ethanol and lactic acid in palm oil-producing countries such as Malaysia and Indonesia.

[**Key words:** oil palm; trunk; sap; sugar; ethanol production; lactic acid production]

Palm oil is the most produced plant oil, with a worldwide production of 4.3 million tons in 2008 (USDA statistics: PS&D online). The combined palm oil production in Malaysia and Indonesia accounts for approximately 88% of the worldwide production (USDA statistics: PS&D online). Since palm oil is cheaper than soybean oil or other oils, it is widely used for industrial purposes, such as in detergents and cosmetics, in addition to foods such as margarine and frying oil. Recently, palm oil has been considered as a material for the production of biodiesel (1,2) and bio-plastics (3,4).

Oil palm (*Elaeis guineensis*) for palm oil production needs to be replanted at an interval of 20 to 25 years in order to maintain oil productivity. The plantation area in Malaysia and Indonesia in 2007 was 4,304,913 ha (5) and nearly 7 million ha (Janurianto, A., presentation at Indonesian Palm Oil Conference and Price Outlook 2010), respectively. Considering the replanting interval, 450,000 ha to 560,000 ha of the oil palm plantation area is expected to be replanted annually during the next 25 years. This means on average 64 million to 80 million old palm trees will be felled every year in the two countries, as approximately 142 oil palms are usually planted in one hectare (6). Consequently, the felled palm trunks can be regarded as one of the most important biomass resources in Malaysia and Indonesia.

Unfortunately, the palm trunk structure is not strong enough for use as lumber, and thus, only the outer part of the trunk, which is relatively strong, is partially utilized for plywood manufacturing. In the plywood production process, the inner part is discarded in large amounts due to its extremely weak physical properties. Meanwhile, it is known that palm sugar and palm wine are produced from sap obtained by tapping the inflorescence of varieties of palm species, such as *Arenga pinnata, Borassus flabellifer, Cocos nucifera, Nypa fruticans* and oil palm (7).

In order to utilize the old palm trunks felled for replanting, especially the inner part, we attempted to produce bioethanol and lactic acid, the material for bio-plastics, from felled trunks. We focused on sugars in the sap of the felled trunk and observed a large quantity of high glucose content sap in the trunk. Other components in the squeezed sap that may affect fermentation, namely, amino acids, organic acids, minerals and vitamins, were also assessed. The results obtained from ethanol and lactic acid production experiments using an industrial yeast strain and a lactic acid bacterium, respectively,

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early show that the oil palm sap squeezed from felled trunks is a romising feedstock for bioethanol and lactic acid.

MATERIALS AND METHODS

Materials Three oil palm trunks (*tenera* type), approximately 25 years old, were tained from a local plywood manufacturer (Business Espri Co., Penang Province, alaysia) (Fig. 1A). The palm trees were felled in the rainy season (December) in Malaysia d kept under roof for less than 5 days before sample preparation. A 7-cm thick disk was moved from the middle part of each trunk, which ranged in length from 10-12 m ig. 1B), and kept at -20 °C before use. The disks (32-42 cm in diameter) were cut into ree parts: inner (a), middle (b), and outer (c) parts, as shown in Figs. 1C and D. Sap was llected by compressing the disks using a laboratory-scale press at 80 MPa. The sap was ntrifuged at 6,000 rpm for 15 min and the supernatant was stored at -20 °C before use.

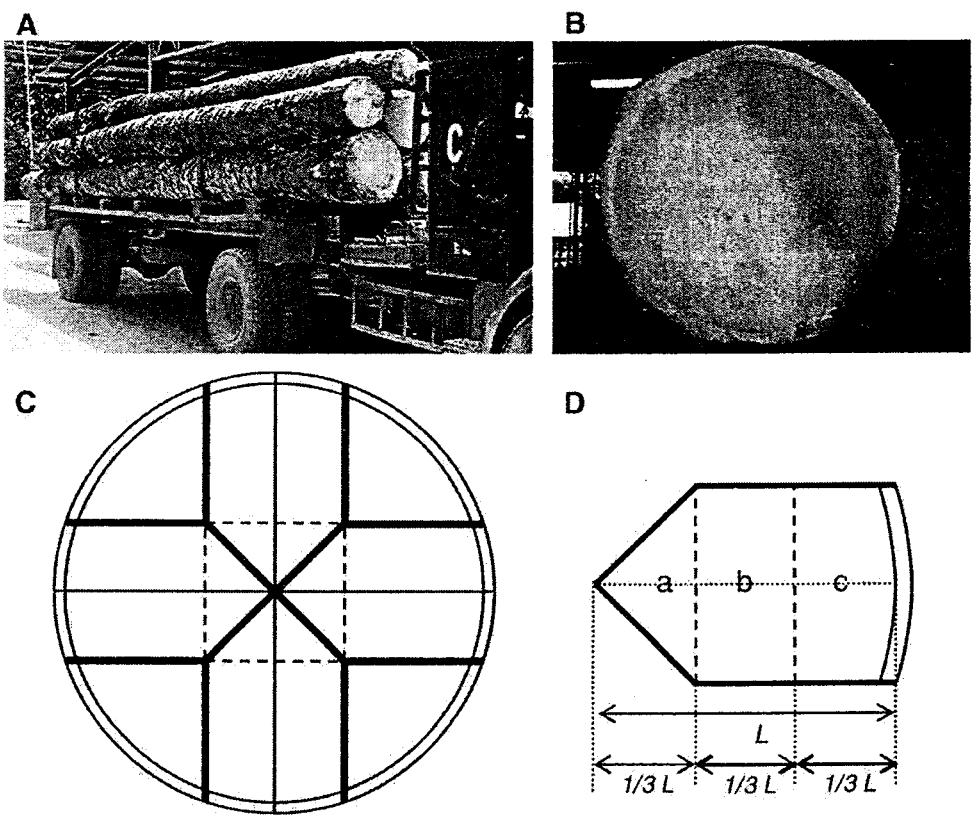
Analysis Moisture content was determined by drying at 105 °C for 48 h. Sugars ntained in the sap were determined by high performance anion-exchange chromatogphy using CarboPac PA (Dionex Corporation, Sunnyvale, CA, USA) with pulsed nperometric detection (HPAEC-PAD). The mobile phase was 2% NaOH at a flow te of 0.6 ml/min at 28 °C. Amino acids were analyzed by an amino acid analyzer (Hitachi 8900). Organic acids were determined by high performance ion exchange chromatography ing Sim-pack SPR-H with a post-column pH-buffered electroconductivity detection ethod (Shimadzu CDD-6A). The mobile phase was 4 mM p-toluenesulfonic acid at a flow te of 0.8 ml/min at 40 °C. Mineral analyses were carried out by inductively coupled plasma omic emission spectroscopy with Vista MP-X (Varian, Inc.). Chloride was determined by ion romatography with the Dionex DX-500. Thiamine (8) and riboflavin (9) were analyzed by PLC with fluorescence detection. Ascorbic acid was analyzed by HPLC with a UV-Vis variable avelength detector. Vitamin B6, pantothenic acid, niacin, inositol, folic acid and biotin were easured by microbiological methods using *Saccharomyces cerevisiae* ATCC9080, *Lactobacilus plantarum* ATCC 8014, *L. plantarum* ATCC 8014, *S. cerevisiae* ATCC 9080, *L. rhamnosus* ATCC 69, and *L. plantarum* ATCC 8014, respectively.

Ethanol fermentation experiments *Saccharomyces cerevisiae* Kyokai no. 7, tained from the National Research Institute of Brewing (NRIB), was used for ethanol rmentation experiments. The yeast was pre-cultured on YPD medium containing 20 g polypepton (Wako Pure Chemical), 10 g yeast extract (Difco), and 20 g glucose per liter. The sap, with and without the addition of polypepton (20 g/L) and yeast extract (10 g/L), was used as the ethanol fermentation medium. Glucose concentrations of the sap media were adjusted to 55 g/L by the addition of distilled water and pH was adjusted to 6.0 with 2N NaOH. The sap media were sterilized with a 0.22-μm membrane filter (Millipore). Pre-cultured yeast cells were inoculated into the sap media at 5% (v/v) and grown at 30 °C without shaking. Reference fermentation was carried out on YPD medium containing 60 g/L glucose. Samples were withdrawn every 12 h from the broth for ethanol and glucose determinations. Ethanol was determined using a gas chromatograph (Shimadzu GC-2014) equipped with a flame ionization detector. A glass column (8 mm × 3.2 m) packed with Chromosorb 103 (60/80 mesh) was used. The chromatogram was run at 185 °C with helium as the carrier gas at a flow rate of 20 ml/min. Glucose was analyzed by Glucose C2 kit (Wako Pure Chemical).

Lactic acid fermentation experiments The homolactic acid bacterium *Lactococcus lactis* ATCC19435 was used for lactic acid fermentation experiments. The bacterium was pre-cultured on MSR medium containing 10 g bactotrypton (Difco), 10 g yeast extract (Difco), 20 g glucose, 2 g K_2HPO_4, 5 g $CH_3COONa \cdot 3H_2O$, 0.2 g $MgSO_4 \cdot 7H_2O$, and 5 mg $MnSO_4 \cdot 4H_2O$ per liter. The sap was diluted 5-fold with distilled water to a final glucose concentration of 16.7 g/L. After adjusting the pH to 7.0 with 2N NaOH, the sap was sterilized with a 0.22-μm membrane filter. Pre-cultured bacterial cells were inoculated into the sap at 5% (v/v) and grown statically at 30 °C. Reference fermentation was carried out on MSR medium containing 18 g/L glucose. Samples were withdrawn every 24 h for lactic acid and glucose determinations. Lactic acid concentrations were determined according to the method described for organic acid analysis.

RESULTS AND DISCUSSION

Moisture content of oil palm trunk Moisture content of parts a, b, and c was approximately 82%, 76% and 68%, respectively (Table 1). Compared to wood timber, whose moisture content is normally between 40% and 50%, oil palm trunk contains far more moisture, indicating the presence of a large quantity of sap. Especially,



G. 1. Images of felled oil palm trunks and diagrams of sample preparation for analyses. (A) Felled trunks carried to a plywood factory. (B) A disk taken from a trunk. (C, D) Disks were t into inner (a), middle (b), and outer (c) parts.

TABLE 1. Moisture content in different parts of oil palm trunk.

Moisture % (w/w)	Part [1]		
	Inner (a)	Middle (b)	Outer (c)
	82.4 ± 1.2	75.9 ± 4.7	67.7 ± 6.9

1) Samples from different parts of disks were prepared according to the method described in Materials and Methods. Results are means ± SD of three determinations.

the inner part of the trunk contained an extremely high level of moisture. From 1 g of the trunk part a, approximately 0.65 g of sap was obtained by the laboratory-scale press used in this experiment.

Sugar composition of oil palm sap Table 2 shows the free sugars determined in the sap from the inner (a), middle (b), and outer (c) parts of the trunk. Glucose was found to be the dominant sugar in all parts, accounting for approximately 86.9%, 86.3% and 65.2% of the total free sugars contained in the inner, middle and outer parts, respectively. In addition to glucose, significant amounts of sucrose and fructose were contained in the sap. By contrast, Eze and Ogan reported that oil palm sap collected in Nigeria, by tapping at the base of the inflorescence, contained sucrose as the dominant sugar (10%, w/v) and glucose and fructose as minor sugars (<1%, w/v) (10). Similar results have been reported on sap collected in Nigeria by tapping the inflorescence of Raphia palm (*Raphia hookeri*), with sucrose as the dominant sugar (11). The discrepancy may be due to the difference in varieties, species and/or cultivating conditions. The other possibility is that the sugar composition of sap collected from felled palm trunk differs from that of sap collected by tapping the base of the inflorescence.

An increasing concentration gradient was observed with most of the sugars, from the outermost to the innermost trunk region, as observed with moisture. In the inner part, the total amount of sugars fermentable by the ethanol-producing yeast, *S. cerevisiae*, and lactic acid bacteria, i.e., glucose, sucrose, fructose and galactose, is 96.7 g/L. Thus, oil palm sap in the inner part of felled trunks can be considered as good feedstock for ethanol and lactic acid.

Chemical properties and composition of oil palm sap Chemical properties and composition of oil palm sap from the inner part of the felled palm trunk were analyzed. The pH of the sap was approximately 5.0 and the specific gravity was 1.07. As for the chemical components of the sap, amino acids, organic acids, vitamins and minerals were analyzed. As shown in Table 3, the total amount of amino acids in the sap was 198.3 μg/g, with serine, alanine, glutamic acid, and aspartic acid as the major amino acids. The amino acid composition is similar to that of sugar cane juice reported by Mee et al. (12). As for organic acids, citric, malic and maleic acids were abundant (Table 4), resulting in slightly acidic sap. Table 5 lists the minerals contained in the oil palm sap. Calcium, magnesium and chloride are contained at high concentrations, which is similar to *Raphia hookeri* palm sap, which contains abundant calcium and magnesium (11). The HPLC analyses and microbioassay determined various kinds of B group vitamins and vitamin C in the sap (Table 6), and are consistent with reports on palm wine in Nigeria (10,13,14). Inositol was found to be contained at a high concentration in the oil palm sap for the first time. Since oil palm sap obtained from felled trunks contains lots of amino acids, minerals, vitamins and organic acids, the oil palm sap is thought to be a good medium for the growth of yeast and lactic acid bacteria.

TABLE 2. Free sugars contained in sap from felled oil palm trunk.

Free sugars	Part		
	Inner (a)	Middle (b)	Outer (c)
	g/L	g/L	g/L
Sucrose	6.5 ± 1.1	3.0 ± 0.4	1.9 ± 0.1
Glucose	85.2 ± 2.5	52.2 ± 3.4	13.1 ± 2.6
Fructose	4.1 ± 1.2	3.1 ± 1.0	2.1 ± 1.7
Xylose	0.7 ± 0.1	0.8 ± 0.1	1.4 ± 1.1
Galactose	0.9 ± 0.1	0.8 ± 0.3	1.0 ± 0.8
Rhamnose	0.4 ± 0.2	0.5 ± 0.2	0.5 ± 0.2
Others	0.3 ± 0.3	0.1 ± 0.1	0.1 ± 0.2
Total	98.1 ± 5.5	60.5 ± 3.3	20.1 ± 1.1

Results are means ± SD of three determinations.

TABLE 3. Amino acids contained in sap from the inner part of felled oil palm trunk.

Amino acids	Concentration (μg/g of sap)
Aspartic acid	17.3
Threonine	7.4
Serine	45.3
Glutamic acid	33.9
Glycine	3.1
Alanine	38.8
Valine	7.6
Methionine	1.7
Isoleucine	5.1
Leucine	1.9
Tyrosine	1.8
Phenylalanine	6.9
Tryptophan	12.1
Lysine	2.3
Histidine	1.6
Arginine	5.8
Proline	5.7
Total	198.3

TABLE 4. Organic acids contained in sap from the inner part of felled oil palm trunk.

Organic acids	Concentration (μg/g of sap)
Succinic acid	30.9
Pyruvic acid	19.0
Malic acid	371.8
Maleic acid	119.1
Lactic acid	1.3
Fumaric acid	8.1
Citric acid	380.6
Acetic acid	39.8
Total	970.6

Ethanol production using oil palm sap Using sap obtained from the inner part of the trunk, ethanol fermentation was carried out with *S. cerevisiae* Kyokai no. 7. Regardless of the addition of polypepton and yeast extract, the yeast rapidly fermented glucose in the sap into ethanol. A representative ethanol fermentation profile of the sap, without addition of nutrients, is shown in Fig. 2. Fermentation was almost complete after 12 h and glucose was thoroughly consumed after 24 h. Meanwhile, the minor sugar components in the sap medium, i.e., sucrose, fructose, and galactose initially found at 4.2 g/L, 2.6 g/L, and 0.6 g/L, respectively, were not detected by HPLC after 24 h. The amount of ethanol produced corresponded to 94.2% of the theoretical yield calculated based on consumption of glucose, sucrose, fructose, and galactose. The ethanol

TABLE 5. Minerals contained in sap from the inner part of felled oil palm trunk.[1)]

Mineral	Concentration (μg/g of sap)
Ca	210
Fe	3.0
Mg	145
Mn	12
Mo	1.5
Na	22
P	12
Si	4.0
Zn	2.5
Cl	535
Total	947

1) Al, As, Ba, Cd, Ce, Co, Cr, Cu, Ga, Ge, Hf, La, Li, Pb, Pd, Sb, Se, Sn, Sr, Ti, V, and Zr were below the level of detection (1 μg/g sap).

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TABLE 6. Vitamins contained in sap from the inner part of felled oil palm trunk.

Vitamin	Concentration (μg/g of sap)
Pyridoxine, pyridoxal, pyridoxiamine	1.1
Pantothenic acid	1.5
Niacin	2.6
Inositol	640
Biotin	0.024
Ascorbic acid	20

Thiamine, riboflavin, folic acid, and cyanocobalamin were not detectable.

Production rate and yield were comparable to the reference fermentation on YPD medium (Fig. 2), indicating that oil palm sap has sufficient nutrients to support ethanol fermentation by *S. cerevisiae*, and does not contain inhibiting substances.

Lactic acid production using oil palm sap *L. lactis* ATCC19435S was grown on sap from the inner part of the trunk to produce lactic acid. As shown in Fig. 3, glucose in the sap was readily converted to lactic acid. Likewise in ethanol fermentation, no additional nutrients were required and no growth inhibition was observed. Glucose, sucrose, fructose, and galactose, at initial concentrations of 16.7 g/L, 1.28 g/L, 1.79 g/L, and 0.18 g/L, respectively, in the medium were completely consumed after 72 h. The lactic acid yield was 89.9% of the theoretical yield based on consumption of these 4 sugars.

The results obtained from ethanol and lactic acid production experiments clearly show that oil palm sap, particularly that obtained from the inner part of the trunk, is good feedstock for bioethanol and lactic acid production. Considering tens of millions of old oil palm trees are felled annually in Malaysia and Indonesia, the sap of felled trunks is a promising and important resource for bioethanol and lactic acid production.

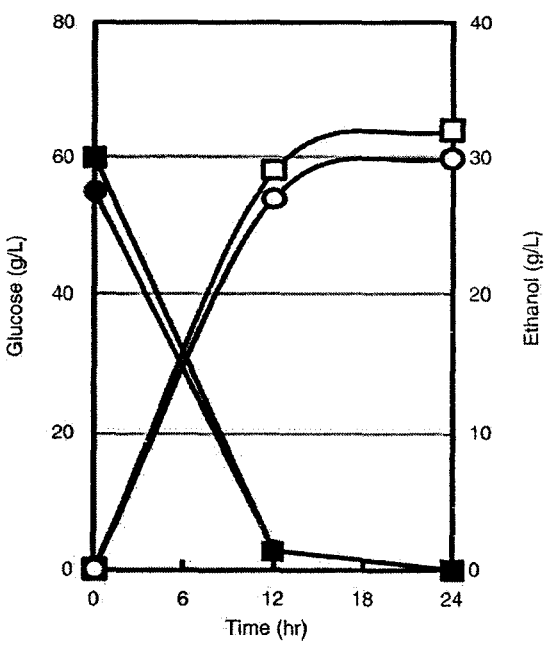

FIG. 2. Time course of ethanol production using felled oil palm trunk sap. *S. cerevisiae* Kyokai no. 7 was grown statically at 30 °C on sap containing 55 g/L glucose and without added nutrients. Reference fermentation was carried out on YPD medium containing 50 g/L glucose. Glucose concentration was determined enzymatically with a Glucose C2 kit. Open circles, ethanol produced from sap; open squares, ethanol produced in reference fermentation; closed circles, glucose in sap culture; closed squares, glucose in reference culture.

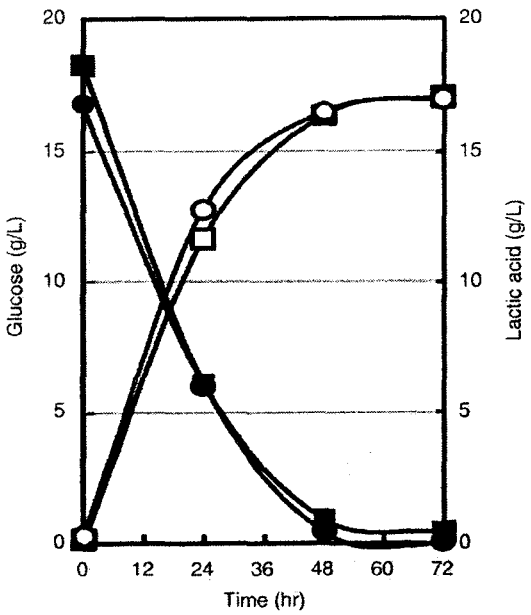

FIG. 3. Time course of lactic acid production using felled oil palm trunk sap. *L. lactis* ATCC19435 was grown statically at 30 °C on sap containing 16.7 g/L glucose and without added nutrients. Reference fermentation was carried out on MSR medium containing 18 g/L glucose. Glucose concentration was determined enzymatically with a Glucose C2 kit. Open circles, lactic acid produced from sap; open squares, lactic acid produced in reference fermentation; closed circles, glucose in sap culture; closed squares, glucose in reference culture.

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Old oil palm trunk: A promising source of sugars for bioethanol production

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ABSTRACT

Oil palm trees are replanted at an interval of approximately 25 years because of decreased oil productivity of old trees. Consequently the felled trunks are the enormous amount of biomass resources in the palm oil producing countries such as Malaysia and Indonesia. In this report, we found that the felled oil palm trunk contains large quantity of sap, which accounts for approximately 70% of the whole trunk weight, and that sugars existing in the sap increased remarkably during storage after logging. Total sugar in the sap increased from 83 mg ml^{-1} to 153 mg ml^{-1}, the concentration comparable to that of sugar cane juice, after 30 days of storage, followed by the gradual decrease. The sugars contained in the sap were glucose, sucrose, fructose and galactose, all of which are fermentable by ordinary industrial yeast strains. The results indicate that old oil palm trunk becomes a promising source of sugars by proper aging after logging and, thus, its sap can be a good feedstock for bioethanol.

1. Introduction

Oil palm (*Elaeis guineensis*) is widely planted for its edible oil in tropical countries such as Malaysia and Indonesia. The production of palm oil is 39 Mt per year in 2007, which is the most produced plant oil in the world [1]. The oil is mainly used for food and related industries, and is also used as a raw material for various products such as detergents and cosmetics. Moreover, a number of research studies have been carried out for biodiesels and bio-plastic materials from the oil in recent years [2–6].

In general, the palm starts bearing oil-contained fruits in 2.5 years after planted and its productivity becomes lower after 20–25 years. Therefore it is necessary to cut the old palms and to replant new seedlings at plantation sites. In Malaysia, about 120,000 ha of oil palm is estimated to be replanted annually from 2006 to 2010 for maintaining the oil productivity [7]. When replanting, old palms are cut and most of them are discarded or burnt at the plantation site. Therefore, efficient ways for utilizing oil palm trunks is desired for ideal oil palm plantation and sustainable palm oil industry.

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It has been traditionally practiced to produce palm sugar and palm wine using sap obtained by tapping the inflorescence of various species of palms including *Arenga pinnata*, *Borassus flabellifer*, *Cocos nucifera*, *Nypa fruticans* and oil palm [8]. Among these palm species, oil palm is considered to produce much smaller amount of tapped sap, or low sugar yield [9]. Oil palm sap was reported to contain approximately 11% sugars with sucrose as a major component accounting for approximately 90% of total sugar [10]. Meanwhile, it has been reported that the 75% methanol extracts of the dried oil palm trunk (OPT) fiber contains 4.9%–7.8% sugars, which correspond to 2.1%–3.4% sugars in the sap assuming that moisture content of OPT is 70% [11]. The ratio of sugars in the methanol extract of the pulverized trunk is significantly different from the one in the tapped sap.

In order to clarify the discrepancy between tapped sap and the methanol extracts, and to evaluate the sap of the felled palm trunks as a source for sugars, we investigated the amount and composition of sugars in the sap squeezed from felled trunks together with moisture contents. We also examined effects of storage of the felled trunks on sugars in the sap. This is the first report that described the amount, composition and change of sugars contained in the sap of felled oil palm trunks. The results clearly show a significant increase of fermentable sugars in the oil palm sap occurs during storage of the trunks after logging, indicating the old and felled oil palm trunks are the promising feedstock for bioethanol.

2. Materials and methods

2.1. Sample preparation

Three oil palms of *tenera* type aged 25 years old were logged at Ara Kuda, Kedah, Malaysia (N5°36', E100°31'). Total height of each palm was approximately 12 m and testing logs (2.5 m long and 36–41 cm in diameter) were taken from the middle part of the whole log as shown in Fig. 1. The log was stored under a roof avoiding direct sunlight and rain at the Penang Campus of Universiti Sains Malaysia. Temperature during the storage was 28–32 °C with humidity of 70–80%.

A disc with 10 cm thickness was sliced from each log after a certain days of storage between 0 and 120 days. To avoid microbial contamination, 5 cm from the end was trimmed before the slicing. Then the disc was cut into three sections; inner (A), intermediate (B) and outer (C) as shown in Fig. 1.

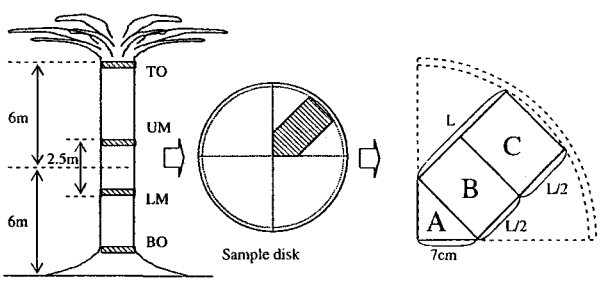


Fig. 1 – Preparation of oil palm trunk samples.

Each sectional sample was placed in an airtight plastic bag and kept in a deep freezer at −20 °C until analysis.

Further sample disks were prepared from the different positions of the trunk according to the height as shown in Fig. 1. Top and bottom parts of the tree were cut and designated TO and BO, respectively. Two more disks were obtained from the end part of the 2.5 m sample log and designated upper middle (UM) and lower middle (LM), respectively. The disks thus prepared were cut into three sections in the same manner as mentioned above.

2.2. Analysis

Moisture content of each OPT sectional sample was determined by drying at 105 °C for 48 h. The sample was cut out from each section at the size of 2 cm × 2 cm × 5 cm. Collection of sap was carried out by squeezing each sample with a laboratory-scale press at 80 MPa. The sap was then centrifuged at 7,000G for 15 min and the supernatant was collected and kept in a deep freezer.

Total sugar content of sap samples was determined by the Dubois method using phenol and sulfuric acid [12]. A filtered sap sample was diluted to 1/3000 with distilled water and 0.2 ml of 5% phenol solution was added to 0.2 ml of the diluted sample, followed by an addition of 1 ml of sulfuric acid. Then the solution was vigorously mixed and cooled at room temperature for 30 min. Absorbance of the solution was recorded at 480 nm. The calibration was carried out with glucose as standard.

Determination of sugar components in each sap was carried out by high performance liquid chromatography (HPLC; Shimadzu LC-20A) with a CAROBO-Sep CHO-682 (7.8 mm I.D., 300 mm, TRANSGENOMIC) column at 80 °C. Distilled water was used for the solvent at a flow rate of 0.4 ml min^{-1} with a refractive index detector. Ribose was used as an internal standard and calibration curves were made for individual sugars, using commercial products purchased from Wako Pure Chemical Industries Ltd.

For quantitative analysis of starch, a small amount of each sectional OPT sample was prepared in powder form by grinding (<0.5 mm) after oven-drying at 105 °C. To remove free sugars, 100 mg of the powdered sample was washed in 10 ml of 80% ethanol at 80 °C for 10 min, which was repeated twice. The total starch assay kit from Megazyme International Ireland Ltd was applied to the extracted powder sample and the absorbance of the sample mixture at 510 nm was recorded. Glucose was employed as a standard for creating the calibration curve.

3. Results and discussion

Total sugar contents in the sap samples from inner (A), intermediate (B), and outer (C) parts of the disks obtained from different height of the oil palm tree are shown in Table 1. Total sugar contents were higher in the inner part than peripheral part except for the bottom most position. The sap obtained from top contained roughly 20%-less sugars compared to the sap from the bottom to middle positions, where vertically even distribution of sugars was observed.

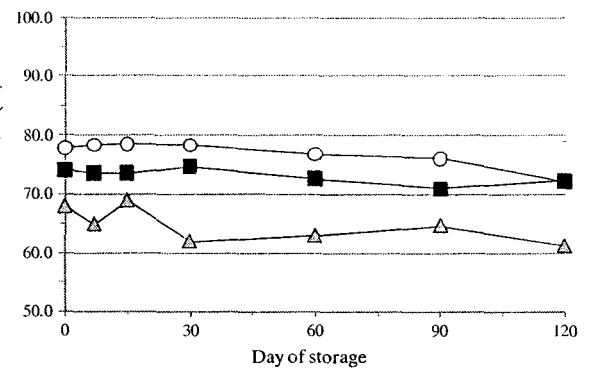
able 1 – Amount of sugars contained in the sap from ifferent heights of oil palm trunk.

	Total sugar(mg ml^{-1})			
	TO	UM	LM	BO
ıner (A)	111.8	129.9	129.2	93.0
ıtermediate (B)	72.7	118.0	94.2	102.8
uter (C)	71.1	103.6	81.6	107.7
verage	85.2	117.2	101.7	101.2

ugars were analyzed by phenol-sulfuric acid method.

Change in moisture content (MC) of each OPT section epared from middle part of trunk was examined during 120 ys of storage as shown in Fig. 2. Immediately after logging, Cs of Part A, B and C were 78, 75 and 67%, respectively. mpared to ordinary wood timber, the MC of OPT is extremely gh, as MC of wood is normally ranging between 40 and 50%. rt A, the most inner of the trunk, contains the highest oisture among the three sections, and the MC becomes lower ward the outer section that is Part C. The trunk consists of rous vascular bundles and powdery parenchyma. The renchyma seems to hold more moisture than the vascular ndles. The difference in MC between the sections may be tributed to the ratio of parenchyma and vascular bundles. In ct we observed more parenchyma in the inner section. uring the 120-day storage, the MC went slightly lower for all ctions. The decrease in MC being marginal (less than 10%), aporation of moisture from the stored logs was presumably evented by hard outer bark of the trunk.

Change in total sugar content of sap from each OPT section as plotted against days of storage as shown in Fig. 3. ncentrations of the sap sugar in Part A, B and C just after gging were 108, 85 and 76 mg ml^{-1}, respectively. During the orage, the concentration unexpectedly increased sharply to come 148, 185 and 134 mg ml^{-1} for A, B and C, respectively, ter 30 days. Beyond 30 days the sugar content decreased



g. 2 – Moisture content of OPT during storage. Open circle dicates inner part A, closed square intermediate part B, d gray triangle outer part C. The data were obtained from il palm trees. SD for part A is between 1.3 and 4.6%. SD r part B is between 3.0 and 6.4%. SD for part C is between and 11.0%. Moisture content (%) = [sample weight et) – sample weight (dry)]/sample weight (wet) × 100.

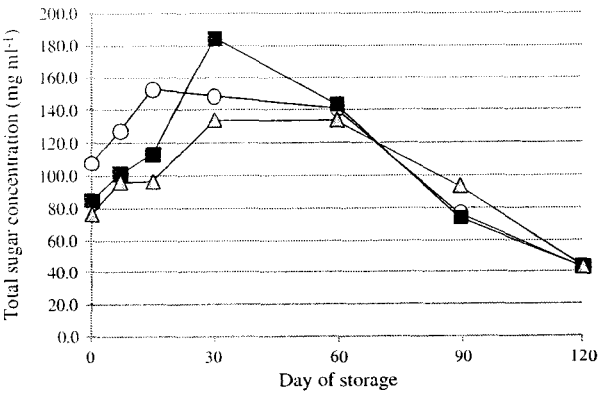


Fig. 3 – Total sugar concentration in OPT sap from part A, B, and C during storage. Open circle indicates inner part A, closed square intermediate part B, and gray triangle outer part C. The data were obtained from 3 oil palm trees. SD for part A is between 8 and 53 mg ml^{-1}. SD for part B is between 2 and 48 mg ml^{-1}. SD for part C is between 4 and 52 mg ml^{-1}.

gradually toward Day 120. An average of sugar concentration through the cross section of the disc was calculated by Eq. (1), assuming that the volume ratio of sections A, B and C is 1:3:5. The average concentration calculated at Day 0 was 83 mg ml^{-1} and it became 153 mg ml^{-1} at Day 30 and then decreased to 43 mg ml^{-1} after 120 days as shown in Fig. 4. Although dispersion in average total sugar content was observed among trunk samples, a distinct changing pattern of sugar concentration in sap, that is, an increase during the first 30 days followed by a decrease thereafter, was recognized.

$$\text{AV Conc} = (\text{Conc A} \times 1 + \text{Conc B} \times 3 + \text{Conc C} \times 5)/9 \quad (1)$$

HPLC sugar analysis of the sap revealed that sucrose, glucose and fructose were major components with galactose and inositol as minor components (less than 0.15% each). At the beginning, sucrose was the most abundant among the

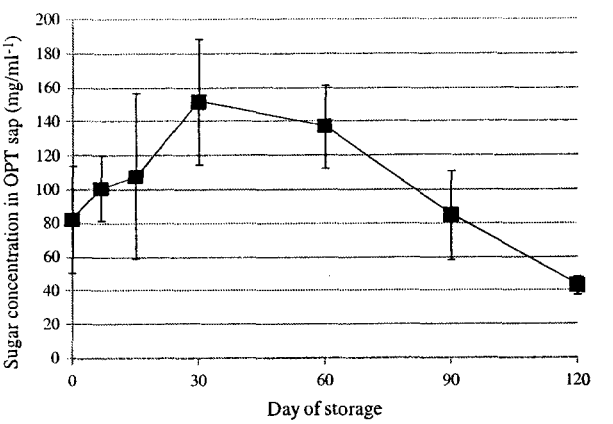


Fig. 4 – Average of total sugar content in OPT sap during storage. The data were obtained from 3 oil palm trees, and calculated according to Eq. (1). Bar, ±SD.

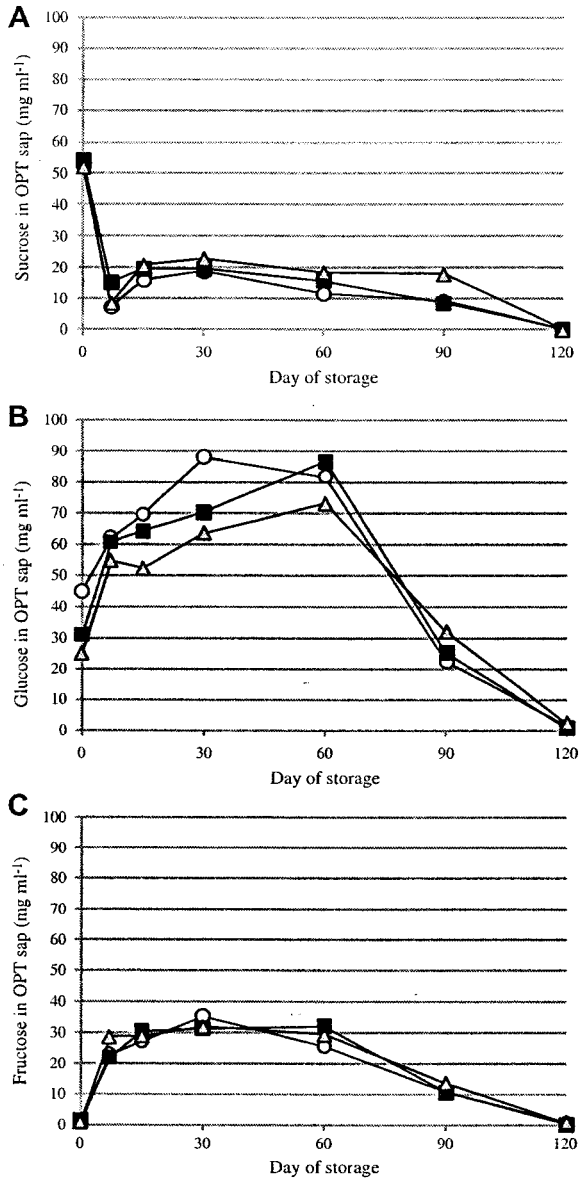


Fig. 5 – Concentration of three main sugars in OPT sap during storage. Open circle indicates inner part A, closed square intermediate part B, and gray triangle outer part C. The data were mean of the values obtained from 2 oil palm trees. A, sucrose; B, glucose; C, fructose.

three major sugars, but decreased rapidly at the very early stage of the storage (Fig. 5 A). In contrast, glucose and fructose increased sharply until Day 30 and stayed high levels for another 30 days (Fig. 5B, C). This suggests that sucrose in the OPT sap was decomposed to the two sugars at this period as sucrose is a disaccharide composed of glucose and fructose. However, the figures clearly showed that the increase of glucose and fructose exceeded the loss of sucrose at 30 and 60 days of storage. The sum of concentrations of three main sugars in the OPT sap was presented in Fig. 6. The changing pattern during the storage shown here is almost identical with

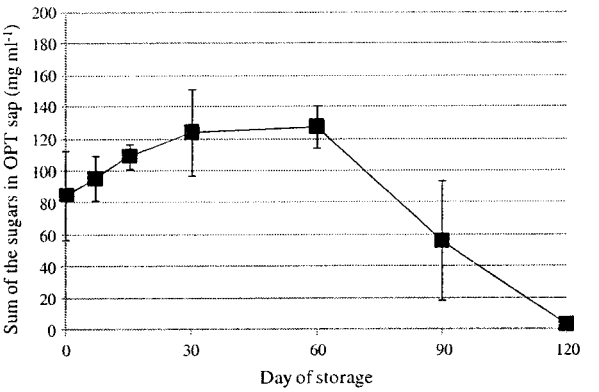


Fig. 6 – Sum of the three main sugar concentrations in OPT sap during storage. The data were mean of the values obtained from 2 oil palm trees, and calculated according to Eq. (1). Bar, ±SD.

Fig. 4, but the maximum concentration of total sugar determined by the phenol-sulfuric acid method is obviously higher than the sum of main sugars. Moreover, the concentrations at Day 120 were apparently different: 43 mg ml^{-1} for total sugar by the phenol-sulfuric acid method, while negligible for sum of main sugars. Meanwhile, broad unknown peaks with very low height were observed in the elution positions for oligosaccharides on HPLC. These results indicate presence of oligosaccharides in the sap of the stored trunks since the phenol-sulfuric acid method counts oligosaccharides in addition to mono- and di-saccharides which can be determined by the HPLC conditions used in this study.

To investigate the cause of the sugar increase, starch contents were determined for OPT samples whose sap was squeezed for the sugar analysis (Fig. 7). At Day 0, the average starch content was 3.5% of dried solid OPT disc and it decreased sharply from the beginning of the storage. Starch content dropped to 0.5% at Day 30 and became negligible after 60 days. Assuming that the moisture content is 70%, the solid is 30 g and the sap is 70 ml when the whole OPT sample is

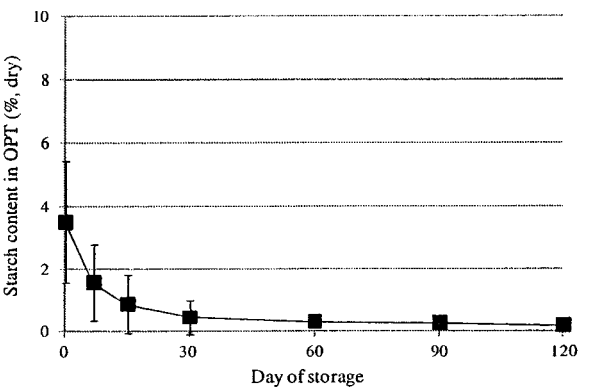


Fig. 7 – Starch content in OPT during storage. The data were mean of the values obtained from 2 oil palm trees, and calculated according to Eq. (1). Bar, ±SD.

00 g. As the concentration of free sugar in the sap increases rom 83 to 153 mg ml^{-1}, the OPT sample gains 4.9 g of sugar in he sap in 30 days. At the same time, starch decreases from .5% to 0.5% of the solid, accounting for 0.9 g loss during this eriod. Starch is likely to be converted to glucose and fructose y the actions of enzymes including starch-degrading nzymes and sucrose metabolism enzymes. It has been eported that these enzymes are induced in plants by various inds of stresses, such as cold stress [13], osmotic stress [14], nd water stress [15], and consequently, monosaccharides nd disaccharides accumulate. We presume the sugar accumulation in the sap of oil palm trunks occurs by induction of he enzymes triggered by the stress of felling. In fact, our reliminary experiments confirmed a significant level of amylase activity in the sap. Thus, a part of the free sugar ncrease is attributed to the starch degradation. However, ossible amount of glucose and fructose generated from tarch is far small, compared to the sugar increase as calculated. This suggests that hydrolysis of cellulose and/or hemicellulose also occurs in the felled trunks to produce monosaccharides. Oligosaccharides indicated by the sugar analyses are thought to be the intermediates of the hydrolysis products from starch and other polysaccharides. Further studies are in progress to determine the hydrolysis of cellulose and hemicellulose during storage of OPT and activities of enzymes involved. The decrease of free sugars after Day 60 is mainly caused by microbial infections. Although about 5 cm from the end of the log was trimmed prior to the disc cutting, fungal penetration was observed after prolonged storage.

To summarize the study here, free sugar content in OPT sap is the maximum (153 mg ml^{-1} for total sugar and 128 mg ml^{-1} for three main fermentable sugars) at 30–60 days of storage after logging and the sap should be squeezed during this period to obtain the highest sugar concentration for further utilization such as the production of bioethanol. Presently, sugar cane juice is used as one of the largest feedstocks for bioethanol. Table 2 compares the sap of old oil palm trunk with sugar cane juice as feedstock for bioethanol. Possible ethanol yield from sap of old palm trunk is calculated to be approximately 9 m^3 ha^{-1}, which exceeds that of sugar cane juice. Oil palm is felled once in 25 years, but the felled trunks are wastes from palm oil industry that has secured profit from oil and related products. In 2007, the plantation areas of mature oil palm are 3,741,000 and 4,540,000 ha for Malaysia and Indonesia [17], respectively. Assuming that 4% of the area is replanted every year, oil palm trunks are logged and discharged from 331,000 ha of the plantation in the two countries. Amount of logged OPT is calculated to be approximately 160 t ha^{-1}, and 9 m^3 ha^{-1} of ethanol can be produced. It means that roughly 3 hm^3 of bioethanol can be produced using the sap of the logged OPT in Malaysia and Indonesia. Unlike sugar cane, bioethanol production using felled OPT will not conflict with food usage and has a great potential as a feedstock for bioethanol.

Table 2 – Comparison of oil palm trunk with sugar cane as bioethanol feedstock.

	Sugar cane	OPT at day 60
Fermentable sugar concentration in juice or sap (%)	14.5[a] [16]	12.8
Moisture content (%)	Approx. 70	68[b]
Amount of sugars contained (g kg^{-1})	102	87
Cane/trunk produced per area (t ha^{-1})	77.6[c] [17]	154–168[d] (136–148 palms ha^{-1} [18])
Possible ethanol yield (m^3 ha^{-1})	6.5[c] [19]	8.7–9.4

a Mean value of the figures shown in reference [16] is cited.
b Calculated from the data in Fig. 2 according to Eq. (1).
c Data for Brazil.
d Calculated assuming the average trunk size is 38 cm in diameter and 10 m long, and the specific gravity is 1.0.

Acknowledgements

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Potential of oil palm trunk sap as a novel inexpensive renewable feedstock for polyhydroxyalkanoate biosynthesis and as a bacterial growth medium

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Premilinary Study of Oil Palm Trunk Sap and Starch Content from Various Cultivars at a Different Storage Time

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ABSTRACT

In this study, sugar content and starch of two cultivar of oil palm namely Dura x Pisifera mix (dura x URT) and Dumpy x Yangambi x AVROS were investigated based on different storage time (0, 15, 30, 45, 60 and 75 days). HPLC method was used to determine the total sugar and individual sugar content. The analysis of total sugar showed sugar content was found to increase after certain of storage time (30 - 60 days) around 11.7-13.7% for a cultivar Dura x Pisifera mix (dura x URT) and 10.4-11.4% for Dumpy x Yangambi x AVROS. Starch content was found to decrease progressively as the storage duration became longer.

Keywords

Oil palm trunks, Oil palm biomass, Starch, Sugar, Storage.

INTRODUCTION

The oil palm or *Elaeis guineensis* Jacq. belongs to the family *Palmae* and has been found to grow wild at West Africa [1]. It produced 90% of biomass from the trunks part and could easily be obtained from replanting activities [2, 3]. The oil palm is a monocotyledon with different properties from that of hardwood and softwood. Basically, oil palm trunks (OPT) consist of parenchyma with fibrous strands and vascular bundle [4, 5]. The parenchyma of oil palm trunks contain abundant of starch [5].

OPT contain high moisture content also known as a sap which could be as high as 500% [4]. This sap contains sugar and could subsequently be converted to ethanol by fermentation. This fermentable sugar can be in the form of sucrose, glucose and fructose.

The main objectives of this study are to analyze the fermentable sugar content and starch content and of the pressed sap samples from felled oil palm trunks at a different cultivars and duration of storage.

MATERIALS AND METHODS

Samples preparation

Oil palm trunks (OPT) with two different types of cultivars were obtained from KLK, Malaysia Northern Branch located in Kedah, Malaysia (Dumpy x Yangambi x AVROS) and MKC Enterprise located in Felda Kelating Jernih, Serting Hilir, at Negeri sembilan, Malaysia (Deli Dura x Pisifera X H&C). The trees were planted in 1981 and 1997, respectively, and were cut at 8 feet length from middle part of OPT. The samples were stored under the shade and were cut into discs according to the schedule of the cutting plan and prepared accordingly based on standard method.

Determination of sugar and starch content

Total sugar content of sap samples was determined by the Dubois method using phenol and sulfuric acid [6]. Absorbance of the solution was recorded at 480 nm. The calibration was carried out with glucose as standard. Determination of sugar components in each sap was carried out by high performance liquid chromatography (HPLC). Distilled water was used for the mobile phase at a flow rate of 0.4 ml min^{-1}.

For analysis of starch, a small amount of each sectional OPT sample was prepared in powder form by grinding and sieve using mesh size 200 before drying. Then, 0.400 g dry sample was weighed and 4.7 ml of 7.2M perchloric acid was added. The samples were then diluted with distilled water and centrifuged before 10ml aliquot were taken for analysis. The amount of starch present was determined by calibrating the absorption at wave length 650 nm with UV spectrophotometer, Shimadzu UV-1201 [7].

RESULT AND DISSCUSSION

The sugar analysis is indicated in Table 1. The results showed the composition of sugars at various storage times. Sucrose, glucose and fructose form as a main free sugar present in OPT. Glucose has a highest constituent of sugar of about 1.82%-7.77%

(Dura x Pisifera mix (dura x URT)). and 2.35% and 5.80% for samples from (Dumpy x Yangambi x AVROS). Other short chain polysaccharides such as maltose, xylose, galactose, arabinose and inositol also present in a small quantity from a total amount of sugar inside the OPT.

Sugar content increased as the storage time increased. The highest total sugar content was found for 60 day storage with 13.9% and 11.72% for sample from Dura x Pisifera mix (dura x URT) and Dumpy x Yangambi x AVROS.

Starch content of Dumpy x Yangambi x AVROS OPT was higher (0.001%-0.65%) compared to Dura x Pisifera mix (dura x URT) (0.01%-0.11%).

Table 1: Summary of sugar and starch content after storage time for different cultivars

			Sugar content					Starch content
			Composition of sugar (%)				Total sugar	
Cultivars	Days	Parts	Sucrose	Glucose	Fructose	Other free sugars	(%)	(%)
Dura x Pisifera mix (dura x URT)	0	A	1.77	3.47	0.39	0.26	5.89	0.34
		B	1.73	2.83	0.39	0.24	5.18	0.37
		C	1.54	1.84	0.66	0.84	4.87	0.39
	15	A	2.43	4.14	2.18	0.58	9.33	0.23
		B	2.96	3.57	2.26	0.55	9.33	0.20
		C	2.21	2.93	2.16	0.52	7.83	0.22
	30	A	1.36	6.06	3.42	0.81	11.6	0.10
		B	1.69	5.25	3.65	1.15	11.7	0.12
		C	1.58	5.28	3.71	1.16	11.7	0.12
	45	A	2.04	6.36	3.55	0.70	12.6	0.07
		B	2.99	5.84	3.68	0.86	13.3	0.07
		C	2.66	5.27	3.68	1.04	12.6	0.05
	60	A	1.95	7.00	3.66	0.79	13.4	0.02
		B	2.79	6.33	3.86	1.02	13.9	0.02
		C	2.67	6.44	3.88	1.01	14.0	0.01
	75	A	1.67	7.78	2.80	0.72	12.9	0.01
		B	1.81	6.14	2.85	0.74	11.5	0.02
		C	3.31	5.02	2.34	0.36	11.0	0.01
Dumpy x Yangambi x AVROS	0	A	3.15	3.85	0.88	0.04	7.92	0.65
		B	5.15	3.74	0.77	0.23	9.88	0.61
		C	3.90	2.35	1.18	0.33	7.76	0.59
	15	A	2.56	4.50	1.23	0.08	8.36	0.33
		B	2.38	5.28	2.35	0.77	10.7	0.31
		C	1.50	4.74	3.46	1.17	10.8	0.28
	30	A	1.46	5.70	2.62	0.10	9.89	0.09
		B	1.00	5.75	2.43	0.73	9.91	0.07
		C	1.31	5.80	2.82	1.13	11.0	0.09
	45	A	4.35	5.13	1.51	0.08	11.06	0.02
		B	2.82	5.53	2.10	0.66	11.10	0.06
		C	2.71	5.37	2.49	1.14	11.72	0.03
	60	A	4.87	5.31	1.54	0.08	11.80	0.04
		B	3.17	5.33	1.83	0.36	10.68	0.04
		C	3.47	5.53	1.83	0.66	11.49	0.04
	75	A	1.23	3.60	2.04	0.03	6.90	0.01
		B	2.68	4.11	1.48	0.22	8.50	0.01
		C	3.90	5.00	1.45	0.39	10.75	0.01

CONCLUSION

In this study, oil palm sap has been analyzed for its sugar amount and composition. The sugar content was found to increase as the storage time increased until 30-60 days. Glucose was the highest sugar content followed by sucrose and fructose. Starch content decreased as the storage time increased.

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EFFECT OF STORAGE TIME ON STARCH CONTENT OF OIL PALM

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ABSTRACT

Starch contents of two cultivars of oil palm namely Deli Dura x Pisifera X H&C and Chemara were investigated based on storage time (0, 15, 30, 45 60 and 75 days). Starch analysis was conducted based on method by Humphrey and Kelly. The starch content was found to decrease progressively as the storage duration became longer. Initial starch content for samples from Chemara cultivar was found to be higher compared to those samples of Deli Dura x Pisifera X H&C type. The starch content was found to be around 7.42-7.24% at day 0 and reduced to around 1.39-1.89% after 75 days of storage.

Keywords: Oil palm trunks, Oil palm trunk, Starch, Storage.

INTRODUCTION

Oil palm or *Elaeis guineensis* has been planted extensively in the last two decades and nowadays it has become major plantation in Malaysia. Three main cultivars of oil palm planted in Malaysia; are *Dura, Pisifera* and *Tenera.* They could be differentiated based on characterization of the fruit. With extensive breeding program, various other cultivars have been produced (Hartley, 1988).

Starch is a major carbohydrate component in the storage tissue inside the plant. It is homopolymer which consist of only one monomer known as D-glucose. It consists a two polymer inside D-glucose; amylose in a linear structure and branches linkages of amylopectin (Copeland et al, 2009; Buleon et al, 1998). The structure of cellulose and starch are quite similar; i.e. the glucose structure in a starch is α-1,4-linkages and for cellulose has β-1,4-linkages and make it a linear and more rigid structure compared to starch (Linde et al, 2008; Balat et al, 2008).

Oil palm trunk consists of a mixture of complex carbohydrate such as cellulose and hemicellulose which contains various amount of reducing sugar such glucose, xylose, sucrose and great amount of starchy material. Previous studies indicated that oil palms contain a lot of starch (Akmar and Kennedy, 2001) and starch in oil palm could be revealed in the parenchymatous tissue (Tomimura, 1992). Presence of starch in this cell is very important in term of mechanism of sugar accumulation in oil palm trunk. It is postulated that the starchy material could be converted into sugar by hydrolyzing action of enzyme as the oil palm trunk were kept for longer duration (Chew et al, 2008; Maruyama et al, 2009; Kosugi et al, 2008). Due to this, it is very important to characterize starch based on duration of storage. In this study we investigated the effect of storage duration on the amount of starch content from two oil palm cultivars.

MATERIALS AND METHODS

Samples preparation

Four oil palm trunks (OPT) with two different types of cultivars were obtained from Advanced Agriecological Research Sdn. Bhd located in Kedah, Malaysia (Chemara) and Sime Darby Sdn Bhd located in Sungai Buloh Estate at Selangor, Malaysia (Deli Dura x Pisifera X H&C). The trees were planted in 1980 and 1977, respectively, and were cut at 8 feet length from middle part of OPT. The samples were stored under the shade and were cut into discs according to the schedule of the cutting plan and prepared accordingly based on standard method for determination of starch content.

Determination of starch content

The samples were prepared by grinding and sieving the dry samples with a size 200 mesh before drying in the desiccators over 97% concentration of sulphuric acid. Then, a 0.400 g dry sample was weighed and 4.7 ml of 7.2M perchloric acid was added. The samples were mixed thoroughly for exactly 10 minutes to ensure good contact between samples and acid. The samples were then diluted with distill water and centrifuged before 10ml aliquot

were taken for analysis. A drop of phenolphthalein was added in the solution followed with 2N NaOH and 2N acetic acid. Then, 2.5ml acetic acid, 0.5ml 10% potassium iodide and 5ml 0.01N potassium iodate was added. The solution was left for 15 minutes. The amount of starch present was determined by calibrating the absorption at wave length 650 nm with UV spectrophotometer, Shimadzu UV-1201 (Humphrey and Kelly, 1961).

RESULT AND DISCUSSION

The starch analysis is indicated in Table 1. The results showed that the composition of starch content for different cultivars at various storage times. Starch content of Chemara OPT was higher starch which was about1.89%-7.42% compared to Deli Dura x Pisifera X H&C type, which was around 1.39%-7.24%. Referring to previous studies on the starch content for a 0 day, as indicated by Tomimura (1992), the starch content for separated sample of vascular bundle and parenchyma and was 2.4% while in parenchyma was 55.5% respectively. In our work starch was found about 7.24-7.42% for both cultivars without separating the samples. Rahim et al. (1987) on the other hand, found that the starch content of oil palm trunk was around 0.304-0.808%. The difference between these two results maybe due to age and species of the tree. Besides that, we presume a different location which could be related to a different area, soil and separated samples as a parenchyma retain a lot of starch, may also contribute to the starch content for these samples (Tomimura, 1992).

Table 1 Summary of starch content after storage time

Cultivars	Starch content in OPT (%,) Days					
	0	15	30	45	60	75
Deli Dura x Pisifera x H&C	7.24	7.29	6.95	1.79	1.46	1.39
Chemara	7.42	7.46	7.32	6.11	1.74	1.89

The duration of storage time 0, 15, 30, 60 and 75 has been chosen to determine the starch content until minimum yield of storage time was obtained. The starch content reduced as the duration of storage increased. Based on the findings of this study, the starch content started to decrease rapidly around day 30-60 and became constantly after 60 days. This probably due to degradation of starch by actions of enzyme and being converted to glucose and other fermentable sugar and a decreasing of starch content is a significant with the increases of the sugar content (Linde et al. 2008; Wang et al, 2000).

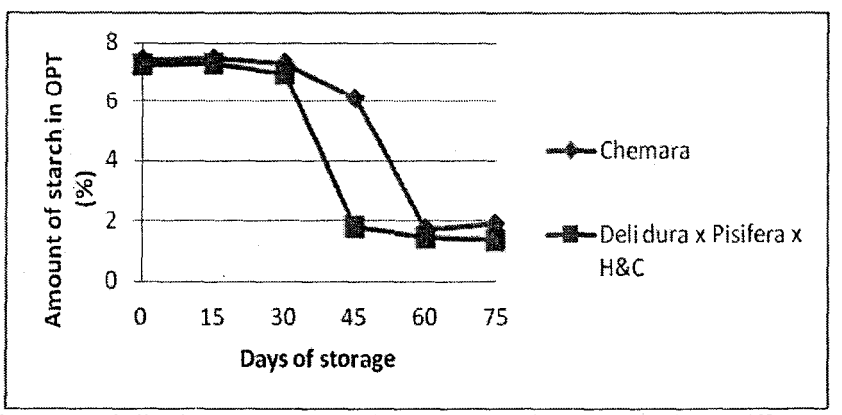

Figure 1 Starch content of oil palm trunks analyzed by Humprey and Kelly method at at different storage time.

It seems that different cultivar of oil palm species behave differently in term of starch content even though anatomically the two cultivars look almost identical. Further work is in progress to investigate this phenomenon for more others cultivars.

CONCLUSION

In this study two different cultivars of oil palm tree; Deli Dura x Pisifera X H&C, and Chemara were used to determine their starch content. The starch content was found to decrease when extended the storage time. It was found that starch content was higher at a 0 day and start to decrease around day 30 - 45 day storage . The oil palm tree from a cultivar Chemara was identified to have higher starch content compared to Deli Dura x Pisifera x H&C type.

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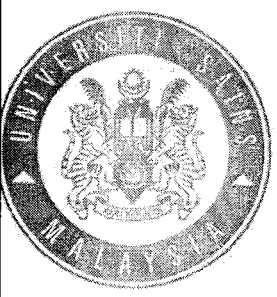

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TABLE OF CONTENT

Content	Page
Acknowledgement	ii
List of Tables	vii
List of Figures	viii
List of Symbols and Abbreviations	xiii
Abstrak	xiv
Abstract	xvii
1. CHAPTER 1 : INTRODUCTION	
1.1 General	1
1.2 Objectives	4
2. CHAPTER 2 : LITERITER REVIEW	
2.1 Oil palm tree	
2.1.1 Overview of Oil Palm (*Elaies guineensis*)	5
2.1.2 The palm oil industry in Malaysia	7
2.2 Breeding of oil palm	9
2.2.1 Development of breeding program	9
2.2.2 Selected oil palm breeding	12
2.3 Oil palm biomass	
2.3.1 Oil palm trunks	14
2.3.2 Growth and morphology of oil palm tree	16
2.3.2.1 Anatomy of Oil Palm trunk	17
2.3.2.2 Physical properties of Oil Palm trunk	20
2.3.2.2.1 Moisture content	20

2.3.2.2.2 Density..... 23
2.3.2.3 Chemical properties of oil palm trunks..... 23
2.4 Sap of oil palm trunks
2.4.1 General..... 25
2.4.2 Collecting sap of oil palm trunks..... 26
2.4.3 Uses of sap of oil palm trunks..... 27
2.4.4 Chemical composition sap of oil palm trunks..... 28
2.4.5 Storage time of oil palm trunks sap 30
2.4.6 Determination of sap content of oil palm trunks..... 32
2.4.6.1 Determination of oil palm sap by High Performance Liquid Chromatography (HPLC)... 32
2.4.6.2 Determination oil palm sap by colometric method..... 36
2.5 Starch content of oil palm trunks
2.5.1 General..... 37
2.5.2 Uses of starch..... 38
2.5.3 Chemical composition starch of oil palm trunks..... 40
2.5.4 Determination of starch content of oil palm trunks..... 42
2.5.4.1 Determination of starch oil palm trunks by Humprey – Kelly method..... 42

3. CHAPTER 3: MATERIAL AND METHOD
3.1 Material..... 43
3.2 Samples preparation..... 44
3.3 Microstructure of oil palm trunks..... 46
3.3.3 Scanning Electron Microscopy (SEM) study of the oil palm cell structure..... 46
3.4 Determination of physical properties of oil palm trunks..... 47
3.4.1 Moisture content of oil palm trunks..... 47
3.4.2 Density of oil palm trunks..... 48
3.5 Determination of sugar content of oil palm sap using HPLC..... 49

3.5.1 Preparation of oil samples sap sugar for HPLC analysis... 49
3.5.2 Preparation of HPLC for sugar analysis..... 50
3.5.3 Preparation of samples sap sugar for colometric..... 53
3.6 Determination of starch content of oil palm trunks by Humprey and Kelly method..... 54

4. CHAPTER 4: RESULT AND DISSCUSION
4.1 Microscopic Oil Palm trunks Structure by Scanning Electron Microscopy (SEM)
4.1.1 Vascular bundle structure..... 56
4.1.2 Parenchyma cell structure..... 58
4.1.3 Fiber structures..... 60
4.2 Physical properties of oil palm trunks
4.2.1 Moisture content of oil palm trunks..... 61
4.2.2 Density of oil palm trunks..... 65
4.3 Analysis of oil palm trunks sap sugar content
4.3.2 Sugar content of oil palm trunks sap by HPLC..... 69
4.3.3 Total sugar content of oil palm trunks sap by colometric method..... 75
4.4 Starch analysis of oil palm trunks by Humprey-Kelly method..... 78
4.5 Correlation between moisture content and sugar content of oil palm trunks..... 84
4.6 Correlation between sugar and starch content of oil palm Trunks..... 86

5. CONCLUSION..... 88
6. RECOMMENDATION..... 91
7. REFERENCE..... 92
8. LIST OF PUBLICATION

i. *Ethanol and Lactic Acid Production using Sap Squeezed from Old Oil Palm Trunks Felled for Replanting.* Kosugi, A., Tanaka, R., Magara, K., Arai, T., Sulaiman, O., Hashim, R., **Hamid, Z.A.A.**, Yahya, M.K.A., Yusof, M.N.M., Ibrahim, W.A., and Mori, Y., (2010). Journal of Bioscience and Bioengineering. 110 (3): 322-325. (Impact factor: 1.749).

ii. *Old Oil Palm Trunk: A Promising Source of Sugars for Bioethanol Production.* Yamada, H., Tanaka, R., Sulaiman, O., Hashim, R., **Hamid, Z.A.A.,** Yahya, M.K.A., Kosugi, A., Arai, T., Murata, Y., Nirasawa, S., Yamamoto, K., Ohara, S., Yusof, M.N.M., Ibrahim, W.A., and Mori, Y., (2010). Biomass and Bioenergy. 34 (11): 1608-1613. (Impact factor: 3.326).

iii. *Potential of oil palm trunk sap as a novel inexpensive renewable feedstock for polyhydroxyalkanoate biosynthesis and as a bacterial growth medium.* Lokesh, B.E., **Hamid, Z.A.A.**, Arai, T., Kosugi, A., Murata, Y., Hashim, R., Sulaiman, O., Mori, Y., and Sudesh, K. Paper in review.

iv. *Effect of Storage Time on Starch Content of Oil Palm.* **Hamid, Z.A.A.,** Sulaiman, O., Hashim, R., Arai, T., Mori, Y., Tanaka, R., Kosugi, A., Murata, Y., and Yamamoto, K. Paper presented at the 2nd International Conference on Environmental Research and Technology (ICERT) conference, Penang, Malaysia on June 2-4th, 2010.

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LIST OF TABLES

Table	Title	Page
Table 1	World production of oil palm	7
Table 2	Moisture content and density of oil palm trunks by the various researchers	21
Table 3	Study on moisture content and density of oil palm trunks	64
Table 4	Comparison of previous study and present study of oil palm sap	73

LIST OF FIGURES

Table	Title	Page
Figure 1	Felled oil palm biomass left at plantation after harvesting activities	2
Figure 2	Oil Palm Tree (*Elaeis guineensis*)	5
Figure 3	Area of oil palm planting in the decades of the last century	8
Figure 4	History and development of Deli dura in Indonesia and Malaysia till 1979	11
Figure 5	The varieties of palm oil fruit from species *Elaeis guineensis*	13
Figure 6	The oil palm trunks biomass after harvesting activities	14
Figure 7	Cross section of oil palm trunks	18
Figure 8	Transverse section of vascular bundles with vessels in oil palm trunk (F), parenchyma ground tissue (PGT), vascular bundle (VB), vessel (V) and phloem(Ph)	19
Figure 9	The pilot plan of squeezing machine to obtain sap of oil palm trunks.	27
Figure 10	Chemical composition in oil palm sap of felled trunks	29
Figure 11	Schematic diagram of High performance Liquid Chromatography (HPLC) unit	32
Figure 12	Structure of Amylose and Amylopectin	41
Figure 13	Information of oil palm samples taken	43
Figure 14	Preparation of oil palm sample	45
Figure 15	Preparation samples for SEM observation	46
Figure 16	Preparation of sap sugar for HPLC analysis.	49
Figure 17	Illustration of High Performance Liquid Chromatography (HPLC)	51
Figure 18	Basic operation of High Performance Liquid Chromatography HPLC	52
Figure 19	Process of Colometric method	53
Figure 20	Humprey and Kelly method	54

Figure 21	Structure of vascular bundle of oil palm trunk taken from transverse section parenchymatous ground tissue (PGT), vessels (V), vascular bundle (VB), bundle (B), fibers (F) and phloem (Ph)	57
Figure 22	Structure of parenchyma cells of oil palm trunk taken from transverse section parenchymatous ground tissue (PGT), bundle (B)	58
Figure 23	Starch in oil palm trunk taken from transverse section. I: Parenchyma without starch content in bottom part of OPT, II: Parenchyma rich with starch content in upper part of OPT, parenchymatous ground tissue (PGT), starch granule (S)	59
Figure 24	Fiber structure in oil palm trunk taken from transverse section, secondary wall (SW), primary wall (PW)	60
Figure 25	Moisture content (%) of oil palm trunks according to the zones for cultivars; A) Dura x Pisifera mix (dura x URT), B) Dumpy x Yangambi x AVROS, C) Deli Dura x Yangambi (D3D x L236T), D) Deli Dura x Pisifera x H&C	62
Figure 26	Density (g/cm^3) of oil palm trunks according to the zones for cultivar; A) Dura x Pisifera mix (dura x URT), B) Dumpy x Yangambi x AVROS, C) Deli Dura x Yangambi (D3D x L236T), D) Deli Dura x Pisifera x H&C	66
Figure 27	Amount of sap sugar content of oil palm trunks taken from cultivars; A) Dura x Pisifera mix (dura x URT), B) Dumpy x Yangambi x AVROS, C) Deli Dura x Yangambi (D3D x L236T) and D) Deli Dura x Pisifera x H&C analyzing with HPLC	70
Figure 28	Average amount of sugars for cultivars Dura x Pisifera mix (dura x URT), Dumpy x Yangambi x AVROS, Deli Dura x Yangambi (D3D x L236T) and Deli Dura x Pisifera x H&C of oil palm trunks taken.	71

Figure 29	Concentration of three main sugars in OPT sap during storage from various cultivars Dura x Pisifera mix (dura x URT), Dumpy x Yangambi x AVROS, Deli Dura x Yangambi (D3D x L236T) and Deli Dura x Pisifera x H&C. A: sucrose; B: glucose; C: fructose	73
Figure 30	Amount of total sap sugar determined by colometric method comparing with HPLC analysis taken from various cultivars; Dura x Pisifera mix (dura x URT), Dumpy x Yangambi x AVROS, Deli Dura x Yangambi (D3D x L236T) and Deli Dura x Pisifera x H&C.	76
Figure 31	Amount of starch at different part of OPT determined by Humprey and Kelly method taken from various cultivars. A) Dura x Pisifera mix (dura x URT), B) Dumpy x Yangambi x AVROS, C) Deli Dura x Yangambi (D3D x L236T), D) Deli Dura x Pisifera x H&C	79
Figure 32	Comparison amount of starch determined by Humprey and Kelly method taken from various cultivars of OPT	81
Figure 33	Transverse section of vascular bundles with vessels for bottom and upper parts of oil palm trunk. I and II – upper part: It is obviously seen the starch content accumulates in the tissue cell. III and IV - bottom part: Less the starch content accumulate in the tissue cell. parenchyma ground tissue (PGT), starch (S), fiber (F)	82
Figure 33	Relationship between sugar content and moisture content of oil palm for cultivars Dura x Pisifera mix (dura x URT), Dumpy x Yangambi x AVROS, Deli Dura x Yangambi (D3D x L236T) and Deli Dura x Pisifera x H&C.	85
Figure 34	Comparison of sugar and starch analysis of oil palm trunks for cultivars; Dura x Pisifera mix (dura x URT), Dumpy x Yangambi x AVROS, Deli Dura x Yangambi (D3D x L236T) and Deli Dura x Pisifera x H&C	87

LIST OF ABBREVIATIONS

AD	Air dry weight
ASTM	American Society for Testing and Materials
AVROS	Algemene Vereniging van Rubberplanters ter Oostkust van Sumatera
CBS	Cooperative Breeding Scheme
CTO	Column Oven
DDI	Distilled De-Ionized
DGU	On-Line Degasser
DP	Degree of polymerization
DOA	Department of Agriculture
FELDA	Federal Land Development Authority
FRIM	Forest Research Institute Malaysia
HPLC	High performance liquid chromatography
KLK	Kuala Lumpur Kepong Berhad
LC	Liquid Chromatography
OPGL	Oil Palm Genetic Laboratory
OPM	Oil palm of Malaya
OPT	Oil Palm Trunk
PORIM	Palm Oil Research Institute of Malaysia
MARDI	Malaysian Agricultural Research and Development Institute
MC	Moisture content
MPOB	Malaysia Palm Oil Board
MPOC	Malaysia Palm Oil Council
NaOH	Sodium Hydroxide
NIFOR	Malaysia and Institute for Oil Palm Research
OD	Oven dry weight
RID	Refractive Index
R&D	Research and Development
SBS	Sabah Breeding Schemes or programme

SEM	Scanning Electron Microscope
SI	System of Units
Socfin	Societe Financiere de Caoutchouces
UPB	United Plantations Berhad
UV-VIS	Ultraviolet/Visible Spectrometry
WIFOR	West African Institute for Oil Palm Research
Wv	Wavelength

LIST OF SYMBOL

%	Percentage
°C	Degree Celsius
g/cm^3	Grams per cubic centimeter
MPa	Megapascal
α	Alpha
m	Meter
mm	Millimeter
ml	Milliliter
rpm	Revolution per minute
g	Gram
psi	Pound force per square inch

ANALISIS KANDUNGAN GULA DAN KANJI BATANG KELAPA SAWIT YANG TELAH DITEBANG MENGIKUT MASA PENYIMPANAN YANG BERBEZA.

Abstrak

Penyelidikan ini adalah untuk mengkaji potensi kebolehgunaan sap batang kelapa sawit sebagai bahan mentah untuk industry bio-ethanol. Sap daripada batang kelapa sawit telah di kaji. Kandungan gula dan kanji telah dianalisis berdasarkan kepelbagaian jenis baka, masa penyimpanan, usia pokok dan lokasi.

Empat jenis baka kelapa sawit telah digunakan untuk penyelidikan ini; Dura x Pisifera campur (Dura x URT), Dumpy x Yangambi x AVROS, Deli Dura x Yangambi (D3D x L236T) dan Deli Dura x Pisifera x H&C. Batang kelapa sawit yg telah ditebang kemudian disimpan pada masa penyimpanan yang berbeza; 0, 15, 30, 45 60 dan 75 hari, sebelum dipotong didalam bentuk disk. Disk itu kemudian telah dibahagi kepada 3 bahagian; dalam (A), tengah (B), dan luar (C). Setiap bahagian kelapa sawit itu kemudian telah di perah untuk mendapatkan sap segar. HPLC dan kaedah phenol sulphuric asid telah digunakan untuk menentukan kandungan jumlah keseluruhan gula dan individu gula. Penentuan kanji pula telah dijalankan dengan menggunakan kaedah Humprey dan Kelly. Struktur anatomi batang kelapa sawit telah di kaji dengan menggunakan SEM dan diambil dari bahagian pemotongan rentas. Penentuan sifat

fizikal telah dibuat iaitu penentuan kandungan lembapan dan ketumpatan berdasarkan standard ASTM.

Penentuan visual menunjukkan batang kelapa sawit mengandungi jumlah kandungan kanji yang banyak terutamanya di bahagian atas pokok kelapa sawit. Sementara sifat fizikal menunjukkan kandungan lembapan untuk semua baka adalah dari 147% hingga 1022% dan meningkat dari bahagian tengah ke luar. Dalam kajian ini, baka Deli Dura x Yangambi (D3D x L236T) telah dikenalpasti mengandungi kandungan lembapan yang tinggi berbanding sampel yang lain. Ketumpatan pula di kaji meningkat dari bahagian luar ke dalam. Baka Dumpy x Yangambi x AVROS telah dikenalpasti mempunyai ketumpatan yang tertinggi berbanding baka yang lain.

Penentuan kandungan gula pula menunjukkan kandungan gula yang dianggarkan melebihi 60% daripada keseluruhan pokok. Gula yang terdapat didalam sap dan meningkat apabila masa penyimpanan di panjangkan selepas penebangan terutamanya selepas hari ke 30 hingga 60 hari penyimpanan. Bahagian dalam dan tengah (bahagian A dan B) telah di kenalpasti mengandungi kandungan jumlah gula keseluruhan yang tinggi dengan kehadiran tiga jenis gula utama glucose, sucrose dan fructose serta kandungan gula yang lain. Baka Cultivar Dura x Pisifera campur (dura x URT) menunjukkan kandungan gula keseluruhan di dalam sap yang tertinggi dengan kepekatan dari 43.82 mg/ml hingga 181.89 mg/ml dengan HPLC dan 38.94 mg/ml hingga 181.74 mg/ml dengan kaedah colometrik.

Keputusan kandungan kanji pula menunjukkan kandungan kanji tinggi di awal masa penyimpanan dan menurun disekitar hari ke 30 hingga 60. Jumlah purata kandungan kanji adalah 0.8% dan kandungan kanji tertinggi telah di jumpai di bahagian dalam (A) dan tengah (B). Baka Dumpy x Yangambi x AVROS telah di kenalpasti mempunyai kandungan kanji yang tertinggi dengan jumlah 0.07% dan 3.21% manakala baka Deli Dura x Yangambi (D3D x L236T) mempunyai kandungan kanji terendah diantara 0.06% dan 1.55% .

Kajian terhadap hubungkait kandungan lembapan dan kandungan gula menjumpai kandungan lembapan yang tinggi akan menyebabkan kandungan gula menurun. Kajian hubungkait kandungan gula dan kanji pula menunjukkan pengurangan kandungan kanji akan member kesan terhadap kandungan gula.

Batang kelapa sawit telah dikenalpasti sebagai bahan mentah yang sesuai digunakan untuk penghasilan bioethanol dengan menggunakan masa penyimpanan dan pemilihan jenis baka yang sesuai. Baka Dumpy x Yangambi x AVROS telah di kenalpasti sebagai baka yang berpotensi di gunakan untuk mencapai tujuan ini.

ANALYSIS OF SAP SUGAR AND STARCH CONTENT OF FELLED OIL PALM TRUNKS AT DIFFERENT STORAGE TIME

Abstract

This research investigated the potential use of oil palm sap as a feedstock for bioethanol production. Sap from squeezed oil palm trunks was investigated. The sugar and starch content were further analyzed based on various cultivars, storage time, age and location.

Four types of cultivars were used for the study that include; Dura x Pisifera mix (Dura x URT), Dumpy x Yangambi x AVROS, Deli Dura x Yangambi (D3D x L236T) and Deli Dura x Pisifera x H&C. The felled oil palm trunks were stored at a different duration of storage time; 0, 15, 30, 45, 60 and 75 days, before they were cut into discs. The disc was divided into 3 parts: inner (A), middle (B), and peripheral (C) parts, respectively. Each parts of the OPT was squeezed to obtain the fresh sap. High Performance Liquid Chromatography (HPLC) and phenol sulphuric acid method were used to determine the total sugar and individual sugar content of the sap. Starch in oil palm samples was analyzed using the method by Humphrey and Kelly. The anatomy structure of oil palm trunks was conducted through the Scanning Electronic Microscopy (SEM) and taken from the transverse section. The physical properties of the sample; moisture content and density, were investigated in accordance to American Society for Testing and Materials (ASTM) standard.

Microscopic investigation result showed that oil palm trunks contain abundant amount of starch especially in the upper part of oil palm tree. Based on the finding of this study on physical properties showed that moisture content for all cultivars are from 147% to 1022% and gradually increased from inner to peripheral zone. In this study, cultivar Deli Dura x Yangambi (D3D x L236T) was identified to contain highest moisture among others samples. The density is higher from peripheral part and progressively lower in the inner part. Cultivar Dumpy x Yangambi x AVROS has been found to be the highest density among to the other cultivars.

Sugar analysis showed the amount of sugar in the oil palm sap approximately more than 60% of the whole tree. Sugars exist in the sap and were found to increase as the storage time extended after felling especially after 30 to 60 storage days. The inner and middle parts (part A and B) were identified to contain highest total sugar content with presence of three main sugars glucose, sucrose and fructose and others sugars. Cultivar Dura x Pisifera mix (dura x URT) shows the highest total sugar in the sap with the concentration from 43.82 to 181.89 mg/ml obtained by HPLC and 38.94 to 181.74 mg/ml obtained by Colometric method.

The starch content started to be high at the initial storage time and decreases around day 30 to 60 of storage. The average amount of starch content was found about 0.8% and the highest starch content was found in the inner part (A) and middle part (B). Cultivar Dumpy x Yangambi x AVROS was recognized to contain highest starch level

about 0.07% and 3.21% and Deli Dura x Yangambi (D3D x L236T) type contain lower starch content, around 0.06% and 1.55% .

Investigation on relationship of moisture content and sugar content found that higher moisture content will give lowest sugar content. Study on relationship of sugar content and starch content also showed that degradation of starch content is significant with the increases of the sugar content.

Oil palm trunks were indicated as a promising feedstock that is suitable for the production of bioethanol with the appropriate storage time and type of cultivar. In this study, cultivars Dumpy x Yangambi x AVROS was found to be the most potential cultivar to be used to achieve this target.

CHAPTER 1
INTRODUCTION

1.1 General Background

Oil palm, *Elaeis guineensis* is tropical tree species and grown well mostly in Africa, South East Asia and in America as a commercially crops for various usages mainly in the manufacturer of food products, soaps and detergents, cosmetics, resins, paints and etc. (Basiron and Weng, 2004; Lam *et al.*, 2009). Oil palm was introduced into Malaysian in 1875 in the early 20th century. This valuable commercial plant were planted extensively and increased tremendously as a growing demand for source of edible oil. Currently, Malaysian has become one of the largest producers and exported approximately over 40% of the total world palm oil production with the total area of oil palm trees planted was more than 4 million hectares (Basiron and Weng, 2004; Chew and Bhatia, 2008). Increasing in demand of palm oil in the world market has stimulated the expansion of rapid replanting of the oil palm tree. Due to this scenario, massive amount of oil palm biomass has been produce as waste after the harvesting. Basically palm tree are felled an average age around 25 years as shown in Figure 1. Improper management of this biomass has become an environmental concern especially for Malaysia.

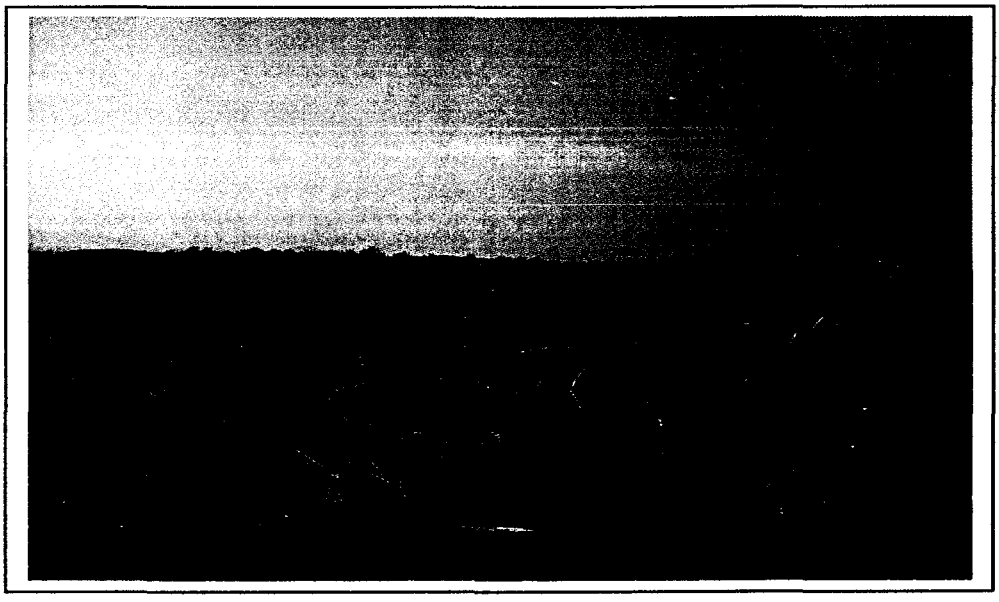

Figure 1: Felled oil palm biomass left at plantation after harvesting activities.

Several studies have been conducted to utilize this biomass into value added products. As a result, almost every portion of this tree also can be converted into usable material especially the oil palm trunks (OPT), which is can be used for the manufacturing plywood and production pulp and paper. Currently, new attention has been given to a production of bioethanol from agricultural crop such as corn, sugarcane, wheat and etc. However, competition between food industries due to source of human food makes production of bioethanol using this agricultural crop make it as international issue on food security. Oil palm biomass looks promising to replace this agricultural crop, due to its availability, lower cost and potential as a source of this bioenergy and has invited numerous researches to discover other usage of this biomass. The production of ethanol using oil palm biomass has been conducted before by using palm oil mill effluent and empty fruit bunches (Alam *et al.*, 2005). Other study have assessed to

convert the empty fruit bunch (EFB) and palm press fiber into sugar by using cellulose enzyme to obtain sugar for further process (Gutierrez *et al.*, 2009). Previous study discovers that acid hydrolysis method can be used to convert empty fruit bunch as a feedstock into sugar (Cheng *et al.*, 2007). Lim *et al.*, (1997) found that oil palm trunks show a promising result to be converted into glucose or sugar by using acid hydrolysis method. The purpose of this research is to provide information and develop a comprehensive method by using oil palm sap as main material to obtain the sugar and also tried to discover an effective cost for ethanol production.

Oil palm tree consist of 90% of biomass, mostly from the trunks part that could easily obtained after replanting activities. Oil palm trunks contain high moisture content also known as a sap which sometimes could be as high as 500% (Husin *et al.*, 1985). This biomass composed a plenteous amount of lignocelluloses, material with major component of starchy material and cellulose that can be converted into sugar subsequently by fermentation into ethanol. However converted starch and cellulose material into sugar are more complicated and required high energy consumption that involves high production costs compared to direct utilization of sugar which are readily available in the oil palm sap (Lin and Tanaka, 2006). Large amount of polysaccharides or fermentable sugars such as sucrose, glucose, fructose, arabinose, mannos and etc are easily found abundantly inside the oil palm sap from the extracted of these lignocelluloses. Therefore, a new method was developed to obtain a large amount of sugar, oil palm sap seem to be a potential source to fulfill this requirement.

1.2 Objective

The objectives of this study are:

1. To analyze the free fermentable sugar content and composition of the pressed sap samples from felled oil palm trunks at a different duration of storage, location and cultivars or clone.
2. To determine the optimum amount sugar and starch content from felled oil palm trunks based on duration of storage, location and cultivars or clone.
3. To identify the potential cultivars contain high concentration of sugar sap in oil palm trunks.

CHAPTER 2
LITERATURE REVIEW

2.1 Oil palm tree

2.1.1 Overview of Oil Palm (*Elaies guineensis*)

The oil palm (also known as *Elaeis guineensis*) is a perennial crop originated from West Africa where is it widely used as a source of edible oil. This crop is woody monocotyledons in the family Arecaceae and the subfamily *Cocoideae*, similar grouped with coconut. The name *Eleais* is derived from Greek word *Elaion* with the meaning oil (Corley and Tinker, 2003). Figure 2 showed the oil palm tree (*Elaeis guineensis*).

Figure 2: Oil Palm Tree (*Elaeis guineensis*)

The African oil palm, *Elaeis guineensis* has been marked by Jacquin in 1973 as a first species from genus *Elaeis*. *Elaeis oleifera* was identified as a second species by Latiff (2000). His research in Advances in oil palm research book, explained the difference of this species with genus *E.guineensis* is their physical properties of the tree. Crossing species between *E.guineensis* and *E.oleifera* ultimately produce a new type of *Elaeis*, namely *Elaeis odora*. However, species from *Elaeis guineensis* has been widely introduced and commercialized until it's became one of the major important crops in South East Asia especially Malaysian and Indonesia (Hartley, 1988).

In 1848, four seedlings from this plant have been brought from two different places: Amsterdam and Mauritius to the Bogor Indonesia as an ornamental plant and spread around Sumatera in 1853 and 1856. This oil palm trees has been planted in some estate in Deli, Sumatera which gave a name to the Deli palm cultivar. In Malaysia, cultivated of oil palm tree has begun in the early 19th century. It has been introduced into Malaysia through the planting of Deli origin also known as Deli dura from Sumatera in the Rantau Panjang, Selangor (Corley and Tinker, 2003; Latiff, 2000). Enhancement of Research and Development (R & D) in Malaysia has brought these types of palms into a first commercial oil palm estate in Tennamaram estate, Selangor in 1917. In 1950's, plantation of oil palm tree has been commercialized and become a new interest due to a stability price of world market and to lessen the dependency on rubber as a main commercial crop at that time (Hartley, 1988). From 1930s to 1980s the rates of planting activities in Malaysia increase tremendously. Until now oil palm has monopolized the agriculture crop in Malaysia.

2.1.2 The palm oil industry in Malaysia

The palm oil industry played an important role in a development of Malaysia economy especially in the agriculture sector. Based on Malaysia Palm Oil Council (MPOC) statistical in 2009, production of oil palm, Malaysia has become second largest producer of palm oil after Indonesia, representing about 41% of a total world market. Presently, both Malaysia and Indonesia dominate over 80% of the world production of oil palm. Production of oil palm industry in Malaysia has risen extensively in recent time from 94 000 tons in year 1960 to 15 million tons in year 2005 particularly in the production of oleo-chemical, bio-fuel industries and oil and fats to fulfill the world demand in various usages. The production of oil palm during the various years of the last century in Malaysia is shown in Table 1.

Table 1: World production of oil palm

Countries	Years (000' tonne)					
	1980	1985	1990	1995	2000	2005
Malaysia	2576	4133	6088	8123	10842	14962
Indonesia	691	1243	2413	4220	7050	14070
Ivory coast	182	-	270	285	278	260
Nigeria	433	386	580	660	740	800
Columbia	74	-	226	388	524	661
Papua New Guinea	35	-	145	223	336	310
Thailand	13	-	232	355	524	685
Brazil	12	29	66	75	108	160
Others	875	1041	1000	5994	5191	1826
Total	4891	6832	11020	20322	25594	33733

Oil world (2010); MPOB (2010)

Based on the above statistic estimation on palm oil plantation area by MPOB (2010), it is postulate there will be about 152,133 hectares of oil palm that will be ready to be replanted in year 2010 and about 140 trees of oil palm in each hectare. This will represent about 21.2 millions of oil palm trees. For each tree, it is estimated to be around 1 tonne of oil palm trunk biomass available which is carried out about 21.2 million tonnes of oil palm trunk biomass available each year that are ready to be used. Therefore, the promising and sustainable amount supplies of this biomass for each year available as long as there are new replantations of oil palm are made. Figure 3 showed the planting oil palm area in Malaysia in the decades of the last century.

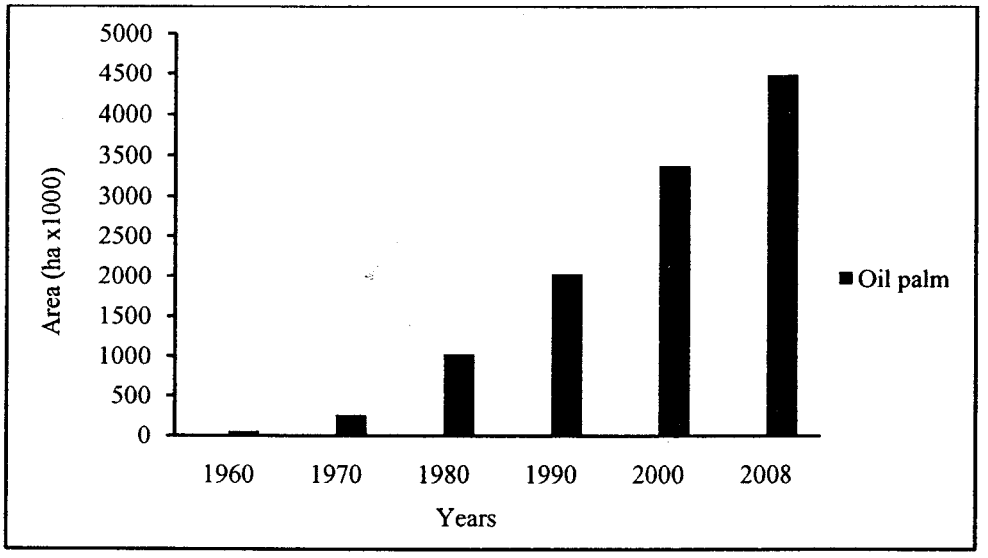

Figure 3: Area of oil palm planting in the decades of the last century (MPOB, 2000; MPOB, 2010).

The availability and consistency of supply of palm oil has been a main factor in stabilizing global market. At the end of 2007, areas under oil palm has climbed up

from 54 000 hectares in year 1960 to 4.24 million hectares. Oil palm was occupied approximately about 60% of agriculture area in Malaysia and its led to a major commercial planting in Malaysia replaced other agriculture crop such a rubber, coconut and cocoa (Basiron, 2008; Basiron, 2002).

2.2 Breeding of oil palm

2.2.1 Development of breeding program

The early oil palm breeding program in Malaysia was started by using Deli Dura seedling discovered by Jack and Jagoe in 1930's which is taken from Bogor Botanical Garden palms (Hartley, 1988). This breeding material was introduced to the Department of Agriculture (DOA) in Serdang and Elmina Estate on 1920's. Due to limited number of parents, many breeding program was established with the objective to improve the high yield bunch with good quality and high oil yield. Besides, the pest and disease tolerance, suitability of seeding to growth well in climate and soil in Malaysia was considered as a serious factor during selection breeding program (Corley and Tinker, 2003). Figure 4 showed the history and development of Deli dura varieties in Indonesia and Malaysia until 1979.

Extension from these programs, the oil palm material from Indonesia and Africa has been brought by United Plantations Berhad (UPB) in 1927's. Oil palm of Malaya (OPM) of Kumpulan Guthrie Berhad (Guthrie) on Ulu Remis and Eleais Estates

and Societe Financiere de Caoutchouces (Socfin) on Johore Labis Estate were established in 1933's. Further, more research organization such Palm Oil Research Institute of Malaysia (PORIM) currently known as Malaysian Palm Oil Board (MPOB) and Malaysian Agricultural Research and Development Institute (MARDI) has been established exclusively in seeking genetic, creation new breeding and seeding by intercrossing seedling from various origin (Khushairi and Rajanaidu, 2000).

The research on palm oil breeding has carried out since 1960s. The research on *Dura* variety with the poor quality and low oil yield in the range of 12-16% has comes out by exploiting this cultivars with Pisifera variety to produced Tenera palm, which result in increased in oil yield up to 25%. From 1960 to 1970, Tenera breeding was planted widely and undertaken the commercial planting of Deli dura material (Hartley, 1988; Kushairi and Rajanaidu, 2000).

Collaborative research centers in Malaysia under 'International Experiment' such as Cooperative Breeding Scheme (CBS) and Sabah Breeding Schemes or programme (SBS) with international research institute, successfully create a new breeding seedling; E206 Dumpy dura (under SBS programme). By 1963, more local research institute such Guthrie, Golden Hope, Dunlop and Pamol involved in the formation of Oil Palm Genetic Laboratory (OPGL) with the same objective to enhance oil palm seeding and breeding quality though research activities. Extensive breeding program was developed in 1964 at the Sabah Department of Agriculture in Ulu Dusun that involve in a large collection of oil palm material from various research centers from

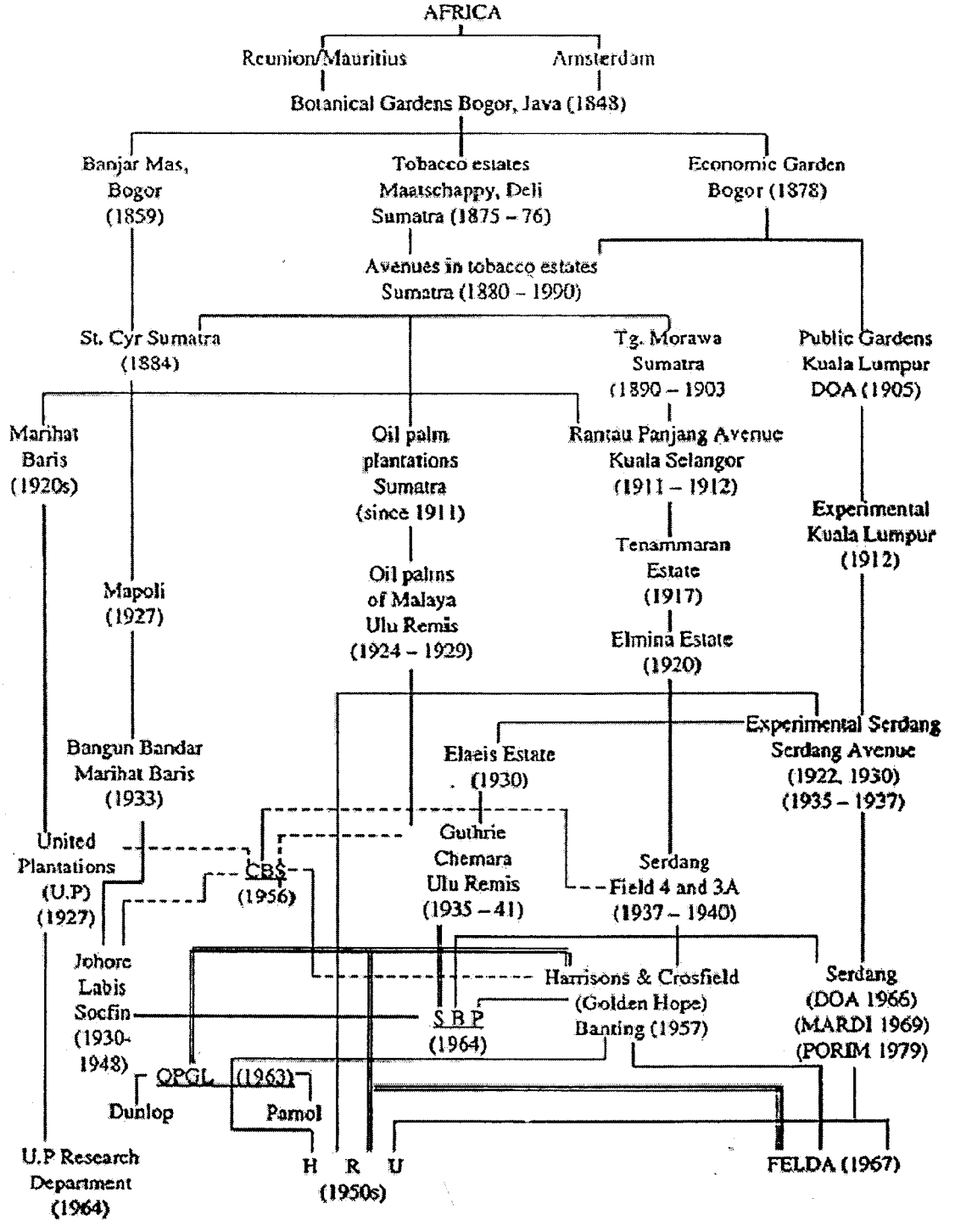

Figure 4: History and development of Deli dura in Indonesia and Malaysia till 1979. (Kushairi and Rajanaidu, 2000).

Malaysia and Institute for Oil Palm Research (NIFOR) for further research and development of oil palm cultivation in the country. Many hybrid breeding has been produced by breeding program such an AVROS, Yangambi, Dumpy and etc, to fulfil the market satisfaction (Corley and Hardon, 1976; Hartley, 1988).

2.2.2 Selected oil palm breeding

Four hybrid breeding, which are Dura x Pisifera mix, Dumpy x Yangambi x AVROS, Deli Dura x Yangambi (D3D x L236T) and Deli Dura x Pisifera x H&C randomly, selected from species *Elaeis guineensis*. Early observation revealed the presence of these cultivars; Dura, Pisfera, Dumpy and Tenera, could be differentiate by outer appearance of the fruit. Hartley (1988) found that the classification of the varieties is related to an anatomy of the palm fruit. The Dura palm consist thick pericarp or exocarp, 2 and 8 mm, thick endocarp (shell) and generally large kernel. The Pisfera has a fruit with thick mesocarp, less endocarp (shell) and small kernel. The Tenera is the product of the cross of Pisfera and Dura. It contains thick mesocarp, thin endocarp and middle sized of kernel. Dumpy palm is a short stem found on Deli plantation at Elimina. The progeny of Dumpy was selected based on the satisfaction crossing outcome, high yielding short stem palm. However due to a low percentage of clean fruit per bunch, this Dumpy palms seed were crosses among different oil palm seedling; Deli Dura, Pisifera or Tenera to obtain a high percentage of fruit bunch and produced a intermediate tall palm stem (Owolalarafe *et al.*, 2007; Kushairi and Rajanaidu, 2000; Opeke, 1982; Basiron *et al.*, 2000). Research in crossing among selected varieties look very

promising especially in term of yield and quality of fruit. The new genes such Yangambi, AVROS, Chemara, H&C and etc is produced from the hybridizations between varieties; Dura x Pisifera, Dura x Tenera and Dura x Dura. Moreover, each and every type of these new genes will present different characters which affect the final outcome (Ascenso, 1965; Hartley, 1988). Figure 5 showed the varieties of palm oil fruit from species *Elaeis guineensis*.

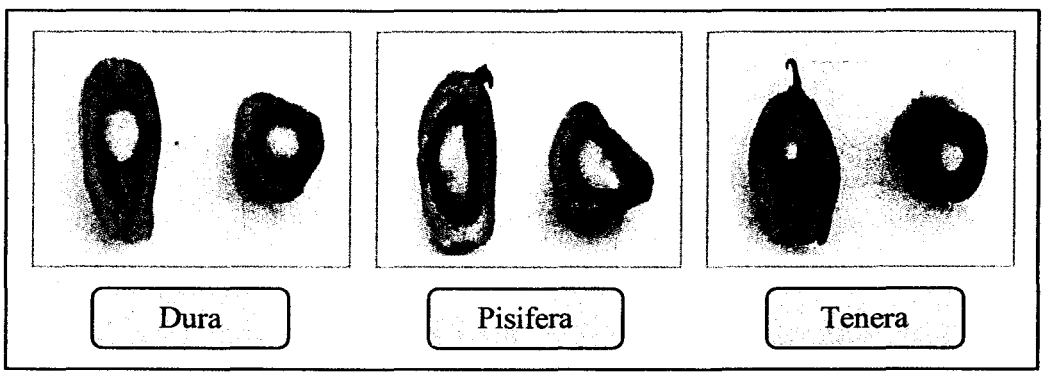

Figure 5: The varieties of palm oil fruit from species *Elaeis guineensis*; Dura, Pisifera and Tenera (Sime Darby, 2010).

Breeding program has been introduced to Division of Agriculture, Malaysia with the main objective to increase the oil yield. The mutation breeding play an important role for further use especially in fuel, nutritional products, food, nutraceuticals and pharmaceuticals industry. The objective of these breeding programs are; 1. Increased the oil yield, 2. Enhanced of oil quality, 3. Produced short palms tree, 4. Resistance to desease, 5. Physiological traits (bunch index, total dry matter and bunch dry matter), 6. Exploitation of GxE interaction (Rajainaidu *et al.*, 2000)

2.3 Oil Palm Biomass

2.3.1 Oil palm trunks

Oil palm industries generate a large amount of oil palm biomass throughout the harvesting and processing activities. As shown in Figure 6, the massive amount oil palm trunks biomass generated after harvesting activities.

Figure 6: The oil palm trunks biomass after harvesting activities.

In 2005, Malaysia alone produced more than 30 million of oil palm biomass includes empty fruit bunch, palm kernel, shells, front and palms trunks that remaining after processing activities. Improper management of this biomass could create serious environmental problems. The oil palm trees have an average economic life span approximately 20 and 25 years depending on the economically harvestable height and non productivity of oil palm fruits. Mature oil palms tree can grow up to 20 feet in height. Oil palm typically is removed from production when they reach 25 feet due to a

difficulty in harvesting. Oil is the main commercial product of the oil palm, however it just contain 10% from the total oil palm biomass while the rest is remaining as a residue (Husin, 2000; Lim *et al.*, 1997).

Traditionally, during harvesting activities, the old and rejected tree was felled. The tree trunks were being burned on the plantation or mulch before being left on the ground as a natural fertilizer to reduce waste, while frond part are left rotting between the rows of palm trees, mainly for soil conservation, erosion control and ultimately the long-term benefit of nutrient recycling. However, it will cause significant losses of organic material and contribute to an environment problem such air and water pollution (Suhaimi and Ong, 2001). Likewise empty fruit bunch, after the oil and kernel have been extracted the fibre from the fruit bunches have been used as a main source of solid combustion to generate power at the mills. However all these afford to manage this biomass still not enough and most of them have no practical way to utilize thus it has become troublesome waste (Balat *et al.*, 2008).

Manufacturing using oil palm biomass as raw material was envisaged a long time ago. Research and development (R&D) program on oil palm trunks was started 20 years ago and it has opened a great opportunity to utilize this biomass into value added product. The product from this residue offered the best prospect as a raw material and could be commercialize that is provided additional revenue to an industry. Through several research work and manipulating the product to enhance the quality, the oil palm trunk successfully could be use to substitute or partially used in the plywood

manufacturing and production of panel product such as particleboard, cement board, medium density fiberboard (MDF) and etc. (Chew and Bahtia, 2008).

Nowadays, Malaysia research has stepped up to another level by convert every part of this biomass into bioethanol product. Exploitation on biomass fuel effectively improves the quality and yet it can be used to generate power plant. As a major part of biomass, oil palm trunks show an excellent potential to replace the other natural source in production of ethanol for the future.

2.3.2 Growth and morphology of oil palm tree

Oil palm or *Elaeis guineensis jacq.* is one of the palmae families. It is single stem plant and may reach to a length of 20 m to 30 m tall. This palm is growing well and produces higher bunch production at a tropical climate like Malaysia which is provide plenty of sunshine with the average annual of rainfall about 2000 mm and yearly temperatures ranging from 25 to 28°C. Soil also one of the important factors that would affect the growth and production of oil palm. Mostly, the hilly soil with the suitability type of soil would give the satisfactory yield and extension of the stem due to the exposure to a sun (Hartley, 1988).

Palm oil is monoecious plants which grows and produce inflorescences either male or female or sometimes for the young palm, during the transitional stage between male and female cycle the hermaphrodite may occur (Latiff, 2000). The

inflorescences grow in the left axils and for both sexes is a compound spadix with 100 to 200 branches, initially enclosed in a spathe or bract that splits 2 weeks prior to anthesis. There are several studies by Owolalarafe *et al.*, (2007), Kushairi and Rajanaidu (2000), Hartley (1988) and etc. that have been made to identify the varieties of the oil palm tree. The classification of oil palm tree into their species is based on characters of fruit (Tomlinson, 1961; Corley and Tinker, 2003).

2.3.2.1 Anatomy of Oil Palm trunk

The oil palm tree is a monocotyledon species of flowering plants. This tropical plant is an unbranched plant and with the single stem. A mature stem growth is an erect and sheltered by persistent frond bases. The stem supports a crown of fronds and at age 12 and 15 years of ages, it may carry 25 to 40 fronds. The fronds contain leaflets which is pinnate with dark green leaf, ranging about 3 to 5 cm. Because the oil palm tree is a non wood tree, it does not comprise cambium, secondary growth, annual rings, ray cells, sap wood and heart wood or branches and knot (Bunting *et al.*, 1934; Killman and Lim, 1985). From the cross section area, the oil palm trunks could be divided into 3 parts; inner, middle and peripheral part as been shown in Figure 7.

According to Hartley (1988), parenchyma tissues were functioned as a storage organ to reserve the photosynthesis product. These tissues are sclerotic; present in mass amount to make it bulk and fulfill the structure of oil palm trunks. Observation before by Tomlinson (1961), shows the ground parenchymatous cells consist of mainly thin – walled spherical cell and the walls of these parenchyma cells gently darker and thicker from pith to peripheral part. In this part, starch grain and silica containing cells could be revealed in abundantly. The parenchyma spongy and lightweight due to its structure that contain a wide air canal exist between the lobed cells which is make it become lacunose and easily absorbs the moisture. These cells were arranged around the vascular bundle and easily separated from each others. Figure 8 showed the anatomy of the oil palm trunks from transverse section of vascular bundles.

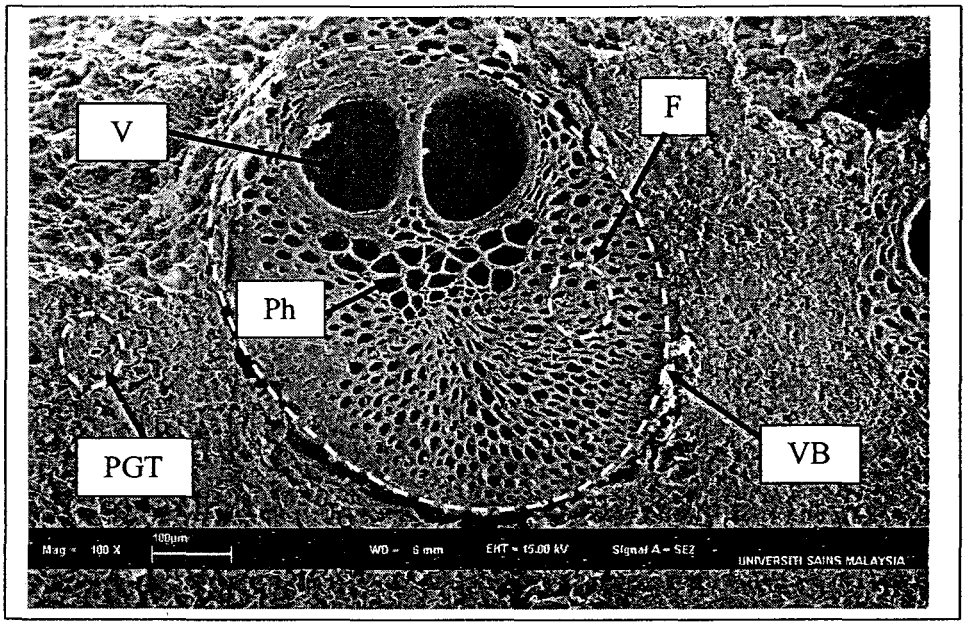

Figure 8: Transverse section of vascular bundles with vessels in oil palm trunk fiber (F), parenchyma ground tissue (PGT), vascular bundle (VB), vessel (V) and phloem(Ph)

The anatomy of the oil palm stem could be explained by division of three major zones; the core zone, center zone and peripheral zone (Corley, 1976). The inner zone, consist a much less congested of vascular bundles compare to central zone. According to Killman and Lim (1985) the growth and development of oil palm stem is dependent on the overall cell division and cell enlargement in the parenchymatous tissues together with enlargement of the fiber of the vascular bundle in this zone. The central part (inner and middle part) is a largest region which is accounts for 80% of the total area. It contained a large and widely scattered vascular bundles embedded in the ground parenchymatous tissue. For peripheral part, it contains a narrow layer of parenchyma and crowded with vascular bundle make it rise to a sclerotic zone. Thus, it provided the main mechanical support to a palm stem. The bark is the most outer part of the palm tree. This part is a narrow cortex with a wide approximately 1.5 cm. Normally, there is very hard peripheral rind surround by soft central zone.

2.3.2.2 Physical properties Properties of Oil Palm trunk

2.3.2.2.1 Moisture content and density

Moisture content is defined as an amount of water retained in trunks tissue. The variation of moisture content oil palm trunks is apparently from bottom to upward and outer to core. The main function of oil palm trunks as a storage organ and it deposit high amount of water and nutrient especially inside the parencymatous tissue (Killman and Lim, 1985; Lim and Khoo, 1986). Table 2 showed the previous study on moisture content and density of oil palm trunks by the various researchers.

Table 2: Moisture content of oil palm trunks by the various researchers

Physical properties	Study on oil palm trunks (OPT)				
	Lim and Khoo (1986)	Gan *et al.*, (2005)	Balfas (2006)	Bakar *et al.*, (1998)	Others researcher
Moisture content (%)	120 to 500	80 to 380	100 to 500	258 to 575	76.5 to 575.4 (Killman and Lim, 1985)
Density (kg/m^3)	190 to 575	141 to 635	210 to 670	110 to 400	230 to 520 (Husin et al., 1986)

The freshly felled oil palm trunks contain high moisture contain and the proportional is uneven between each parts. Inner part contains high moisture content compared to peripheral part. Different trunks height also shows difference moisture content which is gradually rising along the trunks height (Lim and Gan, 2005; Balfas, 2006). Balfas (2006) indicate in his study that the moisture content from bottom to upward is in the range from 60 to 170%.

Lim and Gan (2005) explained in their paper that the differences moisture content for each zone or height can be explained by the different ratio between parenchymatous tissue and vascular bundle distribution within the oil palm trunks. The peripheral part contain abundant amount of vascular bundle compared to middle and less in inner part. The properties of vascular bundle are less hygroscopic compared to

parenchyma naturally spongy and have a high capacity in water absorption to store in the tissue cell (Killman and Lim, 1985; Husin, 2000).

Density is an important properties and strongly influence the mechanical properties of the palm tree. The density for each zone (inner, middle and peripheral) in the oil palm trunks is different radially and vertically and it has been recorded by Killman and Lim (1985), Lim and Gan (2005) and Balfas (2006). The previous study on density of oil palm trunks by the various researchers is shown in Table 2.

According to Killman and Lim (1985), the variation in density of the oil palm trunks is dependent on several factors such as anatomical structure; distribution of vascular bundle, fibers, dense of cell wall parenchyma tissue and fibrous sheaths arrangement. Generally, the density in the inner part is lower than the peripheral part due to distribution of fibrous vascular bundles are much less congested in the inner and middle part compared to a peripheral part.

Variation density in term of height the relation between height and density was not clear. However according to Balfas (2006), the density value decreased proportionally with the trunks height and it may due to the growth of new tissue at the upper part of palm tree. The younger vascular bundle and cell wall with small size make this part less dense than bottom part which contains former tissue of the palm tree (Bakar *et al.*, 1999).

2.3.2.3 Chemical properties of oil palm trunks

The oil palm biomass in a trunk compose large amount of living tissue also known as lignocelluloses. These lignocelluloses consist of three basic polymers; cellulose, hemicelluloses and lignin with small amount of extractive and ash content. Cellulose is a homopolymer and its fibers provide the mechanical strength to a tree. Hemicelluloses or polyose is a mixture various of monosaccharides such as glucose, xylose, mannose, galactose, arabinose, fructose and 4-0-methyl glucuronic acid. Lignin is a complex structure, resistance to microbial or many chemical agent and functional as a bound cellulose fiber to form lignocellulosic structure (Balat *et al.*, 2008; Murai *et al.*, 2009).

In this research, we will concentrate on hemicelluloses as a carbohydrate reserve. Husin (2000) in his study indicates that carbohydrate contain appreciable amount of short chain of polysaccharides and starchy material. All of these materials were obtained from photosynthesis process which is essentially converts the water and carbon dioxide to carbohydrate by absorbing solar energy through chlorophyll. Carbohydrate produced from this process could be convert into polysaccharides and other material such as starch and could be revealed abundantly in the parenchymatous tissue (Hartley, 1988; Corley and Tinker, 2003).

Polysaccharides in oil palm trunks including glucose released from cellulose and hemicelluloses derived from a various monosaccharides such as mannose, galactose, xylose and arabinose suitable to be utilized for the production of bio-ethanol (Balat *et*

al., 2008). The studies on conversion of oil palm biomass into the reducing sugar with the different technique and method have been investigated before, however it is very limited. An earlier study has shown nearly 10% of free sugar has been accounted and constituents of 3 main free sugar; sucrose, glucose and fructose distributed along the trunks which is the center part was contributed to the higher proportion of free sugar (Husin, 2000; Balat *et al.*, 2008; Murai *et al.*, 2009).

Starch is a major carbohydrate component in the storage tissue inside the plant. It is homopolymer which consist of only one monomer known as D-glucose. It consists a two polymer inside D-glucose; amylose in a linear structure and branches linkages of amylopectin (Buleon *et al.*, 1998; Copeland *et al.*, 2009). The structure of cellulose and starch are quite similar; i.e. the glucose structure in a starch is α-1,4-linkages and for cellulose has β-1,4-linkages and make it a linear and more rigid structure compared to starch (Linde *et al.*, 2008; Balat *et al.*, 2008). Previous studies by Rahim and Abdul Razak (1987), shows that chemical composition varies with height and zone. The core and central zone composed enormous amount of carbohydrate compound which is accumulated in the parenchymatous tissue.