

Old Oil Palm Trunks: A Promising Sources of Sugar for Biomass Refinery

Akihiko Kosugi

Japan International Research Center for Agricultural Sciences (JIRCAS),

Present Address: 1-1 Ohwashi, Tsukuba, Ibaraki 305-8686, JAPAN

e-mail: akosugi@affrc.go.jp

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. We found that old oil palm trunks which were felled for replanting contained large quantity of sap with high glucose content. Especially, in the inner part of the trunk, sap accounted for more than 80% of the whole trunk weight. Glucose concentration in the sap from the inner part was 85.2g/L and decreased towards the outer part. Based on these findings we tried to ferment the sap to produce ethanol and lactic acid using sake brewing yeast strain, *Saccharomyces cerevisiae* Kyokai no.7 and a homolactic acid bacterium, *Lactobacillus lactis* ATCC19435. Ethanol was produced from the sap without addition of nutrients at comparable rate and yield to the reference fermentation on YPD medium with glucose as a carbon source. Likewise, the sap was readily converted to lactic acid with almost the same efficiency as the reference fermentation on MSR medium with glucose as a substrate. On the other hand, we also found that sugars existing in the sap increased remarkably during storage after logging. Total sugar in the sap increased from 83 mg/ml to 153 mg/ml, the concentration comparable to that of sugar cane juice. These results strongly 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 biomass refinery.*

Keywords: *Elaeis guineensis*, oil palm, trunk, sap, sugar, ethanol production, lactic acid production

INTRODUCTION

Palm oil is the most produced plant oil with the worldwide production of 43 million tons in 2008 (USDA statistics: PS&D online). 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 others, it is widely used for industrial purposes such as detergent and cosmetics in addition to food such as margarine and frying oil. Recently palm oil is also considered for the material to produce biodiesel and bioplastics. 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. Plantation area in Malaysia and Indonesia in 2007 is 4,304,913 ha 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 planted 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 (1). Consequently, the felled palm trunks can be regarded as one of the most important biomass resources in Malaysia and Indonesia.

However, the palm trunk structure is not strong enough for the use of lumber, and, thus, only outer part of the trunk, which is relatively strong, is partly utilized for plywood manufacturing (Fig 1A). In the plywood production process, inner parts are discharged in

large amounts because of its extremely weak physical property (Fig 1B). Meanwhile, it has been known that palm sugar and palm wine are produced from sap obtained by tapping the inflorescence of varieties of palm species including *Arenga pinnata*, *Borassus flabellifer*, *Cocos nucifera*, *Nypa fruticans* and oil palm (2). In order to utilize the old palm trunks felled for replanting, we made an attempt to produce bioethanol and lactic acid, the material for bioplastics, from felled trunks. We focused on sugars in the sap of the felled trunk and found that large quantity of sap with high glucose content existed in the trunk. This is the first report that described the amount, composition and change of sugars contained in the sap of felled oil palm trunks (3, 4). 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 biomass refinery.

MATERIALS AND METHODS

Sample preparation Three oil palm trunks aged approximately 25 years were obtained from a local plywood manufacturer, Business Espri Co., in Penang Province, Malaysia. A disk with 7 cm thickness was taken from the middle part of each trunk ranging 10-12 m long. Then the disks ranging 32-42 cm in diameter were cut into three parts, i.e. inner (a), middle (b), and outer (c) parts, as shown in Fig.1C and D. Sap was collected by squeezing the disks with a laboratory-scale press at 80MPa. The sap was centrifuged at 6,000rpm for 15 min and the supernatant was stored at -20°C before use. To investigate effects of storage of the felled trunks, we also obtained three oil palms of *tenera* type aged 25 years old; they 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 also cut into three sections; inner (A), intermediate (B) and outer (C).

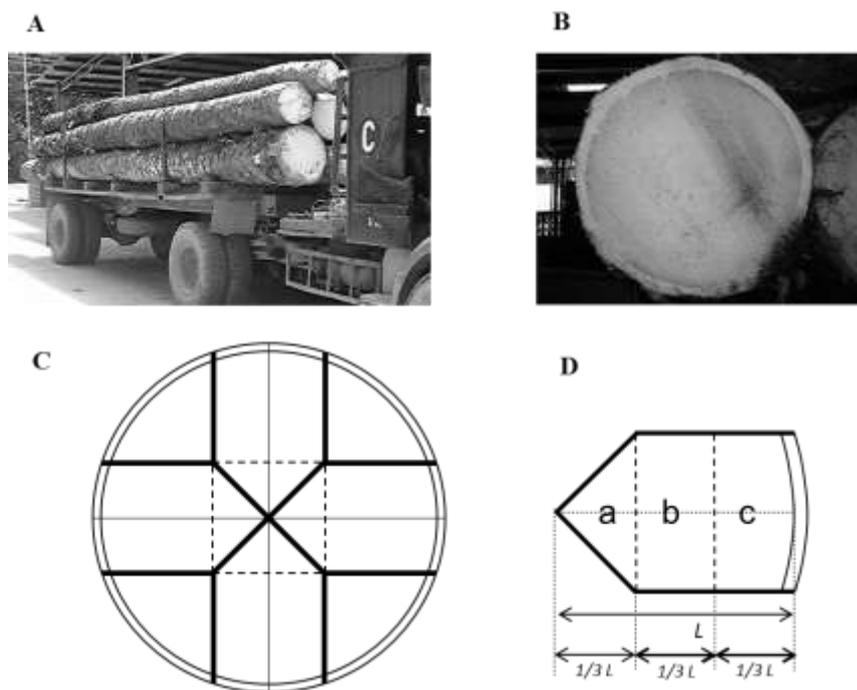


Fig. 1. Pictures of felled oil palm trunks and diagrams of sample preparation for analyses (3). (A) Felled trunks carried to a plywood factory. (B) A disk taken from a trunk. (C, D) Disks were cut into inner (a), middle (b), and outer (c) parts.

Analysis Moisture content was determined by drying at 105°C for 48 hrs. Sugars contained in the sap were determined by high performance anion-exchange chromatography using CarboPac PA (Dionex Corporation, Sunnyvale, CA, USA) with pulsed amperometric detection (HPAEC-PAD). The mobile phase was 2% NaOH at a flow rate of 0.6 ml/min at 28°C. In addition to HPLC method, total sugar content of sap samples was determined by the Dubois method using phenol and sulfuric acid. A filtered sap sample was diluted to 1/3,000 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. Amino acids were analyzed by an amino acid analyzer (Hitachi L-8900). Organic acids were determined by high performance ion exchange chromatography using Sim-pack SPR-H with post-column pH-buffered electro-conductivity detection method (Shimadzu CDD-6A). The mobile phase was 4 mM p-toluene sulfonic acid at a flow rate of 0.8 ml/min at 40°C. Mineral analyses were carried out by inductively coupled plasma atomic emission spectroscopy with Vista MP-X, Varian Inc. Chloride was determined by ion chromatography with the Dionex model DX-500. Thiamine and riboflavin were analyzed by HPLC with fluorescence detection. Ascorbic acid was analyzed by HPLC with a UV-Vis variable wavelength detector. Vitamin B6, pantothenic acid, niacin, inositol, folic acid and biotin were measured by microbiological methods.

Fermentation experiments *Saccharomyces cerevisiae* Kyokai no. 7 obtained from the National Research Institute of Brewing (NRIB) was used for ethanol fermentation experiments. The yeast was pre-cultured on YPD medium containing per liter polypepton (Wako pure chemical), 20 g; yeast extract (Difco), 10 g; and glucose, 20 g. The sap with or without addition of polypepton (20g/L) and yeast extract (10g/L) was used for ethanol fermentation medium. Glucose concentrations of the sap media were adjusted to 55 g/L by adding distilled water and the pH was adjusted to 6.0 with 2N NaOH. The sap media were sterilized with a 0.22- μ m membrane filter (Millipore). Ethanol was determined by using a gas chromatograph (Shimadzu GC-2014) equipped with a flame ionization detector. A glass

column (8 mm by 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). A homolactic acid bacterium, *Lactococcus lactis* ATCC19435, was used for lactic acid fermentation experiments. The bacterium was pre-cultured on MSR medium containing per liter bacto trypton (Difco), 10 g; yeast extract (Difco), 10 g; glucose, 20g; K₂HPO₄, 2 g; CH₃COONa · 3H₂O, 5 g; MgSO₄ · 7H₂O, 0.2 g; MnSO₄ · 4H₂O, 5 mg. The sap was diluted 5-fold with distilled water to make glucose concentration 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. Samples were withdrawn every 24 hrs for determination of lactic acid and glucose. Lactic acid concentration was measured according to the method described for organic acid analysis.

RESULTS AND DISCUSSION

1. *Moisture content of trunk.*

Moisture contents of part a, b, and c were approximately 82.4±1.2%, 76±4.7% and 68±6.9%, respectively (Table 1). Compared to wood timber whose moisture content is normally between 40 and 50%, oil palm trunk contains far more moisture, indicating that large quantity of sap exists in the trunk. Especially the inner part contained extremely high moisture. From 1 g of part a of the trunk, approximately 0.65 g of sap was obtained by the laboratory-scale press used in this experiment.

2. *Sugar composition of the sap prepared from inner, middle and outer parts of oil palm trunk.*

Table 1 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.

Table 1 Free sugars contained in the sap from a felled oil palm trunk (3).

| Free sugars | Area ¹⁾ | | |
|-------------|--------------------|-------------------|------------------|
| | Center (a) g/L | Middle (b) g/L | Outer (c) 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 |
| Others | 0.7±0.3 | 0.6±0.1 | 0.6±0.2 |
| Total | 98.1±5.5 | 60.5±3.3 | 20.1±1.1 |

1) The sap was obtained by squeezing from each area that was delivered from strips in Figure 1 (D).

Results are means of three determinations ± SD.

3. *Chemical properties and compositions of the sap from inner part of the felled palm trunk.*

Chemical properties and compositions of the oil palm sap were analyzed using the sap collected from the inner part of the felled palm trunk. The pH of the sap was approximately 5.0 and the specific gravity was 1.07. As for chemical components of the sap, amino acids, organic acids, vitamins and minerals were analyzed. Total amount of amino acids in the sap was 198.3 $\mu\text{g/g}$ with serine, alanine, glutamic acid, and aspartic acid as major amino acids. Total amount of organic acids in sap was 970.6 $\mu\text{g/g}$ with abundant citric, malic and maleic acids, resulting in slight acidity of the sap. Calcium, magnesium and chloride are contained at high concentrations of 210 and 145 $\mu\text{g/g}$ (There must be three figures for concentrations of calcium, magnesium and chloride.), respectively. The HPLC analyses and micro-bioassay determined various kinds of B group vitamins and vitamin C in the sap. Inositol was found to be contained at a high concentration 640 $\mu\text{g/g}$ in the oil palm sap for the first time. Since the oil palm sap squeezed 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.

4. Ethanol and lactic acid production from oil palm sap.

Using the sap obtained from inner part of the trunk, ethanol and lactic acid fermentations were carried out with *S. cerevisiae* Kyokai no. 7 and *L. lactis* ATCC19435S, respectively. For ethanol production, regardless of addition of polypepton and yeast extract, the yeast rapidly fermented glucose in the sap into ethanol. A representative ethanol fermentation profile on the sap without addition of nutrients is shown in Fig. 2 (a). The fermentation almost completed after 12 hrs and glucose was thoroughly consumed after 24 hrs of fermentation. Meanwhile, the minor sugar components, i.e. sucrose, fructose, and galactose which were contained initially at 4.2g/L, 2.6g/L, and 0.6 g/L, respectively, in the sap medium, were not detectable by HPLC after 24 hrs. 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 production rate and yield were comparable to the reference fermentation on YPD medium, indicating that the oil palm sap has enough nutrients to support ethanol fermentation by *S. cerevisiae* and does not contain inhibiting substances. As shown in Fig. 2 (b), 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 which were initially contained at 16.7g/L, 1.28g/L, 0.79g/L, and 0.18g/L, respectively, in the medium were completely consumed after 72 hrs of fermentation. The yield of lactic acid was 89.9% of the theoretical yield based on consumption of these 4 sugars.

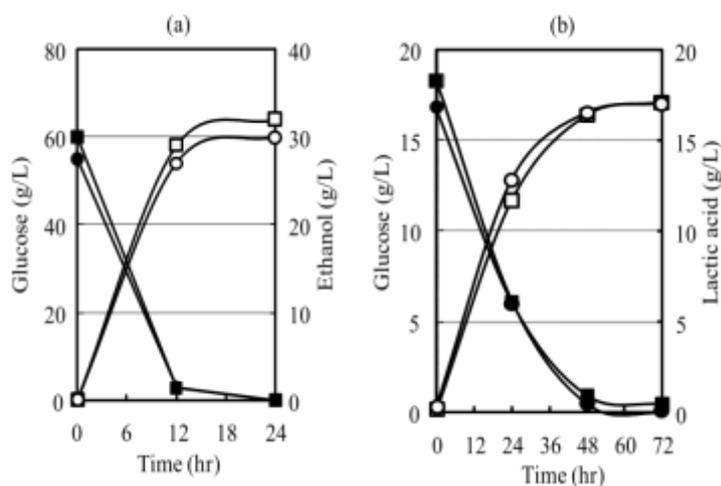


Fig. 2. Time course of ethanol and lactic acid productions using the sap from felled oil palm trunk (3). (a), *S. cerevisiae* Kyokai no. 7 was grown statically at 30°C on the sap containing 55 g/L glucose without addition of nutrients. Reference fermentation was carried out on YPD medium containing 60 g/L glucose. Glucose was determined enzymatically with 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. (b), *L. lactis* ATCC19435 was grown statically at 30°C on the sap containing 16.7 g/L glucose without addition of nutrients. Reference fermentation was carried out on MSR medium containing 18 g/L glucose. Glucose was determined enzymatically with 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.

5. Change in moisture content and total sugar content during storage of oil palm trunks.

Change in moisture content of each oil palm trunk section prepared from middle part of trunk was examined during 120 days of storage. Immediately after logging, moisture contents of Part A, B and C were 78, 75 and 67%, respectively. An average of sugar concentration through the cross section of the disc was calculated by the Equation 1 (EQ1), assuming that the volume ratio of sections A, B and C is 1:3:5.

$$AV\ Conc = (Conc\ A \times 1 + Conc\ B \times 3 + Conc\ C \times 5) / 9 \dots EQ1$$

Part A, the most inner of the trunk, contains the highest moisture among the three sections, and the moisture content becomes lower toward the outer section that is Part C. Change in total sugar content of sap from each oil palm trunk section was plotted against days of storage. Concentrations of the sap sugar in Part A, B and C just after logging were 108, 85 and 76 mg/ml, respectively. During the storage, the concentration unexpectedly increased sharply to become 148, 185 and 134 mg/ml for A, B and C, respectively, after 30 days. 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). The sum of concentrations of three main sugars in the oil palm trunk sap was presented in Fig. 2.

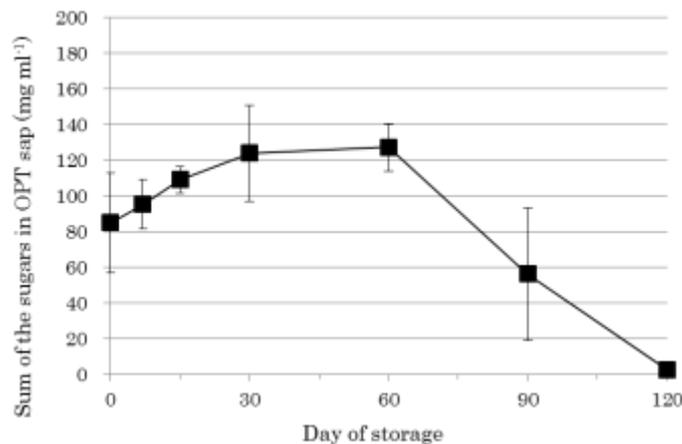


Fig. 2. Sum of the three main sugar concentrations by HPLC analysis in OPT sap during storage. The data were mean of the values obtained from 2 oil palm trees (4).

6. Potentiality of old oil palm as a feedstock for biomass refinery.

To summarize the study here, free sugar content in oil palm trunk sap is the maximum (153 mg/ml for total sugar and 128 mg/ml 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. When the sap of old oil palm trunk compares with sugar cane juice as feedstock for bioethanol, possible ethanol yield from sap of old palm trunk is calculated to be approximately 9 m³/ha, 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 (4, 5). In 2007, the plantation areas of mature oil palm are 3,741,000 and 4,540,000 ha for Malaysia and Indonesia, respectively (4, 5). 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 oil palm trunk is calculated to be approximately 160 t/ha, and 9 m³/ha of ethanol can be produced. It means that roughly 3 hm³ of bioethanol can be produced using the sap of the logged oil palm trunk in Malaysia and Indonesia (5). Unlike sugar cane, bioethanol

production using felled oil palm trunk will not conflict with food usage and have a great potential as a feedstock for biomass refinery (6).

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