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Research article

Ethanol fermentation by the thermotolerant yeast, *Kluyveromyces marxianus* TISTR5925, of extracted sap from old oil palm trunk

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Abstract: Palm sap extracted from old oil palm trunks was previously found to contain sugar and nutrients (amino acids and vitamins). Some palm saps contain a low content of sugar due to differences in species or in plant physiology. Here we condensed palm sap with a low content of sugar using flat membrane filtration, then fermented the condensed palm sap at high temperature using the thermotolerant, high ethanol-producing yeast, *Kluyveromyces marxianus*. Ethanol production under non-optimum conditions was evaluated. Furthermore, the energy required to concentrate the palm sap, and the amount of energy that could be generated from the ethanol, was calculated. The condensation of sugar in sap from palm trunk required for economically viable ethanol production was evaluated.

Keywords: *Elaeis guineensis*; oil palm trunk; waste; bioethanol; thermotolerant yeast; *Kluyveromyces marxianus*

1. Introduction

The oil palm (*Elaeis guineensis*) is one of the most rapidly expanding equatorial crops globally. Plantations are replanted every 25 years due to decreased oil productivity [1]. At least 120,000 hectares of oil palm plantation was estimated to be replanted annually in Malaysia from 2006 to 2010 [2], and 450,000 hectares of oil palm plantation are expected to be replanted annually in Indonesia in the next 25 years. Palm trunks are not used as lumber due to their high moisture content (70–80% based on total mass), resulting in significant warping after drying [3]. The only current practical use for felled oil palm trunks is as plywood [4]. Recently, palm sap squeezed from old oil palm trunks (OPTs) was shown to contain large quantities of sugar, and ethanol was produced from this sugar by fermentation by *Saccharomyces cerevisiae* [5,6]. Sap containing a high content of available sugars (glucose 85.2 g/L) was found in the inner part of the OPT and found to also contain a significant amount of amino acids (serine and glutamic acid), minerals such as calcium and manganese, and several organic acids (such as malic acid and maleic acid). This sap was therefore used directly as a fermentable medium for ethanol or lactic acid production, without supplementation with nitrogen or minerals [6], and could be used as feedstock for bioethanol production without the need for complicated pretreatment such as the saccharification of lignocellulose. However, some palm saps contained a low content of sugar, depending on the species of oil palm or the physiology of the plant. Here we condensed sugar in low-sugar-content sap using flat membrane filtration in order to efficiently utilize the sap.

S. cerevisiae is used as an fermentation yeast for ethanol production in the brewing industry. However, several unique yeasts have been isolated from natural habitats that exhibit unique characteristics for fuel ethanol production using various bioresources as substrates under various conditions [7–9]. *Kluyveromyces marxianusis* is an thermotolerant yeast for bioethanol production at elevated temperatures [10]. The advantages of thermotolerant yeast for industrial ethanol production are: 1. The high fermentation activity of thermotolerant yeasts at elevated temperature decreases the chances of bioreactor contamination. 2. The optimum temperature for *S. cerevisiae* is 28–32 °C whereas *K. marxianus* has a wide optimum temperature range, from 28 to 45 °C. 3. Cooling and distillation costs are decreased when fermentation is conducted at 40 °C. 4. Increased enzyme activity at higher temperature should decrease the need for added enzyme and thus decrease the overall costs for exogenous enzyme [11].

We isolated the thermotolerant yeast, *K. marxianus* TISTR5925, from rotting fruit in Thailand. This yeast strain can produce ethanol by hydrolyzing cassava pulp, the waste product from starch factories [12]. In this study, we compared the growth of three thermotolerant yeast strains, *K. marxianus* NCYC578, NCYC2791, and TISTR5925 with three non-thermotolerant yeast strains, *S. cerevisiae* kyokai7, NCYC3233, and S288c at elevated temperature. In addition, we studied the fermentation of condensed sap from oil palm trunk by *K. marxinanus* TISTR5925 and *S. cerevisiae* Kyokai7 as representative strains of the two species at temperatures from 37 °C to 50 °C. The energy required to concentrate the sugar in the sap by flat membrane filtration was measured. To evaluate ethanol production from palm sap, the total energy required to prepare and concentrate the palm sap was taken as the input energy, and the potential energy obtained from the produced ethanol and the effective energy obtained from the solid residue after squeezing sap from oil palm trunk were taken as the output energy. The Net Energy Ratio (NER) is the proportion of the output energy to the input energy; the NER for different contents of sugar in the sap were compared to identify the sugar

content required for economically-viable ethanol production.

2. Materials and Method

2.1. Preparation of sap from old oil palm trunk

OPTs 23–25 years old were harvested from several plantations in Johor province (1o 28′0″ North, 103°45′0″ East), Malaysia. In initial studies, 26 oil palm (*E. guinesis*) trees were cut in Malaysia and their sap was squeezed [13]. At this time, logged trunks were cut into 1.2 m lengths, and then debarked to the trunk core (1.2 m long × 20 cm diameter). Cores from three trees were prepared. The trunk cores (approx. 30 kg) were shredded and squeezed and palm sap was obtained using the method of Murata et al. [13]; a total of 17 kg sap and 10.1 kg solid residue after extraction of sap was obtained from one trunk core [13]. The squeezed sap and the residues of oil palm trunk were stored at −20 °C to avoid contamination of microbes. After thawing and analysis sugar content, the sap was used for ethanol fermentation and the effective energy obtained from combustion of the residue was estimated.

The content of various sugars in the sap was measured by HPLC (TOSOH, Osaka, Japan) using a CARBOSep CHO-682 LEAD column (Transgenomic Inc., Omaha, NE, USA), a temperature of 80 °C, a flow rate of 0.4 mL/min, and a refractive index (RI) detector (TOSOH RI-8020). The sugar components were identified by comparing their retention times with those of authentic standards.

2.2. Membrane filtration of the palm sap with thin sugar

When the total sugar in the sap was less than 5%, the sap was condensed to at least 10% total sugar using a flat membrane filtration system built by Sanko Ltd. (Kanagawa, Japan) and consisting of a membrane filtration device (LabStak M20; Alfa Laval, Lund, Sweden) and a high pressure pump (Wanner Hydracell G10-X; Wanner Engineering, Minneapolis, MN, USA). The palm sap was pumped into the membrane filtration device using a high pressure pump and then condensed by the reverse osmosis membrane (FILMTEC membrane, BW30-365 high rejection brackish water RO element, Dow Chemical Co., Minneapolis, MN, USA) in the LabStak M20.

2.3. Growth of the yeasts on YPD plates and ethanol production from oil palm sap

K. marxianus TISTR5925 was isolated from rotting fruit from a local market in Thailand [12]. *S. cerevisiae* Kyokai7 was purchased from the National Research Institute of Brewing (Hiroshima, Japan), and *S. cerevisiae* NCYC3233, and *K. marxiaus* NCYC587 and NCYC2791, were purchased from the National Collection of Yeast Cultures (Norwich, United Kingdom). Yeast extract, peptone, and agar were purchased from Difco Laboratories (Detroit, MI, USA). Yeast extract peptone dextrose (YPD) medium comprised 1% yeast extract, 2% peptone, and 2% glucose. Liquid YPD medium was used to prepare plates by adding 2% agar. 10% YPD (1% yeast extract, 2% peptone, 10% glucose) was used as a control fermentation medium for comparison with palm sap and for pre-cultivation. All chemicals, except for the medium, were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

K. marxianus (NCYC587, NCYC2791, TISTR5925) and *S. cerevisiae* (Kyokai7, NCYC3233, S288c) were precultured in liquid 10% YPD medium at 30 °C for 24 h, then streaked on YPD plates and incubated at 30 °C and 45 °C. Of the six strains, TISTR5925 and Kyokai7 were used as representative strains of *K. marxianus* and *S. cerevisiae,* respectively, to ferment palm sap. Precultures of these strains (3 mL) were centrifuged for 5 min at 4,000 x g and the pellets were washed twice with sterile water. The cells were resuspended in 3 mL palm sap (without added nitrogen sources), and ethanol fermentation was conducted at temperatures between 37 °C to 50 °C. Each fermentation was repeated at least three times. The ethanol produced and glucose consumed were monitored simultaneously by HPLC using an Aminex HPX87-H column (300 mm × 7.8 mm; Bio-Rad, Hercules, CA, USA). The ethanol and glucose peaks were identified by comparing the retention times with those of standards [14]. The column was operated at 65 °C and a flow rate of 0.6 ml/min using sulfuric acid (5.0 mM) as the mobile phase. The data were processed using Chromato-PRO (Runtime Instruments, Kanagawa, Japan).

2.4. Measurement of energy consumed for flat membrane filtration

The power (kW) required to concentrate the sap was measured using an electric power meter (Clamp on Power Hi Tester 3169-01, HIOKI E.E. Corp. Ueda, Nagano, Japan). The input energy was then converted into thermal terms using equation 1:

The energy required for sap content (mega joule: MJ)

= (Average power (kW) x running time (h) x 3.6 MJ)/0.4 (Equation 1)

Units and factors:

*a Average power (kW): Average of the power required to concentrate the palm sap

*b kWh: = 3.6 MJ = 3.6×10^6 joule

*c 0.4: the conversion coefficient used in Japan for electric power produced from petroleum oil

The output energy was estimated from the energy in the ethanol produced from the palm sap. The effective heating value for ethanol was obtained by subtracting the heat of vaporization of water from the heating value of pure ethanol, and used to estimate the net energy ratio. The effective heating value of ethanol is 21.2 MJ L^{-1}. The power required to drive the other devises (rotary lathe, shredder, and mill) to shred and squeeze the oil palm trunk were taken from Murata et al. [13].

3. Results and Discussion

3.1. Condensation of palm sap with thin sugar by flat membrane filtration

We developed a system consisting of a shredder and mills for squeezing palm sap from debarked old oil palm trunk cores, and the saps were extracted from several oil palm trunks [13]. The sugar components in the sap were identified by their HPLC peaks as sucrose, glucose and fructose (Figure 1A Untreated sap). The major sugar was glucose (28.8 g/L), followed by fructose (around 2.4 g/L); little sucrose was detected (Table 1 Untreated sap). In contrast, the major sugar in unfermented sap from oil palm tree grown in Nigeria is sucrose, with both glucose and fructose present as minor sugars [15,16]. In our earlier research, glucose, sucrose and fructose were contained in the sap from oil palm trunks [10,20]. A significant amount of sucrose was detected in oil palm sap from one trunk

(Figure 1 and Table 1, Condensed sap 2) but little (Figure 1 and Table 1, Condensed sap 1) or none (Table 1, Condensed sap 3) in oil palm sap from other trunks, indicating that the content of specific sugars depends on the species of oil palm, its physiological state, and cultivation conditions.

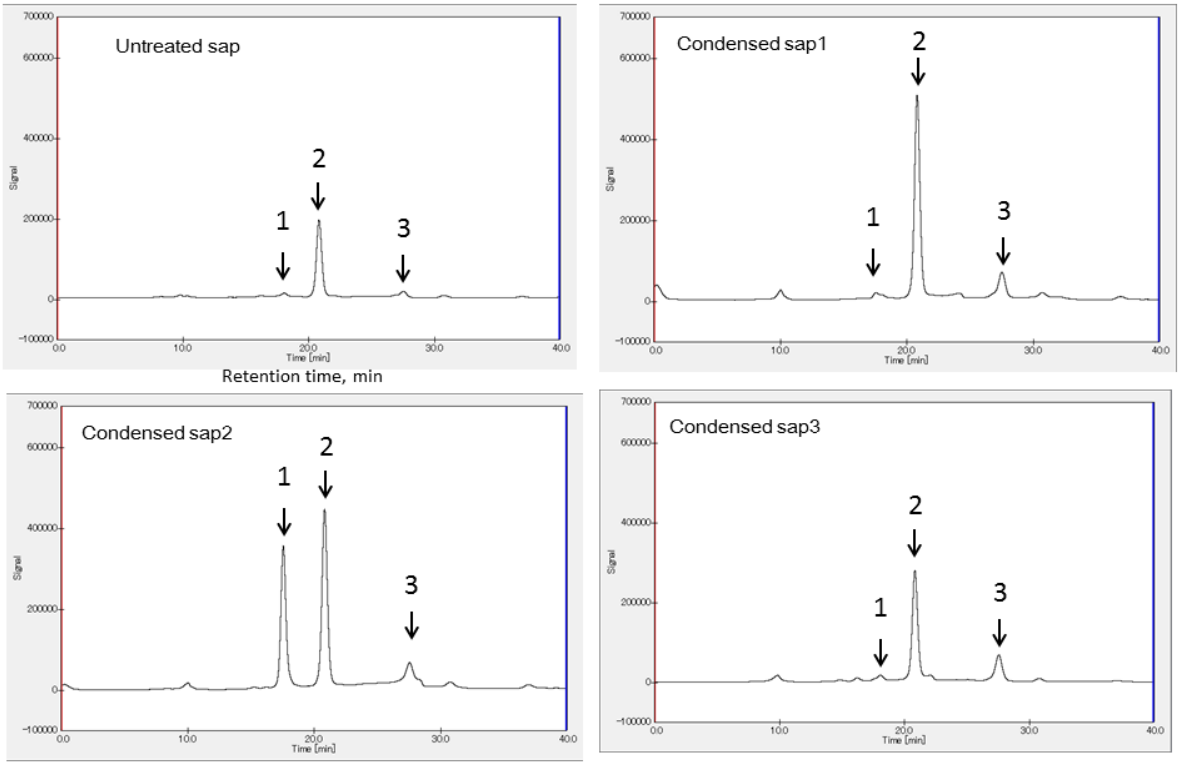

Figure 1. Sugar peaks in sap squeezed from oil palm trunks. HPLC analysis of sugar components in sap squeezed from oil palm trunks. untreated sap, condensed sap 1, condensed sap 2, condensed sap3. Peak 1: sucrose, Peak 2: glucose, Peak 3: fructose. No peak corresponding to sucrose was observed in condensed sap 1 and condensed sap3 but a peak corresponding to sucrose was observed in condensed sap 2. The X axis shows the refractive index, and the Y axis shows the retention time of the sugar.

Sucrose and starch are the major products of photosynthetic CO_2 fixation. Triose phosphate (e.g., glycerol aldehyde-3-phosphate; 3PGA) is produced during CO_2 fixation and is transported from the chloroplast to the cytosol, where it enters the sucrose biosynthetic pathway. Sucrose is biosynthesized by the phosphatase reaction via formation of sucrose-6-phosphate from fructose-6-phosphate and uridine diphosphate glucose (UDP-glucose) [17]. In contrast, starch is synthesized via an elongation reaction of adenine diphosphate glucose (ADP-glucose) from triose phosphate remaining in the chloroplast. Starch and sucrose synthesis is regulated by the inorganic phosphate (Pi) content in the cytosol [18]. Triose phosphate transporter, located in the chloroplast envelope membrane, exchanges triose phosphate for Pi between the stroma and cytoplasm. If the level of Pi in the cytosol is decreased, triose phosphate is not exported from the chloroplast stroma to the cytosol. Consequently, triose phosphate remains in the stroma and enters the starch biosynthesis pathway, and starch accumulates in the chloroplasts [17,18]. Starch synthesis is also favored by high

light conditions, high temperature, and by the presence of triose phosphate [18]. Therefore, starch and sucrose synthesis are regulated in a complex fashion by the physiological condition of the oil palm tree. Facilitation of starch degradation results in high-glucose sap, whereas facilitation of sucrose synthesis produces sap rich in sucrose. Since sucrose is degraded into glucose and fructose by intrinsic invertase, high sucrose palm sap would also contain glucose and fructose. We found that the sap extracted from old palm trunks grown in Johor province contained almost glucose, but the few trunks had the sap contained sucrose except for glucose (Table1 Condensed sap2).

Several squeezed saps had low contents of sugar (31.2 g/L), and the ethanol produced from these saps would be low content (less than 15.6 g/L). Therefore, the sap with thin sugar showed the insufficient yield on ethanol production, because of waste of much energy than the stored in ethanol for distillation. We used a flat membrane filtration approach to condense the sugars and allow efficient ethanol production. Three palm saps containing less than 30–40 g/L sugar were condensed 3 to 4-fold (from 18 L to 5.4 L), then the sucrose, glucose, and fructose were quantified (Table 1). Condensed sap 2 exhibited a large sucrose peak (50.7 g/L, Table 1 Condensed sap 2), whereas condensed sap1 contained 2.5 g/L sucrose, and condensed sap 3 contained too little sucrose to quantify (Table 1 Condensed sap 3). Sucrose is a disaccharide comprising glucose and fructose joined by a glycosidic linkage, and is the major transportable carbohydrate in plants. Since saps squeezed from different trunks contained very different amounts of sucrose, the amount of sucrose in palm sap depends on plant growth conditions.

Table 1. The sugar components in palm sap.

				g/L
	Sucrose	Glucose	Fructose	Total
Untreated sap	-	28.8	2.4	31.2
Condensed sap 1	2.5	74.7	16.8	94.0
Condensed sap 2	50.7	65.5	17.8	134.0
Condensed sap 3	-	42.6	16.6	59.2

3.2. Selection and Evaluation of thermotolerant yeasts for ethanol production from the sap

We incubated *K. marxianus* (TISTR5925, NCYC587, NCYC2791) and *S. cerevisiae* (Kyokai7, S288c, NCYC3233) on YPD plates at 30 °C and 45 °C and compared their growth. As shown in Figure 2, the *K. marxianus* strains showed good growth at 45 °C, whereas the *S. cerevisiae* strains essentially did not grow (Figure 2).

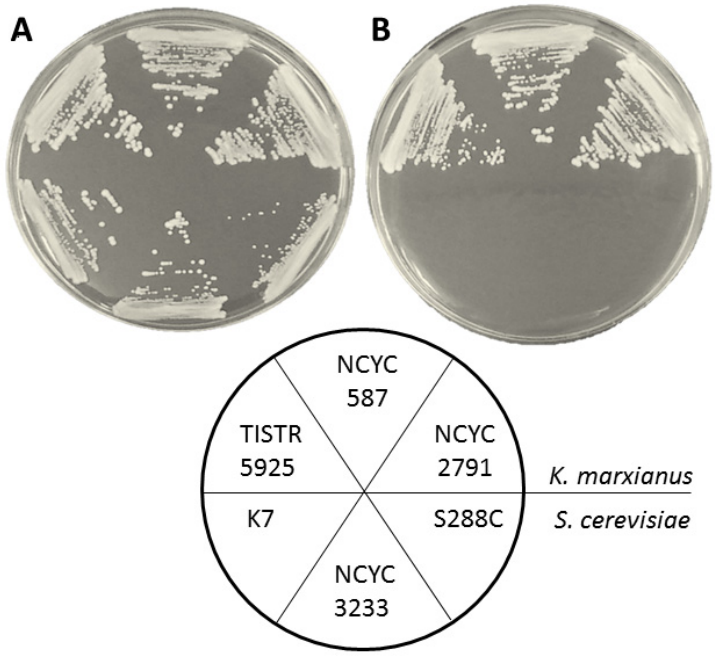

Figure 2. Growth of *S. cerevisiae* and *K. marxianus* on YPD plates at 30 °C and 45 °C. *S. cerevisiae* strains (Kyokai7, S288c, and NCYC3233) and *K. marxianus* (TISTR5925, NCYC587, and NCYC2791) were streaked on YPD plates, and incubated at 30 °C (A) and 45 °C (B).

There are several reports regarding bioethanol production by yeasts other than the representative species, *S. cerevisiae* [7–9]. We isolated the thermotolerant yeast, *K. marxianus* TISTR5925, in Thailand, and observed good ethanol production due to its high fermentation activity at various temperatures and its ability to ferment various sugars [12].

Condensed palm sap no.1 (Table 1) was fermented by the representative yeast strains, TISTR5925 and Kyokai7, at various temperatures. TISTR5925 consumed all the sugars and produced ethanol at temperatures between 37 °C and 45 °C, but not at 50 °C (Figure 3a and b), whereas Kyokai7 did not produce ethanol efficiently at 42 °C and 45 °C, and sugars remained in the condensed sap (Figure 3c and d). *K. marxianus* TISTR5925 showed high fermentation activity from 37 °C to 45 °C because of its thermotolerance, whereas *S. cerevisiae* Kyokai7 showed little fermentation at 42 °C and 45 °C due to heat shock stress. The ethanol yields for *K. marxianus* TISTR5925 and *S. cerevisiae* Kyokai7 at each temperature were calculated and compared. The theoretical value for the complete conversion of the sugar in the palm sap (96.3 g/L) to ethanol is 49.2 g/L (Table 2). The ethanol yield at each temperature was calculated as the ratio with this theoretical value (Table 2a and b). *K. marxianus* TISTR5925 provided high ethanol yields, from 74.8% to 92.2%, at temperatures up to 45 °C, whereas *S. cerevisiae* Kyokai7 provided low ethanol yields (from 49.6% to 68.0%) above 42 °C. Yeast cells are killed by temperature fluctuations result in decreased ethanol production [11]. The thermotolerant yeast, *K. marxianus* TISTR5925, holds promise for ethanol production from oil palm sap at high temperature because of its fermentation activity over a range of temperatures.

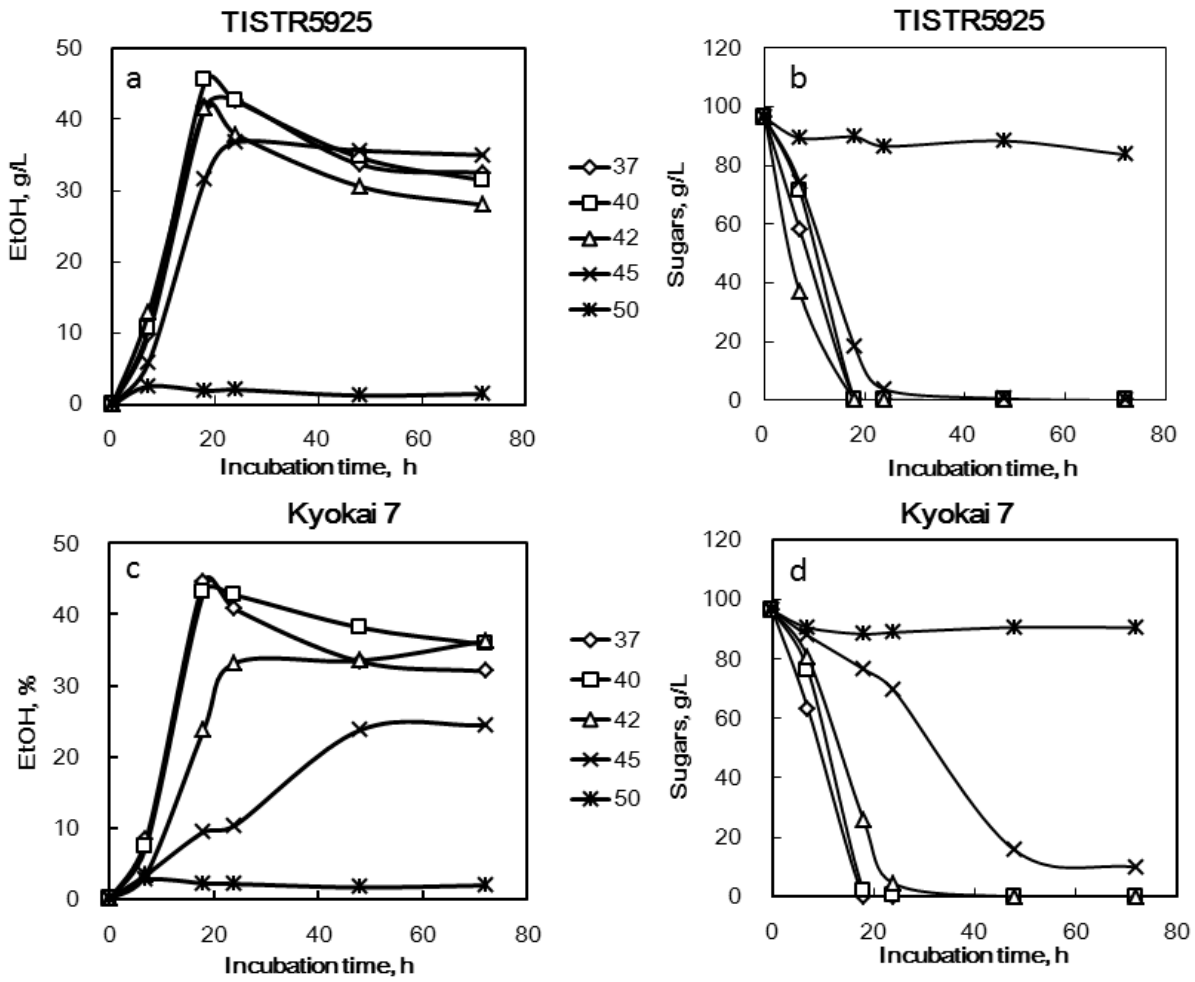

Figure 3. Fermentation of palm sap by TISTR5925 and Kyokai7. Palm sap was condensed to 10% sugars by flat membrane filtration. Both strains were precultured on 10% YPD medium, resuspended in condensed palm sap, and incubated at various temperatures. The experiments were repeated three times and the average values were plotted.

Besides, the ethanol productivity from 0 h to 7 h of *K. marxianus* TISTR5925 was faster maximum 3.7 folds at 42 °C than ones by *S. cerevisiae* Kyokai7 (Table 2). This quick fermentation showed the advantage on the prevention of contamination and downsizing of fermentation tank, therefore, the fermentation by thermotolerant yeast would lead to cost saving in plant construction.

3.3. Net energy ratio for the ethanol production using the flat membrane filtration and the fermentation with the thermotolerant yeast

The energy required to condense the sap was estimated. The content of 18 L (sugar content: 31.2 g/L) of sap to 5.4 L (sugar content: 96.3 g/L) took 1.8 h and required 1.22 kWh of electricity (calculated from equation 1, Materials and Methods), or 10.9 MJ (Table 3a). The consumed power in order to condense sugar in sap depended on the initial content of sugar. If the initial sugar content in sap was too low, the much more energy could be wasted for condensation of palm sap.

Table 2. Fermentation of palm sap.

a. TISTR5925

Temperature, °C	Time, h						Max ethanol, g/L	Ethanol yield*1, %	Ethanol productivity*2, g/L/h
	0	7	18	24	48	72			
37	0.0	9.8	41.7	42.7	33.7	32.5	42.7	86.7	1.40
40	0.0	10.7	45.4	42.6	34.6	31.5	45.4	92.2	1.52
42	0.0	12.9	41.6	37.9	30.6	28.0	41.6	84.5	1.84
45	0.0	5.9	31.6	36.8	35.6	35.0	36.8	74.8	0.84
50	0.0	2.5	1.9	2.1	1.2	1.5	2.5	5.1	0.36

b. Kyokai 7

Temperature, °C	Time, h						Max ethanol, g/L	Ethanol yield*1, %	Ethanol productivity*2, g/L/h
	0	7	18	24	48	72			
37	0.0	8.4	44.5	40.8	33.4	32.0	44.5	90.4	1.20
40	0.0	7.4	43.0	42.7	38.2	35.8	43.0	87.3	1.06
42	0.0	3.5	23.7	33.2	33.5	36.3	33.5	68.0	0.49
45	0.0	3.4	9.4	10.3	23.7	24.4	24.4	49.6	0.49
50	0.0	2.7	2.2	2.1	1.6	1.9	2.7	5.5	0.39

*1 Glucose (1.00 g/L) is converted to a theoretical value of 0.511g/L ethanol.
If the sugar content of palm sap was 96.26 g/L, it was converted to 49.23 g/L ethanol (theoretical value).
The ethanol yield at each temperature was calculated as the ratio with the theoretical value (49.23 g/L).
*2 Ethanol productivity from 0 h to 7 h was calculated as initial rate.

We estimated the initial content of sugar in the saps to range from 41.2 g/L to 91.2 g/L to evaluate profitability for ethanol production from palm sap, and we calculated the amount of electricity required to concentrate the sap sugars to 96.3 g/L (Table 3 Initial sugar). The required energy was found to range from 0.9 MJ to 10.9 MJ, depending on the initial content of sugar in the sap (Table 3 Sap condensation). The energy required for squeezing the sap and for ethanol fermentation was estimated as 5.8 MJ and 0.85 MJ, respectively (Table 3 Sap squeezing and EtOH fermentation). The total input energy was calculated as the sum of the joules required for squeezing and condensing the sap and for ethanol production (Table 3 Total input energy) and was found to depend on the initial content of sugar in the sap.

The sugars in sap squeezed from oil palm trunks were analyzed by HPLC as described in Materials and Methods. Saps with low sugar content (31.2 g/L) were condensed, from 17 L to 5.4 L, using a flat membrane filter. Fermentation of condensed palm sap (96.3 g/L; Table 2) by *K. marxianus* (Figure 3) provided ethanol (45.4 g/L); the volume of ethanol was calculated as 0.31 L according to the equation in Table 4 (Table 4 EtOH). The stored energy in the ethanol was calculated as 6.7 MJ from the energy content of ethanol (21.2 MJ/L) and the volume (0.31 L; Table 4 Energy from EtOH). If the residue obtained after squeezing sap from the oil palm trunk was used as a fuel for the distillation boiler, the amount of residue after extracting sap (17 kg) from an oil palm log (30 kg) was 10.1 kg [13] with a potential energy of 75.8 MJ (Table 4 Potential energy). We estimated that

20% of this energy could be utilized, giving an effective energy of 15.2 MJ (Table 4 Effective energy) [13].

Consequently, total output energy was calculated as 21.8 MJ (the sum of the energy in the produced ethanol and the effective energy from the residue; Table 4 Total output energy). The output energy depended on the volume of the sap and EtOH (Figure 4 Sap and EtOH), which in turn were associated with the initial content of sugar (Table 3 Initial sugar). Therefore, the output energy depended on the initial sugar content.

Table 3. Energy required to produce EtOH from condensed sap (Input energy).

	Sap squeezing *1, MJ	Initial sugar content *2, g/L	Final sugar content, g/L	Difference between initial and final sugar content *3	Sap condensation *4, MJ	EtOH fermentation *5, MJ	Total input energy *6, MJ
a	5.8	31.2	96.3	65.1	10.9	0.85	17.6
b	5.8	41.2	96.3	55.1	9.3	0.85	15.9
c	5.8	51.2	96.3	45.1	7.6	0.85	14.2
d	5.8	61.2	96.3	35.1	5.9	0.85	12.5
e	5.8	71.2	96.3	25.1	4.2	0.85	10.9
f	5.8	81.2	96.3	15.1	2.5	0.85	9.2
g	5.8	91.2	96.3	5.1	0.9	0.85	7.5

*1 The energy required to squeeze sap is cited from Murata et al., 2013.
*2 Sugar content before condensation by flat membrane filtration
*3 Difference between initial and final sugar content in sap: Final sugar content, 96.3g/L − Initial sugar, g/L
*4 The energy required to condense sap, 10.9MJ was used for
different content of sugar 65.1 g/L (= 96.3g/L − 31.2 g/L).
Energy required to condense the palm saps were calculated as follows.
Energy for sap condensation, MJ = 10.9 MJ × (Difference from b to g)/65.1g/L
*5 The energy required for EtOH production is cited from Murata et al., 2013.
*6 Total input energy = (Sap squeezing, MJ) + (Sap condensation, MJ) + (EtOH production, MJ)

The ratio of output energy to input energy was calculated (Table 4 Net Energy Ratio). A high initial sugar content provided a high ethanol output energy. An initial sugar content of 31.2 g/L required a total input energy of 17.6 MJ (Table 3a), whereas a sugar content of 96.3 g/L (5.5 L) provided 0.31 L of ethanol (6.7 MJ; Table 4a). This value is less than the 17.6 MJ required as total input energy for sap content and ethanol production. A sustainable process requires that the input energy required for ethanol production should not exceed the output energy that can be extracted from the biomass. An initial sugar content in sap of 61.2 g/L (Table 3c, Initial sugar) required a total input energy of 12.5 MJ to squeeze and concentrate the sap, and to produce ethanol**.** The output energy from the 0.62 L of ethanol produced was 13.1 MJ (Table 4d Energy from EtOH), providing an NER of output energy to input energy of 1.04 (data not shown). Therefore, positive energy efficiency requires a sugar content in the raw sap above 61.2 g/L (Table 3d and Table 4d).

The biomass residue should be used for energy production in order to improve the energy efficiency of the process [19]. Squeezing an oil palm log (20 cm diameter, 1.2 m long, approx. 30 kg) provided 17 L of sap and 10.1 kg of residue. The potential energy of this residue was calculated to be 75.8 MJ from the low heat value (LHV) of residue containing 50% water (Table 4 Potential energy),

and the effective energy was estimated to be 20% of the potential energy of the residue (Table 4 Effective energy).

An effective energy of 15.2 MJ was estimated, and this energy was added as output energy (Table 4 Total output energy). Adding the effective energy (15.2 MJ) using the residue as fuel during distillation provides a total output energy of 13.1 MJ + 15.2 MJ = 28.3 MJ (Table 4c total output energy). Consequently, the energy efficiency of ethanol production from oil palm sap was improved by using the residue as fuel (Table 4 Net Energy Ratio). In this study the themotolerant yeast, *K. marxianus* TISTR5925, was used to ferment palm sap and was shown to have several advantageous features compared to *S. cerevisiae* due to its thermotolerance. Ethanol fermentation conducted between 35 °C to 40 °C reduces costs by an estimated 3,000,000 yen per year, for 30,000 kL ethanol produced per year [2]. Therefore, less energy is required to produce ethanol using thermotolerant yeast due to decreased the size of fermentation tank and distillation tower cooling costs, thus improving the energy efficiency of the process.

Table 4. The energy from ethanol and the residue (Output), and Net Energy Ratio (NER) of output to input energy.

	Folds of sugar content in sap *1	Sap*2, L	EtOH *3, L	Energy from EtOH *4, MJ	Effective energy from the residues*5, MJ	Total output energy *6, MJ	Total input energy *7, MJ	Net Energy Ratio *8
a	3.1	5.5	0.31	6.7	15.2	21.9	17.6	1.2
b	2.3	7.3	0.41	8.8	15.2	24.0	15.9	1.5
c	1.9	9.0	0.52	10.9	15.2	26.1	14.2	1.8
d	1.6	10.8	0.62	13.1	15.2	28.3	12.5	2.3
e	1.4	12.6	0.72	15.2	15.2	30.4	10.9	2.8
f	1.2	14.3	0.82	17.3	15.2	32.5	9.2	3.5
g	1.1	16.1	0.92	19.5	15.2	34.7	7.5	4.6

*1 Fold of sugar content in sap = 96.3g/L /(Initial sugar, g/L; Citation From Table 3)
*2 Sap, L = 17 L/(folds of sugar content)
*3 Ethanol was quantified by HPLC as 45.4 g/L from the fermentation data in Table 2
Ethanol (L) was calculated as follows; EtOH, L = (0.0454kg/L × Sap volume, L)/0.789 kg/L
*4 Energy in ethanol; 21.2 MJ L^{-1}
Energy from EtOH, MJ = (21.2 MJ/L) × (each EtOH, L)
*5 Average weight of residue after squeezing sap was 10.1 kg.
Low heat value (LHV) of residue with 50% moisture was estimated as 7.5 MJ kg^{-1}.
Potential heat, MJ = (Residue after squeezing; 10.10 kg) x 7.5 = 75.8 MJ
Effective energy was estimated as 20% of potential heat of residue.
Effective energy of the residues = 75.8 MJ × 0.2 = 15.2 MJ
*6 Total energy = (Energy from EtOH, MJ) + (Effective heat of residues, MJ)
*7 Values cited from Table 3
*8 Net Energy Ratio = (Total output energy) / (Total input energy)

4. Conclusion

Sap with a low content of sugar was obtained by squeezing old oil palm trunks. The sugar in the

sap was condensed and fermented into ethanol using *K. marxianus* TISTR5925. The input energy required to condense the sap and for ethanol production, and the output energy contained in the heat energy of the ethanol, were calculated and compared. At a sugar content above 61.2 g/L, the output energy exceeded the input energy.

We estimated the energy from combustion of the residue after squeezing sap from oil palm trunk. Using the residue as fuel during distillation and improving fermentation by using thermotolerant yeast can increase the efficiency of ethanol production from oil palm sap.

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Conflict of Interests

The authors declare no conflict of interests.

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