Enhancement of bioethanol production from palm sap (*Arenga pinnata* (Wurmb) Merr) through optimization of *Saccharomyces cerevisiae* as an inoculum

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Abstract

Availability of fossil fuels is increasingly limited, so the search for alternative fuels is important. In accordance with the current scenario, bioenergy research emphasizes the bioethanol production from plants that are abundant and available throughout the year, such as palm sap (*Arenga pinnata* MERR). The palm sap is a type of palm tree that grows in tropical forests, particularly in South Asia and Southeast Asia. More than 3,000 species of palm exist, and they are categorised as multipurpose trees because they can be used as raw materials for various products, such as sugar, fermented drinks, syrup, palm wine, vinegar, alcohol and bioethanol. This study was aimed to examine bioethanol production from palm sap through optimization of *Saccharomyces cerevisiae* as an inoculum. The research sample was obtained from local farmers in Pusuk, Lombok Island, West Nusa Tenggara, and Indonesia. The research

parameters included the pH change, colour, and ethanol content. The results showed that the pH change of the palm sap during storage was caused by the growth of microorganisms to produce organic acids by releasing hydrogen ions. As pH decreased, L* and b* values also decreased significantly, but not significantly change in a* values was observed. Glucose changed to ethanol during fermentation. The higher the percentage of inoculum used, the higher the volume of ethanol obtained. The bioethanol content of palm sap prior to fermentation was 32.3%, and it increased to 75.6% after 24 hours of incubation.

Keywords: palm sap, bioethanol, fermentation, distillation, inoculum

1. Introduction

Palm sap (*Arenga pinnata* MERR) is a type of palm tree that grows in tropical forests, particularly in South Asia and Southeast Asia [1], [2], [3]. More than 3,000 species of palm exist, and they are categorised as multipurpose trees because they can be used as raw materials for various products, such as sugar, fermented drinks, syrup, palm wine, vinegar, alcohol and bioethanol [4], [5].

Some parts of the palm tree are used to meet human needs; for example, the fruit can be used for cocktails or desserts; and the fibre is used to make ropes, filters, brooms and roofs [6]. Starch, the inner part of the tree, is the raw material for noodle production. Young leaves can be consumed as a salad or cooked as an ingredient for soups. Stems (wood) can be processed for the manufacture of furniture, and roots are used as traditional medicine because they are believed to dissolve bladder stone [7].

The national palm tree cultivation programme for industrial purposes has been implemented since 2007 in Indonesia [8], [9]. The land area for palm cultivation in Indonesia is approximately 60,482 hectares, and its potential for sap production can reach 303.76 million litres per year [10]. If production is 50% of the total population, then sap production can reach

210.600 litres/hectare/year [11]. Every 10 litres of sap can produce an average of 3.5 litres of ethanol [12]. Accordingly, 1 hectare of palm trees can produces of 73,710 litres of ethanol. If production is adequate, then the potential of biofuel from palm sap can reach 2 million kilolitres per year. Thus, biofuel exhibits considerable potential and must be developed.

The quality of palm sap is determined by the pH value [13]. The palm sap with a pH value of 6–7 is considered good quality for use in ethanol production [14]. However, new palm sap tapped with neutral pH is easily damaged; hence, the sap must be processed immediately after harvesting [15], [16], [17]. *Saccharomyces cerevisiae* microorganisms in palm sap can trigger an acidic reaction [18], [19]. This condition poses a challenge to maintaining sap quality at neutral pH (4.0).

The bioethanol production from renewable raw materials has attracted attention today because it can be used as an alternative fuel [20], [21]. Bioethanol is the most popular biofuel because it is environmentally friendly [22]. Bioethanol production for the first generation in general still uses food as raw material [23] [24]. The second generation of bioethanol has replaced it by using biomass as a feedstocks [25], [26]. The second generation technology has encouraged the development of bioethanol production with lower production costs and better environmental impact [27], [28]. Palm sap is the most promising bioethanol raw material for bioethanol production because it is available throughout the year and is abundant [29], [30].

The success factors to the bioethanol production from the palm sap include initial treatment, fermentation method, and refining [31], [32]. Busic et al. [33] asserted that the most influential factor in obtaining bioethanol content from the palm sugar is tapping and distribution during processing because palm sugar is easily damaged by environmental conditions. Kismurtono [34] also explained that the bioethanol content produced from fermented palm sap is strongly influenced by the quality of raw materials used [35], [36], [37]. Meanwhile, according to Sebayang et al. [38], the purity of ethanol can be increased through a distillation process using zeolite as an adsorbent. Bioethanol production from the banana stems

for power generation applications is very profitable economically and environmentally friendly [39].

The bioethanol production from the palm sap is a relatively new technology [40]. Bioethanol has the potential as a vehicle fuel because it has a high calorific value [41]. This high calorific value results in a long burning time [42]. Fuel substitution requires an ethanol level above 95% [43]. In the current study, anaerobic fermentation is performed to produce ethanol at levels that can be used as substitutes for environment-friendly fuels [44], [45].

The research focusing on the processing of palm sap into bioethanol has been conducted by researchers, like [46], but the bioethanol content obtained is still less than 95%, so it cannot be used as a substitute for vehicle fuel. Thus, it is still important to do research to produce bioethanol that can be used as an environmentally friendly substitute for biofuels. These research use a method of fermentation and multilevel distillation to produce biofuels that can be used as biofuel substitutes that are environmentally friendly. Anaerobic fermentation method uses microbial culture made from a mixture of palm sap and Saccharomyces cerevisiae as an inoculum [47], [48]. The increase of ethanol yield up to 99.5% was done by stratified distillation [49], [50], [51]. Based on the arguments above, the purpose of this study was enhancement of bioethanol production from palm sap through optimization of saccharomyces cerevisiae as an inoculum. This research is very important as information to the bioethanol industry to develop palm sap as a raw material for making biofuels.

2. Materials and methods

2.1. Samples and tools

The raw material used was palm sap obtained from farmers in Pusuk, Lombok Island, West Nusa Tenggara Province, Indonesia. To accelerate the growth of the culture, microbes are used. Cerevisiae as additional nutrition in preparing inoculum. The tools used were fermenters, distillation system, pH meter and Hunter Lab.

105

106 *2.2. Preparation of palm sap*

107 The palm sap was heated approximately 50-60 °C for 10 minutes to produce glucose
108 and to destroy bacteria that were not beneficial for fermentation. Thereafter, the palm sap
109 culture was stored for 24 hours to decrease the temperature to 28 °C [52].

110

111 *2.3. Preparation of inoculum culture*

112 To prepare the inoculum culture, 500 millilitres of palm sap was heated at 60 °C for 10-
113 15 minutes and then cooled at the room temperature. After cooling, the palm sap was inoculated
114 with *S. cerevisiae* and bread yeast. Then, it was incubated at 30 °C for 24 hours to be used as
115 microbial culture. This culture was mixed with NPK as an additional nutrient to accelerate
116 microbial growth.

117

118 *2.4. pH testing*

119 Determination of pH is done by measuring the temperature of the sample first. Then
120 the temperature of the pH meter was turned on, adjusted, and allowed to stabilize for 15-30
121 minutes. The electrodes were rinsed with distilled water and dried using a tissue. The electrode
122 is dipped into the sample until the scale reading is stable [53].

123

124 *2.5. Colour testing*

125 The change in palm sap colour was recorded in clean plastic bottles at different time
126 intervals (ASTM D 1500-03). The colours were analysed qualitatively and quantitatively using
127 Adobe Photoshop. The colour value of Adobe Photoshop was set to L* a* b* by using the
128 following equation [54]:

129 $$L^* = \frac{Lightness}{255} \times 100 \quad (1)$$

$$a^* = \frac{240a}{255} - 120 \quad (2)$$

$$b^* = \frac{240b}{255} - 120 \quad (3)$$

where, L^* = 0 (black), L^* = 100 (white), a^* ($-a$ = greenness, $+a$ = redness) and b^* ($-b$ = blueness, $+b$ = yellowness).

2.6. Fermentation process

The palm sap was anaerobically fermented in the bottles at room temperature [55]. The palm sugar was added to the inoculum in varying concentrations of 5, 7, 10, 12, and 15% (v/v). The culture was incubated for 48, 60, and 72 hours at 30 °C. The fermentation process was performed with different volume concentrations of the palm sap, inoculum and microbes as shown in the Table 1. Reaction was accelerated by stirring the culture every 6 hours for 5 minutes. The fermentation results were centrifuged at a spinning speed of 10,000 rpm for 10 minutes at 30 °C. The parameter observed was ethanol content.

Table 1. Treatment of fermentation methods

Treatment	Volume of palm sap (%)	Volume of inoculum (%)	Nutrient (%)
F1	95	5	15
F2	93	7	15
F3	90	10	15
F4	88	12	15
F5	85	15	15

2.7. Distillation process

The water and ethanol were separated via distillation using a vacuum system with a pressure of less than 1 atm and the temperature range of 140-150 °C (ASTM D 86-04b) [56]. The fraction with the lowest boiling point can evaporate firstly and will be at the top of the column, whilst the fraction with a high boiling point will remain at the bottom of the column.

2.8. Measuring ethanol content

Ethanol content was measured using the pycnometer method [57]. Firstly, a sample of 100 millilitre was placed in a distillation flask. Then, 100 millilitre of distilled water was added and distilled at 80 °C. The distillate was stored in an Erlenmeyer flask to a volume of 50 millilitre. The distillate was then inserted into a pycnometer to meet the pycnometer. Excess distillates at the top of the capillary tube were cleaned. The pycnometer containing the distillate was weighed, and the weight was recorded. The same procedure was performed on distilled water for comparison. The results of ethanol density calculations were converted using ethanol specific gravity conversion tables. Ethanol density was calculated using the following equation [58]:

$$F = g\left(m_b - \frac{\rho_{a-m_b}}{\rho_b}\right) \quad (4)$$

where, subscript b = the bottle, a = air, ρ = density, m = mass, and g = acceleration due to gravity.

2.9. Viscosity measurement

The viscosity sample was measured using a gravity capillary viscometer in the temperature range of 20-30 °C (ASTM D 88-94). The mathematical viscosity equation can be written [59]:

$$F = \eta A \frac{V}{L} \quad (5)$$

where, F = force on the surface of the liquid, η = coefficient of fluid viscosity (Ns/m^2), A = liquid area (m^2), V = moving wall velocity (m / s), L = distance of the two surfaces (m).

2.10. Measurement of Calorific Value

The calorific value of combustion was measured using a bomb calorimeter type IKA C-5000 (ASTM D 2014-96) [60]. The reaction that occurs in the bomb calorimeter can produce heat which is absorbed by the water and the bomb, so that no heat is wasted into the air. The calorific value of combustion can be written as:

$$r_{\text{eaction}} = -(q_{\text{air}} + \text{q}_{\text{bomb}}) \quad (6)$$

The amount of heat absorbed by water can be calculated using the formula:

$$Q_{water} = m.c.\Delta T \quad (7)$$

where, m = mass of water (g), c = heat type of water (J/kg°C), and ΔT = temperature change (°C).

The amount of heat absorbed by the bomb calorie meter can be calculated using the formula:

$$q_{water} = c_{\text{bomb}}.\Delta\text{T} \quad (8)$$

where, c_{bomb} = heat capacity of bomb (J/g°C) and ΔT = temperature change (°C).

2.11. Data analysis

The research data were analysed using one-way analysis of variance [61]. If the F-count value is greater than the F-table, then a significant difference exists. Statistical significance between sample treatments was defined at $p<0.05$. Mean differences were evaluated with Duncan's Multiple Range Test (DMRT), which was analysed using SPSS for Windows version 16.0.

3. Results and discussion

3.1. The pH content

The pH value of palm sap used in this study was 5.6, but the value decreased after storage (Fig. 1). Based on the Figure 1 was showed that the pH of palm sap decreases during storage due to the interaction between palm sap and environmental air and the subsequent fermentation process. A similar finding was reported by Elijah *et al.* [62] that the decrease in the pH of palm sap is caused by acid fermentation in alcohol. Devi *et al.* [63] also explained that palm sap naturally contains microbes that can produce amylase enzymes, which eventually become alcohol.

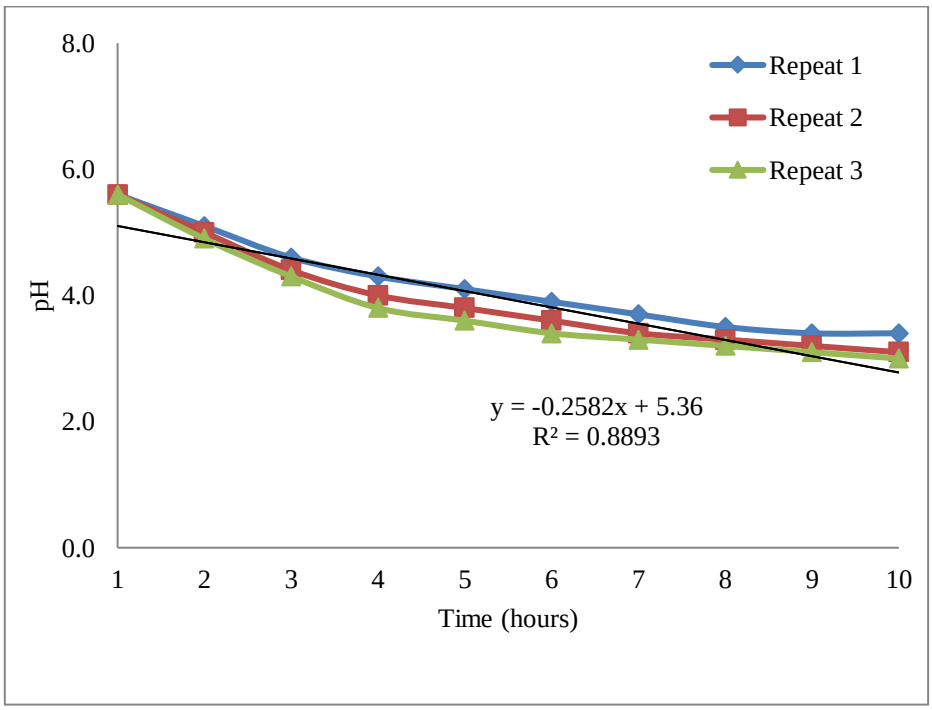

Fig. 1. The pH change with storage time variation

The palm sap is highly sensitive to environmental temperature and it easily damaged. The most easily detected damage indicator is pH value. Some researchers have also reported that the pH of palm sap drops from 7.41-6.02 after 20 hours of storage [18], from 6.70-6.34 [15]. The trend of decreasing pH in this study was apparently lower than those reported by previous researchers. Other researchers have also reported changes in pH along with the length

of storage time, such as sweet sorghum sap from pH 5.16 changing to 4.34 in 48 hours of storage [64], sugarcane with pH changed from 5.30-4.50 in 96 hours [65]. The change in the pH of palm sap is also related to the activity of microorganisms. The growth of microorganisms that can rapidly reduce pH by producing organic acids. When acid increases, pH decreases. When acids release hydrogen ions, vegetative cell bacteria can multiply well under low acid conditions. The decrease in pH by lactic acid bacteria in palm sap has also been reported by Manel et al. [66].

Temperature is an extremely influential factor in the pH decrease of palm sap. In the current study, a high storage temperature indicated a considerable decrease rate in pH presumably because changes in sugar levels to acidic levels accelerate at high temperature. Ansar *et al.* [67] reported that palm sap stored at 40 °C exhibits a higher decrease in pH compared with palm sap stored at 29 °C. The lowest decrease in pH value of palm sap is obtained at a storage 10 °C. Lasekan and Abbas [68] have also reported that palm sap stored at 28 °C has decreased pH faster than that stored at 15 °C.

3.2. Changes in colour (L a* b* values)*

The results show that the L* value decreased significantly ($p<0.05$) from 56.0-47.3, and the b* value decreased significantly ($p<0.05$) from 8.7-7.6. By contrast, no significant change was observed in the value of a* ($p>0.05$) during storage. These results indicate that when pH decreases, the colour values (L* and b*) of palm sap change with storage time length. It has been reported also by Manel et al. [66] that the palm colour changed from the original palm colour (pure) into milk white during the fermentation process.

The effect of colour change during storage before fermentation can be used as an indicator of palm sap quality. The results of the current study exhibit that pH value contains a function of lightness (L*) and yellowness (a*) but is not correlated with redness (b*). This finding agrees with the results of Victor and Orsat [69] that a high pH causes *A. pinnata* to

239 appear more transparent, whereas a low pH causes the palm sap to appear cloudy. Comparison
240 of the pH and colour values of L* a* b* results of this study with some previous studies are
241 shown in the Table 2.

242

243

244 Table 2. Comparison of the pH and colour values of L* a* b* results

Varieties of palm sap	pH	Color interval			Ref.	Tools
		L*	a*	b*		
Borassus flabellifer Linn	4.19-5.23	61.49-87.53	1.46-3.52	12.41-19-31	[17]	Hunter Lab Color flex
A. Pinnata Merr	4.883-6.387	44.5-54.8	1.2-1.6	6.5-9.8	[69]	Minolta Reader
Phoenix dactylifera L.	6.86±0.05	72.01±0.07	0.64±0.02	15.04± 0.02	[70]	Lovibond Tintometer PFX 195
Palm sap	4.8-7.1	47.3-56.0	7.6-8.7	34.0-46.0	This study	Chroma Meter-CR-400)

245

246

247 *3.3. Ethanol content*

248 Fermentation can convert glucose in palm sap into ethanol due to the reduced glucose
249 levels in ethanol. This finding agrees with the results of Mustafa et al. [71] and Trivedi et al.
250 [72]. The higher the percentage of inoculum used, the higher the ethanol content obtained (Fig.
251 2). The ethanol content of palm sap prior to fermentation was 32.3%, and it increased to 75.6%

after 24 hours of fermentation.

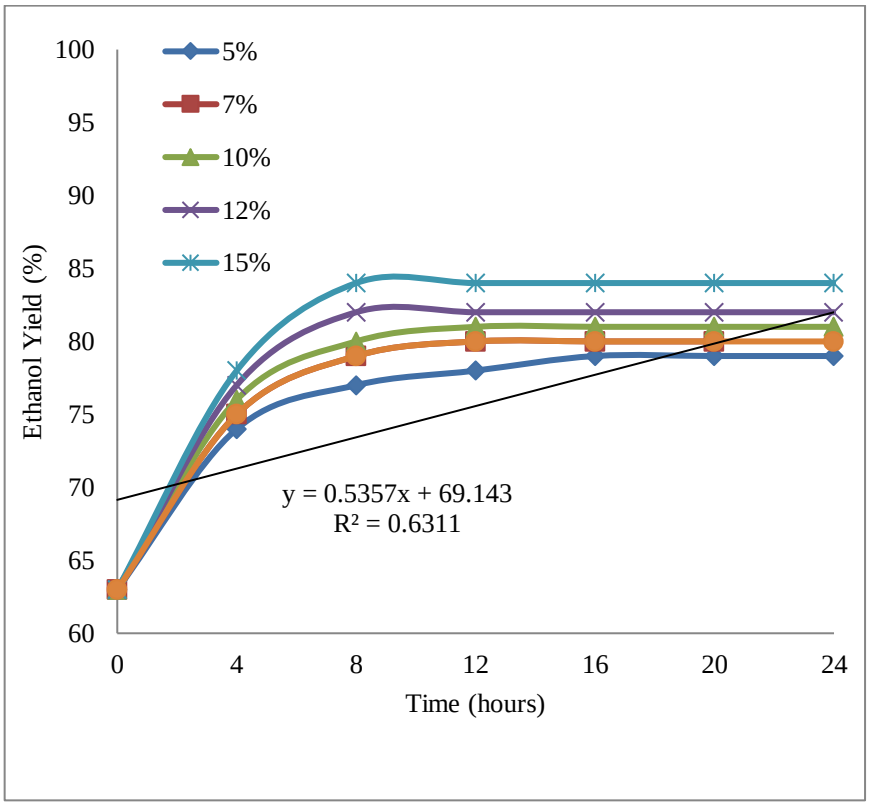

Fig. 2. Profile of ethanol concentration during anaerobic fermentation at varying inoculum concentrations

The analysis variance results showed that the F-count value is greater (368.893) than the F-table (3.885). Thus, inoculum concentration significantly affects ($p<0.05$) ethanol yield. The DMRT analysis determines that inoculum concentration variation significantly influences the obtained ethanol content. The higher the inoculum concentration, the higher the obtained ethanol content. This result was consistent with that of Mojovic *et al.* [73] that the content of fermented ethanol depends on the quality of sap used and the type of microbes for the inoculum.

The result of analysis of variance was known that the value of F-count is greater (368.89) than F-table (3.88) (Table 3), so it can be explained that inoculum concentration variation of significantly affect ($P<0.05$) to ethanol yield was obtained.

Table 3. Analysis of variance

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	10624.13	2	5312.07	368.89	1.68E-11	3.88
Within groups	172.8	12	14.4			
Total	10796.93	14				

The time of fermentation (incubation period) also significantly influences the ethanol yield formed. The longer the fermentation time, the higher the ethanol yield obtained. The same results have been revealed by Oguri et al. [36] who explain that the longer the fermentation process, the chances of ethanol to formed is also higher. The result of this fermentation is still a mixture of ethanol and water, so it must be separated by the distillation method. The same thing has been revealed by Hashem and Darwish [74] who state that the fermentation process usually requires an incubation period of 12-72 hours and depends on the number and type of microorganisms used to initiate fermentation.

In order to increase the ethanol content of fermented products, distillation was carried out to obtain higher ethanol levels. The distillation process was carried out with a vacuum system. In this system the fraction that has the lowest boiling point will experience evaporation first. In this study, the maximum yield obtained was still low at around 75.6%. This is allegedly because the raw materials used have been contaminated before fermentation. The same thing has been reported by Amigun and Musango [75] that the obtaining process of ethanol yield highly depends on raw materials and environmental conditions at the time of tapping.

4. Conclusion

Changes in the pH value of palm sap after tapping are caused by the growth of microorganisms that produce organic acids by releasing hydrogen ions. As the pH decreased,

the values of L* and b* also decreased significantly, but there was no significant change in the values of a*. Palm juice converts glucose into ethanol during fermentation. The higher the percentage of inoculum used, the higher the ethanol yield volume. The ethanol content of palm sap before distillation was 32.3% and increased to 75.6% after distillation. The physicochemical properties of bioethanol still need to be studied comprehensively by conducting trials on various types of motorized vehicles.

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Enhancement of bioethanol production from palm sap (Arenga pinnata (Wurmb) Merr) through optimization of Saccharomyces cerevisiae as an inoculum

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Abstract:	Availability of fossil fuels is increasingly limited, so the search for alternative fuels is important. In accordance with the current scenario, bioenergy research emphasizes the bioethanol production from plants that are abundant and available throughout the year, such as palm sap. The palm sap is a type of palm tree that grows in tropical forests, particularly in South Asia and Southeast Asia. More than 3,000 species of palm exist, and they are categorised as multipurpose trees because they can be used as raw materials for various products, such as sugar, fermented drinks, syrup, palm wine, vinegar, alcohol and bioethanol. This study was aimed to examine bioethanol production from palm sap through optimization of Saccharomyces cerevisiae as an inoculum. The research sample was obtained from local farmers in Pusuk, Lombok Island, West Nusa Tenggara, and Indonesia. The research parameters included the pH change, colour, and ethanol content. The results showed that the pH change of the palm sap during storage was caused by the growth of microorganisms to produce organic acids by releasing hydrogen ions. As pH decreased, L* and b* values also decreased significantly, but not significantly change in a* values was observed. Glucose changed to ethanol during fermentation. The higher the percentage of inoculum used, the higher the volume of ethanol obtained. The bioethanol content of palm sap prior to fermentation was 32.3%, and it increased to 75.6% after 24 hours of incubation.
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Opposed Reviewers:	
Response to Reviewers:	Authors would like to thank the Reviewers and Editors due their appropriate and

	constructive suggestions as well as their proposed corrections, which have been utilized in improving the quality of this manuscript.

June 17, 2021

Professor Marc André Meyers

Editor-in-Chief of Journal of Materials Research and Technology

Dear Professor,

I wish to submit a manuscript entitled “Enhancement of bioethanol production from palm sap (*Arenga pinnata* (Wurmb) Merr) through optimization of Saccharomyces cerevisiae as an inoculum” for possible consideration.

Finally I wish to affirm the manuscript has been prepared in accordance with instructions to authors. I also hereby affirm that the content of this manuscript or a major portion thereof has not been published in a refereed journal, and it is not being submitted for publication elsewhere.

Thank you very much and I shall wait for your kind response.

Best regards,

Dr. Ansar

Reviewers and/or Editors' comments and Author Respond

Ms. Ref. No.: JMRT-D-20-01698

Title: Enhancement of production of bioethanol from palm sap (Arenga pinnata MERR) through optimization of Saccharomyces cerevisiae as an inoculum

Authors : Ansar, Nazaruddin, Atri Dewi Azis, Ahmad Fudholi

Date : June 16, 2021

Reviewers and/or Editors' comments:

Reviewers Comments	Author Respond and Revision
Reviewer #1: Arenga pinnnate Wurmb. Merr is the correct one - the title sugar palm sap is the correct one - in the title and keywords over 40 references are not enough for good review. Get 70-100 references. A lot of work have been done. It seemed the review is not deep enough Strange the title is review but it has results and discussion.	The title was changed based on reviewer 1's suggestion. References were added (Total references is 75)
Reviewer #2 1. Generally, the manuscript is must be improved by native speakers and revise carefully to avoid any mistaken in grammatical errors. 2. In the introduction section, the authors can add bioethanol as fuel type which used in this study. In this regard, use the following references: * Production process and optimization of solid bioethanol from empty fruit bunches of palm oil using response surface methodology. Processes 7 (10), 715 * Enzymatic hydrolysis using ultrasound for bioethanol production from durian (durio zibethinus) seeds as potential biofuel. Chemical Engineering Transactions 56, 553-558 * Experimental investigation, techno-economic analysis and environmental impact of bioethanol production from banana stem. Energies 12 (20), 3947 3. The authors can add detail for optimization in this study 4. Please provide the data of psychochemical properties of bioethanol, the devices to measure the properties with standard. 5. Please authors can add the information of ASTM standards that author used in this study. 6. The authors can add the error analysis from the graph.	1. The manuscript is thoroughly revised, and all possible grammatical error has been corrected with improved. 2. The references were added in introduction section, such as Ref. [38], [39], [42], etc. 3. The viscosity measurement section and measurement of calorific value were added. 4. The physicochemical properties of bioethanol still need to be studied comprehensively by conducting trials on various types of motorized vehicles. 5. Line 126, in colour testing section: (ASTM D 1500-03). Line 148, in distillation process section (ASTM D 86-04b) [56]. Line 176, in Measurement of Calorific Value section: (ASTM D 2014-96) [60]. 6. The error analysis from the graph was added.

7. A recommendation is required at the end of the conclusion based on your findings.	7. The recommendation was added in conclusion section.

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Enhancement of bioethanol production from palm sap (*Arenga pinnata* (Wurmb) Merr) through optimization of *Saccharomyces cerevisiae* as an inoculum

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Abstract

Availability of fossil fuels is increasingly limited, so the search for alternative fuels is important. In accordance with the current scenario, bioenergy research emphasizes the bioethanol production from plants that are abundant and available throughout the year, such as palm sap. The palm sap is a type of palm tree that grows in tropical forests, particularly in South Asia and Southeast Asia. More than 3,000 species of palm exist, and they are categorised as multipurpose trees because they can be used as raw materials for various products, such as sugar, fermented drinks, syrup, palm wine, vinegar, alcohol and bioethanol. This study was aimed to examine bioethanol production from palm sap through optimization of

Saccharomyces cerevisiae as an inoculum. The research sample was obtained from local farmers in Pusuk, Lombok Island, West Nusa Tenggara, and Indonesia. The research parameters included the pH change, colour, and ethanol content. The results showed that the pH change of the palm sap during storage was caused by the growth of microorganisms to produce organic acids by releasing hydrogen ions. As pH decreased, L* and b* values also decreased significantly, but not significantly change in a* values was observed. Glucose changed to ethanol during fermentation. The higher the percentage of inoculum used, the higher the volume of ethanol obtained. The bioethanol content of palm sap prior to fermentation was 32.3%, and it increased to 75.6% after 24 hours of incubation.

Keywords: palm sap, bioethanol, fermentation, distillation, inoculum

1. Introduction

Palm sap (*Arenga pinnata* (Wurmb) Merr) is a type of palm tree that grows in tropical forests, particularly in South Asia and Southeast Asia [1], [2], [3]. More than 3,000 species of palm exist, and they are categorised as multipurpose trees because they can be used as raw materials for various products, such as sugar, fermented drinks, syrup, palm wine, vinegar, alcohol and bioethanol [4], [5].

Some parts of the palm tree are used to meet human needs; for example, the fruit can be used for cocktails or desserts; and the fibre is used to make ropes, filters, brooms and roofs [6]. Starch, the inner part of the tree, is the raw material for noodle production. Young leaves can be consumed as a salad or cooked as an ingredient for soups. Stems (wood) can be processed for the manufacture of furniture, and roots are used as traditional medicine because they are believed to dissolve bladder stone [7].

The national palm tree cultivation programme for industrial purposes has been implemented since 2007 in Indonesia [8], [9]. The land area for palm cultivation in Indonesia

is approximately 60,482 hectares, and its potential for sap production can reach 303.76 million litres per year [10]. If production is 50% of the total population, then sap production can reach 210.600 litres/hectare/year [11]. Every 10 litres of sap can produce an average of 3.5 litres of ethanol [12]. Accordingly, 1 hectare of palm trees can produces of 73,710 litres of ethanol. If production is adequate, then the potential of biofuel from palm sap can reach 2 million kilolitres per year. Thus, biofuel exhibits considerable potential and must be developed.

The quality of palm sap is determined by the pH value [13]. The palm sap with a pH value of 6–7 is considered good quality for use in ethanol production [14]. However, new palm sap tapped with neutral pH is easily damaged; hence, the sap must be processed immediately after harvesting [15], [16], [17]. *Saccharomyces cerevisiae* microorganisms in palm sap can trigger an acidic reaction [18], [19]. This condition poses a challenge to maintaining sap quality at neutral pH.

The bioethanol production from renewable raw materials has attracted attention today because it can be used as an alternative fuel [20], [21]. Bioethanol is the most popular biofuel because it is environmentally friendly [22]. Bioethanol production for the first generation in general still uses food as raw material [23], [24]. The second generation of bioethanol has replaced it by using biomass as a feedstocks [25], [26]. The second generation technology has encouraged the development of bioethanol production with lower production costs and better environmental impact [27], [28]. Palm sap is the most promising bioethanol raw material for bioethanol production because it is available throughout the year and is abundant [29], [30].

The success factors to the bioethanol production from the palm sap include initial treatment, fermentation method, and refining [31], [32]. Busic et al. [33] asserted that the most influential factor in obtaining bioethanol content from the palm sugar is tapping and distribution during processing because palm sugar is easily damaged by environmental conditions. Kismurtono [34] also explained that the bioethanol content produced from fermented palm sap is strongly influenced by the quality of raw materials used [35], [36], [37].

Meanwhile, according to Sebayang et al. [38], the purity of ethanol can be increased through a distillation process using zeolite as an adsorbent. Bioethanol production from the banana stems for power generation applications is very profitable economically and environmentally friendly [39].

The bioethanol production from the palm sap is a relatively new technology [40]. Bioethanol has the potential as a vehicle fuel because it has a high calorific value [41]. This high calorific value results in a long burning time [42]. Fuel substitution requires an ethanol level above 95% [43]. In the current study, anaerobic fermentation is performed to produce ethanol at levels that can be used as substitutes for environment-friendly fuels [44], [45].

The research focusing on the processing of palm sap into bioethanol has been conducted by researchers, like [46], but the bioethanol content obtained is still less than 95%, so it cannot be used as a substitute for vehicle fuel. Thus, it is still important to do research to produce bioethanol that can be used as an environmentally friendly substitute for biofuels. These research use a method of fermentation and multilevel distillation to produce biofuels that can be used as biofuel substitutes that are environmentally friendly. Anaerobic fermentation method uses microbial culture made from a mixture of palm sap and Saccharomyces cerevisiae as an inoculum [47], [48]. The increase of ethanol yield up to 99.5% was done by stratified distillation [49], [50], [51]. Based on the arguments above, the purpose of this study was enhancement of bioethanol production from palm sap through optimization of saccharomyces cerevisiae as an inoculum. This research is very important as information to the bioethanol industry to develop palm sap as a raw material for making biofuels.

2. Materials and methods

2.1. Samples and tools

The raw material used was palm sap obtained from farmers in Pusuk, Lombok Island, West Nusa Tenggara Province, Indonesia. To accelerate the growth of the culture, microbes

are used. Cerevisiae as additional nutrition in preparing inoculum. The tools used were fermenters, distillation system, pH meter and Hunter Lab.

2.2. Preparation of palm sap

The palm sap was heated approximately 50-60 °C for 10 minutes to produce glucose and to destroy bacteria that were not beneficial for fermentation. Thereafter, the palm sap culture was stored for 24 hours to decrease the temperature to 28 °C [52].

2.3. Preparation of inoculum culture

To prepare the inoculum culture, 500 millilitres of palm sap was heated at 60 °C for 10-15 minutes and then cooled at the room temperature. After cooling, the palm sap was inoculated with *S. cerevisiae* and bread yeast. Then, it was incubated at 30 °C for 24 hours to be used as microbial culture. This culture was mixed with NPK as an additional nutrient to accelerate microbial growth.

2.4. pH testing

Determination of pH is done by measuring the temperature of the sample first. Then the temperature of the pH meter was turned on, adjusted, and allowed to stabilize for 15-30 minutes. The electrodes were rinsed with distilled water and dried using a tissue. The electrode is dipped into the sample until the scale reading is stable [53].

2.5. Colour testing

The change in palm sap colour was recorded in clean plastic bottles at different time intervals (ASTM D 1500-03). The colours were analysed qualitatively and quantitatively using Adobe Photoshop. The colour value of Adobe Photoshop was set to L* a* b* by using the following equation [54]:

$$L^* = \frac{Lightness}{255} \times 100 \quad (1)$$

$$a^* = \frac{240a}{255} - 120 \quad (2)$$

$$b^* = \frac{240b}{255} - 120 \quad (3)$$

where, L^* = 0 (black), L^* = 100 (white), a^* ($-a$ = greenness, $+a$ = redness) and b^* ($-b$ = blueness, $+b$ = yellowness).

2.6. Fermentation process

The palm sap was anaerobically fermented in the bottles at room temperature [55]. The palm sugar was added to the inoculum in varying concentrations of 5, 7, 10, 12, and 15% (v/v). The culture was incubated for 48, 60, and 72 hours at 30 °C. The fermentation process was performed with different volume concentrations of the palm sap, inoculum and microbes as shown in the Table 1. Reaction was accelerated by stirring the culture every 6 hours for 5 minutes. The fermentation results were centrifuged at a spinning speed of 10,000 rpm for 10 minutes at 30 °C. The parameter observed was ethanol content.

Table 1. Treatment of fermentation methods

Treatment	Volume of palm sap (%)	Volume of inoculum (%)	Nutrient (%)
F1	95	5	15
F2	93	7	15
F3	90	10	15
F4	88	12	15
F5	85	15	15

2.7. Distillation process

The water and ethanol were separated via distillation using a vacuum system with a pressure of less than 1 atm and the temperature range of 140-150 °C (ASTM D 86-04b) [56]. The fraction with the lowest boiling point can evaporate firstly and will be at the top of the column, whilst the fraction with a high boiling point will remain at the bottom of the column.

2.8. Measuring ethanol content

Ethanol content was measured using the pycnometer method [57]. Firstly, a sample of 100 millilitre was placed in a distillation flask. Then, 100 millilitre of distilled water was added and distilled at 80 °C. The distillate was stored in an Erlenmeyer flask to a volume of 50 millilitre. The distillate was then inserted into a pycnometer to meet the pycnometer. Excess distillates at the top of the capillary tube were cleaned. The pycnometer containing the distillate was weighed, and the weight was recorded. The same procedure was performed on distilled water for comparison. The results of ethanol density calculations were converted using ethanol specific gravity conversion tables. Ethanol density was calculated using the Equation (1)-(3) [58]:

$$F = g\left(m_b - \frac{\rho_{a-m_b}}{\rho_b}\right) \quad (4)$$

where, subscript b is the bottle, a is air, ρ is density, m is mass, and g is acceleration due to gravity.

2.9. Viscosity measurement

The viscosity sample was measured using a gravity capillary viscometer in the temperature range of 20-30 °C (ASTM D 88-94). The mathematical viscosity equation can be written [59]:

$$F = \eta A \frac{V}{L} \quad (5)$$

where, F is force on the surface of the liquid, η is coefficient of fluid viscosity (Ns/m^2), A is liquid area (m^2), V is moving wall velocity (m / s), and L is distance of the two surfaces (m).

2.10. Measurement of Calorific Value

The calorific value of combustion was measured using a bomb calorimeter type IKA C-5000 (ASTM D 2014-96) [60]. The reaction that occurs in the bomb calorimeter can produce heat which is absorbed by the water and the bomb, so that no heat is wasted into the air. The calorific value of combustion can be written as:

$$r_{\text{eaction}} = -(q_{\text{air}} + \text{q}_{\text{bomb}}) \quad (6)$$

The amount of heat absorbed by water can be calculated using the Equation (7):

$$Q_{water} = m.c.\Delta T \quad (7)$$

where, m is mass of water (g), c is heat type of water (J/kg°C), and ΔT is temperature change (°C).

The amount of heat absorbed by the bomb calorie meter can be calculated using the Equation (8):

$$q_{water} = c_{\text{bomb}}.\Delta\text{T} \quad (8)$$

where, c_{bomb} is heat capacity of bomb (J/g°C) and ΔT is temperature change (°C).

2.11. Data analysis

The research data were analysed using one-way analysis of variance [61]. If the F-count value is greater than the F-table, then a significant difference exists. Statistical significance between sample treatments was defined at $p<0.05$. Mean differences were evaluated with Duncan's Multiple Range Test (DMRT), which was analysed using SPSS for Windows version 16.0.

3. Results and discussion

3.1. The pH content

The pH value of palm sap used in this study was 5.6, but the value decreased after storage (Fig. 1). Based on the Fig. 1 was showed that the pH of palm sap decreases during storage due to the interaction between palm sap and environmental air and the subsequent fermentation process. A similar finding was reported by Elijah *et al.* [62] that the decrease in the pH of palm sap is caused by acid fermentation in alcohol. Devi *et al.* [63] also explained that palm sap naturally contains microbes that can produce amylase enzymes, which eventually become alcohol.

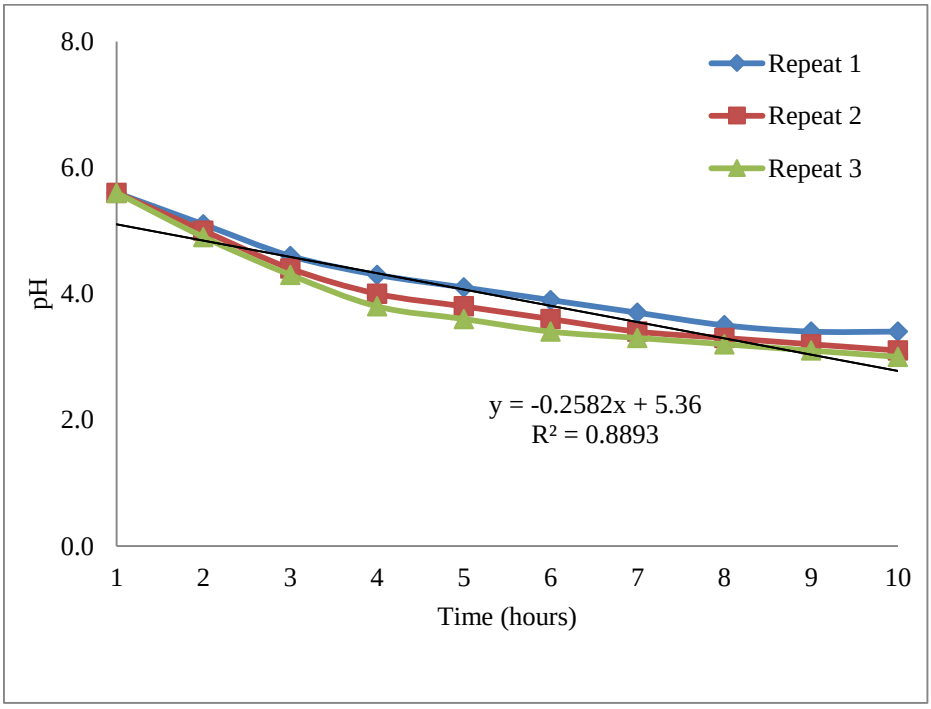

Fig. 1. The pH change with storage time variation

The palm sap is highly sensitive to environmental temperature and it easily damaged. The most easily detected damage indicator is pH value. Some researchers have also reported that the pH of palm sap drops from 7.41-6.02 after 20 hours of storage [18], from 6.70-6.34 [15]. The trend of decreasing pH in this study was apparently lower than those reported by previous researchers. Other researchers have also reported changes in pH along with the length

of storage time, such as sweet sorghum sap from pH 5.16 changing to 4.34 in 48 hours of storage [64], sugarcane with pH changed from 5.30-4.50 in 96 hours [65]. The change in the pH of palm sap is also related to the activity of microorganisms. The growth of microorganisms that can rapidly reduce pH by producing organic acids. When acid increases, pH decreases. When acids release hydrogen ions, vegetative cell bacteria can multiply well under low acid conditions. The decrease in pH by lactic acid bacteria in palm sap has also been reported by Manel et al. [66].

Temperature is an extremely influential factor in the pH decrease of palm sap. In the current study, a high storage temperature indicated a considerable decrease rate in pH presumably because changes in sugar levels to acidic levels accelerate at high temperature. Ansar et al. [67] reported that palm sap stored at 40 °C exhibits a higher decrease in pH compared with palm sap stored at 29 °C. The lowest decrease in pH value of palm sap is obtained at a storage temperature of 10 °C. Lasekan and Abbas [68] have also reported that palm sap stored at 28 °C has decreased pH faster than that stored at 15 °C.

3.2. Changes in colour (L a* b* values)*

The results show that the L* value decreased significantly ($p<0.05$) from 56.0-47.3, and the b* value decreased significantly ($p<0.05$) from 8.7-7.6. By contrast, no significant change was observed in the value of a* ($p>0.05$) during storage. These results indicate that when pH decreases, the colour values (L* and b*) of palm sap change with storage time length. It has been reported also by Manel et al. [66] that the palm colour changed from the original palm colour (pure) into milk white during the fermentation process.

The effect of colour change during storage before fermentation can be used as an indicator of palm sap quality. The results of the current study exhibit that pH value contains a function of lightness (L*) and yellowness (a*) but is not correlated with redness (b*). This finding agrees with the results of Victor and Orsat [69] that a high pH causes *A. pinnata* to

appear more transparent, whereas a low pH causes the palm sap to appear cloudy. Comparison of the pH and colour values of L* a* b* results of this study with some previous studies are shown in the Table 2.

Table 2. Comparison of the pH and colour values of L* a* b* results

Varieties of palm sap	pH	Color interval			Ref.	Tools
		L*	a*	b*		
Borassus flabellifer Linn	4.19-5.23	61.49-87.53	1.46-3.52	12.41-19-31	[17]	Hunter Lab Color flex
A. Pinnata Merr	4.883-6.387	44.5-54.8	1.2-1.6	6.5-9.8	[69]	Minolta Reader
Phoenix dactylifera L.	6.86±0.05	72.01±0.07	0.64±0.02	15.04±0.02	[70]	Lovibond Tintometer PFX 195
Palm sap	4.8-7.1	47.3-56.0	7.6-8.7	34.0-46.0	This study	Chroma Meter-CR-400)

3.3. Ethanol content

Fermentation can convert glucose in the palm sap into ethanol due to the reduced glucose levels in ethanol. This finding agrees with the results of Mustafa et al. [71] and Trivedi et al. [72]. The higher the percentage of inoculum used, the higher the ethanol content obtained (Fig. 2). The ethanol content of palm sap prior to fermentation was 32.3%, and it increased to 75.6% after 24 hours of fermentation.

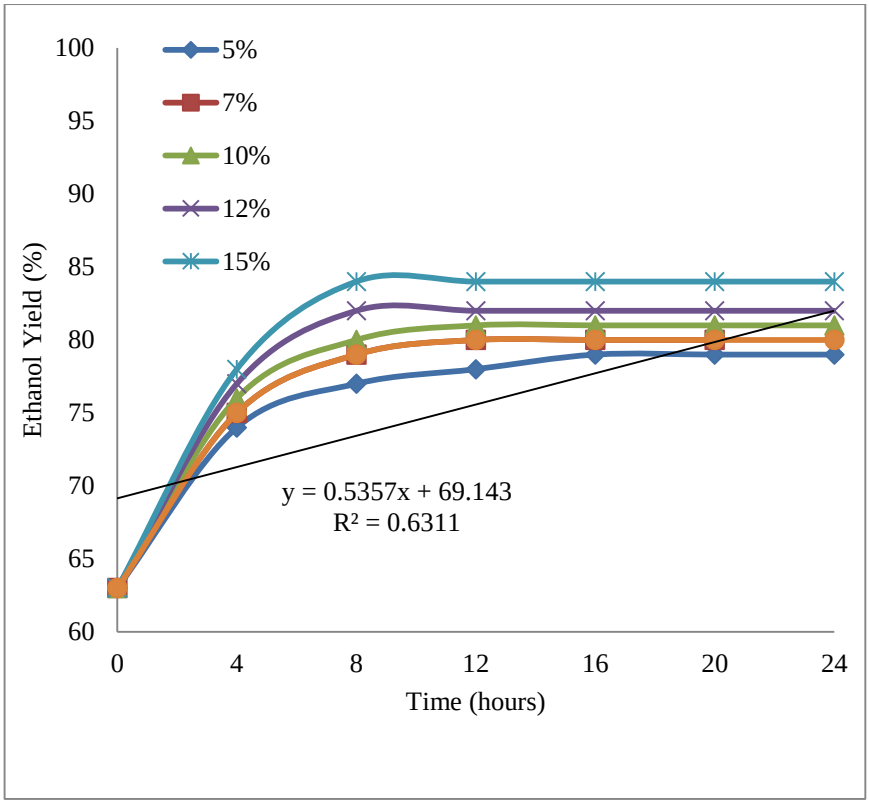

Fig. 2. Profile of ethanol concentration during anaerobic fermentation at varying inoculum concentrations

The analysis variance results showed that the F-count value is greater (368.893) than the F-table (3.885). Thus, inoculum concentration significantly affects ($p<0.05$) ethanol yield. The DMRT analysis determines that inoculum concentration variation significantly influences the obtained ethanol content. The higher the inoculum concentration, the higher the obtained ethanol content. This result was consistent with that of Mojovic et al. [73] that the content of fermented ethanol depends on the quality of sap used and the type of microbes for the inoculum.

The result of analysis of variance was known that the value of F-count is greater (368.89) than F-table (3.88) (Table 3), so it can be explained that inoculum concentration variation of significantly affect ($P<0.05$) to ethanol yield was obtained.

Table 3. Analysis of variance

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	10624.13	2	5312.07	368.89	1.68E-11	3.88
Within groups	172.8	12	14.4			
Total	10796.93	14				

The time of fermentation (incubation period) also significantly influences the ethanol yield formed. The longer the fermentation time, the higher the ethanol yield obtained. The same results have been revealed by Oguri et al. [36] who explain that the longer the fermentation process, the chances of ethanol to formed is also higher. The result of this fermentation is still a mixture of ethanol and water, so it must be separated by the distillation method. The same thing has been revealed by Hashem and Darwish [74] who state that the fermentation process usually requires an incubation period of 12-72 hours and depends on the number and type of microorganisms used to initiate fermentation.

In order to increase the ethanol content of fermented products, distillation was carried out to obtain higher ethanol levels. The distillation process was carried out with a vacuum system. In this system the fraction that has the lowest boiling point will experience evaporation first. In this study, the maximum yield obtained was still low at around 75.6%. This is allegedly because the raw materials used have been contaminated before fermentation. The same thing has been reported by Amigun and Musango [75] that the obtaining process of ethanol yield highly depends on raw materials and environmental conditions at the time of tapping.

4. Conclusion

Changes in the pH value of palm sap after tapping are caused by the growth of microorganisms that produce organic acids by releasing hydrogen ions. As the pH decreased, the values of L* and b* also decreased significantly, but there was no significant change in the

values of a*. Palm juice converts glucose into ethanol during fermentation. The higher the percentage of inoculum used, the higher the ethanol yield volume. The ethanol content of palm sap before distillation was 32.3% and increased to 75.6% after distillation. The physicochemical properties of bioethanol still need to be studied comprehensively by conducting trials on various types of motorized vehicles.

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Declaration of interests

Title of Manuscript:
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    - Enzymatic hydrolysis using ultrasound for bioethanol production from durian (durio zibethinus) seeds as potential biofuel. Chemical Engineering Transactions 56, 553-558
    - Experimental investigation, techno-economic analysis and environmental impact of bioethanol production from banana stem. Energies 12 (20), 3947
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Enhancement of bioethanol production from palm sap (*Arenga pinnata* (Wurmb) Merr) through optimization of *Saccharomyces cerevisiae* as an inoculum

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Abstract

Availability of fossil fuels is increasingly limited, so the search for alternative fuels is important. In accordance with the current scenario, bioenergy research emphasizes the bioethanol production from plants that are abundant and available throughout the year, such as palm sap. The palm sap is a type of palm tree that grows in tropical forests, particularly in South Asia and Southeast Asia. More than 3,000 species of palm exist, and they are categorised as multipurpose trees because they can be used as raw materials for various products, such as sugar, fermented drinks, syrup, palm wine, vinegar, alcohol and bioethanol. This study was aimed to examine bioethanol production from palm sap through optimization of *Saccharomyces cerevisiae* as an inoculum. The research sample was obtained from local farmers in Pusuk, Lombok Island, West Nusa Tenggara, and Indonesia. The research

parameters included the pH change, colour, and ethanol content. The results showed that the pH change of the palm sap during storage was caused by the growth of microorganisms to produce organic acids by releasing hydrogen ions. As pH decreased, L* and b* values also decreased significantly, but not significantly change in a* values was observed. Glucose changed to ethanol during fermentation. The higher the percentage of inoculum used, the higher the volume of ethanol obtained. The bioethanol content of palm sap prior to fermentation was 32.3%, and it increased to 75.6% after 24 hours of incubation.

Keywords: palm sap, bioethanol, fermentation, distillation, inoculum

1. Introduction

Palm sap (*Arenga pinnata* (Wurmb) Merr) is a type of palm tree that grows in tropical forests, particularly in South Asia and Southeast Asia [1], [2], [3]. More than 3,000 species of palm exist, and they are categorised as multipurpose trees because they can be used as raw materials for various products, such as sugar, fermented drinks, syrup, palm wine, vinegar, alcohol and bioethanol [4], [5].

Some parts of the palm tree are used to meet human needs; for example, the fruit can be used for cocktails or desserts; and the fibre is used to make ropes, filters, brooms and roofs [6]. Starch, the inner part of the tree, is the raw material for noodle production. Young leaves can be consumed as a salad or cooked as an ingredient for soups. Stems (wood) can be processed for the manufacture of furniture, and roots are used as traditional medicine because they are believed to dissolve bladder stone [7].

The national palm tree cultivation programme for industrial purposes has been implemented since 2007 in Indonesia [8], [9]. The land area for palm cultivation in Indonesia is approximately 60,482 hectares, and its potential for sap production can reach 303.76 million litres per year [10]. If production is 50% of the total population, then sap production can reach

210.600 litres/hectare/year [11]. Every 10 litres of sap can produce an average of 3.5 litres of ethanol [12]. Accordingly, 1 hectare of palm trees can produces of 73,710 litres of ethanol. If production is adequate, then the potential of biofuel from palm sap can reach 2 million kilolitres per year. Thus, biofuel exhibits considerable potential and must be developed.

The quality of palm sap is determined by the pH value [13]. The palm sap with a pH value of 6–7 is considered good quality for use in ethanol production [14]. However, new palm sap tapped with neutral pH is easily damaged; hence, the sap must be processed immediately after harvesting [15], [16], [17]. *Saccharomyces cerevisiae* microorganisms in palm sap can trigger an acidic reaction [18], [19]. This condition poses a challenge to maintaining sap quality at neutral pH.

The bioethanol production from renewable raw materials has attracted attention today because it can be used as an alternative fuel [20], [21]. Bioethanol is the most popular biofuel because it is environmentally friendly [22]. Bioethanol production for the first generation in general still uses food as raw material [23], [24]. The second generation of bioethanol has replaced it by using biomass as a feedstocks [25], [26]. The second generation technology has encouraged the development of bioethanol production with lower production costs and better environmental impact [27], [28]. Palm sap is the most promising bioethanol raw material for bioethanol production because it is available throughout the year and is abundant [29], [30].

The success factors to the bioethanol production from the palm sap include initial treatment, fermentation method, and refining [31], [32]. Busic et al. [33] asserted that the most influential factor in obtaining bioethanol content from the palm sugar is tapping and distribution during processing because palm sugar is easily damaged by environmental conditions. Kismurtono [34] also explained that the bioethanol content produced from fermented palm sap is strongly influenced by the quality of raw materials used [35], [36], [37]. Meanwhile, according to Sebayang et al. [38], the purity of ethanol can be increased through a distillation process using zeolite as an adsorbent. Bioethanol production from the banana stems

for power generation applications is very profitable economically and environmentally friendly [39].

The bioethanol production from the palm sap is a relatively new technology [40]. Bioethanol has the potential as a vehicle fuel because it has a high calorific value [41]. This high calorific value results in a long burning time [42]. Fuel substitution requires an ethanol level above 95% [43]. In the current study, anaerobic fermentation is performed to produce ethanol at levels that can be used as substitutes for environment-friendly fuels [44], [45].

The research focusing on the processing of palm sap into bioethanol has been conducted by researchers, like [46], but the bioethanol content obtained is still less than 95%, so it cannot be used as a substitute for vehicle fuel. Thus, it is still important to do research to produce bioethanol that can be used as an environmentally friendly substitute for biofuels. These research use a method of fermentation and multilevel distillation to produce biofuels that can be used as biofuel substitutes that are environmentally friendly. Anaerobic fermentation method uses microbial culture made from a mixture of palm sap and Saccharomyces cerevisiae as an inoculum [47], [48]. The increase of ethanol yield up to 99.5% was done by stratified distillation [49], [50], [51]. Based on the arguments above, the purpose of this study was enhancement of bioethanol production from palm sap through optimization of saccharomyces cerevisiae as an inoculum. This research is very important as information to the bioethanol industry to develop palm sap as a raw material for making biofuels.

2. Materials and methods

2.1. Samples and tools

The raw material used was palm sap obtained from farmers in Pusuk, Lombok Island, West Nusa Tenggara Province, Indonesia. To accelerate the growth of the culture, microbes are used. Cerevisiae as additional nutrition in preparing inoculum. The tools used were fermenters, distillation system, pH meter and Hunter Lab.

105

106 *2.2. Preparation of palm sap*

107 The palm sap was heated approximately 50-60 °C for 10 minutes to produce glucose
108 and to destroy bacteria that were not beneficial for fermentation. Thereafter, the palm sap
109 culture was stored for 24 hours to decrease the temperature to 28 °C [52].

110

111 *2.3. Preparation of inoculum culture*

112 To prepare the inoculum culture, 500 millilitres of palm sap was heated at 60 °C for 10-
113 15 minutes and then cooled at the room temperature. After cooling, the palm sap was inoculated
114 with *S. cerevisiae* and bread yeast. Then, it was incubated at 30 °C for 24 hours to be used as
115 microbial culture. This culture was mixed with NPK as an additional nutrient to accelerate
116 microbial growth.

117

118 *2.4. pH testing*

119 Determination of pH is done by measuring the temperature of the sample first. Then
120 the temperature of the pH meter was turned on, adjusted, and allowed to stabilize for 15-30
121 minutes. The electrodes were rinsed with distilled water and dried using a tissue. The electrode
122 is dipped into the sample until the scale reading is stable [53].

123

124 *2.5. Colour testing*

125 The change in palm sap colour was recorded in clean plastic bottles at different time
126 intervals (ASTM D 1500-03). The colours were analysed qualitatively and quantitatively using
127 Adobe Photoshop. The colour value of Adobe Photoshop was set to L* a* b* by using the
128 following equation [54]:

129 $$L^* = \frac{Lightness}{255} \times 100 \quad (1)$$

$$a^* = \frac{240a}{255} - 120 \quad (2)$$

$$b^* = \frac{240b}{255} - 120 \quad (3)$$

where, L^* = 0 (black), L^* = 100 (white), a^* ($-a$ = greenness, $+a$ = redness) and b^* ($-b$ = blueness, $+b$ = yellowness).

2.6. Fermentation process

The palm sap was anaerobically fermented in the bottles at room temperature [55]. The palm sugar was added to the inoculum in varying concentrations of 5, 7, 10, 12, and 15% (v/v). The culture was incubated for 48, 60, and 72 hours at 30 °C. The fermentation process was performed with different volume concentrations of the palm sap, inoculum and microbes as shown in the Table 1. Reaction was accelerated by stirring the culture every 6 hours for 5 minutes. The fermentation results were centrifuged at a spinning speed of 10,000 rpm for 10 minutes at 30 °C. The parameter observed was ethanol content.

Table 1. Treatment of fermentation methods

Treatment	Volume of palm sap (%)	Volume of inoculum (%)	Nutrient (%)
F1	95	5	15
F2	93	7	15
F3	90	10	15
F4	88	12	15
F5	85	15	15

2.7. Distillation process

147 The water and ethanol were separated via distillation using a vacuum system with a
148 pressure of less than 1 atm and the temperature range of 140-150 °C (ASTM D 86-04b) [56].
149 The fraction with the lowest boiling point can evaporate firstly and will be at the top of the
150 column, whilst the fraction with a high boiling point will remain at the bottom of the column.
151
152 *2.8. Measuring ethanol content*
153 Ethanol content was measured using the pycnometer method [57]. Firstly, a sample of
154 100 millilitre was placed in a distillation flask. Then, 100 millilitre of distilled water was added
155 and distilled at 80 °C. The distillate was stored in an Erlenmeyer flask to a volume of 50
156 millilitre. The distillate was then inserted into a pycnometer to meet the pycnometer. Excess
157 distillates at the top of the capillary tube were cleaned. The pycnometer containing the distillate
158 was weighed, and the weight was recorded. The same procedure was performed on distilled
159 water for comparison. The results of ethanol density calculations were converted using ethanol
160 specific gravity conversion tables. Ethanol density was calculated using the following equation
161 [58]:
162 $$F = g\left(m_b - \frac{\rho_{a-m_b}}{\rho_b}\right) \quad (4)$$
163 where, subscript b = the bottle, a = air, ρ = density, m = mass, and g = acceleration due to
164 gravity.
165
166 *2.9. Viscosity measurement*
167 The viscosity sample was measured using a gravity capillary viscometer in the
168 temperature range of 20-30 °C (ASTM D 88-94). The mathematical viscosity equation can be
169 written [59]:
170 $$F = \eta A \frac{V}{L} \quad (5)$$

where, F = force on the surface of the liquid, η = coefficient of fluid viscosity (Ns/m^2), A = liquid area (m^2), V = moving wall velocity (m / s), L = distance of the two surfaces (m).

2.10. Measurement of Calorific Value

The calorific value of combustion was measured using a bomb calorimeter type IKA C-5000 (ASTM D 2014-96) [60]. The reaction that occurs in the bomb calorimeter can produce heat which is absorbed by the water and the bomb, so that no heat is wasted into the air. The calorific value of combustion can be written as:

$$r_{\text{eaction}} = -(q_{\text{air}} + q_{\text{bomb}}) \quad (6)$$

The amount of heat absorbed by water can be calculated using the formula:

$$Q_{water} = m.c.\Delta T \quad (7)$$

where, m = mass of water (g), c = heat type of water (J/kg°C), and ΔT = temperature change (°C).

The amount of heat absorbed by the bomb calorie meter can be calculated using the formula:

$$q_{water} = c_{\text{bomb}}.\Delta T \quad (8)$$

where, c_{bomb} = heat capacity of bomb (J/g°C) and ΔT = temperature change (°C).

2.11. Data analysis

The research data were analysed using one-way analysis of variance [61]. If the F-count value is greater than the F-table, then a significant difference exists. Statistical significance between sample treatments was defined at $p<0.05$. Mean differences were evaluated with Duncan's Multiple Range Test (DMRT), which was analysed using SPSS for Windows version 16.0.

3. Results and discussion

3.1. The pH content

The pH value of palm sap used in this study was 5.6, but the value decreased after storage (Fig. 1). Based on the Figure 1 was showed that the pH of palm sap decreases during storage due to the interaction between palm sap and environmental air and the subsequent fermentation process. A similar finding was reported by Elijah *et al.* [62] that the decrease in the pH of palm sap is caused by acid fermentation in alcohol. Devi *et al.* [63] also explained that palm sap naturally contains microbes that can produce amylase enzymes, which eventually become alcohol.

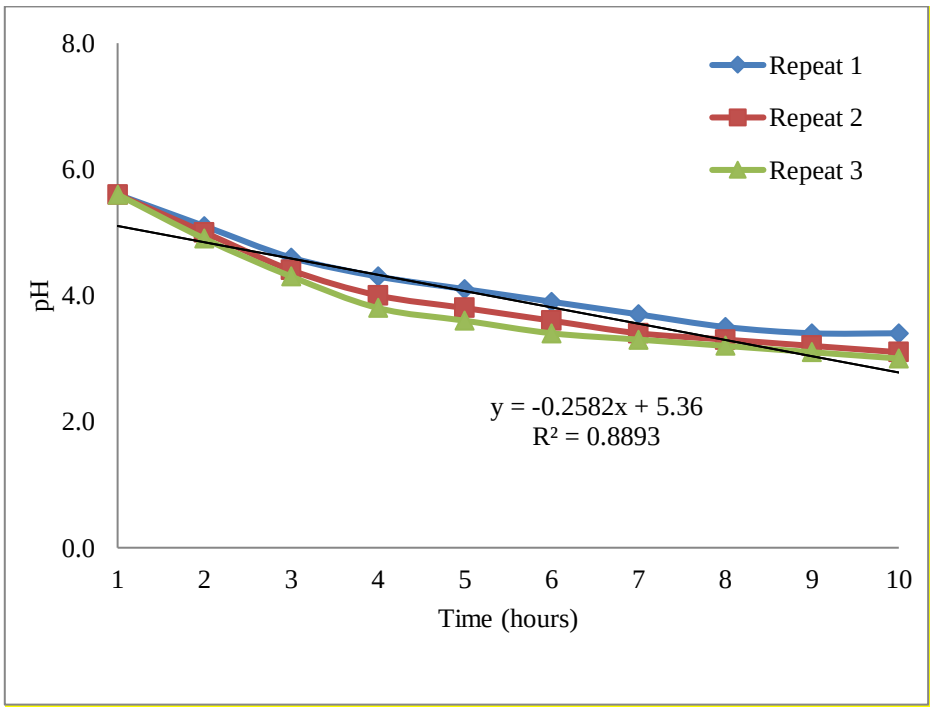

Fig. 1. The pH change with storage time variation

The palm sap is highly sensitive to environmental temperature and it easily damaged. The most easily detected damage indicator is pH value. Some researchers have also reported that the pH of palm sap drops from 7.41-6.02 after 20 hours of storage [18], from 6.70-6.34 [15]. The trend of decreasing pH in this study was apparently lower than those reported by previous researchers. Other researchers have also reported changes in pH along with the length

of storage time, such as sweet sorghum sap from pH 5.16 changing to 4.34 in 48 hours of storage [64], sugarcane with pH changed from 5.30-4.50 in 96 hours [65]. The change in the pH of palm sap is also related to the activity of microorganisms. The growth of microorganisms that can rapidly reduce pH by producing organic acids. When acid increases, pH decreases. When acids release hydrogen ions, vegetative cell bacteria can multiply well under low acid conditions. The decrease in pH by lactic acid bacteria in palm sap has also been reported by Manel et al. [66].

Temperature is an extremely influential factor in the pH decrease of palm sap. In the current study, a high storage temperature indicated a considerable decrease rate in pH presumably because changes in sugar levels to acidic levels accelerate at high temperature. Ansar et al. [67] reported that palm sap stored at 40 °C exhibits a higher decrease in pH compared with palm sap stored at 29 °C. The lowest decrease in pH value of palm sap is obtained at a storage temperature of 10 °C. Lasekan and Abbas [68] have also reported that palm sap stored at 28 °C has decreased pH faster than that stored at 15 °C.

3.2. Changes in colour (L a* b* values)*

The results show that the L* value decreased significantly ($p<0.05$) from 56.0-47.3, and the b* value decreased significantly ($p<0.05$) from 8.7-7.6. By contrast, no significant change was observed in the value of a* ($p>0.05$) during storage. These results indicate that when pH decreases, the colour values (L* and b*) of palm sap change with storage time length. It has been reported also by Manel et al. [66] that the palm colour changed from the original palm colour (pure) into milk white during the fermentation process.

The effect of colour change during storage before fermentation can be used as an indicator of palm sap quality. The results of the current study exhibit that pH value contains a function of lightness (L*) and yellowness (a*) but is not correlated with redness (b*). This finding agrees with the results of Victor and Orsat [69] that a high pH causes *A. pinnata* to

239 appear more transparent, whereas a low pH causes the palm sap to appear cloudy. Comparison
240 of the pH and colour values of L* a* b* results of this study with some previous studies are
241 shown in the Table 2.

242

243 Table 2. Comparison of the pH and colour values of L* a* b* results

Varieties of palm sap	pH	Color interval			Ref.	Tools
		L*	a*	b*		
Borassus flabellifer Linn	4.19-5.23	61.49-87.53	1.46-3.52	12.41-19-31	[17]	Hunter Lab Color flex
A. Pinnata Merr	4.883-6.387	44.5-54.8	1.2-1.6	6.5-9.8	[69]	Minolta Reader
Phoenix dactylifera L.	6.86±0.05	72.01±0.07	0.64±0.02	15.04±0.02	[70]	Lovibond Tintometer PFX 195
Palm sap	4.8-7.1	47.3-56.0	7.6-8.7	34.0-46.0	This study	Chroma Meter-CR-400)

244

245

246 *3.3. Ethanol content*

247 Fermentation can convert glucose in the palm sap into ethanol due to the reduced
248 glucose levels in ethanol. This finding agrees with the results of Mustafa et al. [71] and Trivedi
249 et al. [72]. The higher the percentage of inoculum used, the higher the ethanol content obtained
250 (Fig. 2). The ethanol content of palm sap prior to fermentation was 32.3%, and it increased to
251 75.6% after 24 hours of fermentation.

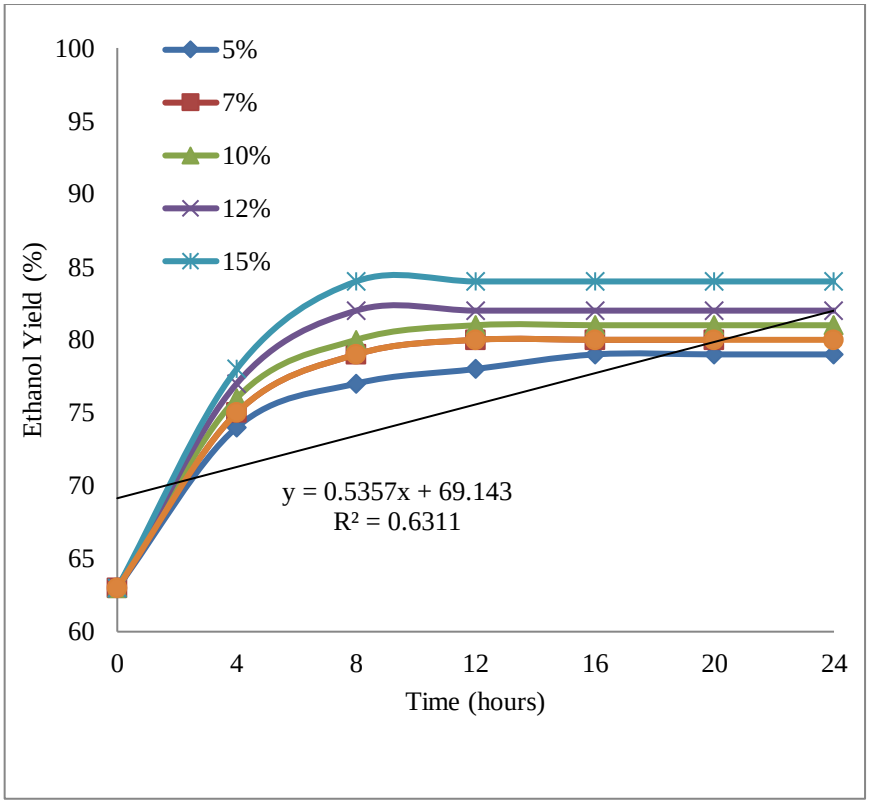

Fig. 2. Profile of ethanol concentration during anaerobic fermentation at varying inoculum concentrations

The analysis variance results showed that the F-count value is greater (368.893) than the F-table (3.885). Thus, inoculum concentration significantly affects ($p<0.05$) ethanol yield. The DMRT analysis determines that inoculum concentration variation significantly influences the obtained ethanol content. The higher the inoculum concentration, the higher the obtained ethanol content. This result was consistent with that of Mojovic et al. [73] that the content of fermented ethanol depends on the quality of sap used and the type of microbes for the inoculum.

The result of analysis of variance was known that the value of F-count is greater (368.89) than F-table (3.88) (Table 3), so it can be explained that inoculum concentration variation of significantly affect ($P<0.05$) to ethanol yield was obtained.

Table 3. Analysis of variance

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	10624.13	2	5312.07	368.89	1.68E-11	3.88
Within groups	172.8	12	14.4			
Total	10796.93	14				

The time of fermentation (incubation period) also significantly influences the ethanol yield formed. The longer the fermentation time, the higher the ethanol yield obtained. The same results have been revealed by Oguri et al. [36] who explain that the longer the fermentation process, the chances of ethanol to formed is also higher. The result of this fermentation is still a mixture of ethanol and water, so it must be separated by the distillation method. The same thing has been revealed by Hashem and Darwish [74] who state that the fermentation process usually requires an incubation period of 12-72 hours and depends on the number and type of microorganisms used to initiate fermentation.

In order to increase the ethanol content of fermented products, distillation was carried out to obtain higher ethanol levels. The distillation process was carried out with a vacuum system. In this system the fraction that has the lowest boiling point will experience evaporation first. In this study, the maximum yield obtained was still low at around 75.6%. This is allegedly because the raw materials used have been contaminated before fermentation. The same thing has been reported by Amigun and Musango [75] that the obtaining process of ethanol yield highly depends on raw materials and environmental conditions at the time of tapping.

4. Conclusion

Changes in the pH value of palm sap after tapping are caused by the growth of microorganisms that produce organic acids by releasing hydrogen ions. As the pH decreased, the values of L* and b* also decreased significantly, but there was no significant change in the

values of a*. Palm juice converts glucose into ethanol during fermentation. The higher the percentage of inoculum used, the higher the ethanol yield volume. The ethanol content of palm sap before distillation was 32.3% and increased to 75.6% after distillation. The physicochemical properties of bioethanol still need to be studied comprehensively by conducting trials on various types of motorized vehicles.

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Enhancement of bioethanol production from palm sap (*Arenga pinnata* (Wurmb) Merr) through optimization of *Saccharomyces cerevisiae* as an inoculum

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Abstract

Availability of fossil fuels is increasingly limited, so the search for alternative fuels is important. In accordance with the current scenario, bioenergy research emphasizes the bioethanol production from plants that are abundant and available throughout the year, such as palm sap. The palm sap is a type of palm tree that grows in tropical forests, particularly in South Asia and Southeast Asia. More than 3,000 species of palm exist, and they are categorised as multipurpose trees because they can be used as raw materials for various products, such as sugar, fermented drinks, syrup, palm wine, vinegar, alcohol and bioethanol. This study was aimed to examine bioethanol production from palm sap through optimization

of *Saccharomyces cerevisiae* as an inoculum. The research sample was obtained from local farmers in Pusuk, Lombok Island, West Nusa Tenggara, and Indonesia. The research parameters included the pH change, colour, and ethanol content. The results showed that the pH change of the palm sap during storage was caused by the growth of microorganisms to produce organic acids by releasing hydrogen ions. As pH decreased, L* and b* values also decreased significantly, but not significantly change in a* values was observed. Glucose changed to ethanol during fermentation. The higher the percentage of inoculum used, the higher the volume of ethanol obtained. The bioethanol content of palm sap prior to fermentation was 32.3%, and it increased to 75.6% after 24 hours of incubation.

Keywords: palm sap, bioethanol, fermentation, distillation, inoculum

1. Introduction

Palm sap (*Arenga pinnata* (Wurmb) Merr) is a type of palm tree that grows in tropical forests, particularly in South Asia and Southeast Asia [1], [2], [3]. More than 3,000 species of palm exist, and they are categorised as multipurpose trees because they can be used as raw materials for various products, such as sugar, fermented drinks, syrup, palm wine, vinegar, alcohol and bioethanol [4], [5].

Some parts of the palm tree are used to meet human needs; for example, the fruit can be used for cocktails or desserts; and the fibre is used to make ropes, filters, brooms and roofs [6]. Starch, the inner part of the tree, is the raw material for noodle production. Young leaves can be consumed as a salad or cooked as an ingredient for soups. Stems (wood) can be processed for the manufacture of furniture, and roots are used as traditional medicine because they are believed to dissolve bladder stone [7].

The national palm tree cultivation programme for industrial purposes has been implemented since 2007 in Indonesia [8], [9]. The land area for palm cultivation in Indonesia

is approximately 60,482 hectares, and its potential for sap production can reach 303.76 million litres per year [10]. If production is 50% of the total population, then sap production can reach 210.600 litres/hectare/year [11]. Every 10 litres of sap can produce an average of 3.5 litres of ethanol [12]. Accordingly, 1 hectare of palm trees can produces of 73,710 litres of ethanol. If production is adequate, then the potential of biofuel from palm sap can reach 2 million kilolitres per year. Thus, biofuel exhibits considerable potential and must be developed.

The quality of palm sap is determined by the pH value [13]. The palm sap with a pH value of 6–7 is considered good quality for use in ethanol production [14]. However, new palm sap tapped with neutral pH is easily damaged; hence, the sap must be processed immediately after harvesting [15], [16], [17]. *Saccharomyces cerevisiae* microorganisms in palm sap can trigger an acidic reaction [18], [19]. This condition poses a challenge to maintaining sap quality at neutral pH.

The bioethanol production from renewable raw materials has attracted attention today because it can be used as an alternative fuel [20], [21]. Bioethanol is the most popular biofuel because it is environmentally friendly [22]. Bioethanol production for the first generation in general still uses food as raw material [23], [24]. The second generation of bioethanol has replaced it by using biomass as a feedstocks [25], [26]. The second generation technology has encouraged the development of bioethanol production with lower production costs and better environmental impact [27], [28]. Palm sap is the most promising bioethanol raw material for bioethanol production because it is available throughout the year and is abundant [29], [30].

The success factors to the bioethanol production from the palm sap include initial treatment, fermentation method, and refining [31], [32]. Busic et al. [33] asserted that the most influential factor in obtaining bioethanol content from the palm sugar is tapping and distribution during processing because palm sugar is easily damaged by environmental conditions. Kismurtono [34] also explained that the bioethanol content produced from

fermented palm sap is strongly influenced by the quality of raw materials used [35], [36], [37]. Meanwhile, according to Sebayang et al. [38], the purity of ethanol can be increased through a distillation process using zeolite as an adsorbent. Bioethanol production from the banana stems for power generation applications is very profitable economically and environmentally friendly [39].

The bioethanol production from the palm sap is a relatively new technology [40]. Bioethanol has the potential as a vehicle fuel because it has a high calorific value [41]. This high calorific value results in a long burning time [42]. Fuel substitution requires an ethanol level above 95% [43]. In the current study, anaerobic fermentation is performed to produce ethanol at levels that can be used as substitutes for environment-friendly fuels [44], [45].

The research focusing on the processing of palm sap into bioethanol has been conducted by researchers, like [46], but the bioethanol content obtained is still less than 95%, so it cannot be used as a substitute for vehicle fuel. Thus, it is still important to do research to produce bioethanol that can be used as an environmentally friendly substitute for biofuels. These research use a method of fermentation and multilevel distillation to produce biofuels that can be used as biofuel substitutes that are environmentally friendly. Anaerobic fermentation method uses microbial culture made from a mixture of palm sap and Saccharomyces cerevisiae as an inoculum [47], [48]. The increase of ethanol yield up to 99.5% was done by stratified distillation [49], [50], [51]. Based on the arguments above, the purpose of this study was enhancement of bioethanol production from palm sap through optimization of saccharomyces cerevisiae as an inoculum. This research is very important as information to the bioethanol industry to develop palm sap as a raw material for making biofuels.

2. Materials and methods

2.1. Samples and tools

The raw material used was palm sap obtained from farmers in Pusuk, Lombok Island, West Nusa Tenggara Province, Indonesia. To accelerate the growth of the culture, microbes are used. Cerevisiae as additional nutrition in preparing inoculum. The tools used were fermenters, distillation system, pH meter and Hunter Lab.

2.2. Preparation of palm sap

The palm sap was heated approximately 50-60 °C for 10 minutes to produce glucose and to destroy bacteria that were not beneficial for fermentation. Thereafter, the palm sap culture was stored for 24 hours to decrease the temperature to 28 °C [52].

2.3. Preparation of inoculum culture

To prepare the inoculum culture, 500 millilitres of palm sap was heated at 60 °C for 10-15 minutes and then cooled at the room temperature. After cooling, the palm sap was inoculated with *S. cerevisiae* and bread yeast. Then, it was incubated at 30 °C for 24 hours to be used as microbial culture. This culture was mixed with NPK as an additional nutrient to accelerate microbial growth.

2.4. pH testing

Determination of pH is done by measuring the temperature of the sample first. Then the temperature of the pH meter was turned on, adjusted, and allowed to stabilize for 15-30 minutes. The electrodes were rinsed with distilled water and dried using a tissue. The electrode is dipped into the sample until the scale reading is stable [53].

2.5. Colour testing

The change in palm sap colour was recorded in clean plastic bottles at different time intervals (ASTM D 1500-03). The colours were analysed qualitatively and quantitatively

using Adobe Photoshop. The colour value of Adobe Photoshop was set to L* a* b* by using the following equation [54]:

$$L^* = \frac{Lightness}{255} \times 100 \quad (1)$$

$$a^* = \frac{240a}{255} - 120 \quad (2)$$

$$b^* = \frac{240b}{255} - 120 \quad (3)$$

where, L^* = 0 (black), L^* = 100 (white), a^* ($-a$ = greenness, $+a$ = redness) and b^* ($-b$ = blueness, $+b$ = yellowness).

2.6. Fermentation process

The palm sap was anaerobically fermented in the bottles at room temperature [55]. The palm sugar was added to the inoculum in varying concentrations of 5, 7, 10, 12, and 15% (v/v). The culture was incubated for 48, 60, and 72 hours at 30 °C. The fermentation process was performed with different volume concentrations of the palm sap, inoculum and microbes as shown in the Table 1. Reaction was accelerated by stirring the culture every 6 hours for 5 minutes. The fermentation results were centrifuged at a spinning speed of 10,000 rpm for 10 minutes at 30 °C. The parameter observed was ethanol content.

Table 1. Treatment of fermentation methods

Treatment	Volume of palm sap (%)	Volume of inoculum (%)	Nutrient (%)
F1	95	5	15
F2	93	7	15
F3	90	10	15
F4	88	12	15
F5	85	15	15

149

150 *2.7. Distillation process*

151 The water and ethanol were separated via distillation using a vacuum system with a
152 pressure of less than 1 atm and the temperature range of 140-150 °C (ASTM D 86-04b) [56].
153 The fraction with the lowest boiling point can evaporate firstly and will be at the top of the
154 column, whilst the fraction with a high boiling point will remain at the bottom of the column.

155

156 *2.8. Measuring ethanol content*

157 Ethanol content was measured using the pycnometer method [57]. Firstly, a sample of
158 100 millilitre was placed in a distillation flask. Then, 100 millilitre of distilled water was
159 added and distilled at 80 °C. The distillate was stored in an Erlenmeyer flask to a volume of
160 50 millilitre. The distillate was then inserted into a pycnometer to meet the pycnometer.
161 Excess distillates at the top of the capillary tube were cleaned. The pycnometer containing the
162 distillate was weighed, and the weight was recorded. The same procedure was performed on
163 distilled water for comparison. The results of ethanol density calculations were converted
164 using ethanol specific gravity conversion tables. Ethanol density was calculated using the
165 Equation (1)-(3) [58]:

166 $$F = g\left(m_b - \frac{\rho_{a-m_b}}{\rho_b}\right) \quad (4)$$

167 where, subscript b is the bottle, a is air, ρ is density, m is mass, and g is acceleration due to
168 gravity.

169

170 *2.9. Viscosity measurement*

171 The viscosity sample was measured using a gravity capillary viscometer in the
172 temperature range of 20-30 °C (ASTM D 88-94). The mathematical viscosity equation can be
173 written [59]:

$$F = \eta A \frac{V}{L} \quad (5)$$

where, F is force on the surface of the liquid, η is coefficient of fluid viscosity (Ns/m^2), A is liquid area (m^2), V is moving wall velocity (m / s), and L is distance of the two surfaces (m).

2.10. Measurement of Calorific Value

The calorific value of combustion was measured using a bomb calorimeter type IKA C-5000 (ASTM D 2014-96) [60]. The reaction that occurs in the bomb calorimeter can produce heat which is absorbed by the water and the bomb, so that no heat is wasted into the air. The calorific value of combustion can be written as:

$$r_{\text{eaction}} = -(q_{\text{air}} + \text{q}_{\text{bomb}}) \quad (6)$$

The amount of heat absorbed by water can be calculated using the Equation (7):

$$Q_{water} = m.c.\Delta T \quad (7)$$

where, m is mass of water (g), c is heat type of water (J/kg°C), and ΔT is temperature change (°C).

The amount of heat absorbed by the bomb calorie meter can be calculated using the Equation (8):

$$q_{water} = c_{\text{bomb}}.\Delta\text{T} \quad (8)$$

where, c_{bomb} is heat capacity of bomb (J/g°C) and ΔT is temperature change (°C).

2.11. Data analysis

The research data were analysed using one-way analysis of variance [61]. If the F-count value is greater than the F-table, then a significant difference exists. Statistical significance between sample treatments was defined at $p<0.05$. Mean differences were evaluated with Duncan's Multiple Range Test (DMRT), which was analysed using SPSS for Windows version 16.0.

3. Results and discussion

3.1. The pH content

The pH value of palm sap used in this study was 5.6, but the value decreased after storage (Fig. 1). Based on the Fig. 1 was showed that the pH of palm sap decreases during storage due to the interaction between palm sap and environmental air and the subsequent fermentation process. A similar finding was reported by Elijah *et al.* [62] that the decrease in the pH of palm sap is caused by acid fermentation in alcohol. Devi *et al.* [63] also explained that palm sap naturally contains microbes that can produce amylase enzymes, which eventually become alcohol.

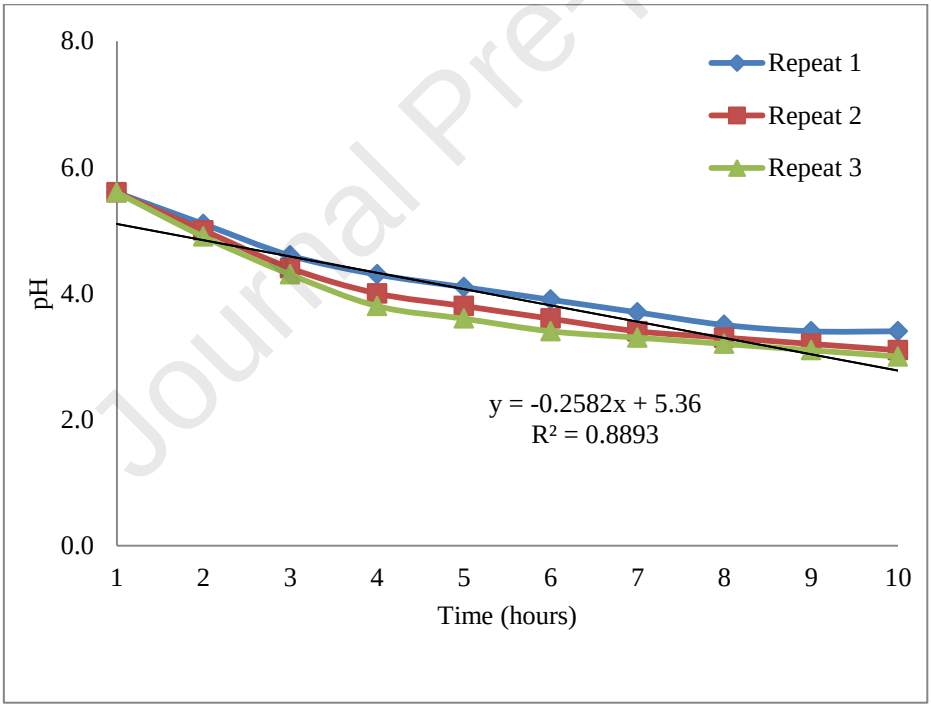

Fig. 1. The pH change with storage time variation

The palm sap is highly sensitive to environmental temperature and it easily damaged. The most easily detected damage indicator is pH value. Some researchers have also reported that the pH of palm sap drops from 7.41-6.02 after 20 hours of storage [18], from 6.70-6.34 [15]. The trend of decreasing pH in this study was apparently lower than those reported by

previous researchers. Other researchers have also reported changes in pH along with the length of storage time, such as sweet sorghum sap from pH 5.16 changing to 4.34 in 48 hours of storage [64], sugarcane with pH changed from 5.30-4.50 in 96 hours [65]. The change in the pH of palm sap is also related to the activity of microorganisms. The growth of microorganisms that can rapidly reduce pH by producing organic acids. When acid increases, pH decreases. When acids release hydrogen ions, vegetative cell bacteria can multiply well under low acid conditions. The decrease in pH by lactic acid bacteria in palm sap has also been reported by Manel et al. [66].

Temperature is an extremely influential factor in the pH decrease of palm sap. In the current study, a high storage temperature indicated a considerable decrease rate in pH presumably because changes in sugar levels to acidic levels accelerate at high temperature. Ansar et al. [67] reported that palm sap stored at 40 °C exhibits a higher decrease in pH compared with palm sap stored at 29 °C. The lowest decrease in pH value of palm sap is obtained at a storage temperature of 10 °C. Lasekan and Abbas [68] have also reported that palm sap stored at 28 °C has decreased pH faster than that stored at 15 °C.

3.2. Changes in colour (L a* b* values)*

The results show that the L* value decreased significantly ($p<0.05$) from 56.0-47.3, and the b* value decreased significantly ($p<0.05$) from 8.7-7.6. By contrast, no significant change was observed in the value of a* ($p>0.05$) during storage. These results indicate that when pH decreases, the colour values (L* and b*) of palm sap change with storage time length. It has been reported also by Manel et al. [66] that the palm colour changed from the original palm colour (pure) into milk white during the fermentation process.

The effect of colour change during storage before fermentation can be used as an indicator of palm sap quality. The results of the current study exhibit that pH value contains a function of lightness (L*) and yellowness (a*) but is not correlated with redness (b*). This

finding agrees with the results of Victor and Orsat [69] that a high pH causes *A. pinnata* to appear more transparent, whereas a low pH causes the palm sap to appear cloudy. Comparison of the pH and colour values of L* a* b* results of this study with some previous studies are shown in the Table 2.

Table 2. Comparison of the pH and colour values of L* a* b* results

Varieties of palm sap	pH	Color interval			Ref.	Tools
		L*	a*	b*		
Borassus flabellifer Linn	4.19-5.23	61.49-87.53	1.46-3.52	12.41-19-31	[17]	Hunter Lab Color flex
A. Pinnata Merr	4.883-6.387	44.5-54.8	1.2-1.6	6.5-9.8	[69]	Minolta Reader
Phoenix dactylifera L.	6.86±0.05	72.01±0.07	0.64±0.02	15.04± 0.02	[70]	Lovibond Tintometer PFX 195
Palm sap	4.8-7.1	47.3-56.0	7.6-8.7	34.0-46.0	This study	Chroma Meter-CR-400)

3.3. Ethanol content

Fermentation can convert glucose in the palm sap into ethanol due to the reduced glucose levels in ethanol. This finding agrees with the results of Mustafa et al. [71] and Trivedi et al. [72]. The higher the percentage of inoculum used, the higher the ethanol content obtained (Fig. 2). The ethanol content of palm sap prior to fermentation was 32.3%,

and it increased to 75.6% after 24 hours of fermentation.

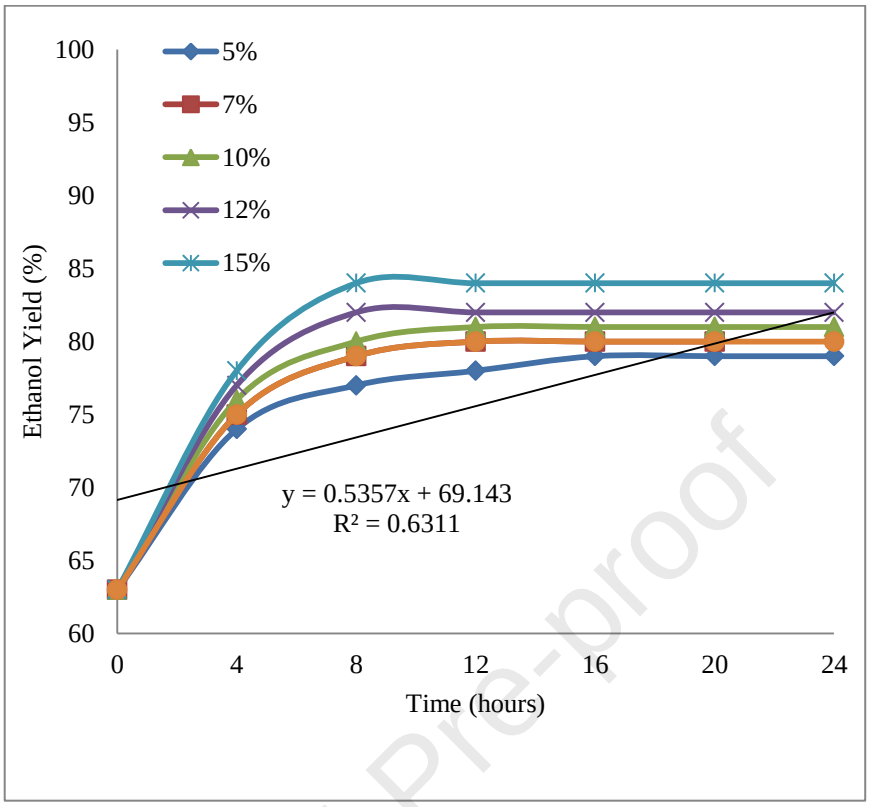

Fig. 2. Profile of ethanol concentration during anaerobic fermentation at varying inoculum concentrations

The analysis variance results showed that the F-count value is greater (368.893) than the F-table (3.885). Thus, inoculum concentration significantly affects ($p<0.05$) ethanol yield. The DMRT analysis determines that inoculum concentration variation significantly influences the obtained ethanol content. The higher the inoculum concentration, the higher the obtained ethanol content. This result was consistent with that of Mojovic et al. [73] that the content of fermented ethanol depends on the quality of sap used and the type of microbes for the inoculum.

The result of analysis of variance was known that the value of F-count is greater (368.89) than F-table (3.88) (Table 3), so it can be explained that inoculum concentration variation of significantly affect ($P<0.05$) to ethanol yield was obtained.

Table 3. Analysis of variance

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	10624.13	2	5312.07	368.89	1.68E-11	3.88
Within groups	172.8	12	14.4			
Total	10796.93	14				

The time of fermentation (incubation period) also significantly influences the ethanol yield formed. The longer the fermentation time, the higher the ethanol yield obtained. The same results have been revealed by Oguri et al. [36] who explain that the longer the fermentation process, the chances of ethanol to formed is also higher. The result of this fermentation is still a mixture of ethanol and water, so it must be separated by the distillation method. The same thing has been revealed by Hashem and Darwish [74] who state that the fermentation process usually requires an incubation period of 12-72 hours and depends on the number and type of microorganisms used to initiate fermentation.

In order to increase the ethanol content of fermented products, distillation was carried out to obtain higher ethanol levels. The distillation process was carried out with a vacuum system. In this system the fraction that has the lowest boiling point will experience evaporation first. In this study, the maximum yield obtained was still low at around 75.6%. This is allegedly because the raw materials used have been contaminated before fermentation. The same thing has been reported by Amigun and Musango [75] that the obtaining process of ethanol yield highly depends on raw materials and environmental conditions at the time of tapping.

4. Conclusion

Changes in the pH value of palm sap after tapping are caused by the growth of microorganisms that produce organic acids by releasing hydrogen ions. As the pH decreased, the values of L* and b* also decreased significantly, but there was no significant change in the values of a*. Palm juice converts glucose into ethanol during fermentation. The higher the percentage of inoculum used, the higher the ethanol yield volume. The ethanol content of palm sap before distillation was 32.3% and increased to 75.6% after distillation. The physicochemical properties of bioethanol still need to be studied comprehensively by conducting trials on various types of motorized vehicles.

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Declaration of interests

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Original Article

Enhancement of bioethanol production from palm sap (*Arenga pinnata* (Wurmb) Merr) through optimization of *Saccharomyces cerevisiae* as an inoculum

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Inoculum

ABSTRACT

Availability of fossil fuels is increasingly limited, so the search for alternative fuels is important. In accordance with the current scenario, bioenergy research emphasizes the bioethanol production from plants that are abundant and available throughout the year, such as palm sap. The palm sap is a type of palm tree that grows in tropical forests, particularly in South Asia and Southeast Asia. More than 3000 species of palm exist, and they are categorised as multipurpose trees because they can be used as raw materials for various products, such as sugar, fermented drinks, syrup, palm wine, vinegar, alcohol and bioethanol. This study was aimed to examine bioethanol production from palm sap through optimization of *Saccharomyces cerevisiae* as an inoculum. The research sample was obtained from local farmers in Pusuk, Lombok Island, West Nusa Tenggara, and Indonesia. The research parameters included the pH change, colour, and ethanol content. The results showed that the pH change of the palm sap during storage was caused by the growth of microorganisms to produce organic acids by releasing hydrogen ions. As pH decreased, L* and b* values also decreased significantly, but not significantly change in a* values was observed. Glucose changed to ethanol during fermentation. The higher the percentage of inoculum used, the higher the volume of ethanol obtained. The bioethanol content of palm sap prior to fermentation was 32.3%, and it increased to 75.6% after 24 hours of incubation.

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1. Introduction

Palm sap (*Arenga pinnata* (Wurmb) Merr) is a type of palm tree that grows in tropical forests, particularly in South Asia and Southeast Asia [1–3]. More than 3000 species of palm exist, and they are categorised as multipurpose trees because they can be used as raw materials for various products, such as sugar, fermented drinks, syrup, palm wine, vinegar, alcohol and bioethanol [4,5].

Some parts of the palm tree are used to meet human needs; for example, the fruit can be used for cocktails or desserts; and the fibre is used to make ropes, filters, brooms and roofs [6]. Starch, the inner part of the tree, is the raw material for noodle production. Young leaves can be consumed as a salad or cooked as an ingredient for soups. Stems (wood) can be processed for the manufacture of furniture, and roots are used as traditional medicine because they are believed to dissolve bladder stone [7].

The national palm tree cultivation programme for industrial purposes has been implemented since 2007 in Indonesia [8,9]. The land area for palm cultivation in Indonesia is approximately 60,482 ha, and its potential for sap production can reach 303.76 million litres per year [10]. If production is 50% of the total population, then sap production can reach 210.600 L/ha/year [11]. Every 10 L of sap can produce an average of 3.5 L of ethanol [12]. Accordingly, 1 ha of palm trees can produces of 73,710 L of ethanol. If production is adequate, then the potential of biofuel from palm sap can reach 2 million kilolitres per year. Thus, biofuel exhibits considerable potential and must be developed.

The quality of palm sap is determined by the pH value [13]. The palm sap with a pH value of 6–7 is considered good quality for use in ethanol production [14]. However, new palm sap tapped with neutral pH is easily damaged; hence, the sap must be processed immediately after harvesting [15–17]. *Saccharomyces cerevisiae* microorganisms in palm sap can trigger an acidic reaction [18,19]. This condition poses a challenge to maintaining sap quality at neutral pH.

The bioethanol production from renewable raw materials has attracted attention today because it can be used as an alternative fuel [20,21]. Bioethanol is the most popular biofuel because it is environmentally friendly [22]. Bioethanol production for the first generation in general still uses food as raw material [23,24]. The second generation of bioethanol has replaced it by using biomass as a feedstocks [25,26]. The second generation technology has encouraged the development of bioethanol production with lower production costs and better environmental impact [27,28]. Palm sap is the most promising bioethanol raw material for bioethanol production because it is available throughout the year and is abundant [29,30].

The success factors to the bioethanol production from the palm sap include initial treatment, fermentation method, and refining [31,32]. Busic et al. [33] asserted that the most influential factor in obtaining bioethanol content from the palm sugar is tapping and distribution during processing because palm sugar is easily damaged by environmental conditions. Kismurtono [34] also explained that the bioethanol content produced from fermented palm sap is strongly influenced by the quality of raw materials used [35–37]. Meanwhile, according to Sebayang et al. [38], the purity of ethanol can be increased through a distillation process using zeolite as an adsorbent. Bioethanol production from the banana stems for power generation applications is very profitable economically and environmentally friendly [39].

The bioethanol production from the palm sap is a relatively new technology [40]. Bioethanol has the potential as a vehicle fuel because it has a high calorific value [41]. This high calorific value results in a long burning time [42]. Fuel substitution requires an ethanol level above 95% [43]. In the current study, anaerobic fermentation is performed to produce ethanol at levels that can be used as substitutes for environment-friendly fuels [44,45].

The research focusing on the processing of palm sap into bioethanol has been conducted by researchers, like [46], but the bioethanol content obtained is still less than 95%, so it cannot be used as a substitute for vehicle fuel. Thus, it is still important to do research to produce bioethanol that can be used as an environmentally friendly substitute for biofuels. These research use a method of fermentation and multilevel distillation to produce biofuels that can be used as biofuel substitutes that are environmentally friendly. Anaerobic fermentation method uses microbial culture made from a mixture of palm sap and *S. cerevisiae* as an inoculum [47,48]. The increase of ethanol yield up to 99.5% was done by stratified distillation [49–51]. Based on the arguments above, the purpose of this study was enhancement of bioethanol production from palm sap through optimization of *S. cerevisiae* as an inoculum. This research is very important as information to the bioethanol industry to develop palm sap as a raw material for making biofuels.

2. Materials and methods

2.1. Samples and tools

The raw material used was palm sap obtained from farmers in Pusuk, Lombok Island, West Nusa Tenggara Province, Indonesia. To accelerate the growth of the culture, microbes are used. Cerevisiae as additional nutrition in preparing inoculum. The tools used were fermenters, distillation system, pH meter and Hunter Lab.

2.2. Preparation of palm sap

The palm sap was heated approximately 50–60 °C for 10 min to produce glucose and to destroy bacteria that were not beneficial for fermentation. Thereafter, the palm sap culture was stored for 24 h to decrease the temperature to 28 °C [52].

2.3. Preparation of inoculum culture

To prepare the inoculum culture, 500 mL of palm sap was heated at 60 °C for 10–15 min and then cooled at the room temperature. After cooling, the palm sap was inoculated with *Saccharomyces cerevisiae* and bread yeast. Then, it was incubated at 30 °C for 24 h to be used as microbial culture. This culture was mixed with NPK as an additional nutrient to accelerate microbial growth.

2.4. pH testing

Determination of pH is done by measuring the temperature of the sample first. Then the temperature of the pH meter was turned on, adjusted, and allowed to stabilize for 15–30 min. The electrodes were rinsed with distilled water and dried using a tissue. The electrode is dipped into the sample until the scale reading is stable [53].

2.5. Colour testing

The change in palm sap colour was recorded in clean plastic bottles at different time intervals (ASTM D 1500-03). The colours were analysed qualitatively and quantitatively using Adobe Photoshop. The colour value of Adobe Photoshop was set to L* a* b* by using the following equation [54]:

$$L^* = \frac{Lightness}{255} \times 100 \quad (1)$$

$$a^* = \frac{240a}{255} - 120 \quad (2)$$

$$b^* = \frac{240b}{255} - 120 \quad (3)$$

where, $L^* = 0$ (black), $L^* = 100$ (white), a^* ($-a$ = greenness, $+a$ = redness) and b^* ($-b$ = blueness, $+b$ = yellowness).

2.6. Fermentation process

The palm sap was anaerobically fermented in the bottles at room temperature [55]. The palm sugar was added to the inoculum in varying concentrations of 5, 7, 10, 12, and 15% (v/v). The culture was incubated for 48, 60, and 72 h at 30 °C. The fermentation process was performed with different volume concentrations of the palm sap, inoculum and microbes as shown in the Table 1. Reaction was accelerated by stirring the culture every 6 h for 5 min. The fermentation results were centrifuged at a spinning speed of 10,000 rpm for 10 min at 30 °C. The parameter observed was ethanol content.

2.7. Distillation process

The water and ethanol were separated via distillation using a vacuum system with a pressure of less than 1 atm and the temperature range of 140–150 °C (ASTM D 86-04b) [56]. The fraction with the lowest boiling point can evaporate firstly and will be at the top of the column, whilst the fraction with a high boiling point will remain at the bottom of the column.

Table 1 – Treatment of fermentation methods.

Treatment	Volume of palm sap (%)	Volume of inoculum (%)	Nutrient (%)
F1	95	5	15
F2	93	7	15
F3	90	10	15
F4	88	12	15
F5	85	15	15

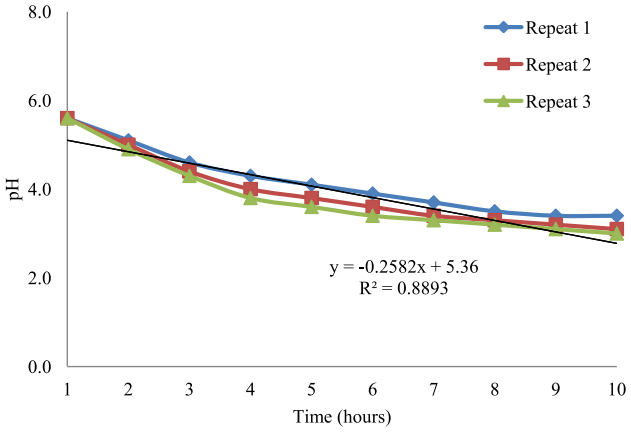

Fig. 1 – The pH change with storage time variation.

2.8. Measuring ethanol content

Ethanol content was measured using the pycnometer method [57]. Firstly, a sample of 100 mL was placed in a distillation flask. Then, 100 mL of distilled water was added and distilled at 80 °C. The distillate was stored in an Erlenmeyer flask to a volume of 50 mL. The distillate was then inserted into a pycnometer to meet the pycnometer. Excess distillates at the top of the capillary tube were cleaned. The pycnometer containing the distillate was weighed, and the weight was recorded. The same procedure was performed on distilled water for comparison. The results of ethanol density calculations were converted using ethanol specific gravity conversion tables. Ethanol density was calculated using the Eqs. (1–3) [58]:

$$F = g\left(m_b - \frac{\rho_{a-m_b}}{\rho_b}\right) \quad (4)$$

where, subscript b is the bottle, a is air, ρ is density, m is mass, and g is acceleration due to gravity.

2.9. Viscosity measurement

The viscosity sample was measured using a gravity capillary viscometer in the temperature range of 20–30 °C (ASTM D 88-94). The mathematical viscosity equation can be written [59]:

$$F = \eta A \frac{V}{L} \quad (5)$$

where, F is force on the surface of the liquid, η is coefficient of fluid viscosity (Ns/m^2), A is liquid area (m^2), V is moving wall velocity (m/s), and L is distance of the two surfaces (m).

2.10. Measurement of calorific value

The calorific value of combustion was measured using a bomb calorimeter type IKA C-5000 (ASTM D 2014-96) [60]. The reaction that occurs in the bomb calorimeter can produce heat which is absorbed by the water and the bomb, so that no heat is wasted into the air. The calorific value of combustion can be written as:

$$r_{reaction} = -\left(q_{air} + q_{bomb}\right) \quad (6)$$

Table 2 – Comparison of the pH and colour values of L* a* b* results.

Varieties of palm sap	pH	Colour interval			Ref.	Tools
		L*	a*	b*		
Borassus flabellifer Linn	4.19–5.23	61.49–87.53	1.46–3.52	12.41-19-31	[17]	Hunter Lab Color flex
A. Pinnata Merr	4.883–6.387	44.5–54.8	1.2–1.6	6.5–9.8	[69]	Minolta Reader
Phoenix dactylifera L.	6.86 ± 0.05	72.01 ± 0.07	0.64 ± 0.02	15.04 ± 0.02	[70]	Lovibond Tintometer PFX 195
Palm sap	4.8–7.1	47.3–56.0	7.6–8.7	34.0–46.0	This study	Chroma Meter-CR-400)

The amount of heat absorbed by water can be calculated using the Eq. (7):

$$Q_{water} = m \cdot c \cdot \Delta T \quad (7)$$

where, m is mass of water (g), c is heat type of water (J/kg°C), and ΔT is temperature change (°C).

The amount of heat absorbed by the bomb calorie meter can be calculated using the Eq. (8):

$$q_{water} = c_{bomb} \cdot \Delta T \quad (8)$$

where, c_{bomb} is heat capacity of bomb (J/g°C) and ΔT is temperature change (°C).

2.11. Data analysis

The research data were analysed using one-way analysis of variance [61]. If the F-count value is greater than the F-table, then a significant difference exists. Statistical significance between sample treatments was defined at $p < 0.05$. Mean differences were evaluated with Duncan's Multiple Range Test (DMRT), which was analysed using SPSS for Windows version 16.0.

3. Results and discussion

3.1. The pH content

The pH value of palm sap used in this study was 5.6, but the value decreased after storage (Fig. 1). Based on the Fig. 1 was showed that the pH of palm sap decreases during storage due to the interaction between palm sap and environmental air and the subsequent fermentation process. A similar finding was reported by Elijah et al. [62] that the decrease in the pH of palm sap is caused by acid fermentation in alcohol. Devi et al. [63] also explained that palm sap naturally contains microbes that can produce amylase enzymes, which eventually become alcohol.

The palm sap is highly sensitive to environmental temperature and it easily damaged. The most easily detected damage indicator is pH value. Some researchers have also reported that the pH of palm sap drops from 7.41 to 6.02 after 20 h of storage [18], from 6.70 to 6.34 [15]. The trend of decreasing pH in this study was apparently lower than those reported by previous researchers. Other researchers have also reported changes in pH along with the length of storage time, such as sweet sorghum sap from pH 5.16 changing to 4.34 in 48 h of storage [64], sugarcane with pH changed from 5.30 to 4.50 in 96 h [65]. The change in the pH of palm sap is also related to the activity of microorganisms. The growth of microorganisms that can rapidly reduce pH by producing organic acids. When acid increases, pH decreases. When acids release hydrogen ions, vegetative cell bacteria can multiply well under low acid conditions. The decrease in pH by lactic acid bacteria in palm sap has also been reported by Manel et al. [66].

Temperature is an extremely influential factor in the pH decrease of palm sap. In the current study, a high storage temperature indicated a considerable decrease rate in pH presumably because changes in sugar levels to acidic levels accelerate at high temperature. Ansar et al. [67] reported that palm sap stored at 40 °C exhibits a higher decrease in pH compared with palm sap stored at 29 °C. The lowest decrease in pH value of palm sap is obtained at a storage temperature of 10 °C. Lasekan and Abbas [68] have also reported that palm sap stored at 28 °C has decreased pH faster than that stored at 15 °C.

3.2. Changes in colour (L* a* b* values)

The results show that the L* value decreased significantly ($p < 0.05$) from 56.0 to 47.3, and the b* value decreased significantly ($p < 0.05$) from 8.7 to 7.6. By contrast, no significant change was observed in the value of a* ($p > 0.05$) during storage. These results indicate that when pH decreases, the colour values (L* and b*) of palm sap change with storage time length. It has been reported also by Manel et al. [66] that the

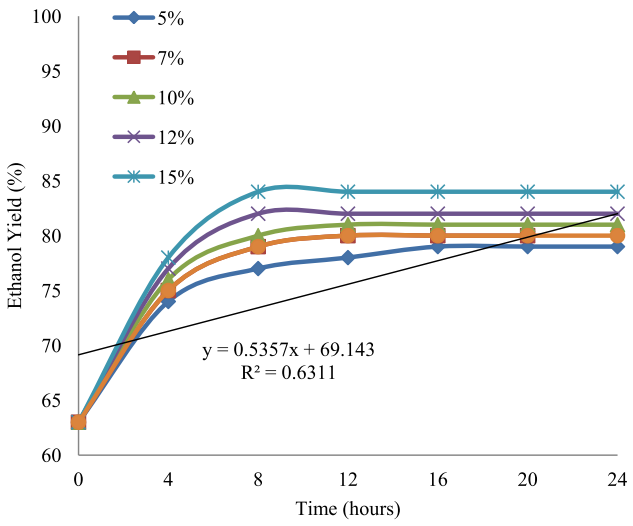

Fig. 2 – Profile of ethanol concentration during anaerobic fermentation at varying inoculum concentrations.

Table 3 – Analysis of variance.

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	10624.13	2	5312.07	368.89	1.68E-11	3.88
Within groups	172.8	12	14.4			
Total	10796.93	14				

palm colour changed from the original palm colour (pure) into milk white during the fermentation process.

The effect of colour change during storage before fermentation can be used as an indicator of palm sap quality. The results of the current study exhibit that pH value contains a function of lightness (L*) and yellowness (a*) but is not correlated with redness (b*). This finding agrees with the results of Victor and Orsat [69] that a high pH causes *A. pinnata* to appear more transparent, whereas a low pH causes the palm sap to appear cloudy. Comparison of the pH and colour values of L* a* b* results of this study with some previous studies are shown in the Table 2.

3.3. *Ethanol content*

Fermentation can convert glucose in the palm sap into ethanol due to the reduced glucose levels in ethanol. This finding agrees with the results of Mustafa et al. [71] and Trivedi et al. [72]. The higher the percentage of inoculum used, the higher the ethanol content obtained (Fig. 2). The ethanol content of palm sap prior to fermentation was 32.3%, and it increased to 75.6% after 24 h of fermentation.

The analysis variance results showed that the F-count value is greater (368.893) than the F-table (3.885). Thus, inoculum concentration significantly affects ($p < 0.05$) ethanol yield. The DMRT analysis determines that inoculum concentration variation significantly influences the obtained ethanol content. The higher the inoculum concentration, the higher the obtained ethanol content. This result was consistent with that of Mojovic et al. [73] that the content of fermented ethanol depends on the quality of sap used and the type of microbes for the inoculum.

The result of analysis of variance was known that the value of F-count is greater (368.89) than F-table (3.88) (Table 3), so it can be explained that inoculum concentration variation of significantly affect ($P < 0.05$) to ethanol yield was obtained.

The time of fermentation (incubation period) also significantly influences the ethanol yield formed. The longer the fermentation time, the higher the ethanol yield obtained. The same results have been revealed by Oguri et al. [36] who explain that the longer the fermentation process, the chances of ethanol to formed is also higher. The result of this fermentation is still a mixture of ethanol and water, so it must be separated by the distillation method. The same thing has been revealed by Hashem and Darwish [74] who state that the fermentation process usually requires an incubation period of 12–72 h and depends on the number and type of microorganisms used to initiate fermentation.

In order to increase the ethanol content of fermented products, distillation was carried out to obtain higher ethanol levels. The distillation process was carried out with a vacuum system. In this system the fraction that has the lowest boiling point will experience evaporation first. In this study, the maximum yield obtained was still low at around 75.6%. This is allegedly because the raw materials used have been contaminated before fermentation. The same thing has been reported by Amigun and Musango [75] that the obtaining process of ethanol yield highly depends on raw materials and environmental conditions at the time of tapping.

4. Conclusion

Changes in the pH value of palm sap after tapping are caused by the growth of microorganisms that produce organic acids by releasing hydrogen ions. As the pH decreased, the values of L* and b* also decreased significantly, but there was no significant change in the values of a*. Palm juice converts glucose into ethanol during fermentation. The higher the percentage of inoculum used, the higher the ethanol yield volume. The ethanol content of palm sap before distillation was 32.3% and increased to 75.6% after distillation. The physicochemical properties of bioethanol still need to be studied comprehensively by conducting trials on various types of motorized vehicles.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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