

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

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15

16 **Abstract**

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

27 parameters included the pH change, colour, and ethanol content. The results showed that the
28 pH change of the palm sap during storage was caused by the growth of microorganisms to
29 produce organic acids by releasing hydrogen ions. As pH decreased, L* and b* values also
30 decreased significantly, but not significantly change in a* values was observed. Glucose
31 changed to ethanol during fermentation. The higher the percentage of inoculum used, the higher
32 the volume of ethanol obtained. The bioethanol content of palm sap prior to fermentation was
33 32.3%, and it increased to 75.6% after 24 hours of incubation.

34

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

36

37 **1. Introduction**

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

43 Some parts of the palm tree are used to meet human needs; for example, the fruit can
44 be used for cocktails or desserts; and the fibre is used to make ropes, filters, brooms and roofs
45 [6]. Starch, the inner part of the tree, is the raw material for noodle production. Young leaves
46 can be consumed as a salad or cooked as an ingredient for soups. Stems (wood) can be
47 processed for the manufacture of furniture, and roots are used as traditional medicine because
48 they are believed to dissolve bladder stone [7].

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

53 210.600 litres/hectare/year [11]. Every 10 litres of sap can produce an average of 3.5 litres of
54 ethanol [12]. Accordingly, 1 hectare of palm trees can produces of 73,710 litres of ethanol. If
55 production is adequate, then the potential of biofuel from palm sap can reach 2 million kilolitres
56 per year. Thus, biofuel exhibits considerable potential and must be developed.

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

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

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

79 for power generation applications is very profitable economically and environmentally friendly
80 [39].

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

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

98

99 **2. Materials and methods**

100 *2.1. Samples and tools*

101 The raw material used was palm sap obtained from farmers in Pusuk, Lombok Island,
102 West Nusa Tenggara Province, Indonesia. To accelerate the growth of the culture, microbes
103 are used. *Cerevisiae* as additional nutrition in preparing inoculum. The tools used were
104 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]:

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

$$130 \quad a^* = \frac{240a}{255} - 120 \quad (2)$$

$$131 \quad b^* = \frac{240b}{255} - 120 \quad (3)$$

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

134

135 *2.6. Fermentation process*

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

143

144 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

145

146 *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]:

$$162 \quad F = g \left(m_b - \frac{\rho_a - m_b}{\rho_b} \right) \quad (4)$$

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]:

$$170 \quad F = \eta A \frac{V}{L} \quad (5)$$

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

173

174 *2.10. Measurement of Calorific Value*

175 The calorific value of combustion was measured using a bomb calorimeter type IKA C-
 176 5000 (ASTM D 2014-96) [60]. The reaction that occurs in the bomb calorimeter can produce
 177 heat which is absorbed by the water and the bomb, so that no heat is wasted into the air. The
 178 calorific value of combustion can be written as:

$$179 \quad r_{\text{reaction}} = - (q_{\text{air}} + q_{\text{bomb}}) \quad (6)$$

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

$$181 \quad Q_{\text{water}} = m \cdot c \cdot \Delta T \quad (7)$$

182 where, m = mass of water (g), c = heat type of water ($\text{J/kg}^\circ\text{C}$), and ΔT = temperature change
 183 ($^\circ\text{C}$).

184 The amount of heat absorbed by the bomb calorimeter can be calculated using the
 185 formula:

$$186 \quad q_{\text{water}} = c_{\text{bomb}} \cdot \Delta T \quad (8)$$

187 where, c_{bomb} = heat capacity of bomb ($\text{J/g}^\circ\text{C}$) and ΔT = temperature change ($^\circ\text{C}$).

188

189 *2.11. Data analysis*

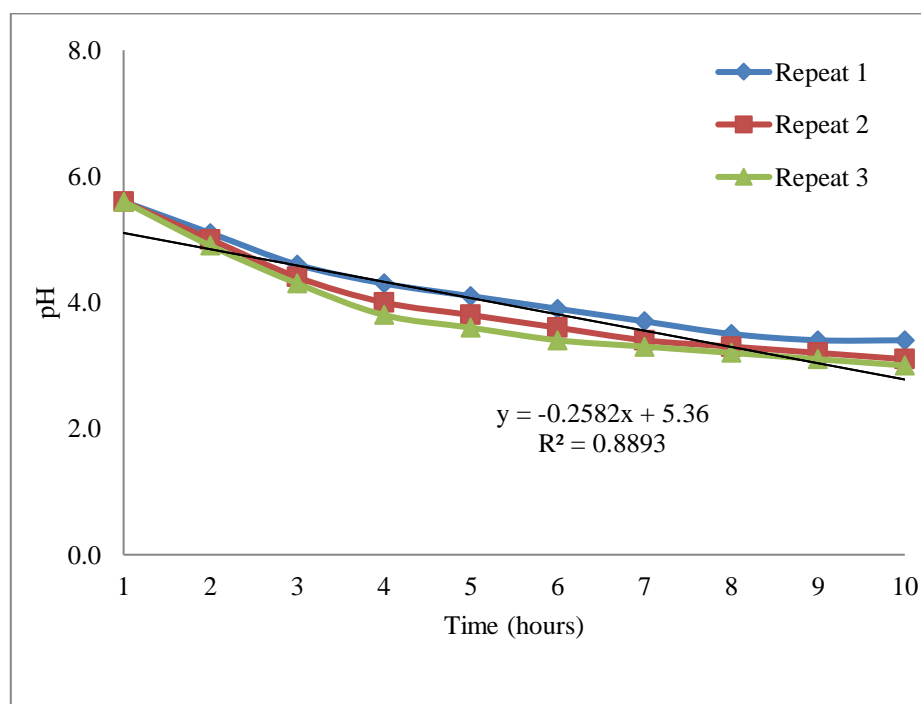
190 The research data were analysed using one-way analysis of variance [61]. If the F-count
 191 value is greater than the F-table, then a significant difference exists. Statistical significance
 192 between sample treatments was defined at $p < 0.05$. Mean differences were evaluated with
 193 Duncan's Multiple Range Test (DMRT), which was analysed using SPSS for Windows version
 194 16.0.

195

196 3. Results and discussion

197 3.1. The pH content

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



205
 206 Fig. 1. The pH change with storage time variation

207
 208 The palm sap is highly sensitive to environmental temperature and it easily damaged.
 209 The most easily detected damage indicator is pH value. Some researchers have also reported
 210 that the pH of palm sap drops from 7.41-6.02 after 20 hours of storage [18], from 6.70-6.34
 211 [15]. The trend of decreasing pH in this study was apparently lower than those reported by
 212 previous researchers. Other researchers have also reported changes in pH along with the length

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

220 Temperature is an extremely influential factor in the pH decrease of palm sap. In the
221 current study, a high storage temperature indicated a considerable decrease rate in pH
222 presumably because changes in sugar levels to acidic levels accelerate at high temperature.
223 Ansar *et al.* [67] reported that palm sap stored at 40 °C exhibits a higher decrease in pH
224 compared with palm sap stored at 29 °C. The lowest decrease in pH value of palm sap is
225 obtained at a storage 10 °C. Lasekan and Abbas [68] have also reported that palm sap stored at
226 28 °C has decreased pH faster than that stored at 15 °C.

227

228 3.2. Changes in colour (L^* a^* b^* values)

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

235 The effect of colour change during storage before fermentation can be used as an
236 indicator of palm sap quality. The results of the current study exhibit that pH value contains a
237 function of lightness (L^*) and yellowness (a^*) but is not correlated with redness (b^*). This
238 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*		
<i>Borassus</i>	4.19-5.23	61.49-87.53	1.46-3.52	12.41-	[17]	Hunter Lab
<i>flabellifer</i>				19-31		Color flex
Linn						
<i>A. Pinnata</i>	4.883-	44.5-54.8	1.2-1.6	6.5-9.8	[69]	Minolta
Merr	6.387					Reader
<i>Phoenix</i>	6.86±0.05	72.01±0.07	0.64±0.02	15.04±	[70]	Lovibond
<i>dactylifera</i>				0.02		Tintometer
L.						PFX 195
Palm sap	4.8-7.1	47.3-56.0	7.6-8.7	34.0-	This	Chroma
				46.0	study	Meter-CR- 400)

245

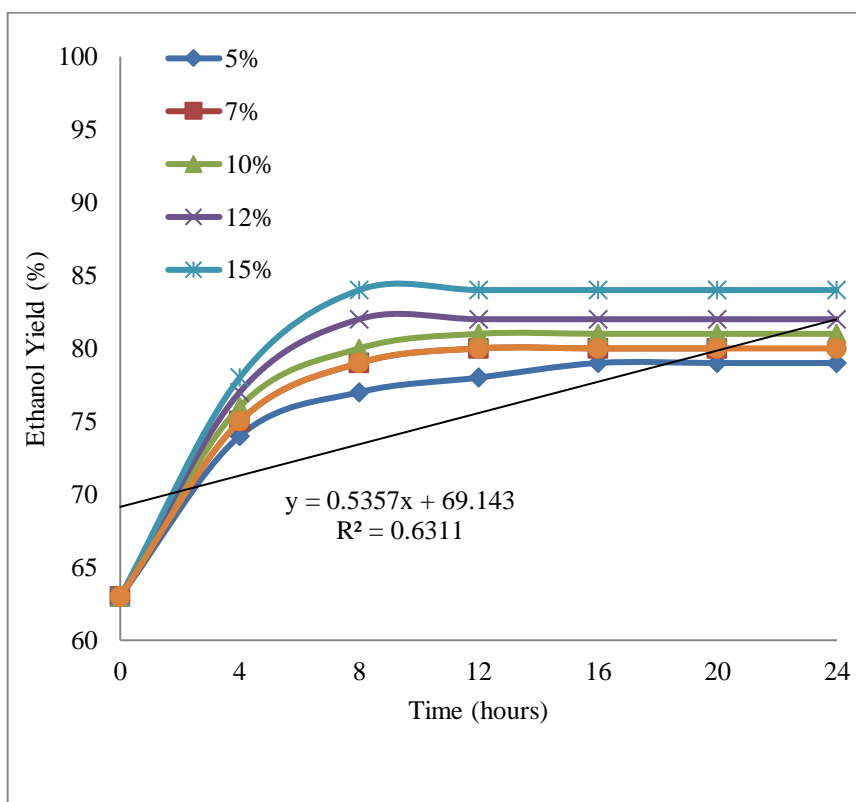
246

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%

252 after 24 hours of fermentation.



253

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

256

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

263 The result of analysis of variance was known that the value of F-count is greater
264 (368.89) than F-table (3.88) (Table 3), so it can be explained that inoculum concentration
265 variation of significantly affect ($P < 0.05$) to ethanol yield was obtained.

266

267

268

269

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				

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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.

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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.

287 4. Conclusion

288

289

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,

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

296

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303 **References**

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

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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 <i>Saccharomyces cerevisiae</i> 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.
Suggested Reviewers:	<p>Mounir El Achaby, Prof Materials Science and Nanoengineering Department (MSN),, Mohammed VI Polytechnic University (UM6P), Lot 660 – Hay Moulay Rachid, 43150 Benguerir, Morocco mounir.elachaby@um6p.ma He is expert in this study, and he has publish in high impact journals</p> <p>S.M. Sapuan, Prof Department of Mechanical and Manufacturing Engineering, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia sapan@upm.edu.my He is expert in this study and has publish in high impact journals</p> <p>Mustafa Balat, Prof Sila Science & Energy Unlimited Company, University Mahallesi, 61000 Trabzon, Turkey mustafabalat@yahoo.com He is expert in bioethanol processing, and he has publish in very high impact journal, and Q1 journals</p>
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
<p>Reviewer #1: Arenga pinnate 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.</p>	<p>The title was changed based on reviewer 1's suggestion. References were added (Total references is 75)</p>
<p>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.</p>	<p>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.</p>

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

3

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18 **Abstract**19 Availability of fossil fuels is increasingly limited, so the search for alternative fuels is
20 important. In accordance with the current scenario, bioenergy research emphasizes the
21 bioethanol production from plants that are abundant and available throughout the year, such as
22 palm sap. The palm sap is a type of palm tree that grows in tropical forests, particularly in
23 South Asia and Southeast Asia. More than 3,000 species of palm exist, and they are categorised
24 as multipurpose trees because they can be used as raw materials for various products, such as
25 sugar, fermented drinks, syrup, palm wine, vinegar, alcohol and bioethanol. This study was
26 aimed to examine bioethanol production from palm sap through optimization of

27 *Saccharomyces cerevisiae* as an inoculum. The research sample was obtained from local
28 farmers in Pusuk, Lombok Island, West Nusa Tenggara, and Indonesia. The research
29 parameters included the pH change, colour, and ethanol content. The results showed that the
30 pH change of the palm sap during storage was caused by the growth of microorganisms to
31 produce organic acids by releasing hydrogen ions. As pH decreased, L* and b* values also
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36

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

38

39 **1. Introduction**

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

45 Some parts of the palm tree are used to meet human needs; for example, the fruit can
46 be used for cocktails or desserts; and the fibre is used to make ropes, filters, brooms and roofs
47 [6]. Starch, the inner part of the tree, is the raw material for noodle production. Young leaves
48 can be consumed as a salad or cooked as an ingredient for soups. Stems (wood) can be
49 processed for the manufacture of furniture, and roots are used as traditional medicine because
50 they are believed to dissolve bladder stone [7].

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

53 is approximately 60,482 hectares, and its potential for sap production can reach 303.76 million
54 litres per year [10]. If production is 50% of the total population, then sap production can reach
55 210.600 litres/hectare/year [11]. Every 10 litres of sap can produce an average of 3.5 litres of
56 ethanol [12]. Accordingly, 1 hectare of palm trees can produces of 73,710 litres of ethanol. If
57 production is adequate, then the potential of biofuel from palm sap can reach 2 million kilolitres
58 per year. Thus, biofuel exhibits considerable potential and must be developed.

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

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

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

79 Meanwhile, according to Sebayang et al. [38], the purity of ethanol can be increased through a
80 distillation process using zeolite as an adsorbent. Bioethanol production from the banana stems
81 for power generation applications is very profitable economically and environmentally friendly
82 [39].

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

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

100

101 **2. Materials and methods**

102 *2.1. Samples and tools*

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

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

107

108 *2.2. Preparation of palm sap*

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

112

113 *2.3. Preparation of inoculum culture*

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

119

120 *2.4. pH testing*

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

125

126 *2.5. Colour testing*

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

$$131 \quad L^* = \frac{\text{Lightness}}{255} \times 100 \quad (1)$$

$$132 \quad a^* = \frac{240a}{255} - 120 \quad (2)$$

$$133 \quad b^* = \frac{240b}{255} - 120 \quad (3)$$

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

136

137 2.6. Fermentation process

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

145

146 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

147

148 2.7. Distillation process

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

153

154 2.8. *Measuring ethanol content*

155 Ethanol content was measured using the pycnometer method [57]. Firstly, a sample of
156 100 millilitre was placed in a distillation flask. Then, 100 millilitre of distilled water was added
157 and distilled at 80 °C. The distillate was stored in an Erlenmeyer flask to a volume of 50
158 millilitre. The distillate was then inserted into a pycnometer to meet the pycnometer. Excess
159 distillates at the top of the capillary tube were cleaned. The pycnometer containing the distillate
160 was weighed, and the weight was recorded. The same procedure was performed on distilled
161 water for comparison. The results of ethanol density calculations were converted using ethanol
162 specific gravity conversion tables. Ethanol density was calculated using the Equation (1)-(3)
163 [58]:

$$164 \quad F = g \left(m_b - \frac{\rho_a - m_b}{\rho_b} \right) \quad (4)$$

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

167

168 2.9. *Viscosity measurement*

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

$$172 \quad F = \eta A \frac{V}{L} \quad (5)$$

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

175

176 2.10. Measurement of Calorific Value

177 The calorific value of combustion was measured using a bomb calorimeter type IKA C-
 178 5000 (ASTM D 2014-96) [60]. The reaction that occurs in the bomb calorimeter can produce
 179 heat which is absorbed by the water and the bomb, so that no heat is wasted into the air. The
 180 calorific value of combustion can be written as:

$$181 \quad r_{\text{reaction}} = - (q_{\text{air}} + q_{\text{bomb}}) \quad (6)$$

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

$$183 \quad Q_{\text{water}} = m \cdot c \cdot \Delta T \quad (7)$$

184 where, m is mass of water (g), c is heat type of water ($\text{J/kg}^\circ\text{C}$), and ΔT is temperature change
 185 ($^\circ\text{C}$).

186 The amount of heat absorbed by the bomb calorimeter can be calculated using the
 187 Equation (8):

$$188 \quad q_{\text{water}} = c_{\text{bomb}} \cdot \Delta T \quad (8)$$

189 where, c_{bomb} is heat capacity of bomb ($\text{J/g}^\circ\text{C}$) and ΔT is temperature change ($^\circ\text{C}$).

190

191 2.11. Data analysis

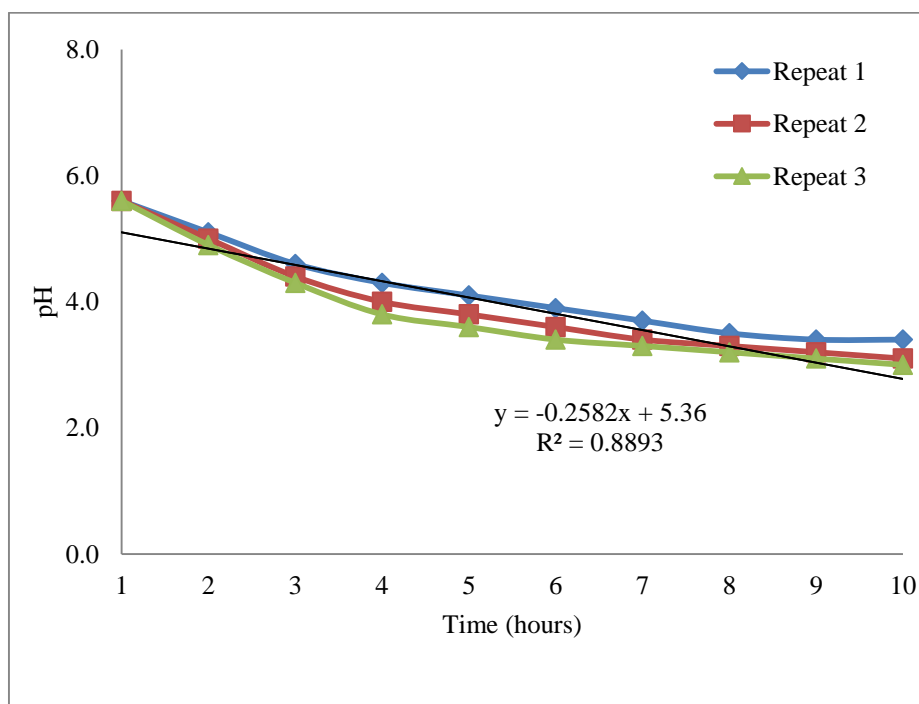
192 The research data were analysed using one-way analysis of variance [61]. If the F-count
 193 value is greater than the F-table, then a significant difference exists. Statistical significance
 194 between sample treatments was defined at $p < 0.05$. Mean differences were evaluated with
 195 Duncan's Multiple Range Test (DMRT), which was analysed using SPSS for Windows version
 196 16.0.

197

198 3. Results and discussion

199 3.1. The pH content

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



207
 208 Fig. 1. The pH change with storage time variation

209
 210 The palm sap is highly sensitive to environmental temperature and it easily damaged.
 211 The most easily detected damage indicator is pH value. Some researchers have also reported
 212 that the pH of palm sap drops from 7.41-6.02 after 20 hours of storage [18], from 6.70-6.34
 213 [15]. The trend of decreasing pH in this study was apparently lower than those reported by
 214 previous researchers. Other researchers have also reported changes in pH along with the length

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

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

229

230 3.2. Changes in colour (L^* a^* b^* values)

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

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

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

244

245 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*		
<i>Borassus</i>	4.19-5.23	61.49-87.53	1.46-3.52	12.41-	[17]	Hunter Lab
<i>flabellifer</i>				19-31		Color flex
Linn						
<i>A. Pinnata</i>	4.883-	44.5-54.8	1.2-1.6	6.5-9.8	[69]	Minolta
Merr	6.387					Reader
<i>Phoenix</i>	6.86±0.05	72.01±0.07	0.64±0.02	15.04±	[70]	Lovibond
<i>dactylifera</i>				0.02		Tintometer
L.						PFX 195
Palm sap	4.8-7.1	47.3-56.0	7.6-8.7	34.0-	This	Chroma
				46.0	study	Meter-CR- 400)

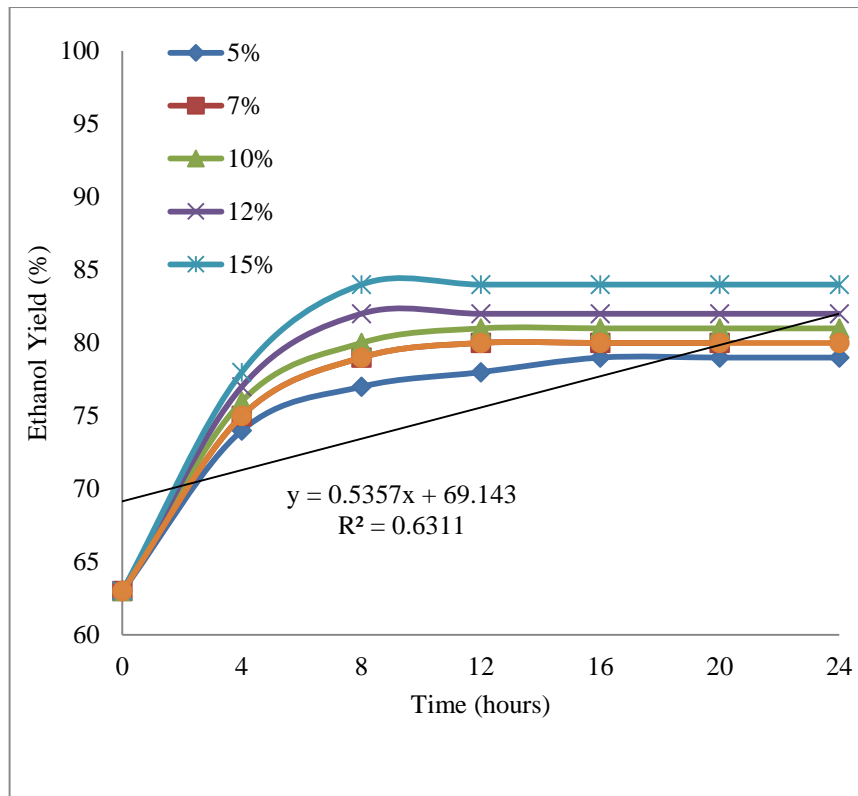
246

247

248

3.3. Ethanol content

249 Fermentation can convert glucose in the palm sap into ethanol due to the reduced
 250 glucose levels in ethanol. This finding agrees with the results of Mustafa et al. [71] and Trivedi
 251 et al. [72]. The higher the percentage of inoculum used, the higher the ethanol content obtained
 252 (Fig. 2). The ethanol content of palm sap prior to fermentation was 32.3%, and it increased to
 253 75.6% after 24 hours of fermentation.



254

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

257

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

264 The result of analysis of variance was known that the value of F-count is greater
265 (368.89) than F-table (3.88) (Table 3), so it can be explained that inoculum concentration
266 variation of significantly affect ($P < 0.05$) to ethanol yield was obtained.

267

268

269

270

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				

271

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

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

287

288 4. Conclusion

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

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

297

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303

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305

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306

307

Declaration of interests

Title of Manuscript:

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

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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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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.
7. A recommendation is required at the end of the conclusion based on your findings.

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

3

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15

16 **Abstract**

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

27 parameters included the pH change, colour, and ethanol content. The results showed that the
28 pH change of the palm sap during storage was caused by the growth of microorganisms to
29 produce organic acids by releasing hydrogen ions. As pH decreased, L* and b* values also
30 decreased significantly, but not significantly change in a* values was observed. Glucose
31 changed to ethanol during fermentation. The higher the percentage of inoculum used, the higher
32 the volume of ethanol obtained. The bioethanol content of palm sap prior to fermentation was
33 32.3%, and it increased to 75.6% after 24 hours of incubation.

34

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

36

37 1. Introduction

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

43 Some parts of the palm tree are used to meet human needs; for example, the fruit can
44 be used for cocktails or desserts; and the fibre is used to make ropes, filters, brooms and roofs
45 [6]. Starch, the inner part of the tree, is the raw material for noodle production. Young leaves
46 can be consumed as a salad or cooked as an ingredient for soups. Stems (wood) can be
47 processed for the manufacture of furniture, and roots are used as traditional medicine because
48 they are believed to dissolve bladder stone [7].

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

53 210.600 litres/hectare/year [11]. Every 10 litres of sap can produce an average of 3.5 litres of
54 ethanol [12]. Accordingly, 1 hectare of palm trees can produces of 73,710 litres of ethanol. If
55 production is adequate, then the potential of biofuel from palm sap can reach 2 million kilolitres
56 per year. Thus, biofuel exhibits considerable potential and must be developed.

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

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

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

79 for power generation applications is very profitable economically and environmentally friendly
80 [39].

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

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

98

99 **2. Materials and methods**

100 *2.1. Samples and tools*

101 The raw material used was palm sap obtained from farmers in Pusuk, Lombok Island,
102 West Nusa Tenggara Province, Indonesia. To accelerate the growth of the culture, microbes
103 are used. *Cerevisiae* as additional nutrition in preparing inoculum. The tools used were
104 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]:

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

$$130 \quad a^* = \frac{240a}{255} - 120 \quad (2)$$

$$131 \quad b^* = \frac{240b}{255} - 120 \quad (3)$$

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

134

135 2.6. Fermentation process

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

143

144 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

145

146 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]:

$$170 F = \eta A \frac{V}{L} \quad (5)$$

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

173

174 2.10. Measurement of Calorific Value

175 The calorific value of combustion was measured using a bomb calorimeter type IKA C-
 176 5000 (ASTM D 2014-96) [60]. The reaction that occurs in the bomb calorimeter can produce
 177 heat which is absorbed by the water and the bomb, so that no heat is wasted into the air. The
 178 calorific value of combustion can be written as:

$$179 r_{\text{reaction}} = - (q_{\text{air}} + q_{\text{bomb}}) \quad (6)$$

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

$$181 Q_{\text{water}} = m \cdot c \cdot \Delta T \quad (7)$$

182 where, m = mass of water (g), c = heat type of water ($\text{J/kg}^\circ\text{C}$), and ΔT = temperature change
 183 ($^\circ\text{C}$).

184 The amount of heat absorbed by the bomb calorimeter can be calculated using the
 185 formula:

$$186 q_{\text{water}} = c_{\text{bomb}} \cdot \Delta T \quad (8)$$

187 where, c_{bomb} = heat capacity of bomb ($\text{J/g}^\circ\text{C}$) and ΔT = temperature change ($^\circ\text{C}$).

188

189 2.11. Data analysis

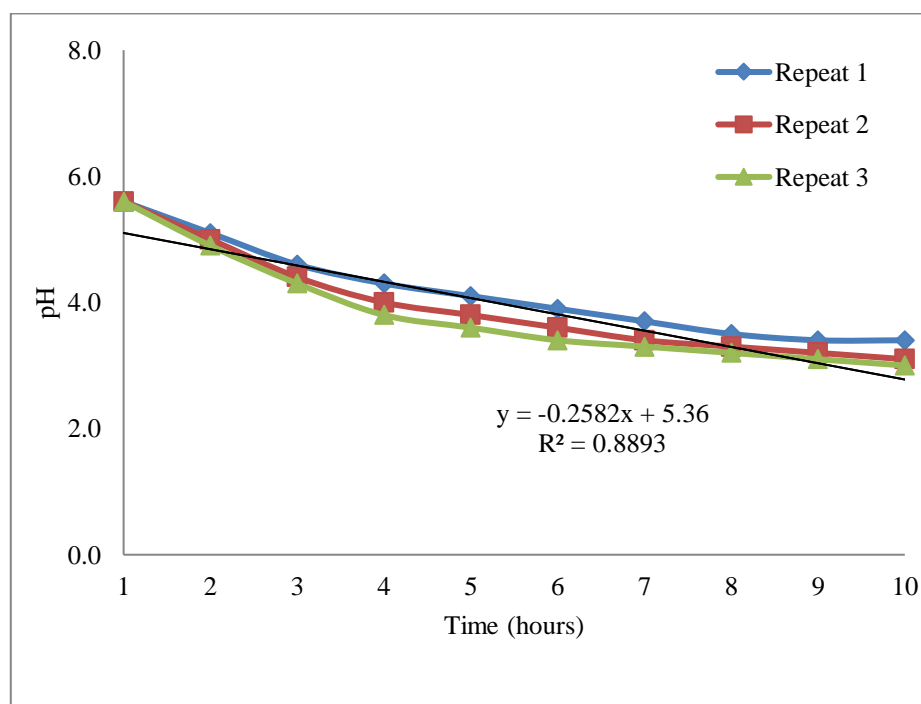
190 The research data were analysed using one-way analysis of variance [61]. If the F-count
 191 value is greater than the F-table, then a significant difference exists. Statistical significance
 192 between sample treatments was defined at $p < 0.05$. Mean differences were evaluated with
 193 Duncan's Multiple Range Test (DMRT), which was analysed using SPSS for Windows version
 194 16.0.

195

196 3. Results and discussion

197 3.1. The pH content

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



205
206 **Fig. 1. The pH change with storage time variation**

207
208 The palm sap is highly sensitive to environmental temperature and it easily damaged.
 209 The most easily detected damage indicator is pH value. Some researchers have also reported
 210 that the pH of palm sap drops from 7.41-6.02 after 20 hours of storage [18], from 6.70-6.34
 211 [15]. The trend of decreasing pH in this study was apparently lower than those reported by
 212 previous researchers. Other researchers have also reported changes in pH along with the length

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

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

227

228 3.2. Changes in colour (L^* a^* b^* values)

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

235 The effect of colour change during storage before fermentation can be used as an
236 indicator of palm sap quality. The results of the current study exhibit that pH value contains a
237 function of lightness (L^*) and yellowness (a^*) but is not correlated with redness (b^*). This
238 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*		
<i>Borassus</i>	4.19-5.23	61.49-87.53	1.46-3.52	12.41-	[17]	Hunter Lab
<i>flabellifer</i>				19-31		Color flex
Linn						
<i>A. Pinnata</i>	4.883-	44.5-54.8	1.2-1.6	6.5-9.8	[69]	Minolta
Merr	6.387					Reader
<i>Phoenix</i>	6.86±0.05	72.01±0.07	0.64±0.02	15.04±	[70]	Lovibond
<i>dactylifera</i>				0.02		Tintometer
L.						PFX 195
Palm sap	4.8-7.1	47.3-56.0	7.6-8.7	34.0-	This	Chroma
				46.0	study	Meter-CR- 400)

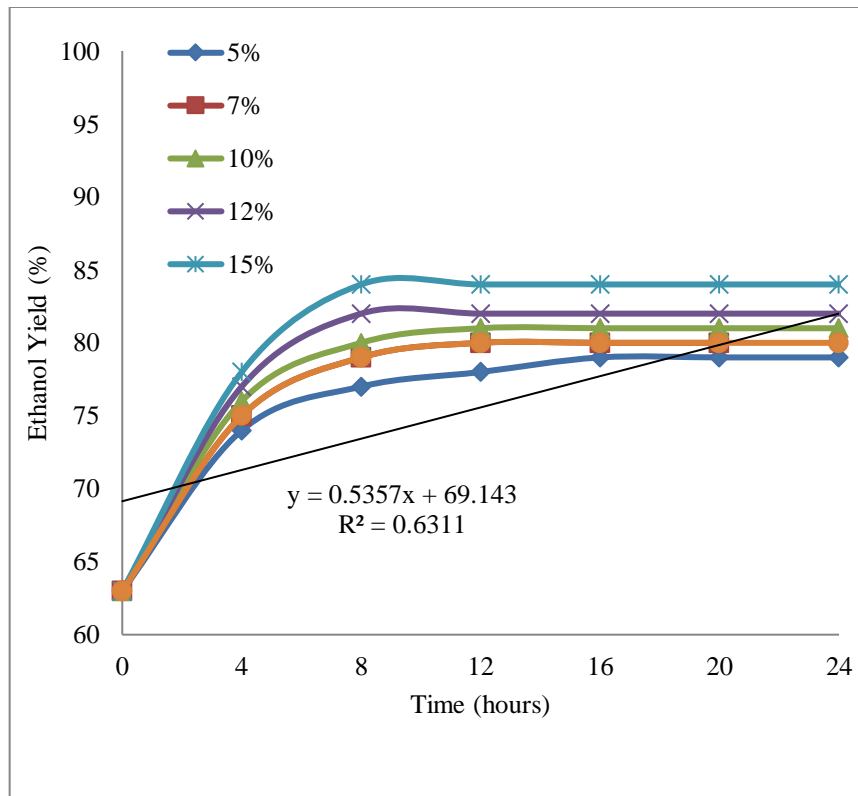
244

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.



252
 253 **Fig. 2. Profile of ethanol concentration during anaerobic fermentation at varying inoculum**
 254 **concentrations**

255
 256 The analysis variance results showed that the F-count value is greater (368.893) than
 257 the F-table (3.885). Thus, inoculum concentration significantly affects ($p < 0.05$) ethanol yield.
 258 The DMRT analysis determines that inoculum concentration variation significantly influences
 259 the obtained ethanol content. The higher the inoculum concentration, the higher the obtained
 260 ethanol content. This result was consistent with that of Mojovic et al. [73] that the content of
 261 fermented ethanol depends on the quality of sap used and the type of microbes for the inoculum.

262 The result of analysis of variance was known that the value of F-count is greater
 263 (368.89) than F-table (3.88) (Table 3), so it can be explained that inoculum concentration
 264 variation of significantly affect ($P < 0.05$) to ethanol yield was obtained.
 265
 266
 267

268

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				

269

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

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

285

286 4. Conclusion

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

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

295

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Journal Pre-proof

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

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

3

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17

18 **Abstract**

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

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

36

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

38

39 1. Introduction

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

45 Some parts of the palm tree are used to meet human needs; for example, the fruit can
46 be used for cocktails or desserts; and the fibre is used to make ropes, filters, brooms and roofs
47 [6]. Starch, the inner part of the tree, is the raw material for noodle production. Young leaves
48 can be consumed as a salad or cooked as an ingredient for soups. Stems (wood) can be
49 processed for the manufacture of furniture, and roots are used as traditional medicine because
50 they are believed to dissolve bladder stone [7].

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

53 is approximately 60,482 hectares, and its potential for sap production can reach 303.76
54 million litres per year [10]. If production is 50% of the total population, then sap production
55 can reach 210.600 litres/hectare/year [11]. Every 10 litres of sap can produce an average of
56 3.5 litres of ethanol [12]. Accordingly, 1 hectare of palm trees can produces of 73,710 litres
57 of ethanol. If production is adequate, then the potential of biofuel from palm sap can reach 2
58 million kilolitres per year. Thus, biofuel exhibits considerable potential and must be
59 developed.

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

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

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

79 fermented palm sap is strongly influenced by the quality of raw materials used [35], [36],
80 [37]. Meanwhile, according to Sebayang et al. [38], the purity of ethanol can be increased
81 through a distillation process using zeolite as an adsorbent. Bioethanol production from the
82 banana stems for power generation applications is very profitable economically and
83 environmentally friendly [39].

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

89 The research focusing on the processing of palm sap into bioethanol has been
90 conducted by researchers, like [46], but the bioethanol content obtained is still less than 95%,
91 so it cannot be used as a substitute for vehicle fuel. Thus, it is still important to do research to
92 produce bioethanol that can be used as an environmentally friendly substitute for biofuels.
93 These research use a method of fermentation and multilevel distillation to produce biofuels
94 that can be used as biofuel substitutes that are environmentally friendly. Anaerobic
95 fermentation method uses microbial culture made from a mixture of palm sap and
96 *Saccharomyces cerevisiae* as an inoculum [47], [48]. The increase of ethanol yield up to
97 99.5% was done by stratified distillation [49], [50], [51]. Based on the arguments above, the
98 purpose of this study was enhancement of bioethanol production from palm sap through
99 optimization of *saccharomyces cerevisiae* as an inoculum. This research is very important as
100 information to the bioethanol industry to develop palm sap as a raw material for making
101 biofuels.

102

103 **2. Materials and methods**

104 *2.1. Samples and tools*

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

109

110 2.2. Preparation of palm sap

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

114

115 2.3. Preparation of inoculum culture

116 To prepare the inoculum culture, 500 millilitres of palm sap was heated at 60 °C for
117 10-15 minutes and then cooled at the room temperature. After cooling, the palm sap was
118 inoculated with *S. cerevisiae* and bread yeast. Then, it was incubated at 30 °C for 24 hours to
119 be used as microbial culture. This culture was mixed with NPK as an additional nutrient to
120 accelerate microbial growth.

121

122 2.4. pH testing

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

127

128 2.5. Colour testing

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

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

$$133 \quad L^* = \frac{\text{Lightness}}{255} \times 100 \quad (1)$$

$$134 \quad a^* = \frac{240a}{255} - 120 \quad (2)$$

$$135 \quad b^* = \frac{240b}{255} - 120 \quad (3)$$

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

138

139 2.6. Fermentation process

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

147

148

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]:

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

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]:

$$174 \quad F = \eta A \frac{V}{L} \quad (5)$$

175 where, F is force on the surface of the liquid, η is coefficient of fluid viscosity (Ns/m²), A is
 176 liquid area (m²), V is moving wall velocity (m / s), and L is distance of the two surfaces (m).

177

178 *2.10. Measurement of Calorific Value*

179 The calorific value of combustion was measured using a bomb calorimeter type IKA C-
 180 5000 (ASTM D 2014-96) [60]. The reaction that occurs in the bomb calorimeter can produce
 181 heat which is absorbed by the water and the bomb, so that no heat is wasted into the air. The
 182 calorific value of combustion can be written as:

$$183 \quad r_{\text{reaction}} = - (q_{\text{air}} + q_{\text{bomb}}) \quad (6)$$

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

$$185 \quad Q_{\text{water}} = m \cdot c \cdot \Delta T \quad (7)$$

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

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

$$190 \quad q_{\text{water}} = c_{\text{bomb}} \cdot \Delta T \quad (8)$$

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

192

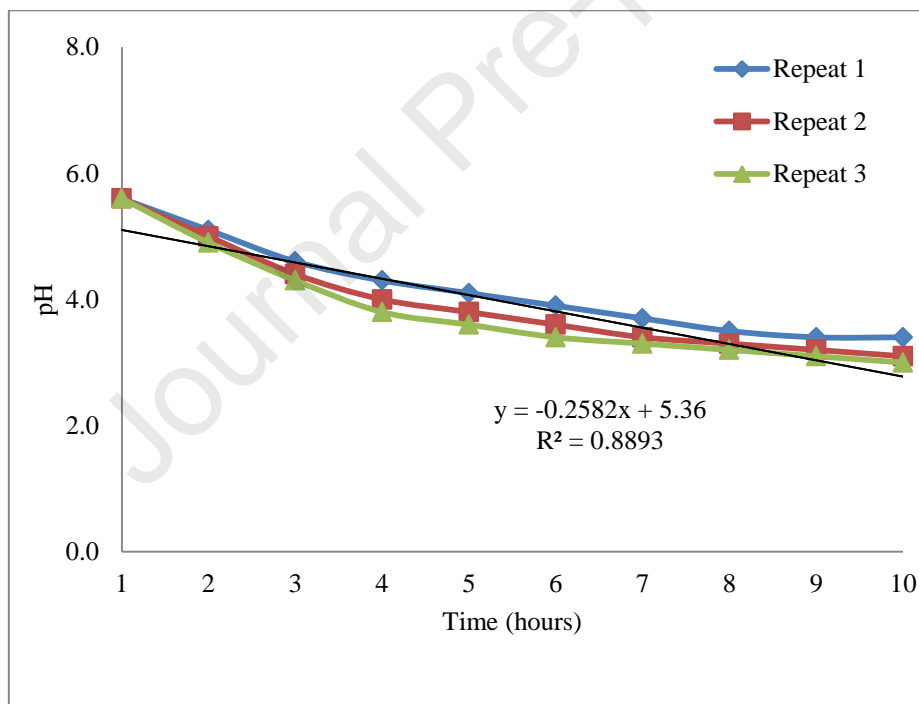
193 *2.11. Data analysis*

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

199

200 **3. Results and discussion**201 *3.1. The pH content*

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



209

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

211

212 The palm sap is highly sensitive to environmental temperature and it easily damaged.
 213 The most easily detected damage indicator is pH value. Some researchers have also reported
 214 that the pH of palm sap drops from 7.41-6.02 after 20 hours of storage [18], from 6.70-6.34
 215 [15]. The trend of decreasing pH in this study was apparently lower than those reported by

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

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

231

232 3.2. Changes in colour (L^* a^* b^* values)

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

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

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

246

247 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*		
<i>Borassus</i>	4.19-5.23	61.49-87.53	1.46-3.52	12.41-	[17]	Hunter Lab
<i>flabellifer</i>				19-31		Color flex
Linn						
<i>A. Pinnata</i>	4.883-	44.5-54.8	1.2-1.6	6.5-9.8	[69]	Minolta
Merr	6.387					Reader
<i>Phoenix</i>	6.86±0.05	72.01±0.07	0.64±0.02	15.04±	[70]	Lovibond
<i>dactylifera</i>				0.02		Tintometer
L.						PFX 195
Palm sap	4.8-7.1	47.3-56.0	7.6-8.7	34.0-	This	Chroma
				46.0	study	Meter-CR-400)

248

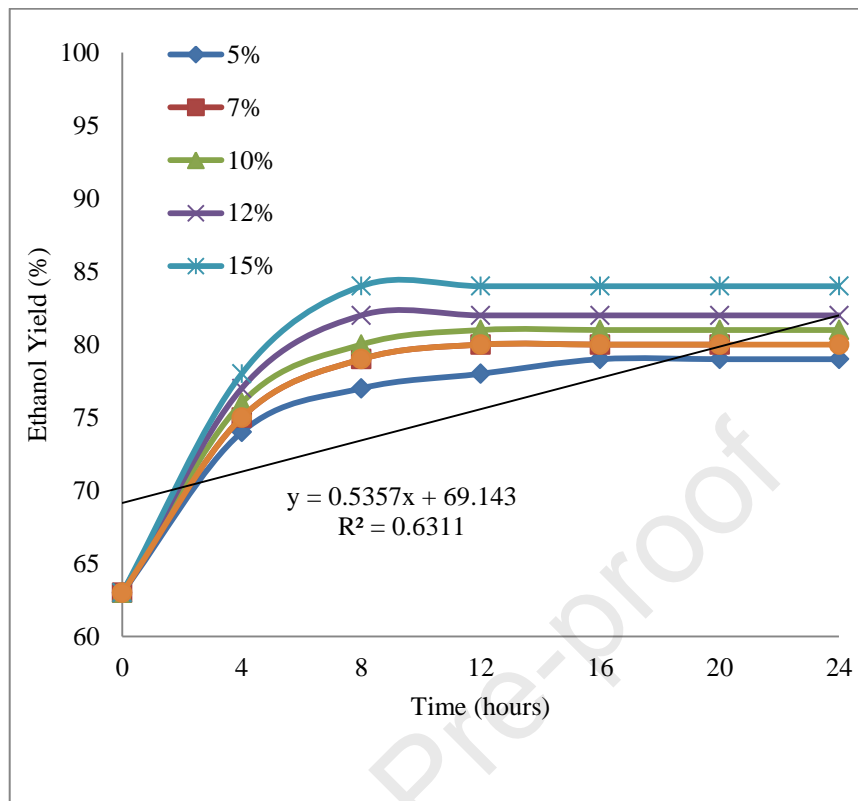
249

250

3.3. Ethanol content

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

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



256

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

259

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

267 The result of analysis of variance was known that the value of F-count is greater
268 (368.89) than F-table (3.88) (Table 3), so it can be explained that inoculum concentration
269 variation of significantly affect ($P < 0.05$) to ethanol yield was obtained.

270

271

272

273

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				

274

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

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

291

292 **4. Conclusion**

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

301

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308

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310

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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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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. © 2021 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

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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 produce 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]:

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

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

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

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

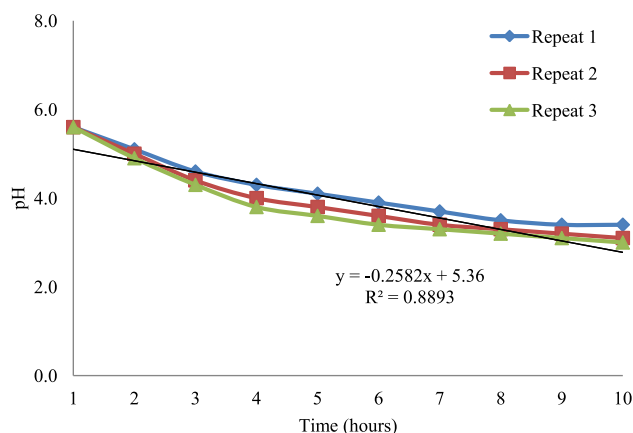


Fig. 1 – The pH change with storage time variation.

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/m²), A is liquid area (m²), 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:

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

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*		
<i>Borassus flabellifer</i> Linn	4.19–5.23	61.49–87.53	1.46–3.52	12.41-19-31	[17]	Hunter Lab Color flex
<i>A. Pinnata</i> Merr	4.883–6.387	44.5–54.8	1.2–1.6	6.5–9.8	[69]	Minolta Reader
<i>Phoenix dactylifera</i> 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):

$$Q_{water} = m \cdot c \cdot \Delta T \tag{7}$$

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):

$$q_{water} = c_{bomb} \cdot \Delta T \tag{8}$$

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

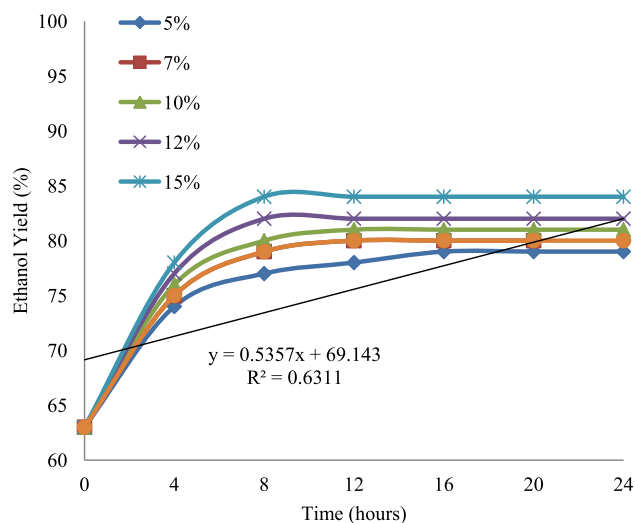


Fig. 2 – Profile of ethanol concentration during anaerobic fermentation at varying inoculum concentrations.

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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