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# Bioethanol from Sweet Potato Clones at Pruning Age and Yeast Concentrations

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***Abstract*—*The use of bioethanol can save premium usage in addition, bioethanol can reduce greenhouse gas emissions by up to 80% from its combustion results so as to decomp additionally greenhouse effects. Raw materials for producing bioethanol from that materials containing glucose, starch, cellulose. The purpose of the study was to find out of tape yeast concentrations on the bioethanol fermentation process of 8 sweet potato clones. The interaction between clone variation and yeast concentration has a noticeable effect on the results of the analysis of starch levels after the fermentation process. Starch levels showed a very significant decrease in the starch levels of fresh raw materials before the fermentation process. The results showed that the interaction between clone variation and yeast concentration had a noticeable effect on the results of the analysis of alcohol levels produced. The highest alcohol content is produced from Sari clones ($_{150\ DAP}$) with a yeast concentration of 15% with a content of 6.59%. The variation of clones and the concentration of yeast interacts manifestly with the results of the analysis of the total sugar content after the fermentation process. The total sugar measured is the total amount of sugar left after the fermentation process. Results showed a significant decrease in total sugar fermentation process by tape yeast showed the absence of starch hydrolysis and a total overhaul of the resulting sugar into alcohol. The concentration of yeast has no effect on the change of pH in the fermentation process, the pH at the end of the fermentation process is in the range of 4.1-6.3.*

*Keywords*—***Bioethanol, fermentation, eco-friendly, sweet potato.***

## I. INTRODUCTION

Bioethanol produced from sweet potatoes has advantages compared to petroleum fuels because the source is renewable and has a high octane value so that the combustion process becomes more complete with $CO_2$ emission levels of 40-80% lower and is environmentally friendly. This potential is quite large to be used as a substitute for gasoline and diesel fuel because it has more potential than cassava. Starch in sweet potatoes can be hydrolyzed into carbohydrate monomer units and can be used by microorganisms in the fermentation process. According to Jena & Kumar Kar (2019) that the production of bioethanol used as biofuel is an important choice that is more environmentally friendly than fossil fuels. In addition, the raw materials used to produce bioethanol are cheaper than fossil products (Bušić et al., 2018). This type of renewable energy can reduce dependence on external oil sources and effectively reduce greenhouse gas emissions (Li et al., 2014). Renewable energy development is a key strategy to sustain economic growth and to improve the environment. Biomass is an alternative renewable fuel for gasoline (Khoo et al., 2013).

Renewable energy is very important and needed by many parties. Alternative energy sources have several advantages, namely renewable and environmentally friendly because they have low $CO^2$ emissions (Kartini & Dhokhikah, 2018). Conversion efficiency increased significantly during sweet potato ripening, where the highest value was reached 25 days after harvest. Among the heating methods, the conventional one is slightly superior in terms of conversion efficiency (9% higher at 25 days) and has better results regarding cost analysis (Schweinberger et al., 2016).

Alternative sources of raw materials that are very important and have the potential to be developed are sweet potato plants which are perennial plants, can thrive in tropical and sub-tropical regions. Able to adapt to adverse environmental conditions, such as drought and infertile soil as well as tolerance to pests and diseases, produce well with low inputs such as fertilizer and water (Lestari et al., 2019). Production of bioethanol made from carbohydrates is generally carried out in several stages which are not simultaneous in separate reactors such as extraction, hydrolysis and fermentation processes using *Sacharomyces cereviceae*.

The development of sweet potatoes as a source of food and feed is important for the development of Bio-industry Concept Agriculture. Clones that are suitable for the benefit of agricultural development with the concept of bio-industry are dual-purpose type clones. Research on 17 sweet potato clones has been successfully classified (Indawan, Lestari, & Thiasari, 2018). Into forage type (3 cultivars), low dual-purpose (3 cultivars), high dual-purpose (7 cultivars), and low root production (4 cultivars) (Lestari & Hapsari, 2015). Described by Indawan et al. (2018), that developed and researched sweet potato clones planted with the addition of biochar as a support for improving soil conditions with the aim of producing optimal sweet potato clones. According to Khaidir et al. (2016), that the concentration of tape yeast affects the pH and levels of the resulting bioethanol, but does not have a significant effect on the yield and density of bioethanol.

## II. MATERIAL AND METHODS

The research was carried out in November 2018 - January 2019, at the Laborotory Industrial Microbiology, Chemical Engineering of Tribhuwana Tungga Dewi University, and Malang State Polytechnic. The test components include: Chemical components of fresh ingredients (starch content, total sugar content, pH). Fermented chemical components (ethanol content, starch content, total sugar content and pH).

The first factor of the tuber samples tested was from harvests treated with stover pruning at 80 DAP, 90 DAP, 120 DAP and 150 DAP, namely: Kuningan Putih ($_{80\ DAP}$), Beta-2 (80 DAP), Kuningan Merah ($_{90\ DAP}$), BIS OP-61 ($_{90\ DAP}$), 73-OP-5 ($_{120\ DAP}$), Beta 2-♀-29 ($_{120\ DAP}$), BIS OP 61-OP 22 ($_{150\ DAP}$), Sari ($_{150\ DAP}$). Second factor: Variation of tape yeast concentration consisted of 3 levels: $B_1$: Yeast concentration 5% (w/w), $B_2$: Yeast concentration 10% (w/w), $B_3$: Yeast concentration 15% (w/w). The test was repeated 3 times to obtain 72 experimental units.

Making bioethanol using a simple anaerobic fermenter with a volume of 500 mL. Each treatment uses 100 g of fresh sweet potato which is mashed using a blender by steaming first. Tape yeast was added to the fermenter with a concentration of 5%, 10%, and 15% (w/w), then 300 ml of sterile distilled water was added. Fermentation was carried out for 10 days at room temperature. Measurement of ethanol content using a Gas Chromatography (GC-14B) Shimadzu FID system. Flame Lonization Detector (FID) is a detector that has maximum reproducibility for various applications. The temperature used is 40-160ºC and the gas used as the carrier gas is Nitrogen ($N_2$). Starch and total sugar content were tested using methods based on AOAC.

The type of acid catalyst used is sulfuric acid ($H_2SO_4$). Saccharomyces cereviseae to convert glucose into ethanol. One of the ways to produce ethanol is by fermenting glucose syrup with microorganisms. Yeast is tolerant to quite high alcohol (12-18% v/v), resistant to high sugar content and remains active in fermenting at 4-32ºC. Analysis procedure (1). Measurement of ethanol content, (2). Measurement of total sugar, (3). Reducing sugar levels. The Somogyi-Nelson method determines glucose levels based on spectrophotometry. Data analysis used Analysis of Variance (ANOVA) with a significant level of 5%.

## III. RESULT AND DISCUSSION

### Starch content, total sugar and pH

Results of analysis of starch content, total sugar, initial pH, alcohol content and fermented starch content, correlation between alcohol content, starch and total sugar, pH fermented from 8 sweet potato clones, can be seen in Table 1 - Table 7 below.

There is instability in the nature of a clone not because of genetic factors, but because of differences between planting locations. Making bioethanol with sweet potato as the basic ingredient is through three stages of process, namely hydrolysis, fermentation and distillation processes. The hydrolysis process is carried out to break down starch into reducing sugars so that it can be fermented into bioethanol. The fermentation process converts glucose into ethanol with the help of the bacterium *Saccharomyces cereviceae*.

Ethanol is a colorless liquid with a characteristic odor. The specific gravity at 15ºC is 0.7937 and the boiling point is 78.32ºC at a pressure of 76 mmHg, the heat of combustion is 328 Kcal. The highest starch content was 23.05% in Kuningan Putih clone (80 DAP) and the lowest was 7.73% in BETA 2-♀-29 clone (120 DAP). The highest total sugar was in clone 73-OP-5 (120 DAP) and the lowest was in Kuningan Merah (90 DAP) of 2.26% (Table 1).

The process of producing bioethanol from materials with the main component of starch will involve saccharification and fermentation processes. Changes in chemical components in the manufacture of bioethanol are relatively the same as the process of making tape from cassava by utilizing inoculums from a succession of microorganisms. Fermentation factors that need to be considered to achieve good and correct process conditions include inoculum concentration, temperature, humidity, and aeration.

*Table 1. Results of analysis of starch content, total sugar and pH of 8 fresh sweet potato clones.*

| No. | Clones | Starch content (%) | Sugar total (%) | pH initial |
|---|---|---|---|---|
| 1. | Kuningan Putih (80 DAP) | 23.05 | 3.79 | 4.8 |
| 2. | Beta 2 (80 DAP) | 18.95 | 4.69 | 5.8 |
| 3. | Kuningan Merah (90 DAP) | 7.98 | 2.26 | 5.4 |
| 4. | BIS OP-61 (90 DAP) | 21.93 | 5.33 | 4.4 |
| 5. | 73-OP-5 (120 DAP) | 16.31 | 6.13 | 5.5 |
| 6. | BETA 2-♀-29 (120 DAP) | 7.73 | 2.07 | 5.9 |
| 7. | BIS OP 61-OP 22 (150 DAP) | 18.67 | 4.78 | 4.4 |
| 8. | Sari (150 DAP) | 18.63 | 3.89 | 4.8 |

Note: DAP = day after planting.

*Table 2. Alcohol content of fermented 8 sweet potato clones with 3 variations of tape yeast concentration.*

| No. | Clones | Concentration yeast (%) | | |
|---|---|---|---|---|
| | | 5% | 10% | 15% |
| 1. | Kuningan Putih (80 DAP) | 5.96 | 5.45 | 6.20 |
| 2. | Beta 2 (80 DAP) | 2.87 | 3.70 | 3.61 |
| 3. | Kuningan Merah (90 DAP) | 0.70 | 0.37 | 0.94 |
| 4. | BIS OP-61 (90 DAP) | 1.55 | 1.46 | 3.15 |
| 5. | 73-OP-5 (120 DAP) | 4.68 | 5.16 | 5.04 |
| 6. | BETA 2-♀-29 (120 DAP) | 2.41 | 2.45 | 2.39 |
| 7. | BIS OP 61-OP- 22 (150 DAP) | 3.16 | 2.90 | 3.53 |
| 8. | Sari (150 DAP) | 5.84 | 6.11 | 6.59 |

Note: DAP = day after planting.

*Table 3. Starch content from the fermentation of 8 sweet potato clones with 3 variations of tape yeast concentration.*

| No. | Clones | Concentration yeast (%) | | |
|---|---|---|---|---|
| | | 5% | 10% | 15% |
| 1. | Kuningan Putih (80 DAP) | 0.27 | 0.62 | 0.66 |
| 2. | Beta 2 (80 DAP) | 0.25 | 0.38 | 0.29 |
| 3. | Kuningan Merah (90 DAP) | 0.09 | 0.28 | 0.41 |
| 4. | BIS OP-61 (90 DAP) | 0.37 | 0.41 | 0.60 |
| 5. | 73-OP-5 (120 DAP) | 0.05 | 0.07 | 0.21 |
| 6. | BETA 2-♀-29 (120 DAP) | 0.21 | 0.22 | 0.72 |
| 7. | BIS OP 61-OP-22 (150 DAP) | 0.63 | 0.70 | 0.67 |
| 8. | Sari (150 DAP) | 0.64 | 0.65 | 0.74 |

Note: DAP = day after planting.

**Correlation between alcohol content and starch content**

Starch content (0.64%, 0.65%, 0.74%) successively at concentrations of 5%, 10%, 15% in the Sari clone (150 DAP) (Table 3). The highest fermented total sugar (1.40%, 1.54%, 1.44%) at different concentration levels (5%, 10%, 15%) was in the BIS OP 61-OP-22 clone (150 DAP), while the lowest total fermented sugar (0.26%, 0.25%, 0.28%) at different concentration levels (5%, 10%, 15%) in the Kuningan Merah clone (90 DAP) (Table 5).

*Table 4. Correlation between alcohol content and starch content from the fermentation of 8 sweet potato clones with 3 variations of tape yeast concentration.*

| | Alcohol content | Starch content | |
|---|---|---|---|
| Alcohol content | 1 | | |
| Starch content | 0.285482273 | 1 | Weak correlation |

*Table 5. Total sugar content of fermented 8 sweet potato clones with 3 variations of tape yeast concentration.*

| No. | Clones | Concentration yeast (%) | | |
|---|---|---|---|---|
| | | 5% | 10% | 15% |
| 1. | Kuningan Putih (80 DAP) | 0.34 | 0.31 | 0.30 |
| 2. | Beta 2 (80 DAP) | 0.39 | 0.35 | 0.28 |
| 3. | Kuningan Merah (90 DAP) | 0.26 | 0.25 | 0.28 |
| 4. | BIS OP-61 (90 DAP)) | 0.73 | 0.74 | 0.44 |
| 5. | 73-OP-5 (120 DAP) | 0.54 | 0.45 | 0.39 |
| 6. | BETA 2-♀-29 (120 DAP) | 0.39 | 0.38 | 0.44 |
| 7. | BIS OP 61-OP-22 (150 DAP) | 1.40 | 1.54 | 1.44 |
| 8. | Sari (150 DAP) | 1.13 | 0.86 | 0.63 |

Note: DAP = day after planting.

**Correlation between alcohol content and total sugar**

The core stage of bioethanol production is the fermentation of sugar, either in the form of glucose, sucrose or fructose by yeast, especially *Saccharomyces* sp or *zymomonas mobillis* bacteria. Produced from the raw material starch or starch $(C_6H_{10}O_5)n$ which is hydrolyzed into glucose and then fermented with the microorganism *Saccharomyces cerevisiae* at a temperature of ± 27-30ºC. Fermented products can contain ethanol up to ± 18%.

There is an interaction effect between the concentration of tape yeast and the length of time of fermentation on the pH and levels of bioethanol produced from the red sweet potato fermentation process. The highest total sugar was 6.13% in clone 73-OP-5 (120 DAP) and the lowest was 2.07% in BETA 2-♀-29 clone (120 DAP). The alcohol content was 5.66% at a concentration of 5% in the Kuningan Putih clone (80 DAP), 6.11% and 6.59% at a concentration of 10% and a concentration of 15% in the Sari clone (150 DAP). Correlation of alcohol content and starch content is weakly correlated. The correlation between alcohol content and total sugar content is very weak, meaning that the variables of sugar content and starch content do not show a linear relationship.

*Table 6. Correlation between alcohol content and total sugar resulting from the fermentation of 8 sweet potato clones with 3 variations of tape yeast concentration.*

| | Alcohol content | Total sugar content | |
|---|---|---|---|
| Alcohol content | 1 | | |
| Starch content | 0.092503483 | 1 | Very weak correlation |

**pH**

The length of time of fermentation has an effect on the pH and content of the bioethanol produced, but has no significant effect on the yield and density of bioethanol produced by fermented red sweet potato. The difference in starch and sugar content between fresh sweet potato and flour is due to the high variability in raw material composition. Total sugar expressed glucose equivalents were similar for the two ingredients: 75.0% and 77.0% w/w dry matter, for fresh sweet potato and flour respectively (Lareo et al., 2013). Described by Ramasamy et al. (2014), that starch is the most valuable component in sweet potato, changes in starch content during storage time directly affect the development of the sweet potato industry. Biodiesel synthesis using bioethanol of different grades showed that the yield and product purity increased with the grade of ethanol used, with values ranging from 63 to 83 wt%, and 50 to 94 wt%, respectively. High alcohol values are needed to make biodiesel and bioethanol production (Jhonprimen et al., 2012). Concentrations of 5%, 10%, 15% showed the highest pH showed a value of 6.3 in the BIS OP 61-OP-22 clone (150 DAP) from the initial pH of 4.4, meaning that there was an increase in the pH value, while the lowest pH value was 4.1 in the Beta 2 clone (80 DAP) from the initial pH: 5.8 there was a decrease in the pH value (Table 7).

*Table 7. The pH of the fermented 8 sweet potato clones with 3 variations of tape yeast concentration.*

| No. | Clones | Yeast concentration (%) | | |
|---|---|---|---|---|
| | | 5% | 10% | 15% |
| 1. | Kuningan Putih (80 DAP) | 5.4 | 5.1 | 5.1 |
| 2. | Beta 2 (80 DAP) | 4.1 | 4.8 | 4.8 |
| 3. | Kuningan Merah (90 DAP) | 5.0 | 5.0 | 5.1 |
| 4. | BIS OP-61 (90 DAP)) | 5.1 | 5.2 | 5.2 |
| 5. | 73-OP-5 (120 DAP) | 5.0 | 5.0 | 5.0 |
| 6. | BETA 2-♀-29 (120 DAP) | 5.0 | 5.0 | 5.1 |
| 7. | BIS OP 61-OP-22 (150 DAP) | 6.3 | 6.3 | 6.3 |
| 8. | Sari (150 DAP) | 5.5 | 5.4 | 5.5 |

Note: DAP = day after planting.

The results showed that starch content was weakly correlated with other ingredients, indicating that starch content did not have a linear relationship, while the percentage of dry content and fermentable sugar content was closely correlated with starch content. The percentage of dry content significantly and positively correlated with fermentable flour and sugar content. Confirmed by Laude et al. (2011), that $CO_2$ emissions decreased by 115% by carbon capture and storage during ethanol production. According to Koga et al. (2013), bioethanol production is the final expression of the quality of fermentation in sweet potato tubers. During the storage and fermentation process, there are many factors that cause changes in the yield of bioethanol from sweet potato genotypes that are different from the storage time. The interaction between clone variation and yeast concentration had a significant effect on the results of analysis of starch content after the fermentation process.

Starch content showed a very significant decrease compared to the starch content of fresh raw materials before the fermentation process. The alcohol content is still relatively low because the purification process has not been carried out. To increase the purity, it is necessary to carry out a distillation process because tape yeast is a mixed inoculum, so it is necessary to pay attention to the conversion of starch and sugar in sweet potatoes into products other than ethanol.

The SFS process from sweet potato hydrolyzate occurs at a temperature of 35ºC with a pH of 4.5 which produces an ethanol concentration of 5.32% (v/v) with an ethanol formation efficiency of 35.79%, a fermentation efficiency of 70.16% and a yield of 11.79%. The SFS process lasts for 48-72 hours at 55-60ºC. The substrate concentration and processing time of SFS in the manufacture of ethanol from sweet potato hydrolyzate was 20% (w/v) with a processing time of 48-72 hours. SSF was performed using fresh sweet potato and flour for different ratios of dry matter to water (w/v). The ratios are 1:2 (according to fresh sweet potato without adding water), 1:5 and 1:8 for fresh sweet potato, and 1:2, 1:3, 1:5 and 1:8 for sweet potato flour. Characterization of ethanol is done by determining some of the physical properties of the selected ethanol and the values obtained are compared with standard data on the physical properties of ethanol.

These properties include Density, Viscosity, and Boiling Point, are as follows: 0.825 g/cm³, 0.00143 Pa.s, and 78.40°C. Showed that ethanol can be produced from starch-containing staple foods through fermentation (Anaele et al., 2020). concluded that the concentration of the amyloglucosidase enzyme 1.2 ml/kg of substrate (3000 U/ml) with a concentration of Sacharomyces ceriviceae 10% (v/v) was the best treatment with a concentration of bioethanol produced 7.48% (v/v), yield 19.89%, product formation efficiency by substrate 47.37%, fermentation efficiency 92.88%, and substrate consumption concentration 15.78 g/L (Zhang et al., 2011). Starch hydrolysis with an acid catalyst requires high temperatures, namely 120ºC-160ºC. The acid will break down the starch molecules randomly and the resulting sugars are mostly reducing sugars. The type of acid catalyst used is sulfuric acid ($H_2SO_4$), and Saccharomyces cereviseae to convert glucose into ethanol. One way to produce ethanol is by fermenting glucose with the microorganism *Saccharomyces cerevisiae.*

*Saccharomyces cerevisiae* is a yeast that is tolerant to fairly high alcohol (12-18% v/v), resistant to high sugar levels and remains active in fermenting at 4-32ºC. As stated by Ayoola et al., (2017) that the dry matter content which was higher than fresh sweet potato did not increase the final ethanol concentration. The availability of ethanol tolerant yeast can improve performance, can be obtained up to 4800 L of ethanol per hectare. Three bioethanol production models were selected to evaluate the life-cycle energy efficiency and environmental impact of sweet potato-based bioethanol (Isah et al., 2019). Bioethanol is produced primarily by the process of fermenting sugars, although it can be prepared by the chemical reaction of ethylene with steam in the following reaction scheme:

$$_2C_2H_4(g) + H_2O\ (l) \rightarrow C_2H_5OH\ (g)$$

The fermentation process on the other hand involves the enzymatic breakdown of simple sugars like glucose by enzymes known as zymases. The scheme of the reaction is as follows:

$$C_6H_{12}O_6 \text{ (s)} \rightarrow 2C_2H_5OH \text{ (aq)} + 2CO_2 \text{ (g)}$$

Starchy substrates are often used for the production of bioethanol, used as a substitute for fossil fuels can be produced either by microbial fermentation of sugars or from petrochemical sources, however the production of bioethanol by microbial fermentation of sugars is the most widely used because of its simplicity.

## IV. CONCLUSION

The test results concluded that the interaction between clone variation and yeast concentration had an effect on the highest alcohol content analysis results from the Sari clone $_{(150\ DAP)}$ with a yeast concentration of 15% with a concentration of 6.59%. Clone variation and yeast concentration interacted with the results of analysis of total sugar content after the fermentation process. The total sugar measured was the total sugar content remaining after the fermentation process, indicating a significant decrease in total sugar content. Yeast concentration had no effect on changes in pH during the fermentation process, the pH at the end of the fermentation process was in the range 4.1-6.3 (clone Beta-2 $_{(150\ DAP)}$ and clone BIS OP-61-OP-22 $_{(150\ DAP)}$.

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