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The Effect of Yeast Amounton Aren Sap to the Patterns of Growth and Development of Yeast During Fermentation

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Abstracts: *Sugar has long been known for farmers as economically valuable crop. But the input of science and technology onarenga is still minimum until now compared with the coconut and oil palm, the arenfamily plants. If there are one hundred mature arenga plants in one hectare of land, the production of ethanol per hectare per day is 125 liters, this value is quite large and economically attractive. The production ofyeast ethanol is influenced by environmental factors in which the yeast grows and evolves, including temperature, pH, sulfate content, Na ion, acetic acid, lactic acid, sugar and ethanol. It also includes the source of carbon, nitrogen, minerals and vitamins which are needed to produce high ethanol by examining the effect of yeast on CO_2 gas produced.Data analysis techniques in this research usedANOVA and Tukey test as further testing. ANOVA or Analysis of Variance, and commonly known as the F-test was used to test the comparison of a value on a wide range of groups (or treatment). The results showed that the addition of 0:25 grams of yeast will produce the lowest CO_2 compared to others in the amount of 7:10 liters. On the other hand, the addition 10.0 gr of yeast will produce the highest CO_2 than others, but these results tend to be similar to the results given in 2.5 g and 6 g of yeast. The results of the ANOVA and further testing showed the optimum results of the yeast addition at 2.5, 6.0, and 10.0 grams of CO_2 will provide the highest value. Forthe effectiveness consideration, namely providing 6.0 g yeast and 10 grams will be too much, then the use of 2.5 grams of yeast has been selected for the next stage.*
Keywords:*Aren sap, Yeast Amount, Yeast Growth Patterns, Yeast Development*

I. Introduction

Arenga pinnata are known as source of sugar in itssap (Aren sap) and grows in many areas in North Sulawesi (Kindangen et al., 1991). Thesugar content onarenga sap whichis in therange 6-16% (Imamkhasani et al., 1989) makes this plant has great potential to be used as a source of raw material in the production of ethanol. The research thatwas conducted on the componentof aren sap in Tomohon, North Sulawesi indicated that the sugar content was in the range of 11 to 16% (Pontoh, 2009).

Aren sap is the liquid extracted from male flower aren tree. This fluid contains around 10-15%.Aren sap can be processed into soft drinks, and alcoholic, sugar syrup, brown sugar and nata de arenga. The tapping process is not hard to do. This activity can be used as the main source of income or as an additional income in rural areas (Anonymous, 2001). Aren trees have male flowers and female flowers. Both flowers can be tapped its nira. Male flowers are always tappedbecause the amount and quality of the results are more satisfying than the female flowers. Male flowers are shorter than female flowers. The Length of male flowers is about 50 cm, while the female flowers reach 175 cm. Male flowers can be tapped at the time they have already produced stamens.

The fermentation ofAren sapto be ethanol is the first step to produce ethanol as a whole. Almost all ethanol industries use yeast (Saccharomyces cereviceae) as an organism to ferment (Jacques et al, 1999, and Pontoh, 2009) that have compared various commercial yeast and yeast isolated from local sources in the fermentation of nila arenga. Fermentation is the process of changing the glucose molecule into ethanol and carbon dioxide. This process occurs through a series of reactions that occur in the cells of microorganisms.

Fermentation is the process of energy production in cells in an-aerobic state (without oxygen). In general, fermentation is one form of an-aerobic respiration, but there is a clearer definition that defines fermentation as respiration in an-aerobic environment. Sugar is a common ingredient in fermentation. Some examples the result offermentation are ethanol, lactic acid and hydrogen, but some other components that can be produced from the fermentation such as butyrate and acetone. (Romano et al, 2008).

The production ofyeast ethanol is influenced by various factors of the environment where the yeast grows and develops (Jacques et al, 1999), these factors include temperature, pH, sulfate content, Na ion, acetic acid, lactic acid, sugar and ethanol. In addition to these factors for yeast nutrients including sources of carbon, nitrogen, minerals and vitamins which are needed to produce high ethanol. The source of nitrogen contained in Aren sap is unclear yet. Yeast is known not beable to use nitrogen from amino acids, so the possibility of the source of nitrogen is not from protein. The amount of available nitrogen in nila aren is not known yet that the addition of nitrogen such as in the form of ammonium sulfate can be done. Likewise, the addition of other

nutrients such as magnesium chloride, calcium chloride and pH and temperature variations will be done so that the results in the form of ethanol fermentation can be maximized.

This study aimed to examine the effect of yeast on CO_2 gas produced. The research was conducted in the Laboratory of Minaesa Institute of Technology.

II. Research Methods

2.1. Tools and materials

1. **Tool**. Alcohol meter, Brix meter, GPS, Erlenmeyer, stirring rod, Bekker glass, bottle, pH meter, digital scales, distillation equipment, hoses, buckets, gallon, hot plate, electric stove, burette, microscopes, pipettes and the tools used to analyze with macro-Kjeldahl, gravimetric, spektrofotmetrik and SSA.
2. **Materials**. Aren sap, yeast, ammonium sulfate, buffer Trisodium Citrate, Calcium Chloride, Magnesium Chloride, HCl, distilled, water and chemicals for chemical composition analysis ofnila aren.

2.2. The Research onthe Patterns of the Growth and Development of Yeast

1. First of all nila sap was heated (sterilized) until the sugar content at 20% and then cooled.
2. Prepare bottles and gallons of water along with a fully charged hose which has been designed for the fermentation process.
3. Weighed each yeast at 0:25 grams, 1.0 grams, 2.5 grams, 6.0 grams and 10 grams
4. 300 ml Aren sap was inserted into bottles that have been sterilized, then added each concentration of yeast and shakento dissolve the yeast, after that the ingredients weresealed with sterilized cover that has been designed so that it could be connected with a hose that leads to the gallon which was full of water and has been measured in ml.
5. Connected the bottle containing the sample that the environment was set at thetemperature of 25°C with a gallon then calculate the CO_2 produced by observing the amount (L) of water coming down.
6. At the time of the CO_2 produced that was shown by the bubblesthat came out of the gallon of water were no longer be seen,the fermentation process was stopped.

2.3. ResearchVariables

Predictor variables / Treatment (X)

Variable X1 = Given Yeast (0.25 g, 1 g, 2.5 g, 6 g, and 10 g)

Dependent Variable / Response (Y)

Variable Y1 = Concentration of CO_2 produced (liters)

2.4. Data Analysis Techniques

Analysis of Variance (ANOVA)

Data analysis techniquesthat were used in this study, namely ANOVA and Tukey test as the further test. ANOVA or Analysis of Variance, and commonly known as the F-test was used to test the comparison of a value on a wide range of groups (or treatment). ANOVA is equivalent to a completely randomized design (CRD) (Yitnosumarto, 1993).

ANOVA techniques are used to test the variability of observations of each group and the mean variability between groups. Through both variability, there will be drawn conclusions about the population mean. F or F_{count}taken from the price of the average sum of mean square between groups divided by the average of the sum of error square. It is more clearly seen in the formula below which is presented on the table of ANOVA (Yitnosumarto, 1993):

Table 1. Formula One Way ANOVA

Source Diversity	Number of Squares	db	Central Squares	F_{-count}
Treatment	$JK_p = n\sum_{i}^{p}(\overline{Y_i} - \overline{Y_{..}})^2$	(p-1)	$KT_p = \frac{JK_p}{(p-1)}$	$\frac{KT_p}{KT_g}$
Error	$JK_g = \sum_{i}^{p}\sum_{j}^{n}(\overline{Y_{ij}} - \overline{Y_{i.}})^2$	p(n-1)	$KT_g = \frac{JK_g}{p(n-1)}$	
Total	$JK_T = \sum_{i}^{p}\sum_{j}^{n}(\overline{Y_{ij}} - \overline{Y_{..}})^2$	(pn-1)		

where: p = many groups / treatment

n = many observations

The hypothesis used in the ANOVA test is:
- H0: suspected that the whole group of the population mean is equal
- Ha: suspected that the whole group of the population mean is different

The basis of the decision is (with α error rate used is 5%):
- If F count> F table 0:05 or the Sig F <α, then H0 is rejected (significant)
- If F arithmetic <F table 0:05 or the Sig F> α, then H0 is accepted (non-significant)

If there is a significant difference, then do further testing procedure. One of the more commonly used test is the Tukey test (Steel and Torrie, 2001). With the approach of the notation (A, B, C, ... and so on), at each level of treatment will be given notation. Treatment with the same notation indicating the similarity between the treatment, whereas treatment with different notation indicates the difference between the treatments.

ANOVA testing to this hypothesis, which is suspected there is a difference of carbon dioxide (CO_2) generated at each given yeast (0:25, 1:00, 2:50, 6:00, and 10.0 g)

Curve Fitting

In this study, each treatment (treatment 5 respectively), using quantitative treatment, namely has numerical distance on a level with the other levels (Yitnosumarto, 1993).

To obtain the estimated value of the optimum point (minimum / maximum) of the effect of each treatment on the response variable can be tested Curve Fitting (curve fitting) (Steel and Torrie, 2001). One form of the model that can be used is a polynomial model as follows:
- Linear model (degree 1), the equation y = b0 + B1X
- Quadratic model (degree 2), the equation y = b0 + B1X + b2x2
- Cubic Model (grade 3), with the equation y = b0 + B1X + b2x2 + b3x3
- Model Kuartik (degree 4), the equation y = b0 + B1X + b2x2 + b3x3 + b4x4

Where y is the response variable (in this study is ethanol, CO2, and the resulting efficiency), and x is the predictor variable / treatment, eg Ammonium Sulfate, x = 0.75 g, 1:50 gr, gr 2:25, 3:00 g, 3.75 g.b0, b1, ..., b4 are regression coefficients obtained from the estimation of Ordinary Least Square (OLS) (Gujarati, 2003). So through the curve fitting tool, the answer will be obtained optimum value, both the minimum and maximum of each response due to treatment (Santoso, 2002).

III. Result and Discussion

This study examined the effect of yeast on CO_2 gas produced. There are five levels of given yeast (bakery yeast/ fermipan), as follows ;
- 0.25 gr/300 ml
- 1.00 gr/300 ml
- 2.50 gr/300 ml
- 6.00 gr/300 ml
- 10.00 gr/300 ml

The following graph presents the effect of yeast on CO_2 gas produced per hour as follows:

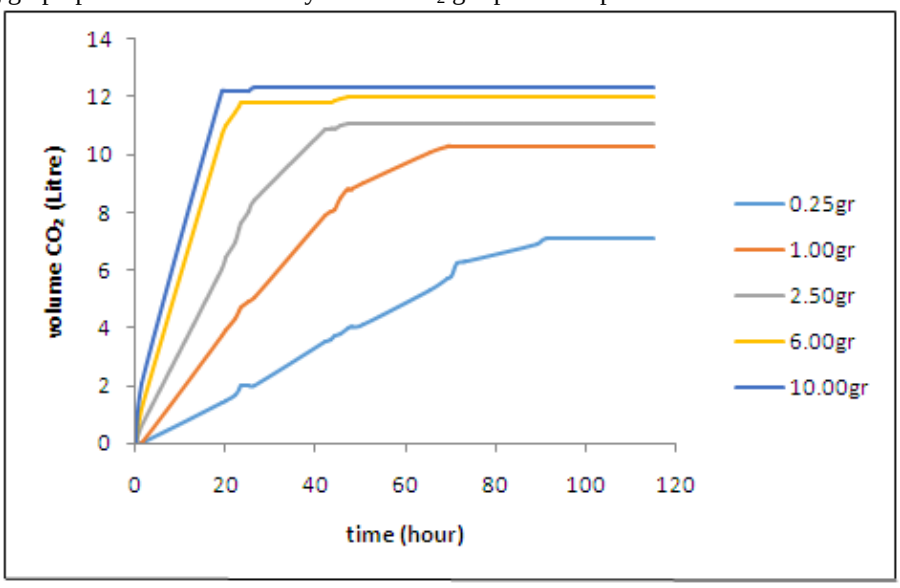

Figure 1. CO_2 gas produced at various levels of given yeast each time

Figure 1 showed the given yeast at 0.25 g, will produce the lowest CO_2 gas that reaches 7:10 liter after 115.30 hours. By giving 1.00 g yeast, will produce CO_2 gas which is higher than the provision of 0:25 g yeast, which reached 10:30 liter. Next, by giving 2.5 g of yeast will produce CO_2 at 11:05 liter. While the given yeast at 6 g and 10 g, will produce the highest CO_2 gas at 12 and 12:30 liter. From 0 to 115.30 hours, sugar is processed into ethanol, with the activeness of the yeast then sugar is running out because it is converted to CO_2 (approximately 49%), and ethanol (approximately 51%). To see the effect of the yeast which will produce the highest CO_2 gas, ANOVA test are presented as follows:

Prior to the ANOVA test, it is conducted prior to the fulfillment of assumptions, namely normalitydata and homogenity of variance. The assumption of normality uses the Kolmogorov-Smirnov test, the data said to be normal if the Sig KS> 0.05. The assumption of homogeneity uses Levene's Test, the data is said to have a diversity of homogeneous if the Sig Levene> 0.05.

Table 2. The Testing Homogeneity and Normality Assumptions on the Given YeastTreatment

Variable	The Assumption of Normality (Sig KS)	Homogeneity Assumption (Sig Levene)
CO_2	0.355	0.187

Source: Processed Primary Data

Table 2 showed the value of the Kolmogorov-Smirnov Test> 0.05, which indicates that the data used in this study is normal distribution. Italso can be seen the Sig Levene's Test> 0.05, which indicates that the assumption of homogeneity of variance are met. It means variance (variation) of the fifth magnitude given yeast (0.25 g, 1:00g, 2:50 g, 6:00g, and 10:00 g) is likely almost the same. Thus the homogeneity and normality assumption underlying the ANOVA are met.

The results of comparative analysis of the CO_2 generated by Analysis of Variance (ANOVA) on the comparison between the amount of given yeast. The big difference in CO_2 being produced in each given yeast is visible if the Sig F <0.05, the error rate is 5%. Test results are presented in Table 3 below.

Table 3. Results of ANOVA of CO_2 produced per given yeast

Source Diversity	Degrees Free	Number of Squares	Central Squares	F-count	Sig F
Treatment	4	52.140	13.035	53.096	0.000
Error	10	2.455	0.246		
Total	14	54.594			

Source: Processed Primary Data

Based on the analysis refering to Table 2, it is showed that the value of F at 53.096 and the Sig F for 0000. Because the Sig F <0.05, it could be concluded that there is a difference of CO_2 produced on each scale of given yeast. This indicates that in each provision given yeast 0:25gr, 1:00 gr, 2:50, 6:00 grams, and 10.0 grams of CO_2 will result in different values.

To determine the amount of the provision of yeast which give different values of CO_2 produced, conducted a further test (Tukey test). If the amount is given the same notation (the samesubset), there are similarities between the amount indicated, otherwise if the amount is given different notation (different subset), indicating there is a difference between the amount. The following is the complete test results:

Table 4. The Advanced Result Test of CO_2Produced per GivenYeast

GivenYeast	CO_2Produced	Notation
0.25 gr	7.10	A
1.00 gr	10.30	B
2.50 gr	11.05	BC
6.00 gr	12.00	C
10.0 gr	12.30	C

The results of further analysis with Tukey test showed that the givenyeast of 0:25 grams will produce the lowest CO_2 compared to others in the amount of 7:10 liters (notation A, and there is no other treatment that has the same notation). On the other hand, the given 10.0 gyeast will produce the highest CO_2 compared to other (notation C), but the results tend to be similar to the results given in yeast 2.5 g and 6 g (shown in Figure 5.10). So that the results of the ANOVA and the further test showed the optimum results of given yeast at 2.5, 6.0, and 10.0 grams of CO_2 will provide the highest value. Because the effectiveness considerations, namely providing 6.0 g yeast and 10 grams are too much, then the use of 2.5 grams of yeast was selected for the next stage. Figure 2 shows the effect of theyeast concentration to the fermentation time:

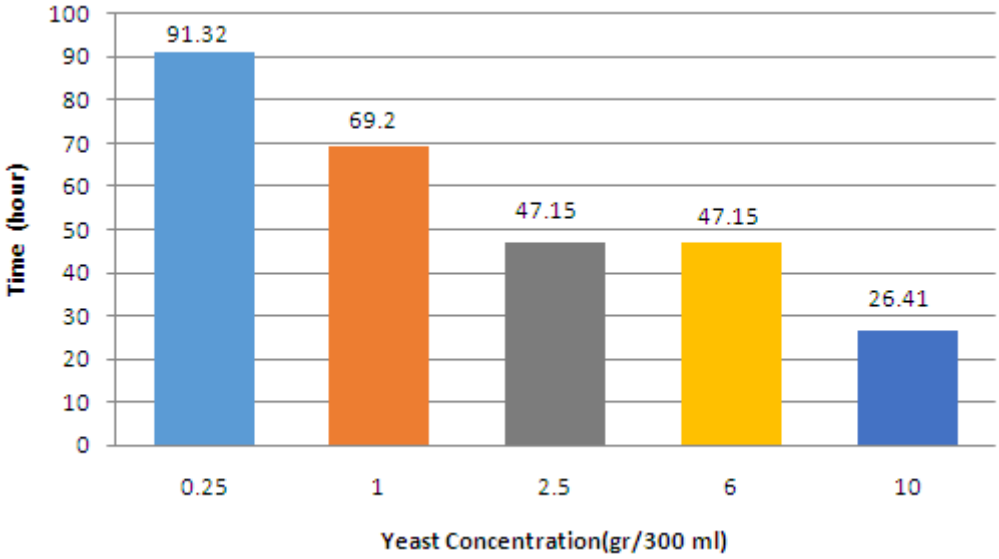

Figure 2. The Effect of Yeast Concentration to the Fermentation Time

Ingeldew (1999) states that until todaythe most efficient microorganism to produce ethanol is yeast. That is why yeast is used widely in the ethanol industry. Producing ethanol yeast cellis influenced by various factors of the environment in which yeast was grown including temperature, pH, sulfate content, Na ion, acetic acid, lactic acid, sugar and ethanol. In addition to these factors for yeast nutrients, such as carbon sources, nitrogen, minerals and vitamins which are needed to produce high ethanol (Lantemona, et.al 2013)

The pattern of development of yeast cells has enormous relevance to the process of ethanol production in the industrial system. In general, the pattern of cell growth will follow the pattern as it is. The number of cells that slightly compared with the available substrate will cause a lag-phase development longer. This will provide an opportunity for another microorganism cells to thrive. In contrast, big of number of yeast cells will shorten the lag-phase time (Lantemona, et.al 2012).

Active dry yeast is easy to be used in the fermentation process. In theindustry of fuel of alcohol and wine, yeast is inoculated in the fermentation tool with recommended value between 1.0-2.0 millionsell / ml per plato (plato = gram of extract (sucrose) per 100 g of solution (Jacques, 1999).

IV. Conclusion

The concentration of yeast affects the growth and development of the yeast during the fermentation of Aren sap. The optimum concentration of yeast is equal to 2.5 grams.

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