The Role of Single Layer Immobilized Cells in Mead Fermentation Rate

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Abstract: The research consisted of the making of honey-must, the making of single layer immobilized cells, and mead fermentation. Mead fermentation carried out with variation of treatments which were control (C) and single layer immobilized cells (SL). The analysis performed were analysis of alcohol content, sugar content, and yeast assimilable nitrogen (YAN) concentration which determine fermentation rate. The result of this research explained that during mead fermentation, sugar content decreased with latest sugar content varied namely 4,30-4,70ºBrix, alcohol content with the latest value namely 8,5-9,5%, and fermentation rate for SL was faster than C namely 0,105 g.ml^{-1}.hr^{-1}.

1 INTRODUCTION

Honey is one of bee's derivative product with various health benefits and also contributes to enhance community's economical state. Mead (as known as honey wine) is one way to innovate honey by fermenting honey solution (Pereira et al., 2013). Mead is probably the oldest fermented beverage in the world that was produced, but its production has declined in recent years partly due to a lack of scientific progress in this field.

Based on studies of wine, wine is considered as a safe and healthy beverage in the moderate amount of consumption because it had a positive effect on the cardiovascular system (German & Walzem, 2000; Snopek et al., 2018; Wurz, 2019). Alcohol that contained in wine included as macro nutrition and worked as an energy source, able to provide calories for biological activities for humans, energy for physical work, and thermogenesis (Joshi et al., 2012). It is also studied before that antioxidant content, especially honey phenolic compounds, increased from mead fermentation process (Wintersteen et al., 2005). In addition, the fermented ethanol compounds in mead can enhance the solubility and extraction strength for beneficial compounds contain in mead (Roldán et al., 2011).

Over past few decades, the use of immobilized cells methods had received attention of researchers and had been successfully applied in the production of alcohol (ethanol), organic acids, enzymes, and fermented food products (beer and wine) (Liouni et al., 2008; Reddy et al., 2008) and proved can overcome some common problems that occur in the fermentation process. During mead fermentation process, some common problems were the inability to obtain the desired alcohol content, a long fermentation process, heterogeneity of the final product (Pereira et al., 2014), and the re-fermentation from the yeast that increase volatile acids thus produced unwanted aroma (Ramalhosa et al., 2011). Immobilized cells also allow easier handling of yeast cells, clear end products, and continuity of use (Kourkoutas et al., 2004; Park & Chang, 2000). The purpose of this research was to study the role of single layer immobilized cells in mead fermentation rate including its alcohol and sugar content.

2 MATERIALS AND METHODS

The main and chemical ingredients that used in this research were honey from *Ceiba pentandra*, *Saccharomyces cerevisiae var. bayanus* (dry yeast), malic acid ($C_4H_6O_5$), formaldehyde (CH_2O), distilled water, sodium alginate, natrium hydroxide (NaOH), and calcium chloride ($CaCl_2$). The

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equipment used in this research were refractometer (Hanna Instrument 96801), pH meter (Hanna Instrument pH 213), and autoclave.

The research steps were the making of honey-must, the making of single layer immobilized cells, and mead fermentation. Honey-must is a solution resulting from dilution of honey in distilled water. At the stage of making honey-must, dilution of honey in distilled water done until the concentration of sugar reach 15ºBrix (Srimeena et al., 2014). Insoluble materials then filtered so the clear honey-must solution was obtained. The honey-must then adjusted to pH around 3,7 using malic acid ($C_4H_6O_5$). The parameters measured before and after honey-must adjustment were sugar content (ºBrix), pH, and yeast assimilable nitrogen concentration (YAN). YAN was determined by the formaldehyde method (Pereira et al., 2014). The next step was honey-must pasteurization by heating at temperature of 65ºC for 10 minutes (Mendes-Ferreira et al., 2010) using a water bath and then cooled it to room temperature.

The making of single layer immobilized cells began with preparing starter yeast culture. The starter yeast culture was prepared by rehydrating two grams of dry yeast in 20 ml of distilled water at 38oC to obtain a suspension of yeast ca. 108 CFUs per ml (Pereira et al., 2014). Sodium alginate was dissolved in distilled water at a concentration of 4% (w/v) and sterilized by autoclaving at temperature of 121ºC for 20 minutes. To inoculate honey-must solution with 106 CFU per ml, the appropriate amount of the yeast suspension was added to sodium alginate solution. This polymer cells mixture was added drop wise to a 180 mM calcium chloride sterile solution and allowed to harden for 30 minutes at 4ºC.

For mead fermentation step, firstly honey-must was divided into 6 polyethylene terephthalate (PET) gallons. Honey-must in the first three gallons were used as control (C) where only 106 CFU per ml yeast added, and the rest three gallons were added with single layer immobilized cells (SL). Each mixture was then incubated at room temperature (Roldán et al., 2011) and batch fermented under anaerobic condition. Each gallons were closed and clamped, the remaining air was removed using a pump. Fermentation was considered complete when the YAN concentration, alcohol content, and sugar content were constant. Measurement of YAN concentration, alcohol content, and sugar content carried out every two days and with thrice replications.

3 RESULTS

The free cells yeast in control (C) and wet beads yeast in single layer immobilized cells (SL) were weighted before being put into honey-must. In addition, the pH value, sugar content (ºBrix), and YAN concentration after adjustment were measured. The initial physicochemical characteristics of honey-must can be seen in Table 1.

Table 1: Initial characteristics of honey-must after adjustment.

	Control	Single Layer
Total yeast weight (g)	26.49±0.02	31.61±0.01
pH	3.72±0.00	3.72±0.00
Sugar content (ºBrix)	15.30±0.00	15.20±0.00
Yeast Assimilable Nitrogen (mg/L)	248.00±0.00	249.10±0.01

The YAN value after honey-must adjustment could be included in high levels (>225 mg/L) so it didn't require additional nutrients. Changes in sugar content (ºBrix), alcohol content (%), and YAN concentration during mead fermentation can be seen in Table 2 and the ANOVA result for each parameters can be seen in Table 3.

Table 2: Sugar content, alcohol content, and yeast assimilable nitrogen concentration during mead fermentation.

Day	Sugar content (ºBrix)		Alcohol content (%)		Yeast assimilable nitrogen concentration (mg/L)	
	Control	Single layer	Control	Single layer	Control	Single layer
1	14.47±0.00	13.4±0.00	0±0.00	0±0.00	256.67±0.00	270.67±0.00
3	14.13±0.00	11.77±0.00	0±0.00	1.83±0.08	275.33±0.00	254.33±0.00
6	12.50±0.00	10.73±0.00	3.17±0.08	4.67±0.08	270.67±0.00	228.67±0.00
8	11.13±0.00	9.73±0.00	4.17±0.08	6.67±0.08	263.67±0.00	212.33±0.00
10	10.57±0.00	7.30±0.01	5.00±0.00	8.60±0.03	254.33±0.00	189.00±0.00

Table 2: Sugar content, alcohol content, and yeast assimilable nitrogen concentration during mead fermentation (Cont.)

Day	Sugar content (°Brix)		Alcohol content (%)		Yeast assimilable nitrogen concentration (mg/L)	
	Control	Single layer	Control	Single layer	Control	Single layer
13	9.70± 0.00	5.53± 0.00	6.00± 0.00	9.17± 0.00	249.67± 0.00	168.00 ±0.00
15	7.57± 0.00	4.37± 0.00	7.00± 0.00	9.43± 0.00	238.00± 0.00	165.67 ±0.00
17	5.53± 0.00	4.30± 0.00	7.70± 0.03	9.50± 0.00	235.67± 0.00	163.33 ±0.00
20	4.97± 0.00	4.30± 0.00	8.00± 0.00	9.50± 0.00	224.00± 0.00	161.00 ±0.00
22	4.90± 0.00	4.30± 0.00	8.27± 0.00	9.50± 0.00	219.33± 0.00	163.33 ±0.00
24	4.87± 0.00	4.27± 0.00	8.47± 0.00	9.50± 0.00	217.00± 0.00	161.00 ±0.00
27	4.73±0.00	4.30± 0.00	8.50± 0.00	9.50± 0.00	214.67± 0.00	161.00 ±0.00
29	4.7± 0.00	4.30± 0.00	8.50± 0.00	9.50± 0.00	214.67± 0.00	158.67 ±0.00
30	4.7± 0.00	4.30± 0.00	8.50± 0.00	9.50± 0.00	212.33± 0.00	158.67 ±0.00
31	4.7± 0.00	4.30± 0.00	8.50± 0.00	9.50± 0.00	212.33± 0.00	158.67 ±0.00
32	4.7± 0.00	4.30± 0.00	8.50± 0.00	9.50± 0.00	212.33± 0.00	158.67 ±0.00

Control (C) hadn't significant difference with single layer immobilized cells (SL). During mead fermentation, the value of sugar content (°Brix) will decrease over time until it finally stabilized. The final value of the sugar content was almost the same, ranging from 4,3 to 4,7. SL had lower latest sugar content compared to C.

Changes in the value of alcohol content hadn't significant difference from C and SL. During mead fermentation, the value of alcohol content (%) increase over time until finally stabilized. The value of alcohol content varied from 8,5 to 9,5%. SL produced higher alcohol content compared to C. From table 2, the relationship between sugar content and alcohol content can be addressed, the higher the alcohol content along with the decrease in sugar content of mead.

Table 3: Analysis of variance (ANOVA) result.

		Sum of squares	Degree of freedom	Mean square	F	Sig.
Sugar content (°Brix)	Between groups	15.60	1	15.60	1.30	0.26
	Within groups	360.04	30	12.00		
	Total	375.64	31			
Alcohol content (%)	Between groups	20.46	1	20.46	2.26	0.14
	Within groups	272.27	30	9.08		
	Total	292.74	31			
Yeast assimilable nitrogen (mg/L)	Between groups	22171.97	1	22171.97	23.00	0.00
	Within groups	28920.53	30	964.02		
	Total	51092.50	31			

Fermentation rate of wine was associated with yeast assimilable nitrogen (YAN) value, where an increase in fermentation rate is associated with a decrease in the number of YAN (Pereira et al., 2014). There was a significant difference in the value of YAN between C and SL. The value of mead fermentation rate is calculated using Michaelis Menten equation (Levenspiel, 1999) which was approached by the linear equation as follows:

$$\frac{1}{V} = \frac{1}{Vmax} + \left(\frac{Km}{Vmax}\right)\frac{1}{[S]} \quad (1)$$

Vmax values (maximum reaction speed) and Km (Michaelis Menten constant) can be obtained from the equation and can be seen in Table 4. When compared, SL had faster fermentation rate than C.

Table 4: Vmax and Km value from mead fermentation.

	Vmax ($g.ml^{-1}.hr^{-1}$)	Km ($g.ml^{-1}$)
Control	0.035	6.641
Single layer	0.105	23.627

4 DISCUSSION

4.1 Effect of Immobilized Cells on Alcohol Content (%) and Sugar Content (Obrix) of Mead

Conversion of sugar into alcohol was an important process in the fermentation of wine. During mead fermentation process, alcohol content formed was related to sugar content. The higher the alcohol content formed, the lower the sugar content value in the mead. Total sugar content (°Brix) during fermentation was an indicator of yeast's activity in fermenting solution (Kaur et al., 2014). When the value of sugar content decreased, it was due to the formation of alcohol.

The alcohol content achieved in this research varied between 8,5-9,5%, where fermentation using immobilized cells achieved higher alcohol content and faster fermentation rate than control. This proved that fermentation using immobilized cells caused yeast cells to have higher alcohol tolerance that control. Besides, this difference was due in immobilized cells, yeast cells were protected from inhibitors and other unfavourable fermentation conditions that caused faster development of yeast cells (Park & Chang, 2000). This result was in accordance with the research conducted before (Yu et al., 2007), where the productivity of alcohol in fermentation was higher in immobilized system compared to the free cells system because yeast cells in the immobilized system consumed sugar faster and efficiently.

4.2 Effect of Immobilized Cells on Mead Fermentation Rates

Mead fermentation rate is associated with the value of yeast assimilable nitrogen (YAN), where there was an increase of fermentation rate associated with a decrease in the amount of YAN (Pereira et al., 2014). Fermentation rate depends in the concentration of the inhibitors such as fatty acids (hexanoic acid, octanoic acid, decanoic acid), protein (enzymes), furfural, and hydroxymethilfurfural (Sroka et al., 2013). High concentration of inhibitors will inhibit the fermentation rate. Inhibitors interact synergistically with high osmotic pressure and an increase in alcohol content during fermentation.

Thus in this research, mead fermentation using immobilized cells resulted in a faster fermentation rate and fermentation time than free cells system because of the yeast cells were protected from inhibitors and other unfavourable fermentation conditions. This result was in accordance with the research before (Navrátil et al., 2001), where the use of immobilized cells can increase the rate of fermentation because yeast cells were protected in the matrix from adverse environmental conditions such as pH, temperature, and inhibitors so their growth were faster. In addition, the reduced intracellular pH value in immobilized cells increases the permeability of cytoplasmic membranes with protons thereby increasing ATP consumption which cause an increase in glucolytic activity and an increase in glucose consumption in the medium (Kourkoutas et al., 2004) thereby increasing the rate of mead fermentation.

5 CONCLUSIONS

During mead fermentation, there were a decrease in sugar content and YAN concentration, as well as an increase in alcohol content. Fermentation using immobilized cells achieved higher alcohol content and faster fermentation rate and fermentation time than control (free cells system).

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