3. RESULTS

This research is conducted in order to investigate the influence of yeast pitching rate and fermentation temperature on the final concentration of ethyl acetate and isoamyl acetate as flavor compounds in beer. In this research, three yeast pitching rates (1, 2, and 3 g/L) and three different fermentation temperatures (10, 12, and 14°C) were applied to first brews of Heineken® wort. After nine days of fermentation and an overnight deep cooling period, the samples were taken for ethyl acetate, isoamyl acetate, and total higher alcohols analysis using gas chromatography instrument with flame ionization detector (FID).

3.1. Volatile Compounds Concentration

Results of esters and total higher alcohol concentration of different fermentation temperature and yeast pitching rate were expressed as mean ± standard deviation (SD) can be seen on Table 2. Based on Table 2, all fermentation temperature gave significant difference in ethyl acetate, isoamyl acetate, and total higher alcohol concentration. It can be seen the concentration of ethyl acetate, isoamyl acetate, and total higher alcohol increased along with fermentation temperature. Yeast pitching rate did not give significant difference for ethyl acetate and total higher alcohol concentration. In isoamyl acetate concentration, there was a significant difference between 2 and 3 g/l, but yeast pitching rate of 1 g/l did not have any significant difference with 2 and 3 g/l.

Table 2. Volatile Compounds Concentration

Fermentation Temperature (°C)	Volatile Compounds (mg/L)	Yeast Pitching Rate		
		1 g/l	2 g/l	3 g/l
10	Ethyl acetate	3.953 ± 0.065^{a1}	4.143 ± 0.252^{a1}	5.054 ± 0.140^{a1}
12		5.953 ± 1.035^{b1}	5.939 ± 2.772^{b1}	5.289 ± 0.135^{b1}
14		8.853 ± 2.310^{c1}	7.675 ± 0.381^{c1}	9.738 ± 1.175^{c1}
10	Isoamyl acetate	2.992 ± 0.159^{a1,2}	2.661 ± 0.329^{a1}	2.954 ± 0.169^{a2}
12		5.134 ± 1.150^{b1,2}	3.367 ± 1.820^{b1}	3.568 ± 0.074^{b2}
14		5.603 ± 2.566^{c1,2}	5.248 ±0.496^{c1}	7.948 ± 1.351^{c2}
10	Total higher alcohol	52.072 ± 2.349^{a1}	49.832 ± 4.544^{a1}	50.200 ± 2.583^{a1}
12		68.436 ± 3.881^{b1}	67.308 ± 4.678^{b1}	57.975 ± 4.804^{b1}
14		64.440 ± 5.198^{c1}	66.187 ± 1.707^{c1}	74.982 ± 8.892^{c1}

Keys:

- All data are expressed in mean and standard deviation
- Data with different number superscript at each column shows significant difference between each yeast pitching rate based on *Two-Way* ANOVA with Duncan's multiple range test ($p < 0,05$)
- Data with different letter superscript at each row shows significant difference between each temperature fermentation based on *Two-Way* ANOVA with Duncan's multiple range test ($p < 0,05$)

3.2. Correlation Analysis

Results of correlation analysis can be seen on Table 3. It can be observed that temperature have very significant correlations with ethyl acetate (0.801), isoamyl acetate (0.640), and total higher alcohol (0.775) concentation. However, there's no significant correlation between yeast pitching rate and all volatile compounds. Strong positive correlations can also be seen between ehtyl acetate, isoamyl acetate, and total higher alcohol concentration. The heat map of correlation analysis result can be seen in Table 4.

Table 3. Correlation Analysis

Parameter	Temperature	Yeast Pitching Rate	Ethyl Acetate	Isoamyl Acetate	Total Higher Alcohol
Temperature	1	0	.801**	0.640**	0.775**
Yeast Pitching Rate	0	1	0.101	0.035	-0.049
Ethyl Acetate	0.801**	0.101	1	0.592**	0.681**
Isoamyl Acetate	0.640**	0.035	0.592**	1	0.704**
Total Higher Alcohol	0.775**	-0.049	0.681**	0.704**	1

Keys:

- All data are the result of pearson correlation test (two-tailed).
- Values with * symbol represent significant correlation with 0.05 significance level
- Values with ** symbol represent significant correlation with 0.01 significance level

Table 4. Heat Map of Correlation Analysis Results

	Temperature	YPR	EA	IAA	THA
Temperature	1	0	0.801743	0.640045	0.770383
YPR	0	1	0.103596	0.031945	-0.04262
EA	0.801742758	0.103596	1	0.590208	0.679228
IAA	0.640045185	0.031945	0.590208	1	0.694477
THA	0.770382772	-0.04262	0.679228	0.694477	1

3.3. Regression Analysis Results

3.3.1. Ethyl Acetate

Results of multiple regression analysis between ethyl acetate, fermentation temperature, and yeast pitching rate can be seen in Table 5. A multiple regression analysis was conducted to predict ethyl acetate concentration based upon the fermentation temperature and yeast pitching rate. An R^2 value of 0.683 was obtained. The ethyl acetate predicted concentration is equal to 0.438 + 0.148 (temperature) + 0.019 (pitch rate), where the temperature is measured in °C and the pitch rate in g/L.

Table 5. Regression analysis results for ethyl acetate

	Unstandardized coefficients (B)
Constant	0.438
Temperature	0.148
Yeast pitching rate	0.019

3.3.2. Isoamyl Acetate

Results of multiple regression analysis between isoamyl acetate, fermentation temperature, and yeast pitching rate can be seen in Table 5. A multiple regression analysis was conducted to predict isoamyl acetate concentration based upon the fermentation temperature and yeast pitching rate. An R^2 value of 0.411 was obtained. The isoamyl acetate predicted concentration is equal to 0.269 + 0.159 (temperature) + 0.008 (pitch rate), where the temperature is measured in °C and the pitch rate ing/L .

Table 6. Regression analysis results for isoamyl acetate

	Unstandardized coefficients (B)
Constant	0.269
Temperature	0.159
Yeast pitching rate	0.008

3.4.The Implementation of Fermentation Temperature Changes in Heineken® Beer

Table 4., shows results of esters concentration analysis from September 2019 to March 2020. It can be observed that there were temperature changes. In October 2019, PT Multi Bintang Indonesia used 9.90°C as fermentation temperature, which increased to 10.00°C in November 2019 and December 2019. Finally, there was further increase in temperature to 10.10°C in January, February, and March 2020. The analyses were done by Heineken Quality Assurance Laboratory (certification of analysis attached in Appendix no. 8).

Table 7. Esters Concentration in Heineken® Beer from October 2019 to March 2020

Reference Beer	Fermentation Temperature (°C)	Ethyl Acetate (mg/L)	Isoamyl Acetate (mg/L)
10 (October 2019)	9.90	21.8	3.52
11 (November 2019)	10.00	21.8	3.57
12 (December 2019)	10.00	22.3	3.68
01 (January 2020)	10.10	21.4	3.4
02 (February 2020)	10.10	23.0	3.81
03 (March 2020)	10.10	23.7	3.84

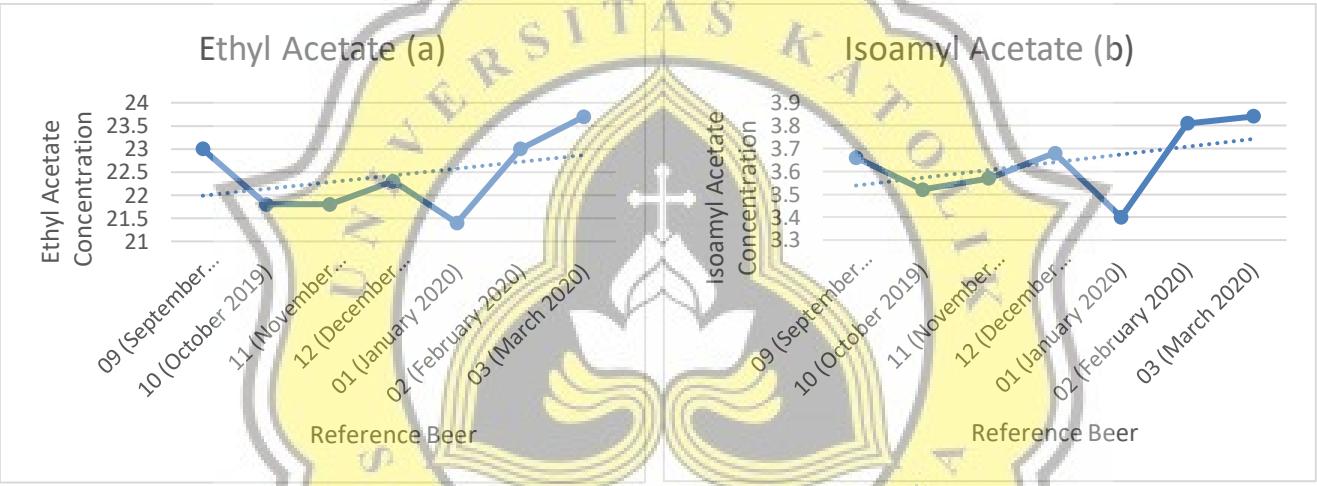

Figure 4. Concentration of Ethyl Acetate (a)., and Concentration of Isoamyl Acetate (b) in Heineken Beer

Figure 4 (a and b). Show the results of Heineken Quality Assurance Laboratory's esters concentration analysis from September 2019 to March 2020. It can be observed that the highest ethyl acetate and isoamyl acetate concentration was found on March 2020, where the fermentation used was 10.1°C. With the exception of September 2019 and January 2020, it can be observed that higher fermentation temperature results in higher esters concentration.