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BIOL 342

Independent Research Project

13th December 2020

Change in the Rate of Yeast Metabolism Based on the Temperature Change

Abstract

Yeasts use alcoholic fermentation as main mode of energy production in anaerobic conditions, producing ethanol as byproduct in this process. This allows them to have variety of applications in human lives ranging from bakery to brewery. With yeast using enzymes and molecular kinetics for this reaction process, temperature can have great impact on the rate of yeast metabolic process. This research aims to find out how temperature change impacts yeast metabolic process by observing amount of carbon dioxide gas produced under five different temperature conditions. The temperature conditions are prepared with approximately 20 °C gradient starting at around 0 °C and rising to approximately 100 °C. In order to compare the differences in gas volume changes in each temperature group, one-way ANOVA analysis followed by Tukey's post hoc test was performed on GraphPad Prism. The one-way ANOVA shows p-value less than 0.0001, indicating existence of significant difference. Following Tukey's post hoc test shows that this significant difference lies in between all temperature groups except in between two lowest temperature and two highest temperature groups. This research, along with further researches for more accurate analysis, can be used to provide information about optimal yeast activity in bakery and brewery industries to minimize financial loss coming from failure to utilize yeast in optimum conditions.

Introduction

One of the most commonly used and easily accessible microorganisms in human lives is yeast. Yeasts are eukaryotic, unicellular microorganism in fungi kingdom (Turker). Under anaerobic condition, yeasts use alcoholic fermentation as main mode of energy production, using energy produced in reaction to produce ATP in itself and producing ethanol and carbon dioxide as byproduct. Due to such properties of yeasts, their application is very common in food industry, especially in process of alcohol brewing and bread leavening, in which alcoholic fermentation is necessary (Turker). Fermentation reaction of yeasts are as following: $C_6H_{12}O_6(s) + H_2O(l) \rightarrow 2\ CH_3CH_2OH(l) + 2\ CO_2(g) +$ Energy (Buratti). In this reaction, monosaccharide glucose is used as reactant for fermentation process. However, it is crucial to note that such monosaccharides are not readily available in nature; most sugar sources available are in form of larger polysaccharides. In other words, it is necessary for yeasts to break down polysaccharide sugar into monosaccharide form in order to start fermentation reaction for ATP production.

There are several enzymes that are responsible for breaking down of polysaccharides into monosaccharides. Maltase is an enzyme that is responsible for breaking down disaccharide

maltose into two monosaccharide glucose molecules (Committee). Invertase is responsible for breaking down of disaccharide sucrose into monosaccharide fructose and glucose (Committee). Only after theses enzymes make glucose available for fermentation reaction, enzyme zymase will use monosaccharides produced by other enzymes to produce ATP, CO_2 and alcohol through fermentation process (Committee).

Since yeasts have such variety usage, it is important to know optimal condition that yeast can function to produce desired fermentation process. On a large-scale brewing or baking process, failure to meet those conditions can lead to huge financial loss. Temperature is one of the most important conditions regarding enzyme, cell metabolism process and molecular kinetics. Therefore, this research will investigate how yeast activities change as their temperature conditions change. Based on above information, it can be hypothesized that as the temperature of the environment that yeasts are in increases, then, due to increased molecular kinetics and enzymatic activity, the metabolic activity of the yeasts will increase as well, until temperature is too high that enzymes in the yeasts start to denature. This hypothesis can be put in a form of statistical hypothesis of null hypothesis (H_0) and alternative hypothesis (H_a) as following:

H_0: The volume of CO_2 produced will show NO DIFFERENCE between different temperature conditions.

H_a: The volume of CO_2 produced will show DIFFERENCE between different temperature conditions.

Materials and Methods

The materials used for this experiment were Ziploc Bags, yeast, white sugar, water, ice, empty plastic containers, pots and kettles, stopwatch, measuring cup, straws, thermometer, and measuring spoon.

First, using measuring cup, 10mL of water was added into an empty Ziploc bag. Top level of the water added was marked with permanent marker. Ziploc bag was marked for every 10mL gradient by adding 10mL of water each time. These marks were used for approximation of CO_2 gas produced later in the experiment. 15 Ziploc bags were prepared this way and divided into five groups with three bags in each group. Each group was labeled “Ice-Cold”, “Cold”, “Warm”, “Hot” and “Boiling-Hot” respectively. This represented the temperature conditions that the yeast in each bag were going to be in. In each bag, one package (~8g) of yeast, one tablespoon of white sugar and one tablespoon of water was added and stirred so they mix well. The temperature of the water added in the bag was determined based on the labels on the bags. Ice water was prepared by filling a plastic container with ice and adding cold tap water. The accurate temperature was measured using thermometer and water prepared in this way was added in bags labeled “Ice-Cold”. Accurate temperature of cold tap water was measured using cold tap water and added in bags labeled “Cool”. In the same way, temperature of hot tap water was measured and added in bags labeled “Warm”. Boiling water was prepared using kettle and mixed with hot tap water in ratio of 1:1. Accurate temperature of the mixture was measured using thermometer and was added in bags labeled “Hot”. Finally, the temperature of water in boiling kettle was measured and added directly into bags labeled “Boiling-Hot”. All Ziploc

bags were slide shut after all its ingredients were added and all the air inside the bag was sucked out using straw. The bags were left for reaction to proceed for one hour and any change observed was recorded.

Approximated change in volume of gas inside the bag was statistically analyzed by one-way ANOVA to see if change in the volume is statistically significant or not. This was determined by looking at p-value. If one-way ANOVA showed that the difference between the temperature groups were significant, Tukey's post hoc test was done to see between which group significant difference was observed.

Results

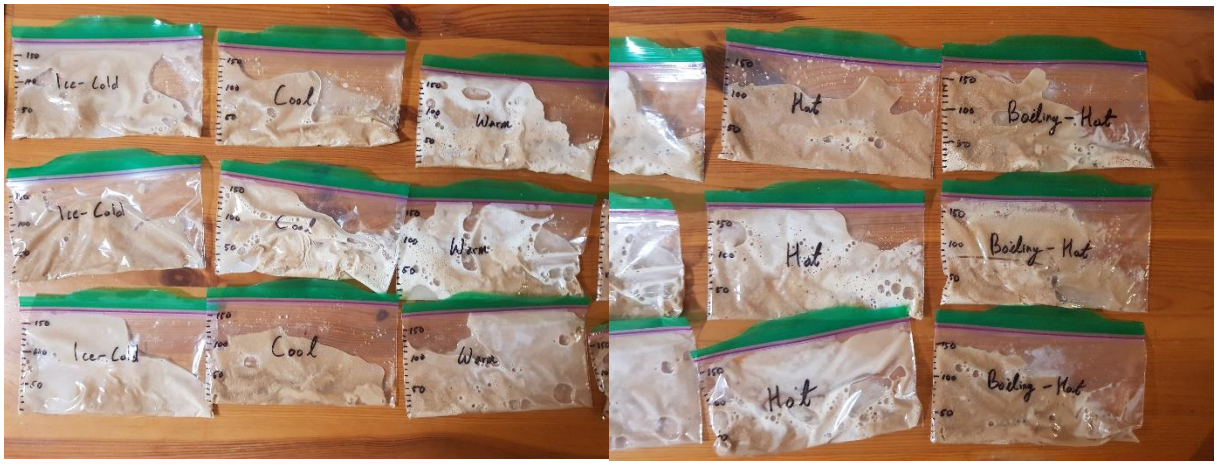

Figure 1. Initial state of Ziploc bags

Figure 2. State of Ziploc bags after 1 hour of reaction

	Bag 1	Bag 2	Bag 3	Average
Ice Cold (1.9 °C)	50 mL	30 mL	70 mL	50 mL
Cool (21.1 °C)	120 mL	80 mL	60 mL	86.67 mL
Warm (43.6 °C)	150 mL	150 mL	140 mL	146.67 mL
Hot (63.3 °C)	0 mL	0 mL	0 mL	0 mL
Boiling-Hot (99.1 °C)	0 mL	0 mL	0 mL	0 mL

Table 1. Collected data on change in gas volume after 1 hour of reaction

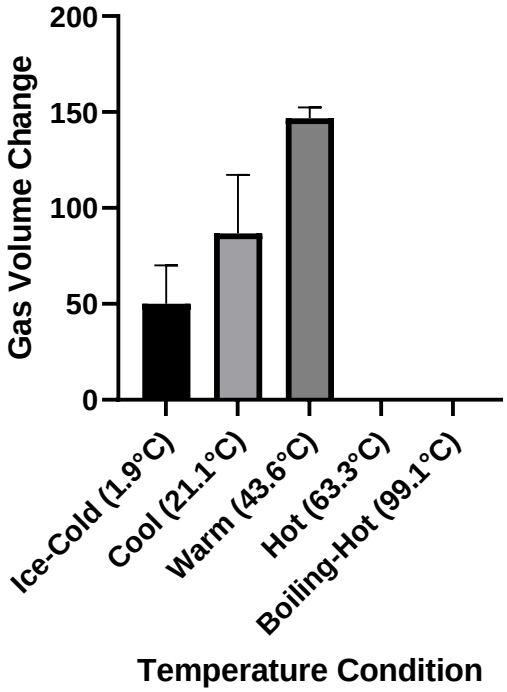

Figure 3. Bar graph showing mean cake volume with SD for five different temperature conditions

The collected data of change in gas volumes in each Ziploc bag was recorded in the GraphPad Prism 8 software. One-way ANOVA was used to analyze the collected data of changes in gas volume for five groups of temperature conditions. Figure 3 shows the mean gas volume changes in a bar graph and the standard deviation in error bar.

ANOVA Summary	
F Value	42.44
P Value	<0.0001
R Squared	0.9444

Table 2. The one-way ANOVA summary showing P value, F and R Squared from analysis of the gas volume changes of Ziploc bags.

Since P-value of one-way ANOVA was less than 0.05, it indicates that there is significant difference among the means of five temperature condition groups. Therefore, Tukey's post hoc test was done to see in between which particular groups the significant difference existed.

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Ice-Cold (1.9°C) vs. Cool (21.1°C)	-36.67	-81.09 to 7.760	No	ns	0.1208
Ice-Cold (1.9°C) vs. Warm (43.6°C)	-96.67	-141.1 to -52.24	Yes	***	0.0002
Ice-Cold (1.9°C) vs. Hot (63.3°C)	50	5.574 to 94.43	Yes	*	0.0263
Ice-Cold (1.9°C) vs. Boiling-Hot (99.1°C)	50	5.574 to 94.43	Yes	*	0.0263
Cool (21.1°C) vs. Warm (43.6°C)	-60	-104.4 to -15.57	Yes	**	0.0085
Cool (21.1°C) vs. Hot (63.3°C)	86.67	42.24 to 131.1	Yes	***	0.0006
Cool (21.1°C) vs. Boiling-Hot (99.1°C)	86.67	42.24 to 131.1	Yes	***	0.0006
Warm (43.6°C) vs. Hot (63.3°C)	146.7	102.2 to 191.1	Yes	****	<0.0001
Warm (43.6°C) vs. Boiling-Hot (99.1°C)	146.7	102.2 to 191.1	Yes	****	<0.0001
Hot (63.3°C) vs. Boiling-Hot (99.1°C)	0	-44.43 to 44.43	No	ns	>0.9999

Table 3. The Tukey's post hoc test summary comparing every temperature group with every other temperature groups.

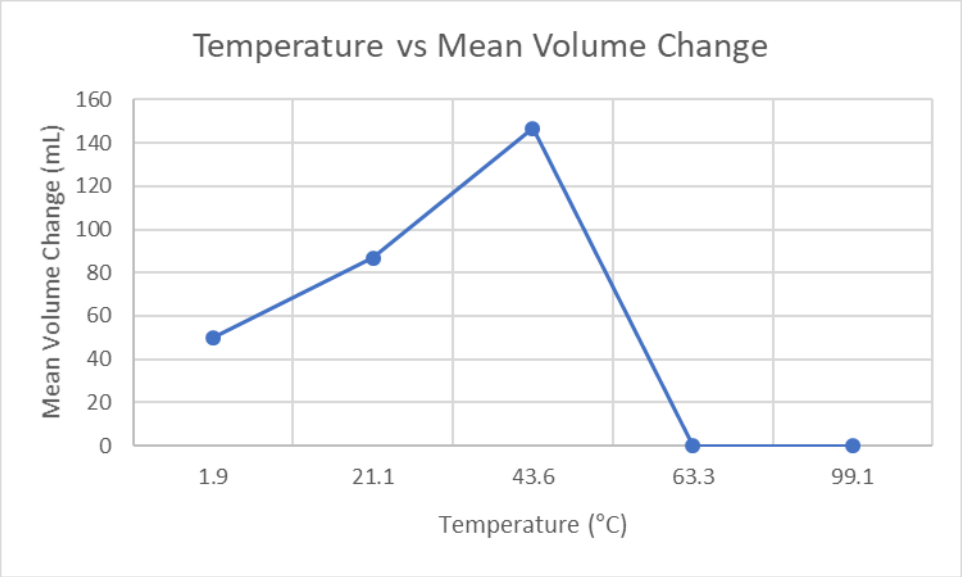

Figure 4. Line graph plotting temperature vs average volume change.

Discussion

According to the results, P value obtained from the ANOVA was found to be less than 0.0001, which is less than 0.05. Therefore, it is possible to reject null hypothesis and support alternative hypothesis, which stated that the volume of CO_2 produced would show difference between different temperature conditions. Consequently, Tukey's post hoc test was done to compare each temperature group with every other temperature groups to see in between which particular groups significant difference lies in. From the Tukey's post hoc test, it was observed that there was no significant difference between "Ice-Cold" (1.9 °C) and "Cool" (21.1 °C) temperature groups nor in between "Hot" (63.3 °C) and "Boiling-Hot" (99.1 °C) groups. Yet, significant difference was observed in between every other groups. From figure 4, it can be noted that there is an increasing trend of gas volume change from temperature 1.9 °C to 43.6 °C, peaks at 43.6 °C and a sudden decrease in between temperature 43.6 °C and 63.3 °C. On temperature ranges greater than 63.3 °C, there seems to be no change in the gas volume at all. From this trend, in can be inferred that the temperature in which enzyme denaturation takes

place lies in this range. This also supports the hypothesis which stated that that as the temperature of the environment that yeasts are in increases, the metabolic activity of the yeasts will increase as well, until temperature is too high that enzymes in yeast start to denature.

The findings made in this research are in congruence with findings in paper of Uden, "Temperature Profiles of Yeasts." (Uden). Uden's paper also state that even though there is varying temperature tolerance range between different yeasts, maximum temperature tolerated was usually around 45 °C (Uden). Some difference lies in the rate in which yeast metabolic activity changes. In Uden's paper, it was observed that the rate of yeast metabolic activity change was in logarithmic scale. In this research, the graph indicates linear increase and decrease in yeast metabolic activity with increasing temperature. This is probably due to limitations in this study.

There were many limitations in this experiment. The first limitation was due to limitation of the equipment. With absence of experimental equipment to accurately measure the volume, the change in the volume of gas could only be approximated. This could have resulted in in accurate volume change to be recorded for analysis. Another limitation lies in the number of replicates for the study. Usually in experiments, more replications lead to more accurate and precise result. Yet, in this experiment, only three repeats on each temperature condition group was prepared. As noted in Uden's paper, various yeasts can have varying ranges of temperature tolerance. Since only three replicates per group was tested, the result is likely to be less accurate and precise. Finally, due to limitations of resources, only five temperature groups were tested. In between each temperature group, about 20 °C of temperature difference exist. This is relatively large range, and since no other temperature was tested in this range, what happens in other temperature points in the range remains unclear in this research. Also, it is difficult to keep temperature condition constant over 1 hour of reaction period. The water used can be cooled down or warmed up in this period.

Even with limitations discussed above, the correlation between the temperature and the yeast metabolic activity was still observed. If this experiment were to be repeated, it would be recommended to use equipment to more accurately measure volume changes and test the effect of temperature over more variety of temperature conditions, with more replications as well.

Conclusion

In conclusion, there were statistically significant difference in volume changes observed between different temperature groups. The significance was observed in between every other groups except in between "Ice-Cold" and "Cool" group and "Hot" and "Boiling-Hot" group. Positive trend was observed in terms of yeast metabolic activity from 1.9 °C to 43.6 °C with decline following in range 43.6 °C to 63.3 °C and no volume changes observed afterwards. This supports the hypothesis which stated which stated that that as the temperature of the environment that yeasts are in increases, the metabolic activity of the yeasts will increase as well, until temperature is too high that enzymes in yeast start to denature. However, given the fact that there were many limitations in this experiment, it would be necessary to repeat this experiment with more accurate measurements, more temperature conditions to be tested and

more replications of each condition for more accurate results.

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