The Effects of Various Caffeine Concentrations on the Carbon Dioxide Production of *Saccharomyces cerevisiae* (Yeast) During Fermentation

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PURPOSE

The objective of this research is to determine the effects of various caffeine concentrations on the amount of carbon dioxide produced by yeast during fermentation. This research aims to understand how caffeine may affect the metabolic rate of cancerous cells. Yeast is a common model organism to human cells. In addition, cancer cell physiology and metabolic fluxes are increasingly similar to those in rapidly fermenting and proliferating yeast. Caffeine is a useful compound in this experiment because it is commonly found in sizable quantities throughout nature.

INTRODUCTION

Caffeine, a natural nervous system stimulant, is commonly found in living organisms such as various fruits, leaves, beans of coffee, cacao, and guarana plants (1). In mammals, caffeine is vasoconstrictive, which causes the narrowing of blood vessels through small muscles in the vessel's walls (12). Caffeine resides in the methylxanthine class, a class of natural bronchodilators that cause the muscles of the lungs and airway to relax (10). Due to caffeine having multiple functions and effects, and is found in many living organisms, this inspired the need to understand how caffeine may affect a metabolic process like fermentation.

Fermentation is the process of converting carbohydrates into ethanol and lactic acids (7). The fermentation process is widespread, used in brewing, baking, the dairy industry, producing vitamin C, fuel production, wastewater treatment, the preservation of food, producing biopolymers, medicines, and providing significant health benefits (7).

Saccharomyces cerevisiae, also known as yeast, is a single-celled organism that is a part of the fungus kingdom, as seen in Figure 3 (6). Yeast uses fermentation to undergo anaerobic respiration, with a standard output being carbon dioxide gas (6). Yeast must be exposed to a type of liquid to hydrate, an example being water, for the fermentation process to occur (3). During fermentation, yeast breaks down the sugar provided by either a liquid or flour into smaller complexes to obtain the energy for growing and multiplying (3). As yeast breaks down sugar, the yeast excretes a liquid that releases carbon dioxide and ethyl alcohol (3).

Yeast contains several enzymes that work under different conditions, including Zymase, Protease, Maltase, and Invertase, which are all present throughout fermentation (2). In this experiment, the yeast used table sugar as its energy source during fermentation. Table sugar is chemically composed of sucrose, a disaccharide consisting of one molecule of glucose and one molecule of fructose, as seen in Figure 4 (4).

Yeast is a model organism because they share many simple biological properties with human cells, such as a nucleus containing DNA packed into chromosomes (11). Most metabolic and cellular processes in humans are also found in yeast (11). For example, cancer cells use glycolysis to activate oncogenes, a gene that transforms normal cells into cancerous cells and depletes tumor suppressors, while yeast uses glycolysis to extract energy from sugar to ferment (11). In addition, yeast cells perform cell division similarly to human cells (13). The relationship between yeast and human cancer cells has been actively studied in recent years. Enzymes involved in lipid metabolism have been found to play a prominent role in cancer cell proliferation, specifically cancers like prostate and breast cancer (5). Most of these enzymes are also found in yeast (9). Above all, cancer cell physiology and metabolic fluxes are increasingly similar to those in rapidly fermenting and proliferating yeast (9). By inhibiting the glycolytic activity of cancerous cells, the progression of tumors and proliferation of cancerous cells is also inhibited (8). It is predicted that as caffeine concentration increases, the amount of CO_2 produced during yeast fermentation will also increase.

H_0 : Differences in the carbon dioxide output of fermenting yeast when exposed to various concentrations of caffeine for ten minutes are due to chance alone.

H_1 : Differences in the carbon dioxide output of fermenting yeast when exposed to various concentrations of caffeine for ten minutes are not due to chance alone.

MATERIALS AND METHODS

The experiment was conducted from February 10th, 2022, through March 3rd, 2022, in the KAMSC Biology room. This room was 71.4 ℉ and consisted of iridescent lights and partly shaded windows.

1. The Vernier software was downloaded to the Windows 10 Surface Pro.
2. One rubber-lined hole on the top of the system container was covered with masking tape.
3. The CO_2 sensor was put into the second rubber-lined hole so that it was facing downwards into the container.
4. Two and a quarter teaspoons of yeast were measured on a scale, using the weigh boat, and set aside.
5. 120 mL of warm water was measured–using a pipette to remove any excess water– then was poured into the 250 mL glass beaker.
6. One half a gram of caffeine powder was measured on a scale using the weigh boat. The caffeine powder was extracted using the scoopula.
7. One teaspoon of table sugar was measured on a scale using the weigh boat.
8. The measured granulated sugar and caffeine were poured into the beaker and stirred with the stirring rod until the granulated sugar and caffeine were dissolved.
9. The Vernier software was opened on the Windows 10 Surface Pro, and the "new experiment" option was selected.
10. The measured yeast was added to the beaker with the dissolved granulated sugar and caffeine. Then, it was stirred three times with the stirring rod.
11. The beaker was quickly placed inside the container within 3 seconds, and the lid was closed with the CO_2 sensor inside the container.
12. After the lid was secured, the "collect data" button was clicked on the top of the Vernier software page on the Windows 10 Surface Pro.
13. The Vernier software was left to run the data collection for 600 seconds (10 minutes).
14. Once the data was collected, the results appeared on a graph. The results were then saved to the Windows 10 Surface Pro for future analysis.
15. Steps 1-14 were repeated for 0 g(control), 1.0 g, 1.5 g, 2.0 g, 2.5 g, and 3.0 g of caffeine. The materials were cleaned and reused for each trial.
16. Steps 1-15 were repeated for a total of three trials of each amount of caffeine. Trial 1 began on February 10, 2022, Trial 2 began on February 13, 2022, and Trial 3 began on February 28, 2022.

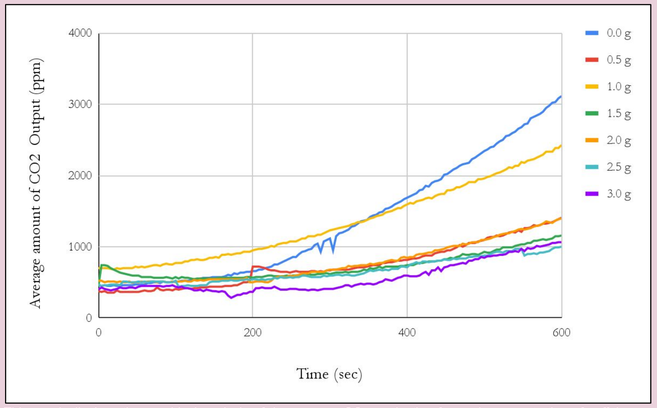
RESULTS

Figure 1 illustrates how the CO_2 output (ppm) decreased as the amount of caffeine added to the yeast, sugar, and warm water solution increased. In all three trials, the overall CO_2 output (ppm) of the yeast decreased for each caffeine concentration compared to the control group, which had 0.0 g of caffeine. As more caffeine was added to the yeast, sugar, and warm water solution, the CO_2 output (ppm) increased at a slower rate.

As seen in Figure 2, the numerical analysis shows that the highest rate of change in CO_2 output (ppm) over 600 seconds occurred in the control group, 0.0 g of caffeine. The lowest rate of change in CO_2 output (ppm) over 600 seconds occurred when 2.5 g of caffeine was added to the yeast, sugar, and warm water solution.

Observations that were made during this experiment include bubbles, smell, color, temperature, and overall volume. When the yeast was added the water and sugar solution became a beige color that was opaque, the solution before yeast was added can be seen in Figure 5. It was observed that for all caffeine concentrations in each trial there was a foam at top of the solution–water, sugar, caffeine, and yeast–in the beaker, as seen in Figure 4. This can also be seen in Figure 6. Although, as caffeine concentration increased, the foam at the top of the beaker seemed to rise less than the beaker with no caffeine, the control. Occasionally, a bubble could be seen rising from the bottom of the beaker where some of the yeast had settled. It was also observed that the beaker had only a mild smell at the beginning of each run, but after reacting in the system container for 10 minutes the solution in the beaker had a very strong smell. In addition, the beaker felt slightly warmer after 10 minutes in the system container than it did when it was placed in the system container. However, as the amount of caffeine added to the beaker increased, the cooler it felt after each 10 minute period.

1 *Average Amount of CO_2 Output (ppm) From Yeast in Various Caffeine Concentrations Over Time*



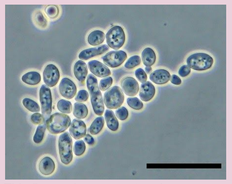
This graph displays the graphical analysis of the average CO_2 production for each concentration of caffeine. Parts per million (ppm) is the amount of CO_2 in a million molecules of air.

2 *Average Rate of Change and Cumulative Change of CO_2 Output During Yeast Fermentation*

Amount of Caffeine	0.0 g	0.5	1.0	1.5	2.0	2.5	3.0
Average Rate of Change (ppm per sec)	5.1899	2.3450	4.0446	1.9285	2.3270	1.6679	1.7728
Cumulative Change (Final - Initial)	2653.6	1029.9	1727.4	717.79	873.56	529.61	666.30

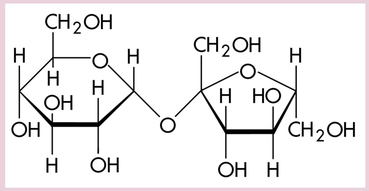
This table gives numerical values of the rate of change and the cumulative change for each of the caffeine concentrations. These values were calculated from the data given by the Vernier probe.

3 *Microscopic Yeast*



This figure shows yeast under a microscope at 400x magnification.

4 *Molecular Makeup of Sucrose*



This figure shows a molecule of sucrose at the molecular level.

4 *Resulting Solution*

This shows the solution of water, sugar, and yeast after a ten minute period (no caffeine added).

5 *Starting Solution*

This shows the solution of water and sugar before the yeast was added.

6 *Solution During*

This shows the solution of water, sugar, and yeast during the ten minute trial.

DISCUSSION

The primary purpose of this experiment was to determine the differences in the carbon dioxide output of fermenting yeast when exposed to various concentrations of caffeine. The results demonstrated that the null hypothesis was rejected. As the amount of caffeine increased, the amount of CO_2 produced decreased. This was concluded because the slope of the lines representing groups with caffeine in Figure 1 is less than it was for the control group; the incline represents the rate of CO_2 production. In addition, the difference between the final and initial concentration of CO_2 in the control was greater than the final and initial concentration of CO_2 in the experimental group with 3.0 g of caffeine as seen in Table 1. Furthermore, as seen in the top row of Table 1, the rate of CO_2 lowered as the amount of caffeine increased. These results conclude that caffeine slows the production of CO_2 production in yeast fermentation.

As with any experiment, errors affected the results of this experiment. One of these errors is the caffeine did not always dissolve completely into the mixture of water and granulated sugar. This error should be resolved in the future by warming the water more and allowing sufficient time for the caffeine and sugar to dissolve completely. Another error is that the amount of yeast was not consistent for all trials. This error occurred because we did not have a correct size measuring spoon; the ¼ of the 2 ¼ teaspoons was approximately half of a half teaspoon. In the future, this can be fixed by measuring out 2 ¼ teaspoons of yeast and finding the mass, keeping the mass consistent. A third error in this experiment was that our system container was not completely air-tight. An air-tight container fit for the CO_2 sensor should be used in the future. A fourth error that affected the results is that when the stirring rod was unintentionally removed from the beaker after stirring in the yeast. This error should be minimized in the future by pouring the water, caffeine, and sugar solution over the yeast and swirling the beaker instead of pouring the yeast into the solution and stirring.

The data that was collected inspires future research and other questions as well. Future research could include more trials of what has been done, along with more caffeine concentrations. Future research could also try to answer how caffeine may inhibit other processes in yeast. This research shows that caffeine affects CO_2 emissions in yeast; how it does that still needs to be researched and if it affects the human body the same way. Different processes in yeast and the caffeine's effect on those processes would also need to be researched further. Additionally, this same setup could be tested with pH levels and at various temperatures to determine whether caffeine still reduces CO_2 production or affects the production of CO_2 as much.

Both the graphical and numerical results of this experiment show that an increase of caffeine causes a decrease in CO_2 production. Considering that yeast is a model organism of a human cell with similar metabolic processes and molecules, it can be assumed that caffeine may have a similar effect on cancerous cells. In a system containing cancerous cells, caffeine will decrease the metabolic rate of the cancerous cells through inhibiting the cancerous cells proliferation rate.

REFERENCES

1. *Caffeine*. The Nutrition Source. (2020, November 12). Retrieved March 2, 2022, from https://www.hsph.harvard.edu/nutritionsource/caffeine/#:~:text=Caffeine%20is%20naturally%20found%20in,added%20to%20beverages%20and%20supplements
2. Committee, T. B. C. C. A. (2015, October 24). *Enzymes*. Understanding Ingredients for the Canadian Baker. Retrieved February 8, 2022, from https://opentextbc.ca/ingredients/chapter/enzymes/
3. CraftyBaking. (n.d.). *Yeast fermentation: How does yeast fermentation work?: Crafty baking: Formerly Baking911: CraftyBaking: Formerly Baking911*. CraftyBaking. Retrieved February 7, 2022, from https://www.craftybaking.com/how-baking-works/yeast#
4. Encyclopedia Britannica, inc. (n.d.). *Sucrose*. Encyclopædia Britannica. Retrieved February 10, 2022, from https://www.britannica.com/science/sucrose
5. Fernández, L. P., Gómez de Cedrón, M., & Ramírez de Molina, A. (1AD, January 1). *Alterations of lipid metabolism in cancer: Implications in prognosis and treatment*. Frontiers. Retrieved March 3, 2022, from https://www.frontiersin.org/articles/10.3389/fonc.2020.577420/full#:~:text=Many%20enzymes%20implicated%20in%20de,formation%20and%20transformation%20(51).
6. Hagman, A., & Piškur, J. (2015, January 24). *A study on the fundamental mechanism and the evolutionary driving forces behind aerobic fermentation in yeast*. PloS one. Retrieved December 6, 2021, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4305316/
7. Jahangir, M. (2020, April 17). *10 reasons why is fermentation important to human*. Microbial Facts. Retrieved February 2, 2022, from https://www.microbialfacts.com/applications-of-fermentation/
8. Jang, M., Kim, S. S., & Lee, J. (2013, October 4). *Cancer cell metabolism: Implications for therapeutic targets*. Nature News. Retrieved March 3, 2022, from https://www.nature.com/articles/emm201385
9. Natter, K., & Kohlwein, S. D. (2013, February). *Yeast and cancer cells - common principles in lipid metabolism*. Biochimica et biophysica acta. Retrieved March 3, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3549488/#:~:text=Enzymes%20involved%20in%20lipid%20metabolism,fermenting%20and%20rapidly%20proliferating%20yeast.
10. NHS. (n.d.). *Bronchodilators*. NHS choices. Retrieved February 4, 2022, from https://www.nhs.uk/conditions/bronchodilators/#:~:text=Bronchodilators%20are%20a%20type%20of,by%20inflammation%20of%20the%20airways
11. Nielsen, J. (2009, October 23). *Systems biology of lipid metabolism: From yeast to human*. FEBS Letters. Retrieved March 3, 2022, from https://www.sciencedirect.com/science/article/pii/S0014579309008424#bib22
12. U.S. National Library of Medicine. (n.d.). *Vasoconstriction: Medlineplus medical encyclopedia*. MedlinePlus. Retrieved February 4, 2022, from https://medlineplus.gov/ency/article/002338.htm#:~:text=Vasoconstriction%20is%20the%20narrowing%20
13. Wellcome Genome Campus. Public Engagement Team. (2021, July 21). *Using yeast in biology*. Stories. Retrieved March 3, 2022, from https://www.yourgenome.org/stories/using-yeast-in-biology

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