The Effects of Water Temperature on CO_2 Production of *Saccharomyces Cerevisiae*

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ABSTRACT

Saccharomyces cerevisiae, also known as yeast, is a unicellular organism used in different practices such as baking and brewing beer. This experiment investigated the effects of water temperature on the growth of *S. cerevisiae*. In this study, four different temperature points were used to find out approximately what temperature is the optimal growth temperature for *S. cerevisiae*; the four temperature points were 10ºC, 20ºC, 27ºC and 35ºC. In order to find how temperature affects the growth of *S. cerevisiae,* balloons were used to trap the CO_2 produced as the products of yeast fermentation. Following a one-way ANOVA test, it was found that the p-value was less than 0.0001. Thus, the results of the experiment were significant, and there was a difference in the mean volume of balloons at the different treatment temperatures. Furthermore, the Dunnett post hoc test revealed that the differences in the mean volume of balloons occurred between the control group (20ºC) and each treatment temperature. The yeast had the most CO_2 production at 35℃, meaning that the yeast had the most growth in this temperature, making it the optimal growth temperature for *S. cerevisiae*.

INTRODUCTION

Yeasts are unicellular eukaryotic organisms. They are used in everyday practices such as baking, brewing beer, as well as scientific research (EFFCA, n.d.). Thus, it is essential to investigate the optimal growth temperature for yeast in order to use yeast effectively and accurately in such practices. The purpose of this experiment is to examine how different temperatures affect the activity of *S. cerevisiae*, commonly known as baker's yeast which is a form of active dry yeast, as measured by CO_2 production. To achieve this, four different temperatures will be used to measure yeast activity: 10ºC, 20ºC, 27ºC and 35ºC. Since *S. cerevisiae* is used mainly during baking bread and is the slowest leavening agent compared to baking powder and baking soda, it is crucial to find the optimal growth temperature for baking yeast to aid bakers to save time during the leavening process (Mill, 2018).

The process where yeast is activated with sugar and water is called fermentation, and within this process, yeast produces CO_2 (Science Buddies, 2014). The chemical reaction is as follows: $C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$ (Food Crumbles, 2016). For this experiment, since the activation of yeast produces CO_2, which is a gas, it can be collected in a balloon in which we can measure its volume as it expands, to quantify yeast activity and growth. For example, a larger volume balloon would indicate that more CO_2 was produced and, therefore, more yeast growth has occurred.

Different types of yeast have varying optimal growth conditions. Although, in general, yeasts optimally grow in a temperature range of 25℃ to 30℃, we would like to examine if the optimal growth temperature of active dry yeast falls within this range (Bullerman, 2003). Since yeast has the ability to grow within a large range of temperatures (0℃ to 50℃), we also wanted to test if active dry yeast continues to grow outside the suggested optimal growth temperatures, and whether these temperatures have any effect on yeast activity and growth (Bullerman, 2003). We chose to use active dry yeast for this experiment as it is a common ingredient in baking and finding the ideal temperature for yeast growth would be helpful knowledge to have for bakers to reach the optimal level of leavening for their products.

We hypothesize that if water temperature affects CO_2 production and, consequently, yeast's growth rate, then yeast at a temperature of 27℃ will result in the most CO_2 production (measured as balloon volume) since 25ºC - 30ºC is the optimal temperature range for yeast growth. We expect that the yeast activity and CO_2 production will be the lowest at 10ºC since yeast does not activate at low temperatures (Mill, 2018). In addition, we expect that at 35ºC, CO_2 production will be lower than at 27ºC since 35ºC is beyond the optimal range for yeast; as a

result, the yeast may not be able to continue fermenting due to the stress of higher than normal temperatures (Mill, 2018).

METHODS AND MATERIALS

To measure the CO_2 production of the yeast, we used balloons. With the balloons, we stretched them out by blowing them up repeatedly and then deflated them and set them aside. This allowed for the balloons to expand more easily if necessary during the experiment. We filled a plastic container, a quarter full with water. This was used as a water bath for our plastic water bottles to maintain a constant temperature during the experiment. We kept iced water and a kettle of hot water on hand to maintain the desired temperature of the water bath.

To prepare our yeast samples, we poured one packet of active dry yeast (~7g) into a 350mL plastic water bottle, along with a teaspoon of granulated sugar. We then added one cup of 20℃ water into the bottle. We gently mixed the bottle and quickly covered the top of the bottle with a balloon. We placed the bottle into the water bath and adjusted the temperature of the water bath to match the temperature of the water in the bottle, which was 20℃. Every 5-10 minutes, we checked the water bath temperature with a thermometer to ensure the temperature was 20℃ +/- 2℃. If the temperature was not within this range, we added hot or cold water to adjust.

After 90 minutes, we recorded the diameter of the spherical portion of the balloon using two rulers placed on either side of the balloon and then measured the distance between the two rulers (Figure 1). We repeated these steps two more times for this temperature, and we used this as the control temperature. We repeated these steps for a water temperature of 10℃, 27℃, and 35℃, where the water bath temperature corresponded to the water temperature of the water placed into the water bottles accordingly. There were a total of three people conducting this

experiment, with two group members having a total of 3 replicates for each temperature and with one group member having one replicate for each temperature. We repeated the entire experiment, so there were a total of two trials. Overall there were seven replicates (n=7) for each treatment group. Figure 2 is an example of how the balloons looked after 90 minutes.

To obtain the final volume of each balloon, we used the formula for the volume of a sphere ($V= 4/3\pi r^2$) added to the volume of a cylinder ($V= \pi r^2 h$). This is because the base of the balloons connected to the bottle was a cylindrical shape and the top of the balloons were a sphere. The geometry and shape of the balloons are demonstrated in Figure 2.

Statistical tests used to analyze the data were a one-way ANOVA test. The post hoc test performed was a Dunnett test and was used to compare the means between the control mean with the mean volume of the other temperatures. To perform our statistical analysis, we used the software GraphPad Prism.

Figure 1: This figure shows how the diameter of the spherical part of the balloon was measured. The black and red lines depict rulers. The red line between the two black lines was how the diameter of the balloon was measured. This figure also depicts the cylindrical base or neck of the balloon.

A) 

B) 

C) 

D) 

Figure 2: The above pictures are of 1 replicate of the balloons at each temperature after 90 minutes. A) is of the balloon at the control temperature 20℃. B) is of the balloon at 10℃. C) is of the balloon at 27℃. D) is of the balloon at 35℃.

RESULTS

As mentioned, our statistical analysis was performed using Prism 9 on GraphPad. Our data followed an approximately normal distribution without any transformations applied. The

one-way ANOVA test obtained a p-value < 0.0001. The alpha level we used was 0.05, as we believed that a 5% risk of denoting a difference between the volumes of the balloons at the tested temperatures when a difference does not exist was appropriate for this experiment. A bar graph showing the mean volume of CO_2 production at each temperature, with 95% confidence interval bars was also created (Figure 3). Since there is no overlap of the 95% confidence interval bars and our obtained p-value is less than our alpha value, the difference in the means of the volumes of the balloons at different temperatures are statistically significant.

A Dunnett post hoc test was performed as we wanted to compare the means of our treatment temperatures to our control temperature. The Dunnett post hoc test revealed a statistically significant difference at an alpha level of 0.05 between the control mean volume (20℃) and the mean volume of the balloons at the temperatures of 10℃, 27℃ and 35℃.

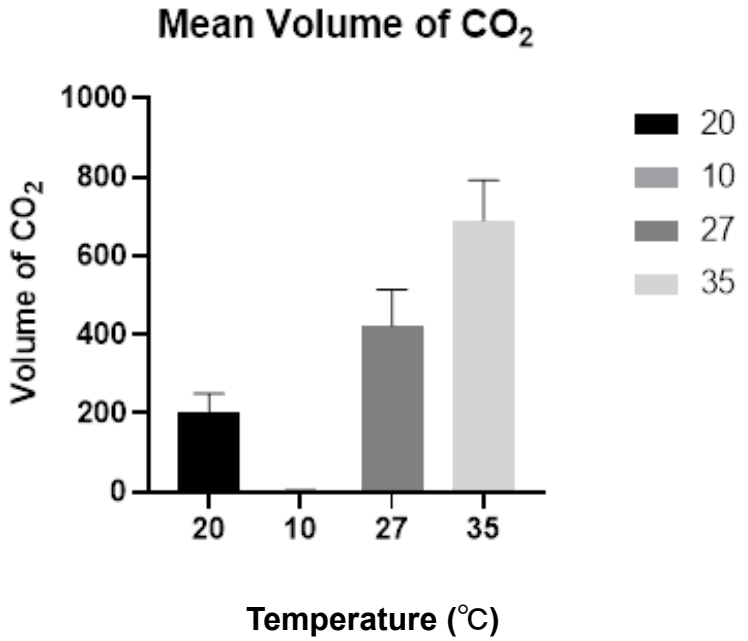

Figure 3: The graph above shows the mean volume of the balloons filled with CO_2 at 20℃ (control), 10℃ 27℃ and 35℃ with n=14 for each temperature. The error bars represent the 95%

confidence interval for each mean. The mean volume of the balloons at 20℃ was 202.1 cm^3, at 10℃ it was 0 cm^3, at 27℃ it was 423.1 cm^3 and at 35℃ it was 691.02 cm^3.

DISCUSSION

The main purpose of this experiment was to determine if water temperature had an effect on yeast growth. After running an ANOVA test, we found the p-value to be < 0.0001. Since the p-value is less than the alpha level of 0.05, we could conclude that our results were statistically significant. As a result, we can reject the null hypothesis (H_0) that the volume of CO_2 produced by *S. cerevisiae* was the same at each temperature. In addition, we can accept the alternative hypothesis (H_A) that the volume of CO_2 produced from *S. cerevisiae* was different at each temperature. Further, in order to determine where this significance lies within our data, we ran a Dunnett post hoc test. This allowed us to determine that the difference lied between the control mean volume (20℃) and the mean volume of the balloons at the temperatures of 10℃, 27℃ and 35℃. In simpler terms, the means at all the treatment temperatures are significantly different than the control mean.

Generally, the optimal temperature for *S. cerevisiae* growth falls within a temperature range of 25℃ to 30℃ (Bullerman, 2003). As a result, we predicted that *S. cerevisiae* will have optimal growth within this temperature range, and specifically at our tested temperature of 27℃. Through this experiment, however, our results differed from this prediction. In this experiment, we found that the optimal growth of *S. Cerevisiae* occurred at the temperature of 35℃, rather than at 27℃, which was outside of this optimal temperature range. This unexpected result could be due to temperature fluctuations over the observed 90 minutes. Although the temperature was checked every 5 minutes, it was difficult to maintain a constant temperature of 35℃ throughout

the entire experiment and for each replicate. As a result, during the experiment, the temperature of the water bath decreased and occasionally dropped close to the optimal range of yeast growth discussed earlier, specifically within 1 - 2 degrees of 30℃, which was the upper limit of this range. Considering this, these temperature fluctuations could be a potential explanation of this result.

On the other hand, through this experiment, we found little to no yeast growth in the treatment group with a temperature of 10℃. Further, this treatment group overall had the lowest volume of CO_2 produced. This result was expected as the temperature of 10℃ falls well below the optimal temperature range. In addition, at this temperature of 10℃ is when yeast metabolism is slowed (Biernacki, 2020). As a result, this slows down the *S. cerevisiae* fermentation process and thus slows down the production of carbon dioxide, which is a product of fermentation, as a result (Maicas, 2020).

Limitations and sources of error

One of the limitations of this experiment was that we could not measure the temperature of the water that was poured into the water bottle. Although we had a water bath, it was unclear if the water bath was able to maintain a constant temperature for the yeast in the bottle. Another limitation of this experiment was the brand of active dry yeast used. While the type of yeast used for each trial was active dry yeast, we were limited in the brands that were available to us. Thus, the two brands that were used in this experiment were Red Star and Fleischmans. Although both brands share the similarity that both were active dry yeast, these different brands can have differences in the possible ingredients they contain, which may affect yeast growth. Nonetheless, in order to account for this variable in our experiment, each trial utilized the same brand of active

dry yeast in order to minimize any confounding variables associated with the use of these different brands.

One of the potential sources of error was the different temperature fluctuations for each replicate at each temperature. Although the environment was kept constant during each trial, there were different degrees of temperature fluctuations during this experiment that we were unable to fully control. As a result, this also could have played a role in the small differences of the resulting volume measured in our replicates at each temperature.

Further research

Since yeast growth, which is shown by the volume of CO_2 produced, was greatest at the temperature of 35℃, further research can be done to test how water temperatures greater than 35℃ affect the growth of *S. Cerevisiae*. Where yeast activity and growth can be tested at varying temperatures in order to determine the optimal temperature of growth. Further, since yeast is important in creating a light and spongy texture in dough during the general baking process, due to its production of CO_2, this knowledge can aid in the enhancement of baking as a result of determining optimal CO_2 production of *S. Cerevisiae* (Cote, n.d.; Baking Industry Research Trust, n.d.).

To extend our experimental design further, we can observe the rate of change in the volume of CO_2 produced by yeast fermentation through the measurement of the volume of the balloon at set time intervals. Further, we can also extend the total time for which we observe the growth of *S. Cerevisiae* at each temperature. This can help determine the optimal time for maximum yeast growth as well as how long the growth phase of *S. Cerevisiae* can be at different temperatures.

CONCLUSION

In this experiment, we assessed the effects of temperature on the production of CO_2 by fermented active dry yeast. Our results concluded that there was a statistically significant difference between the mean volumes at each temperature. A Dunnett post-hoc test revealed that the difference between the mean volumes is between the control volume mean and each treatment temperature. Considering our results found that the greatest volume of CO_2 produced was obtained from the treatment group with a temperature of 35℃, further research can be done in order to determine the optimal temperature for *S. Cerevisiae* growth.

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APPENDIX A: RAW DATA

A.1: Group Member 1

TRIAL 1

	Temperature (°C)											
	20°C (CONTROL)			**10°C**			**27°C**			**35°C**		
Time (mins)	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
90	Sphere: D = 6.5cm Cylinder: D = 1.5cm H = 4.5cm	Sphere: D = 8.3cm Cylinder: D = 1.5cm H = 4.5cm	Sphere: D = 7.2cm Cylinder: D = 1.5cm H = 4.5cm	Sphere: D = 0 cm Cylinder: D = 0 cm H = 0 cm	Sphere: D = 0 cm Cylinder: D = 1.5cm H = 3.5cm	Sphere: D = 0 cm Cylinder: D = 1.5cm H = 4.5cm	Sphere: D=10.5cm Cylinder: D = 1.5cm H = 4.5cm	Sphere: D = 10cm Cylinder: D = 1.5cm H = 4.5cm	Sphere: D=10.7cm Cylinder: D = 1.5cm H = 4.5cm	Sphere: D=11.8cm Cylinder: D = 1.5cm H = 4.5cm	Sphere: D=11.3cm Cylinder: D = 1.5cm H = 4.5cm	Sphere: D=11.9cm Cylinder: D = 1.5cm H = 4.5cm

TRIAL 2

	Temperature (°C)											
	20°C (CONTROL)			**10°C**			**27°C**			**35°C**		
Time (mins)	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
90	Sphere: D = 7.5cm Cylinder: D = 1.5cm H = 4.5cm	Sphere: D = 8cm Cylinder: D = 1.5cm H = 4.5cm	Sphere: D = 7cm Cylinder: D = 1.5cm H = 4.5cm	Sphere: D = 0 cm Cylinder: D = 1.5cm H = 4cm	Sphere: D = 0 cm Cylinder: D = 1.5cm H = 4.5cm	Sphere: D = 0 cm Cylinder: D = 1.5cm H = 4.5cm	Sphere: D = 10cm Cylinder: D = 1.5cm H = 4.5cm	Sphere: D = 9.5cm Cylinder: D = 1.5cm H = 4.5cm	Sphere: D=10.5cm Cylinder: D = 1.5cm H = 4.5cm	Sphere: D = 12cm Cylinder: D = 1.5cm H = 4.5cm	Sphere: D=11.5cm Cylinder: D = 1.5cm H = 4.5cm	Sphere: D=12.2cm Cylinder: D = 1.5cm H = 4.5cm

A.2: Group Member 2

Trial 1

	Temperature (°C)											
	20°C (CONTROL)			**10°C**			**27°C**			**35°C**		
Time (mins)	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
90	Sphere: D = 7 cm Cylinder: D = 1.5 cm H = 4.6 cm	Sphere: D = 6 cm Cylinder: D = 1.4 cm H = 4.8 cm	Sphere: D = 6.4 cm Cylinder: D = 1.5 cm H = 4 cm	Sphere: D = 0 cm Cylinder: D = 0 cm H = 0 cm	Sphere: D = 0 cm Cylinder: D = 0 cm H = 0 cm	Sphere: D = 0 cm Cylinder: D = 0 cm H = 0 cm	Sphere: D = 8.6 cm Cylinder: D = 1.6 cm H = 4 cm	Sphere: D = 8.6 cm Cylinder: D = 1 cm H = 3 cm	Sphere: D = 8.4 cm Cylinder: D = 1.1 cm H = 1.3 cm	Sphere: D = 9.3 cm Cylinder: D = 1.5 cm H = 2.3	Sphere: D = 10.5 cm Cylinder: D = 1.7 cm H = 2.8 cm	Sphere: D =10 cm Cylinder: D = 1.4 cm H = 3 cm

Trial 2

	Temperature (°C)											
	20°C (CONTROL)			**10°C**			**27°C**			**35°C**		
Time (mins)	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
90	Sphere: D = 6.4 cm Cylinder: D = 1.5 cm H = 4.4 cm	Sphere: D = 6.2 cm Cylinder: D = 1.3 cm H = 3.4 cm	Sphere: D = 6 cm Cylinder: D = - H = -	Sphere: D = 0 cm Cylinder: D = 0 cm H = 0 cm	Sphere: D = 0 cm Cylinder: D = 0 cm H = 0 cm	Sphere: D = 0 cm Cylinder: D = 0 cm H = 0 cm	Sphere: D = 8.3 cm Cylinder: D = 1.5 cm H = 3.5 cm	Sphere: D =8 cm Cylinder: D = 1.5 cm H = 3.5 cm	Sphere: D =8.4 cm Cylinder: D = 1.7 cm H = 3.6 cm	Sphere: D = 9.8 cm Cylinder: D = 4 cm H = 1.4 cm	Sphere: D = 10.6 cm Cylinder: D = 4 cm H = 1.4 cm	Sphere: D = 9.8 cm Cylinder: D = 4 cm H = 1.4 cm

A.3 Group Member 3

Trial 1

	Temperature (°C)											
	20°C (CONTROL)			10°C			27°C			35°C		
	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
90	Sphere: D = 8.8cm Cylinder: D = 1.4cm H = 5.1cm	Sphere: D = N/A Cylinder: D = N/A H = N/A	Sphere: D = N/A Cylinder: D = N/A H = N/A	Sphere: D = 0cm Cylinder: D = 0cm H = 0cm	Sphere: D = N/A Cylinder: D = N/A H = N/A	Sphere: D = N/A Cylinder: D = N/A H = N/A	Sphere: D = 6.6cm Cylinder: D = 1.4cm H = 5.1cm	Sphere: D = N/A Cylinder: D = N/A H = N/A	Sphere: D = N/A Cylinder: D = N/A H = N/A	Sphere: D = 10.7cm Cylinder: D = 1.4cm H = 5.1cm	Sphere: D = N/A Cylinder: D = N/A H = N/A	Sphere: D = N/A Cylinder: D = N/A H = N/A

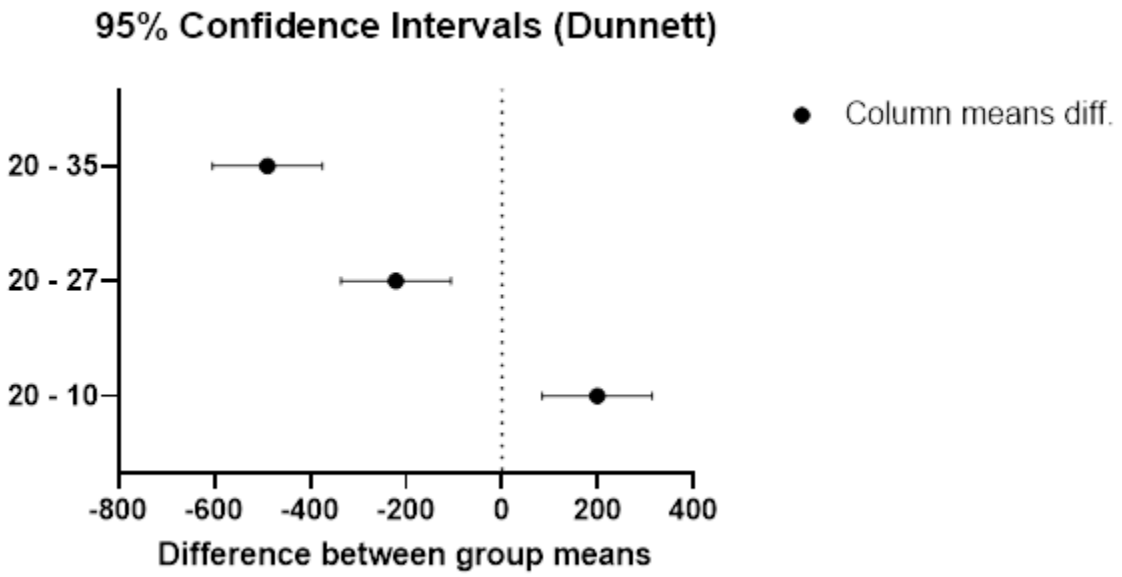
Trial 2

	Temperature (°C)											
	20°C (CONTROL)			10°C			27°C			35°C		
Time (mins)	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
90	Sphere: D = 8.5cm Cylinder: D = 1.4cm H = 5.1cm	Sphere: D = N/A Cylinder: D = N/A H = N/A	Sphere: D = N/A Cylinder: D = N/A H = N/A	Sphere: D = 0cm Cylinder: D = 0cm H = 0cm	Sphere: D = N/A Cylinder: D = N/A H = N/A	Sphere: D = N/A Cylinder: D = N/A H = N/A	Sphere: D = 10.1cm Cylinder: D = 1.4cm H = 5.1cm	Sphere: D = N/A Cylinder: D = N/A H = N/A	Sphere: D = N/A Cylinder: D = N/A H = N/A	Sphere: D = 10.6cm Cylinder: D = 1.4cm H = 5.1cm	Sphere: D = N/A Cylinder: D = N/A H = N/A	Sphere: D = N/A Cylinder: D = N/A H = N/A

APPENDIX B: FINAL BALLOON VOLUMES

20 ℃ Final Volume (cm^3)	10℃ Final Volume (cm^3)	27℃ Final Volume (cm^3)	35℃ Final Volume (cm^3)
145	0	276.1	498.9
129.3	0	335.4	629.8
113.1	0	311.57	498.9
142	0	294.9	425
114.8	0	274.3	612.5
144.3	0	313.4	528.2
151.8	0	614.1	868.3
307.4	0	531.6	763.5
203.4	7.9	649.4	890.1
361	6.2	160	912
228.9	7.9	560	804.3
276.05	7.1	531.6	958.8
187.6	7.9	456.9	653
325	7.9	614.1	631

APPENDIX C: DUNNETT POST-HOC TEST



Appendix C: A Dunnett post hoc test was performed to compare the means of the control temperature (20℃) with each treatment temperature; this figure shows the difference between those means. From left to right on the graph the difference of means between the control and the treatment temperatures are: -488.9 (between 20℃ and 35 ℃), -221.0 (between 20℃ and 27℃), and 198.9 (between 20℃ and 10℃). Since none of the 95% confidence intervals of the three points on the graph contain zero, the means of the balloon volumes at 10℃, 27℃ and 35℃ are all significantly different from the control mean.