Does the optimum temperature for the rate of reaction of the enzyme catalase, as measured by oxygen production, vary between plant and animal cells, when comparing: leek, potato, chicken liver, pork liver and fish liver; and does it make a difference if the animal is homeothermic or poikilothermic?

> Biology Extended Essay Candidate Session Number: Candidate Name: Word count: 3967

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# **Acknowledgements**

I would firstly like to thank my supervisor \_\_\_\_\_\_\_\_\_\_\_ for providing me with the best support and guidance. I am also very appreciative of the help that our lab technician, \_\_\_\_\_\_\_\_\_\_\_, gave me during my experiment. My mother also contributed by purchasing the sources of catalase that was required for my experiment. 

## **Abstract**

The research question for this essay is: Does the optimum temperature for the rate of reaction of the enzyme catalase, as measured by oxygen production, vary between plant and animal cells, when comparing: leek, potato, chicken liver, pork liver and fish liver; and does it make a difference if the animal is homeothermic or poikilothermic? This investigation is carried out through a comparison of optimum temperatures by an experiment where the enzyme catalase breaks down hydrogen peroxide. Catalase is an enzyme that breaks down hydrogen peroxide, a toxic substance in cells, into water and oxygen. The rate of reaction of each source of catalase at a range of temperatures is calculated using the volume of oxygen produced in one minute. This rate of reaction shows the point at which the enzyme reaches its optimum temperature before denaturing.

Sources of catalase being compared are; potato, leek, chicken liver, pork liver, and fish liver. Pork and chicken are homeothermic animals that differ greatly from fish liver, a poikilothermic animal.

The experiment illustrated a fairly clear relationship between the catalase source body temperature or atmosphere temperature and the optimum temperatures calculated. Pork and chicken resulted in the highest optimum temperatures and fish liver catalase had the lowest optimum temperature. The optimum temperature for catalase does vary between plant and animal cells when taking into consideration homeothermic and poikilothermic animals. Homeothermic animals have a more definite optimum temperature for catalase because their body temperature is constant. Poikilothermic animals have an indefinite optimum temperature for catalase because of their varying body temperatures. However, the plant catalase was inconclusive. The graphs of my results show the exact production of oxygen and depict the optimum temperature of each source. Although this experiment did not result in an exact optimum temperature for each source of catalase tested.

Word count: 299

## **Introduction**

The research question for this essay is:

Does the optimum temperature for the rate of reaction of the enzyme catalase, as measured by oxygen production, vary between plant and animal cells, when comparing: leek, potato, chicken liver, pork liver and fish liver; and does it make a difference if the animal is homeothermic or poikilothermic?

My investigation will include determining the optimum temperature of catalase from experimental results and research using the sources of catalase listed above. 

Most chemical reactions have an energy barrier that must be overcome before the reaction can take place. This energy is the energy of activation. Catalysts are used to lower the activation energy needed so that reactions can occur more easily. Enzymes are catalysts that are specific to one substrate. A substrate and an enzyme form a complex that requires less energy for the reaction. This compound quickly breaks down to form reaction products. ("Enzyme")

Up to a point the rate of enzyme reaction increases as the temperature increases due to the collision theory. It increases up to a certain point and then the enzyme is denatured, this point is the optimum temperature.

I will be testing the enzyme catalase, as it is present in all eukaryotic cells in the peroxisomes. Catalase is an enzyme that catalyzes the reaction by which hydrogen peroxide is broken down to produce water and oxygen. ("Catalase") Peroxisomes are organelles bound by a single membrane present in eukaryotic cells. They are responsible for various metabolic reactions and these reactions produce hydrogen peroxide  $(H_2O_2)$  as a byproduct. Peroxisomes in the liver break down toxins such as alcohol and other harmful compounds by transferring hydrogen from the toxins to oxygen. "The  $H_2O_2$  formed by peroxisome metabolism is itself toxic", (Campbell and Reece 111) but peroxisomes contain catalase to break down this toxic substance.

The decomposition of hydrogen peroxide by catalase to produce water and oxygen is shown by the equation below.

$$
2H_2O_2 \rightarrow 2H_2O + O_2
$$

("How does catalase break")

As catalysts, all enzymes have an influence on the rate of reactions. "A set of reactants in the presence of an enzyme will form product(s) at a faster rate than without the enzyme." (Damon  $67$ ) In my investigation I will be focusing on the optimum temperature of catalase. This must be tested by using the rate of reaction at different temperatures. As temperature increases, the rate of reaction also increases. This optimum temperature can vary depending on the roll of the enzyme and the source of the enzyme.

Plants and animals all contain catalase but are adapted to various temperatures. Plants grow in the ground, which has a much lower average temperature than the body temperature of animals. Leek and potato are two species, which are grown in cold weather climates.

I am testing two mammal sources of catalase, homeotherms, and one fish source, poikilothermic. The main difference between poikilotherms and homeotherms is that homeotherms have warm blood that maintains a set temperature and poikilotherms change depending on environment. The metabolisms of poikilotherms are ever changing and the metabolisms of homeotherms are usually the same. (Youngbergco2011wp)

The liver of a chicken, pig and fish will be used, as this is the organ in animals that contains the highest concentration of catalase. Leek and potato will be used as the plant species.

The rate of reaction can be measured to determine at which temperature the catalase carries out the reaction most efficiently. Rate can be calculated by:

> amount of reactant used or amount of product formed rate of reaction  $=$ time taken

> > ("Rates of Reaction")

In the decomposition of hydrogen peroxide by catalase the products formed are oxygen gas and water. The product formed in the rate equation will be the volume of oxygen produced in the reaction within 60 seconds.

My hypothesis is that the optimum temperature for catalase rate of reaction will be higher in the chicken and pig liver than in the potato, leek and fish sources of catalase. This is because the environment by which the catalase is adapted to, determines the optimum temperature of that catalase. Since fish is a poikilothermic animal the metabolism varies depending on the environment and therefore the catalase will function at various temperatures. Fish liver will be lower than the mammal catalase optimum temperature because water temperatures are never as high as body temperature. Chicken and pig are homeothermic animals that regulate their body temperature naturally and will therefore have a constant temperature of metabolic activity.

I chose this topic out of curiosity for the subject. After conducting several enzyme labs in the classroom I wanted to take these further and create a larger experiment and from there it grew into an Extended Essay topic.

## **Investigation**

### **Variables**

Independent: Source of catalase - will include chicken liver, pork liver, fish liver, potato and leek. These sources of catalase must be prepared by blending and straining.

Dependent: Optimum temperature for catalase activity, this will be measured by finding the rate of reaction for each temperature. The highest rate of enzyme activity will signify the optimum temperature for that source of catalase, measured by the production of  $O_2$ .



#### Controlled variables:



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### **Materials**

Below is a list of apparatus and materials needed for 5 trials with one source of catalase. All amounts of material were the same for each test.



## **Method**

#### *Method Part 1 - Preparing the catalase solutions*

- 1. With each of the liver types, add 80g of liver with 20ml of distilled water.
- 2. With each of the plant types, add 80g of the plant with 20ml of distilled water.
- 3. Blend the water and catalase source together with a hand blender until there are no lumps
- 4. Strain the fluid with cheesecloth.
- 5. Put the catalase solutions in plastic sealed containers and label them.
- 6. Store the catalase solutions in the freezer. The very low temperatures do not affect catalase solutions.

#### *Method Part 2*

- 1. Pour the entire mixture of the first source of catalase into a  $100 \text{cm}^3$  glass beaker.
- 2. Place this beaker in the first water bath of  $30^{\circ}$ C and place a thermometer and a pipette in the beaker.
- 3. With the second 100cm<sup>3</sup> beaker pour approximately 60ml of 20vol  $H_2O_2$ (hydrogen peroxide)
- 4. Place this beaker in the same water bath as the catalase solution  $(30°C)$ , and place a thermometer and a pipette in this beaker
- 5. Monitor the beakers in the water bath until both the hydrogen peroxide and the catalase solution are at the same correct temperature.
- 6. While the solutions are getting to temperature set up one boiling tube with a delivery tube and a plug in a boiling tube rack.
- 7. Connect the delivery tube to the gas syringe and ensure it is a tight seal.
- 8. Press the end fitting all the way into the glass barrel to empty the gas syringe ready for the trial.
- 9. Place a boiling tube stopper and the stop clock directly next to the boiling tube.
- 10. When the solutions are at the correct temperature stir them and then measure, using the pipettes and the two  $5 \text{cm}^3$  measuring cylinders, 1ml of the catalase solution into one measuring cylinder and  $2ml$  of  $H_2O_2$  into the second measuring cylinder.
- 11. After the solutions have been removed from the water baths, transfer them quickly to minimize temperature change.
- 12. First pour the  $1 \text{cm}^3$  of catalase solution into the boiling tube attached to the gas syringe.
- 13. Then add the  $2cm^3$  of  $H_2O_2$  to the same boiling tube and immediately after, plug the boiling tube using the stopper with one hand and start the stop clock with the other hand.
- 14. Repeat the steps above using the water baths at 10, 20, 30, 40, 50, and 60 $^{\circ}$ C, using a fresh set of equipment. Use ice and cold water in a large plastic tub for the 10°C water bath.
- 15. Repeat steps 1-13 using the catalase solutions prepared in method 1, leek, potato, chicken liver, fish liver and pork liver. With each catalase source repeat the experiment 5 times at each temperature. Use a fresh set of glassware for each trial.

Each catalase source at each temperature is repeated to allow the collection of sufficient and relevant data.

### **Uncertainties**

There are several apparatus uncertainties that must be taken into account for the method of the experiment.



#### **Results**

In this section, the results of my investigation are shown in both raw data and processed data forms. The raw data values, volume of oxygen produced in each trial, can be seen in *Appendix: 1* 

#### **Observations**

#### *Leek:*

For this source of catalase there was a gradual increase in oxygen produced throughout the 60 seconds. Some amount of effervescence was formed, very bright green color. 

#### *Potato:*

After the solution was freshly blended and filtered there was sediment that would settle at the bottom of the container. The liquid part of the solution was a light brown color. The potato produced the least amount of effervescence *Chicken liver:*

This was a vigorous reaction. The gas produced increased rapidly in the first 10 seconds and then stayed constant for the remainder of the time. At temperatures, such as  $40^{\circ}$ C – 60°C the gas would begin to retract by about 1ml. This could have been due to the change in temperature as the gas cooled off. At similar high temperatures the liver solution would begin to solidify. *Pork liver:*

This had a more gradual increase of gas produced throughout the 60 seconds. This could be due to lesser concentration of catalase in the solution. Other observations were the same as chicken liver at higher temperatures.

#### *Mackerel liver:*

This had a very rapid increase in the oxygen produced. The maximum amount was reached in the first ten seconds of combining the hydrogen peroxide and the catalase solution. Effervescence was shown and it was a yellow/brown color. The fish liver was much smoother and had fewer clumps than the mammal liver sources of catalase.

## **Processed data**

(see example calculations in Appendix 2.)

Table showing the average volume of oxygen produced in one minute at each temperature for each of the catalase sources.



The table below shows the rate of reaction at each temperature for each source. The red highlighted cells indicate the highest rate that was reached. The highest rate is what determines the optimum temperature.



Below is a graph comparing the rates of the four different catalase sources. The optimum temperature can be observed where the highest point of the line is.





Graphs showing the average volume of oxygen produced at each temperature for each source of catalase.



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(all graphs generated by excel)

## **Discussion and Reasoned Argument**

As stated before, the research question for this essay is:

Does the optimum temperature for the rate of reaction of the enzyme catalase, as measured by oxygen production, vary between plant and animal cells, when comparing: leek, potato, chicken liver, pork liver and fish liver; and does it make a difference if the animal is homeothermic or poikilothermic?

"Up to a point, the rate of an enzymatic reaction increases with increasing temperature, partly because substrates collide with active sites more frequently when the molecules move rapidly."(Campbell and Reece 154) The point to which the rate of an enzymatic reaction increases is the optimum temperature for the enzyme.

There is a point on each graph where the rate of reaction reaches a maximum point and then begins to decrease with the denaturation of the enzyme. Denaturation is caused by the active site changing shape due to the high temperatures and not allowing the substrate to bind with the enzyme therefore decreasing the rate of reaction. Therefore the sources of catalase with a lower optimum temperature, their catalase active site changes shape more easily.



The relationship between rate of reaction of enzymes and temperature is shown in the graph to the left. "Chemists have a rule of thumb that a  $10^{\circ}$ C increase in temperature gives a doubling of the reaction rate. This rule is loosely derived from the Arrhenius equation." (Oswald) This is depicted in my results when comparing the graphs above. The potato and leek show the most noticeable rate curves and the optimal temperature can be seen when the rate drops significantly at a certain temperature. The chicken liver and pork liver show the general trend of the increase

### (Oswald)

in rate, following the Arrhenius equation, but the complete denaturation of the enzyme cannot be as easily observed because of the temperatures that were used. Fish liver had a complete opposite curve, as the rate of reaction decreases from 10 to 20 degrees and then stays constant until the denaturation of the enzyme at 50 degrees is shown.

Fish liver showed the most unique curve compared to the other sources of catalase. There has been research to prove that living organisms "sustain relatively similar rates of metabolic activity at widely different temperatures" (Somero [abstract]). This is due to the adaptation or acclimation of enzymes, which has allowed them to operate normally at different temperatures where they would

normally be denatured if not adapted. This evidence supports the idea of poikilothermic – "the internal body temperature varies according to the ambient temperature" ("Learn About Animal Adaptations") and therefore affects the metabolic rate of the fish. Since the Mackerel fish, which I used in my experiment, are found in a wide range of depths and water temperatures ranging from  $6^{\circ}$ C-20 $^{\circ}$ C (Bigelow and Schroeder) their poikilothermic characteristics cause their metabolic reactions to stay constant as the environmental temperature fluctuates. This can be seen, as the rate of reaction does not fluctuate between 20 and 50 degrees. Since the highest rate of enzymatic reaction was at  $10^{\circ}$ C it would be useful to conduct a further experiment at lower temperatures. The fish liver catalase denatured at  $60^{\circ}$ C and there was almost no oxygen produced. This was the only source where the oxygen produced became almost zero.

All other sources of catalase increased in rate of reaction as temperature increased. This is because of the factor of temperature increasing the kinetic energy of the molecules, causing more collisions and therefore more substrates to bind with the enzyme.

Chicken has an average body temperature of  $40.6-41.7^{\circ}$ C. "Birds" are homeothermic – they produce and dissipate heat to maintain a relatively constant body temperature. The internal body temperature of birds shows more variability than mammals, and therefore there is no absolute body temperature" ("Poultry Production Manual"). This fluctuation of temperatures in birds is why the rate of catalase reaction did not increase or decrease by great amounts when temperatures varied. When comparing the chicken liver and the pork liver, the chicken liver had one increase from 10 to 20 $^{\circ}$ C and then stayed moderately stable until denaturation, with only slight increase at  $50^{\circ}$ C; whereas the pork liver had a gradual increase in rate of reaction and did not stay constant at any point. The chicken liver depicts a straighter line in the volume of oxygen produced and the pork liver depicts a curved line before the enzyme is denatured.

Pigs have an average body temperature of 38.7-40°C ("Temperature and humidity index"). Both the literature values for body temperature and my findings of optimum temperature for catalase show that the chicken has a higher temperature than the pig. This trend was also shown in my data when comparing the optimum temperatures of both these sources of catalase. Although my investigation does not allow for the precise optimum temperature to be found the general trends can be shown in comparing the different sources.

The leek source of catalase has the largest difference in rate after the denaturing of the enzyme. The liver types and potato showed a more gradual change in rate. This trend on the graph is most likely due to the amounts of catalase that were present in the solution. Tables in *Appendix*: 1 show that the volume of oxygen produced for animal species and leek were the highest compared to the very low volumes of oxygen produced by the potato. To determine if this is true I would need to determine the amount of active enzyme in the sample. This oxygen difference could be due to the potato being a storage organ containing a high level of starch and leek being a leaf source of catalase. The plant sources generally have a lower optimum temperature because plants do not produce a body temperature like

mammals do. Although potato had the same optimum temperature as pork liver so plant catalase is inconclusive.

## **Conclusion**

From the graphs above and the calculations of averages I have deduced the following optimum temperatures for each source of catalase.

Pork liver catalase has an optimum temperature of  $40^{\circ}$ C Chicken liver catalase has an optimum temperature of  $50^{\circ}$ C Fish liver catalase has an optimum temperature of  $10^{\circ}$ C Leek catalase has an optimum temperature of  $30^{\circ}$ C Potato catalase has an optimum temperature of  $40^{\circ}$ C

The data shows that an increase in temperature causes an increase in rate of reaction in all tested sources of catalase except for fish liver cells because of the significantly lower body temperature and poikilothermic characteristics.

My hypothesis was supported to an extent, by my findings. I predicted that the chicken and pork liver catalase would have a higher optimum temperature than the plant and fish liver catalases and that the optimum temperatures would match the general temperature of the environment that the catalase is adapted to. It is shown by the trends on the graph that the optimum temperature of chicken liver was significantly higher than all other sources of catalase and the leek catalase was the lowest optimum temperature. The optimum temperature of  $40^{\circ}$ C for the potato did not support my hypothesis as I expected this value to be much lower. However, the potato produced the smallest volume of oxygen, which suggests a very low concentration of catalase.

In conclusion I have found that the source of catalase does affect the optimum temperature of the catalase rate of reaction. Previous studies show that "catalase activity of toad liver (the cold blooded animal) has its optimum at  $15^{\circ}$ C, but that of cow liver (the warm blooded animal) at  $40^{\circ}$ C." (Mitsuda and Yasumatsu 201) This study also tested if these optimum temperatures were dependent on enzyme purity but they found that these optimum temperatures of catalases were independent of enzyme purity. (Mitsuda and Yasumatsu 200) Both, my findings and findings in other research show that the optimum temperatures are similar to the animal's body temperature or in the case of fish, a poikilothermic animal, similar to its atmosphere. I have determined that there is a difference in optimum temperature depending if the catalase animal source is homeothermic or poikilothermic. 

This investigation could be taken further comparing more species of plants and animals and specifically determining the reason for such a high optimum temperature for potato that was skewed by the very low amount of oxygen produced. The comparison of more cold-blooded animals could improve this investigation. The optimum temperatures could also become more precise if more temperatures with smaller intervals were tested. And the unknown amount of enzyme in the sample was a big problem in determining the optimum temperature.

## **Evaluation**

There are many limitations and errors that have had an impact on my results and conclusions. This includes both random and systematic errors.

The purity and concentration of catalase is one major effecting factor because of the very clear difference in the amount of oxygen produced with each catalase source. In the mammal liver there was generally more oxygen produced than the potato and fish liver. The leek cells had a very high volume of oxygen and therefore contained more catalase per gram. This doesn't dramatically affect the optimum temperature found because of a study by Mitsuda, that enzyme purity is independent of its optimum temperature. It is more likely that not all of the catalase will be denatured at high temperatures if the solution is larger and not heated thoroughly. This cannot be improved easily, but catalase can be extracted from the sources with great precision. My experiment would have concluded more precisely of I had used sources of catalase that would react to produce similar volumes of oxygen. 

The original sources and storage time of the plants and liver was unknown in my experiment and this could have caused denaturation of the enzyme as some of the liver was frozen thawed and refrozen several times, while other sources of liver and plants were fresh. This was not kept constant for each of the sources and it is an unknown factor affecting the precision of my results. This could be improved by keeping all sources consistent and buying them fresh and local.

I could have improved the accuracy of my results and averages by increasing the number of trials. I could have also tested several different livers and plants of the same species to ensure that it was not just a fault in one source.

I only tested a range of 6 temperatures, which limited my accuracy in determining the optimum temperature. I could have improved my conclusion by testing several more temperatures by making the intervals smaller, for example intervals of  $5^{\circ}$ C rather then  $10^{\circ}$ C.

One of the greatest errors in my experiment was the unknown concentration of enzyme because my catalase solutions were not purified or crystalized. The concentration of catalase I was using from each source varied because of the other substances present in the tissues. It was clear from the results that the liver had the highest concentrations of catalase. This is difficult to improve without sophisticated equipment, although it is possible to crystalize catalase in a pure form and would have led me to more exact optimum temperatures.

This evaluation has helped me to determine if my conclusion is reliable or not while investigating the optimum temperature for the rate of reaction of the enzyme catalase between plant and animal cells, when comparing: leek, potato, chicken liver, pork liver and fish liver; and the difference between homeothermic or poikilothermic animals. 

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# **Appendices**

## **Appendix 1**

## *Volume of oxygen produced in reaction between catalase from different cells and H<sub>2</sub>O<sub>2</sub>*



### Potato



#### Chicken Liver



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## Pork Liver



### Mackerel Liver



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### **Appendix 2**

**Calculation for average volume of oxygen produced at each temperature** 

$$
Average = \frac{sum\ of\ 5\ trials\ (ml)}{5}
$$

*Example*:

$$
Average = \frac{26 + 24 + 25 + 22 + 23 ml}{5}
$$

 $Average = 24 ml$ 

The values for the average gas produced at each temperature with each source of catalase are shown in Table 2.1 on page 8.

*Calculation for the rate of reaction at each temperature for each source of catalase* 

 $Rate = \frac{volume\ of\ oxygen\ produced}{\angle Q}$ 60

*Example:* 

$$
Rate = \frac{26.8 \text{ml}}{60 \text{s}}
$$

 $Rate = 0.447$  (ml of oxygen/minute)