

Research Question: How does temperature affect the rate of browning (enzymatic) in apple discs immersed in sucrose solution.

## Introduction

Enzymatic browning is a biochemical process in which plant (fruit or vegetable) tissues take on a brown color when exposed to oxygen. This experiment was conducted to test the affect temperature on the rate of browning of a *Malus domestica* or more commonly known as the Fuji apple, immersed in sucrose solution. The aim of the investigation was to see how the rate of browning is increased or decreased at different temperature as well as exploring the reasons behind such occurrences. Additionally, there are several to why everyone tries to prevent the process of browning as this browning cannot only make the food look unappealing, soften it and cause it to lose flavor, but it can also severely limit the shelf life of the product decreasing its value. <sup>1</sup>Some foods can benefit from enzymatic browning. Foods that have a more desirable flavor or color as a result of enzymatic browning include cocoa, tea, coffee, raisins, and prunes however others foods mainly fruits such as apples (*Malus*), bananas (*Musa acuminata*) and pears (*pyrus communis*) have an undesirable colour and flavor as a result of browning. This experiment looks at the scientific theory behind browning and the biological benefits to this specific process.

## Design

**\*Independent Variable: The Temperature of the sucrose solutions**

**\*Dependent Variable: The rate of browning of the apple discs**

Controlled Variables	Process that validates the control of this variable
Size of the apples discs	Completed using a cork borer with a thickness of 5mm
Concentration of Sucrose (stock solution)	Refilling the beaker with the bottle labeled 10% Sucrose Solution
Volume of Sucrose Solution	Pour 7ml of sucrose solution into each test tube using a 10ml measuring cylinder $\pm$ 0.2ml
Duration of test tubes in each water bath	Keeping a stopwatch by each water bath as the test tubes must only be in the bath for 17 minutes
Volume of Solution in each cuvette	Using a 10ml measuring cylinder 5ml of solution will be poured into each cuvette
Number of samples per temperature	The number of sample will be constant, 5 test tubes for test temperature shall be used. In order for me to calculate an appropriate mean and to calculate the standard deviation (this will improve my accuracy)

<sup>1</sup> [fairfld61.files.wordpress.com/2010/08/bsaac1-6-enzymatic-browning.ppt](http://fairfld61.files.wordpress.com/2010/08/bsaac1-6-enzymatic-browning.ppt)

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Independent and Dependent Variables:

\*The independent variable will be monitored with thermostatic water baths & the dependent variable will be monitored by the colorimeter.

Data collection range:

Remember that the range of data that needs to be collected would be five samples from five different temperatures ranging from relatively warm to hot. The temperature of the water baths are as follows : 30°C, 40°C, 50°C, 60°C, 70°C  $\pm$  0.1°C. This range has been chosen as this range will be able to show at which temperature, the rate has sped up or slowed down and at which temperature the enzyme has denatured.

**Null Hypothesis:** The rate of browning is not increased by an increase in the temperature in which it is place.

### Hypothesis:

When fruits or vegetables are peeled or cut, enzymes contained in the plant cells are released. The presence of oxygen from the air, the enzyme polyphenolase causes the conversion of phenolic compounds to melanins (brown pigment). Polyphenoloxidases, in plants, are usually found in the chloroplasts, although they can be released from here ripening. The enzymes contain copper at their active site. This metal ion enables them to oxidize the phenolic group.<sup>2</sup>

Moreover, the substrate for the enzyme is polyphenols and it is a phenolic compound. The reaction that occurs as a result of the presence of oxygen is called enzymatic browning and it occurs at warm temperatures when the pH is between 5.0 and 7.0. <sup>3</sup>The reduction in browning is dependent on the type of substance and its concentration, soaking in water is all that is needed however, sucrose solution in this case was more desirable. There is a general correlation between the temperature and the rate of browning: by increasing the temperature of the sucrose solution in which apple discs are placed, the rate of browning will increase until the polyphenol oxidase is inactivated. Therefore, I predict that the test tubes that will be placed in the water bath of 70 °C, will the apple discs with the fastest rate of browning. Additionally, when the solution is placed into the colorimeter, the absorbance percentage will be higher than the other discs of other temperatures. From this, it will also be presumed that the discs in the water bath of 30°C will have the least rate of browning and so the absorbance will not be as high due to the fact that not much browning has actually taken place.

### Apparatus Required:

#### Preparation

- |                          |                                |
|--------------------------|--------------------------------|
| - Three test tube racks  | - 10 ml Measuring Cylinder     |
| - Twenty-Five test tubes | - Sucrose Solution in a beaker |
| - One apple              | - Stop watch                   |
| - Cork Borer             | - Labels                       |
| - Ruler                  | - Pencil                       |
| - Knife                  |                                |
| - Pipette                |                                |

<sup>2</sup> Polyphenol Oxidase Causes Browning of Fruits and Vegetables

<http://www.suite101.com/content/polyphenol-oxidase-causes-browning-of-fruits-and-vegetable-a243450#ixzz1E8GdAmFO>

<sup>3</sup> [www.curriculumsupport.education.nsw.gov.au/.../apple\\_browning.pdf](http://www.curriculumsupport.education.nsw.gov.au/.../apple_browning.pdf)

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- Tile

### Retrieving the Juice

- Filter Paper
- Another set of Twenty-Five test tubes
- Crucible set ( with a pestol)
- Twenty- Five cuvettes
- Three Stands
- Pipette
- Measuring Cylinder
- Colorimetre
- 

### Method

- 1) Take one apple
- 2) Collect 25 cuvettes and 3 bases to use for colorimeter (5ml in each one)
- 3) Then collect 25 test-tubes and three test tube rack
- 4) Using a 10ml measuring cylinder pour in 7 ml of sucrose solution (10%)  $\pm$  0.2ml into each test tube then label each with the appropriate temperature
- 5) Remember there are 5 test tubes for each temperature
- 6) The temperature of the water baths are as follows: 30°C, 40°C, 50°C, 60°C, 70°C  $\pm$  0.1°C.
- 7) Measure the diameter of the cork borer (uncertainty should be 1mm)
- 8) Place the apple on the tile and using a knife peel off some of the skin
- 9) Use the cork borer to cut out a cylinder of the apple then use a ruler (uncertainty at  $\pm$ 1mm) and scalpel to cut cylinder into 25 discs of 5 mm in thickness.
- 10) Place one disc into one test tube
- 11) Ready the stop watches, and place them by each water bath
- 12) After putting the test tubes into water baths, start the stop watches, wait and time for 17 minutes
- 13) While waiting, ready 25 more test tubes, and put filter paper in each test tube
- 14) After 17 minutes have passed, one by one decant the bathing liquid from each and every test tube into the crucible and crush the disc. After one disc has been crushed put it into the filter paper and wait for the solution to drip into the test tube. This should be completed for every sample for each temperature.
- 15) Once a reasonable amount of solution is available (this may take some time) the next step can be undertaken
- 16) Using a pipette and a measuring cylinder collect 5ml  $\pm$  0.2ml and pour the solution into the cuvettes. Remember to label cuvettes, so you are able to identify the sample number and the temperature this is very important as if the cuvettes are not labeled this will lead to inaccuracies.
- 17) Then check for the absorbency and the transmittance. Remember to find the right wavelength for the solution for the best results and always remember to calibrate the colorimeter with a cuvette filled with distilled water before starting as this ensures accurate results. The wavelength used in this experiment was 430nm
- 18) Draw up a table for the data in order to record your data ( $\pm$  2 s.f.)

### Justification of apparatus

Preparation

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- 25 test tubes are needed as there are five temperatures and I need five sample, the same goes for the 25 cuvettes for the colorimeter
- The cork borer and ruler are used as they improve accuracy. Using them ensure that all the discs are of the same size.
- I do not need much solution and so using a small 10 ml measuring cylinder rather than a large one means a small uncertainty to deal with. Labels and stopwatches are also necessary and improve the accuracy as well.

#### Retrieving the Juice

- The colorimeter shows us the absorption percentage
- Filter paper is needed to remove solid bits of the solution
- The crucible is used instead of a blender as my discs were much too small to blend, crushing them using a crucible and pestle would be more efficient and work better. This way I was easily able to filter.

### Data Collection and Processing

#### Qualitative data

##### Description of the physical state of the solution

30 °C	40°C	50°C	60°C	70°C
Extremely Pale Yellow almost clear	Slightly Pale Yellow	Very yellow	Yellow	Very Pale Yellow almost clear

From this we can derive that as the temperature increases, the colour of the solution becomes more yellow until the temperature of 50°C, however after this temperature the colour of the solution become slightly clear or in other words the yellowness of the solution starts to decrease.

#### Quantitative data

This table shows the percentage absorption of the 5 samples for each of the temperature measured using a colorimeter. The wavelength was 430nm as this the wavelength at this I would get the most accurate results.

Percentage Absorption 2 s.f. ( $\pm 0.001\%$ )					
Temperature $\pm 0.1$ °C	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
30 °C	0.300	0.292	0.296	0.274	0.302
40°C	0.281	0.338	0.383	0.356	0.300
50 °C	0.387	0.345	0.390	0.400	0.501
60°C	0.285	0.301	0.309	0.297	0.315
70 °C	0.055	0.040	0.031	0.036	0.039

The anomalies are highlighted in the colour red. The reason to the arrival of anomalies would most likely be that when the crushing of the disc was taking place, some crushed more than others, however the arrival of the identified anomalies are evaluated in more depth in the Conclusion and Evaluation. I have concluded these two results to be anomalies because

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though they are within the range of the data collected, they are not the expected results as they are slightly off. In general, the qualitative data correlates with quantitative data.

### Mean of Temperatures calculated with anomalies

Temperature $\pm 0.1$ °C	Mean (2 s.f.)
30 °C	0.293
40°C	0.332
50 °C	0.405
60°C	0.301
70 °C	0.040

Calculation of Standard deviation

$$s = \sqrt{\frac{\sum(x - \bar{x})^2}{N - 1}}$$

Where

S= Standard Deviation

$x$  = Each value in the sample

$\bar{x}$  = Mean of all the Values

N= Number of Samples

### Standard Deviation calculated with anomalies

Temperature $\pm 0.1$ °C	Standard Deviation (2 s.f.)
30 °C	0.011
40°C	0.041
50 °C	0.058
60°C	0.012
70 °C	0.009

### The Graph for Average Percentage Absorption for Apple discs at each Temperature (with anomalies)

To calculate the error bars for standard deviation and use them in the graph below, we must do the following:

Apply standard deviation, using the follow formula:

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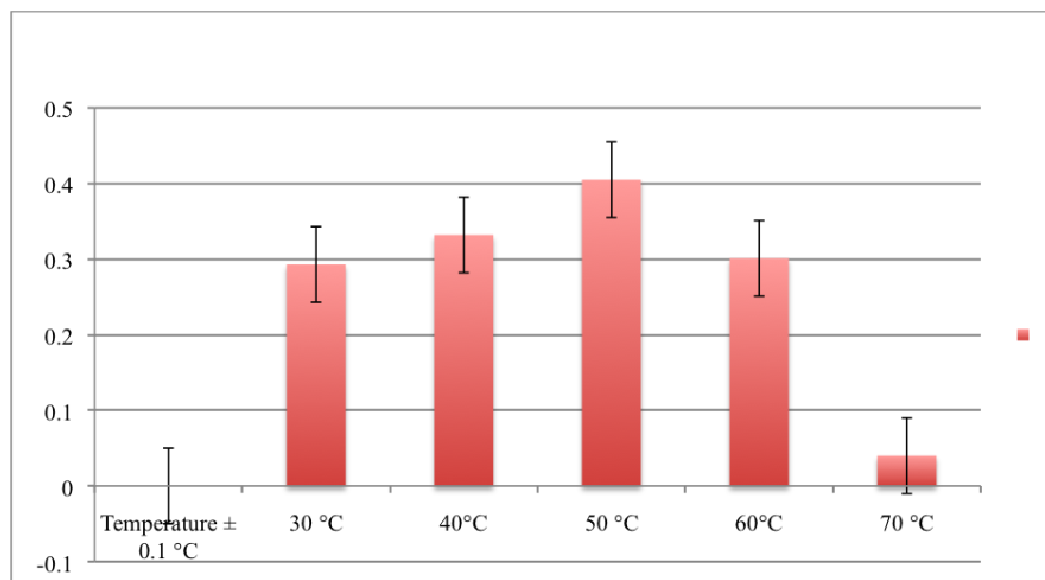
$$S.D. = \sqrt{\frac{\sum_{s=1}^m \sum_{i=1}^n (y_{is} - M)^2}{(n_y - 1)}}$$

$$M = \frac{\sum_{s=1}^m \sum_{i=1}^n y_{is}}{n_y}$$

4

s = series number □ I = point number in series s □ m = number of series for point y in chart  
□ n = number of points in each series □  $y_{is}$  = data value of series s and the I<sup>th</sup> point □  $n_y$  = total number of data values in all series □ M = arithmetic mean.

The advantage of using such error bars is that because they show how the data are spread. Range error bars encompass the lowest and highest values. In this case, we shall be able to see whether a single result fits within the normal range. We shall also be able to make inferences from the data (i.e., to make a judgment whether the rate at different temperatures are significantly different, or whether the differences might just be due to random change or chance).



This graph shows the rate increased greatly from 40°C to 50°C and that this would be the optimum temperature for the increase of the rate of browning as after this 50°C, the rate slowly starts to decrease but there is significant decrease in rate from 60°C to 70°C. The standard deviations here show that there is a large spread of data and shows us the difference between the estimated mean and the true value. Ninety-five percentage of the values lie within  $\pm 0.02$  of the Standard deviation and this is true for my graph. The temperature 60°C has the error bar of the shortest length and thus it has a standard deviation which means that the data points are considered close to the expected value. However the temperature 70°C seems to have the error of the longest length and thus has a high standard deviation which indicates a broad range of possible values relative to the expected value.

<sup>4</sup> Excel workbook formula

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### Mean of Temperature without anomalies

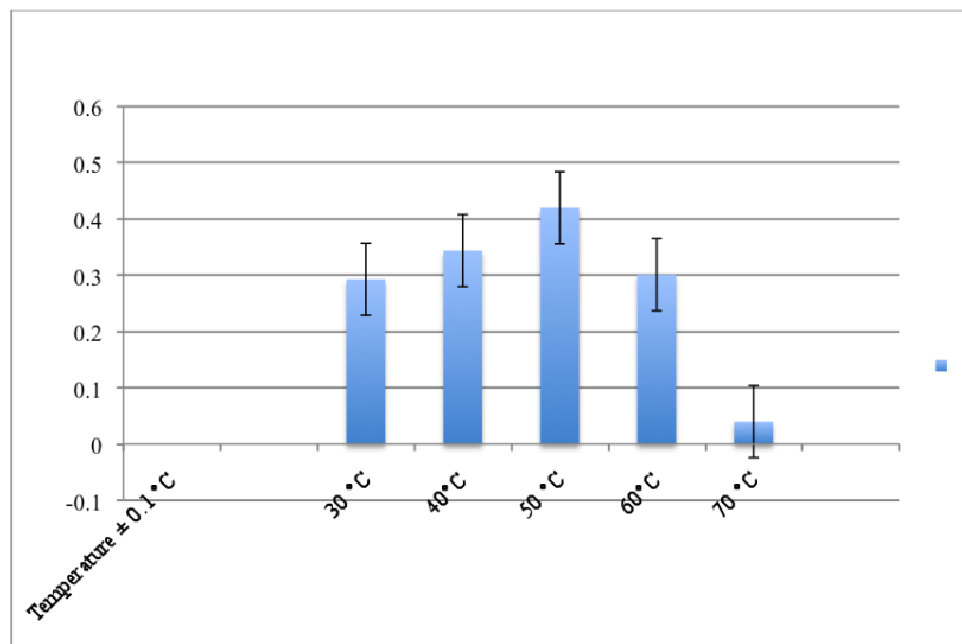
Temperature $\pm 0.1$ °C	Mean (2 s.f.)
30 °C	0.293
40°C	0.344
50 °C	0.420
60°C	0.301
70 °C	0.040

### Standard Deviation without anomalies

Temperature $\pm 0.1$ °C	Standard Deviation (2 s.f.)
30 °C	0.011
40°C	0.035
50 °C	0.055
60°C	0.012
70 °C	0.009

The values in orange are values that have been reduced as an a result of excluding and ignoring the anomalies. They differ from the mean and standard deviation of the data that includes the anomalies.

### The Graph for Average Percentage Absorption for Apple discs at each Temperature (without anomalies)

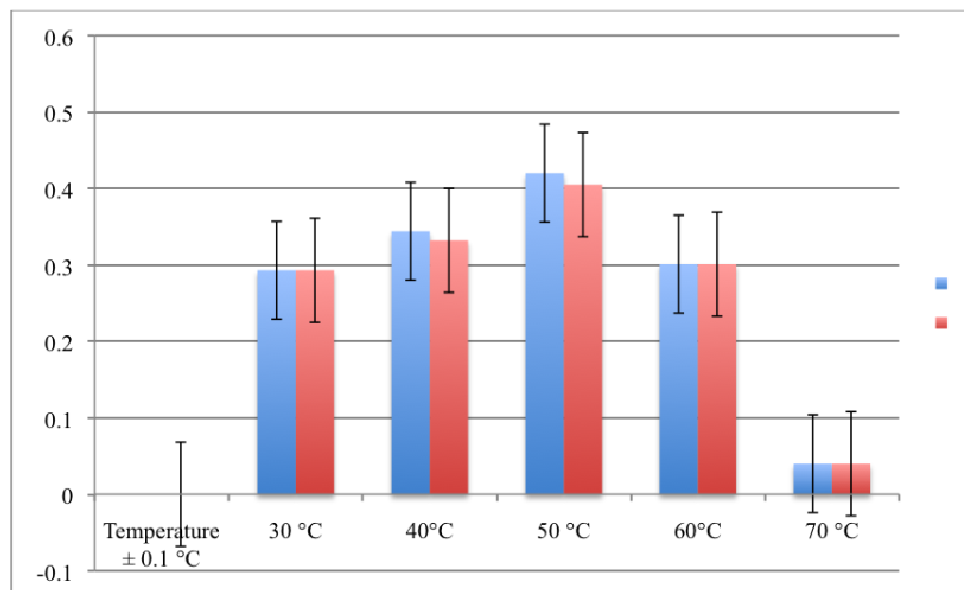


This graph shows that 50°C is the optimum temperature and beyond this temperature the rate of browning will no longer increase but in fact decrease just as the graph. This graph has been added as to the standard deviation and the error bars of data that excludes its anomalies. The

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length of all the errors bars are quite long and so this shows by excluding the anomalies A high standard deviation exists which indicates a broad range of possible values relative to the expected value.

### Comparison between the Two Graphs



Surprisingly, the error bars for the data that excludes anomalies is much longer than the data that includes the anomalies. This is quite unexpected as unusual as one would assume the data that excludes the anomalies would have a low standard deviation and thus be more accurate and closer to true value (mean). However, the data that excludes anomalies seems to have a larger spread of data.

### Conclusion and Evaluation

Weakness/ Limitation	Evaluation + Significance weakness	Possible improvements
<p>Design and method of the placing the test – tubes into the water baths:</p> <p>Effectiveness of the use of equipment –</p> <p>a) Temperature of water baths</p> <p>2. Procedure</p> <p>a) Thickness of apple discs</p>	<p>Additionally temperature of the water baths were slightly off by <math>\pm 0.1^{\circ}\text{C}</math> and so some test tubes received a little less heat and some a little more.</p>	<p>Additionally, may be the water baths can be turned off long before the experiment takes place, as this is the only way to ensure that the exact temperature needed is met.</p>



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b) and Filtering	Crushing	<p>The discs were of the same diameter as I has used the corker borer, however there was the uncertainty of the ruler and so the thickness for all the discs were not the same and this was evident in the appearance of the discs yet not much could be done. These would lead the results to be larger than expected.</p>
c) Colorimetre		<p>Using something to ensure that when the cylinder from the cork borer is cut, it stays in the same position, and perhaps aligning the knife to the title would also be a good idea as this would improve the accuracy of the cutting</p>
		<p>Next time, more precise cutting is required and more time to cut, and when crushing with the pestol, I should aim to try to crush the discs as evenly as possible to attain an average amount of juice. I should try to crush the discs as much as possible, to make for juice available to measure in colorimetre.</p>
3. Time Management		
a) Placing five test tubes into each water bath for about 17 minutes each.		<p>Perhaps if filtered correctly next time, the volume would be the same however if not, then I should try to even out the volume in the cuvette, to keep the experiment fair and accurate.</p>
		<p>The fluctuation may have occurred due to the fact there were some droplets of solution around the rim of top of cuvette, this can resolved by</p>

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	<p>percentage, had small fluctuations instead of a steady absorption. This had lead, to smaller than expected results.</p> <p>I had placed an stopwatch at each water bath however it would be impossible to take out all five samples from each temperature at the exact time even if the water baths were right next to each other. And so the test tubes were taken out at a time ranging from 17 -17.50 seconds. Some test tubes were in the water baths for slightly longer and so this would affect the results I received as the results would be larger than expected.</p>	<p>using a pipette to pour the solution in carefully.</p> <p>Perhaps, next time I can ask other people to help me take out the five samples at the same time rather than me taking them out one by one and hence increasing the time at which some samples remained in the water baths, this would improve the accuracy of my results.</p>
Data Quality and precision	<p>Ruler <math>\pm 1\text{mm}</math>  10ml measuring cylinder <math>\pm 0.2\text{ml}</math>  Temperature <math>\pm 0.1^{\circ}\text{C}</math>  Time <math>\pm 0.1\text{s}</math>  Colorimetre <math>\pm 0.001\%</math></p>	<p>These manufacturing errors, and can be reduced by taking averages over a large number of samples and observations. These random errors do have constant affect in all experiments.</p>

**Comparison and Explanation of the Graph which shows the both the inclusion and exclusion of anomalies:**

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This graph alludes to us that, the standard deviation of the data that excluded the anomalies was higher than that included the anomalies. The mean of the data that excluded the anomalies was also higher, which meant the spread of data was larger than that of the data that included the anomalies.

### **Final Conclusion (with theory):**

To conclude, I can reject my null hypothesis for it was not true and except my hypothesis, which is true to a certain extent as in my hypothesis I did not take into account the denaturation of the enzyme. According to my results, the enzyme denatured at 50°C as this was the optimum temperature at which the rate of browning had increased the most, after this temperature the rate decreased significantly meaning that the enzyme's shape had change and could no longer function as it did not fit in the active site (lock and key).

Enzymes are globular proteins, which act as a catalyst in chemical reactions and their structure is very delicate and can be damaged by various substances and conditions. Denaturation is the changing of the structure of an enzyme, so that it no longer can carry out its function and this change is usually a permanent one.

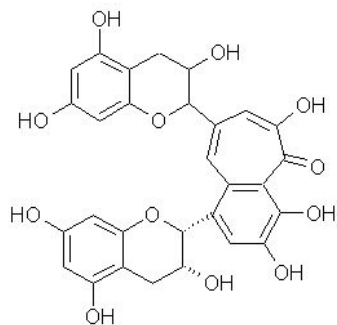
The substrates bind to a special region on the surface of the enzyme called the active site. This active site, catalyses a chemical reaction involving substrates. Enzyme substrate specificity means most enzymes are specific, they catalyse only a few different reactions. There are only a small number of substrates. This means that if the enzyme denatures, then nothing can be replaced, the rate being measured shall decrease as the reaction can longer take place.

As the temperature was increased, a number of bonds in the protein molecule were weakened. As these bonds were weakened and broken, the protein obtained a more flexible structure. If heating had ceases before 40°C, the protein would have been able to readily refold to its original structure. As heating continued, some of the hydrogen bonds that stabilized helical structure were broken. As these bonds are broken, water can interact with and form new hydrogen bonds with the amide nitrogen and carbonyl oxygens of the peptide bonds. The presence of water further weakens nearby hydrogen bonds.

Additionally, as temperature increases beyond its optimum temperature, there are more collisions of particles due to an increase in kinetic energy provided (heat) within enzyme, and the speed of these collisions are extremely fast which also cause the enzyme to denature and change shape.

In all, temperature affects the rate of browning to an extent, as the rate does increase with the temperature but does denature and hence stops the increase. The temperature that this enzyme works best at is between 40°C -50°C. However my conclusion is not extremely strong due to my inaccuracies that occurred during the experiment and my distinct anomalies.

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Structure of a Phenol enzyme <sup>5</sup>

Word Count: 3,394 Words

<sup>5</sup> <http://www.food-info.net/uk/colour/enzymaticbrowning.htm>