The Effect of Vitamin C on the Decomposition of Hydrogen Peroxide

Research Question: How does changing the concentration of vitamin C affect the ability of liver catalase to break down hydrogen peroxide?

Aim: To investigate the effect of different concentrations of vitamin C on the efficiency of liver catalase during the decomposition of hydrogen peroxide as measured by the changes in temperature and the volume of froth produced during the reaction.

Ex
The research question is reasonably focused but it could have given the source of the liver and the range of vitamin C concentrations.

Personal Engagement:

I have always had a keen interest in biochemistry, especially in terms of the processes in the human body. Hence, I was motivated to pursue further research into the beneficial effects of vitamin C in the body after performing prior experiments with this substance that interested me greatly. In addition, in terms of personal health, it could be determined whether it would be beneficial to the body, in terms of destroying various free radicals, if we increase the intake of vitamin C in our daily diet.

PE
Appears contrived.

PE
Some justification for the investigation.

Introduction:

Vitamin C, also known as ascorbic acid, has a well-known reputation in being one of the most important components of the daily nutrients needed by humans. It is a micronutrient found in several kinds of food, especially fruits and vegetables; more specifically, it is a water-soluble vitamin necessary for several metabolic processes in the body, including growth, development, and repair of tissues. ("Vitamin C Benefits").

EX
Background material relevant.
Com
Citations given though they could be more complete.

One of the key roles that vitamin C plays is that of an antioxidant. These protect the body against free radicals, or reactive oxygen species (ROS), which are molecules containing at least one unpaired electron ("Radical"). These electrons render the molecules highly reactive; hence, there is a risk of damage that could be inflicted upon the intricate chemical systems that exist in the human body. ROS have been frequently linked to one of the causes of cancer, as their instability could lead to the mutation of cells (Bowen). Similarly, catalase is a common enzyme found in most living organisms which act as an antioxidant. It is one of the fastest reacting enzymes in the body: it can convert millions of hydrogen peroxide molecules into water and oxygen gas in one second. There is an even distribution of catalase throughout all of the body, but the strongest concentration is in the liver, due to the fact that the liver is the primary detoxifying organ in the body. (Goodsell).

Catalase acts primarily against hydrogen peroxide, a byproduct of respiration in our bodies as well as taken in from our diet and other outside sources. Hydrogen peroxide at high concentrations is generally regarded as cytotoxic; meaning it is detrimental to cells (Halliwell). This is due to the fact that hydrogen peroxide itself is an ROS as a result of the reduction of oxygen (Bowen). Though it is important in destroying mutated cells or killing foreign bacteria, it could also negatively affect healthy cells that are required for processes in the human body (Halliwell). Hence, the role of catalase in keeping hydrogen peroxide at manageable levels is vital. The decomposition of hydrogen peroxide with the catalase enzyme is shown as such (Goodsell):

$$2H_2O_2 \xrightarrow{Catalase} 2H_2O + O_2$$

This experiment will analyze whether the combination of two antioxidants will increase their detoxifying properties by mixing various concentrations of vitamin C with bovine liver catalase, and subsequently allowing this solution to react with a fixed concentration of hydrogen peroxide.

Hypothesis:

If vitamin C concentration increases, then hydrogen peroxide will decompose at a higher rate. This could be due to the fact that both vitamin C and catalase are antioxidants (Weil; Goodsell); thus the mixture of both could positively affect the rate of hydrogen peroxide decomposition.

Variables:

The independent variable, or the variable in the experiment that will be changed, is the concentration of vitamin C. The range of the independent variable, or the varying concentrations of vitamin C that will be used, will follow as such: 1%, 0.75%, 0.5%, 0.25%, 0.1%, and 0%.

The dependent variables, or the variables that will be changed as a result of the experiment, will be the volume of froth produced by the substrate-enzyme solution as well as the temperature of the solution.

Ex
What is the link between the froth or the temperature change and the reaction being followed?

Controlled Variables

Variable	Why it should be controlled	Method to control it
Temperature	As temperature increases up to a certain point, so does the reaction rate. High temperatures could also denature the	Ensure that the experiment is done at room temperature (25°C) using a thermometer.

	enzyme.	
Concentration of catalase	Higher concentration of enzymes results in a higher rate of enzyme activity.	Ensure that the same concentration of liver catalase is used by creating the 2% catalase solution and using this same solution in the entire experiment.
Concentration of hydrogen peroxide	Higher concentration of substrate results in a higher rate of enzyme activity.	Ensure that the same concentration of hydrogen peroxide is used by using a 6% solution from a lab supply company and using this same solution in the entire experiment.
Volume of catalase	Higher volume of enzyme would result in a higher rate of enzyme activity.	Ensure that the same volume of liver catalase is used by measuring out 2 cm^3 of catalase using a measuring cylinder during each trial.
Volume of hydrogen peroxide	Higher volume of substrate would result in a higher rate of enzyme activity.	Ensure that the same volume of hydrogen peroxide is used by measuring out 2 cm^3 of hydrogen peroxide using a measuring cylinder during each trial.
Volume of vitamin C (ascorbic acid)	Higher volume of vitamin C may result in differing rates of reaction.	Ensure that the same volume of vitamin C is used by measuring out 2 cm^3 of vitamin C using a measuring cylinder during each trial.
Time to take recording	The reaction may change as the liver solution concentration changes so recordings should be taken at fixed intervals.	Note down the temperature and volume of bubbles after 30 seconds.
pH	As pH increases up to a certain point, so does the reaction rate. High pH could also denature the enzyme.	The solutions used will not be changed so the pH will remain the same. To monitor this, a pH probe could be used in all the solutions to ensure that they are at the same pH.

Ex
Was it actually used?
No data.

Ex
But it is not buffered.

Exposure to light	Vitamin C is very sensitive and may change concentrations when exposed to light.	Perform the experiment in a dim room with ambient light from the outside and no overhead lighting.

Ex
Aware of need to control most important variables.

Apparatus:

- 1.3000 g ascorbic acid ($C_6H_8O_6$) powder
- 750.00 cm^3 distilled water
- 10.00 g bovine liver
- 60.0 cm^3 6% hydrogen peroxide (H_2O_2)
- Temperature probe (± 0.1 °C)
- 5x 10 cm^3 measuring cylinders (± 0.5 cm^3)
- 5 cm^3 syringes (± 0.1 cm^3)
- Blender
- Stopwatch (± 0.05 seconds)

An
Measurement uncertainties presented.

Method:

Combining 50.00 cm^3 of distilled water with 0.5000 grams of ascorbic acid powder created a 1% solution of vitamin C. Concentrations of 0.75%, 0.5%, 0.25%, and 0.1% of vitamin C were then produced in the same method, with corresponding measurements shown in the table below.

Concentration (%)	**Volume of Distilled Water** (cm^3) (± 0.5 cm^3)	**Mass of ascorbic acid powder** (g) (± 0.001 g)
1.00	50.0	0.500
0.75	50.0	0.375
0.50	50.0	0.250
0.25	50.0	0.125
0.10	50.0	0.050

10.000 grams of cow liver were blended together with 500.0 cm^3 of distilled water in order to create a 2% liver catalase solution. 2.0 cm^3 of this solution was then placed in a 10 cm^3 measuring cylinder using a syringe, along with 2.0 cm^3 of the 1% ascorbic acid solution. The total volume of the two solutions was recorded, and initial temperature of the solution was measured using a temperature probe.

A syringe was used to add 2.0 cm^3 of the 6% hydrogen peroxide solution to one of the test tubes containing the catalase solution. After 30 seconds, the volume and the temperature of the substrate-enzyme complex was noted. Four more solutions of liver catalase and 1% ascorbic acid were created, and subsequently combined with the hydrogen peroxide for a total of 5 trials for this particular concentration of vitamin C.

Ex
Sufficient data collected.

The same method was then carried out for the other concentrations of vitamin C, with a total of 5 trials for each concentration.

Ex
Safety, ethical and environmental issues addressed.

Risk Assessment, Environmental Issues, and Ethics:

The bovine liver used to produce the catalase solution was taken from the cow after the animal had been slaughtered so no pain was inflicted onto the animal during removal.

Hydrogen peroxide is a corrosive chemical; thus, safety glasses should always be worn during the experiment, and containers of the chemical must be properly labeled. Excess hydrogen peroxide and vitamin C solutions will be disposed in a residue beaker to ensure of proper disposal of dangerous chemicals.

Results:

Raw Data

Concentration of vitamin C against temperatures of liver catalase-hydrogen peroxide solution

	Temperature (°C) (± 0.1 °C)									
	Trial 1		Trial 2		Trial 3		Trial 4		Trial 5	
Concentration of Vitamin C (%)	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
1.00	25.2	26.2	25.5	26.2	25.2	26.0	25.0	26.1	25.3	26.2
0.75	23.1	25.5	22.9	25.7	23.9	25.9	24.4	26.2	23.3	25.5
0.50	24.4	27.2	24.8	28.1	24.7	27.9	24.9	27.8	24.6	27.7
0.25	20.1	24.2	25.2	29.4	23.4	29.2	25.2	29.6	24.9	29.4
0.10	23.4	28.3	23.5	28.2	23.0	27.8	23.1	27.9	22.5	27.3
0.00	21.2	30.8	21.5	30.4	20.4	28.2	21.3	29.4	20.2	28.5

Concentrations of Vitamin C against volume of froth created in liver catalase-hydrogen peroxide solution

Com
Unconventional presentation of the independent variable.

	Volume of froth (cm^3) (± 0.5 cm^3)									
	Trial 1		Trial 2		Trial 3		Trial 4		Trial 5	
Concentration of Vitamin C (%)	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
1.00	4.0	8.0	4.0	7.0	4.0	8.0	4.0	7.0	4.0	7.5
0.75	4.0	10.0	4.0	11.0	4.0	10.0	4.0	9.5	4.0	10.0
0.50	4.0	17.0	4.0	16.0	4.0	15.0	4.0	16.0	4.0	16.0
0.25	4.0	22.0	4.0	20.0	4.0	19.0	4.0	21.0	4.0	20.0
0.10	4.0	26.0	4.0	27.0	4.0	26.0	4.0	25.0	4.0	27.0
0.00	4.0	40.0	4.0	38.0	4.0	39.0	4.0	39.0	4.0	38.0

An
Relevant qualitative observations.

Qualitative Data

- Solution of catalase and vitamin C, which was originally a cloudy pink-brown shade, became paler in color as it reacted with the hydrogen peroxide.
- At lower concentrations of vitamin C, volume of froth was produced very rapidly at first, the rate of froth formation slowing down during the 30 seconds of the trial

Processed Data

Percentage change of temperatures in liver catalase-hydrogen peroxide solution

	Percentage change in temperature (%) (± 0.01%)						
Concentration of Vitamin C (%)	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Average	Standard Deviation
1.00	3.97 [1]	2.75	3.17	4.40	3.56	3.57	0.65
0.75	10.39	12.23	8.37	7.38	9.44	9.56	1.87
0.50	11.48	13.31	12.96	13.65	12.60	12.80	0.84
0.25	20.40	16.67	20.51	17.46	18.07	18.62	1.75
0.10	20.94	20.00	20.87	20.78	21.33	20.78	0.49
0.00	45.28	41.40	38.24	38.03	41.09	40.81	2.95

An
Appropriate processing as the initial temperatures vary.

An
Standard calculations

Sample Calculation

An/Com
Processing can be followed.

(Note: Average and Standard Deviation were done using Excel.)

[1] Trial 1, Concentration 1%: % change $= \frac{\text{Final Temperature - Initial Temperature}}{\text{Initial Temperature}} \times 100$

% change $= \frac{26.2°C - 25.2°C}{25.2°C} \times 100$

% change = 3.97%

Percentage change in volume of froth in liver catalase-hydrogen peroxide solution

	Percentage change in volume of froth (%) (± 0.01%)						
Concentration of Vitamin C (%)	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Average	Standard Deviation
1.00	100.00 [1]	75.00	100.00	75.00	87.50	87.50	12.51
0.75	150.00	175.00	150.00	137.50	150.00	152.50	13.74
0.50	325.00	300.00	275.00	300.00	300.00	300.00	17.73
0.25	450.00	400.00	375.00	425.00	400.00	410.00	28.52
0.10	550.00	575.00	550.00	525.00	575.00	555.00	20.96
0.00	900.00	850.00	875.00	875.00	850.00	870.00	20.95

Sample Calculation

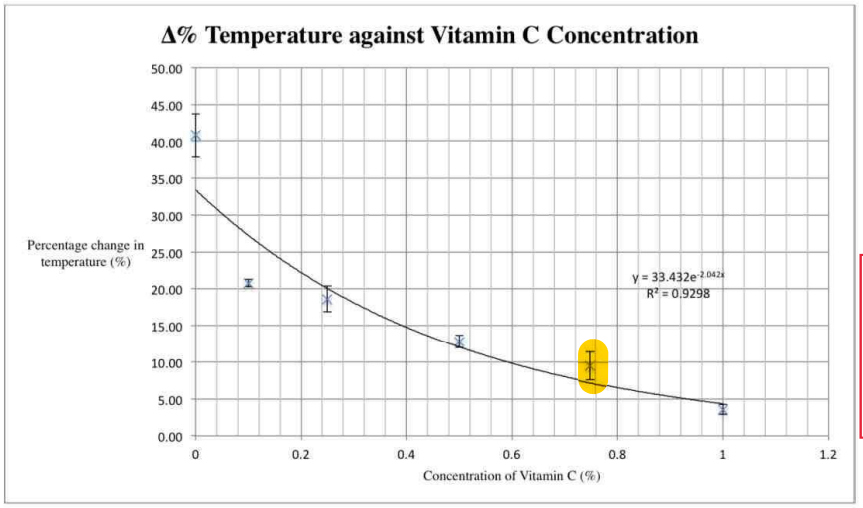
(Note: Average and Standard Deviation were done using Excel.)

[1] Trial 1, Concentration 1%: % change = $\frac{\text{Final Volume - Initial Volume}}{\text{Initial Volume}} \times 100$

% change = $\frac{8.0\ \text{cm3} - 4.0\ \text{cm3}}{4.0\ \text{cm3}} \times 100$

% change = 100.0 %

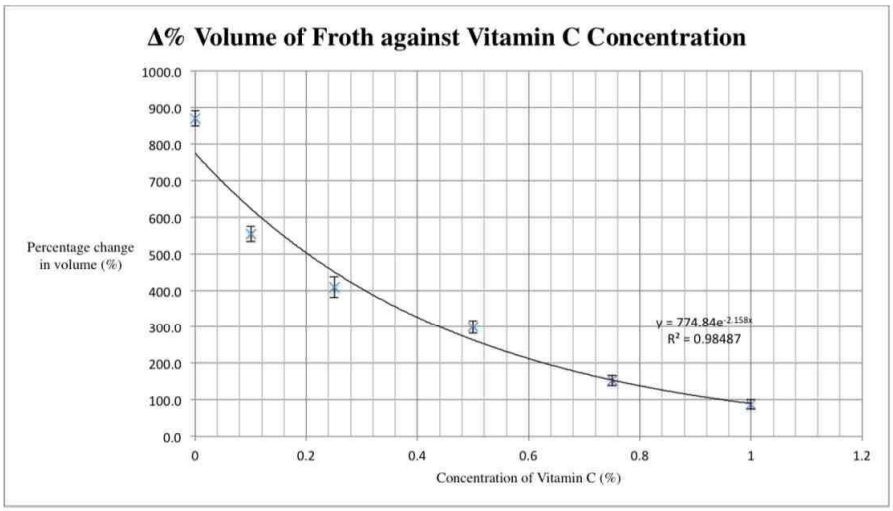
Graphs



An
Dot to dot with error bars would be acceptable and, given the amount of data, probably more legitimate.

An/Com
What do the error bars represent? Presumably the standard deviation.

Error Bars = ± 1 standard deviation



Error Bars = ± 1 standard deviation

Rate of reaction based on changes in temperature in liver catalase-hydrogen peroxide solution

Concentration of Vitamin C (%)	Reaction rate based on temperature (°C s^{-1}) (± 0.010 °C s^{-1})						
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Average	Standard Deviation
1	0.033 [1]	0.023	0.027	0.037	0.030	0.030	0.005
0.75	0.080	0.093	0.067	0.060	0.073	0.075	0.013
0.5	0.093	0.110	0.107	0.113	0.103	0.105	0.008
0.25	0.137	0.140	0.160	0.147	0.150	0.147	0.009
0.1	0.163	0.157	0.160	0.160	0.160	0.160	0.002
0	0.320	0.297	0.260	0.270	0.277	0.285	0.024

An Calculation of rate appropriate. However if data logging has been used a slope from the initial reaction rate would be better.

Sample Calculation

(Note: Average and Standard Deviation were done using Excel.)

[1] Trial 1, Concentration 1%: Rate of reaction = $\frac{\text{Final Temperature - Initial Temperature}}{\text{Time of reaction}}$

Rate of reaction = $\frac{26.2°C - 25.2°C}{30 \text{ seconds}}$

Rate of reaction = 0.033 °C s^{-1}

Rate of reaction based on changes in froth volume in liver catalase-hydrogen peroxide solution

	Reaction rate based on volume of froth ($cm^3\ s^{-1}$) (± 0.010 $cm^3\ s^{-1}$)						
Concentration of Vitamin C (%)	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Average	Standard Deviation
1.00	0.133 [1]	0.100	0.133	0.100	0.117	0.117	0.017
0.75	0.200	0.233	0.200	0.183	0.200	0.203	0.018
0.50	0.433	0.400	0.367	0.400	0.400	0.400	0.024
0.25	0.600	0.533	0.500	0.567	0.533	0.547	0.038
0.10	0.733	0.767	0.733	0.700	0.767	0.740	0.028
0.00	1.200	1.133	1.167	1.167	1.133	1.160	0.028

Sample Calculation

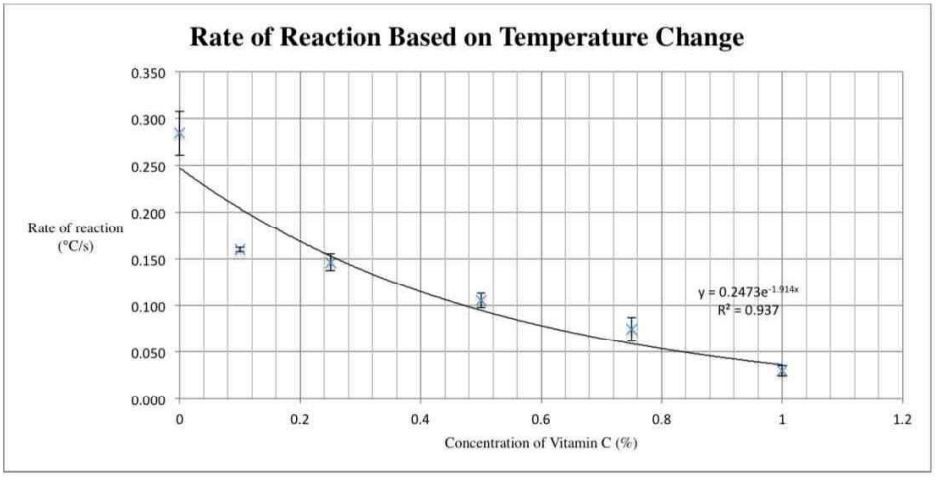
(Note: Average and Standard Deviation were done using Excel.)

[1] Trial 1, Concentration 1%: Rate of reaction = $\frac{\text{Final Volume - Initial Volume}}{\text{Time of reaction}}$

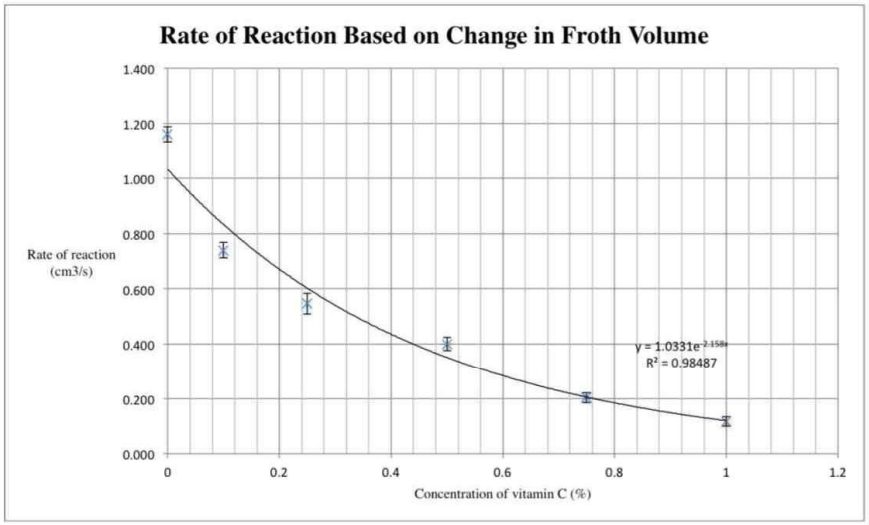
Rate of reaction = $\frac{8\ cm3 - 4\ cm3}{30\ seconds}$

Rate of reaction = 0.133 $cm^3\ s^{-1}$

Graphs



Error Bars = ± 1 standard deviation



Error Bars = ± 1 standard deviation

Data Analysis & Conclusion

Both graphs for percentage change as well as rate of reaction shows similar trends in that the data experiences a decrease that reaches towards a plateau: as concentration of vitamin C increases, the rate of reaction significantly decreases. The plateau shows that increasing the vitamin C concentration will not affect the rate of reaction after a certain period of time.

Ev
Not quite sure what is meant here.

In terms of comparing the values between temperature change and froth volume, the trend is even more evident in that there is a rather large difference between the reaction rate in terms of froth volume at 0% vitamin C concentration and at 1% vitamin C concentration. However, the error bars for the temperature change at 0% concentration are significantly larger than the others; hence, this may have skewed the trend line to show a weaker correlation in comparison to the correlation between change in froth volume and concentration of vitamin C.

An
Error bars discussed but more detail needed.

There are no major overlaps in terms of error bars; hence, this shows that each data point is significantly different from one another. With the exception of the temperature change and rate of reaction based on temperature at 0% vitamin C, the error bars are rather small, showing that there is not a large amount of variation in the data. The correlation coefficients of each of the graphs are over 0.9, showing that there is

An
Over-optimistic interpretation of the data. Non-overlapping error bars SUGGEST a significant different but a test is required to confirm it.

An
R_2 values are not correlation coefficients. They are coefficients of determination. They can give a measure of the quality of fit.

An
Correct interpretation of R_2 nevertheless.

a good fit between the data points and the trend lines. All of these show that the data could stand as reliable, as there is no significant variation between trials and there is an observed trend.

Vitamin C seems to inhibit the effects of catalase, as shown by the decrease in reaction rate with an increase in vitamin C concentration during the decomposition of hydrogen peroxide; thus, the hypothesis put forth was not corroborated. After further research, however, studies have shown that vitamin C could also act as a pro-oxidant in addition to its role as an antioxidant. In this case, the micronutrient plays the role of creating hydrogen peroxide in the body, which could potentially be beneficial to several physiological processes in the body, like the relaxing of muscle lining in the arteries. Research also states that it could be possible that vitamin C begins losing its antioxidant properties at high concentrations. This points to the fact that during the reaction, there is a high possibility that vitamin C aided in the production of more hydrogen peroxide, which caused the decomposition to occur slower due to the increase in the concentration of hydrogen peroxide (Klingenhoeffer, 2015)

Ev
Conclusion supported by the data.

Ev
Explanation provided.

Ev
Set in a scientific context.

To conclude, vitamin C and catalase do not work together as hypothesized, as after a certain concentration, vitamin C begins to play a different role than what was previously recognized, switching from the role of antioxidant to that of a pro-oxidant. In this case, the ROS created by vitamin C could be an advantage to the defense mechanism of our bodies; however, it is also clear that a corresponding increase in the concentration of catalase would also be necessary in order to balance out the levels of hydrogen peroxide in the body, as research has shown that hydrogen peroxide at high levels could be toxic to the body.

Ev
Insufficient data to support this.

Evaluation

Source of Error	Why?	Improvements
The volume of oxygen produced was difficult to determine, as measuring the froth using the measuring cylinders was inaccurate due to the nature of the bubbles.	This would result in inaccurate measuring, and subsequently data.	Use advanced apparatus like a gas pressure probe, which would give accurate data as to how much oxygen is produced.
More variation in the concentrations of vitamin C was needed.	This is important in order to see a more coherent trend, as the limited range in concentration could potentially skew the trend line.	Create concentrations of over 1%, for example, 1.5% and 2%, in addition to concentrations closer to zero, like 0.01%.
Pure catalase was not used in the experiment.	As the catalase that was used was taken from blended bovine liver, there could potentially be other chemicals that could affect the experiment.	Use a pure extract of liver catalase with a known concentration, ordered from a lab supply company.

Room temperature would often fluctuate from the initial 25°C.	The substrate-enzyme complexes would have different temperature starting points, thus affecting the activity of the enzyme and subsequently the data.	Perform the experiment in a water bath at 25°C.

Ev
Feasible and sensible suggested improvements.
Adding a buffer would help.

Ideas for Further Research

With the information gained from this experiment, further research could be done to test at which concentrations or conditions vitamin C becomes a pro-oxidant rather than an anti-oxidant. According to research, vitamin C shows pro-oxidative behavior when paired with certain iron ions and other metal ions (Naidu 4); hence, an experiment could be done to see the effect of various concentrations of iron on the pro-oxidative behavior of vitamin C. Further research, through the use of various past experiments and journals, could be done to study the effects of vitamin C in various biological macromolecules, such as DNA, proteins, and lipids, and whether the pro-oxidative effects of vitamin C could potentially aid or harm them.

Furthermore, catalase in other organisms, such as potatoes and carrots, could be used in order to test whether vitamin C would have the same pro-oxidative effect on varying concentrations of catalase in different organisms.

Ev
Logical extension proposed.

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