Optimum Temperature and pH for Digestive Enzymes

A Science Paper Presented By:

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Abstract

The purpose of the project was to determine the best environment (temperature and pH) for enzymes to work in the digestive tract. Boiled eggs were used for the protein to measure the change in mass. The enzymes were purchased from Science Kit and prepared according to directions. The pH was adjusted with drops of hydrochloric acid and sodium hydroxide. The temperatures were established in a refrigerator, on the counter top and in an incubator. The change in mass was determined by initial massing and remassing in 24 hours after filtering. The results showed that the pepsin at pH = 2 did better at lower temperatures while the trypsin at pH =2 did better at higher temperatures. The alternative hypothesis was not accepted for this study.
Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purpose of the Study</td>
<td>1</td>
</tr>
<tr>
<td>Problem Stated</td>
<td>1</td>
</tr>
<tr>
<td>Review of Literature</td>
<td>1</td>
</tr>
<tr>
<td>Hypothesis</td>
<td>3</td>
</tr>
<tr>
<td>Experimental Design</td>
<td>4</td>
</tr>
<tr>
<td>Materials</td>
<td>4</td>
</tr>
<tr>
<td>Photographs</td>
<td>5</td>
</tr>
<tr>
<td>Procedure</td>
<td>5</td>
</tr>
<tr>
<td>Data Table</td>
<td>6</td>
</tr>
<tr>
<td>Results and Observations</td>
<td>6</td>
</tr>
<tr>
<td>Statistics</td>
<td>7</td>
</tr>
<tr>
<td>Graphs</td>
<td>8</td>
</tr>
<tr>
<td>Conclusion</td>
<td>9</td>
</tr>
<tr>
<td>Future Study</td>
<td>10</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>10</td>
</tr>
<tr>
<td>Literature Cited</td>
<td>10</td>
</tr>
<tr>
<td>Appendix</td>
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Purpose of the Study:

The purpose of the project is to determine which digestive enzyme (pepsin or trypsin) will have the greatest effect by change in mass of a protein at different pH levels and different temperatures. The idea for the study came from an Internet site. Also, a previous lab in a mini science course gave the experimenter knowledge that enzymes speed up the break down of proteins. The information is important to help understand the human body and how digestion works, especially the effect of enzymes on digestion.

Statement of the Problem:

Which type of protease (pepsin or trypsin) affects the digestion of a protein the most at different pH levels and different temperatures (°C) by measuring the change in mass in grams of the protein following exposure for 24 hours?

Review of Literature:

Enzymes are important proteins present in every living cell. “They are organic catalysts capable of speeding chemical reactions without themselves being changed in the process.” More than one thousand enzymes are now known, each capable of catalyzing certain reactions. “Enzymes are associated with several cellular organelles such as mitochondria, plastids, the nucleus, and the endoplasmic reticulum.” Common reactions that include enzymes are photosynthesis, cell respiration, and the making of proteins.

Enzymes do not produce reactions that would not otherwise occur, but their actions speed up and favor certain reactions. Because of this, there is a type of regulation
in the way the reactions occur. Some may be performed in one minute while others may take a thousand years to occur. Only a small amount of enzymes are needed to make great changes in the reaction (Tufty, 1973).

Proteins are one of the three main classes of food that provide energy for the body. Proteins exist in every cell and are essential to plant and animal life. Humans obtain protein from different types of foods like cheese, eggs, fish meat and milk. Proteins make up a big part of each human cell. They are important in building, maintaining, and repairing tissues in the human body, especially bone cartilage and muscles (Ahrens, 2001).

There are many “sporadic” and genetic diseases that are caused by the lack of proteins. Humans must include a certain amount of amino acids in the diet. Otherwise, diseases may occur. These include cystic fibrosis, Alzheimer’s, Parkinson’s and other body imbalances in the elderly. “A unified view of the molecular cellular pathogenesis of these conditions has led to search for chemical chaperones that can slow, arrest or revert disease progression.” Molecules and reactions are now appearing to be linked to treatment of disease (www.nature.com and www.users.rnc.com). Perhaps understanding how enzymes play a role will benefit and reduce these diseases. Otherwise, humans may continue to live with the negative symptoms of disease.

One symptom of improper protein balance is the loss of hair. Crash diets are associated with hair loss due to the exclusion of proper protein. When this occurs, the body will save protein by shifting hair growth into a resting phase. Increased shedding can happen for two to three months when protein imbalance occurs. But, the condition can be reversed with a proper diet. Other symptoms may include tooth decay, muscle
loss, decreased bone mass, etc. Without a doubt, proteins are important and the enzymes that work to break them down so the body can use them are important as well.

Prevention of diseases and the possible side effects should make all humans concerned about getting an adequate balanced diet. Research has led to many links to enzymes and the bodily functions. This is the best way to prevent negative outcomes on the human body (Conrad, 2007).

**Hypothesis:**

The null hypothesis is that if a protein is tested with pepsin and trypsin at different pH levels and different temperatures, then there will be no significant difference in the change in mass for the protein.

The alternative hypothesis is that if a protein is tested with pepsin and trypsin at different pH levels and different temperatures, then the pepsin at pH of 2 at the highest temperature will have the greatest change in mass (grams).
**Experimental Design:**

Independent Variables: type of protease, pH and temperature

Dependent Variable: change in mass in grams

Control: none defined, true comparison (It was determined in a science lab that enzymes do speed up the process.)

Constants: same size test tubes, time allotted, balance, filter procedure, type of protein, amount of solvent, type of thermometer

Retests: 10 for pepsin pH =2 at 15°C
    10 for pepsin pH =2 at 25°C
    10 for pepsin pH =2 at 35°C
    10 for pepsin pH =8 at 15°C
    10 for pepsin pH =8 at 25°C
    10 for pepsin pH =8 at 35°C
    10 for trypsin pH =2 at 15°C
    10 for trypsin pH =2 at 25°C
    10 for trypsin pH =2 at 35°C
    10 for trypsin pH =8 at 15°C
    10 for trypsin pH =8 at 25°C
    10 for trypsin pH =8 at 35°C

**Quantitative Measure:** change in mass in grams at different pH levels and temperatures

**Materials:**

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<th>Quantity/Description</th>
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<td>distilled water</td>
<td>pH meter</td>
</tr>
<tr>
<td>beaker</td>
<td>safety goggles</td>
</tr>
<tr>
<td>HCl</td>
<td>NaOH</td>
</tr>
<tr>
<td>paper towels</td>
<td>weighing paper</td>
</tr>
<tr>
<td>funnel</td>
<td>thermometers</td>
</tr>
<tr>
<td>ring stand/ring clock</td>
<td>volumetric flasks to make solutions</td>
</tr>
<tr>
<td>120 cubes egg</td>
<td>test tube racks</td>
</tr>
<tr>
<td>30 grams pepsin</td>
<td>wax pencil</td>
</tr>
<tr>
<td>30 grams trypsin</td>
<td>graduated cylinder</td>
</tr>
<tr>
<td>polyethylene gloves</td>
<td>digital balance (0.000 g)</td>
</tr>
<tr>
<td>scalpel</td>
<td>refrigerator</td>
</tr>
<tr>
<td>refrigerator</td>
<td>incubator</td>
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<td>paper towels</td>
<td>filter paper</td>
</tr>
<tr>
<td>volumetric flasks to make</td>
<td></td>
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<tr>
<td>solutions</td>
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Procedure:

Prepare Solutions and Eggs:
1. Prepare egg white (albumen) by boiling eggs 10 minutes. Cool, shell and cut eggs into 2.5 gram cubes.
2. Prepare with teacher 0.01 M HCl by adding 50 ml of 0.1M HCl and diluting to 500 ml in volumetric flask.
3. Prepare with teacher 0.001M NaOH by adding 5 ml of 0.1M NaOH and diluting to 500 ml in volumetric flask.
4. Prepare pepsin by adding 4 grams to a flask and diluting to 200 ml with distilled. Make twice and adjust pH with HCL and NaOH to pH of 2 and 8 using pH meter.
5. Prepare trypsin by adding 10 grams pancreatin and diluting to 200 ml. Make twice and adjust pH.

Testing Procedure:
1. Use a wax pencil to label all test tubes with correct enzyme, temperature and pH (120 total).
2. Place the tubes in separate test tube racks according to the group.
3. Pour 10 ml of pepsin solution pH of 2 in 30 test tubes.
4. Pour 10 ml of pepsin solution pH of 8 in 30 test tubes.
5. Pour 10 ml of trypsin solution pH of 2 in 30 test tubes.
6. Pour 10 ml of trypsin solution pH of 8 in 30 test tubes.
7. Place a 2.5 gram cube of egg white in each test tube. Make sure to mass each cube before testing. Record the mass accurately for each retest.
8. Place each test tube rack in the correct environment (15ºC in refrigerator, 25ºC on counter, 35ºC in incubator).
9. Allow 24 hours to pass.
10. Set up a ring stand, ring, funnel, beaker and filter.
11. Pour the first solution from the test tube into the filter paper slowly.
12. Label each filter and set aside to dry.
13. Repeat filtering for all retests.
14. When the paper is dry, remass and record the change in mass by subtracting the mass of the filter paper and determining the remaining mass as compared to the original mass of the egg.
15. Clean all equipment and prepare a data table.
## Data Table of Change of Mass in Grams for a Protein Tested with Different Enzymes (Pepsin and Trypsin) at Different pH Levels and Different Temperatures

<table>
<thead>
<tr>
<th>Retests</th>
<th>15°C</th>
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<tr>
<td></td>
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<tr>
<td>1</td>
<td>1.9</td>
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<td>2.1</td>
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<td>2</td>
<td>1.7</td>
<td>2.2</td>
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<td>3</td>
<td>1.7</td>
<td>2.2</td>
<td>1.1</td>
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<td>4</td>
<td>1.8</td>
<td>2.2</td>
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<td>5</td>
<td>1.7</td>
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<td>1.5</td>
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<tr>
<td>Std. Dev.</td>
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<td>0.16</td>
<td>0.29</td>
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### Results and Observations:

The results showed that the lowest average change mass occurred with pepsin at 25º C at a pH of 2 with a 1.3 gram change. The highest average change in mass occurred with pepsin at pH of 2 at 15º Celsius with a 2.1 gram change. Looking at overall averages, pepsin seemed to work better at a pH of 2 while trypsin worked better at a pH of 8. Both enzymes seemed to work better at the lower temperature if you consider the overall averages for the different temperatures. The standard deviations are relatively small which indicates similar data results for the retests. It was observed that the enzymes had distinct odors and that a slight color change occurred in the egg white.
Statistics: Tukey HSD Results for p values

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<tr>
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<th>pH 8</th>
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<td>0.0005</td>
<td>0.00013</td>
</tr>
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<td>T vs P at 25º</td>
<td>0.1595</td>
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<tbody>
<tr>
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<td>0.2179</td>
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<td>T vs T 25º to 35º</td>
<td>0.0233</td>
<td>0.8784</td>
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<tr>
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<td>P vs P 15º to 25º</td>
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<td>0.0002</td>
</tr>
<tr>
<td>P vs P 25º to 35º</td>
<td>0.2179</td>
<td>0.2179</td>
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<tbody>
<tr>
<td>pH 2 to pH 8 T vs T 15º to 25º</td>
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<td>0.0060</td>
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<tr>
<td>pH 2 to pH 8 T vs T 25º to 35º</td>
<td>0.1321</td>
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<tr>
<td>pH 2 to pH 8 T vs T 35º to 35º</td>
<td>0.6169</td>
<td>0.8400</td>
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Graph 1: Scatter Graph for Difference in Mass (Grams) for Protein Exposed to Different Enzymes at 15 Degree Celsius

- pH 2 Trypsin
- pH 2 Pepsin
- pH 8 Trypsin
- pH 8 Pepsin
Graph 2: Scatter Graph for Difference in Mass (Grams) for Protein Exposed to Different Enzymes at 25 Degree Celsius

Graph 3: Scatter Graph for Difference in Mass (Grams) for Protein Exposed to Different Enzymes at 35 Degree Celsius
Conclusion:

The alternative hypothesis stated that if a protein is tested with pepsin and trypsin at different pH levels and different temperatures, then the pepsin at pH of 2 at the highest temperature will have the greatest change in mass (grams). The hypothesis is not supported by the data and therefore is not accepted. There was a statistical difference in the data for pepsin compared to trypsin at the lower temperatures for pH of two. Temperature did seem to play a major role for each enzyme because there was also a significant difference when comparing pepsin to pepsin and trypsin to trypsin at the different temperatures. Pepsin seemed to be more affected by the changes. This study is important to understanding better how enzymes function in the body. Enzymes may be a key part of overcoming some diseases.
Future Study:

The project could be extended and bettered by:

1. limiting the time the protein is exposed.
2. completing more retests.
3. testing at more temperatures.
4. testing more enzymes.
5. testing with a churning motion as in the digestive system.

The project applies to the real world by offering information about how enzymes work in the digestive system to help break down proteins for use.

Acknowledgements:

I would like to thank the people that helped make my project a learning experience. First, I would like to thank Mrs. Freeman for her direction and encouragement. I would like to thank my mom and dad for supporting me and buying the materials. Thank you very much!

Literature Cited:


Conrad, Carol. Personal Interview. February 26, 2007


No Author. “Therapeutic Approaches to Protein” (2004), June 2004. www.nature.com/


Appendix

ANOVA: Between Groups Design

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PAIRWISE COMPARISONS [Q=TukeyHSD: *=p<0.05 **=p<0.01]

- AvB t(18)=4.25  p< 0.0005  Q=0.0000
- AvC t(18)=0.60  p< 0.5585  Q=0.0000
- AvD t(18)=1.94  p< 0.0678  Q=0.0000
- AvE t(18)=1.28  p< 0.2165  Q=0.0000
- AvF t(18)=4.94  p< 0.0001  Q=0.0000
- AvG t(18)=0.44  p< 0.6617  Q=0.0000
- AvH t(18)=0.41  p< 0.6889  Q=0.0000
- BvC t(18)=2.79  p< 0.0121  Q=0.0000
- BvD t(18)=3.12  p< 0.0060  Q=0.0000
- BvE t(18)=6.06  p< 0.0001  Q=0.0000
- BvF t(18)=11.18 p< 0.0001  Q=0.0000
- BvG t(18)=3.14  p< 0.0057  Q=0.0000
- BvH t(18)=4.76  p< 0.0002  Q=0.0000
- CvD t(18)=0.87  p< 0.3972  Q=0.0000
- CvE t(18)=1.68  p< 0.1099  Q=0.0000
- CvF t(18)=4.63  p< 0.0002  Q=0.0000
- CvG t(18)=0.16  p< 0.8784  Q=0.0000
- CvH t(18)=0.94  p< 0.3590  Q=0.0000
- DvE t(18)=3.69  p< 0.0017  Q=0.0000
- DvF t(18)=8.95  p< 0.0001  Q=0.0000
- DvG t(18)=1.12  p< 0.2766  Q=0.0000
- DvH t(18)=2.45  p< 0.0246  Q=0.0000
- EvF t(18)=3.81  p< 0.0013  Q=0.0000
- EvG t(18)=1.58  p< 0.1321  Q=0.0000
- EvH t(18)=0.86  p< 0.4009  Q=0.0000
- FvG t(18)=4.69  p< 0.0002  Q=0.0000
- FvH t(18)=4.51  p< 0.0003  Q=0.0000
- GvH t(18)=0.80  p< 0.4313  Q=0.0000

DESCRIPTIVE DETAILS

Fa  ph 2 T ph 2 P FaL3  FaL4  FaL5  FaL6  FaL7  FaL8
### ANOVA: Between Groups Design

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**PAIRWISE COMPARISONS [Q=TukeyHSD: *=p<0.05 **=p<0.01]**

- AvB \(t(18)=3.81\) \(p<0.0013\) \(Q=0.0000\)
- AvC \(t(18)=1.58\) \(p<0.1321\) \(Q=0.0000\)
- AvD \(t(18)=0.86\) \(p<0.4009\) \(Q=0.0000\)
- AvE \(t(18)=2.48\) \(p<0.0233\) \(Q=0.0000\)
- AvF \(t(18)=1.08\) \(p<0.2963\) \(Q=0.0000\)
- AvG \(t(18)=1.94\) \(p<0.0684\) \(Q=0.0000\)
- AvH \(t(18)=0.86\) \(p<0.4009\) \(Q=0.0000\)
- BvC \(t(18)=4.69\) \(p<0.0002\) \(Q=0.0000\)
- BvD \(t(18)=4.51\) \(p<0.0003\) \(Q=0.0000\)
- BvE \(t(18)=5.76\) \(p<0.0001\) \(Q=0.0000\)
- BvF \(t(18)=4.75\) \(p<0.0002\) \(Q=0.0000\)
- BvG \(t(18)=5.24\) \(p<0.0001\) \(Q=0.0000\)
- BvH \(t(18)=4.51\) \(p<0.0003\) \(Q=0.0000\)
- CvD \(t(18)=0.80\) \(p<0.4313\) \(Q=0.0000\)
- CvE \(t(18)=0.74\) \(p<0.4698\) \(Q=0.0000\)
- CvF \(t(18)=0.63\) \(p<0.5391\) \(Q=0.0000\)
- CvG \(t(18)=0.25\) \(p<0.8061\) \(Q=0.0000\)
- CvH \(t(18)=0.80\) \(p<0.4313\) \(Q=0.0000\)
- DvE \(t(18)=1.65\) \(p<0.1161\) \(Q=0.0000\)
- DvF \(t(18)=0.20\) \(p<0.8400\) \(Q=0.0000\)
- DvG \(t(18)=1.12\) \(p<0.2785\) \(Q=0.0000\)
- DvH \(t(18)=0.00\) \(p<1.0000\) \(Q=0.0000\)
- EvF \(t(18)=1.47\) \(p<0.1595\) \(Q=0.0000\)
- EvG \(t(18)=0.51\) \(p<0.6169\) \(Q=0.0000\)
- EvH \(t(18)=1.65\) \(p<0.1161\) \(Q=0.0000\)
- FvG \(t(18)=0.93\) \(p<0.3641\) \(Q=0.0000\)
- FvH \(t(18)=0.20\) \(p<0.8400\) \(Q=0.0000\)
- GvH \(t(18)=1.12\) \(p<0.2785\) \(Q=0.0000\)
ANOVA: Between Groups Design

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PAIRWISE COMPARISONS [Q=TukeyHSD: *=p<0.05 **=p<0.01]

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