

The Effect of Enzymes on Food Stuff

Stuff

Degradation of Egg White Proteins in the Presence of the enzyme – Pepsin

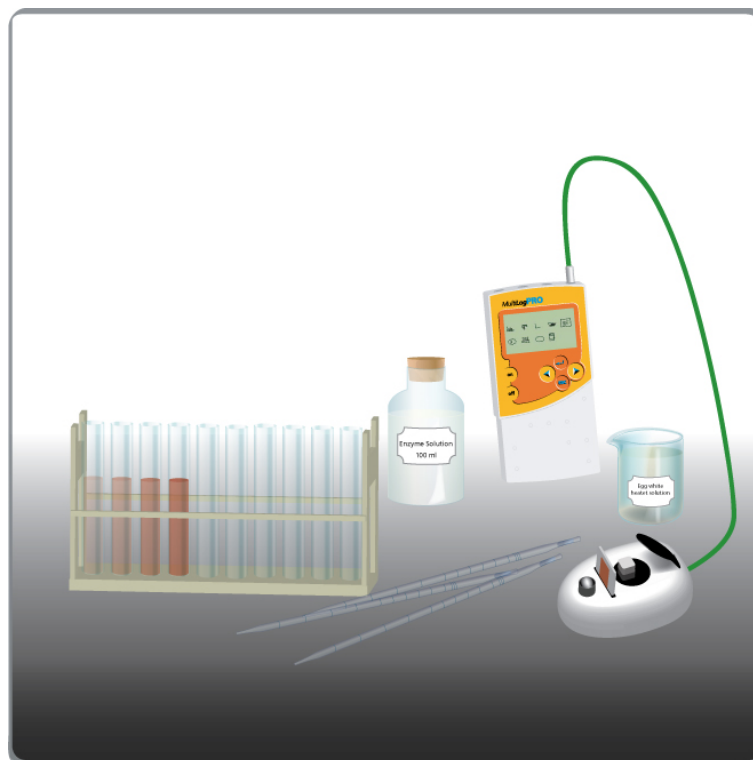


Figure 1

Introduction

Pepsin, trypsin and chymotrypsin are three enzymes that degrade proteins found in our food stuff. Each of the three enzymes cleave the proteins at a different location and together they degrade the proteins to short peptides and to the building blocks: amino acid, which are readily absorbed by the intestine lining.

Pepsin is produced in the mucosal lining of the stomach. It is secreted in an inactive form, trypsinogen, and are then converted to an active form, pepsin, under very low pH conditions: 1.0-3.0. Pepsin optimal activity is obtained at this range of pH
Pepsin is used in the preparation of cheese and other protein-containing foods.



In this experiment, degradation of egg white proteins by pepsin is followed using a colorimeter sensor. Egg white proteins are first heated to create a turbid solution. Upon degradation the solution becomes clear.

Equipment

- One Egg White
- 100ml 0.2N HCl solution
- 20ml Pepsin solution (use a powder of pepsin of about 525 Units/mg solid, 4770 Units/mg protein. Dissolve the powder in distilled water. For optimal activity the concentration of enzyme may vary between 0.1% and 0.5%. It should be checked in advance)
- Bunsen burner
- Thermometer (or a Temperature sensor)
- 400-600ml flask
- 5ml and 1ml pipettes
- Stand with 10 tubes
- Colorimeter sensor
- MultiLogPRO

Equipment Setup Procedure



1. Connect the MultiLogPro to the serial port of the computer.
2. Turn the MultiLogPro on.
3. Connect the colorimeter sensor to the I/O 1 port of the MultiLogPro
4. Assemble the equipment as illustrated in figure 1 below.
5. Set the MultiLogPro up according to the setup specified below. You can set up the MultiLogPro either by using the MultiLogPro keypad or using the

Setup Wizard in MultiLab (click Setup Wizard  on the main toolbar).

MultiLogPRO Set Up

- Input 1: Colorimeter
- Rate: Every second
- Recording time: 08:20 MM:SS (500 Samples)


Experimental Procedure

1. Prepare the Egg White Solution:
 - a. add 40ml distilled water to 10ml Egg white.
 - b. Mix it rapidly with a fork and filter it through 4 layers of gauze.
 - c. Heat the solution up to 55-60°C (not above this temperature!!) with constant stirring till a turbid solution is obtained. At this stage the solution should resemble diluted milk.
 - d. This solution is the **substrate** used in the experiment. Keep it in a small flask.
2. Calibrate the colorimeter:
 - a. Use the Red Filter.
 - b. Prepare a blank solution: to 3ml distilled water, add 1ml enzyme solution.
 - c. Pour the blank into a cuvette and insert it into the colorimeter. Close the cover well.
 - d. Start the MultiLogPro either from the MultiLogPRO Panel or from MultiLab: click Run  on the main toolbar.
 - e. Turn the knob till you receive 100% transmission.
 - f. Stop the MultiLogPRO.
3. Measure the pH of the reaction solution:
 - a. Add to a tube - 2.4ml Egg white solution
0.6ml 0.2N HCl
1.0ml of water
 - b. Measure the pH of the solution using the pH sensor.
It should be in the range of 2.0-3.0.
If necessary, adjust the pH by changing the volume of 0.2N HCl you add.
4. Measure Rate of Protein Degradation:
 - a. Add to the cuvette - 2.4ml Egg white solution 0.6ml 0.2N HCl
 - b. Start the MultiLogPRO either from the MultiLogPRO Panel or from MultiLab: click Run  on the main toolbar.
 - c. Add to the cuvette 1ml Enzyme solution.
 - d. Mix well with a wooden stick and insert the cuvette immediately into the colorimeter. Close the cover well.

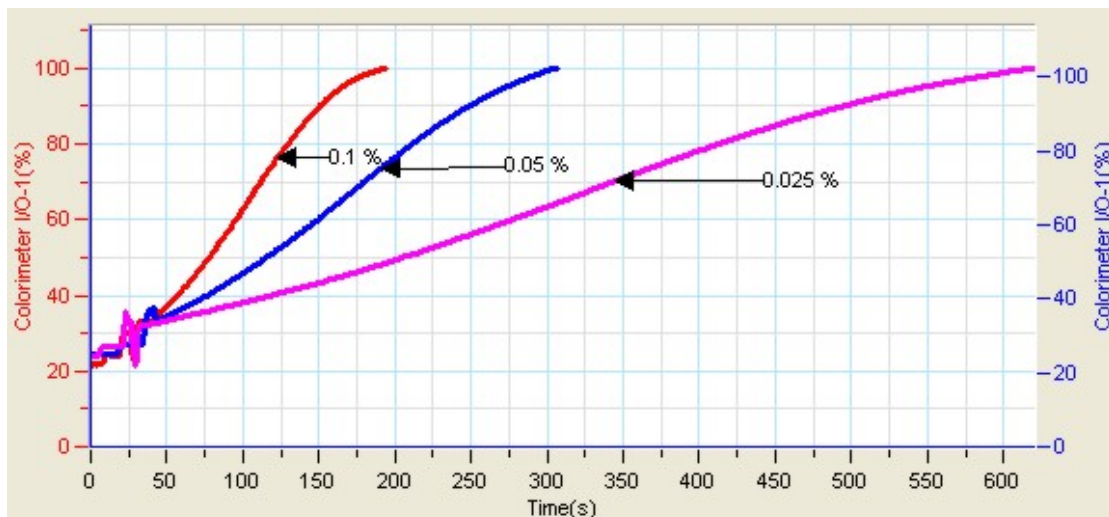


- e. Follow changes in light transmittance registered on the computer monitor during the experiment.
4. Repeat section 3 with at least 2-4 different enzyme concentrations.

Data Analysis

1. The rate of Protein degradation is calculated from the rate of change in light transmission.
Apply a linear fit to the curve you received with each enzyme (or substrate) concentration:
 - a. Use the cursors to select the desired range
 - b. Click Linear fit  on the main toolbar. The fit equation will be displayed in the information bar at the bottom of the graph window
The slope of the fit line is the net reaction rate.

An example of the graphs obtained in this experiment, is shown below:



2. Prepare a graph describing the relation between Enzyme (or Substrate) concentrations and the rate of Protein degradation.

Questions

1. Describe the graph you prepared showing the relation between enzyme (or substrate) concentration and rate of protein degradation.
2. What is the effect of an increase in enzyme concentration on the rate of protein degradation?
3. What is the effect of an increase in substrate concentration on the rate of protein degradation?

4. Assume, what will be the rate of degradation of another protein by pepsin?
5. How will a change in pH affect the rate of degradation of egg white proteins by pepsin?

Further Suggestions

1. Measure the effect of pH on pepsin activity in the range of 1-10: use either buffer solutions or add different volumes of 0.2N HCl or 0.2N Na_2CO_3 .
2. Measure the effect of temperature on pepsin activity: incubate substrate and enzyme mixture (at concentrations giving optimal activity) at different temperature. Every 1-2 min take out samples and measure their transmittance using a colorimeter.