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Section A07, Thu 9-11:50 am

Partner: Sh. G

Lab 5 (Determining enzyme activity of turnip)

Purpose/ Objective:

The purpose of this lab is to measure the enzyme activity of a turnip. We used blanching method to determine different sample's absorbance in different time periods in this lab.

Introduction:

Enzymes are large biological molecules responsible for the thousands of metabolic processes that happen in our life. They are highly selective catalyst. They accelerate both the rate and specificity of metabolic reactions. Without digestive enzymes, our food would sit in our stomach for weeks. The purpose of enzymes is to break down large molecules (to smaller pieces). For humans, digestive enzymes break down the foods we eat so the nutrients, vitamins, and minerals can be absorbed into the blood stream and carried throughout the body.

Foods also contains enzyme. Enzymes catalyze chemical reactions. During the preservation of foods, these reactions can be detrimental and shorten the storage life of a product; this happen even in frozen food because enzyme continue to function at low temperature. Quality attributes related to color, flavor, texture, odor and nutritional value can all be affected by enzymatic action and therefore inactivation of enzymes is essential to maintenance of a high quality frozen food product. Enzymes are protein and can be denature by high pH, high salt and high temperature. Once they denature they are no longer active. Blanching is a mild heat treatment used to inactivate enzyme before freezing. Blanching is a process that a vegetable or fruit, is plunged into boiling water, removed after a specific time, and finally plunged into iced water or placed under cold running water. Preserving the food is very important so the food doesn't get bad and give us food poison, so it is very important to know how to inactivate the enzyme of the food so it doesn't change the color of them. In some cases we get favorable color change with the help of enzyme like dried fruit and cider but in other cases like fresh fruit is bad

because it make is brown. Sometimes we can slow down the work of enzyme by adding acid like lemon juice or taking the oxygen away from fruit and vegetable.

Procedure:

The procedure followed for the experiment is found in "Principles of Food Composition Laboratory Manual" (2013) Experiment 2, Acidity in Foods, pages 49-55

Modifications: the time in sec for the absorbance is different in lab manual and the lab procedure. (for example 20 sec and 25 sec…)

Data/ result:

Table 1. Absorbance and times.

Time (sec)	Abs 0 sec	Abs 15 sec	Abs 30 sec	Abs 15 sec	Abs 60 sec	Abs 90 sec	Abs 120 sec
5	0.330	0.239	0.216	0.239	0.139	0.090	0.160
10	0.520	0.380	0,352	0.380	0.240	0.164	0.020
15	0.690	0.509	0,490	0.509	0.347	0.244	0.025
20	0.830	0.634	0.616	0.634	0.420	0.317	0.030
25	0.980	0.756	0.722	0.756	0.516	0.385	0.035
30	1.100	0.855	0.835	0.855	0.598	0.450	0.040
35	1.240	0.955	0.930	0.955	0.678	0.502	0.046
40	1.350	1.035	1.015	1.035	0.744	0.564	0.058
45	1.460	1.13	1.10	1.13	0.815	0.622	0.063
50	1.540	1.21	1.20	1.21	0.885	0.676	0.071
55	1.640	1.29	1.27	1.29	0.950	0.726	0.076
60	1.700	1.37	1.34	1.37	1.010	0.780	0.830
90	1.99	1.70	1.66	1.70	1.350	1.070	0.129
120	1.99	1.90	1.85	1.90	1.560	1.30	0.174

Figure 1.1 graphs of absorbance versus time (second)

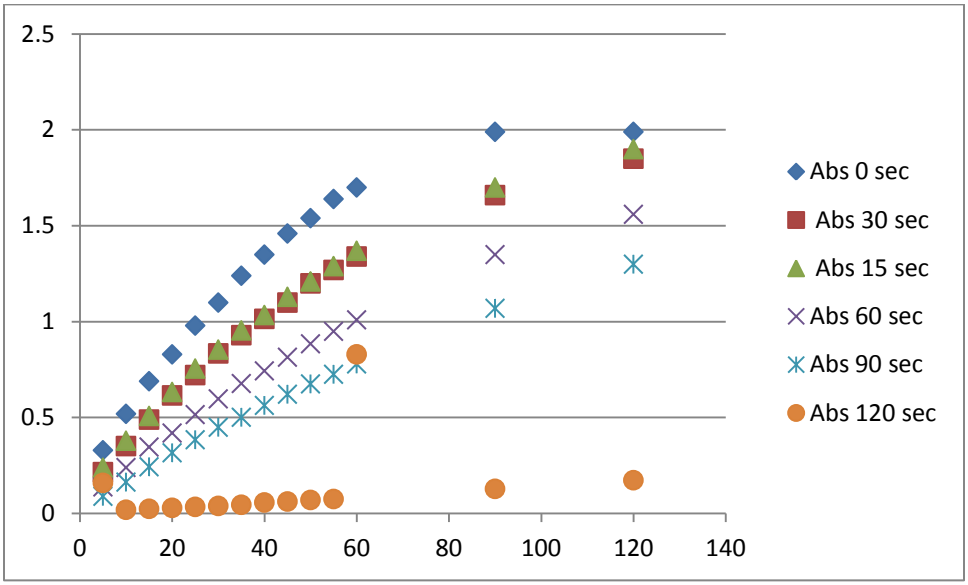


Figure 1. 2 graph of Absorbance versus time (fitted slope, less than 40 seconds for linear slope)

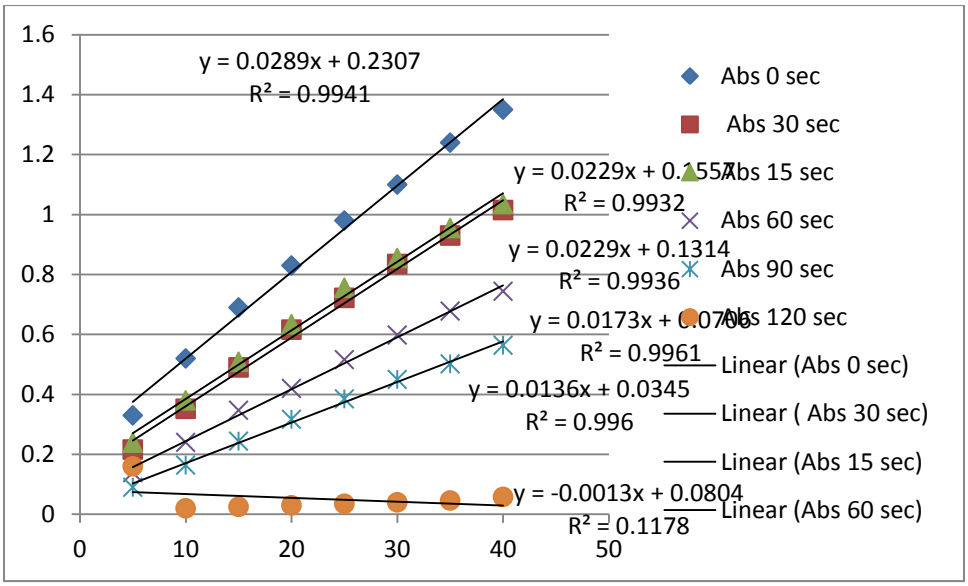
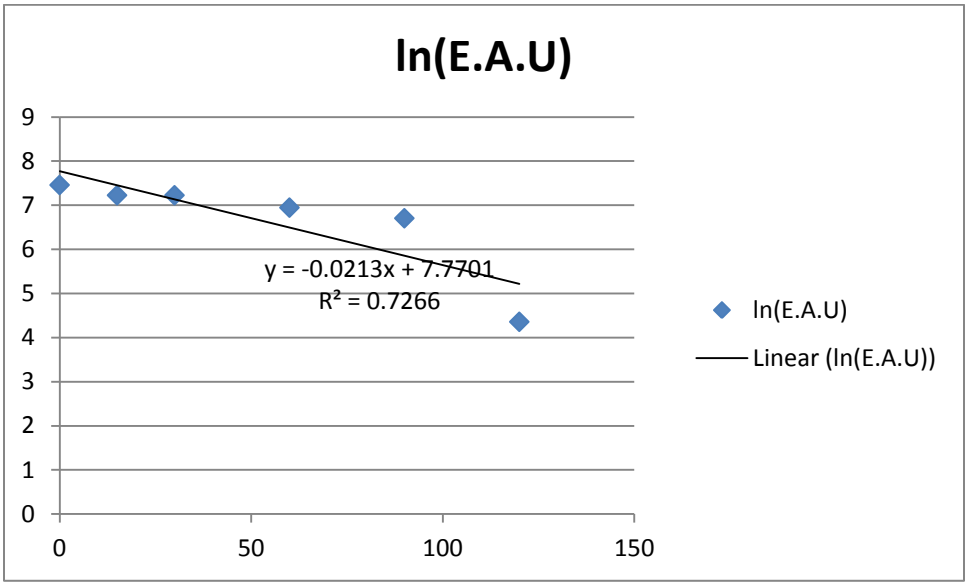


Table. 2. The slope. Slopes and enzyme activity (EAU)

blanching time	Slope (au/sec)	au/min	E.A.U	ln (E.A.U)
0	0.0289	1.734	1734	7.458186
15	0.0229	1.374	1374	7.225481
30	0.0229	1.374	1374	7.225481
60	0.0173	1.038	1038	6.945051
90	0.0136	0.816	816	6.704414
120	0.0013	0.078	78	4.356709

Figure 2. Graph of ln of enzyme activity (EAU) versus blanching time (sec)



Calculation:

Sample calculation

Enzyme activity sample

010 a.u/min * 1 EAU /0.001 au/ min

To convert the slop (sec to slop in min

Multiply the slope by 60

0.0289 au/sec * 60 = 1.734

Then 1.734 au/min * 1000 to get EAU = 1734 EAU

Half life calculation:

Using equation from page 51 lab manual:

If [A}0/[A]t = 2

Then e^ (kt)= 2

Kt = ln 2= 0.693

K is the slope (constant, form the figure 2)

T(1/2) = 0.693/ 0.7266

Half life (t1/2)= 0.9537 sec

Discussion:

Blanching is a method to shock the enzyme; first we put the sample in hot water and then put it in ice bath. Blanching causes enzyme inactivity by denaturing the structure of the protein. The more time the sample is heated the more it gets deactivated. The sample that is lease heated has the highest enzyme activity. We had to do different time blanching and then get their absorbance. The sample that was zero heated had the highest enzyme activity and very difficult to get their absorbance. In the half life formula K is a constant and represents the slop. 40 seconds was the best because as we see in our graph till 40 sec the line is linear and after that we see a curve. Turnips were cut the same size so all the pieces had approximately the same amount of enzyme. They were cut into uniform ¼ - ½. We tried to cut them the same size and we used ruler.

By looking at the figure 1, we can easily say that the enzyme activity will be reduced with the more time of blanching in boiling water. By looking at figure 2, the half –life of an enzyme represent the time that is needed to denature half of the enzyme. The structure of protein and enzyme is very important in the time of blanching. Some proteins need more temperature to get denature than others.

Conclusion:

Blanching method was used in this lab to determine the enzyme activity of the turnip. The more sample was heated the lease enzyme activity we saw. Enzyme is protein and heat can denature the 3 dimensional structure of it so the activity of it will decrease. Higher and more time temperature causes the protein (enzyme) to get more denatures and deactivate. Half life of enzyme is the ½ of the time that takes an enzyme gets denature. This lab was easy and inexpensive to do. We learned how to inactive enzyme activity in vegetable and fruits that we don't want them to turn brown or bad.

Question:

1. The half life of thee enzyme represent the time that will take for an enzyme to be half denatured and the structure changes.
2. Yes, we used boiling water to blanch the turnips. The 100 degree of water is used in order to blanch and denature the enzymes in the turnip.
3. Most blanched sample has the least enzyme activity. We measured the longest time blanching sample at the beginning. This sample gives us slower absorbance time so we can get used to working with the machine.