

Food Science and Technology 605
 Lab: Enzymatic Browning: Kinetics of Polyphenoloxidase

Objectives

1. To review some basic concepts of enzyme kinetics
2. To gain some experience in the preparation of a crude enzyme extract
3. To perform an enzyme assay
4. To study the kinetics of polyphenoloxidase

Apparatus and Instruments

1. Visible spectrophotometer and cuvetts
2. Pipettes, 2 and 5 ml
3. Pipetor, 250 – 1000 μ l
4. Test tubes
5. Parafilm
6. Small blender
7. Whatman no.1 filter paper
8. Knife
9. Ice bucket
10. Erlenmeyer flask, 125 ml
11. Beaker, 100 and 600 ml
12. Graduated cylinders, 50 ml
13. Paper towels
14. Forceps
15. Hot plate

Reagents and Materials

1. Small potatoes (held in refrigerator overnight)
2. Ice (in the ice bucket)
3. Buffer A: sodium phosphate buffer, 0.1M, pH 6.8, containing 0.1M NaF
4. Buffer B: sodium phosphate buffer, 0.1M, pH 6.8
5. Dopa: 4mg/ml in buffer B

Table 8.1 Volumes of buffer, substrate, and enzyme extract for PPO assays

Reagent	Tube Number ^a											
	1	2	3	4	5	6	7	8	9	10	11	12
Buffer B ^b , ml	2.5	2.4	2.3	2.2	2.1	2.0	1.9	0.8	0.7	0.6	0.5	0.4
Dopa solution ^d , ml	-	0.1	0.2	0.3	0.4	0.5	0.6	2.0	2.0	2.0	2.0	2.0
Enzyme extract, ml	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.2	0.3	0.4	0.5	0.6

^a Total volume in each tube should equal 3.0 ml

^b 0.1M phosphate buffer, pH 6.8

^d 4mg dopa / ml buffer B, pH 6.8

Preparation of Crude Enzyme Extract

1. Peel a cold, raw potato and cut into small pieces
2. Rapidly weigh about 10g potato and mix with 50 ml ice cold buffer A
3. Grind the mixture in a blender for about 1 min
4. Filter the mixture with Whatman no.1 paper into an iced 125ml Erlenmeyer flask and hold on ice until needed

Enzyme Assay

1. Transfer the volumes of buffer B and dopa solution shown in Table.8.1 to plastic cuvettes labeled 1 through 12, taking care to pipette as accurately as possible.
2. Set the wavelength on the spectrophotometer to 475nm and zero the instrument against distilled water. Re-zero after each assay.
3. When everything is set, initiate the reaction by adding the enzyme extract. Mix well and begin recording absorbance reading immediately. To do this, one person should make and record the readings while the other watches the clock and indicates when readings should be taken. Take readings at 15s intervals for 2 min for each tube. Record all readings on a data sheet.

Data Treatment

1. Make a plot of absorbance versus time for each tube. Estimate the rate of reaction in each tube from the linear portion of the absorbance versus time curve.
2. Determine reaction velocities for tubes 2-7. Express reaction velocity as millimoles IQ per liter per minute.
3. Construct a plot of velocity versus [S] for tubes 2-7.
4. Construct a Lineweaver-Burk plot from data for tubes 2-7. Determine K_M and V_{Max} for your enzyme.
5. Determine the enzyme activities (rates) for tubes 8-12. Plot activity versus volume of enzyme extract used. Is your plot linear? If it is, is this what you expected? Why? If it is not, what might be a plausible explanation?