

Blanching effectiveness

Objectives

To practice testing for blanching adequacy

Apparatus and Instruments

1. Visible spectrophotometer and cuvettes
2. Pipettes, 1, and 2ml
3. Test tubes
4. Parafilm
5. Small blender
6. Whatman no.1 filter paper
7. Knife
8. Ice bucket
9. Erlenmeyer flask, 125ml
10. Beaker, 600ml
11. Graduated cylinders, 50ml
12. Forceps
13. Hot plate
14. water baths, 30, 60, and 80°C

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, pH 6.8
6. Guaiacol solution (1% v/v in 95% ethanol)
7. Hydrogen peroxide (0.5% v/v)

Blanching of the raw potatoes

1. Blanch a potato at the assigned temperature (in the proper water bath or in the beaker with boiling water) for the following times 0, 2.5, 5, 10, 20, 30 minutes
2. Immediately put the potato on ice to cool it down

Preparation of Crude Enzyme Extract

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

Enzyme Assay

For *Polyphenoloxidase*:

1. Transfer 2.0ml of buffer B and 0.5 ml of dopa solution to cuvettes
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 0.5ml of the enzyme extract to the prepared cuvet. Invert to mix and begin recording absorbance readings 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).
4. Take readings at 15s intervals for 2min for each tube. Record all readings in the table.

Blanching temperature = ___ °C

Absorbance recorded every 15 seconds.

Time of absorbance reading	Blanching time (min)					
	0	2.5	5	10	20	30
0"						
15"						
30"						
45"						
1' 00"						
1' 15"						
1' 30"						
1' 45"						
2' 00"						

For *Peroxidase*:

1. Transfer 0.6ml of buffer B and 0.6ml of the enzyme extract to a test tube
2. Incubate for 5 minutes at 30°C
3. Transfer the content of the test tube to a cuvet
4. Set the wavelength on the spectrophotometer to 410 nm and zero the instrument against distilled water. Re-zero after each assay.
5. When everything is set, initiate the reaction by adding 1.2ml of the hydrogen peroxide solution and 0.6ml of the guaiacol solution to the prepared cuvet. Invert to mix and begin recording absorbance readings immediately
6. Take readings at 15s intervals for 2min for each tube. Record all readings in the table.

Blanching temperature = ___ °C

Absorbance recorded every 15 seconds.

Time of absorbance reading	Blanching time (min.)					
	0	2.5	5	10	20	30
0''						
15''						
30''						
45''						
1' 00''						
1' 15''						
1' 30''						
1' 45''						
2' 00''						