University "Dunarea De Jos", Faculty of Food Science and Engineering, Galati

Kinetic study of the thermal & high pressure inactivation of horseradish peroxidase- HRP

Student's Research Session, Galati- mai 2013

Grounds

- The kinetics of peroxidase thermal inactivation in purified horseradish extract is reported by many recent researches (citation).
- The actuality of this topic is also confirmed by the high number of publications having horseradish as a central topic (~10,000 articles in Science Direct data base).
- However there is room for more in depth studies on the high pressure thermal inactivation of HRP crude extract

Objectives

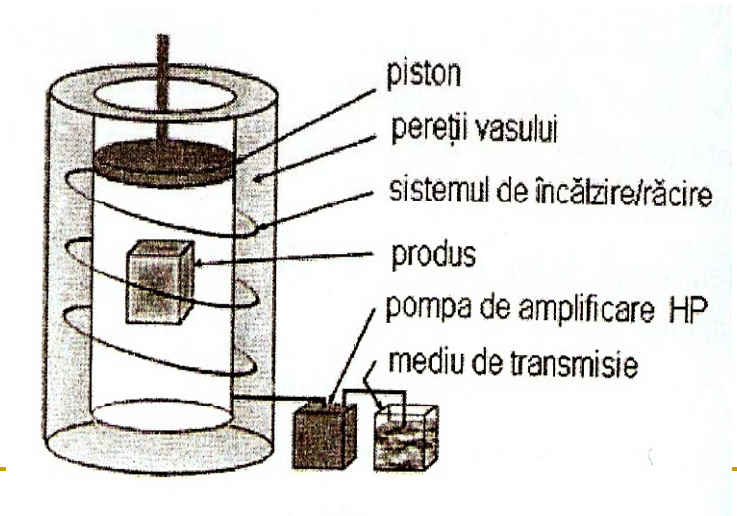
- Separation and partial purification of horseradish peroxidase.
- Kinetic study of thermal inactivation of horseradish peroxidase.
- Kinetic study of the combined treatment: high pressure and thermal treatment of horseradish peroxidase.
- Mathematical modeling of the inactivation.

Materials

- Horseradish extract
- 0.2 M potasium phosphate buffer (pH=6,6)
- 0.1 mM Disodic E.D.T.A
- 10g/kg P-phenylenediamine
- 15g/kg Hydrogen peroxide

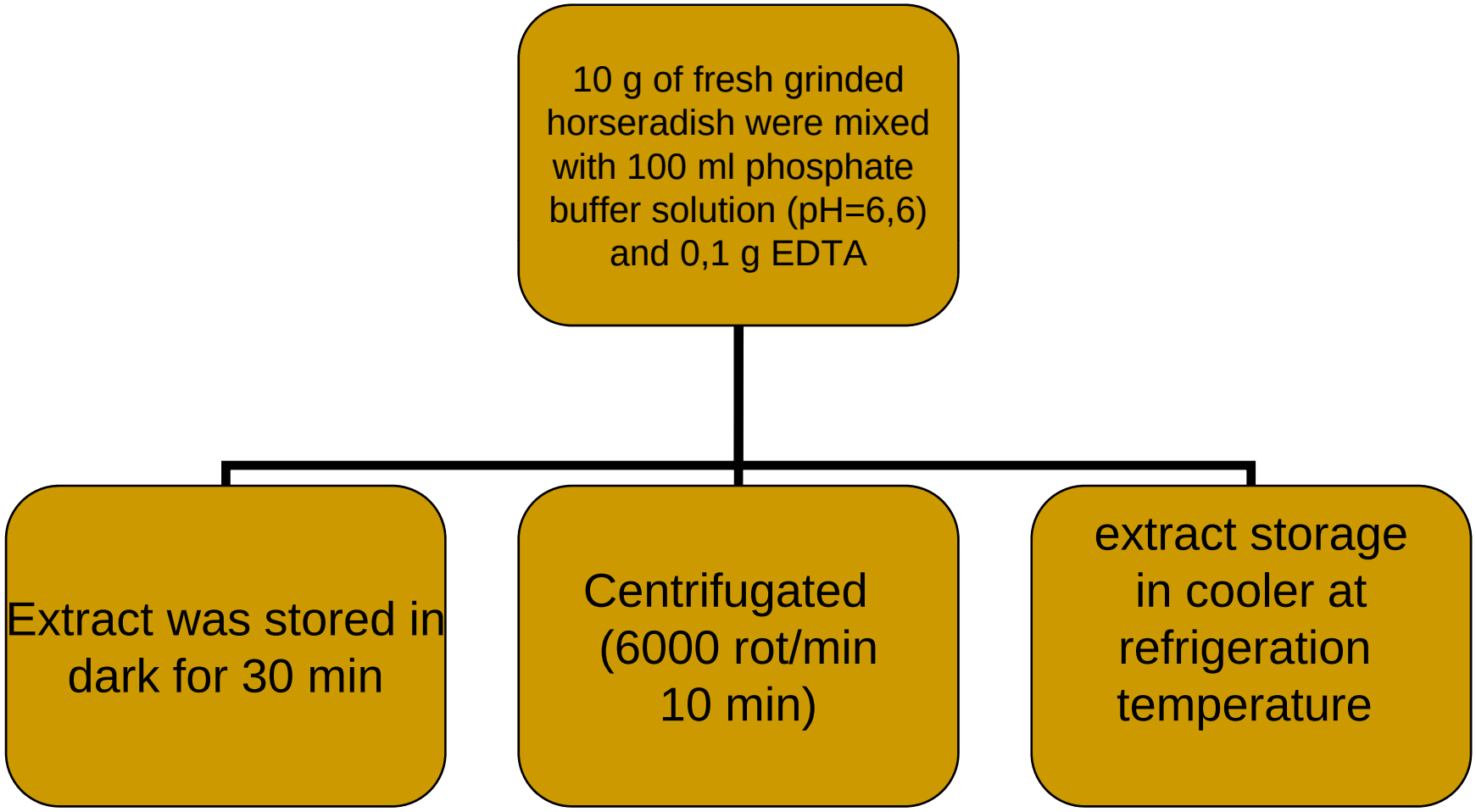
Methods

- *Spectrophotometric method:*
- Enzyme relative activity was determined as the absorbance at 485nm
- *High pressure processing equipment*



- It consists of four main parts:
- Pressure vessel and pressure sealing
- The pressure generating system
- Heating and cooling system
- The sample treated

Crude extract

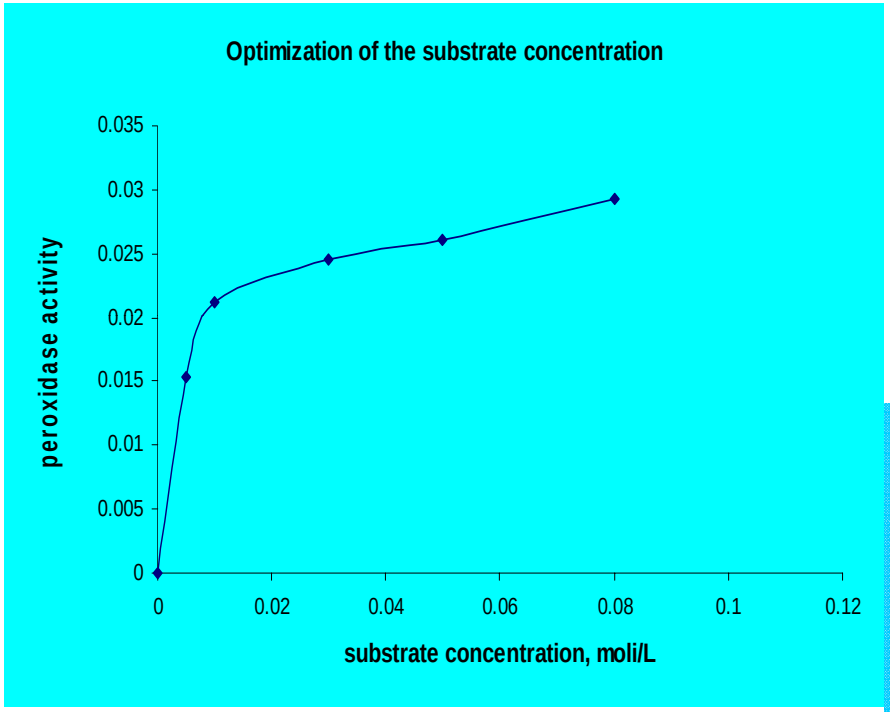


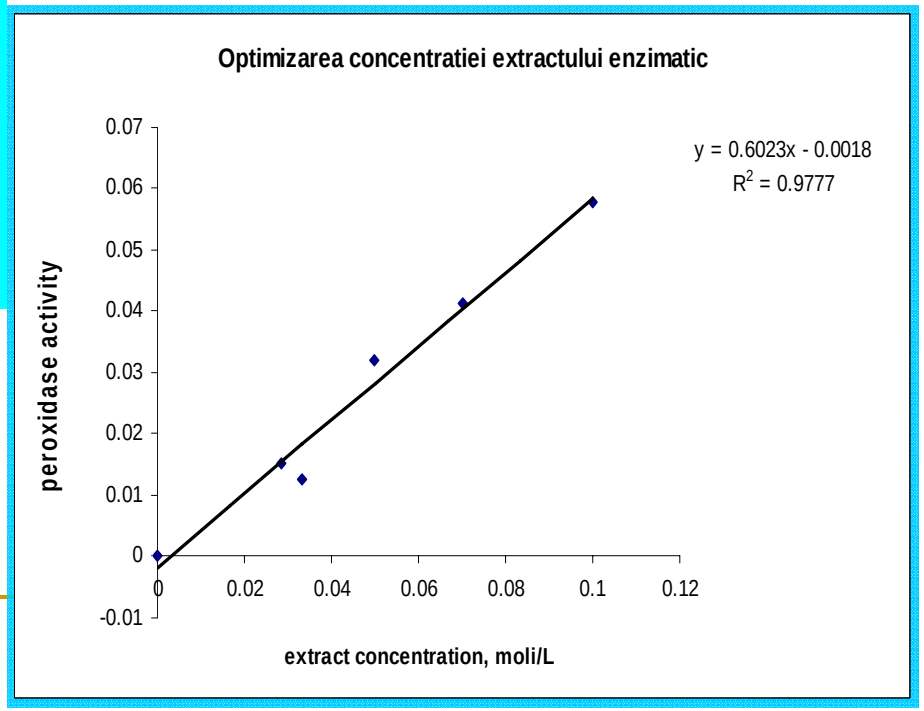
Results and Discussion

Kinetic inactivation studies: data-analysis

Formulation of an inactivation model

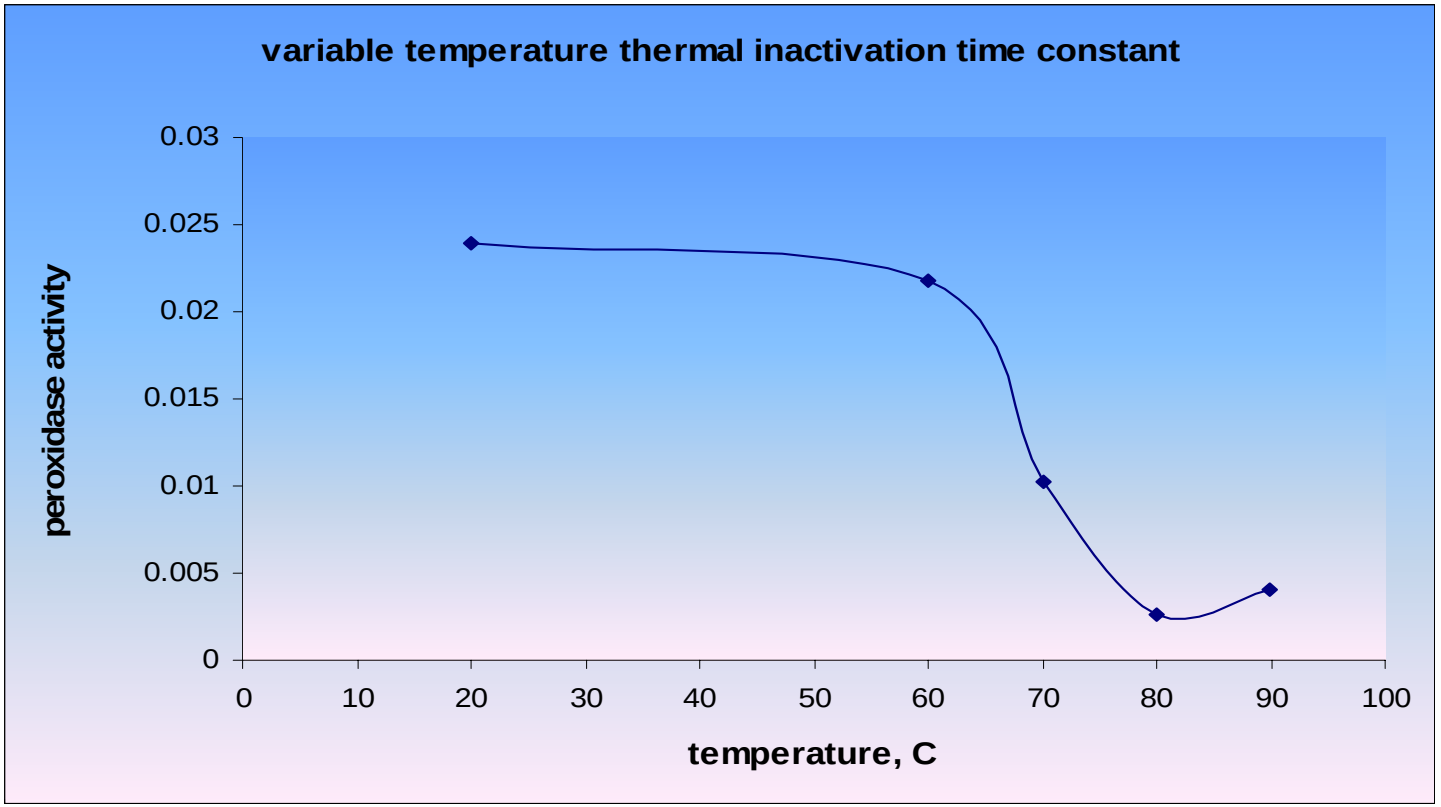
optimization – detection limits



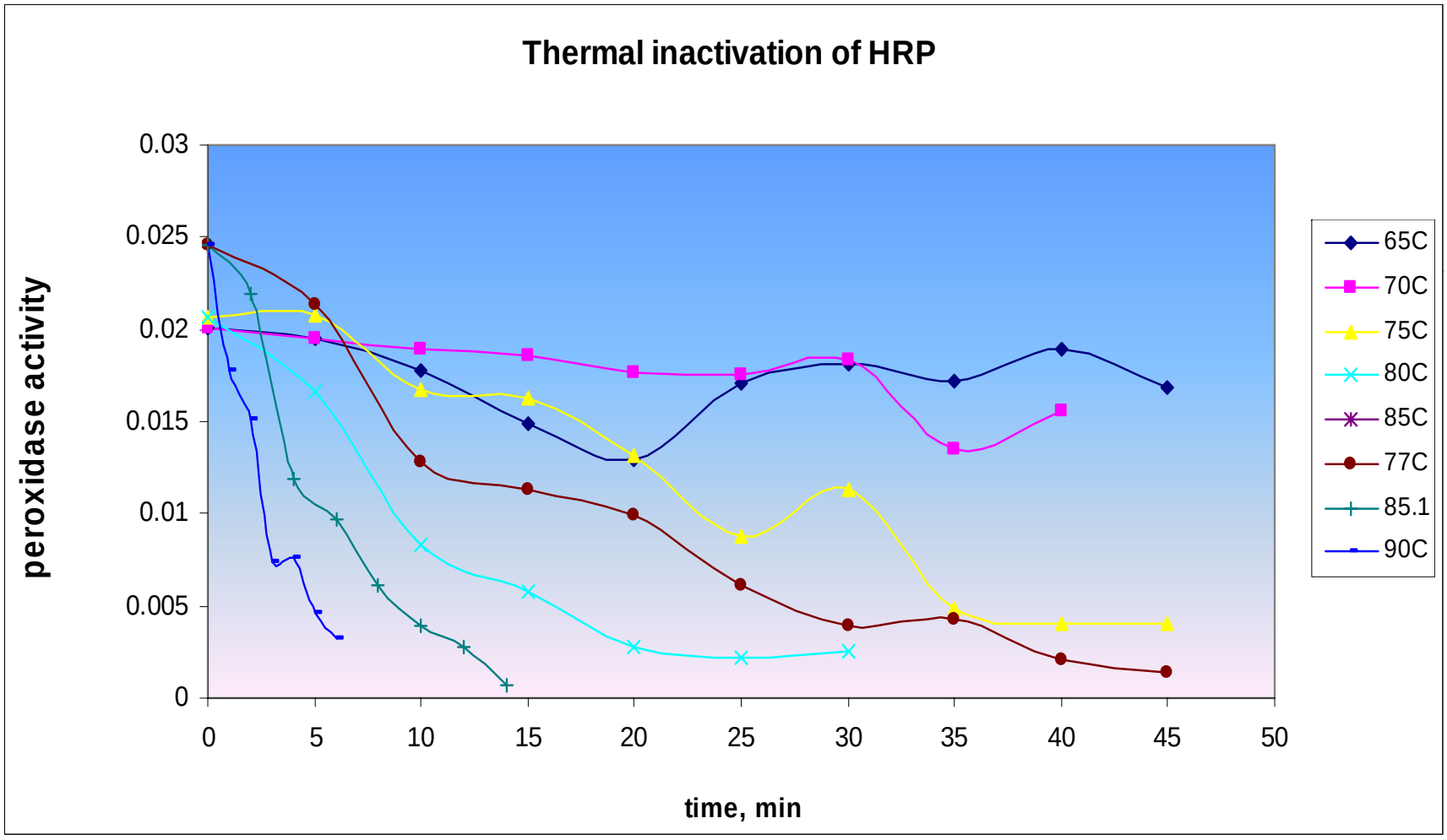


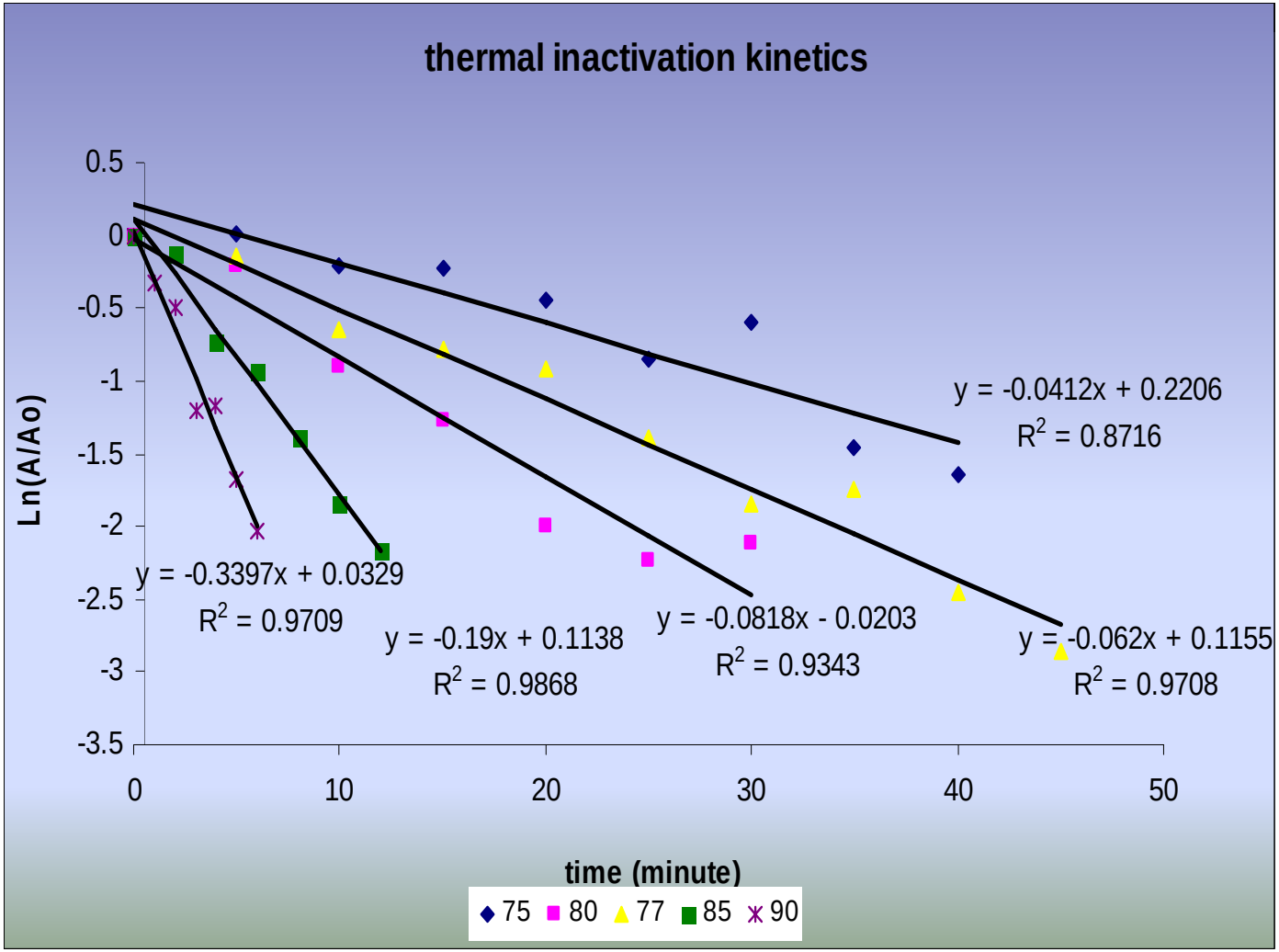
Kinetic of thermal inactivation

a. *HRP activity dynamics with temperature*



b. Thermal inactivation kinetics of HRP



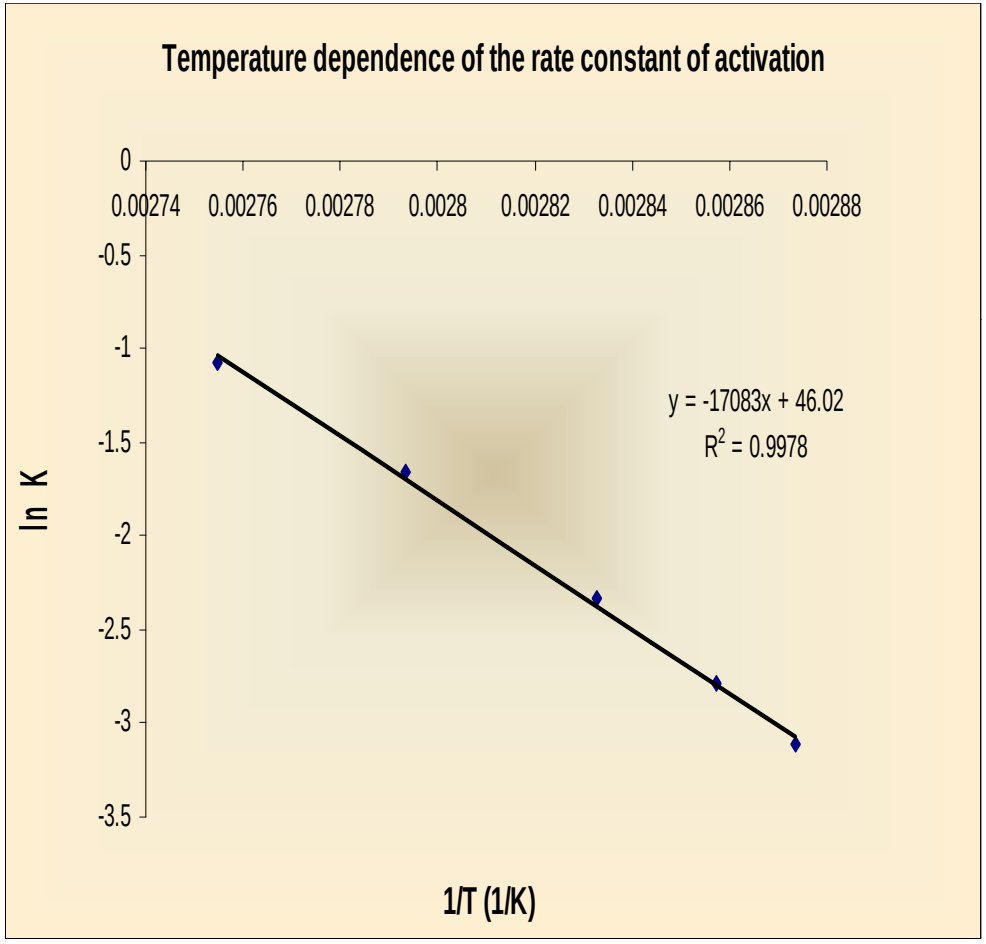

thermal inactivation kinetics
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
Ln(A/Ao)
0
10
20
30
40
50
time (minute)
y = -0.0412x + 0.2206
R2 = 0.8716
y = -0.3397x + 0.0329
R2 = 0.9709
y = -0.19x + 0.1138
R2 = 0.9868
y = -0.0818x - 0.0203
R2 = 0.9343
y = -0.062x + 0.1155
R2 = 0.9708
75
80
77
85
90

Kinetic parameters

- *Activation energy*

Ea= 142.0281 kJ/mol

- suggest a high dependence of the inactivation rate constant to temperature
- there is a good correlation between the experimental points and regression (R = 0.99)



inactivation kinetics at combined treatment