Immobilization and catalytic properties of pumpkin seed lipase on chitosan

Pimwouth Natisoontorn

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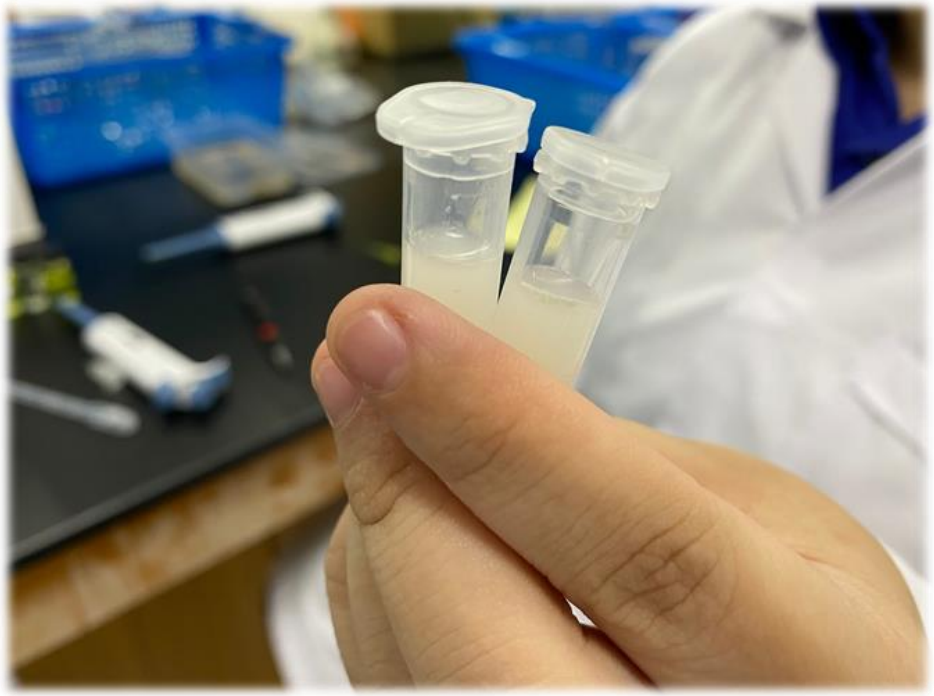
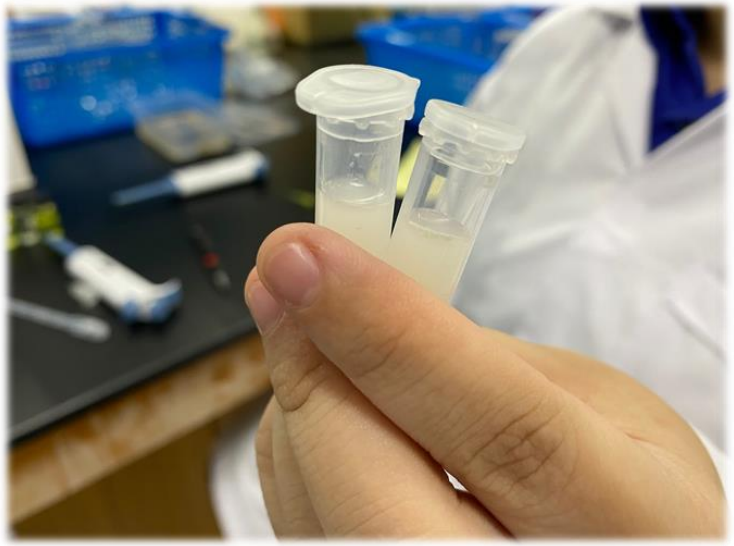
Outline

- Introduction
- Methodology
- Results
- Conclusion



Lipase

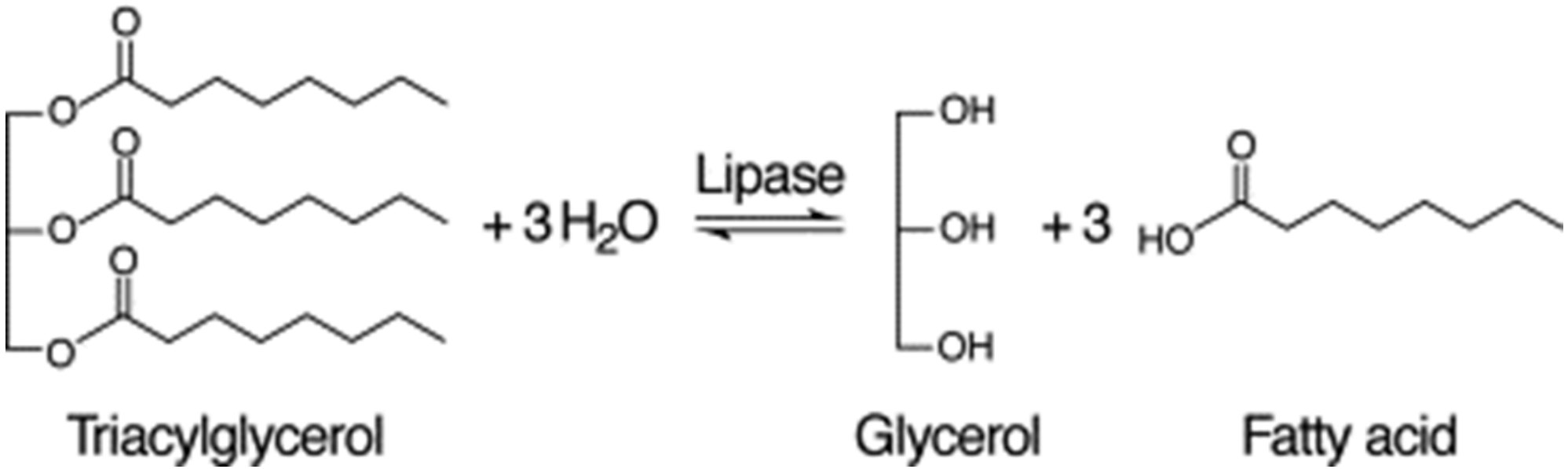


Application of Lipase

Introduction

Enzyme from plants

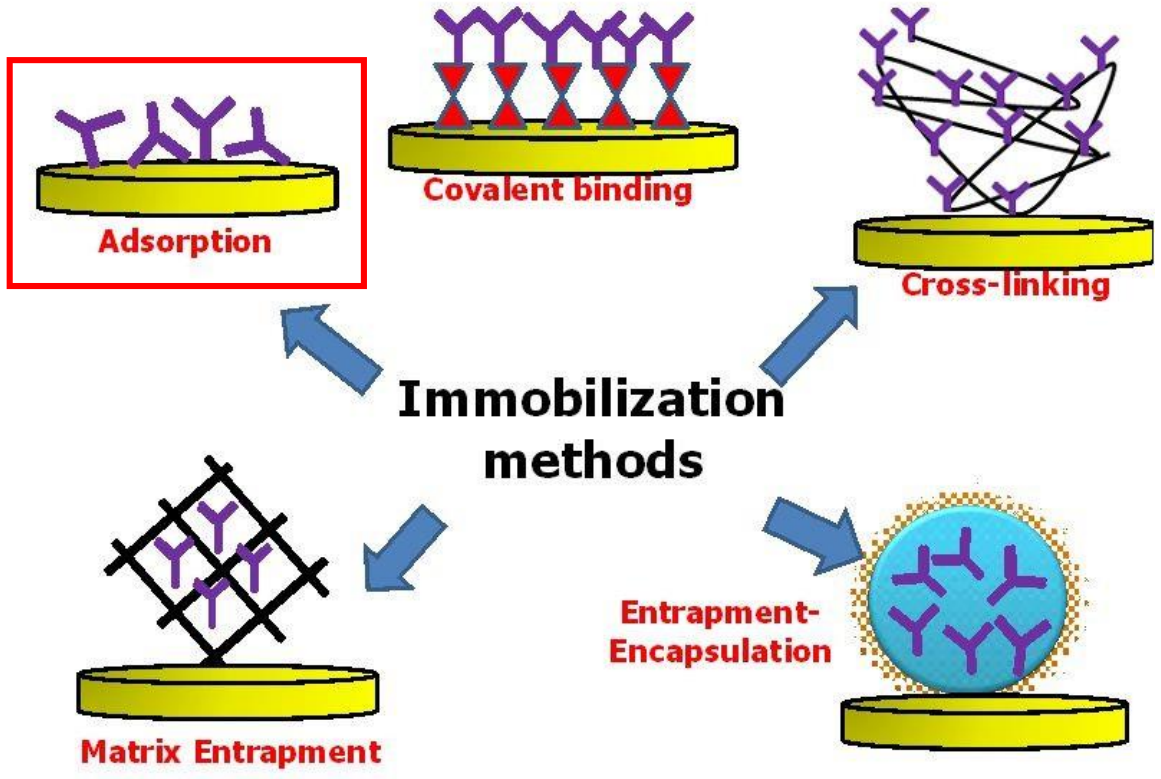
-> Low production cost. We extract from Pumpkin Seeds



Introduction

Immobilizing enzyme

-> Is to bind enzymes into insoluble solid



> OUR PROJECT <

focuses on

Chitosan-immobilized

lipase!

https://www.intechopen.com/books/biosensors-micro-and-nanoscale-applications/nanomaterials-for-advancing-the-health-immunosensor

Problem

Which pH and temperature do crude extracted lipase and immobilized lipase have highest activity?

Hypothesis

The immobilized enzyme is less effective, but it can withstand more severe conditions Ex. pH, Temperature

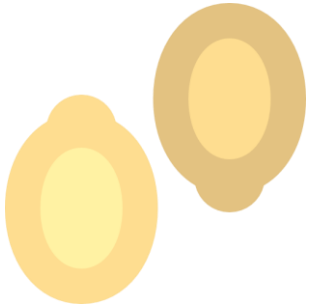
Objectives

- To extract lipase from germinated pumpkin seeds
- To study lipase activity at various pH and temperatures
- To study properties of lipase and immobilized lipase

Project scope

- We extract the enzyme from germinated pumpkin seeds
- We use physical adsorption method on Chitosan

Part1: Lipase Extraction



Germinate Pumpkin seeds for 3 days

Hexane

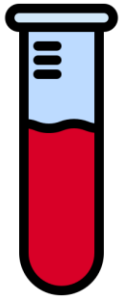
shake 1 hr

Defatted Seed

Centrifuge 10000x g

shake 30 min

Phosphate Buffer

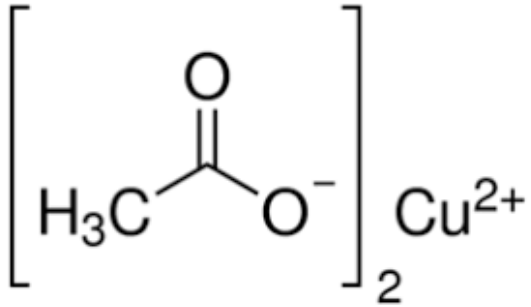


Distilled Water

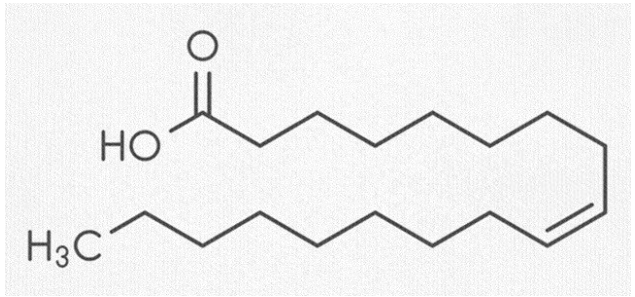
Marcel AVRAMIUC (2016)

Part 2 : Lipase Activity Assay

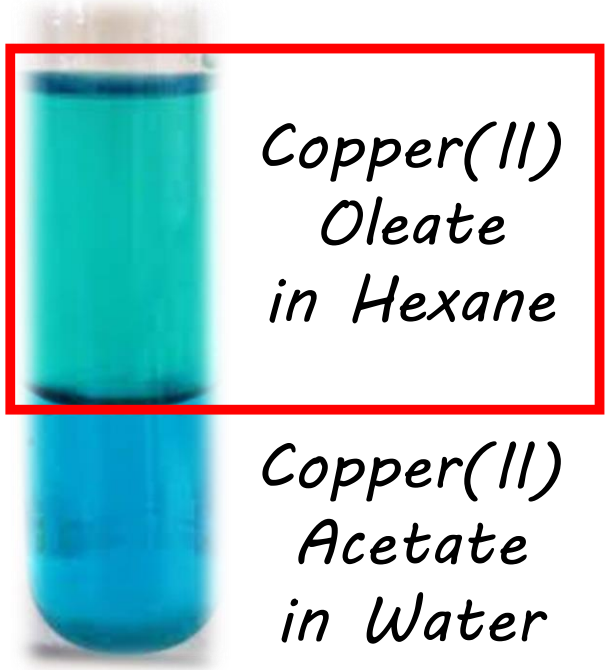
Copper Acetate Test

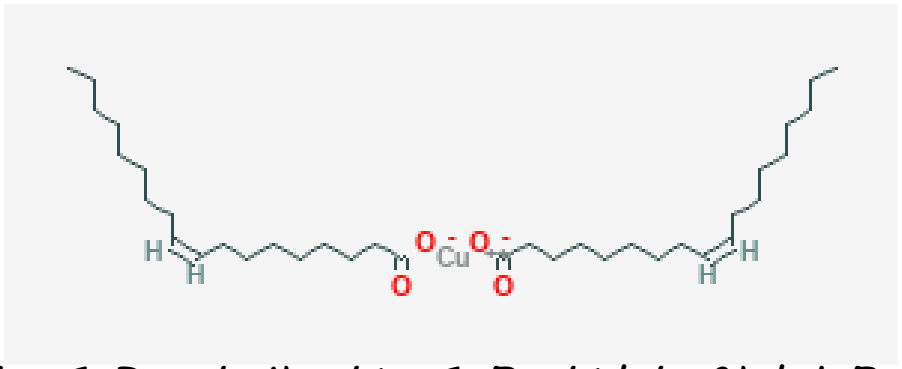


Copper(II) Acetate



Oleic Acid



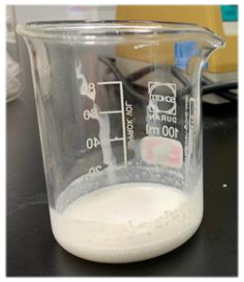


Copper(II) Oleate
Stable & Clear

(Anuradha Balan,1 Darah Ibrahim,1 Rashidah Abdul Rahim,2 and Fatimah Azzahra Ahmad Rashid2, 2012)

Part 2 : Lipase Activity Assay

Activity Assay Process



Emulsion

+

Phosphate Buffer

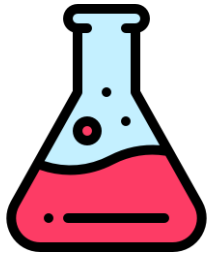
+

Calcium Chloride

+

Lipase

shake 30 min

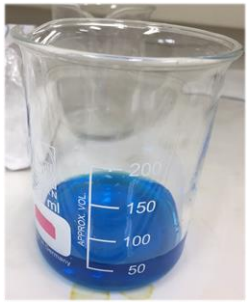


HCl

+

Hexane

Centrifuge 5 min



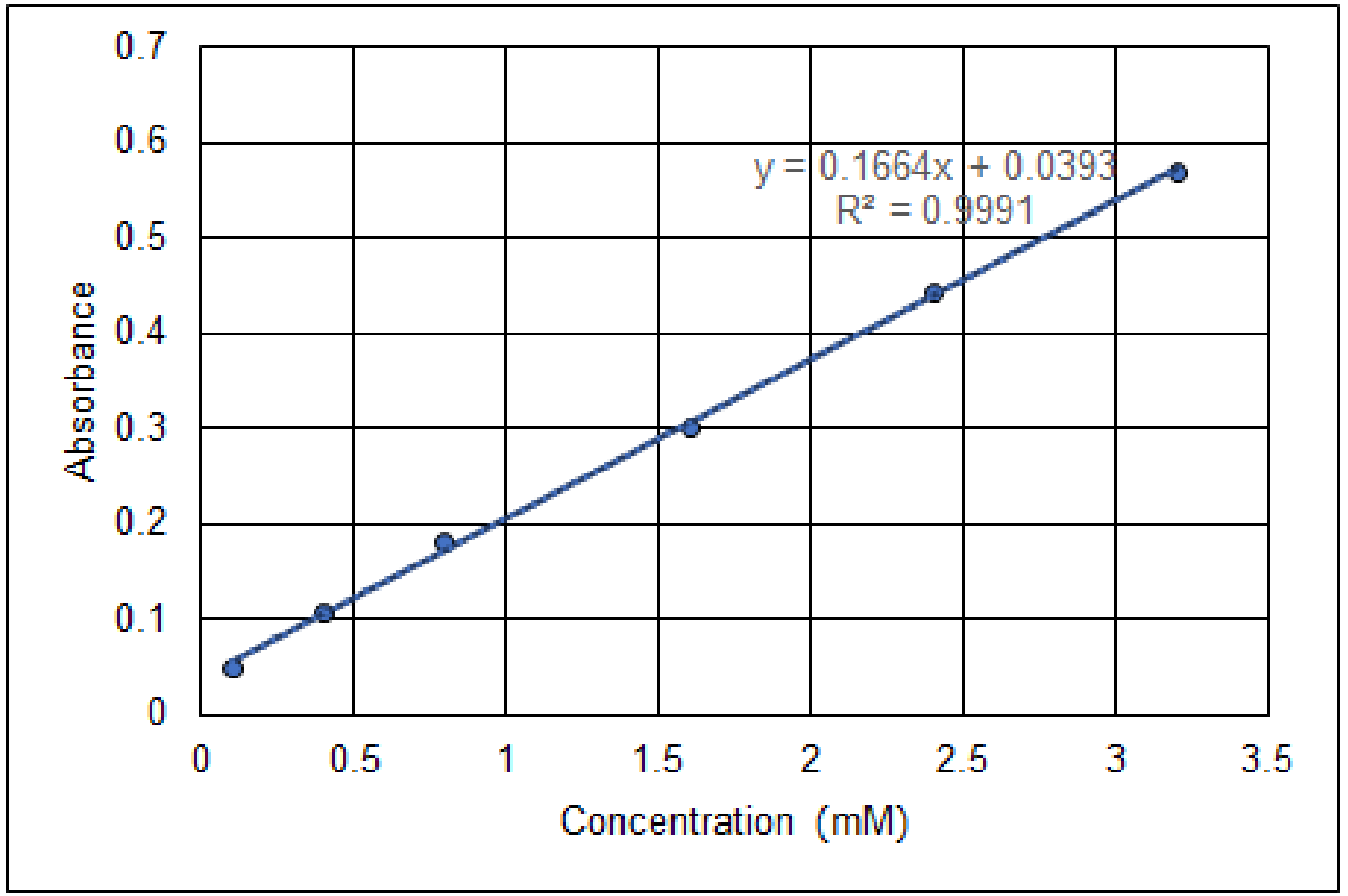
Copper Reagent

Centrifuge 2 min

UV-Vis Spectrophotometer At 700 nm

Part 2 : Lipase Activity Assay

Standard Graph of Oleic acid



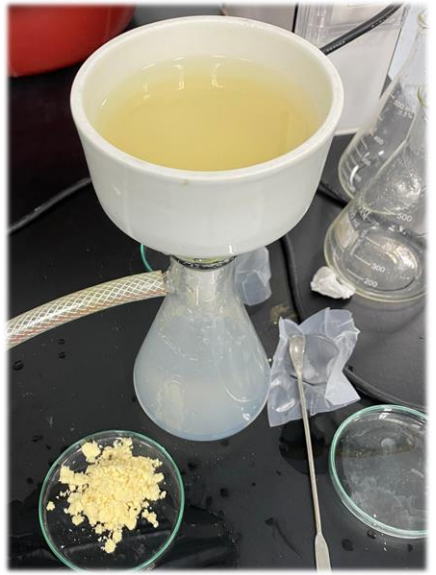
Part 3 : Immobilization on Chitosan

Lipase

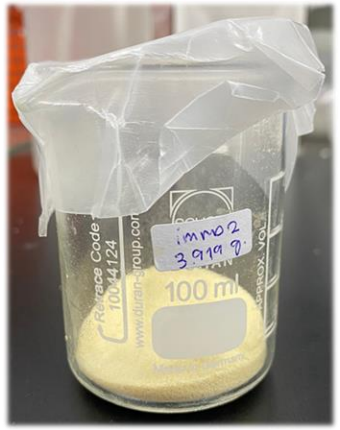
Chitosan

Phosphate Buffer

Stir at 350rpm for 7-8 hr



Filter and Rinse with Water



Dry at 50°C for 12 hr

Pereira, E. B., Zanin, G. M. & Castro, H. F. (2003).

Part 4 : Protein Assay

Lowry Protein Assay

Step 1: Protein + Cu^{2+} → Cu^{2+}-peptide bond complex (purple)

Step 2: Folin reagent + Cu^{2+}-peptide bond complex → Reduced folin reagent (blue)

Part 4 : Protein Assay

Lowry Protein Assay

Step 1: Protein + Cu^{2+} → Cu^{2+}-peptide bond complex *(purple)*

Step 2: Folin reagent + Cu^{2+}-peptide bond complex → Reduced folin reagent *(blue)*

→ Solution Color

More sensitive than biuret = **More accuracy**

Part 4 : Protein Assay

Protein Assay Method

Solution D
A+B+C

Immobilized
Enzyme

Vortex

Wait for 10mins

Folin-Ciocalteu
Solution

Vortex

Wait for 30mins

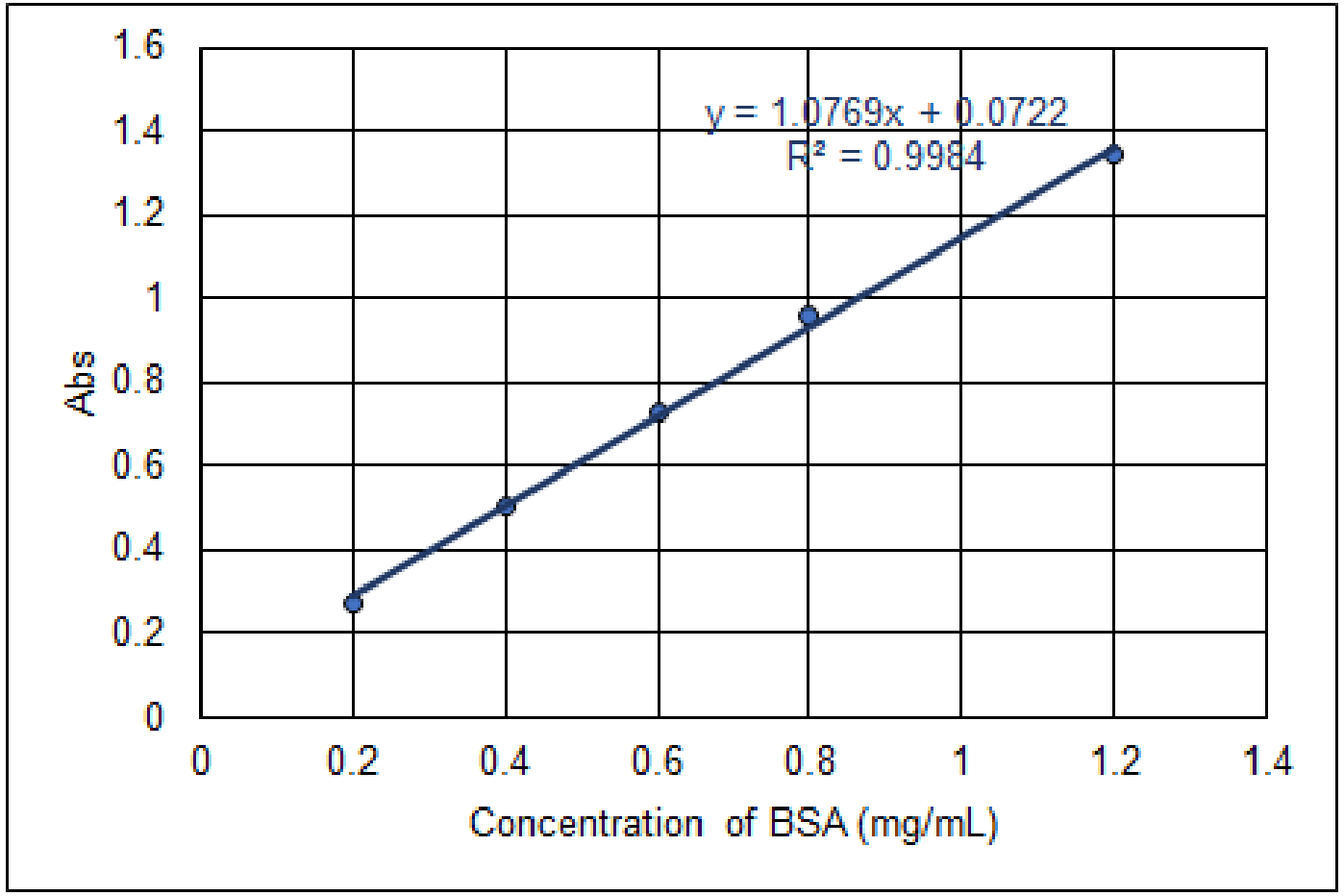
UV-Vis
Spectrophotometer
At 660nm

A : Copper(II) Sulfate 0.1 M
B : Sodium Tartrate 0.1 M
C : Sodium Hydroxide 0.1 M + Sodium Carbonate 0.2 M

(Djagal W. Marseno, Retno Indrati and Yoshiyuki Ohta, 1976)

Part 4 : Protein Assay

Standard Graph of BSA



Data Analysis

Unit of Lipase

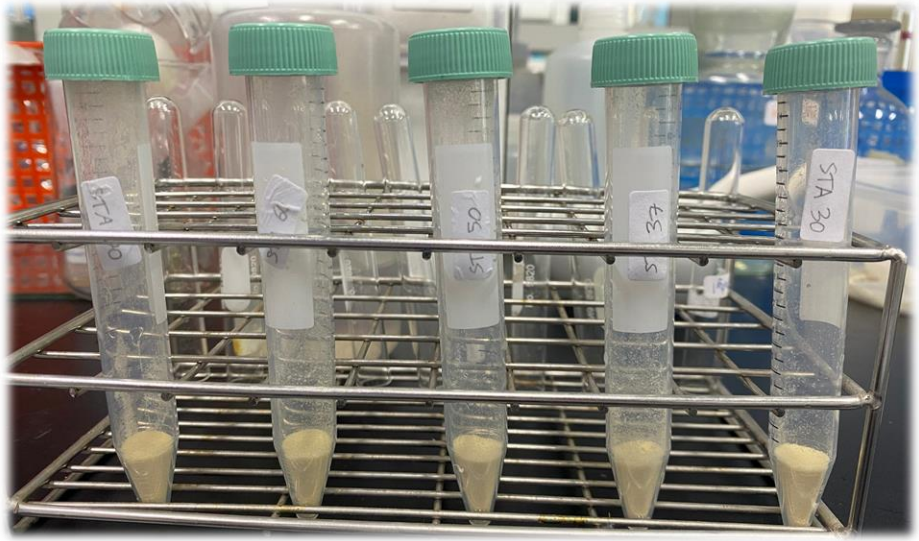
1 unit = quantity of enzyme that liberates the equivalent of 1µmol of free fatty acid per minute

$$\text{Unit} = \frac{C \times V}{\text{min} \times 1000}$$

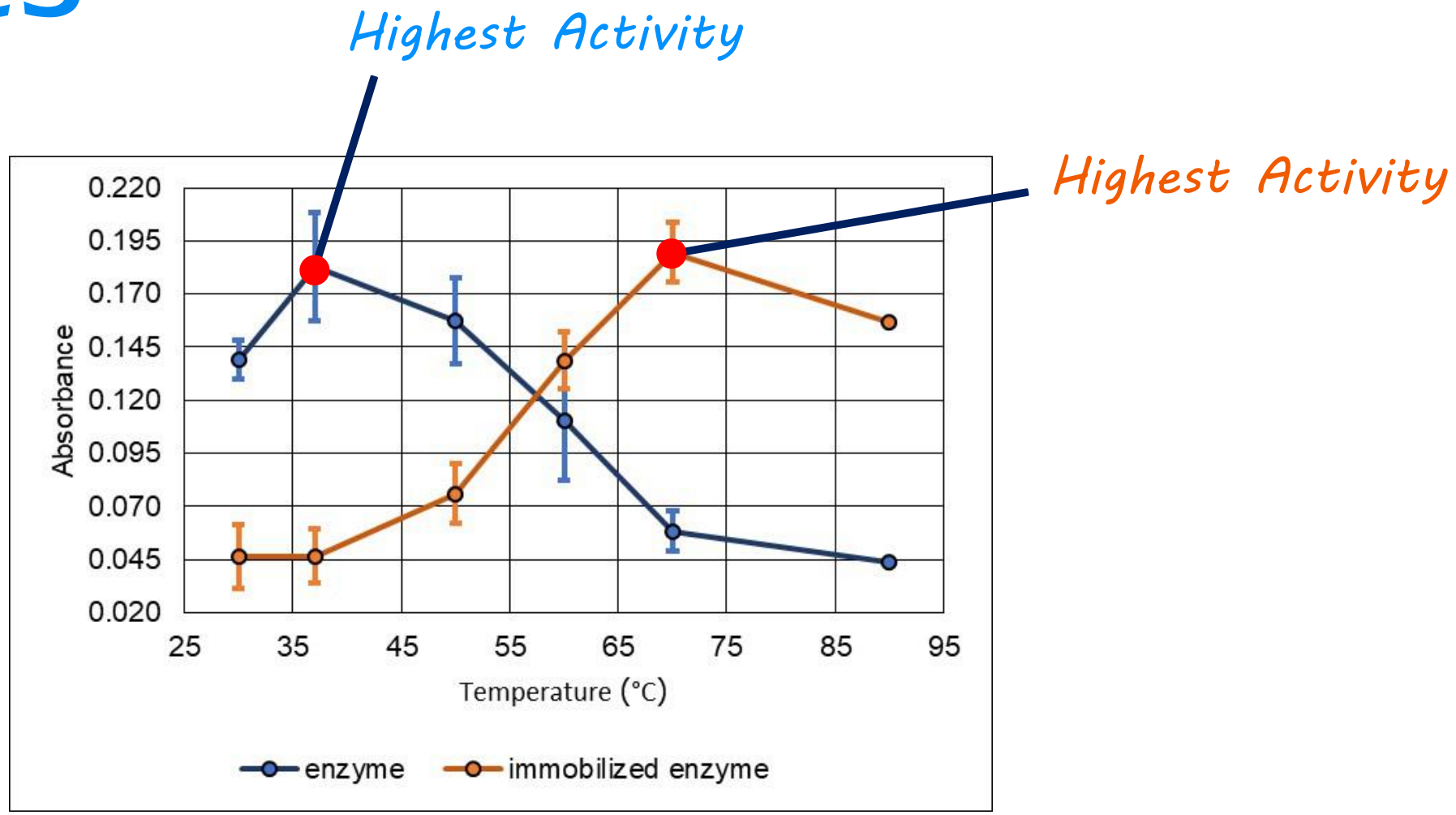
C = Concentration of Oleic acid from the incubation (µM)

V = Volume of substance used during incubation (mL)

min = Incubation time

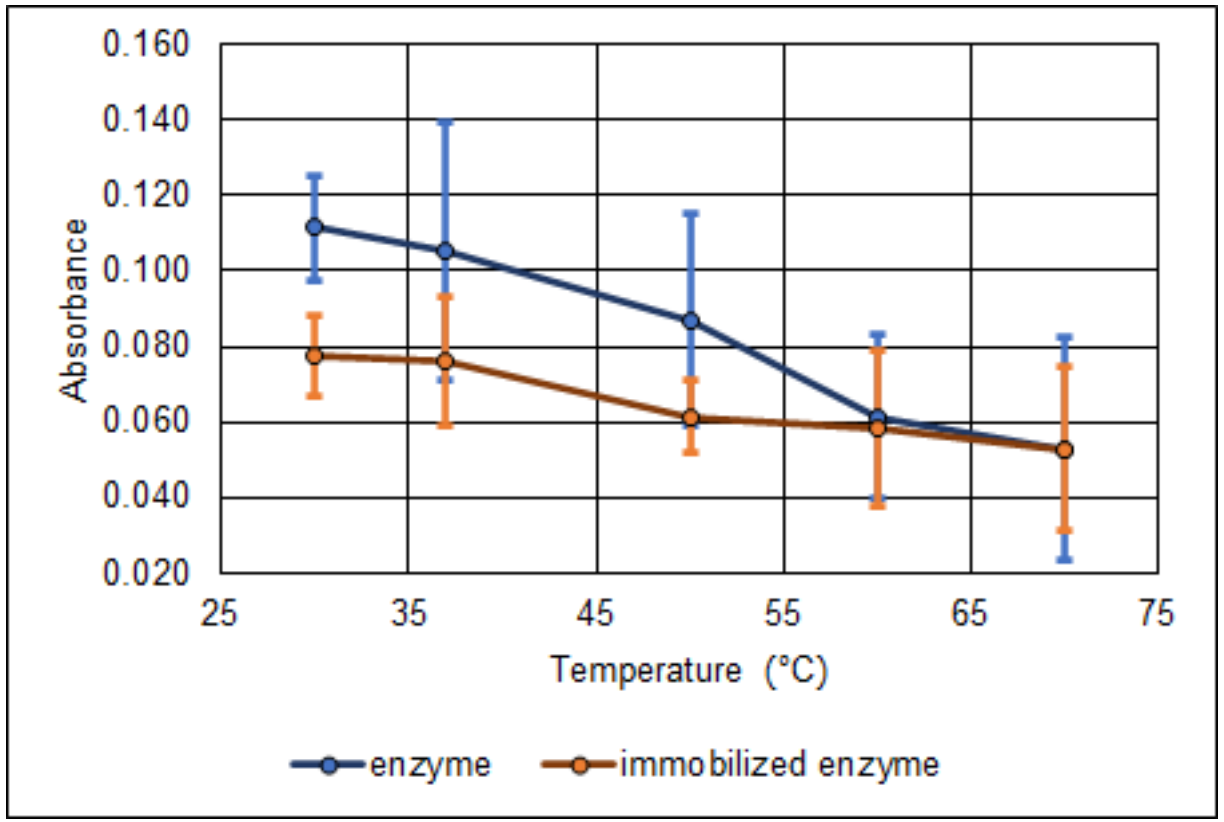


Results



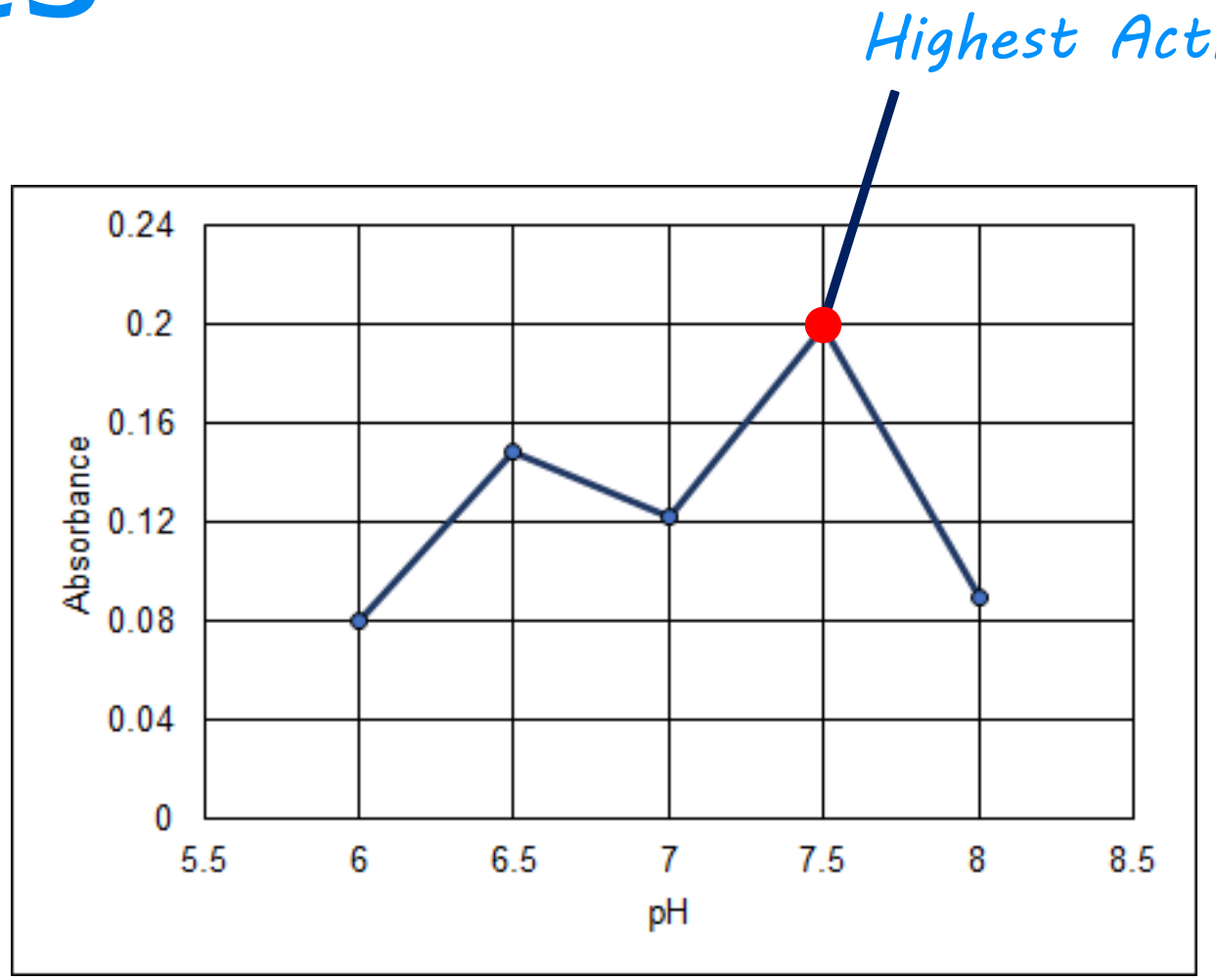
Absorbance at different incubating temperature

Results



Absorbance after putting the enzyme in different water temperature for 1hr

Results



Absorbance in different pH of Phosphate Buffer during incubation

Conclusion

- Crude extracted lipase from germinated pumpkin seeds have 0.559 Unit of lipase per gram of peeled pumpkin seeds

- Crude extracted enzyme has an optimal pH, temperature at 7.5, 37 °C respectively and immobilized enzyme has an optimum temperature at 70 °C

- Chitosan can bind 22.4274 % of protein and has high activity

References

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Thank You