Immobilization of Peroxidase from Castor Leaves for Application As Hydrogen Peroxide Strip Test

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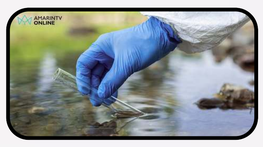
Abstract

This study aimed to investigate the immobilization of peroxidase extracted from castor leaves (*Ricinus communis* L.) onto filter paper by physical adsorption for application as simple hydrogen peroxide strip test. Peroxidase was extracted by 0.1 M sodium acetate-acetic acid buffer, pH 6.0. The enzyme was partially purified using aqueous-two phase partitioning system. Enzyme activity and protein concentration were then evaluated giving a specific activity of 1.616 u/mg protein. The hydrogen peroxide strip test was prepared by dropping a reagent mixture consisting of peroxidase at 6.118 mu, 1% (w/v) *o*-Dianisidine, and 0.1 M sodium acetate-acetic acid buffer at pH 6.0 onto filter paper before allowing to dry for 10 minutes. The prepared strips were tested with hydrogen peroxide in the concentration range of 0, 9,250, 18,500, 27,750, and 36,963 ppm. Results showed that the apparent color intensity on the paper increased with the increasing hydrogen peroxide concentration. The color change could be observed both by the naked eye and semi-quantitatively by analyzing the color intensity using the ColorConverter application. These results demonstrated peroxidase from castor leaves can be applied in strip test and used further to develop methods for water quality assessment and environmental analysis.

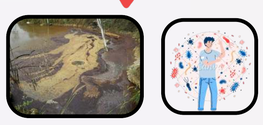


Introduction

Problem



residual H_2O_2



Impact

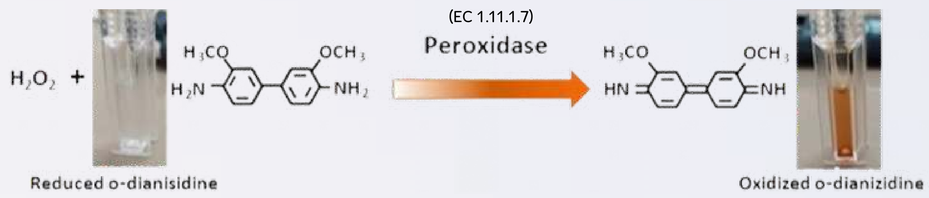


Figure 1. Oxidation of the molecular chromophore (o-dianisidine) by H_2O_2 and peroxidase, and the resultant color change from colorless to brown

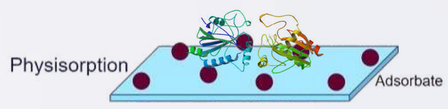


Figure 2. Peroxidase Immobilization

Objectives

- To investigate the immobilization of peroxidase extracted from castor leaves onto filter paper via physical adsorption
- To apply the paper with immobilized peroxidase as a simple hydrogen peroxide strip test

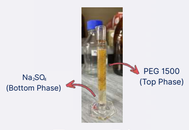
Methodology

Extraction and Partial Purification of Peroxidase


Castor Leaves →

Homogenization in Extraction Buffer →

Filtration & Centrifugation →

Crude Enzyme →


Aqueous Two-Phase System (PEG 1500 / Na_2SO_4)

Peroxidase Activity Assay

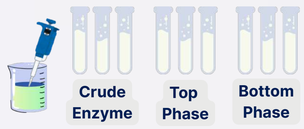
Reaction Mixture (Sodium Acetate Buffer + *o*-Dianisidine + H_2O_2) →


→

Absorbance 460 nm

Determination of Protein Concentration in the Enzyme Solution

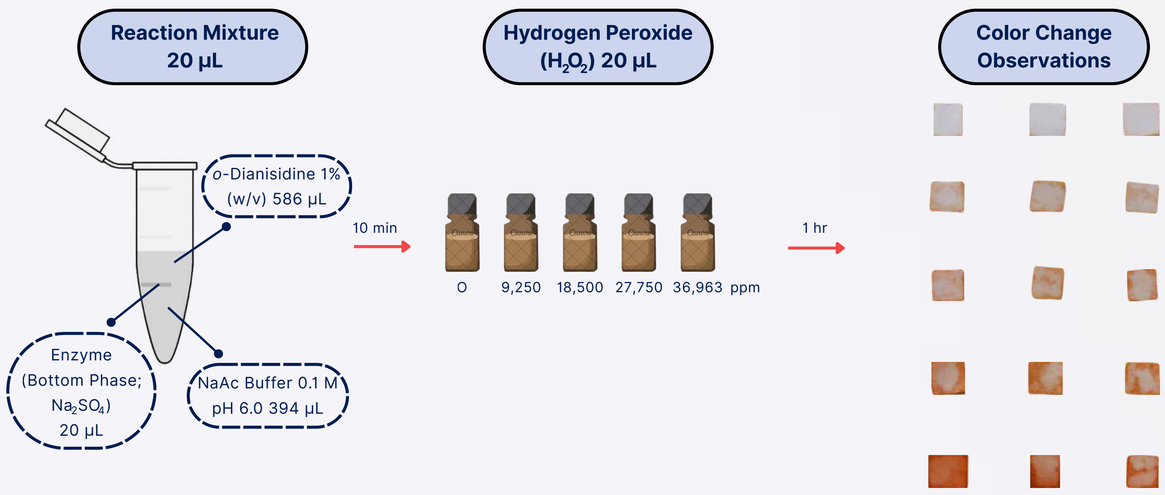

Preparing Bradford Reagent →


Preparing BSA Standard Curve →


Adding Bradford Reagent (Standards & Samples) →

Absorbance 595 nm

Hydrogen peroxide strip test



Results and Discussion

Determination of Peroxidase Activity and Protein Content

Table 1. Peroxidase Activity, Protein Concentration, and Specific Activity

Sample	Peroxidase Activity (unit/mL)	Protein Concentration (mg/mL)	Specific Activity (unit/mg)
Crude Enzyme	11.051	29.698	0.372
Top Phase (14% PEG 1500)	2.170	3.733	0.581
Bottom Phase (12% Na_2SO_4)	15.293	9.461	1.616

- The bottom phase showed the highest specific activity when compared with crude enzyme and top phase.
- The salt-rich bottom phase provides higher water content, supporting enzyme solubility and structural stability.
- The bottom phase was selected for hydrogen peroxide strip test development.

Hydrogen peroxide strip test at different concentrations



- The color intensity on the strip increased with the increasing hydrogen peroxide concentration.
- The color change occurred due to peroxidase-catalyzed oxidation of o-dianisidine, producing a reddish-brown color.
- Color intensity was analyzed using the ColorConverter application, and RGB values decreased as the strip became darker.

ColorConverter

Conclusion

- The bottom phase showed the highest specific peroxidase activity and was selected for strip test development.
- Peroxidase from castor leaves produced a clear color change as hydrogen peroxide concentration increased.
- The developed strip test can be used for preliminary visual detection based on color change.
- The tested concentration range was relatively high when compared with typical environmental levels. Therefore, future work should focus on improving sensitivity to allow detection at lower concentrations.

Acknowledgement

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Laboratory Members at CB2409

References

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