Factors Affecting The Catalysis of Castor Plant Peroxidase

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ABSTRACT

Peroxidase is a group of enzymes that catalyze the oxidation of various substrates using hydrogen peroxide (H_2O_2) as an electron acceptor. This enzyme is commonly found in plants and is known for its versatility in catalyzing a wide range of reactions. The objectives of this study were to extract peroxidase from castor leaves using an aqueous two-phase system (ATPS) and to investigate factors affecting its catalytic activity, including pH, temperature and metal ions. The extraction process was performed using an aqueous two-phase system consisting of polyethylene glycol (PEG 1500) and ammonium sulfate in a ratio of 1.4 g to 1.2 g in a total volume of 10 ml of crude enzyme extract. Results revealed that the total enzymatic activity in the bottom phase containing the salt solution was higher than that in the top phase, indicating that the lower phase retained most of the active peroxidase. The purification fold of the enzyme was 3.77. The enzyme exhibited maximum activity at pH 5.0 in a temperature range of 30°C to 50°C. The enhanced activity at mildly acidic pH is attributed to the stability of the heme group under these conditions, which maintains the active site's structural integrity, allowing efficient electron transfer during catalysis. Metal ions including $FeCl_3$ (74%), $ZnCl_2$ (49%), $CoCl_2$ (40%), $MgCl_2$ (37%) and $CaCl_2$ (31%) were found to inhibit enzymatic activity from the most to the least. This inhibition was likely due to the interaction of metal ions with amino acid side chains, resulting in conformational changes that disrupt the active site, thereby preventing substrate binding. The properties of castor peroxidase reported here are useful for future applications of this enzyme in industrial and biotechnological processes such as bioremediation, the synthesis of bioactive compounds and analytical biochemistry.

INTRODUCTION

Peroxidase is a group of enzymes that catalyze the oxidation of substrates by using hydrogen peroxide as an electron acceptor. These enzymes are capable of oxidizing various substrates, such as phenols or aromatic amines, and are commonly found in plants with a wide range of catalytic activities. Castor leaves (*Ricinus communis*) contain phenolic compounds that act as antioxidants and enzymes such as peroxidase and catalase, which play significant roles in biological research, industry, and agriculture. Peroxidase enzymes in castor leaves contribute to the biosynthesis of lignin and suberin, reinforcing the cell wall structure and acting as a barrier against pathogens and unfavorable environmental conditions. Peroxidase in castor leaves exhibits optimal activity within a pH range of 5.0–7.0, making it effective in mildly acidic to neutral conditions. It demonstrates sensitivity to specific substrates, including guaiacol, pyrogallol, and ascorbate, which are commonly used in biochemical reactions to assess peroxidase activity. Additionally, this enzyme shows resistance to a wide range of temperatures, highlighting its stability and potential applicability in various industrial and environmental processes.

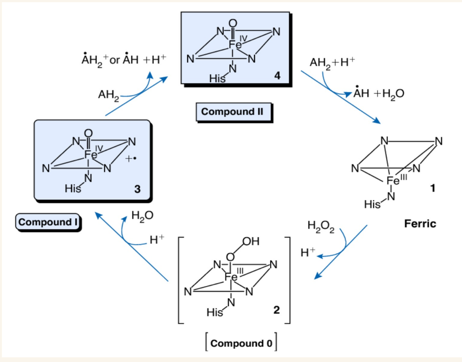


Figure 1 The catalytic mechanism of peroxidase

Figure 2 Castor leaves

EXPERIMENTS

Determination of activity and purification of peroxidase

Preparation of Enzyme Extract → Enzyme Separation Using An Aqueous Two-Phase System → A reaction mixture was prepared by combining 1% H_2O_2, 4% guaiacol, 0.1 M sodium acetate buffer at pH 6.0 and enzyme extract. → (3 minutes) 5% TCA was added to terminate the reaction → The absorbance was measured at 470 nm

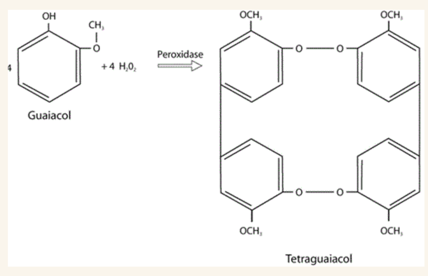
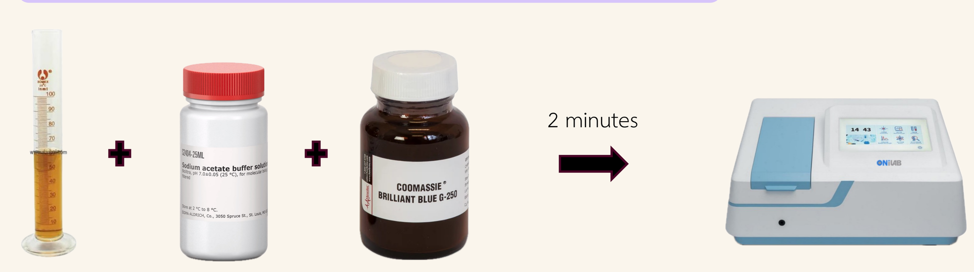


Figure 3 Oxidation of guaiacol to tetraguaiacol

Determination of protein content



The enzyme solution was mixed with 0.1 M sodium phosphate buffer at pH 6.0 and Coomassie Brilliant Blue reagent → (2 minutes) The absorbance was measured at 595 nm

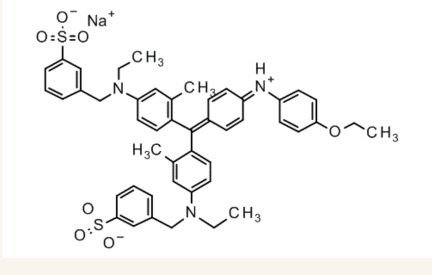


Figure 4 Structure of Coomassie Brilliant Blue G-250

Characterization of Enzyme

A reaction mixture was prepared by combining 1% H_2O_2, 4% guaiacol, 0.1 M citrate-phosphate buffer and enzyme extract → (3 minutes) 5% TCA was added to terminate the reaction → The absorbance was measured at 470 nm

Effect of pH on peroxidase activity

0.1 M citrate-phosphate buffer with pHs ranging from 3 to 9 were used.

Effect of temperature on peroxidase activity

0.1 M citrate-phosphate buffer was used at pH 5 and the reaction was allowed to proceed for 3 minutes at 30, 40, 50, 60, 70 and 80°C.

Effect of metal ions on peroxidase activity

The reaction mixture was prepared by mixing 1% H_2O_2, 4% guaiacol, and 0.1 M citrate-phosphate buffer at pH 5 containing 10 mM $FeCl_3$, $MgCl_2$, $CaCl_2$, $CoCl_2$ or $ZnCl_2$. The reaction was allowed to proceed for 3 minutes at 30 °C.

RESULTS

Determination of activity and purification of peroxidase

Table 1 Summary of purification table for castor peroxidase

Sample	Peroxidase activity (unit/ml)	Protein (mg/ml)	Specific activity (unit/mg)	Purification fold
Crude	8.25	9.44	0.87	1.00
Upper Phase	0.32	3.94	0.08	0.09
Lower Phase	29.04	4.21	6.90	7.90

Example Calculation

$$\text{peroxidase activity (unit/ml)} = \frac{(A/t) \times V_t \times D_f \times 1000}{\varepsilon \times S_v \times S_f \times P}$$

$$= \frac{(0.585/3) \times 3 \times 150 \times 1000}{26.6 \times 400 \times 1 \times 1}$$

= 8.25 unit/ml

Determination of protein content

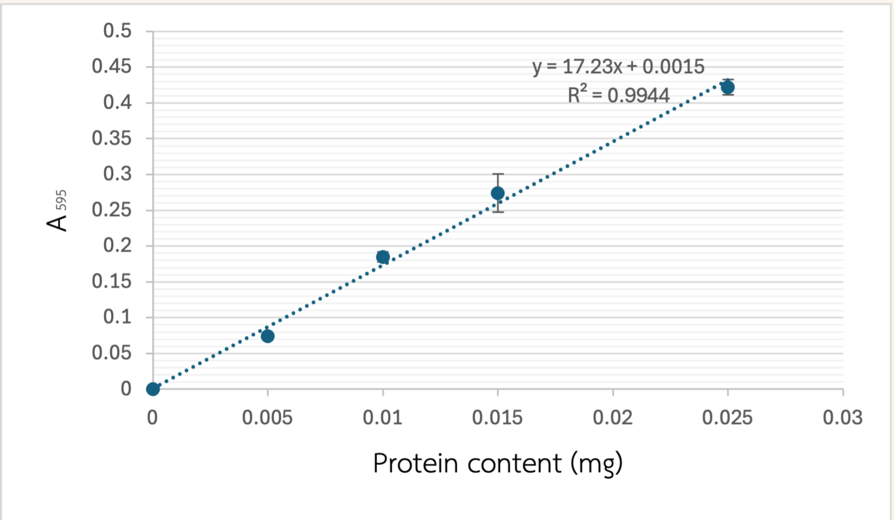


Figure 5 Shows the relationship between protein content and A_{595}

From the equation y = 17.23x + 0.0015
Substitute y = absorbance
0.273 = 17.23x + 0.0015
x = 0.0158 mg
Therefore, protein = 0.0158 × dilution factor / sample volume in each tube
= 0.0158 × 30 / 0.05
= 9.44 mg/ml

$$\text{Specific activity (unit/mg)} = \frac{\text{peroxidase activity}}{\text{protein content}}$$

$$= \frac{\text{peroxidase activity}}{\text{protein content}}$$

$$= \frac{8.25}{9.44}$$

= 0.87 units/mg

Characterization of castor peroxidase

Effect of pH on peroxidase activity

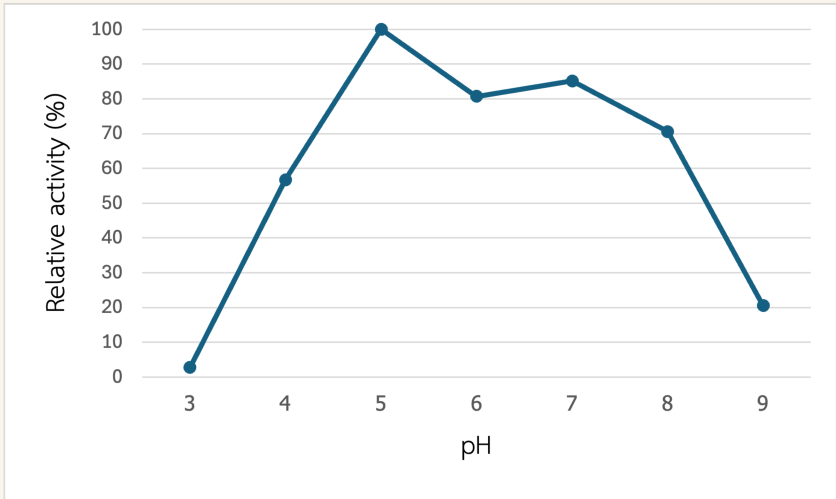


Figure 6 shows that at pH 5, the relative activity is the highest.

Effect of temperatures on peroxidase activity

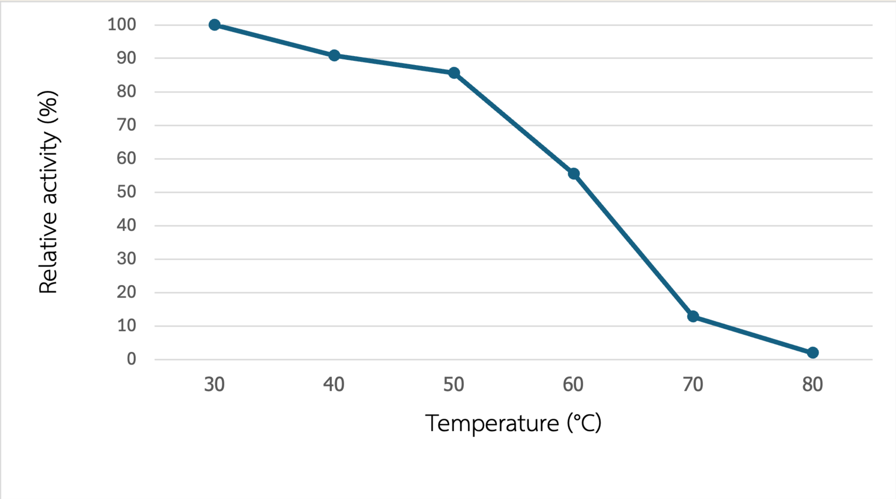


Figure 7 shows that at 30°C, the relative activity is the highest.

Effect of metal ions on peroxidase activity

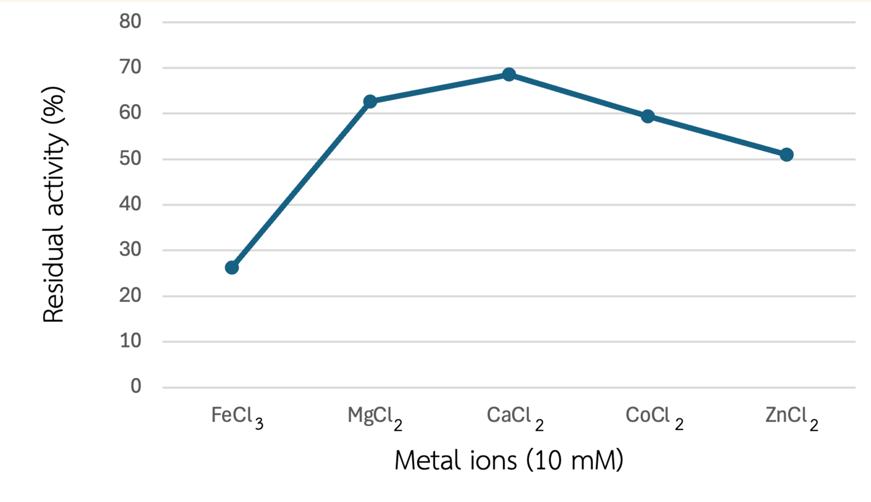


Figure 8 shows that $FeCl_3$ has the greatest inhibitory effect on enzymatic activity.

CONCLUSIONS

The lower phase of the ATPS extract from castor showed the highest specific activity among the tested conditions. The highest relative activity was observed at pH 5, while the optimum temperature for relative activity was 30°C. Among the tested metal ions, Fe^{3+} exhibited the strongest inhibitory effect on enzyme activity, reducing the residual activity to 26.27%, followed by Zn^{2+}, Co^{2+}, Mg^{2+} and Ca^{2+}. These findings indicate that pH, temperature and metal ions significantly influence peroxidase activity.

REFERENCES

Ilesanmi, O.S., & Adedugbe, O.F. (2023). Novel peroxidase from bitter leaf (Vernonia amygdalina): Purification, biochemical characterization and biotechnological applications. *Biocatalysis and Agricultural Biotechnology 49:102662. https://doi.org/10.1016/j.bcab.2023.102662.*

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