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Some Metal Activators of Peroxidase from Peel of Watermelon in the Oxidation of Guaiacol

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Authors' contributions

This work was carried out in collaboration between both authors. Author IOM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author OAA managed the study's analyses and managed the literature searches. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: To identify some activators of peroxidase from the peel of watermelon in the oxidation of guaiacol.
Study Design: *In vitro* enzyme assay.
Place and Duration of Study: This study was done in the Department of Biochemistry, Faculty of Life Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria in November 2023.
Methodology: The effects of some metallic chloride on the activity of peroxidase from peel of watermelon in the oxidation of guaiacol was done in the absence and presence of varying concentrations of the chloride salts of Cu, Fe, K and Mg using standard procedures.
Results: Results showed that the optimum salt concentration for enzyme activation for chlorides of Cu, Fe, K and Mg were 0.5 mM, 0.5 mM, 1.5 mM and 1 mM respectively within a salt concentration range of 0.5 mM to 2 mM.

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Conclusion: From this study, it can be concluded that chlorides of Cu, Fe, K and Mg are activators of peroxidase from peel of watermelon. However, chlorides of Cu and Fe are better activators of the enzyme since they activate the enzyme at a comparatively lower concertation when compared with chlorides of K and Mg. The Chloride of K had the least activation potential. The identification of chlorides of Cu, Fe, K and Mg as activators of peroxidase from peel of watermelon is of great importance as the search for cheaper and alternative sources of peroxidases for various applications continues.

Keywords: Watermelon; peel; activators; guiacol.

1. INTRODUCTION

Peroxidases belong to the class of oxidoreductases [1]. They are ubiquitous [2] and are widely found in plants, animals and microorganisms. Their function is to protect the cells against the effects of oxidative stress and cell damage due to H_2O_2 [3]. The presence or absence of the heme group is a determinant in the classification of peroxidases as heme or non-heme peroxidases, both of which have different functions [4]. The function of peroxidases is mainly the oxidation of molecules at the expense of hydrogen peroxide [5]. Peroxidases are very crucial enzymes that are involved in several biochemical processes some of which include its use in dye degradation [6] treatment of effluent [7] and mycotoxins degradation [8].

One very common substrate used for studying peroxidase activity is guaiacol. The oxidized form of guaiacol is a brown-colored product which can be easily monitored for the activity of peroxidase at 470 nm using a spectrophotometer.

The peel of watermelon (*Citrullus lanatus*) which is often discarded as waste could be a very good source of peroxidase. Fruit peels could also be a sustainable source of enzymes, thus contributing to waste reduction and the development of eco-friendly biotechnological applications.

The catalytic efficiency of peroxidases can be significantly influenced by the presence of metal ions. This influence could be activation or inhibition of the peroxidase activity. The metal ions used in this study are chlorides from Cu, Fe, K and Mg. Understanding the effects of these metal ions on the peroxidase activity from watermelon peel could provide valuable insights into the functional properties and industrial applications of peroxidase from watermelon peel.

This study therefore aims to investigate the role of copper, iron, potassium, and magnesium as activators of peroxidase extracted from the peel of watermelon in the oxidation of guaiacol. This would contribute to a wider and better understanding of the peroxidase derived from watermelon peel and their practical application in biotechnology.

2. MATERIALS AND METHODS

Disodium hydrogen phosphate, sodium dihydrogen phosphate, Dimethyl sulphoxide, Guaiacol, hydrogen peroxide (30 %), sodium acetate, acetic acid, were all purchased from SchauLab S.L. (Spain) and Loba Chimine Pot. Ltd. (India). The metallic chlorides and all other reagents used were all of analytical grades and purchased from Sigma-Aldrich (Dorset, Poole, United Kingdom). All kinetic measurements were carried out using a spectrophotometer.

2.1 Methods

2.1.1 Collection of plants materials

The Watermelon (*Citrullus lanatus*) used in this study was purchased from a local market at Ekpoma, Esan West Local Government Area, Edo State, Nigeria. They were well washed with distilled water in the laboratory for further laboratory processes.

2.1.2 Preparation of crude enzyme

10 g of peel from the watermelon fruit was weighed and washed with distilled water. The homogenization of the peel was done using a blender. This process was done with the peel in 100 mL of 0.1 M sodium phosphate buffer of pH 7.0. Filtration of the homogenate was done using a muslin cloth. The filtrate was then centrifuged (Centrifuge 800B, Pec-Medical U.S.A) at 4000 rpm for 30 minutes. The supernatant was decanted into plain sample bottles and labeled as "crude extract" which was stored frozen for analysis.

2.1.3 Estimation of guaiacol oxidation by crude peroxidase isolated from watermelon peel with varying salt concentration

The kinetics of peroxidase from the peel of watermelon in the oxidation of guaiacol was investigated in the absence and presence of varying concentrations of chloride salts of Cu Fe, K and Mg by spectrophotometrically monitoring the oxidation of guaiacol to produce tetraguaiacol at a wavelength of 470nm according to the standard procedures. The salt concentrations varied from 0.5 mM to 2 mM. Each of the reaction mixtures used in the kinetic study contained: 2.3 mL of 0.6 M sodium acetate buffer (pH 5.4), 0.2 mL of 0.02 mM Guaiacol, 0.1 mL of crude extract, 0.2 mL of varying concentration chloride salt, 0.2 mL of 2 mM of H2O2 added last to start the reaction. The final concentration of H_2O_2 in the 3 mL assay was 0.13 mM. The total volume of the reaction mixture was 3 mL. The absorbance was read every 2 seconds for one minute at 25^{o}C after adding hydrogen peroxide using a stop-clock. The control had no metal ion but replaced with distilled water.

2.1.4 Determination of Initial reaction rate (Vo)

The initial reaction rate of the crude peroxidase from the peel of watermelon was determined by calculating., change in absorbance per second (Δ absorbance/second), then dividing by the molar absorptivity for Guaiacol oxidation product (ε= 26,000 M-1cm-1), multiplied by the sample path length (1.00 cm for cuvette used). The result was expressed in mM/second. All assays were done in five replicates. The effects of varying concentrations of the salts were determined graphically using the mean values obtained per assay

3. RESULTS AND DISCUSSION

Fig. 1 shows the effect of varying $CuCl_2$ concentrations on the activity of peroxidase from the peel of watermelon for the oxidation of guaiacol. Results shows that $CuCl_2$ activated the enzyme within a salt concentration range of 0.5 mM to 2 mM. A $CuCl_2$ concentration of 0.5 mM optimally activated the enzyme. Further increase in salt concentration decreased the activity of the enzyme. Fig. 2 shows the effect of $FeCl_2$ on the activity of the peroxidase from watermelon peel. Results also show that $FeCl_2$ is an activator of this enzyme with the concentration of 0.5 mM $FeCl_2$ showing an optimum enzyme activation. Fig. 3 shows the effect of KCl on the activity of peroxidase from the peel of watermelon. Results also show that KCl is an activator of the peroxidase with an optimal KCl concentration of 1.5 mM for activation of enzyme. Similarly, $MgCl_2$ was seen to be an activator of the enzyme and this was optimum at 1 mM $MgCl_2$ concentration.

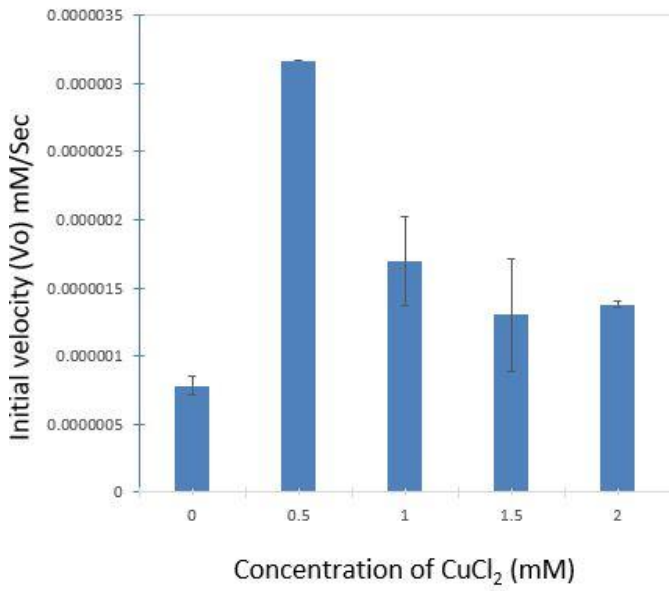

Fig. 1. Effect of $CuCl_2$ concentrations on the peroxidase activity in watermelon peel

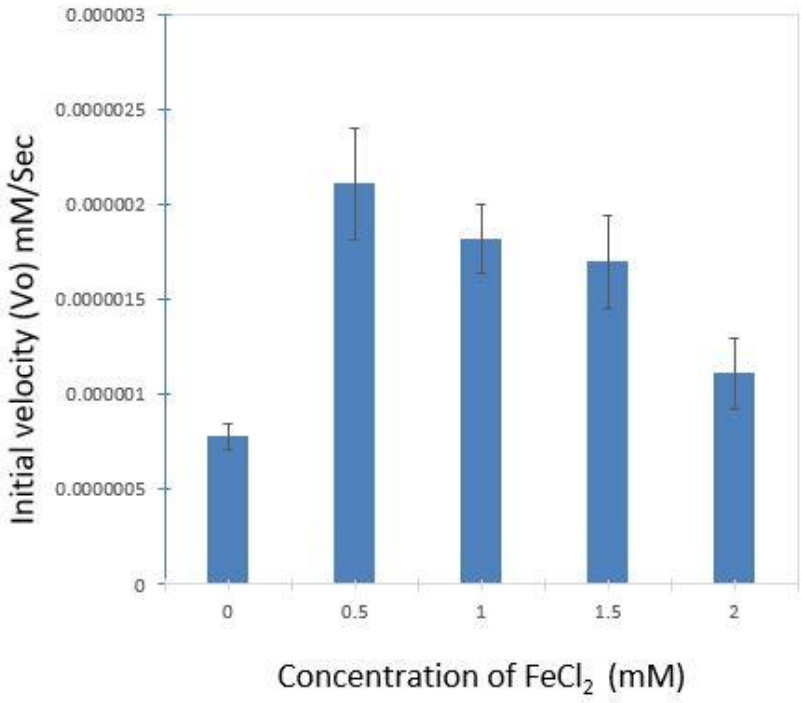

Fig. 2. Effect of $FeCl_2$ concentrations on the peroxidase activity in watermelon peel

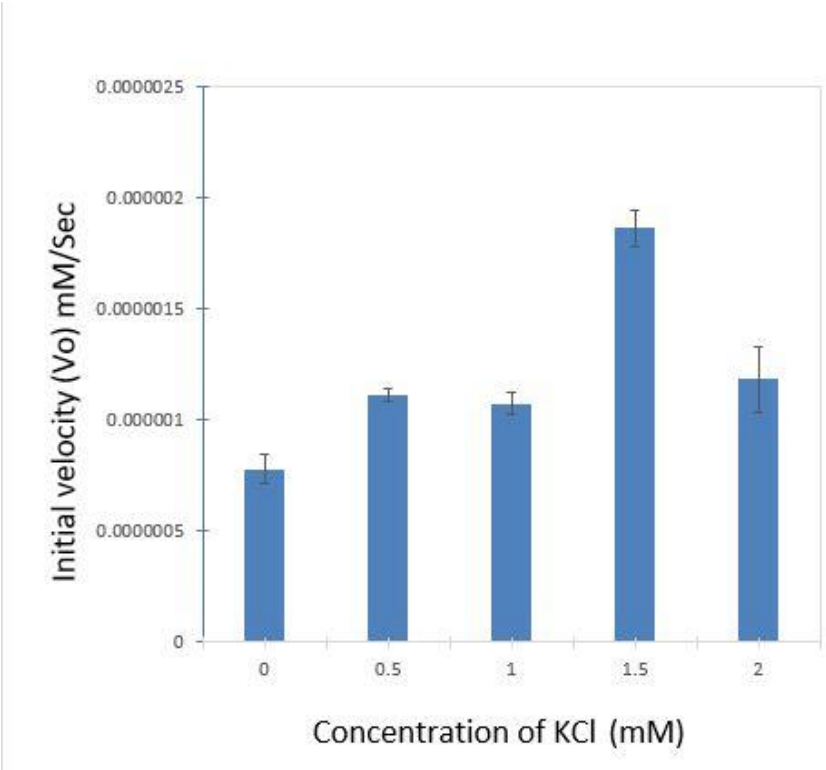

Fig. 3. Effect of KCl concentrations on the peroxidase activity in watermelon peel

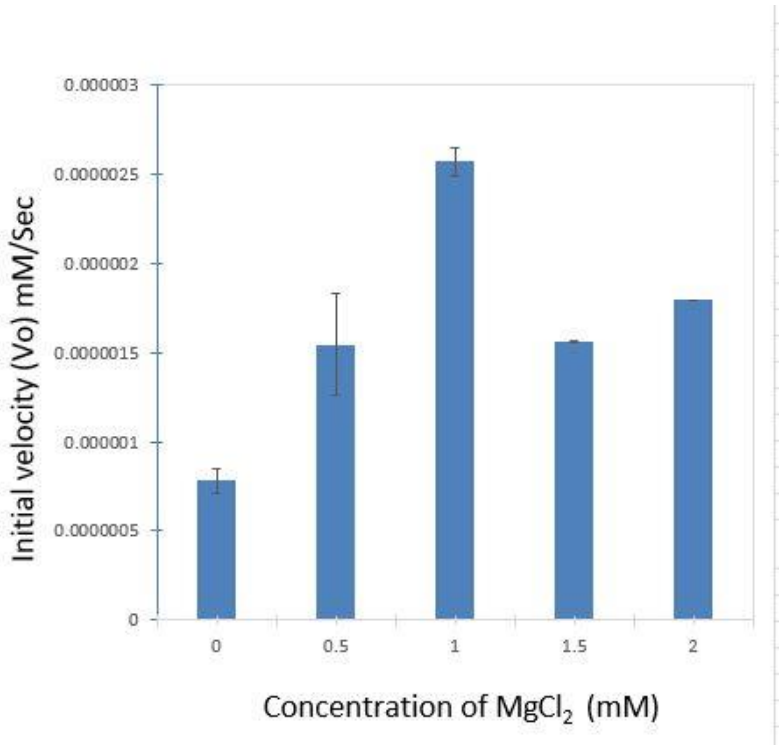

Fig. 4. Effect of $MgCl_2$ concentrations on the peroxidase activity in watermelon peel

Peroxidases are species-specific [9]. In a previous study on the effects of copper ions on peroxidase activity [10], it was reported that increasing copper concentration increased peroxidase activities in leaves and roots of *Astragalus neo-mobayenii*. Results from this study showed a similar activating property of $CuCl_2$ on peroxidase from peel of watermelon. However, the optimal activating concentration of $CuCl_2$ was 0.5 mM. Concentrations higher than 0.5 mM up to 3 mM resulted in a proportionate decrease in enzyme activity even though all concentrations were significantly higher than the control group. The activating effect of $FeCl_2$ on the peroxidase from watermelon peel was also similar to the findings of previous research [11] where the peroxidase activity was found to significantly increase in the gills, liver, kidney and brain of Fish (*Cirrhina mrigala*) after exposure to iron as compared to a control group. Thus the peroxidase from watermelon peel appears to have similar characteristic in the presence of copper when compared with peroxidases from other sources. The observed increase in activity of peroxidase from watermelon peel was similar to the findings in previous research [12] where it was reported that KCl increased peroxidase activities in two rice varieties. Report from previous research [13] on the effect of $MgCl_2$ on peroxidase activity is similar to the findings in this research where $MgCl_2$ was found to be an activator of the enzyme thus sharing similarities with peroxidase from other sources in this regard.

4. CONCLUSION

This study explored the effects of chlorides of some metal ions on the activity of peroxidase from the peel of watermelon during guaiacol oxidation. Results showed that $CuCl_2$ and $FeCl_2$ optimally activated the enzyme at 0.5 mM, while KCl and $MgCl_2$ were most effective at 1.5 mM and 1 mM, respectively. Higher concentrations of these ions reduced enzyme activity. These findings suggest that these metal ions could significantly enhance peroxidase activity, offering potential applications in biotechnological processes requiring controlled oxidation reactions. The study therefore highlights the importance of optimizing metal ion concentrations for effective enzyme function and future industrial applications.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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