***Original Research Article***

# Some activators of peroxidase from peel of watermelon in the oxidation of guaiacol


## 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 between 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 in the absence and presence of varying concentrations of the chloride salts of Cu, Fe, K and Mg. This was determined spectrophotometrically by monitoring the oxidation of guaiacol to produce a brown tetraguaiacolwhich was monitored at a wavelength of 470nm. The various salt concentrations were varied between 0.5mM and 2 mM. Each of the reaction mixtures used in this kinetic study comprised; 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 $H_2O_2$ added last to start the reaction. The final concentration of $H_2O_2$ in the 3 mL assay was 0.13mM. The total volume of the reaction mixture was 3 mL. The time course was determined by reading the absorbance every 2 seconds for one minute after adding hydrogen peroxide using a stop-clock. The initial velocity of the enzyme was tereafer calculated. The control had no metal ion but replaced with an equivalent volume of distilled water.

**Results**: Results showed that within a salt concentration range of 0.5 mM to 2 mM, chlorides of Cu, Fe, K and Mg activated the peroxidase from the peel of watermelon. Each salt had its own concentration which resulted in an optimal activity of the enzyme. However, the various concentrations of the salts used were able to activate the peroxidase from the peel of watermelon.

**Conclusion:**Since peroxidases are specie specific, understanding the factors that affect the activity of these peroxidases are of great importance. In this study, 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

Enzymes are proteins (with the exception of some catalytic RNA molecules) that possess catalytic activity. There are six major classes of enzymes according to the reactions they catalyze. They are oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases. Each of these classes have subclasses. Peroxidases, belong to the class of

oxidoreductases [1]. Peroxidases (E. C. 1.11.1.X) decompose various peroxides (ROOH) mainly hydrogen peroxide to oxidize organic and inorganic substrates. [1]. Peroxidases are ubiquitous [2]. Peroxidases 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]

Peroxidase is a heme or iron-porphyrin protein which belongs to a large family of enzymes called oxidoreductases. The function of peroxidases is mainly the oxidation of molecules at the expense of hydrogen peroxide [5]. Peroxidases are characterized by their catalytic ability to act on the oxidation of a wide variety of compounds, such as phenols [1]. Peroxidase has several industrial applications some of such are its use in dye degradation[6] treatment of effluent [7] and mycotoxins degradation[8]. Considering the several applications of peroxidase and the high cost of commercially available peroxidase, the search for cheap and alternative sources of peroxidase in inevitable. This study evaluates the interactions of peroxidase from the peel of watermelon with some cations. This will create an insight on the effect of some cations on the activity of peroxidase from the peel of watermelon in the oxidation of guaiacol.

## 2. MATERIAL 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 UV-780 recording spectrophotometer.

### 2.2 Methods

#### 2.2.1 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.

#### 2.2.2. 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.

#### 2.2.2 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 presence of varying concentrations of chloride salts of Cu Fe, K and Mg was determined spectrophotometrically by monitoring the oxidation of Guaiacol to produce

tetraguaiacol at a wavelength of 470nm. The salt concentrations varied from 0.5 mM and 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 H2O2 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 after adding hydrogen peroxide using a stop-clock. The control had no metal ion but replaced with distilled water

### 2.2.3. Determination of Initial reaction rate (Vo)

The initial reaction rate of the crude peroxidase from the peel of watermelon was determined by calculating., 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

Figures 1 shows the effect of varying $CuCl_2$ concentrations on the activity of peroxidase from the peel of watermelon in 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. Figure 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. Figure 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.

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 (Cirrhinamrigala) 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.

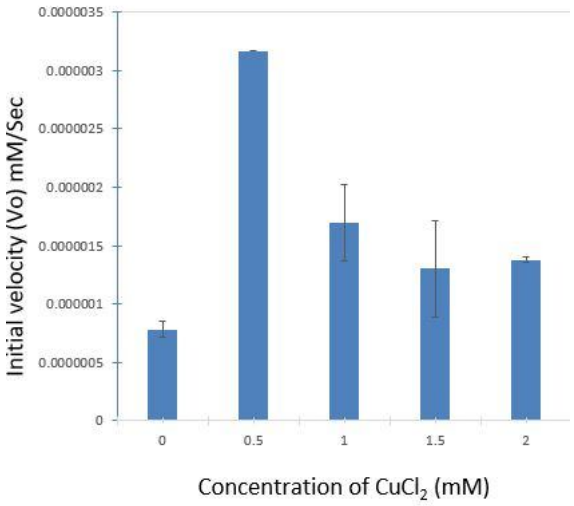


Figure 1: Effect of $CuCl_2$ concentrations on the peroxidase activity in watermelon peel

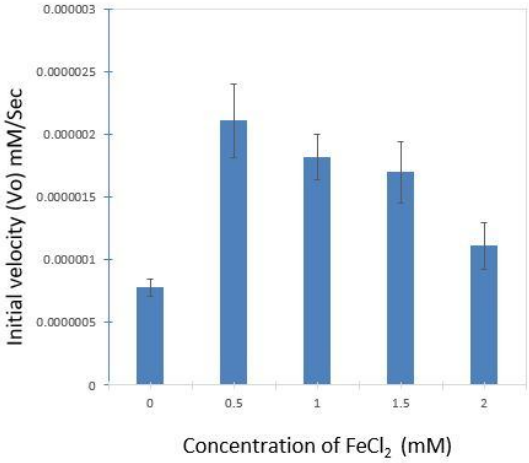


Figure 2: Effect of $FeCl_2$ concentrations on the peroxidase activity in watermelon peel

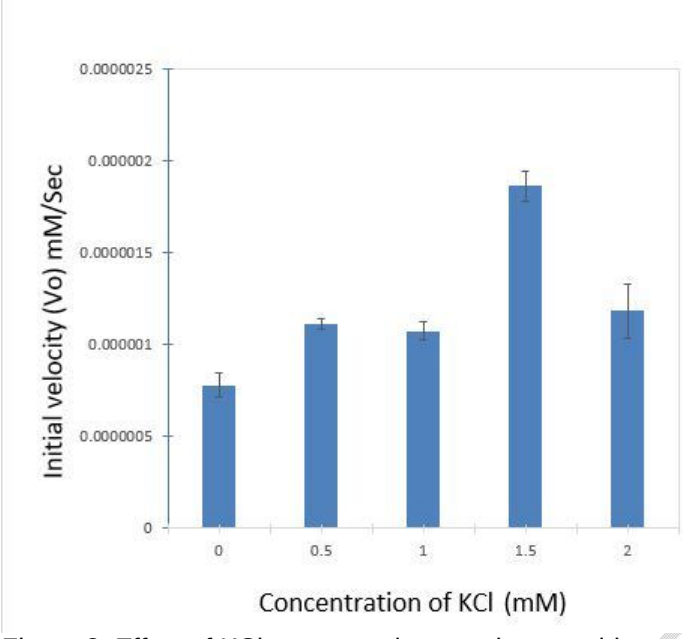


Figure 3: Effect of KCl concentrations on the peroxidase activity in watermelon peel

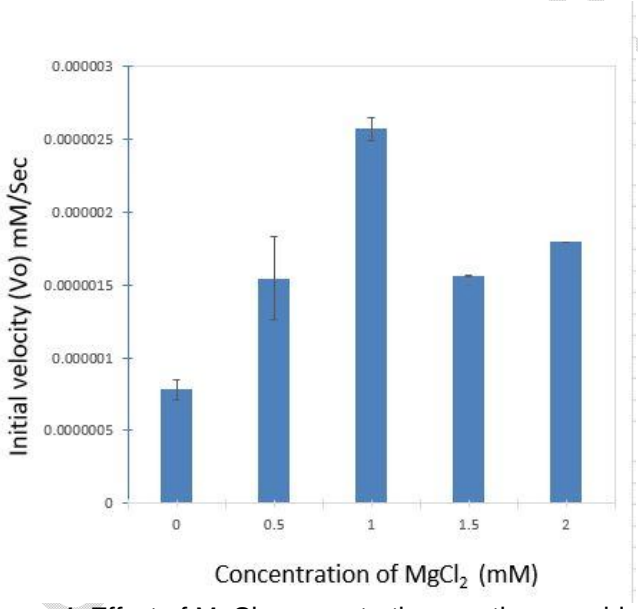


Figure 4: Effect of $MgCl_2$ concentrations on the peroxidase activity in watermelon peel

## 4. CONCLUSION

The specie specific nature of peroxidase is a major reason for the search for alternative sources of peroxidase which are cheaper and possess characteristic comparable with commercially available peroxidases. In this study, the chlorides of copper, iron potassium and magnesium were all found to be activators of the peroxidase from watermelon peel.

Each of the metallic chlorides had their unique concentration for optimal enzyme activity. This information contributes to knowledge on the effect of these metallic chloride on the activity of peroxidase from watermelon peel and it is of great industrial importance.

## 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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