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Some Activities of Peroxidase from Mango (*Mangifera indica* L. Var. *Mapulehu*) Kernel

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ABSTRACT

Mango (*Mangifera indica*) belonging to Anacardiaceae family is a tropical fruit-bearing plants of Asian origin which grows well in tropical regions and it is considered as the king of fruits. Peroxidases (E.C.1.11.1.7) (POD) are a group of oxidoreductases that catalyse the reduction of peroxides, such as hydrogen peroxide and the oxidation of a variety of organic and inorganic compounds. POD activity has been identified in plants, microorganisms and animals where they play important roles. POD are used for clinical and industrial purposes but like any other enzyme, their activity is affected by a number of factors. Hence, in this study, the effect of enzyme and substrate concentrations on peroxidase activity of filtered extract of ground mango (*Mangifera indica)* kernel suspension (400 g L^{-1}) was studied colorimetrically at 420 nm. Pyrogallol 5% w/v as substrate, filtered extract of crude enzyme from the kernel and phosphate buffer (pH 6.0) were employed in the analysis. Experimentally, first order reaction rate was observed with varied volumes of enzyme source up to 1.6 mL at a constant 1.0 mL of the substrate. On the other hand, holding the enzyme extract constant at 0.4 mL, peroxidase activity varied proportionately with substrate concentration up to 3.0 mL. Statistically, the mean of triplicate values were taken for each concentrations of enzyme and substrate taken. The result was subjected to regression analysis. From lineweaver-Burk plot, K_m of 1.48 mM and V_{max} of 0.29 units g^{-1} mango kernel were derived for peroxidase of mango (*Mangifera indica*) kernel using pyrogallol as substrate in this study. This study therefore identified limits of enzyme and substrate concentrations suitable to assay peroxidase activity. Hence, peroxidase in Mango (*M. indica*) kernel can be isolated, purified, characterised and quantitatively made available for industrial and medical use.

Key words: Mango, enzyme, substrate, peroxidase, pyrolgallol

INTRODUCTION

Peroxidases (E.C.1.11.1.7) (POD) are haem proteins and contain iron (III) protoporphyrin IX (ferriprotoporphyrin IX) as the prosthetic group. These are a group of oxidoreductases that catalyse the reduction of peroxides, such as hydrogen peroxide and the oxidation of a variety of organic and inorganic compounds (Koksal, 2011). POD is a major system for the enzymatic removal of H_2O_2 and peroxidative damage of cell walls is controlled by the potency of antioxidative peroxidase enzyme system. POD is found in many plant-based foods (Ziaebrahimi *et al*., 2007) and plays roles in food quality, including deterioration of colour and flavour (Benjawan *et al*., 2006). Peroxidase activity has been identified in plants, microorganisms and animals where peroxidases play important roles (Habib *et al*., 2003; Koksal, 2011; Anand *et al*., 2011).

Peroxidase can spoil fruits and vegetables at sub-zero temperatures and low moisture levels. Peroxidase is used along with glucose oxidase to determine glucose concentration of biological material. It has an edge over catalase because catalase is usually inactivated more easily by denaturation factors than peroxidase. Peroxidase test is used more often and are considered the most sensitive indices of the adequacy of blanching. Enzyme activities in foodstuffs are determined to quantify the potential extent of deterioration, the benefit of any treatments given and specific constituents.

Enzymes (like peroxidase) that catalyze phenol oxidation may be classified as phenolase which are oligomers in food and contain one copper prosthetic group per subunit. This oxidation of phenols is responsible for browning reactions in most fruits (Jimenez-Atienzar *et al*., 2007).

Although there are generally four types of browning reactions in foods namely; maillard, caramelization, ascorbic acid oxidation and phenolase browning, of these four, only the phenolase browning is enzyme-catalysed oxidation browning reaction (Yu *et al*., 1994). Enzymatic browning is rare in intact tissue of fruits, since phenolics substrates and phenolase are separated. Enzymatic browning is very common at the cut surface of light-colour fruits. The cut surface may rapidly change to brown colour due to the oxidation of phenols to orthoquines which in turn quickly polymerize to form brown pigment or melanins (Jiang *et al*., 2004). Mango (*Mangifera indica*) belonging to Anacardiaceae family is a tropical fruit-bearing plants of Asian origin (Wauthoz *et al*., 2007) which grows in tropical regions. It is considered as the king of fruits (Jagarlamudi *et al*., 2011). The mango fruits are very popular world-wide and are relished for the tasty fleshy mesocarp. The seeds (shell plus kernel) are found littered on streets as waste and as source of pollution in many countries because the seed kernel is not currently utilized for any commercial purpose (Maisuthisakul, 2008). Many polyphenolics contained in mango have shown anticancer activity (Noratto *et al*., 2010).

The composition of several varieties of Mango kernel had been reported. Arogba *et al*. (1998) reported the Polyphenol Oxidase (PPO) activities of Mango kernel. At the moment, there is a gross underutilization of the kernel as food, apparently due to the presence of inherent antinuritional factors like tannin.

This study described effect of enzyme and substrate concentrations on peroxidase activity of Mango (*M. indica*) kernel using 5% pyrogallol as substrate and 40% extract of *M. indica* kernel as crude enzyme source.

MATERIALS AND METHODS

Collection of sample: Fresh and ripe Mango (*Mangifera indica*) fruits were harvested directly from mango tree in Kogi State, Nigeria in the month of June, 2010 and used immediately for each determination.

Preparation of crude mango kernel peroxidase extract: The modified method of Arogba *et al*. (1998) was adopted. Fourty grams of white kernel, obtained after cracking the shell manually were crushed with prechilled (0°C) 0.01 M phosphate buffer (pH 6.0, 60 mL) using a pestle and mortar and made up to 100 mL with buffer in a measuring cylinder. The macerate was immediately filtered using suction through coarse filter (Whatman No. 4) paper into an Erlenmeyer flask (250 mL) placed on ice. No brown colour was observed in the crude extract before use.

Assay for peroxidase

Effect of enzyme concentration on peroxidase activity of *M. indica* kernel: The peroxidase activity of *M. indica* kernel was determined using colorimetric method based on the initial rate of increase in absorbance at 420 nm.

A constant volume of 1.0 mL of 5% (w/v) pyrogallol was mixed with varying amounts of crude enzyme extract ranging from 0.4 to 5.0 mL in the presence of a constant volume of hydrogen peroxide (0.16 mL). Phosphate buffer (pH 6.0) at 25°C was added to make up a total volume of 9.5 mL. A portion of the mixture was rapidly transferred into a 1.0 cm pathlength cuvette. Absorbance was read after one minute of reaction using a digital colorimeter (Genesys 20, thermospectronic, England).

Before taking absorbance readings, the instrument was zeroed using the same combination of reagents above but without the enzyme extract. Replicate readings were taken for both test and blank determinations. The average of the replicate readings was taken.

In our study, one unit of peroxidase activity was defined as the amount of the enzyme extract causing a change in absorbance of 0.001 per min at 420 nm.

Effect of substrate concentrations on peroxidase activity of *M. indica* kernel: A 5% solution of pyrogallol varying in concentration from 1.0 to 9.0 mL was employed to study the effect of substrate concentrations.

In a test tube, the following were added; a constant 0.16 mL 0.5% hydrogen peroxide, a constant 0.4 mL enzyme extract and pyrogallol solution (as mL of 0.39 M) of varying volumes. 0.1 M phosphate buffer (pH 6.0) was used to make a total volume of 9.5 mL, then a portion of the mixture was quickly transferred to a 1 cm path length cuvette. After zeroing the instrument, the absorbance was then taking after one minute of reaction. A replicate determination was also conducted and the average was taken.

The absorbance and substrate concentration values were subjected to regression analysis using the Regression Eq. 1 and 2:

$$\sum y = a_1 \sum x + na_o \quad (1)$$

$$\sum xy = a_1 \sum x^2 + a_o \sum x \quad (2)$$

where, x and y are the variables.

$$(a_o) = (n\Sigma xy-(\Sigma x)(\Sigma y))/(n\Sigma x^2-(\Sigma x)^2)$$

$$(a_1) = (\Sigma y-b\ (\Sigma x))/n$$

Where:

a_o = The slope of the regression line
a_1 = The intercept point of the regression line at the y axis
n = No. of values
Σ = Summation

The result was used to plot the double reciprocal curve of activity against substrate concentrations using line weaver-Burk equation. K_m and V_{max} were calculated from the plot of 1/activity against 1/substrate concentration.

The intercept on x-axis gave 1 km^{-1} and that of y-axis gave 1/Vmax while slope = km/Vmax.

Statistical analysis: Results were expressed as mean values of three separate determinations using SPSS and were then subjected to regression equation to statistically analyse the association between the two variables.

RESULTS AND DISCUSSION

Figure 1 shows the effect of enzyme concentrations on peroxidase activity of mango (*M. indica*) kernel obtained by plotting peroxidase activity as change in absorbance per minute against enzyme concentrations (as mL of 40% w/v crude extract).

The peroxidase activity in 40% (w/v) mango (*M. indica*) kernel crude extract using 1 mL 5% (w/v) of pyrogallol as substrate (pH 6.0) experimentally gave, first order reaction rate with varied volumes of enzyme source from 0.4mL up to 1.6 mL at a constant 1.0 mL of the substrate. A curve was then observed as from enzyme concentrations beyond 1.6 to 3.0 mL with a plateau noticed at enzyme concentrations from above 3 mL of 40% (w/v) crude extract (Fig. 1).

On the other hand, holding the enzyme extract constant at 0.4 mL, peroxidase activity varied proportionately with substrate concentrations from 1.0 up to 3.0 mL (Fig. 2). There was also a corresponding increase in peroxidase activity at substrate concentrations of 4.0-5.5 mL which gave a curve to the right but with reduced reaction velocity as the concentration of substrate approaches 5.5 mL. Beyond this point (5.5-9.0 mL), a plateau was noticed (Fig. 2).

The results after been subjected to regression analysis, gave the equation y = 11.998×-8.486. Then from lineweaver-Burk plot (Fig. 3), the intercept on x-axis gave km^{-1} with a value of 1.48 mM for *M. indica* kernel peroxidase using pyrogallol as substrate and the intercept on the y-axis gave 1/Vmax having a corresponding value of 0.29 enzyme units/g.

Enzyme activity is affected by factors such as temperature, pH, specificity, ionic strength, inhibitors, co-enzyme and co-factors, enzyme and substrate concentrations (Van Ginkel *et al.*, 2001). Enzymes are very shape specific proteins that would not study efficiently if the enzyme structure was altered or denatured. Substrate concentrations and enzyme concentrations do not denature the enzymes but affect enzyme reaction rates by limiting the amount of reactants (substrate and enzyme) available to produce the product.

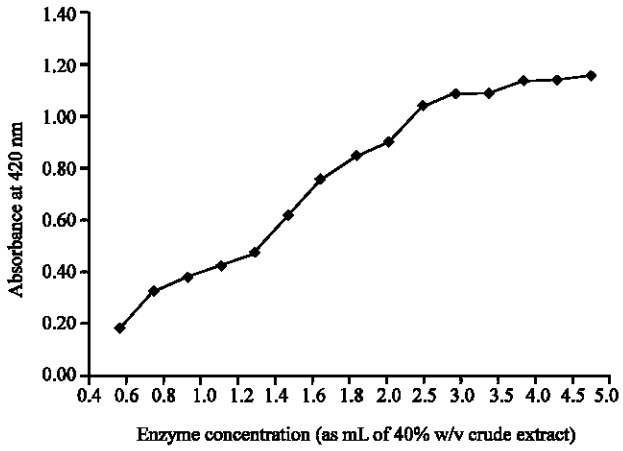

Fig. 1: Effect of enzyme concentrations on peroxidase activity of *M. indica* kernel

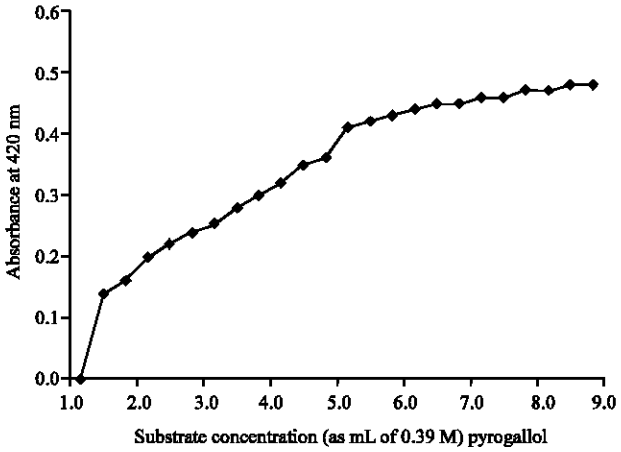

Fig. 2: Effect of substrate concentrations on peroxidase activity of *M. indica* kernel

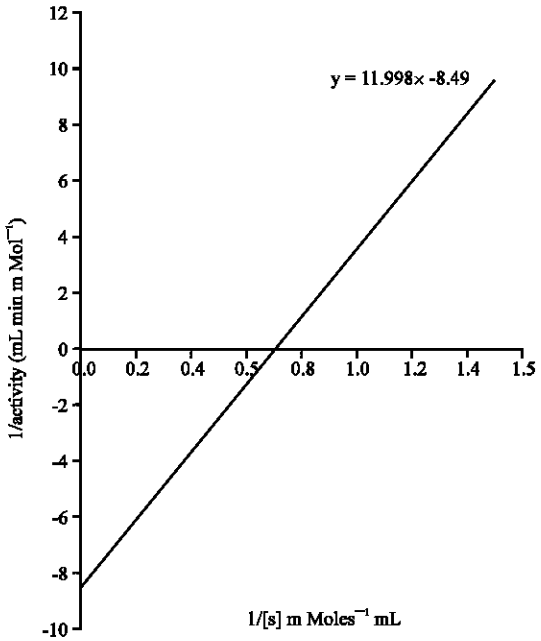

Fig. 3: Double reciprocal curves of peroxidase activity against substrate concentrations of mango (*M. indica*) kernel extract

Palmer (2001) reported that it is rather difficult, if not impossible and meaningless, to define the quantity of enzyme present in preparations in direct terms of concentration. But that it is more convenient and reliable measuring the enzyme activity.

Consequently, peroxidase activity in 40% (w/v) mango (*M. indica*) kernel crude extract was studied in this study using 5% (w/v) of pyrogallol as substrate at pH 6.0 as shown in Fig. 1. The curve was not sigmoidal in shape; hence, peroxidase in *M. indica* kernel could not be described as an allosteric enzyme. The plateau of the curve which was noticed is indicative of the optimum Enzyme-Substrate concentration (ES). Without corresponding increase in substrate concentrations, the velocity of reaction at this point was almost zero hence, the plateau a view also upheld by other researchers (Aaron *et al.*, 2011).

In the substrate concentration test, it could be seen that higher substrate concentrations also resulted in higher product concentrations. The reaction rate which continued to increase with the increasing concentrations of substrate used up to a substrate concentration of 5.0 mL 0.39 M pyrogallol, as shown in Fig. 2, is indicative of the fact that adding more of the substrate would increase the reaction rate and that eventually the maximum reaction rate that can be achieved by a certain enzyme level would be the reaction rate at the saturation point.

Km, expressed as Michealis-Menten constant, is a measure of the affinity between an enzyme and substrate and also describes rate of dissociation of the Enzyme-Substrate complex (ES). Large Km value represents low affinity and weak enzyme-substrate association whereas, low Km is indicative of a strong enzyme-substrate complex and high affinity. This study showed Km value of 1.48 mM for *M. indica* kernel peroxidase using pyrogallol as substrate and a Vmax of 0.29 enzyme units/g kernel. Similar research study by various authors on Kinetic parameters for horseradish peroxidase calculated from Lineweaver-Burk plots gave a Km of 0.8 mM using pyrogallol as substrate (Yamaguchi *et al*., 2004); a K_m of 8×10^{-5} and V_{max} of 1.53 when hydroquinone was used as substrate (Shukla *et al*., 2004). Yu *et al*. (1994) also reported a Km of 58 μM and Vmax of 3.36 units/nmol for French bean peroxidase using pyrogallol as substrate. Ghamsari *et al*. (2007) also reported a Km of 10.50 mM and a Vmax of 28.50 nmol min^{-1} mg $prot^{-1}$ as Kinetic parameters detected for guaiacol-dependent peroxidase activity in *Crocus sativus*. Hence, the affinity of peroxidase for its substrate could be directly related to the available hydroxyl groups in the substrate. Consequently, Km and Vmax values for peroxidase activity may vary with type of substrates, source and purity of the enzyme.

CONCLUSION

This study identified limits of enzyme and substrate concentrations suitable to assay peroxidase activity. The Km of 1.48 mM using pyrogallol is a useful diagnostic value of peroxidase in *M. indica* kernel. Peroxidase in Mango (*M. indica*) kernel can be isolated, purified, characterised and quantitatively made available for industrial and medical use.

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