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SOME PROPERTIES OF POLYPHENOL OXIDASE EXTRACTED FROM COLOCASIA ESCULENTA

Wodu, E. [1], Frank-Oputu, A[1]., Eboh, A. S. [1], Ogbomade, R. S. [2], Daufa, E. B. [1] and Omubo, I. D. [1]

[1]Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. [2]College of Health Technology, Otuogidi, Bayelsa State, Nigeria

Abstract

Crude polyphenol oxidase was isolated from *Colocasia esculenta* and the effects of temperature, pH and some metal ions were investigated. The activity of polyphenol oxidase was measured by measuring the increase in absorbance at 420nm using a spectrophotometer. The results revealed that pH and temperature optima were 6.0 and 40°C respectively and the enzyme is stable around neutral pH. The activity of polyphenol oxidase extracted from *Colocasic esculenta* was significantly ($p<0.05$) increased in the presence of Cu^{++} and Mg^{++} with relative activities of 373.70±7.98% and 161.90±10.22% respectively. Copper is an activator of polyphenol oxidase was isolated from *Colocasia esculenta.* Polyphenol oxidase activity can be activatied and or inhibited using appropriate metal ion or the pH and temperature of the medium.

Key words: *Colocasic esculenta*, Polyphenol oxidase, Catechol, Metal ions, pH.

Introduction

Oxidative browning occurs in foods as a consequence of the action of polyphenol oxidase (PPO; EC 1.14.18.1) activities. When fresh foods are cut open or infected such that cellular compartments of enzymes and substrates are broken down, rapid oxidation of phenolics produces a dark brown by-product known as melanins (Daun *et al*., 2004). In the presence of atmospheric oxygen, polyphenol oxidases catalyze the o-hydroxylation of monophenols to o-diphenols and the further oxidation of o-diphenols to o-quinones leading to the formation of a dark brown pigment (Gawlik-Dziki *et al*., 2008).

Polyphenol oxidases are widely distributed in plants as implicated by earlier studies and are located mainly in the thalakoid membrane of mitochondria and chloroplasts (Benjamin and Montgomery, 1973). Polyphenol oxidases have been studied in tuber such as *Colocasia esculenta* (Lee and Park, 2007), potato (Thygesen *et al*., 1995; Manohan and Wai, 2012), yam (Yapi *et al*., 2014; Chikezie, 2015); fruits such as quince (Yagar and Sagiroglu, 2002), grape (Munoz *et al*., 2004), banana (Fatemh *et al*., 2008) etc.

The activity of polyphenol oxidases have been shown to be influenced by some metal ions. However, the aim of this study was to extract polyphenol oxidase from C*olocasia esculenta* and to elucidate the effect of temperature, pH and some metal ions on its activity.

Materials and Methods

All reagent used were of analytical grade and does not need further purification. Freshly harvested tuber of *Colocasia esculenta* was washed to remove dirt. The cormel was peeled and the tuber was cut into tiny cube sizes.

Preparation of Acetone Powder

Exactly 200g of the tiny cubes was weighed and soaked overnight in acetone in a freezer. The cubes were homogenized with a warring blender and the homogenate filtered through double layer of cheese cloth. The residue was dried at ambient temperature to obtain the acetone powder.

Preparation of Enzyme Extract

Exactly 50g of acetone powder was dissolved in 250ml sodium phosphate buffer. The solution was centrifuged (universal centrifuge, 320R Hectti) at 4000rpm for 20 minutes at 4°C to obtain the aqueous crude extract.

Acetone Precipitation

To precipitate the enzyme, equal volume of acetone was added to the supernatant bit by bit with gentle stirring for 30 minutes in ice bath. The mixture was centrifuged at 4000rpm for 20 minutes at 4°C to obtain the precipitate, which was dissolved in 20ml of extraction buffer.

Ammonium Sulphate Precipitation

Exactly 4.128g of ammonium sulphate was introduced gradually into 20ml of enzyme solution in ice bath with gentle stirring for 30 minutes. The mixture was centrifuged at 4000rpm for 20 minutes at 4°C. The precipitate was re-dissolved in 20ml of extraction buffer and was used as the partially purified preparation of the enzyme.

Total Protein

Protein estimation was done in all preparations by the Bradford method (1976).

Enzyme Assay

Polyphenol oxidase activity was determined by the method of Liu *et al*., (2005) with slight modification. The 4ml reaction mixture contained 1.5 ml of 40 mM catechol and 2.3 ml of 0.1 mM phosphate buffer (pH 6.8). Then, 0.2 ml of crude enzyme was added to the test tube and mixed thoroughly. The increase in absorbance was measured at 420 nm with a UV-spectrophotometer (Spectrum lab 755a). The reaction time for PPO was 3 min, and the activity was expressed in units: one unit = 0.001ΔA420/min/mg protein.

Kinetic Properties

Colocasia esculenta polyphenol oxidase activity was assayed with catechol as substrate (10, 20, 30 and 40mM). The enzyme reaction proceeded at pH 6.8 and 25°C. The enzyme's kinetic behaviour was explained by the Michaelis-Menten equation, and

kinetic parameters (Km and Vmax) were estimated by the Linewaver-Burk plot (Lineweaver & Burk 1934).

Effects of pH on Polyphenol Oxidase activity and stability

The effect of pH was investigated using 0.1 M sodium acetate buffer for the pH range of 3.0 – 5.0 and 0.1 M sodium phosphate buffer for pH 6.0 – 9.0. To determine the effect of pH on polyphenol oxidase stability, 0.1ml of enzyme solution was incubated in 0.9ml of various buffer solutions (pH 3.0–9.0) for 10 hours at $4^{O}C$, and the residual activity was measured. The enzyme activity was measured according to the standard procedure. Residual polyphenol oxidase activity was determined in the form of percent residual polyphenol oxidase activity at the optimum pH.

Effect of Temperature

To determine the optimum temperature, polyphenol oxidase activity was assayed at various reaction temperatures (20–80°C) controlled by a circulation water bath. The residual activity (%) at different temperatures was compared with that obtained at optimal temperature.

Metal ion effect

The enzyme was incubated with 10mM of each metal ion for 10minutes. Metal used for the study were Cu^{++}, Na^{+}, Zn^{++}, Co^{++}, K^{+} and Mg^{++}. Standard assay procedure was followed and the reaction mixture without metal ion represented 100% activity.

Statistical analysis

The SPSS statistical analysis system was used for analysis of the data. All the assays of polyphenol oxidase from *Colocasia esculenta* tuber were in triplicate determinations. The data collected were presented as means ± standard deviations and also relative activity in percentage (%). The statistical significance was assessed by one-way analysis of variance. Significant differences ($P \leq 0.05$) among treatments were detected using Duncan's multiple range tests.

Results and Discussions

The partially purified preparation of polyphenol oxidase extracted from *Colocasia esculenta* was purified 2.69 fold±0.50 with a specific activity of 1.65±0.12 units min^{-1} mg^{-1} protein and the enzyme yield was 62.76% ±7.59.

Metal ions have been shown to modulate (inhibit/activate) the activity of polyphenol oxidases. The results from the present investigation (figure 1) on *Colocasia esculenta* tuber polyphenol oxidase revealed that Cu^{++} and Mg^{++} significantly ($p<0.05$) increase the activity of *Colocasia esculenta* polyphenol oxidase. Relative activity of polyphenol oxidase in the presence of Cu^{++} was 373.70%±7.98 while for Mg^{++} was 161.90%±10.22. The significant increase in the enzyme activity due to the presence of copper is not surprising since polyphenol oxidases are copper containing enzymes and copper is essential for catalytic activity. Similar increase of polyphenol oxidase activity in the presence of Cu^{++} was reported by Kong *et al*., (2000) for *Thermomicrobium roseum* polyphenol oxidase and Liu *et al*., (2004) for *Bacillus thuringiensis* polyphenol oxidase. Copper is responsible for the redox processes that involve electron transfer, dioxygen chemistry and reduction of nitrogen oxides, thus it can serve as a structural support for the larger protein (polyphenol oxidase) molecule that it is a part of, thereby affecting the activity of the protein (Mishra and Gautam, 2016). Na^{+} and K^{+} ions decreased the activity of *Colocasia esculenta* polyphenol oxidase by 64.43%±4.90 and 70.33%±2.75 respectively. Inhibition of polyphenol oxidase by Na^{+} had been reported by some researchers. Liu *et al*., (2005) reported that sodium chloride exhibits a strong inhibition of apple polyphenol oxidase activity. Sodium chloride, has been shown to be useful in prevention of the darkening in freshly peel fruits and vegetables. However, Chong *et al*., (2011) reported that sodium chloride in generally a weak inhibitor of *Musa acuminata* polyphenol oxidase at low to moderate concentrations.

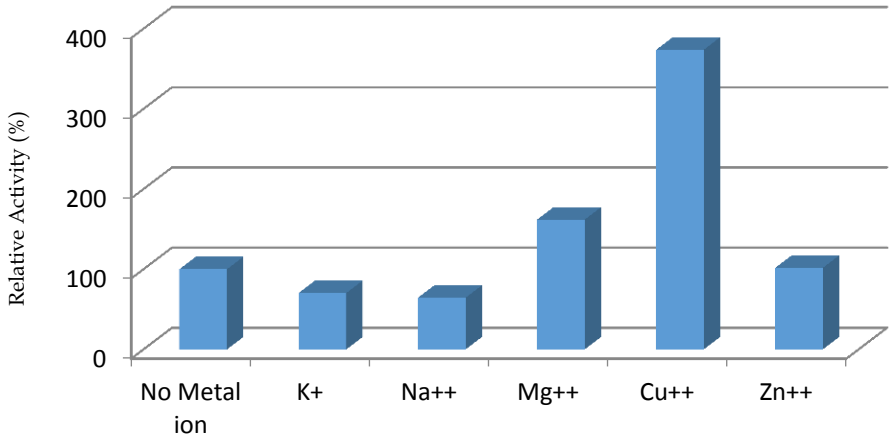

Figure 1. Polyphenol oxidaseactivity in the presence of six metal ions

Optimum pH

As depicted in figures 2 the plot of the relative activity (%) versus pH showed a single peak corresponding to pH 6.0, hence it is the optimum pH of polyphenol oxidase extracted from *Colocasia esculenta*. Optimum pH for maximum activity of polyphenol oxidase in plants is within the range 4.0-7.0, depending on the extraction method, and localization of the enzyme in the plant cell (Alward and Haisman, 2010) and the substrate used in the assay (Alward and Haidman, 1969; Armok, 2010). However, optimum pH 7.5 had been reported by Wong and Lee, (2014) for cassava leave PPO and 8.4 and 8.0 by Oluwatosin and Olusola (2016) for polyphenol oxidase from the peel and flesh of red apple, respectively.

The pH range at which an enzyme shows highest activity is called pH stability. Increase or decrease in pH above or below the range of stability, leads to decrease in enzyme activity. polyphenol oxidase extracted from *Colocasia esculenta* was unstable in acidic pH but was more stable between pH 6.0-7.0 as shown in Figures 3. This finding is in line with that reported by Kavrayan and Aydemir (2001) in which peppermint PPO was found to be stable between pH 6.0 and 7.0.

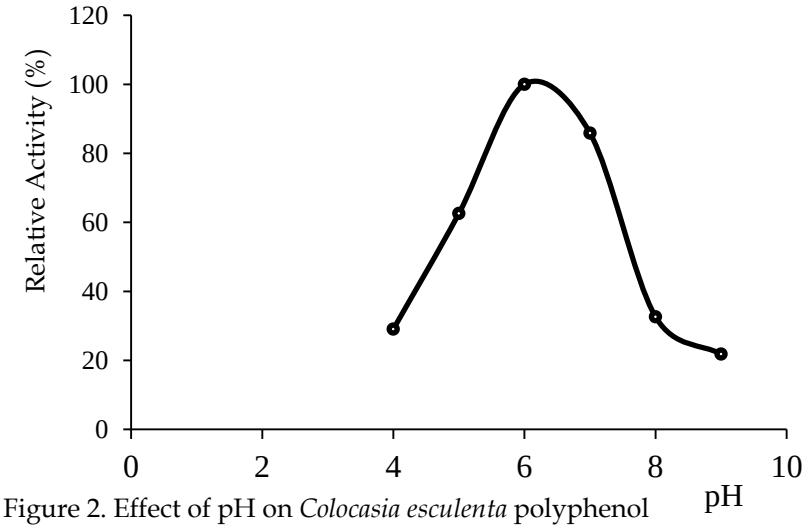

Figure 2. Effect of pH on *Colocasia esculenta* polyphenol oxidase activity

Figure 3. pH stability of *Colocasia esculenta* polyphenol oxidase activity

Optimum Temperature

Optimum temperature for polyphenol oxidase extracted from *Colocasia esculenta* was 40°C as can be seen in figure 4. This same value had been reported as optimum temperature by Bello and sule, (2002) for polyphenol oxidase from *Carica* Papaya and Pumpkin. Higher temperature of 50-60°C was reported by Saeidian, (2013) for tomato polyphenol oxidase, and 50°C for garden egg and bush mango (Bello and Sule, 2012). However lower temperatures of 20°C as optimum temperature for cassavas leaf

polyphenol oxidase was reported by Wong and Lee, (2014) and 30°C for guava (Bello and Sule, 2012).

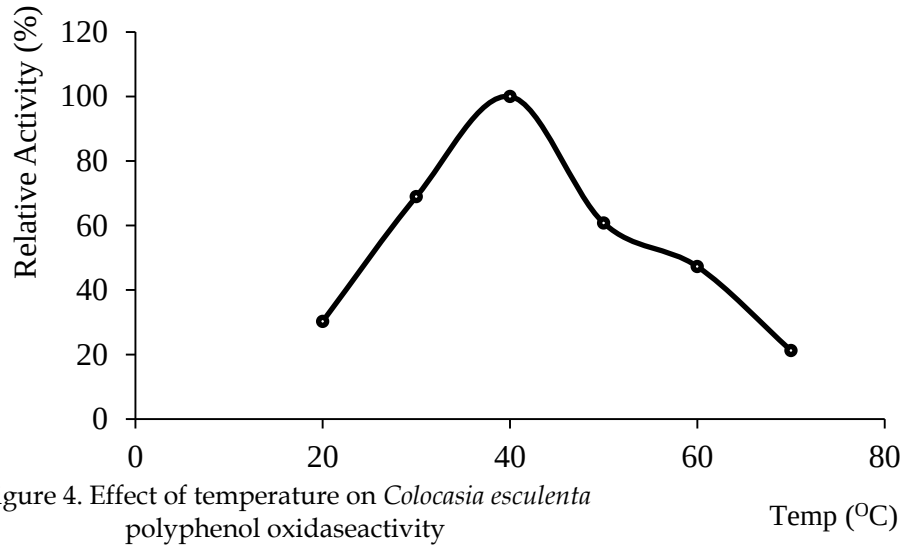

Figure 4. Effect of temperature on *Colocasia esculenta* polyphenol oxidaseactivity

Conclusion

This investigation concluded that the activity of polyphenol oxidase can be controlled. Copper and magnesium ions may be a good activator of some polyphenol oxidases, whereas sodium and potassium ions on the other hand may prevent food browning due to the action of polypheno; ozidase.

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