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# Thermal Stability of Polyphenol Oxidase Extracted from Three Varieties of *Ipomoea batatas* Tubers

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***Abstract:*** **The copper containing enzyme, polyphenol oxidase implicated in enzymatic browning was investigated in tubers of three varieties of *Ipomoea batatas*. The optimum temperature and stability of the enzyme were studied. The enzyme activity was measured by monitoring the increase in absorbance for 3 minutes. The activity was taken as the slope of the absorbance versus time graph. The effect of temperature on enzyme stability was determined through a temperature range of 20 - 70ºC. The results revealed that orange flesh and brown peel varieties showed maximum activity at 40ºC, while purple peel variety exhibited maximum activity at both 35 - 40ºC. Polyphenol oxidase extracted from the three varieties retained over 90% activity at 40º C even after 60mins of incubation. The enzyme from the three varieties was not stable at 20ºC, 60º C and 70ºC. Conclusively therefore, the temperature at which the enzyme is less stable may be exploited in the control of undesirable browning of *Ipomoea batatas* tubers due to polyphenol oxidase, while also considering the effect of that temperature on the tubers.**

***Keywords:*** **Polyphenol oxidase, *Ipomoea batatas*, Optimum temperature, Stability, Residual activity.**

## 1. INTRODUCTION

The copper containing enzyme, polyphenol oxidase is largely responsible for enzymatic browning of fruits and vegetables. It takes advantage of atmospheric oxygen and catalyses the o-hydroxylation of monophenols to o-diphenols which are then further oxidized to o-quinones resulting in the formation of a dark brown pigment (Gawlik-Dziki *et al*., 2008).

Temperature is one important factor that influence enzyme activity. For most enzymes the amount of product formed per unit time increases with temperature until it gets to a temperature where the enzyme is less stable. Above this temperature, there is a precipitous and usually irreversible drop in activity (Zubay *et al*., 1995) because the tertiary structure of a protein held together by weak forces is easily broken at increased temperature (Bonner, 2018).

The temperature dependence of polyphenol oxidase has been studied in senescent plantain puree (N'Guessan *et al*., 2018), cassava leaves (Wong and Lee, 2014) and *Solanum aethiopicum*, *Carica papaya*, *Cucurbita pepo*, *Psidium guajava* and *Irvingia gabonnensis* fruits (Bello and Sule, 2012). This work reports the investigation on the effects of thermal treatment on polyphenol oxidase extracted from tubers of three varieties of *Ipomoea batatas*.

## 2. MATERIALS AND METHODS

**Enzyme Extract Preparation.** *Ipomoea batatas* tubers were washed and cut into tiny cubes. Thirty grams (30g) of each variety were homogenized in ice cold potassium phosphate buffer (0.1M, pH 6.8). The filtrate was collected using a double layer of cheese cloth. Aqueous extract was obtained by centrifuging the filtrate for 25mins at 4000rpm and at 4°C.

**Acetone Precipitation**

To precipitate the enzyme protein, cold acetone was added to the supernatant above in bits in the ratio 1.5:1. The mixture was gently stirred in ice bath for 60 minutes and centrifuged for 25mins at 4000rpm and at 4°C. The precipitate dissolved in 10ml of buffer was used as crude enzyme.

**Protein Estimation and Enzyme Assay**

Bradford method (1976) was used to estimate protein concentration. The reaction mixture of 3ml contained 2.8ml of substrate (40mM catechol prepared in 0.1M phosphate buffer, pH 6.8) and 0.2 ml of enzyme solution. The increase in absorbance was monitored for 3mins at 30sec interval. The initial velocity was calculated from the slope of absorbance versus time graph. One unit of enzyme activity represent the amount of enzyme that increased the absorbance by 0.001 units per minute.

**Effect of Temperature**

Polyphenol oxidase was assayed at temperatures 10 –70°C controlled in a circulating water bath. The relative activity (%) at different temperatures was obtained by comparing them with that obtained at optimal temperature.

The effect of temperature on the enzyme stability was determined as follows: Exactly $1cm^3$ of enzyme preparation was incubated at temperatures 20 - 70ºC for 60mins. The tubes were immediately cooled in ice bath to stop further loss of activity. The standard enzyme assay was performed using the heated enzyme. Residual activity (%) was calculated relative to the unheated enzyme.

**Statistical analysis**

SPSS software was used for data analysis. The statistical significance was assessed by one-way analysis of variance. Significant differences ($P \leq 0.05$) among means were detected using Duncan's multiple range tests.

## 3. RESULTS

**Optimum temperature**

The dependence of polyphenol oxidase activity on temperature is shown in the plot of the enzyme's relative activity versus temperature (figure 1). The temperature at which *Ipomoea batatas* polyphenol oxidase exhibited maximum activity (optimum temperature) given in table 1 revealed that orange flesh and brown peel varieties have same optimum temperature of 40ºC, while purple peel variety had range 35 - 40ºC.

**Thermal stability**

Polyphenol oxidase from the three varieties showed over 90% activity at 40ºC even after 60mins of incubation (figures 2-4).

At 30ºC and 50ºC polyphenol oxidase from orange flesh variety was relatively stable and retained 80% activity upon incubation for 30mins after which there was a significant ($p<0.05$) drop in activity. The enzyme was not stable at 20ºC, 60ºC and 70ºC.

Polyphenol oxidase from purple peel and brown peel varieties retained a minimum of 70% activity after incubation for 60mins at 30ºC and 50ºC. At 20ºC brown peel variety retained a minimum of 70% activity upon incubation for 30mins after which a significant (p 0.05) decrease in activity was seen. As with orange flesh variety, purple peel and brown peel polyphenol oxidase were not stable at 20ºC, 60ºC and 70ºC.

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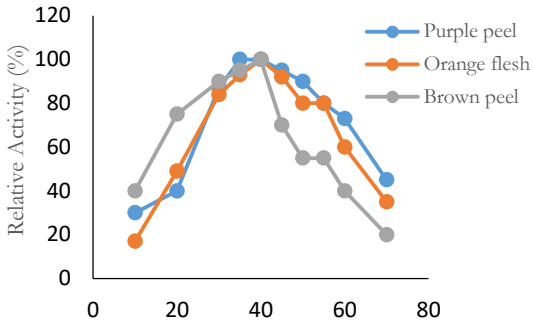


Fig: 1 Plot of relative activit versus temperature for optimum temperature determination

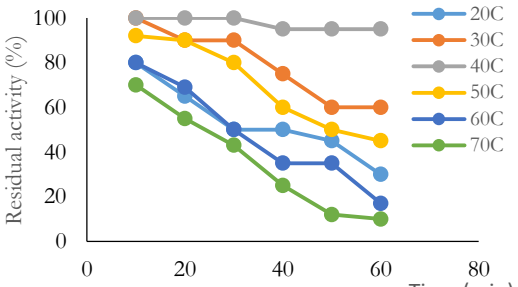


Fig. 2: Plots of residual activiy versus time of incubation for orange lesh variety PPO

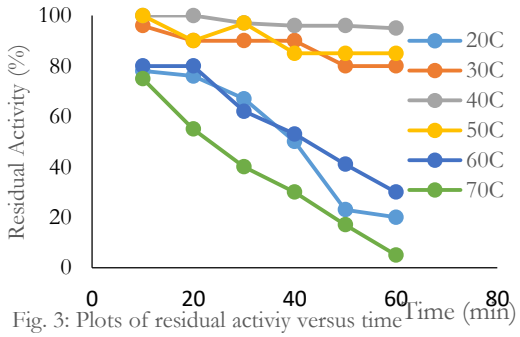


Fig. 3: Plots of residual activiy versus time of incubation for brown peel variety PPO.

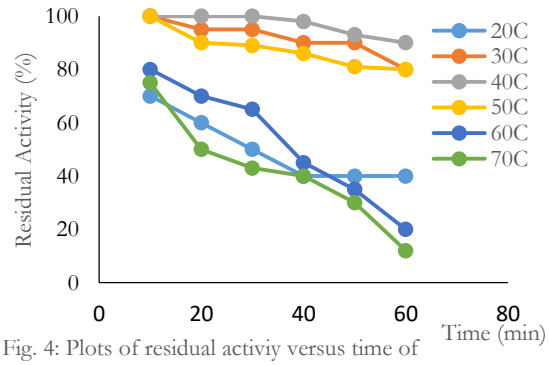


Fig. 4: Plots of residual activiy versus time of incubation for purple peel variety PPO.

**Table 1: Temperature optimum for polyphenol oxidase from *Ipomoea batatas* varieties**

| *Enzyme Source* | *Optimum Temp. (ºC )* |
|---|---|
| Ipomoea batatas *variety* | |
| *Orange flesh* | 35-40 |
| *Purple peel* | 40 |
| *Brown peel* | 40 |

## 4. DISCUSSION

The effect of temperature on enzyme activity is seen in the bell-shaped curve for a plot of activity versus temperature. The highest point on the curve represent the optimum temperature which varies for an enzyme depending on the source and other factors. Optimum temperature for polyphenol oxidase similar to those reported this work have been documented. Optimum temperature of 40ºC for polyphenol oxidase was reported by Murillo *et al*., (2016) Nagai and Suzuki, (2001, 2003), Silva and Koblitz, (2010), Bello and Sule, (2012), Dogru and Erat, (2012) and Wodu *et al*., (2018) for *Averrhoa carambola* juice, Chinese cabbage, soybean sprouts, *Spondias spp*, *Carica papaya and Cucurbita pepo*, parsley and *Colocasia esculenta* respectively. Optimum temperature of 35ºC reported in this work was also reported by Sikora *et al*., (2019) for *Len culinaris* Medik polyphenol oxidase. Although Deepaa and Wong (2012) had reported 30ºC as optimum temperature for purple sweet potato polyphenol oxidase which is a little different from 35ºC reported in the present study. This difference may be due to the plant location, extraction procedure, etc. Optimum temperature as low as 20ºC for polyphenol oxidase extracted from apricot and apple (Mahmood *et al*., 2009) and *Manihot esculenta crantz* leaves (Wong and Lee, 2014) had been reported. Higher Optimum temperature 50ºC was reported by by Serradell *et al*., (2000) for strawberry polyphenol oxidase, while 55ºC was reported by Navarro *et al*., (2014) for persimmon polyphenol oxidase.

Results from the thermal stability studies revealed that polyphenol oxidase extracted from the three *Ipomoea batatas* varieties showed some similarities and differences. They were all very stable at 40ºC and retained above 90% of their

initial activity even after 60mins of incubation. However, orange flesh variety was least stable at other incubation temperatures. Polyphenol oxidase from purple peel and brown peel varieties retained at least 70% of their initial activities even after incubation at 30ºC and 50ºC for 60mins, whereas orange flesh variety retained at least 70% of its initial activity upon incubation at both temperatures for 30mins. Enzymes in this report were largely unstable at 20ºC, 60ºC and 70ºC. The decrease observed in activity in higher temperatures is as a result of unfolding of the proteins tertiary structure (Kumar *et al*., 2008). Similar decrease had been reported by Mahmood *et al*., (2009) and Wong and Lee, (2014). Wohlt *et al*., (2021) also reported thermal inactivation of banana polyphenol oxidase at 60ºC and 70ºC. Polyphenol oxidase extracted from different samples have been reported to display different levels of stability upon heating. Heat labile *Allium* polyphenol oxidase was reported by Arslan *et al*., (1997) to be entirely inactivated at 40ºC. Lettuce polyphenol oxidase on the other hand retained 72% of its activity when incubated at 50ºC for 60mins (Martin-Diana *et al*., 2005).

## 5. CONCLUSION

In conclusion, the activity of polyphenol oxidase from tubers of *Ipomoea batatas* varieties like other enzymes depend on temperature. The enzymes which were very stable around 40ºC were unstable at 20ºC, 60ºC and 70ºC. The temperature at which the enzymes are less stable may be exploited in the control of undesirable browning of *Ipomoea batatas* tubers due to polyphenol oxidase, while also considering the effect of that temperature on the tubers to be processed.

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