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Optimum pH and pH Stability of Polyphenol Oxidase Extracted from Tubers of Three Varieties of *Ipomoea batatas* (Sweet Potato) Commonly Cultivated in Bayelsa State, Nigeria.

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Abstract.
The effect of pH on the activity and stability of polyphenol oxidase extracted from tubers of three varieties of *Ipomoea batatas* (sweet potato) namely orange flesh, purple peel and brown peel was investigated. The enzyme was assayed by monitoring the increase in absorbance for 3 minutes. pH effect was determined with 0.2 M acetate buffer (pH range between 3.0–5.5) and 0.2 M sodium phosphate buffer (pH range between 6.0–9.0). Optimum pH values for orange flesh, purple peel and brown peel were found to be 7.0, 6.5 and 7.0 respectively. The enzymes extracted from orange flesh variety of *Ipomoea batatas* was stable at the pH range of 6.5-7.5, while purple peel and brown peel varieties were stable at the pH range of 6.0 -7.5. Any increase or decrease of pH from these ranges would cause decrease in the activity of the enzyme, hence the use of pH can be a good way of controlling the undesirable colouration it causes it in foods.
Keywords: Optimum pH, pH stability, Polyphenol oxidase, Three varieties *Ipomoea batatas.*

Introduction.

Polyphenol oxidase is one of the enzymes largely responsible for enzymatic browning of fruits and vegetables. Its role in food browning results from the formation of O-quinones from the oxidation of phenolic substrates. The resulting instable O-quinone reacts with other O-quinone molecules and with protein or amino acids to form a dark brown pigment (Mason and Peterson, 1965). The formation of these dark brown polymers is undesirable because it decreases the visual and nutritional quality of the plant and affects their market value (Eskin and Shahidi, 2013).

The study of an enzyme's dependence on pH is an important determinant on the efficiency of the enzyme. The enzyme activity increases with pH, gets to reach its maximum at its optimum pH and decreases with further increase in pH, and ultimately falls to zero activity. A plot of the enzyme's activity as a function of pH resembles a bell-shaped curve (Bisswanger, 2016).

Most enzymes are irreversibly denatured in solutions with very high and very low pH due to attack on their tertiary structure (Whitaker, 1994). This means therefore, that pH can be used to control enzyme activity.

Polyphenol oxidases have been studied in many plants such as apple (Deepaa and Wong, 2012; Aydin *et al*., 2015), apricot (Arslan *et al*., 1998; Mahmood *et al*., 2009; Deepaa and Wong, 2012), banana (Ünal, 2007), nettle (Gülçin *et al*., 2005), broccoli (Gawlik-Dziki *et al*., 2007), peppermint (Kavrayan and Aydemir, 2001), hot pepper (Arnmok *et al*., 2010) sweet

potato (Deepaa and Wong (2012), ispir sugar bean (Sakiroglu *et al*., 2013), onion (Dogan *et al*., 2013), eggplant (Deepaa and Wong, 2012; Mishra *et al*., 2012), loquat fruit (Zhang and Shao, 2015) Ataulfo mango (Cheema and Sommerhalter, 2015). and *Carica papaya* (Bello *et al*., 2021)

Polyphenol oxidase activity have also been studied in *Ipomoea batatas*, however, pH dependence of *Ipomoea batatas* polyphemol oxidase have not been exhaustively reported. This work, thus elucidates the optimum pH and pH stability of polyphenol oxidase extracted from three varieties of *Ipomoea batatas*.

Materials and Methods.

Preparation of Enzyme Extract.

Freshly harvested tubers of *Ipomoea batatas* were washed to remove dirt. The tubers were cut into tiny sizes. Exactly 30g each of the tiny cubes of each variety were homogenized in ice cold 0.2M potassium phosphate buffer (pH 6.8). The homogenate was filtered through double layer of cheese cloth. The filtrate was further centrifuged (universal centrifuge, 320R Hectti) at 4000rpm for 25mins at 4°C to obtain the aqueous crude extract.

Acetone Precipitation.

The enzyme was precipitated by adding ice cold acetone in bits to the supernatant in the ratio 1.5:1, with gentle stirring for 60 minutes in ice bath. The mixture was centrifuged at 4000rpm for 25mins at 4°C to obtain the precipitate, which was re-dissolved in 10ml of extraction buffer and was used as the enzyme source.

Total Protein.

Protein estimation was done in all preparations by the Bradford method (1976). Bovine serum albumin was used as standard.

Enzyme Assay.

Polyphenol oxidase activity was assayed by monitoring the increase in absorbance at 420nm. The 3ml reaction mixture contained 2.8ml of 40mM substrate (prepared in 100mM phosphate buffer, pH 6.8) and 0.2 ml of enzyme solution. The increase in absorbance was monitored for 3mins using a UV-spectrophotometer (Spectrum lab 755a). The blank consisted of 3 ml of substrate solution in phosphate buffer. The initial velocity was calculated from the slope of the absorbance versus time graph. One unit of PPO activity was defined as the amount of enzyme that caused a 0.001 increase of absorbance per minute.

Effects of pH on Polyphenol Oxidase activity.

Optimum pH of polyphenol oxidase was done by assaying for the enzyme activity in a pH range of 4.0 – 9.0. Buffer solutions used for this study were 0.2 M acetate buffer for the pH range of 3.0 – 5.5 and 0.2 M phosphate buffer for pH 6.0 – 9.0. The enzyme activity was assayed by using the standard reaction mixture and changing the buffer.

To determine the effect of pH on PPO stability, 0.1 ml of enzyme solution was incubated in 0.9 ml of various buffer solutions (pH 4.0 – 9.0) for 10hrs at 4^0C, and the residual activity was measured. The enzyme activity was measured as described above. Residual polyphenol oxidase activity was expressed in the form of relative activity (%) of the pH optimum.

Kinetic Properties

Ipomoea batatas polyphenol oxidase activity was determined with catechol as substrate (10mM to 40mM). The enzyme reaction proceeded at pH 6.8 and ambient temperature. The

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

Results and Discussion.

pH as a factor that affects the rate of enzyme catalysed reaction cannot be overemphasized. The optimum pH is that in which an enzyme exhibits highest activity. Thus, any change in H^+ concentration above or below this pH tend to decrease the activity of the enzyme. As indicated from this study, polyphenol oxidase isolated from purple peel sweet potato had optimum pH value of 6.5, while those from orange flesh and brown peel varieties had same value of 7.0 as depicted in figure 1 and table 1. Researchers have shown that polyphenol oxidases extracted from plants showed maximum activity near neutral pH. The optimum pH of 6.5 estimated for purple peel variety polyphenol oxidase had also been reported by Bello *et al*., (2021) for *Carica papaya* PPO, Zhang and Shao, (2015) for Loquat fruit PPO and Dogan *et al*., (2013) for onion balm PPO. Also, optimum pH of 7.0 estimated for polyphenol oxidase extracted from orange flesh and brown peel varieties had been reported by Mahmood *et al*., (2009) for apricot, apple and eggplant PPO, Deepaa and Wong (2012) for sweet potato PPO and Arnmok *et al*., (2010) for hot pepper PPO.

The optimum pH values estimated for the polyphenol oxidase extracted from the three varieties of sweet potatoes fall within the range of 4.0 - 8.0 as have been reported by some researchers. Although, higher optimum pH value of 8.4 was reported for red apple peel PPO by Olusola and Oluwatosin, (2016).

The optimum pH of polyphenol oxidases from different sources varies due to certain factors. These factors as observed by some researchers include the type of substrate used (Whitaker, 1994; Paul and Gowda, 2000; Arnnok *et al*., 2010), the method of extraction and cellular location of the enzyme (Alward and Haidman, 1969). It was also reported by Danilo *et al*., (2021) that the variation of pH optimum for PPOs may be due to the difference in primary structure of the enzyme obtained from different sources. This is because the protonation of ionizable amino acid affects intermolecular interactions, which ultimately translates to different conformations of the enzyme at different pH values (Ben-Shalom *et al*.,1977; Jukanti, 2017). Different optimum pH values resulting from the different substrates used may be due to difference in binding capacity of the substrates as they interact with the enzyme active site under acidic and alkaline conditions (Jukanti, 2017).

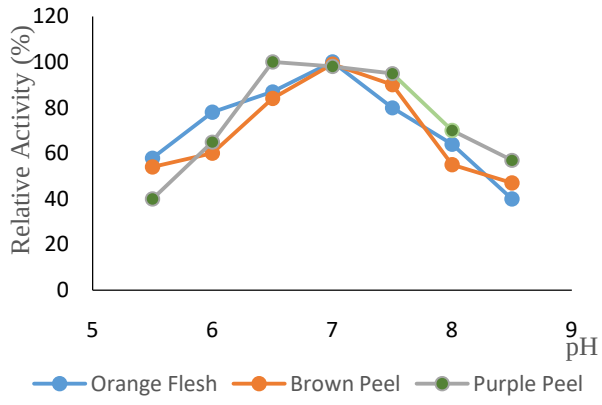

Fig 1: Optimum pH of polyphenol oxidase activity

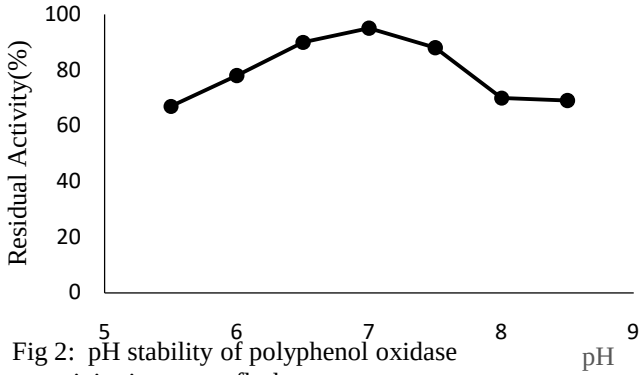

Fig 2: pH stability of polyphenol oxidase activity in orange flesh sweet potato

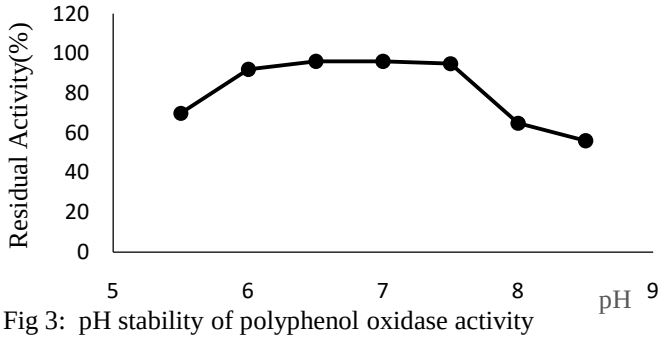

Fig 3: pH stability of polyphenol oxidase activity in brown peel sweet potato

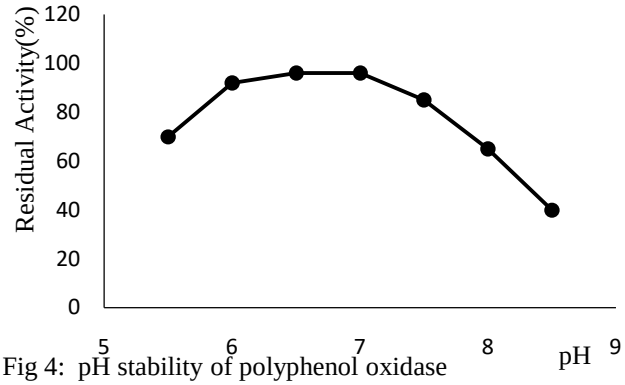

Fig 4: pH stability of polyphenol oxidase activity in purple peel sweet potato

The pH range in which an enzyme shows highest activity is referred to as pH stability. However, any increase above and decrease below this range results in decrease in the enzyme activity. As inferred from the present study, polyphenol oxidase extracted from the three varieties of sweet potato investigated were stable at slightly acidic to slightly alkaline pH. Orange flesh sweet potato polyphenol oxidase is stable between pH 6.5 - 7.5, whereas purple peel and brown peel polyphenol oxidases are stable between pH 6.0 - 7.5 as shown in figures 2 - 4.

As with the observation from the present investigation, most plant polyphenol oxidases are stable near neutral pH. Polyphenol oxidase extracted from garden egg, pawpaw and pumpkin are most stable near neutral pH (Bello *et al*., 2011). Also pH stability of 6.0 - 7.0 was reported by Kavrayan and Aydemir, (2001) for peppermint polyphenol oxidase, and Wodu *et al*., (2018) for *Colocasia esculenta* polyphenol oxidase.

Table 1: Optimum pH and pH stability of the PPO extracted from *Ipomoea batatas* varieties.

Enzyme Source	Optimum pH	pH Stability
Ipomoea batatas variety		
Orange flesh	7.0	6.5 -7.5
Purple peel	6.5	6.0 - 7.5.
Brown peel	7.0	6.0 - 7.5

The investigation concluded that polyphenol oxidase extracted from the three varieties of *Ipomoea batatas* (sweet potato) exhibited optimum pH near neutral. Also all three enzymes are stable at pH range near neutral pH similar to Polyphenol oxidases extracted from other plant sources.

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