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OPTIMUM pH AND pH STABILITY OF CRUDE POLYPHENOL OXIDASE (PPO) EXTRACTED FROM FIVE FRUIT SAMPLES COMMONLY CONSUMED IN KANO STATE, NIGERIA

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ABSTRACT
The effect of pH on the activity and stability of crude polyphenol oxidase (PPO) extracted from garden egg (Solanum aethiopicum), pawpaw (Carica papaya), pumpkin (Cucurbita pepo), guava (Psidium guajava) and bush mango (Irvingia gabonnensis) fruits were studied. Catechol at concentration of 20 mM was used as a substrate while sodium acetate buffer (0.2 M), pH range between 3.0– 5.5 and sodium phosphate buffer(0.2 M) , pH range between 6.0– 8.5 were used to determine the effect of pH on the PPO activity. Optimum pH values were found to be 6.0,6.5,6.0, 4.5 and 4.0/or 8.0 for the enzyme extracted from Solanum aethiopicum,, Carica papaya, Cucurbita pepo, Psidium guajava and Irvingia gabonnensis respectively. The enzyme was found to be stable at the pH range of 5.0-7.5 for the enzyme extracted from garden egg, 6.0-8.0 for that from pawpaw, 4.5-7.0 for that from pumpkin, 4.0-6.5 for that from guava and 3.5-5.5 and 7.0-8.0 for that from bush mango respectively. Increase or decrease of pH from the ranges would cause decrease in the activity of the enzyme, and can be a good way of controlling undesirable changes caused by it in foods.

Keywords: Optimum pH, pH stability, Polyphenol oxidase, Common fruits.

INTRODUCTION

Polyphenol oxidase (monophenol, dihydroxyphenylalanine:oxygen oxidoreductase, EC1.14.18.1; PPO) is a bifunctional, copper-containing enzyme widely distributed in the phylogenetic scale. In the presence of molecular oxygen, the enzyme catalyses both the o-hydroxylation of monophenols to give o-diphenols (cresolase activity) and the further oxidation of o-diphenols to o-quinones (catecholase activity). The o-quinones thus generated are very unstable and rapidly react with themselves and with amino acids or proteins, to form brown or black pigments (Mason and Peterson, 1965; Matheis and Whitaker, 1984; Garcia- Carmona *et al*., 1988) that are responsible for melanisation in animals and browning in plants. This browning phenomenon is generally undesirable in food technology because of the unpleasant appearance and the concomitant development of off flavour. Owing to its technological importance, therefore, numerous studies have been devoted to the inhibition of the enzyme from different sources by different chemical compounds (Ferrar and Walker, 1996,Marcos,*et a*l.,2008 and Wong, *et al*.,1971).

Polyphenol oxidase catalyses two basic reactions: hydroxylation to the *o*-position adjacent to an existing hydroxyl group of the phenolic substrate (monophenol oxidase activity), and oxidation of diphenol to *o*-benzoquinones (dipehnol oxidase activity). Both reactions utilize molecular oxygen as a co-substrate. Whether a single enzyme system exhibits both mono- and di- phenol oxidase activities is still unclear. However, when both monophenol- and diphenol oxidases are present in plants, the ratio of monophenol to diphenol oxidase activity is usually 1:10 or as low as 1:40 (Nicolas *et al*. 1994).

The two classes of enzymes can also be distinguished by their optimum pH and other criteria such as their differential susceptibilities towards some inhibitors, e.g. thiourea, phenylthiourea, cetyltrimethylammonium bromide, polyvinylpyrrolidone or carbon monoxide (Albisu *et al.,* 1989).The use of highly specific inhibitors of tyrosinase, such as tropolone or 4-hexylresorcinol, helped the discrimination of this enzyme from laccases and peroxidases (Dawley and Flurkey, 1993). Although the latter enzymes (EC 1.11.1.7) are known to oxidize a wide range of substrates including polyphenols (Vamos-Vigyazo, 1981), they are highly specific for hydrogen peroxide as the oxygen donor and cannot operate in the presence of catalase (International Union of Biochemistry, 1978). Since this enzyme is a key enzyme in catalysing browning reaction, this work studied the optimum pH and stability of crude PPO from five fruits (garden egg, pawpaw, pumpkin, guava and bush mango) commonly consumed in Kano State. It is hoped that, the result could be used as a guide to control unwanted discoloration in the products from these fruits and provide pH range that can be used in further studies of this enzyme.

MATERIALS AND METHODS

Extraction of PPO

Fruits were sliced horizontally into halves with a sharp knife, seeds were removed and the fruit cavities were cleaned in case of pawpaw. Each half was cut into four equal slices, and the processed fruit samples were stored at 5^0 C over night.

Prepared fruit sample (50g) was homogenised using pestle and mortar for 1 minute in 400 cm^3 of cold acetone. The homogenates were filtered quickly under vacuum of a Buchner funnel. The filterate was suspended in 150cm^3 of 0.1M sodium phosphate buffer pH (6.5) and stirred for 30 minutes at 0^0C. The suspension was centrifuged at 10000rpm for 30 minutes at 4^0C, and the homogenate contained the extracted PPO (Ying and Zhang, 2008).

PPO assay

Polyphenol oxidase activity was determined by measuring the increase in absorbance at 410 nm with a spectrophotometer. The sample cuvette contained 2.0 cm^3 of catechol (10-80mM), 0.9 cm^3 of 0.2 M sodium acetate buffer pH 4.0 and 0.1 cm^3 of enzyme solution. Each sample was assayed in triplicate. Reference cuvette (blank) contained 2.0 cm^3 of the same substrate solution and 1.0 cm^3 of 0.2 M sodium acetate buffer (Ying and Zhang, 2008).

Effect of pH on PPO Activity and Stability

Two kinds of buffer solutions were used for this study: 0.2 M sodium acetate buffer for the pH range of 3.0–5.5 and 0.2 M sodium phosphate buffer for pH 6.0–8.5. Catechol (20 mM) was used to determine the effect of pH on PPO activity. To determine the effect of pH on PPO stability, 0.1 cm^3 of enzyme solution was incubated in 0.9 cm^3 of various buffer solutions (pH 3.0–8.5) for 10 h at 4^0C, and the residual activity was measured at 2 and 10 hours, respectively. The enzyme activity was measured according to the method described by Ying and Zhang (2008). Residual PPO activity was determined in the form of percent residual PPO activity at the optimum pH.

RESULTS

Effect of pH on the activity of crude PPO activity from garden egg, pawpaw, pumpkin, guava and bush mango at 25^0C are depicted in Figures 1-5. From the figure it can be seen that; the optimum pH for the crude enzyme ranges from 4.0 for PPO extracted from bush mango to 6.5 for that from pumpkin while the pH at which these enzymes are stable upon incubation after 2 and 10 hours is between 3.5 in bush mango to 8.0 in pumpkin respectively (Figures 6-10; Table 1).

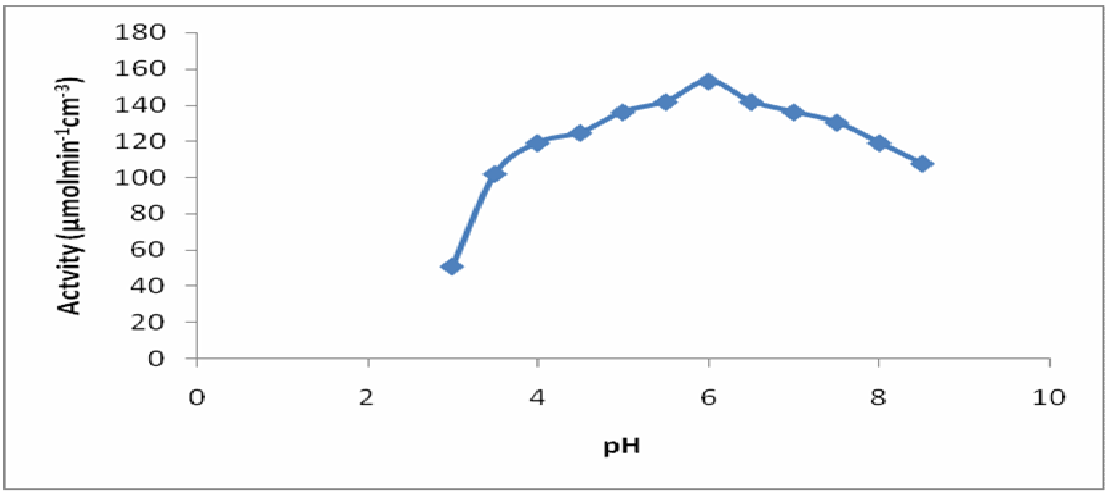

Figure 1: Activity of crude PPO extracted from garden egg (*Solanum aethiopicum*) fruit against pH

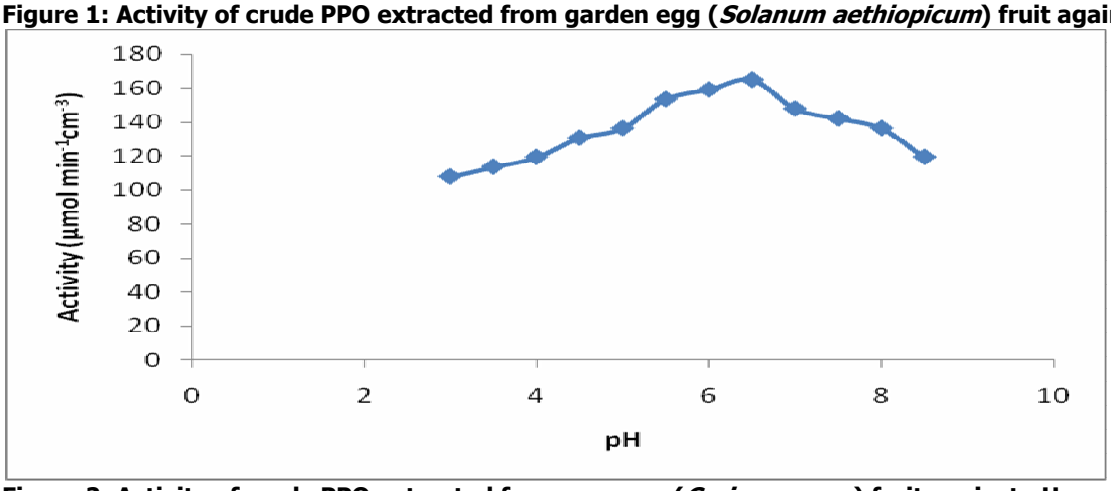

Figure 2: Activity of crude PPO extracted from pawpaw (*Carica papaya*) fruit against pH.

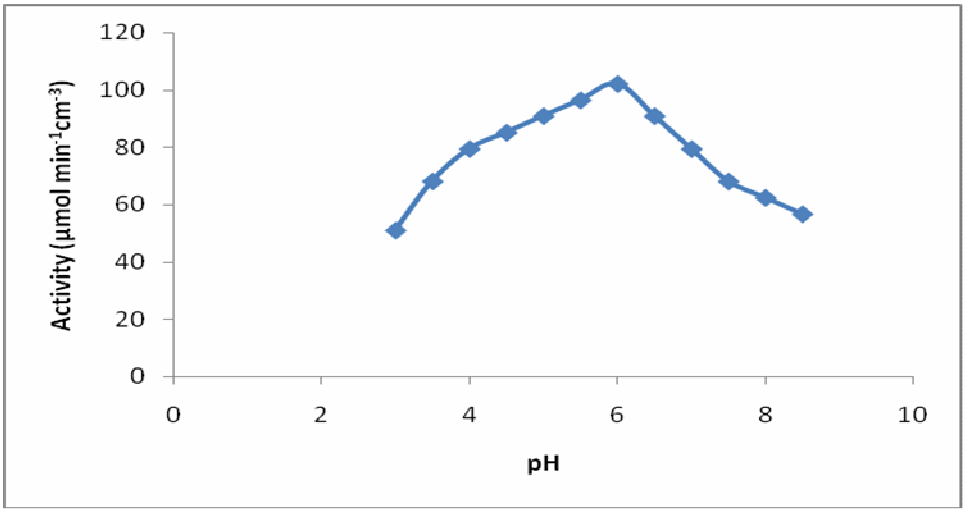

Figure 3: Activity of crude PPO extracted from pumpkin(*Cucurbita pepo*) fruit against pH.

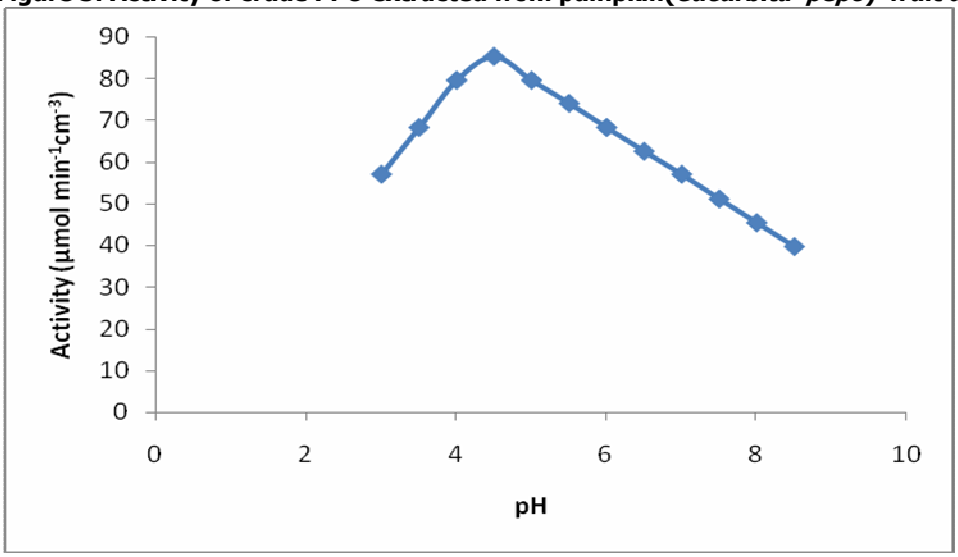

Figure 4: Activity of crude PPO extracted from guava (*Psidium guajava*) fruit against pH.

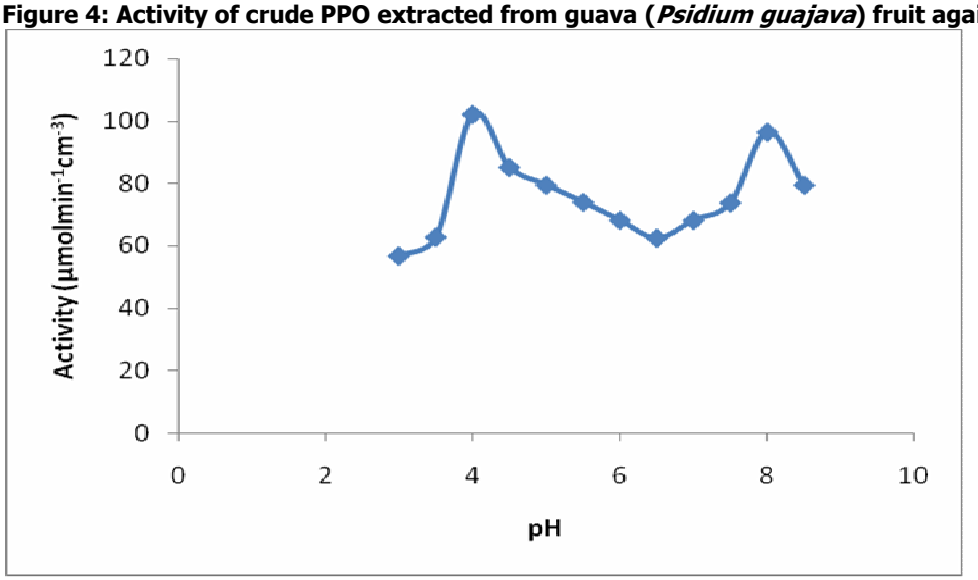

Figure 5: Activity of crude PPO extracted from bush mango *(Irvingia gabonnensis)* fruit against pH.

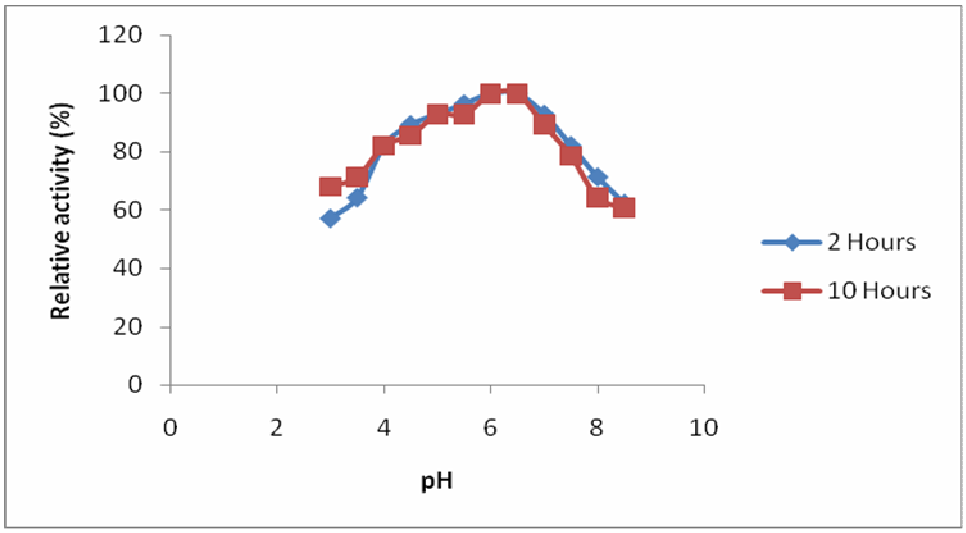

Figure 6: % Relative activity of crude PPO extracted from garden egg (*Solanum aethiopicum*) against pH after 2 and 10 hours incubation.

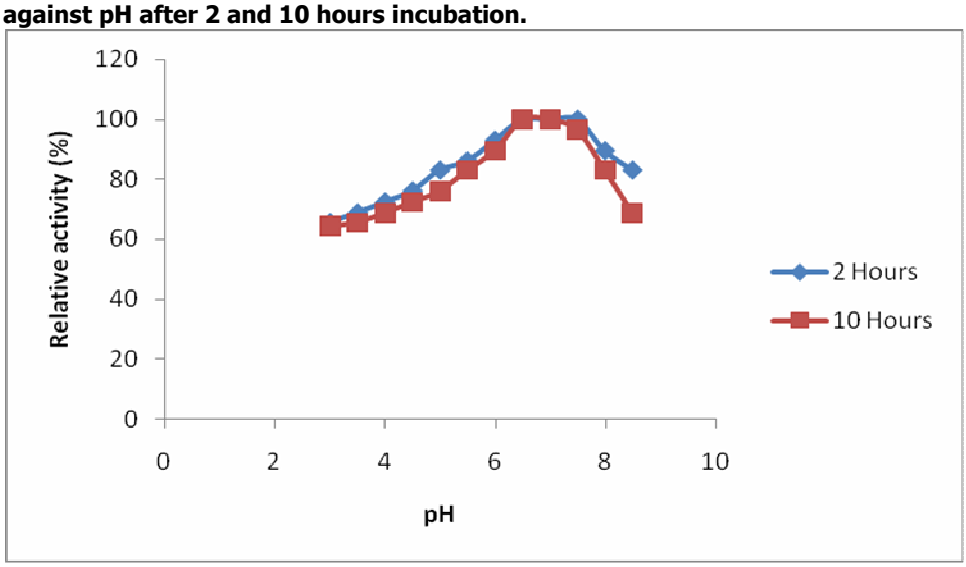

Figure 7: % Relative activity of crude PPO extracted from pawpaw (*Carica papaya*) against pH after 2 and 10 hours incubation.

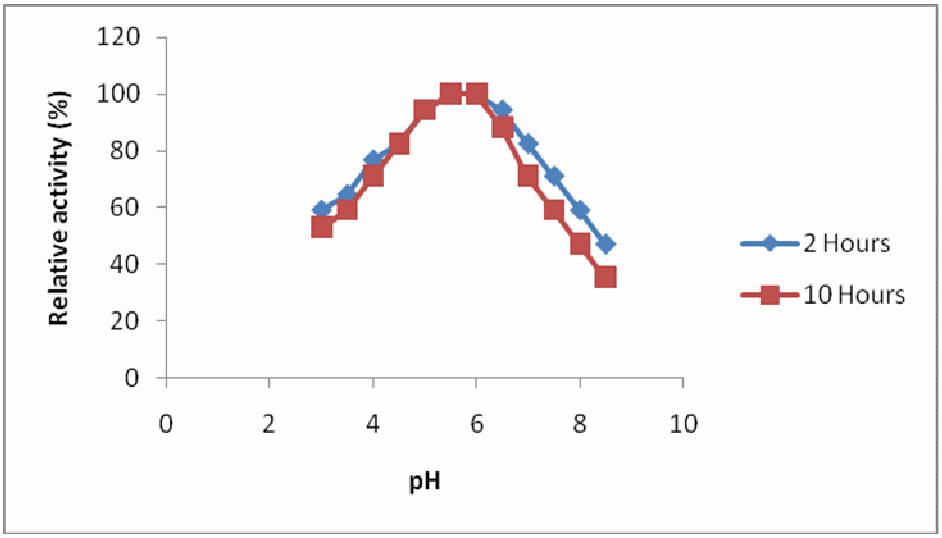

Figure 8: % Relative activity of crude PPO extracted from pumpkin (*Cucurbita pepo*) against pH after 2 and 10 hours incubation.

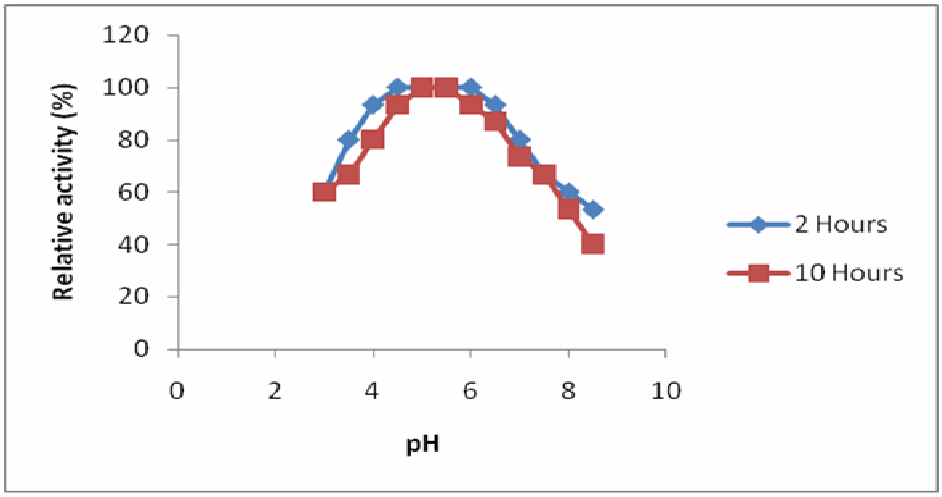

Figure 9: % Relative activity of crude PPO extracted from guava *(Psidium guajava*) against pH after 2 and 10 hours incubation.

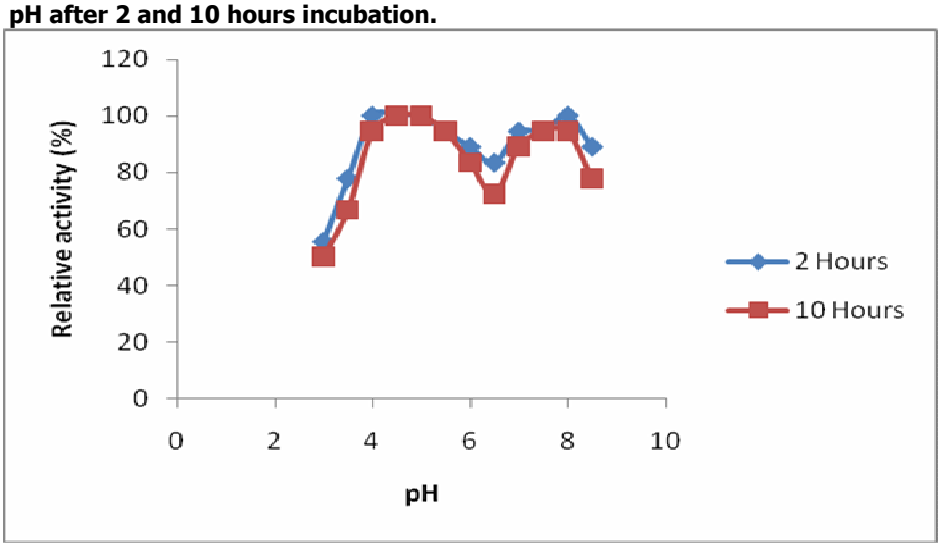

Figure 10: % Relative activity of crude PPO extracted from bush mango (*Irvingia gabonnensis)* against pH after 2 and 10 hours incubation.

Table 1: The optimum pH and pH stability of the crude PPO extracted from five fruit samples.

Enzyme source	Optimum pH	pH stability
Solanum aethiopicum	6.0	5.0-7.5
Carica papaya	6.5	6.0-8.0
Cucurbita pepo	6.0	4.5-7.0
Psidium guajava	4.5	4.0-6.5
Irvingia gabonnensis	4.0 & 8.0	3.5-5.5 &7.0-8.0

DISCUSION

pH is among the factors which affects the rate of an enzyme catalysed reaction. An optimum pH is the pH at which an enzyme shows its maximum activity. Therefore for any enzyme, when the $[H^+]$ concentration of the reaction medium is increased above or decreased below the optimum pH, the activity tends to decrease. From the result obtained in this study, the optimum pH for crude PPO extracted from garden egg and pumpkin, pawpaw and guava were found to be 6.0, 6.5 and 4.5 while that from bush mango was found to show two optimum pH 4.0 and 8.0 as depicted in Figures 1-5 and Table 1. Undesirable browning in products of these fruits can be prevented by altering the pH below or above the optima. This result is supported by the work of Aylward and Haisman (1969) who reported that; the optimum pH for maximum PPO activity in plants varied from approximetly 4.0 to 7.0, depending on the extraction methods, substrate used for assay and localization of the enzyme in the plant cell. In most cases PPO enzymes from different plants have only one pH optimum such as those from potato 5.0 (Balasingam and Ferdinand, 1970), medlar 6.5 (Barbaros *et al*., 2002), longan fruit 7.0 (Jiang, 1999). However, the result of this work reveals that PPO extracted from bush mango shows two optimum pH values of 4.0 and 8.0 as shown in Figure 5, but with maximum activity at 4.0.

This observation (possession of two optimum pH values) might have resulted from the presence of isoenzymes which is supported by the fact that PPOs from some other plants have two pH optima, such as those from apple (Shannon and Pratt, 1967) and sweet cherry (Pifferi *et al*., 1974). The result of this work is also in line with the reports of Segel (1976) and Tipton and Dixon (1983) who stated that, ionizable groups of the protein structure of enzymes are affected by the pH of the food medium. These groups must be in the appropriate ionic form in order to maintain the conformation of the active site, bind substrates, or catalyse the enzymatic reaction. Changes in the ionization status of enzymes are generally reversible. Irreversible denaturation can however occur under conditions of extreme pH. The stability of the substrate is also affected by changes in pH, since substrates can undergo chemical breakdown under extreme conditions of pH. Degraded substrates often behave as enzyme inhibitors, since they share the molecular features of the substrate.

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. Crude PPO extracted from garden egg, pawpaw and pumpkin showed instability in acidic pH but was more stable near neutral pH as shown in Figures 6-10 and Table 1. This observation agrees with the findings of Kavrayan and Aydemir (2001) in which peppermint PPO was found to be stable between pH 6.0 and 7.0. PPO from bush mango also shows two ranges of pH stability but more activity was observed at range of 3.5-5.5(figure 10). This is possibly due to the presence of isozymes. Therefore, these results support earlier reports which indicate that most plant PPOs are unstable in acidic pH with exception of the isoform from bush mango (Rivas and Whitaker, 1973 and Barbaros *et al*., 2002). In conclusion this work can be used as reference pH range at which purification or extraction of PPO from the five fruits studied can be made, as well as in inhibiting their activity.

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