

# Thermal and pH stabilities of partially purified polyphenol oxidase extracted from *Solanum melongenas* and *Musa sapientum* fruits

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### ABSTRACT

Background: Enzyme activity depends largely on environmental conditions such as temperature and pH. **Objectives:** The stabilities of polyphenol oxidase (PPO) extracted from Solanum melongenas and Musa sapientum fruits pre-incubated in varying thermal and pH conditions were carried out. Materials and Methods: Enzyme activity was measured using spectrophotometric methods. The reaction mixture contained 3.5 mL of 0.20 M phosphate buffer (pH = 6.8), 1.0 mL of 0.75 mM catechol, and 0.5 mL of enzyme solution. Results: PPOS. melongenas and PPOM. sapientum gave different temperature and pH optima. The temperature-activity profile of PPOS. melongenas and PPOM. sapientum showed a strong positive correlation (r = 0.907363). At pH = 10.0, PPOM. sapientum activity represented 65.3% decay in enzyme activity, whereas PPOS. melongenas represented 79.3% decay in enzyme activity. PPOS. melongenas and PPOM. sapientum stability at pre-incubated temperatures of 20, 50 and 60°C and pH values of 3.5, 6.0 and 8.0 were measured. Residual activities of PPOS. melongenas and PPOM. sapientum showed strong positive correlations under the same experimental thermal conditions, with the exception at  $20^{\circ}$ C (r = 0.693375). Specifically, pre-incubation of PPOM, sapientum for t = 90 min at 60°C caused 18.4% decay in relative activity of PPOM, sapientum. At t = 90 min, pre-incubation of PPOM. sapientum, in pH = 3.5 caused decay in activity within the range of 30.8-36.1%, whereas PPOM. sapientum pre-incubated in pH = 6.0 and pH = 8.0 gave decay in activity within the range of (1.5-9.8%) and (2.7-6.5%), respectively. Conclusion: PPOS. melongenas and PPOM. sapientum showed relatively higher stabilities as the incubation pH tended toward alkaline conditions, whereas the two experimental temperatures (20 and 60°C) promoted destabilization.

KEY WORDS: Musa sapientum, pH, polyphenol oxidase, Solanum melongenas, temperature

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INTRODUCTION

Polyphenol oxidase (PPO) is a collection of ubiquitous plant enzymes (EC 1.10.3.2, catechol oxidase or diphenol oxygen oxidoreductase [1,2]; EC. 1.14.18.1; monophenol

plant enzymes (EC 1.10.3.2, catechol oxidase of diphenol oxygen oxidoreductase [1,2]; EC. 1.14.18.1; monophenol oxidase, cresolase, and tyrosinase [3,4]) responsible for undesirable browning reactions of fruits and vegetables. However, studies have shown that many plant PPOs lack monophenol oxidase (cresolase) activity, restricting potential substrates of enzymes to diphenolic compounds such as catechol, 3, 4-dihydroxyphenylalanine, and chlorogenic acid [5,6]. Enzymatic browning is associated with oxidation of phenolic compounds in the presence of molecular oxygen to corresponding quinone intermediates that polymerize to form melanin and off-color pigments 7]. The kinetic properties of PPO extracted from various plant sources have been reported by several authors [8-10]. PPO is a copper-metalloenzyme located in the chloroplast thylakoid membrane [11] and can exist in an active or latent state [12]. PPO enzymes extracted from various plant tissues exhibit different characteristics, and exist in multiple molecular forms (isoforms) [13-15]. Isoenzymic forms of PPOs are identified according to their physical, chemical or enzymatic properties such as electrophoretic mobility, temperature and pH optima, substrate specificity and isoelectric index (pI)[16].

Enzyme activity depends largely on environmental conditions such as temperature and pH. Thermal and pH stabilities of PPO, as being reported here, describes the capacity of pre-incubated enzymes to withstand thermal and pH induced unfolding at specified experimental temperature and pH conditions. This study seeks to establish the thermal and pH conditions that promote stability of the PPOs extracted from *Solanum melongenas* and *M. sapientum* fruits.

### MATERIALS AND METHODS

### **Collection and Preparation of Fruit Samples**

Fresh and disease free fruits of *S. melongenas* and *Musa* sapientum were harvested from a private botanical garden in Umuoziri-Inyishi, Imo State, Nigeria between 17<sup>th</sup> and 30<sup>th</sup> of July, 2012. The fruits were identified and authenticated by Dr. F. N. Mbagwu at the Herbarium in the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. The two fruits were washed under continuous current of distilled water for 5 min and air dried at room temperature. The stalk (*S. melongenas*) and rind (*M. sapientum*) were removed manually. The samples were stored at  $-4^{\circ}$ C until used for analyses.

#### **Extraction and Purification of PPO**

Extraction and partial purification of PPO was according to the methods of Madani et al. [4], with minor modifications. 10 g of the sample was homogenized in external ice bath, using Ultra-Turrax T25 (Janke and Kunkel, Staufen, Germany) homogenizer set in 80 mL of 0.1 M phosphate buffer (pH = 6.8) containing 10 mM ascorbic acid for 180 s at intervals of 60 s. The homogenate was quickly squeezed through two layers of clean cheesecloth into a beaker kept in ice. The crude extract was rinsed with 200 mL of acetone  $(-20^{\circ}C)$  to eliminate phenolic compounds [15,17]. The sample was centrifuged at 32,000 g for 20 min at 4°C. Solid ammonium sulfate (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> was added to the supernatant to obtain 80% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> saturation and precipitated proteins were separated by centrifugation at 32,000 g for 30 min at 4°C. The precipitate was re-dissolved in 10 mL distilled water and ultra-filtered in a Millipore stirred cell with a 10-kDa membrane (Millipore 8050, Milan, Italy). The filtrate was dialyzed (cellulose membrane, Medicell Intl. Ltd., 6-27/32) at 4°C against distilled water for 24 h with four changes of the water during dialysis. The dialyzed sample constituted the partial purified PPO extract and was used as the enzyme source from the corresponding segments of the two fruits. Protein concentrations were determined by the methods of Bradford [18], using bovine serum albumin as standard at  $\lambda_{max} = 595$  nm. One unit of PPO activity was defined as the amount of enzyme that causes an increase in absorbance of 0.001/mL/min under the condition of the assay [19].

### **Determination of PPO Activity**

PPO activity was measured immediately after the extract was partially purified. Enzyme assay was according to the methods of Qudsieh *et al.* [20], with minor modifications [9]. Enzyme activity was determined by measuring the increase in absorbance using a spectrophotometer (U-2000 Hitachi, Japan) at 24°C. The reaction mixture contained 3.5 mL of 0.20 M phosphate buffer (pH = 6.8), 1 mL of 0.75 mM catechol, and 0.5 mL of enzyme solution in a final volume of 5 mL. The mixture was quickly transferred into a cuvette and the change in absorbance was monitored at  $\lambda_{max} = 540$  nm at a regular interval of 30 s.

The rate of the reaction was calculated from the initial linear slope of activity curves.

## Measurement of Temperature and pH Optima of PPO Activity

Activity of PPO was measured in assay mixture containing 0.75 mM catechol under varying temperatures within the range of 20-70°C. The enzyme activity was measured using 0.20 M phosphate buffer under varying pH conditions within the range of 5-10.

### Effect of Temperature and pH on PPO Activity and Stability

Purified enzymes extracted from *S. melongenas* and *M. sapientum* fruits were pre-incubated in varying temperatures of 20, 50 and 60°C. At regular time intervals of 30, 60 and 90 min, aliquots of the enzyme solution was withdrawn and assayed for PPO activity. The residual PPO activity was measured according to the following experimental conditions (PPO<sub>S. melongenas</sub>: pH<sub>optimum</sub>  $\approx$  7.0 at T°C<sub>optimum</sub>  $\approx$  30; PPO<sub>M. sapientum</sub>: pH<sub>optimum</sub>  $\approx$  7.0 at T°C<sub>optimum</sub>  $\approx$  30; PPO activity pre-incubated in varying pH values of 3.5, 6.0, and 8.0 were carried out. The residual PPO activity was measured according to the following experimental conditions (PPO<sub>S. melongenas</sub>: pH<sub>optimum</sub>  $\approx$  7.0 at T°C<sub>optimum</sub>  $\approx$  30; PPO activity pre-incubated in varying pH values of 3.5, 6.0, and 8.0 were carried out. The residual PPO activity was measured according to the following experimental conditions (PPO<sub>S. melongenas</sub>: pH<sub>optimum</sub>  $\approx$  7.0 at T°C<sub>optimum</sub>  $\approx$  30; PPO<sub>M. sapientum</sub>: pH<sub>optimum</sub>  $\approx$  7.0 at T°C<sub>optimum</sub>  $\approx$  40). Residual PPO activity was determined in the form of percent residual PPO activity at the temperature and pH optima.

### RESULTS

The fractionation steps and corresponding purification indices of the two PPO extracts is summarized in Table 1.

At the end of the purification steps, specific enzyme activity of  $PPO_{S.\ melongenas}$  and  $PPO_{M.\ sapientum}$  increased within the range of 0.314-8.11 U/mg protein and 0.292-7.65 U/mg protein respectively. Final enzyme activity of  $PPO_{S.\ melongenas}$  was 0.154 U, whereas  $PPO_{M.\ sabientum}$  gave 0.130 U [Table 1].

Temperature-activity profile of PPO<sub>S. melongenas</sub> and PPO<sub>M.</sub> sapientum is presented in Figure 1. The temperature-activity profile of the two PPOs showed a typical bell-shaped curve. The PPO<sub>S. melongenas</sub> T°C<sub>optimum</sub>  $\approx 30$ ; PPO<sub>S. melongenas</sub> activity = 0.096  $\pm$  0.02 U, whereas PPO<sub>M. sapientum</sub> T°C<sub>optimum</sub>  $\approx 40$ ; PPO<sub>M. sapientum</sub> activity = 0.086  $\pm$  0.02 U. The temperature-activity profile of PPO<sub>S. melongenas</sub> and PPO<sub>M. sapientum</sub> showed a strong positive correlation (r = 0.907363). At experimental temperature of 70°C, PPO<sub>S. melongenas</sub> activity = 0.021  $\pm$  0.01 U; PPO<sub>S. melongenas</sub> = 0.042  $\pm$  0.03 U, which represented 78.1% and 51.1% decay in enzyme activity, respectively.

The pH-activity profile of the two PPOs showed a typical bell-shaped curve. The PPO<sub>S. melongenas</sub> and PPO<sub>M. sapientum</sub> gave  $pH_{optimum} \approx 7.0$ . However, pH-activity profile of PPO<sub>S. melongenas</sub> exhibited two peak

values; pH  $\approx$  7.0 and pH  $\approx$  8.5. At experimental maximum pH = 10.0, PPO<sub>M. sapientum</sub> activity = 0.034 U, representing 65.3% decay in enzyme activity, whereas PPO<sub>S. melongenas</sub> = 0.025 U representing 79.3% decay in enzyme activity.

Tables 2 and 3 showed residual activities of PPO<sub>S. melongenas</sub> and PPO<sub>M. sapientum</sub> incubated in varying temperature and pH. The residual activity of PPO<sub>S. melongenas</sub> ranged between 0.086  $\pm$  0.02 U and 0.067  $\pm$  0.03 U; PPO<sub>M. sapientum</sub> was between 0.080  $\pm$  0.03 U and 0.070  $\pm$  0.02 U. A cursory look at Table 2 showed that the decreasing levels of PPO<sub>S. melongenas</sub> and PPO<sub>M. sapientum</sub> depended on temperature and duration of incubation. Residual activities of PPO<sub>S. melongenas</sub> and PPO<sub>M. sapientum</sub> showed a strong positive correlations under the same experimental temperature conditions, with the exception at 20°C, which gave a weak positive correlation (r = 0.693375) [Table 4].

Similarly, PPO<sub>S. melongenas</sub> and PPO<sub>M. sapientum</sub> incubated in varying pH conditions exhibited decreasing residual levels of PPO activity with the progress of experimental time. Specifically, residual level of PPO<sub>S. melongenas</sub> activity under the three experimental pH was in the order pH = 6.0 > pH = 8.0 > pH = 3.5 within the duration of the experiment (30 < t < 90). Table 4 showed PPO<sub>S. melongenas</sub> and PPO<sub>M. sapientum</sub> activities displayed strong positive correlations under the same pH conditions.

 $\label{eq:pre-incubation of PPO_{S.\,melongenas}} at 50^{\circ}C \ caused \ the \ lowest \ decay \\ in \ relative \ activity \ compared \ to \ PPO_{S.\,melongenas} \ pre-incubated \ at \ 20$ 

and 60°C. PPO<sub>S. melongenas</sub> pre-incubated at 60°C exhibited lower decay in relative activity at incubation period t = 30 min and t = 90 min, compared to PPO<sub>S. melongenas</sub> pre-incubated at 20°C. Conversely, PPO<sub>S. melongenas</sub> pre-incubated at 60°C showed higher decay in activity compared to the enzyme pre-incubated at 20°C; at t = 60 min [Figure 2].

Pre-incubation of  $PPO_{M. sapientum}$  at the three experimental temperatures caused increasing decay in the relative activity of the enzyme with the progress of time, which was in the order 20°C > 60°C > 50°C [Figure 2]. However, the increasing





S. melongenas						M. sapientum			
Enzyme fraction	E <sub>A</sub> (U)	T <sub>P</sub> (mg)	Specific E <sub>A</sub> (U/mg)	% Yield	E <sub>A</sub> (U)	T <sub>P</sub> (mg)	Specific E <sub>A</sub> (U/mg)	% Yield	
Crude homogenate	0.308	0.980	0.314	100	0.234	0.802	0.292	100	
Centrifuged at 32,000 g	0.215	0.072	2.99	69.8	0.167	0.082	2.04	71.4	
80% (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.194	0.032	6.06	62.9	0.151	0.043	3.51	64.5	
Ultra-filtration	0.162	0.022	7.36	52.6	0.133	0.029	4.59	56.8	
Dialysis	0.154	0.019	8.11	49.9	0.130	0.017	7.65	55.6	

Table 1: Properties of PPO extracted from S. melongenas and M. sapientum fruits at various purification steps

EA: Enzyme activity, To: Total protein, PPO: Polyphenol oxidase, S. melongenas: Solanum melongenas, M. sapientum: Musa sapientum

Table 2:	Residual	activity	of PP0	S. meloi	ngenas and	PPO M.	sapientum	incubated	in varying	temperature

		Enzyme activity $V_o$ (U)							
		S. melongenas		M. sapientum					
Time (min)	30	60	90	30	60	90			
20°C	0.079±0.03	0.076±0.02	$0.067 \pm 0.03$	$0.077 \pm 0.02$	$0.072 \pm 0.03$	0.072±0.03			
50°C	0.086±0.02	$0.083 \pm 0.01$	$0.082 \pm 0.02$	$0.080 \pm 0.03$	$0.079 \pm 0.02$	0.076±0.02			
60°C	0.082±0.01	0.075±0.02	0.073±0.02	0.076±0.03	0.073±0.02	0.070±0.02			

Values are means of three determinations ± SD; PPO: Polyphenol oxidase, *S. melongenas: Solanum melongenas, M. sapientum: Musa sapientum*, SD: Standard deviation

Table 3: Residual a	activity of PPO	<i>S. melongenas</i> and PPO	M. sapientum incuba	ited in varying	JрН
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Time (min)	Enzyme activity $V_o$ (U)							
		S. melongenas		M. sapientum				
	30	60	90	30	60	90		
pH=3.5	$0.067 \pm 0.03$	$0.052 \pm 0.03$	$0.033 \pm 0.03$	0.068±0.03	$0.065 \pm 0.03$	$0.063 \pm 0.03$		
pH=6.0	$0.143 \pm 0.02$	$0.140 \pm 0.02$	$0.138 \pm 0.02$	$0.097 \pm 0.02$	$0.092 \pm 0.02$	$0.088 \pm 0.02$		
pH=8.0	$0.127 \pm 0.01$	$0.124 \pm 0.01$	$0.122 \pm 0.01$	0.095±0.01	0.094±0.01	0.092±0.01		

Values are means of three determinations ±SD; PPO: Polyphenol oxidase, *S. melongenas: Solanum melongenas, M. sapientum: Musa sapientum*, pH: Phosphate, SD: Standard deviation

decays in activities of PPO<sub>M. sapientum</sub> pre-incubated at 20°C and 60°C was not significantly different (P < 0.05). Specifically, pre-incubation of PPO<sub>M. sapientum</sub> for t = 90 min at 60°C caused 18.4% decay in relative activity of PPO<sub>M. sapientum</sub> [Figure 3].

Pre-incubation of PPO<sub>*M. sapientum*</sub> in pH = 3.5 caused decay in relative enzyme activity between the range of 56.3% and 78.8% within the experimental time (30 < t < 90) min. Decay in relative activity was significantly different among the PPO<sub>*M. sapientum*</sub> incubated at the three experimental pH conditions, which was in the order pH = 3.5 > pH = 8.0 > pH = 6.0 [Figure 4].

The decay in relative activity of  $PPO_{M. sapientum}$  pre-incubated in pH = 6.0 and pH = 8.0 were not profound compared

Table 4: Correlation coefficient between residual activities of PPO *S. melongenas* and PPO *M. sapientum* incubated in varying temperature and pH

Correlation coefficient (r)								
Temperatur	re (°C)		рН					
20	50	60	3.5	6.0	8.0			
0.693375	0.846154	0.952217	0.983342	0.984018	0.953821			

PPO: Polyphenol oxidase, *S. melongenas: Solanum melongenas, M. sapientum: Musa sapientum;* pH: Phosphate



Figure 2: Percentage decay in enzyme activity of polyphenol oxidase extracted from Solanum melongenas incubated at varying temperature



Figure 3: Percentage decay in enzyme activity of polyphenol oxidase extracted from *Musa sapientum* incubated at varying temperature

to pH = 3.5 pre-incubation. At the end of experimental t = 90 min, pre-incubation of PPO<sub>*M. sapientum*</sub>, in pH = 3.5 caused decay in relative activity within the range of 30.8-36.1%, whereas PPO<sub>*M. sapientum*</sub> pre-incubated in pH = 6.0 and pH = 8.0 gave moderate decays in relative activities, which was within the range of (1.5-9.8%) and (2.7-6.5%), respectively [Figure 5].

#### DISCUSSION

From the present study, PPO extracted from the two fruits showed different pH and temperature optima (PPO<sub>S. melongenas</sub>: pH<sub>optimum</sub>  $\approx$  7.0 at T°C<sub>optimum</sub>  $\approx$  30; PPO<sub>M. sapientum</sub>: pH<sub>optimum</sub>  $\approx$  7.0 at T°C<sub>optimum</sub>  $\approx$  40) [Figures 1 and 6]. Worthwhile to note, PPO<sub>M. sapientum</sub> T°C<sub>optimum</sub>  $\approx$  40, was same as PPO extracted from lily Carica papaya and Cucurbita pepo [21]. Other reports by several authors [17, 22-26] gave diverse temperature and pH optima of PPOs extracted from various plant tissues. Specifically, Zheng *et al.* [27], using 10 mM catechol as substrate reported that Vitis vinifera "Thompson seedless PPO activity pH<sub>optimum</sub>  $\approx$  6.0 and T°C<sub>optimum</sub>  $\approx$  25. Nakamura *et al.* [28], stated that T°C<sub>optimum</sub> and pH<sub>optimum</sub> of PPO extracted from Koshu V. vinifera were approximately 25°C and 6.0, respectively. Aylward and







Figure 5: Percentage decay in enzyme activity of polyphenol oxidase extracted from *Musa sapientum* incubated at varying pH



Figure 6: pH profile of polyphenol oxidase activity extracted from Solanum melongenas and Musa sapientum fruits

Haisman [29] and Sellés-Marchart et al. [30], reported that differences in optimum pH for PPO activity depended on plant sources, extraction methods, and purities of enzyme, buffers, and substrates. However, most plants show maximum PPO activity near neutral pH values [31,32]. These previous reports are consistent with the present report (PPO<sub>S. melongenas</sub>:  $\text{pH}_{\text{optimum}} \approx 7.0$ ;  $\text{PPO}_{M. sapientum}$ :  $\text{pH}_{\text{optimum}} \approx 7.0$ ) [Figure 6]. Remarkably, pH-activity profile of  $\text{PPO}_{S. melongenas}$  exhibited two peak values; pH  $\approx$  7.0 and pH  $\approx$  8.5, which was an indication of the presence of isoenzyme based on similar reports by Bello et al. [22]. Using catechol as substrate, Bello and Sule [33], reported variable T°C<sub>optimum</sub> of PPO extracted from wide varieties of Tropical fruits and vegetables. Accordingly, *Solanum* aethiopicum: T°C<sub>optimum</sub>  $\approx$  50°C; C. papaya: T°C<sub>optimum</sub>  $\approx$  40°C; C. pepo  $T^{\circ}C_{\text{optimum}} \approx 50^{\circ}$ C; Psidium guajava  $T^{\circ}C_{\text{optimum}} \approx 30^{\circ}$ C; Irvingia gabonnensis  $T^{\circ}C_{\text{optimum}} \approx 50^{\circ}$ C. It is worthwhile to note that PPO  $T^{\circ}C_{\text{optimum}}$  is dependent on substrate type [25]. Notably, Ziyan and Pekyardimic [34] had earlier reported the effect of seven different substrates on the T°C<sub>optimum</sub> of Pyrus communis PPO.

Pre-incubation of PPO<sub>S. melongenas</sub> and PPO<sub>M. sapientum</sub> under the two experimental conditions of temperature and pH gave diverse activity, which was a reflection of divergent level of PPO stability. The time dependent decay in PPO activity of two fruit extracts [Figures 2-5] showed also the divergent capacities of enzymes to withstand destabilizing effects of unfavorable temperature and pH conditions. This finding was an obvious indication that the three-dimensional structure and functionality of enzymes are inextricably connected with pH and temperature conditions [35]. In a similar study, Lacki and Duvnjak [36] have reported that changes in pH level from 5.0 to 3.2 caused loss of PPO stability of white-rot fungus Trametes versicolor, which was comparable to that observed when the pre-incubation temperature was increased from 50°C to 70°C. In another study, Yemenicioglu and Cemeroglu [37], showed the effect of ripening on thermal stability of Prunus armeniaca PPO and posited that thermal stability of PPO depended on the cultivar and stage of ripening and the presence of isoenzyme as reported by Yemenicioglu et al., [23].

The study by Mahmood et al. [25] showed that PPO from different plant sources exhibited different thermal stabilities. The present study shows that decreasing levels of PPO<sub>S, melonenas</sub> and  $PPO_{M. sapientum}$  activity depended on temperature and duration of incubation [Table 2], which was a reflection of level of thermal stability of the two PPO extracts [24,27,33]. The decay in  $PPO_{S. melongenas}$  and  $PPO_{M. saplentum}$  activity was more profound at 20°C and 60°C [Figures 2 and 3]. The present finding is in similarity with the reports of Chisari et al. [38] in which they noted that PPOs from melon varieties (Amarillo and Charentias) were nearly completely inactivated after 30 min of incubation at 60°C (94% loss of enzyme activity). Mizobutsi et al., [24] have reported that Litchi chinensis pericarp incubated at a temperature of 60°C for 10 min reduced the enzyme activity to scarcely detectable level. Furthermore, studies by Zheng et al., [27] have reported that Thompson seedless grape PPO exhibited thermal stability between 10°C and 25°C, but showed significant activity loss at temperatures higher than 40°C and was completely inactivated at 70°C for 10 min. They further stated that thermal inactivation of PPO showed a first-order kinetic with an activation energy (E<sub>1</sub>) of 146.1  $\pm$  10.8 kJ/mol at pH = 6.0. Therefore, it is worthwhile to note that PPOs from different plant sources exhibited divergent thermal stabilities [33].

Similarly, the relationship between stability of PPO and pH showed a time depended decay in enzyme activity [Figures 4 and 5]. Nakamura et al., [28] had earlier noted that PPO extracted from Koshu V. vinifera was stable in the alkaline pH range (between pH = 7.0 and pH = 11.0). Again, Mizobutsi et al., [24] have reported that L. chinensis pericarp pre-incubation up to 35 min; at pH 2.5 or 9.5 caused completely inactivated the enzyme. They further stated that the acid pH was more effective destabilization agent. Likewise, reports of Gaoa et al., [26] showed that PPO of leaf extract of Cleome gynandra L exhibited optimal activity at pH = 8.0, and further noted a progressive PPO stability from pH 3.0 to 9.0. In similar characteristics, PPO<sub>S. melongenas</sub> and PPO<sub>M. sapientum</sub> showed relatively lower decay in activity when pre-incubation pH tended towards alkaline conditions, whereas decay in activity was profound at acidic pH conditions. The relatively high decay of PPO<sub>S. melongenas</sub> and PPO<sub>M. sapientum</sub> activities pre-incubated at pH = 3.5 [Figures 4 and 5] was an indication that acidic pH promoted enzyme destabilization, which provided strong evidence that denaturalization pH of the PPOs was near pH  $\approx$  3.5. However, the propensity of acidic pH to cause destabilization the enzyme extracts was in the order  $PPO_{S. melongenas} > PPO_{M. sapientum}$ . Comparable reports on characterization of PPO from *L. chinensis* pericarp according to Liu et al., [17] showed that incubation of the enzyme at pH = 3.1 for 1 day caused 49.50% loss in PPO activity, and only 2.43% of the activity remained after 12 days of incubation, indicating that L. chinensis pericarp PPO was very unstable at pH = 3.1. They further posited that the PPO activity decayed more moderately when incubated at pH = 4.5 than when incubated at pH = 3.1. Furthermore, Bello *et al.*, [22] have reported that crude PPO extracted from S. aethiopicum, C. papaya and C. pepo showed instability in acidic pH, but was more stable near neutral pH, which was in agreement with the findings of Kavrayan and Aydemir [39] in which Mentha piperita PPO was found to be stable between pH 6.0 and 7.0.

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