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

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Enzyme activity depends largely on environmental conditions such as temperature and pH. The stability of polyphenol oxidase (PPO) extracted from *Solanum melongenas* and *Musa sapientum* fruits pre-incubated in varying thermal and pH conditions were carried out. Enzyme activity was measured by 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. $PPO_{S.\ melongenas}$ and $PPO_{M.\ sapientum}$ gave different temperature and pH optima. The temperature-activity profile of $PPO_{S.\ melongenas}$ and $PPO_{M.\ sapientum}$ showed a strong positive correlation (r = 0.907363). At pH = 10.0, $PPO_{M.\ sapientum}$ activity represented 65.3% decay in enzyme activity, whereas $PPO_{S.\ melongenas}$ represented 79.3% decay in enzyme activity. $PPO_{S.melongenas}$ and $PPO_{M.\ sapientum}$ 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 $PPO_{S.melongenas}$ and $PPO_{M.\ sapientum}$ showed a strong positive correlations under the same experimental thermal conditions, with exception at 20°C (r = 0.693375). Specifically, pre-incubation of $PPO_{M.sapientum}$ for t = 90 min at 60°C caused 18.4% decay in relative activity of $PPO_{M.\ sapientum}$. At t = 90 min, pre-incubation of $PPO_{M.\ sapientum}$, at pH = 3.5 caused decay in activity within the range of 30.8-36.1%, whereas $PPO_{M.\ sapientum}$ 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. $PPO_{S.melongenas}$ and $PPO_{M.\ sapientum}$ showed relatively higher stabilities as the incubation pH tended towards alkaline conditions, whereas the two experimental temperatures (20 and 60°C) promoted destabilization.

Key words: Polyphenol oxidase, temperature, pH, *Solanum melongenas* and *Musa sapientum.*

INTRODUCTION

Polyphenol oxidase (PPO) is a collection of ubiquitous plant enzymes [EC 1.10.3.2, catechol oxidase or diphenol oxygen oxidoreductase (Klabunde et al., 1998; Fawzy, 2005); EC. 1.14.18.1; monophenol oxidase, cresolase and tyrosinase (Mayer, 2006; Madani et al., 2011)] 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 the enzymes to diphenolic compounds such as catechol, 3, 4-dihydroxyphenylalanine, and chlorogenic acid (Steffens et al., 1994; Escobar and Shilling, 2008). 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-colour pigments (da Silva and Koblitz, 2010). The kinetic properties of PPO extracted from various plant sources have been reported by several authors (Gowda and Paul, 2002; Chikezie, 2006; Gouzi et al., 2010).

PPO is a copper-metalloenzyme located in the chloroplast thylakoid membrane (Sommer et al., 1994) and can exist in an active or latent state (Mayer and Harel 1979). PPO enzymes extracted from various plant tissues exhibit different characteristics, and exit in multi-

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ple molecular forms (isoforms) (Marshall et al*.,* 2000; Altunkaya and Gokmen, 2011; Ünal et al., 2011). 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) (Yoruk and Marshall, 2003).

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. Fruits of *Solanum melongenas* (eggplant) and *Musa sapientum* (Banana) are highly cherished and consumed in Nigeria and the world over. The economic benefits from the sales of these fruits are profound. However, spoilage of these fruits with financial losses is most often associated with the initiation of browning reactions associated with post-harvest activities.

Unavoidably, most of the fruits are bruised and injured during the course of transportation, storage and preservation. In an effort to control the browning process, the present study sought to establish the thermal and pH conditions that promote stability of the PPOs extracted from *S. melongenas* and *M. sapientum* fruits. Insights into the nature of environmental factors that promote stability of PPO could serve as point of reference for the conception of environmental conditions as instruments for control and mitigation of the browning process that has been implicated in fruit spoilage, deterioration and unacceptability.

MATERIALS AND METHODS

Collection and preparation of fruit samples

Fresh and disease free fruits of *S. melongenas* and *M. sapientum* were harvested from a private botanical garden in Umuoziri-Inyishi, Imo State, Nigeria between 17th -30th 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°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*.* (2011) with minor modifications. Ten grams (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°C) to eliminate phenolic compounds (Liu et al*.,* 2007; Ünal et al*.,* 2011). The sample was centrifuged at 32000 *g* for 20 min at 4°C. Solid ammonium sulphate $(NH_4)_2SO_4$ was added to the supernatant to obtain 80% $(NH_4)_2SO_4$ saturation and precipitated proteins were separated by centrifugation at 32000 *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 4 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 (1976) using bovine serum albumin as standard at λ_{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^{-1} min^{-1} under the condition of the assay (Oktay et al*.,* 1995).

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*.* (2002) with minor modifications (Chikezie, 2006). 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 λ_{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 ($PPO_{S.\ melongenas}$: $pH_{optimum}$≈ 7.0 at T °$C_{optimum}$≈ 30; $PPO_{M.\ sapientum}$: $pH_{optimum}$ ≈ 7.0 at T °$C_{optimum}$≈40), at the given time intervals. At the same time intervals, measurement of PPO activity pre-incubated in varying pH values of 3.5, 6.0 and 8.0 were carried out. The residual PPOactivity was measured according to the following experimental conditions ($PPO_{S.\ melongenas}$: $pH_{optimum}$≈ 7.0 at T °$C_{optimum}$≈ 30; $PPO_{M.\ sapientum}$: $pH_{optimum}$ ≈ 7.0 at T °$C_{optimum}$≈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

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

Enzyme fraction	*S. melongenas*				*M. sapientum*			
	E_A (U)	T_P (mg)	Specific E_A (U/mg)	% Yield	E_A (U)	T_P (mg)	Specific E_A (U/mg)	% Yield
Crude homogenate	0.308	0.980	0.314	100	0.234	0.802	0.292	100
Centrifuged at 32000 *g*	0.215	0.072	2.99	69.8	0.167	0.082	2.04	71.4
80% $(NH_4)_2SO_4$	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

E_A, Enzyme activity; T_P, total protein.

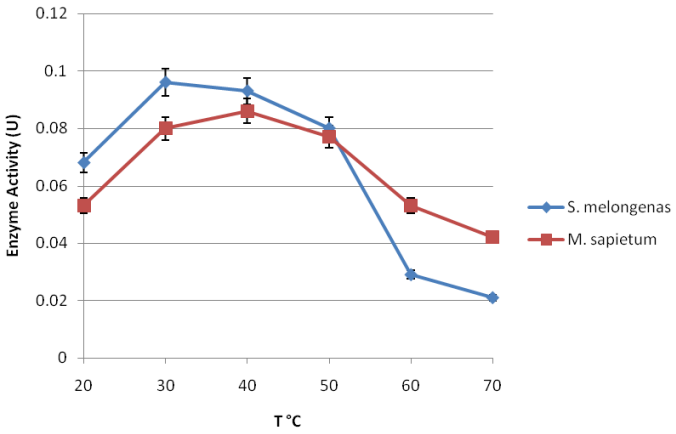

Figure 1. Temperature-activity profile of PPO activity extracted from *S. melongenas* and *M. sapientum* fruits.

within the range of 0.314 to 8.11 U/mg protein and 0.292 to 7.65 U/mg protein respectively. Final enzyme activity of $PPO_{S.melongenas}$ was 0.154 U, whereas $PPO_{M.\ sapientum}$ gave 0.130 U (Table 1).

Temperature-activity profile of $PPO_{S.melongenas}$ and $PPO_{M.\ sapientum}$ is presented in Figure 1. The temperature-activity profile of the two PPOs showed a typical bell-shaped curve. The $PPO_{S.melongenas}$ T °$C_{optimum}$ ≈ 30; $PPO_{S.\ melongenas}$ activity = 0.096±0.02 U, whereas $PPO_{M.\ sapientum}$ T °$C_{optimum}$ ≈ 40; $PPO_{M.\ sapientum}$ activity = 0.086±0.02 U. The temperature-activity profile of $PPO_{S.melongenas}$ and $PPO_{M.\ sapientum}$ showed a strong positive correlation (r = 0.907363). At experimental temperature of 70°C, $PPO_{S.melongenas}$ activity = 0.021±0.01 U; $PPO_{S.\ melongenas}$ = 0.042±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 $PPO_{S.melongenas}$ and $PPO_{M.\ sapientum}$ gave $pH_{optimum}$ ≈ 7.0. However, pH-activity profile of $PPO_{S.\ melongenas}$ exhibited two peak values; pH ≈ 7.0 and pH ≈ 8.5. At experimental maximum pH = 10.0, $PPO_{M.\ sapientum}$ activity = 0.034 U, representing 65.3% decay in enzyme activity, whereas $PPO_{S.\ melongenas}$ = 0.025 U representing 79.3% decay in enzyme activity. Tables 2 and 3 show the residual activities of $PPO_{S.\ melongenas}$ and $PPO_{M.\ sapientum}$ incubated at varying temperature and pH. The residual activity of $PPO_{S.melongenas}$ ranged between 0.86±0.02 and 0.067±0.03 U; $PPO_{M.\ sapientum}$ was between 0.080±0.03 and 0.070±0.02 U. A cursory look at Table 2 shows that the decreasing levels of $PPO_{S.\ melongenas}$ and $PPO_{M.\ sapientum}$ depended on temperature and duration of incubation. Residual activities of $PPO_{S.melongenas}$ and $PPO_{M.\ sapientum}$ showed a strong positive correlations under the same experimental temperature conditions, with exception at 20°C, which gave a weak positive correlation (r = 0.693375) (Table 4).

Similarly, $PPO_{S.melongenas}$ and $PPO_{M.\ sapientum}$ incubated at varying pH conditions exhibited decreasing residual levels of PPO activity with the progress of experimental time. Specifically, residual level of $PPO_{S.melongenas}$ 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 shows $PPO_{S.\ melongenas}$ and $PPO_{M.\ sapientum}$ activities displayed a strong positive correlations under the same pH conditions.

Pre-incubation of $PPO_{S.melongenas}$ at 50°C caused the lowest decay in relative activity compared to $PPO_{S.\ melongenas}$ pre-incubated at 20 and 60°C. $PPO_{S.melongenas}$ pre-incubated at 60°C exhibited lower decay in relative activity at incubation period t = 30 min and t = 90 min, compared to $PPO_{S.\ melongenas}$ pre-incubated at 20°C. Conversely, $PPO_{S.melongenas}$ pre-incubated at 60°C showed higher decay in activity compared to the enzyme pre-incubated at 20°C; at t = 60 min (Figure 3). 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 >60 >50°C (Figure 3). However, the increasing decays in activities of $PPO_{M.\ sapientum}$ pre-incubated at 20 and 60°C was not significantly different (p< 0.05). Specifically, pre-incubation of $PPO_{M.sapientum}$ for t = 90 min at 60°C caused 18.4% decay in relative activity of $PPO_{M.\ sapientum}$ (Figure 4). Pre-incubation of $PPO_{M.sapientum}$ in pH = 3.5 caused decay in relative enzyme activity between the range of 56.3-78.8% within the experimental time (30 <t < 90) min. Decay in relative activity was significantly different among the $PPO_{M.\ sapientum}$ incubated at the three experimental pH conditions,

Table 2. Residual activity of $PPO_{S.melongenas}$ and $PPO_{M.\ sapientum}$ incubated in varying temperature.

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

Table 3. Residual activity of $PPO_{S.melongenas}$ and $PPO_{M.\ sapientum}$ incubated in varying pH.

Time (min)	Enzyme activity V_o(U)					
	S. melongenas			*M. sapientum*		
	30	60	90	30	60	90
pH = 3.5	0.067±0.03	0.052±0.03	0.033±0.03	0.068±0.03	0.065±0.03	0.063±0.03
pH = 6.0	0.143±0.02	0.140±0.02	0.138±0.02	0.097±0.02	0.092±0.02	0.088±0.02
pH = 8.0	0.127±0.01	0.124±0.01	0.122±0.01	0.095±0.01	0.094±0.01	0.092±0.01

Values are means of three determinations ± S.D.

Table 4. Correlation coefficient between residual activities of $PPO_{S.melongenas}$ and $PPO_{M.\ sapientum}$ incubated in varying temperature and pH.

Correlation coefficient (*r*)					
Temperature (°C)			pH		
20	50	60	3.5	6.0	8.0
0.693375	0.846154	0.952217	0.983342	0.984018	0.953821

which was in the order pH = 3.5 > pH = 8.0 > pH = 6.0 (Figure 5).

The decay in relative activity of $PPO_{M.sapientum}$ pre-incubated in pH = 6.0 and pH = 8.0 were not profound compared to pH = 3.5 pre-incubation. At the end of experimental *t* = 90 min, pre-incubation of $PPO_{M.\ sapientum}$, in pH = 3.5 caused decay in relative activity within the range of 30.8-36.1%, whereas $PPO_{M.\ sapientum}$ 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 6).

DISCUSSION

From the present study, PPO extracted from the two fruits showed different pH and temperature optima ($PPO_{S.\ melongenas}$: $pH_{optimum}$≈ 7.0 at T $°C_{optimum}$≈ 30; $PPO_{M.\ sapientum}$: $pH_{optimum}$ ≈ 7.0 at T $°C_{optimum}$≈40) (Figures 1 and 2). Worthwhile to note, $PPO_{M.sapientum}$ T$°C_{optimum}$ ≈ 40, was same as PPO extracted from lily *Carica papaya* and *Cucurbita pepo* (Ying and Zhang, 2008). Other reports by several authors (Liu et al*.,* 2007; Bello et al*.,* 2011; Yemenicioglu et al*.,* 1999; Mizobutsi et al*.,* 2010; Mahmood et al*.,* 2009; Gaoa et al*.,* 2011) gave diverse temperature and pH optima of PPOs extracted from various plant tissues. Specifically, Zheng et al*.* (2012) using 10 mM catechol as substrate reported that *Vitis vinifera Thompson Seedless* PPO activity $pH_{optimum}$≈ 6.0 and $temperature_{optimum}$≈ 25. Nakamura et al. (1983) stated that T$°C_{optimum}$ and $pH_{optimum}$ of PPO extracted from Koshu *V. vinifera* were approximately 25 and 6.0°C, respectively. Alyward and Haisman (1969) and Sellés-Marchart et al. (2006) 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 (Jime´nez-Atie´nzar et al*.,* 2004; Dogan and Dogan, 2004). These previous reports are consistent with the present report ($PPO_{S.\ melongenas}$: $pH_{optimum}$≈ 7.0; $PPO_{M.\ sapientum}$: $pH_{optimum}$ ≈ 7.0) (Figure 2). Remarkably, pH-activity profile of $PPO_{S.melongenas}$ exhibited two peak values; pH ≈ 7.0 and pH ≈ 8.5, which was an indication of the presence of isoenzyme based on similar reports by Bello et al*.* (2011). Using catechol as substrate, Bello and Sule (2012), reported variable T $°C_{optimum}$ of PPO extracted from wide varieties of tropical fruits and vegetables. Accordingly, *S. aethiopicum*: T $°C_{optimum}$≈ 50°C; *C. papaya*: T $°C_{optimum}$≈ 40°C; *C. pepo* T $°C_{optimum}$ ≈ 50°C; *Psidium guajava*: T $°C_{optimum}$ ≈ 30°C;

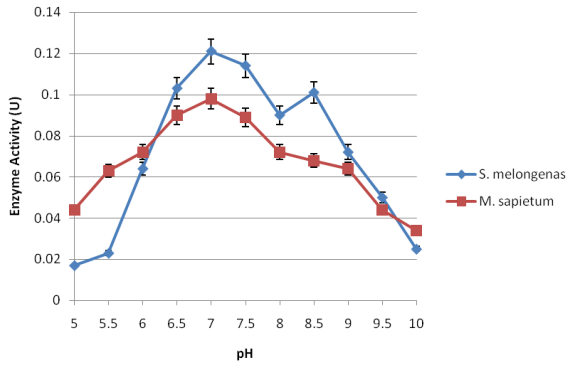

Figure 2. pH profile of PPO activity extracted from *S. melongenas* and *M. sapientum*fruits.

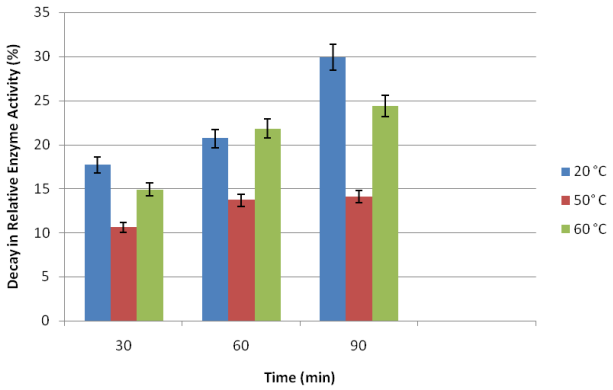

Figure 3. Percentage decay in enzyme activity of PPO extracted from *S. melongenas* incubated at varying temperature.

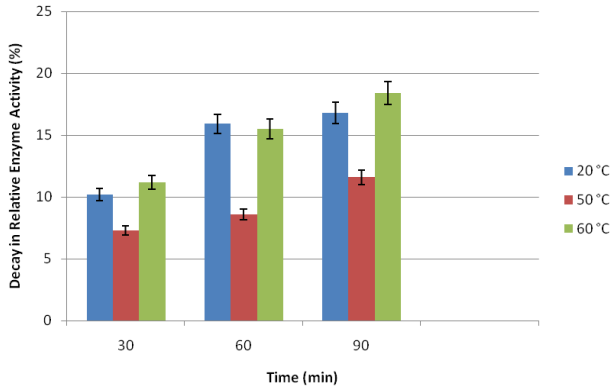

Figure 4. Percentage decay in enzyme activity of PPO extracted from *M. sapientum* incubated at varying temperature.

Irvingia gabonnensis: T °$C_{optimum}$≈ 50°C. It is worthwhile to note that PPO T °$C_{optimum}$ is dependent on substrate type (Mahmood et al., 2009). Notably, Ziyan and Pekyrdimic (2004) had earlier reported the effect of seven different substrates on the T °$C_{optimum}$ of *Pyrus communis* PPO.

Pre-incubation of $PPO_{S.melongenas}$ and $PPO_{M.\ sapientum}$ 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 3, 4 5 and 6) showed also the divergent capacities of the enzymes to withstand destabilizing effects of unfavourable 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 (Rodwell and Kennelly, 2003). In a similar study, Lacki and Duvnjak (1999) 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 to 70°C. In another study, Yemenicioğlu and Cemeroğlu (2003), showed the effect of ripening on thermal stability of *P. 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. (1999).

The study by Mahmood et al. (2009) showed that PPO from different plant sources exhibited different thermal stabilities. The present study shows that decreasing levels of $PPO_{S.\ melongenas}$ 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 (Mizobutsi et al., 2010; Bello and Sule, 2012; Zheng et al., 2012). The decay in $PPO_{S.melongenas}$ and $PPO_{M.\ sapientum}$ activity was more profound at 20 and 60°C (Figures 3 and 4).

The present finding is in similarity with the reports of Marcos et al. (2008) in which they noted that PPOs from melon varieties (*Amarillo* and *Charentias*) were nearly com-pletely inactivated after 30 min of incubation at 60°C (94% loss of enzyme activity). Mizobutsi et al. (2010) reported that *Litchi chinensis* pericarp incubated at temperature of 60°C for 10 min reduced the enzyme activity to scarcely detectable level.

Furthermore, studies by Zheng et al. (2012) reported that Thompson seedless grape PPO exhibited thermal stability between 10 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_a) of 146.1±10.8 kJ/mol at pH = 6.0. Therefore, it is worthwhile to note that PPOs from different plant sources exhibited divergent thermal stabilities (Bello and Sule, 2012).

Similarly, the relationship between stability of PPO and pH showed a time depended decay in enzyme activity (Figures 5 and 6). Nakamura et al. (1983) had earlier noted that PPO extracted from Koshu *V. vinifera* was

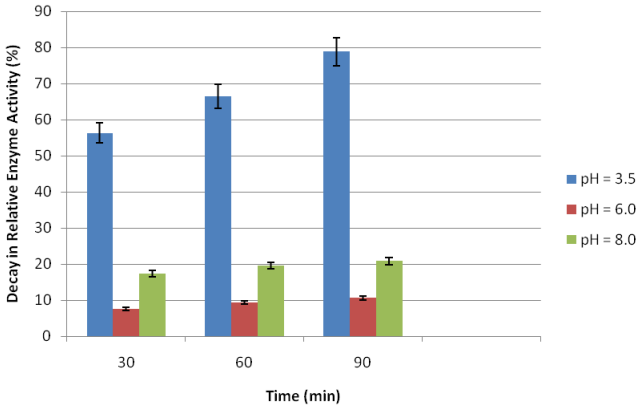

Figure 5. Percentage decay in enzyme activity of PPO extracted from *S. melongenas* incubated at varying pH.

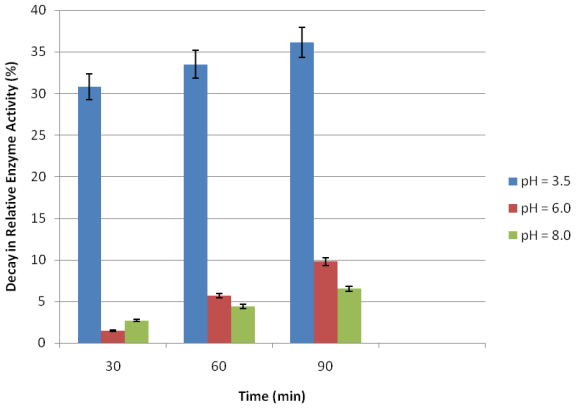

Figure 6. Percentage decay in enzyme activity of PPO extracted from *M. sapientum* incubated at varying pH.

stable in the alkaline pH range (between pH = 7.0 and pH = 11.0). Again, Mizobutsi et al. (2010) reported that *L. chinensis* pericarp pre-incubation up to 35 min, at pH 2.5 or 9.5 caused complete inactivation of the enzyme. They further stated that the acid pH was more an effective destabilization agent. Likewise, reports of Gaoa et al. (2011) 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, $PPO_{S.melongenas}$ and $PPO_{M. sapientum}$ 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 $PPO_{S.melongenas}$ and $PPO_{M. sapientum}$ activities pre-incubated at pH = 3.5 (Figures 5 and 6) was an indication that acidic pH promoted enzyme destabilization, which provided strong evidence that denaturalization pH of the PPOs was near pH ≈ 3.5. However, the propensity of acidic pH to cause destabilization of 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. (2007) 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. (2011) 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, whichis in agreement with the findings of Kavraya and Aydemir (2001) in which *Mentha piperita* PPO was found to be stable between pH 6.0 and 7.0.

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