Biochemistry and Molecular Biology
2016; 1(1): 1-10
http://www.sciencepublishinggroup.com/j/bmb
doi: 10.11648/j.bmb.20160101.11

Partial Purification and Characterisation of Polyphenol Oxidase from Two Commonly Consumed Eggplants (*Solanum mellongena depressum* and *Solanum gilo*) in Nigeria

David Morakinyo Sanni

Department of Biochemistry, Federal University of Technology, Akure, Nigeria

Email address:
moraksanni@yahoo.co.uk

To cite this article:
David Morakinyo Sanni. Partial Purification and Characterisation of Polyphenol Oxidase from Two Commonly Consumed Eggplants (*Solanum mellongena depressum* and *Solanum gilo*) in Nigeria. *Biochemistry and Molecular Biology.* Vol. 1, No. 1, 2016, pp. 1-10.
doi: 10.11648/j.bmb.20160101.11

Received: May 31, 2016; **Accepted**: June 12, 2016; **Published**: July 6, 2016

Abstract: Polyphenol oxidase (PPO) catalyzes in the presence of oxygen, the oxidation of mono- and di- phenols to o-quinones. The browning in fruits, vegetables and their processed products occurs as a result of this oxidation reaction. PPO in this study was isolated and characterized from two species of garden egg; *Solanum mellongena depressum* and *Solanum gilo*. Extracts were purified using a combination of ammonium sulphate precipitation, ion exchange chromatography on DEAE sephadex and gel filtration on Sephadex G-200 column. The effect of pH and temperature were carried out, pH and thermal stability, kinetic and substrate specificity, the effect of inhibitors and activator on the enzyme activity was investigated. About 6% protein yield for both species and a purification fold of 30.0 and 41.0 for *S. depressum* and *S. gilo* respectively was achieved. The optima pH of activity were found to be 4.0 – 4.5 and 7.0 for *S. depressum* and 4.0 and 8.0 for *S. gilo* while 4.5 and 8.0 were obtain in presence of SDS, the activity of the PPO increased in the presence of small concentration of SDS in all the pH investigated. The temperature optimum for both species was observed at 30°C. The PPO were stable at 30°C retaining about 88% and 87% of initial activity after 60minutes for *S. depressum* and *S. gilo* respectively while PPO from *S. depressum* was inactivated after 40min and 80°C and 70°C. A minimal remaining activity of 5% was observed at 80°C after 60min incubation time. PPO was fairly stable at pH 6.0 – 8.0 retaining a percentage remaining activity of 40.3% to 50% for *S depressum* and 45.5% to 52.1% for *S. gilo*. The PPO exhibited a marked activity towards o-diphenol and lower activities for monophenols. Ascorbic acid, EDTA and SDS inhibited enzymatic activity while the Km values using catechol, DOPA and catechin as substrate were 0.3mM, 0.095mM and 1.09mM for *S. depressum* and 1.9mM, 0.414mM and 0.56mM for *S. gilo*. PPO from *S. depressum* exhibited a higher enzymatic activity compared to *S. gilo* while *S. gilo* retained a fairly more stable activity.

Keywords: Polyphenol Oxidase, *Solanum spp.*, Enzyme, Activity

1. Introduction

Polyphenol oxidase (PPO; 1, 2 benzenediol: oxygen oxidoreductase; EC 1.10.3.1), also known as tyrosinase, polyphenolase, phenolase, catechol oxidase, cresolase, or catecholase is widely found in nature [1]. Polyphenol oxidase (PPO) is typically present in the majority of plant tissues and has received much attention from researchers in the field of plant physiology and food science, because of its involvement in adverse browning of plant products. Enzymatic browning occurs as a result of the oxidation by PPO of phenolic compounds to quinones and their eventual (non enzyme-catalyzed) polymerization to melanin pigments. It appears that oxidative browning reactions, proceeding in many foods of plant origin, generally cause deterioration in food quality by changing nutritional and organoleptic properties. These reactions significantly diminish consumer acceptance, storage life and value of the plant products. In

addition to its general occurrence in plants, PPO is also found in seafood (crustacean) products, such as shrimp [2]. PPO has been studied extensively for more than a century from numerous plant sources; attention is given to significant variations in physicochemical properties of the enzyme from different plant sources but comparison of the same plant of different species has not been established.

The garden eggplant is an economic flowering plant belonging to the family *Solanaceae*, apart from being a source of vegetable, has numerous health benefits which are essential for the overall development of human body and its acceptability cuts across religious, tribal, cultural and ethnic groups in Nigeria, they are also widely cultivated in America and Europe. They provide the body with water soluble vitamins which are rich in thiamin (Vitamin B1) required for normal growth and proper functioning of the heart and Nervous system, Niacin (Vitamin B6) needed for cellular respiration. Because of the economic and health importance of garden egg and the fact that some people eat it raw, some cooked in the form of sauce while others make salad from it, this research involves the purification and characterization of PPO from the two species of garden egg *Solanum mellongena depressum* and *Solanum gilo* and to establish and ascertain the specie with better and favourable physicochemical properties for storage.

2. Methodology

2.1. Sample Collection and Preparation of Crude Extracts

Freshly harvested *Solanum gilo* (white) *and* Solanum mellongena depressum (green) were purchased from *Oja Oba* on *Oba Adesida* Road, Akure, Ondo State, Nigeria. About 200g of each species was homogenized with 600ml ice cold 25mM phosphate buffer (pH 6.8) containing 10mM ascorbic acid using a warring blender for 3min with a 60 seconds resting period to avoid local elevation in temperature. The mixture was filtered using four layers of cheese cloth. The filtrate obtained was centrifuged in a refrigerating centrifuge at 6,000 rpm for 30 min at 4°C. The supernatant obtained was stored in a refrigerator and used as crude extracts for further studies.

2.2. Determination of Polyphenol Oxidase Activity

PPO activity was determined according to the method described by Sanni and Adekunle, [3] measuring the initial rate of Quinone formation, as indicated by an increase in absorbance at 420 nm, using visible spectrophotometer. Change in absorbance was recorded after incubation at room temperature for 10 minutes. The sample cuvette contained 0.7 ml of 10mM catechol solution in 0.1 M phosphate buffer (pH 6.8) and 0.3 ml of the enzyme solution. The blank samples contain 0.7 ml of 10mM catechol and 0.3 ml of 0.1 M buffer solution. One unit of PPO activity was defined as the amount of enzyme that caused a change in absorbance of 0.001 per minute.

$$\text{Unit of polyphenol oxidase activity} = \frac{\text{Change in absorbance of 0.001 (unit/ml)}}{\text{10 minutes}}$$

$$\text{Specific activity} = \frac{\text{Unity of activity (unit/ml/mg)}}{\text{Amount of protein (mg/ml)}}$$

2.3. Partial Purification of Crude Extracts Polyphenol Oxidase

2.3.1. Ammonium Sulphate Precipitation

About 500ml of crude extract from each species was brought to 30 to 80% ammonium sulphate $(NH_4)_2SO_4$ saturation by slowly dissolving solid ammonium sulphate using magnetic stirrer. The precipitate was separated by centrifugation at 6,000 rpm for 30 minutes. The precipitate was dissolved in 40ml of 0.1M phosphate buffer (pH 6.8) and dialyzed at 4°C overnight with three changes of buffer. The dialyzed samples were used as the polyphenol enzyme source in further analysis.

2.3.2. Ion Exchange on DEAE Sephadex

Dialysed enzyme solution was placed on a DEAE-Sephadex column (2.5×40 cm) and eluted with 0.01M Potassium phosphate buffer pH 6.8 and sequentially followed by 0.025M, 0.05M, 0.075M and 0.1M (flow rate: 20 ml/hr). The eluate from DEAE sephadex column was concentrated by saturated solution of Sucrose (4 M) and further purified by gel filtration.

2.3.3. Gel Filtration

Gel filtration of the enzyme was carried out on a Sephadex G-200 column (1.5× 70 cm; flow rate: 10ml/h) with 0.1M potassium phosphate buffer pH 6.8. The fractions containing the enzyme activity were pooled and used for further investigation.

2.4. Enzyme Characterisation

2.4.1. Effect of pH on Polyphenol Oxidase Activity in the Absence and Presence of SDS

Effect of pH on PPO activity in presence and absence of SDS was carried out using buffer of pH range of 4.0-9.0, 0.1 M acetate (pH 4.0-5.0), 0.1 M phosphate (pH 6.0-7.0) and 0.1 M Tris/HCl (pH 8.0-9.0). The reaction mixture in presence of SDS contains 0.69 mM of the detergent while PPO activity was assayed according to standard assay procedure.

2.4.2. Effect of pH on Polyphenol Oxidase Stability

The effect of pH on stability was determined by preparing four fold enzyme solutions with buffer solution between pH 4.0 to 9.0 at room temperature. The enzyme solutions were in catechol for 5 hours and the initial enzymatic activity was measured after mixing using a vortex mixer while the residual PPO remaining activity were determined every hour throughout the 5 hours incubation time.

2.4.3. Effect of Temperature on Polyphenol Oxidase Activity

The assay mixture containing the PPO and Catechol was incubated for 10 minutes at different temperature of 30 to 80°C at 10°C temperature interval. The absorbance reading was taken at 420 nm according to standard assay procedure.

2.4.4. Thermal Stability

Thermal stability of the purified PPOs were determined at various temperatures by incubating the enzyme solution in 0.1 M Phosphate buffer pH 6.8 for 1 hour between the temperatures range of 30 to 80°C (at intervals of 10°C). The initial enzymatic activity was obtained and residual PPO activity were determined at 10 minutes time interval for 1 hour incubation period and compared with the initial enzyme activity.

2.4.5. Substrate Specificity

Substrate specificity was determined by using eight substrates (Catechol, DOPA, Tyrosine, Catechin, Epicatechin, Gallic acid, Protocatechuic acid and ρ-coumaric acid). 5 mM of each substrate was prepared in 0.1 M phosphate buffer (pH 6.8). Enzyme activity was assayed according to standard assay procedure.

2.4.6. Effect of Inhibitors and Activator on the Enzyme Activity

Polyphenol oxidase activity was measured in the presence of ascorbic acid, EDTA and SDS at three concentrations (1, 5, 10 mM) prepared in 10 mM Catechol (pH 6.8) and the residual activity measured according to standard assay procedure.

2.4.7. Kinetic Parameters

Michaelis Menten constant (K_m) and maximum reaction velocity (V_{max}) were determined using three different substrates (Catechol, DOPA and Catechin). Substrate solutions were prepared in 0.1 M Phosphate buffer (pH 6.8) at different initial concentrations (1, 5, 10, 15 and 20 mM). PPO activity was assayed according to standard assay procedure. The data were processed using Lineweaver-Burk plot.

3. Results and Discussion

PPO from the two species of garden egg; *S. depressum*and *S. gilo* purified by the combination of ion-exchange chromatography on DEAE sephadex A50 and Gel filtration chromatography on sephadex G200 with a fold purification of 30 and 41 from *S. depressum* (table 1 and figure 1a,) and *S. gilo* (table 2 and figure 2a,) repectively and 6% purification yield in both species and a specific activity of 1254U/mg and 1161.6U/mg for *S. depressum* and *S. gilo* respectively showed the presence of Polyphenol oxidase.

Table 1. *Purification scheme of PPO from S. depressum.*

	Volume (ml)	Protein conc (mg/ml)	Activity (U/ml)	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Fold	Yield (%)
Crude	570	2.5	103.4	1425.0	58938.0	41.4	1	100
Ammonium sulphate ppt	30	0.9	415.9	27.0	12477.0	462.1	11	21
DEAE Sephadex A50	20	0.4	277.5	7.5	5550.0	738.0	18	9
Sephadex G200	13	0.2	250.8	2.6	3260.4	1254.0	30	6

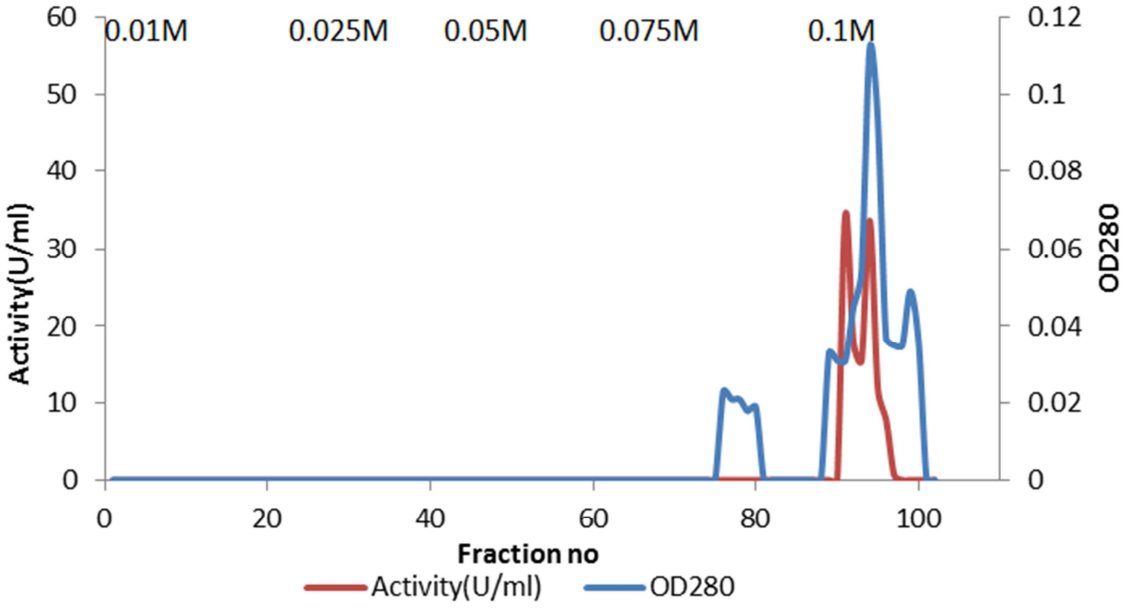

Figure 1a. *Elution profile of PPO from Solanum mellongena depressum (green) using DEAE sephadex chromatography column (2.5×40 cm). Elution with Potassium phosphate buffer, pH 6.8, 0.01M, 0.025M, 0.05M, 0.075M and 0.1M (flow rate: 2 ml/min).*

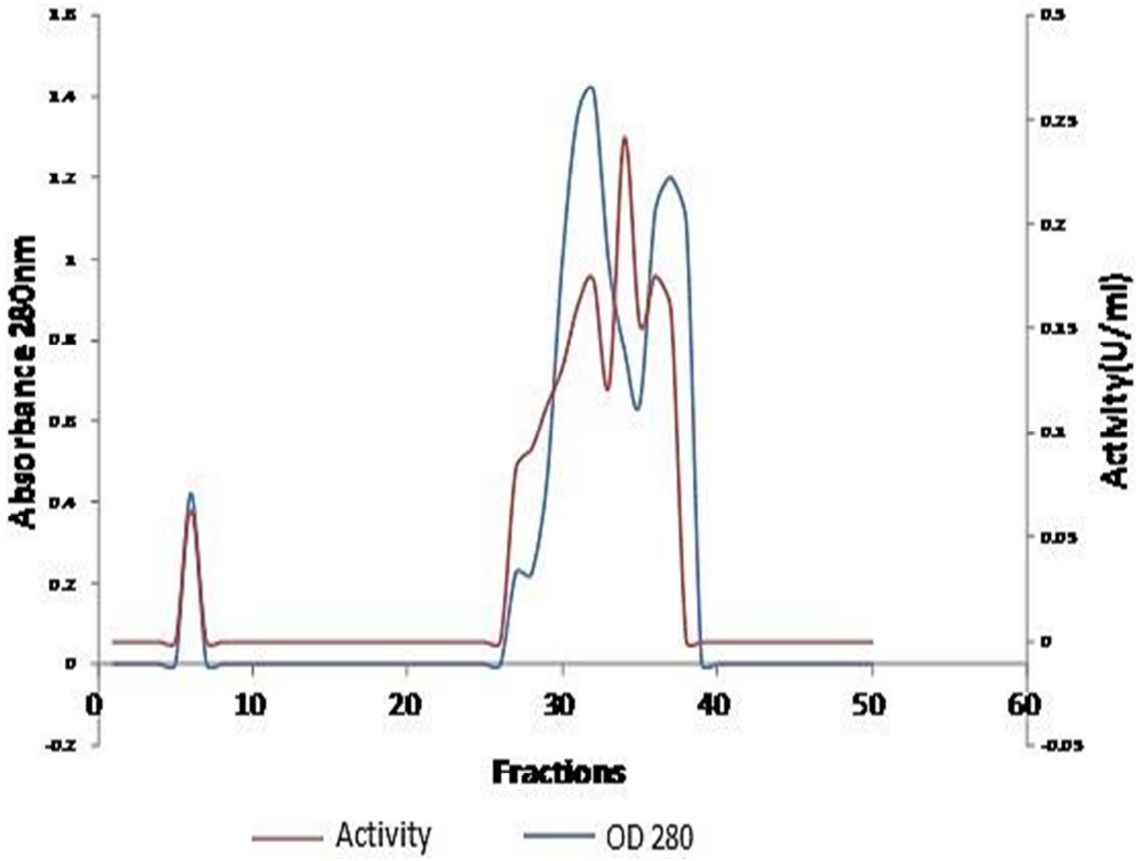

Figure 1b. *Elution profile of PPO from Solanum mellongena depressum in Sephadex G-200 column (1.5x70cm; flow rate 10ml/h) with 0.1M Potassium phosphate buffer pH 6.8.*

Table 2. *Purification scheme of PPO from Solanum gilo.*

	Volume (ml)	Protein conc. (mg/ml)	Activity (U/ml)	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Fold	Yield (%)
Crude	530	2.4	68.5	1272.0	36305.0	28.5	1	100
Ammonium sulphate ppt	30	0.7	200.7	21.3	6021.0	282.7	10	17
DEAE Sephadex A50	13	0.5	194.7	6.5	2531.1	389.4	14	7
Sehadex G200	8	0.3	290.4	2.0	2323.2	1161.6	41	6

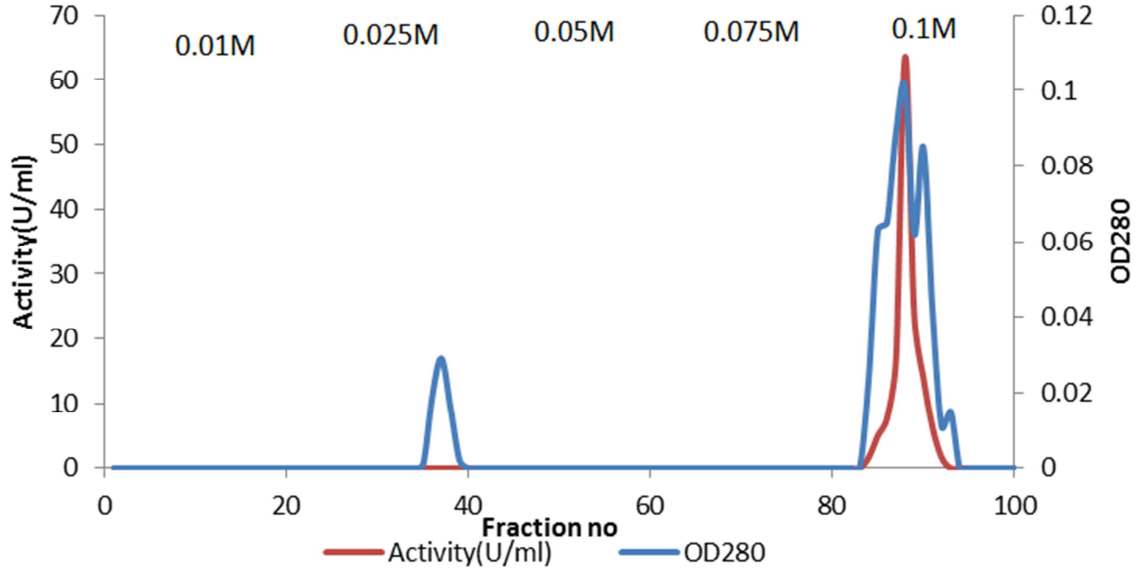

Figure 2a. *Elution profile of Polyphenol oxidase from Solanumgilo using anion exchange chromatography column (2.5×40 cm). Elution with Potassium phosphate buffer, pH 6.8, 0.01M, 0.025M, 0.05M, 0.075M and 0.1M (flow rate: 2 ml/min).*

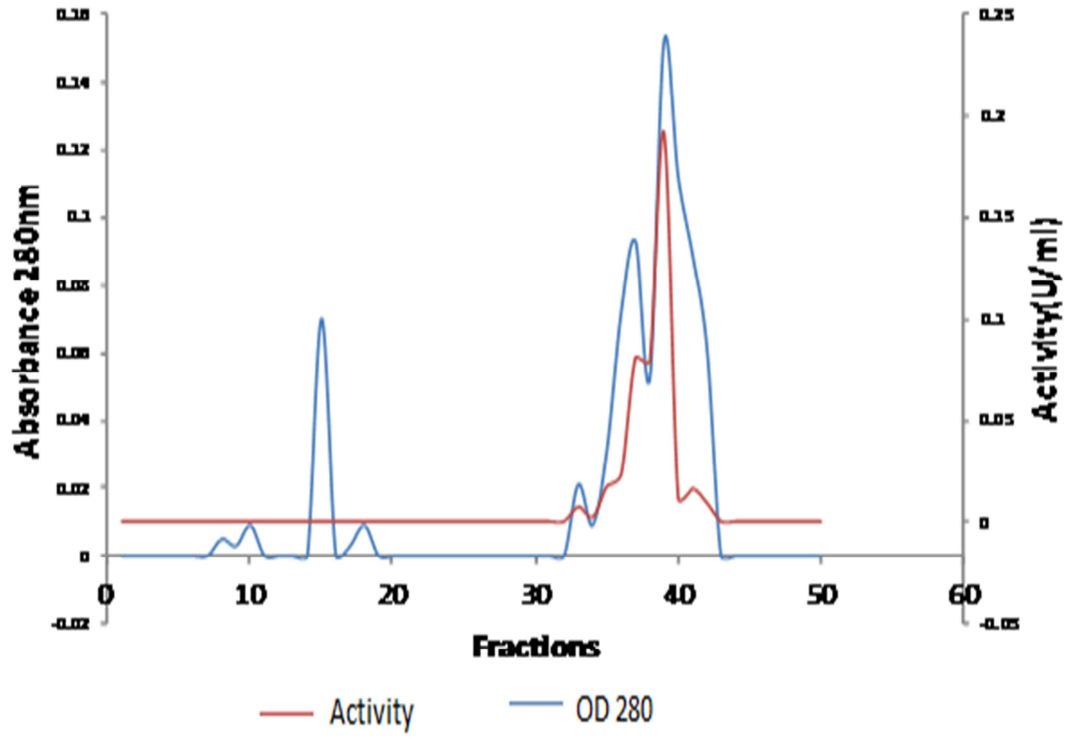

Figure 2b. *Elution profile of PPO from Solanum gilo in Sephadex G-200 column (1.5x70cm; flow rate 10ml/h) with 0.1M Potassium phosphate buffer pH 6.8.*

Mishra *et al.,* [4] also confirmed the presence of PPO in garden egg (*Solanum melongena*) as well as in other plants; potato [5], grape [6], chestnut [7] and blue berry [8].

PPO from this study was observed to reveal enzymatic activity within the pH ranges: 4.0 – 8.5. This observation is within the reported pH optimum range of 4.0 – 8.0 by Yoruk and Marshall [9] for plants PPOs. The observed more than one optimal pH had been reported by Heimdal *et al.,* [10] for Lettuce. This may probably be due to the presence of more than one isoform of the enzyme in two species of garden egg [11]. Oba *et al.,* [12] also observed two isoforms of PPO from banana bud extracts which exhibited variation in their PPO activities. Other plants have been reported to exhibit pH optimum at acidic region such as Cherry and strawberry [13], Apple and grape [14]. However kiwifruit [15], and spinach [16] were found to revealed optimum at alkaline pH.

The result further revealed a shift in the optimum pH of the PPO from the two species of garden egg in presence of 0.69mM SDS. The activity of the enzyme increases in the presence of this low concentration of SDS at pH range of 4.0 – 9.0 with a marked activation of 66.67% and 30.17% for *S. depressum* and *S. gilo* at pH 8.5.

Table 3. *Polyphenol oxidase percentage activation by SDS at varying pH.*

pH	*S. depressum (%)*	*S. gilo (%)*
4.0	1.14	5.63
4.5	4.55	4.69
5.0	6.98	6.15
6.0	16.05	7.69
6.5	10.34	14.10
6.8	14.44	9.30
7.0	2.75	17.20
8.0	35.87	-2.05
8.5	66.67	30.17

Different authors have reported enzymatic activation by SDS. Marques *et al.,* [14] also observed alteration in general behaviour of apple PPO with changing pH in the presence of SDS, as it is inhibited at acidic pH and activated at pH above 5.0 in the presence of 3.5mM SDS. Plants such as *Prunus* fruits [13], peach [17], strawberry [18] and broad bean [19] PPOs also had similar report, where SDS causes a shift in the pH optimum of the enzyme from low to higher pH values. The ability of SDS to activate the enzyme alters both its enzymatic and physical characteristics and suggests that a limited conformational change due to binding of small amounts of SDS may induce or initiate the activatition of latent enzyme [20]. Also, most enzymes lose their biological activity upon treatment with SDS because of the drastic alteration of the tertiary and quaternary structure ofprotein [17].

pH stability study revealed above 50% PPO activity was still retained for PPO from *S. depressum* and *S. gilo* (figure 4a and b) at pH 7 after 5 hours of incubation.

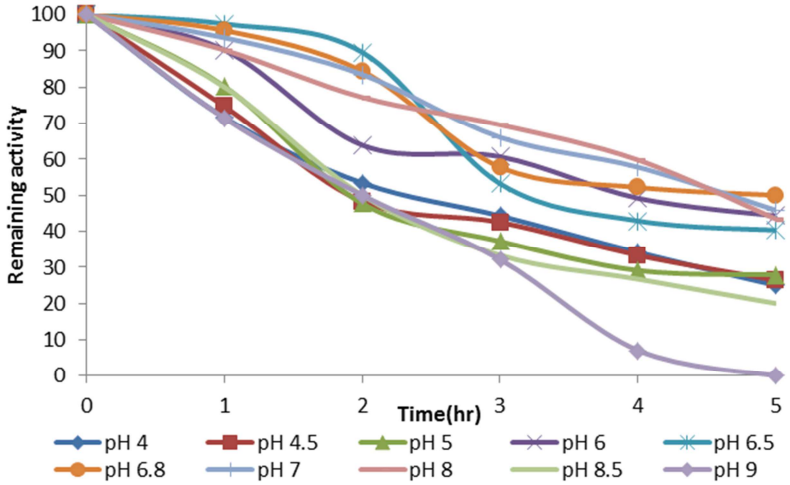

Figure 3a. *Effect of pH on the stability of PPO from S. depressum. At pH 9 the enzyme loses its activity completely within 4.8hrs compared to other pH ranges observed. Enzyme activity is more stable at pH 8.*

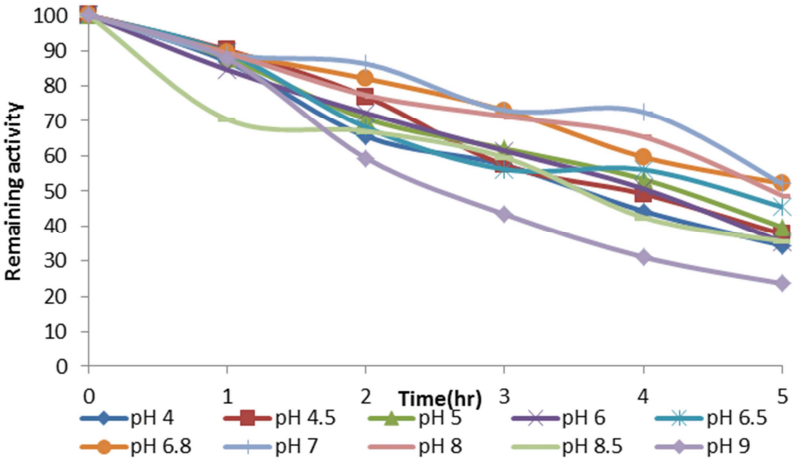

Figure 3b. *Effect of pH on the stability of PPO from S. gilo. At pH 8.5 between 0-1hr and pH 9 between 2-5hrs an observed sharp decrease in enzyme activity compared to other pH ranges. Enzyme activity seems to be more stable at pH 7.*

The influence of temperature on the enzyme activity of PPO for *S. depressum* and *S. gilo* are illustrated in figure 4a and b as shown below.

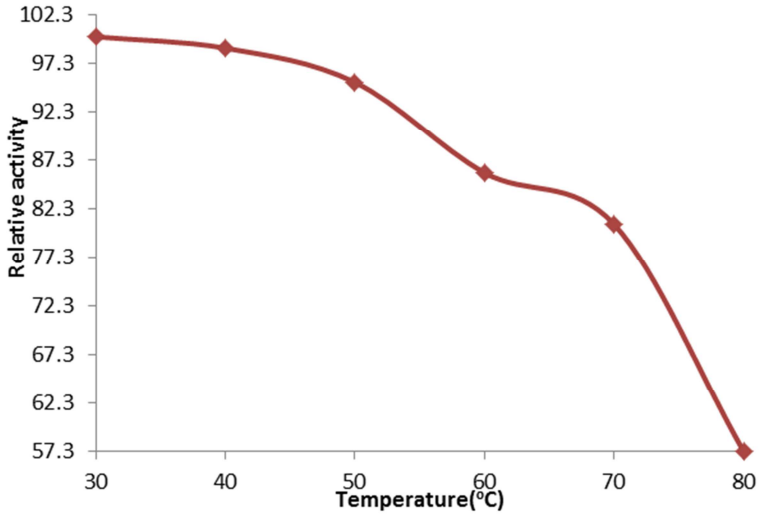

Figure 4a. *Effect of temperature on PPO from S. depressum. At 30-40°C the enzyme activity was stable but decreases drastically at 70-80°C.*

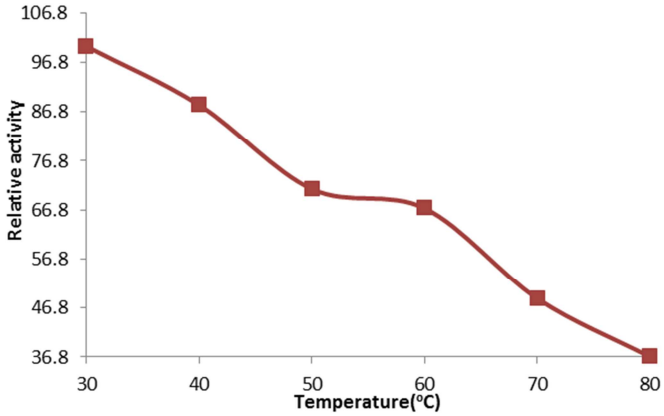

Figure 4b. *Effect of temperature on PPO from Solanumgilo. At 50-60°C the enzyme activity was stable though decreases as temperature increases.*

The optimum temperature of 30°C obtained for PPO in *S. depressum*and *S. gilo* has been reported for PPOs from apple [21], banana [22] and mango [23] though a higher temperature optimum of 45°C were reported for PPOs from cocoa bean [24] and sunflower [25]. Other plants PPOs showed a wide range in their optimaltemperature; lettuce, 25-35°C [10] and grape, 25-45°C [26]. However, PPOs from strawberry [18] and cucumber [27] have relatively high optimum temperature compared to those from other plant sources. Optimum temperature of the enzyme activity is affected by the substrate used in the assay, as reported by Sakiroglu*et al.,* [28] where dog-rose extract PPO activity toward monophenols exhibits higher optimal temperature than toward di- and triphenols considered. Integrity of the delicate three-dimensional structure of the enzyme molecule is subjected to disruption and denaturation at high temperatures. These variations in temperature may also alter the solubility of oxygen, one of the substrates required for PPO to perform its catalytic activity [29].

The thermal inactivation study revealed a similarity in the thermal stability of PPO from *S. depressum* and *S. gilo* (figure 5a and b) with a percentage remaining activity of 88% and 87% at 30°C, 36% and 38% at 40°C, 31% and 29% at 50°C respectively.

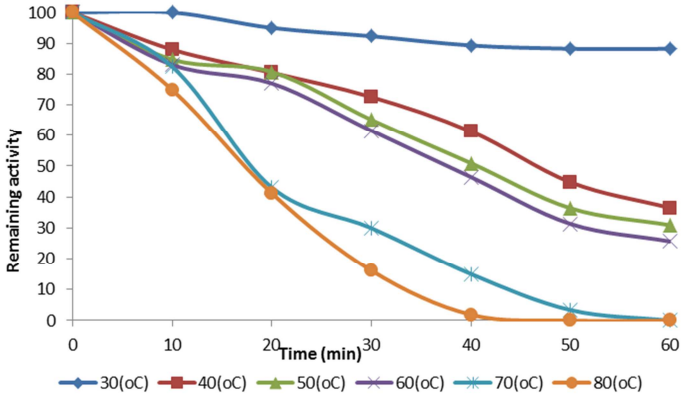

Figure 5a. *Effect of temperature on the stability of PPO from S. depressum. At 30°C the enzyme activity was stable but decreases as the temperature increases.*

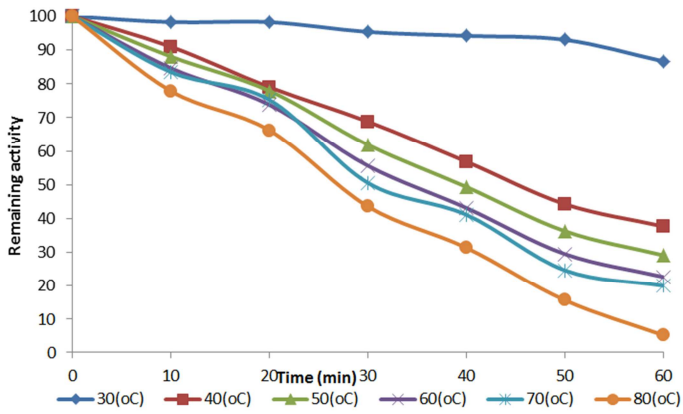

Figure 5b. *Effect of temperature on the stability of PPO from S. gilo. At 30°C the enzyme activity was stable but started decreasing after 1hr, though with increased temperature enzyme activity decreases proportionally.*

However, at higher temperature there was complete inactivation of PPO from *S. depressum* after 40 and 60 minutes incubation time at 70°C and 80°C respectively while *S. gilo* still retained a minimal activity of 20% and 5% at 70°C and 80°C after 60 minutes. The PPO showed a better thermal stability compared to PPOs from lettuce [10] and cocoa bean [31] which are relatively heat stable. Heat treatment up to 70°C for 5 min did not affect Lettuce PPO activity while at 90°C no activity remained after 5 min [10]. PPO in mango skin is also relatively thermostable, requiring more than 15 min at 80°C for 50% loss of activity [23]. Thermal stability of PPO may also be influenced by nature of phenolic substrate used during determination [30].

The substrates specifity on PPO obtained from *S. depressum* and *S. gilo* revealed a higher activity toward o-diphenols with lower activities toward the monophenols such as tyrosine and coumaric acid.

The activity of apple PPO on tyrosine was found to be much lower than on o-diphenols [21] and was suggested to be typical for PPOs. Activity toward o-diphenols only was found for PPOs from grape [24], field bean seed [32] strawberry [30] and sunflower seed [25]. Lee *et al.,* [31] observed that substrate specificity of PPO is also dependent on species and cultivars. For example, in the case of DeChaunac grape PPO, caffeic acid is oxidized at much faster rates than other structurally related substances. Oba *et al.,* [12] further observed that PPO isoforms in a tissue of interest may also exhibit differential substrate specificities and variations in their relative activities toward monophenols and o-diphenols.

Table 4. *Substrate specificity of PPO from S. depressum and S. gilo.*

	Relative activity (*S. depressum*)%	**Relative activity (*S. gilo*)%**
Di/tri-phenols		
Catechol	100.0	100.0
Dopa	97.1	105.0
Catechin	50.0	100.0
Epicatechin	43.7	60.9
Gallic acid	47.8	59.4
Protocatechuric acid	23.3	43.5
Monophenols		
Tyrosine	23.8	25.8
ρ-coumaric acid	17.6	26.5

**Catechol was used as reference substrate.

Table 5. *Effect of inhibitors on PPO from S. depressum and S. gilo.*

	1mM		**5mM**		**10mM**	
	S. depressum	***S. gilo***	***S. depressum***	***S. gilo***	***S. depressum***	***S. gilo***
Reference	100	100	100	100	100	100
Ascorbic acid	27	61.8	16.4	34.7	16.4	34.7
EDTA	43.4	63.2	27.6	53.9	19.7	38.2
SDS	29.6	7.9	0	0	0	0

The PPO from *S. depressum* and *S. gilo* were found to be inhibited by Ascorbic acid, EDTA and SDS as seen in table 5 above. Ascorbic acid is commonly used as an antibrowning agent in the manufacturing of fruit juices, purees, frozen sliced fruits, and canned fruits and vegetables [9]. López-Nicolás *et al*., [33] found ascorbic acid in combination with citric acid to be a more effective inhibitor than ascorbic acid alone. This increased inhibition is likely due to the increased stability of ascorbic acid in an acidic environment as well as inhibition of the acidic environment on the catalytic activity of the enzyme. EDTA however is a chelating agent. The active site of PPO is comprised of two copper atoms through which the enzyme interacts with its phenolic substrates and oxygen. Chelating agents have the ability to react with metals through chelation, making copper at the active site unavailable, thereby inhibiting PPO. Also, resistance of PPO to SDS as observed in this study is perhaps due to presence of disulfide bonds strengthening PPO structure [34]. Degree of SDS activation varies greatly with plant materials. For example, PPO activity increased about 65 to 119-fold in broad bean [35], 19-fold in grape [36], 25-fold in peach [17], 10-fold in banana [37], 4-fold in potato leaf [38] and 7-fold in mango [23] by SDS under the defined experimental conditions. In general, these compounds diminish or inhibit the browning reaction rate by means of eliminating from the reaction an active reaction element(s). PPO from different sources may react similarly with inhibitor compounds. However, effectiveness of inhibitors against different PPOs could significantly vary and, therefore, specific control measures for individual systems would also be needed [39].

Reaction rate at different catechol, DOPA and catechin concentrations showed that polyphenol oxidase from *S. depressum* and *S. gilo* obeys Michealis-Menten kinetics. Table 6 shows summary of kinetic parameters.

Table 6. *The summary of the Kinetic parameters.*

Specie	**Parameter**	**Catechol**	**DOPA**	**Catechin**
S. depressum	K_m (mM)	0.3	0.095	1.09
	V_{max} (mM/min)	256.0	238.1	131.60
S. gilo	K_m (mM)	1.9	0.414	0.56
	V_{max} (mM/min)	163.9	172.4	123.50

K_m values 0.3mM, 0.095mM and 1.09mM for *S. depressum* and 1.9mM, 0.414mM and 0.56mM for *S. gilo* from substrates respectively obtained using Lineweaver-Burk plot, which in comparison with the other PPO from different fruitshad lower affinity. Marques *et al.,* [14] observed that K_m value for apple was 34.0mM catechol and 3.1mM 4-Methylcatechol while $K_m$values for Grape are 3.2mM Chlorogenic acid 4.3mM Catechin [36]. Plant PPOs are capable of oxidizing a wide range of o-diphenols, the primary substrates with varied oxidation rates and affinities

of different orders of magnitude and the native concentrations of natural phenols vary from plant to plant. The variability of V_{max} values amongst the species was related to the absolute concentration of PPO in their respective extracts, since absolute concentration of enzyme (E) shows direct proportionality with maximum enzyme activity (V_{max}). This result conforms to previous reports stated which noted significant differences and marked variations in PPO kinetic parameters between enzyme extract of different species of plants and their substrates.

4. Conclusion

This study revealed the activity of PPO in *S. depressum* and *S. gilo* and its physicochemical properties. The enzyme was active in all the pH range between 4.0 – 8.5 for both species with the same optimum temperature of 30°C. The PPOs exhibited similar thermal characteristic with a remaining activity of 88% and 87% at 30°C, 36% and 38% at 40°C, 31% and 29% at 50°C for *S. depressum* and *S. gilo* respectively after a period of an hour. Higher activity was observed towards o-diphenols and lower enzyme activity towards monophenols, while EDTA, ascorbic acid and SDS showed varying level of PPO inhibition from *S. depressum* and *S. gilo*.

References

[1] Whitaker, J. R. (1996). Polyphenol oxidase. In Food *Chemistry,* (O. R. Fennema, ed.) pp. 492-494, Marcel Dekker, New York.

[2] Simpson, L., Neckelmann, N., de la Cruz, V. F., Simpson, A. M., Feagin, J. E., Jasmer, D. P., and Stuart, K. (1987). J. Biol. Chem. 262: 6182-6196.

[3] Sanni, D. M. and Adekunle, A. A. (2012). Comparative physicochemical properties of polyphenol oxidase from white and red kolanuts (*Cola nitida* subspecies *alba* and *rubra*). J. Sustainable Technology, 3 (1): 9-17

[4] Mishra, B., Gautam, S. andArun Sharma, A. (2012). Purification and characterization of polyphenol oxidase (PPO) from eggplant (*Solanummelongena*). Food Chemistry, 134: 1855–1861.

[5] Cho, Y. K. and Ahn, H. K. (1999) Purification and characterization of polyphenol oxidase from potato: 1. Purification and properties. Journal of Food Biochemistry, 23: 577–592.

[6] Rapeanu, G., Van Loey, A., Smout, C. and Hendrickx, M. (2006), Biochemical characterization and process stability of polyphenoloxidase extracted from Victoria grape (*Vitisvinifera ssp. Sativa*). Food Chemistry*,* 94: 253-261.

[7] Lu, S. M, Tong, G. P., Long, Y. and Feng, H. (2005). Partial purification and characterization of polyphenol oxidase from fresh-cut Chinese water chestnut. J. Food Biochem. 30: 123–137.

[8] Kader, F., Rovel, B., Girardin, M. and Metche, M. (1997). Mechanism of Browning in Fresh Highbush Blueberry Fruit corymbosum (*Vaccinium L*). Partial Purification and Characterisation of Blueberry Polyphenol Oxidase. J. Sci. Food Agric. 73: 513–516.

[9] Yoruk, R. and Marshall, M. R. (2003). A survey on the potential mode of inhibition for oxalic acid on polyphenol oxidase. J. Food Sci. 68: 2479-2485.

[10] Heimdal, H., Larsen, L. M. and Poll, L. (1994). Characterization of polyphenol oxidase from photosynthetic and vascular lettuce tissues *(Lactucu saliva).* J. Agric. Food. Chem. 42: 1428-1433.

[11] Nozue, M., Souri, M., Arakawa, D. and Kojima, M. (1998). Purification and characterization of two isoforms of chlorogenic acid oxidase from sweet potato cells in suspension culture. J. Plant Physiol. 153: 552-557.

[12] Oba, K., Iwatsuki, N., Uritani, I., Alvarez, A. M. and Garcia, V. V. (1992). Partial purification and characterization of polyphenol oxidase isozymes in banana bud. Biosci. Biotech. Biochem. 56: 1027-1030.

[13] Fraignier, M., Marques, L., Fleuriet, A. and Macheix, J. (1995). Biochemical and immunochemical characteristics of polyphenol oxidase from different fruits of *Prunus.* J. Agric. Food Chem. 43: 2375-2380.

[14] Marques, L., Fleuriet, A. and Macheix, J. (1995). Characterization of multiple forms of polyphenoloxidase from apple fruit. Plant Physiol. Biochem. 33: 193-200.

[15] Park, E. Y. and Luh, B. S. (1985). Polyphenol oxidase of kiwi fruit. Journal of Food Sci., 50: 678 - 684.

[16] Sheptovitsky, Y. G. and Brudvig, G. W. (1996). Isolation and characterization of spinach photosystem I1 membrane-associated catalase and polyphenol oxidase. Biochemistry 35: 16255- 16263.

[17] Laveda, F., Nunez-delicado, E., Garcia-carmona, F. and Sanchez-ferrer, A. (2001). Proteolytic activation of latent paraguaya peach PPO. Characterization of monophenolase activity. J. Agric. Food Chem. 49: 1003-1008.

[18] Serradell, M. D. L. A., Rozenfeld, P. A., Martinez, G. A., Civello, P. M., Chaves, A. R. and Anon, M. C. (2000). Polyphenoloxidase activity from strawberry fruit *(Frugariaxananassa,* Duch., cv Selva) characterization and partial purification. J. Sci. Food Agric. 80: 1421- 1427.

[19] Moore, B. M. and Flurkey, W. H. (1990). Sodium dodecyl sulfate activation of a plant polyphenoloxidase. Effect of sodium dodecyl sulfate on enzymatic and physical characteristics of purified broad bean polyphenoloxidase. J. Biol. Chem. 265: 4982-4988.

[20] Espin, J. C. and Wichers, H. J. (1999). Activation of a latent mushroom (*Agaricus bisporus*) tyrosinase isoform by sodium dodecyl sulfate (SDS). Kinetic properties of the SDS-activated isoform. J. Agric. Food Chem. 47: 3518-3525.

[21] Zhou, P., Smith, N. L. and Lee, C. Y. (1993). Potential purification and some properties of Monroe apple peel polyphenol oxidase. J. Agric. Food Chem. 41: 532-536.

[22] Yang, C. P., Fujita, S., Ashrafuzzaman, M., Nakamura, N., Hayashi, N. (2000). Purification and characterization of polyphenol oxidase from banana (*Musa sapientum*L.) pulp. J. Agric. Food Chem. 48: 2732–2735.

[23] Robinson, S. P., Loveys, B. R. and Chacko, E. K. (1993). Polyphenol oxidase enzymes in the sap and skin of mango fruit. Aust. J. Plant Physiol. 20: 99-107.

[24] Lee, P. M., Lee, K. and Karim, M. I. A. (1991). Biochemical studies of cocoa bean polyphenol oxidase. J. Sci. Food Agric. 55: 251-260.

[25] Raymond, J., Rakariyatham, N. and Azanza, J. L. (1993). Purification and some properties of polyphenol oxidase from sunflower seeds. Phytochemistry, 34: 927-931.

[26] Valero, E. and Garcia-carmona, F. (1998). pH-dependent effect of sodium chloride on latent grape polyphenol oxidase. J. Agric. Food. Chem. 46: 2447–2451.

[27] Miller, A. R., Kelley, T. J. and Mujer, C. V. 1990. Anodic peroxidase isoenzymes and polyphenol oxidase activity from cucumber fruit: tissue and substrate specificity. Phytochemistry 29: 705-709.

[28] Sakiroglu, H., Kufrevioglu, O. I., Kocacaliskan, I., Oktay, M. and Onganer, Y. (1996). Purification and characterization of Dog-rose (Rosa *dumlis* Rechst.) polyphenol oxidase. J. Agric. Food Chem. 44: 2982-298.

[29] Whitaker J. R. (1994). Principles of Enzymology for the Food Sciences, Second Ed., pp. 271-556, Marcel Dekker, New York.

[30] Wesche-Ebeling, P., and Montgomery, M. W. (1990). Strawberry polyphenol oxidase: its role in anthocyanin degradation. J. Food Sci. 55: 731–734.

[31] Lee, C. Y., Smith, N. L. and Pennesi, A. P. (1983), Polyphenol oxidase from De Chaunac grapes. Journal of Science of Food and Agriculture, 34: 987-991.

[32] Paul B. and Gowda L. R. (2000). Purification and characterization of a polyphenol oxidase from the seeds of field bean *(Dolichos lablab)*. J. Agric. Food Chem. 48: 3839-3846.

[33] López-Nicolás, J. M., Núñez-Delicado, E., Sánchez-Ferrer, Á. and García-Carmona, F. (2007): Kinetic model of apple juice enzymatic browning in the presence of cyclodextrins: the use of maltosyl-β-cyclodextrin as secondary antioxidant. Food Chemistry, 101: 1164–1171.

[34] Mari, S., Marques, L., Breton, F., Karamanos, Y. and Macheix, J. (1998). Unfolding and refolding of active apple polyphenol oxidase. Phytochemistry, 49: 1213-1217.

[35] Jimenez, M. and Garcia-carmona, F. (1996). The effect of sodium dodecyl sulphate on polyphenol oxidase. Phytochemistry, 42: 1503-1509.

[36] Sanchez-ferrer, A., Bru, R. and Garcia-carmona, F. (1989). Novel procedure for extraction of a latent grape polyphenol oxidase using temperature-induced phase separation in Triton X-114. Plant Physiol. 91: 1481-1487.

[37] Sojo, M. M., Nunez-delicado, E., Garcia-carmona, F. and Sanchez-ferrer, A. (1998). Monophenolase activity of latent banana pulp polyphenol oxidase. J. Agric. Food Chem. 46: 4931-4936.

[38] Sanchez-ferrer, A., Laveda, F. and Garcia-carmona, F. (1993). Partial purification of soluble potato polyphenol oxidase by partitioning in aqueous two-phase system. J. Agric. Food. Chem. 41: 1219-1224.

[39] Ferrar, P. H and Walker, J. R. L. (1996). Inhibition of diphenol oxidases; a comparative study. Journal of Food Biochemistry, 20: 15-30.