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Original Article

STUDIES ON KINETIC PARAMETERS AND BIOCHEMICAL CHARACTERISTICS OF POLYPHENOL OXIDASE PURIFIED FROM JACKFRUIT (*ARTOCARPUS HETEROPHYLLUS*) WASTE

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ABSTRACT

Objectives: Polyphenol oxidase activity was extensively studied in jackfruit for its role in enzymatic browning. PPO and the phenolic compound play a vital role in defensive mechanism against pest and diseases. Thus, to facilitate further studies in jack fruit waste, Polyphenol oxidase [PPO] was purified and characterized.

Methods: Partial Purification of PPO from waste done through a sequential process of ammonium sulfate precipitation, dialysis and ion-exchange chromatography [DEAE- Cellulose]. Then the partially purified PPO was subjected to check various parameters like molecular weight and kinetic activity, the following characteristics of enzyme are checked: SDS-PAGE, pH, temperature, thermal stability, heat inactivation, metal ions, surfactants and inhibitor.

Results: Purified PPO resulted in ~23 folds enriched in the specific activity of 1360 [µkat/mg] and it was found to be the monomer with a molecular weight of 63 kDa revealed by Coomasie Brilliant Blue staining. PPO exhibited optimum activity at pH 7.0 and temperature 20^{o}C. PPO showed the maximum stability between pH 6.4- 7.6 at 10 ^{o}C - 40 ^{o}C. PPO showed the enzyme activity towards Diphenol> Triphenol> Monophenol, the substrate specificity was especially high towards the catechol at 0.1 M. The PPO activity was activated by Mn^{2+}, Triton X- 100, EDTA, Sorbic acid and Citric acid, but inhibited by L- cysteine, Ascorbic acid, SDS, Cetyl trimethyl ammonium bromide [CTAB], K^{+}, Zn^{2+}, Ca^{2+} and Mg^{2+}. Kinetic constant for PPO was found to be k_m= 15.82 mM and V_{max}= 2182 U/ml min using catechol as substrate.

Conclusion: Partial Purification of PPO from waste done through a sequential process of ammonium sulfate precipitation, dialysis and ion-exchange chromatography [DEAE- Cellulose]. The best substrate for PPO was identified as catechol [diphenol] and best inhibitor was L-cysteine and ascorbic acid.

Keywords: Polyphenol oxidase, PPO purification, Catechol, Ascorbic acid and L- Cysteine.

INTRODUCTION

Polyphenol oxidases [PPO] [EC.1.14.18.1] are oxidoreductases that catalyses both the hydroxylation of monophenols and oxidation of o-diphenols to o-quinones. It has a dinuclear copper center able to insert an oxygen atom at ortho position of the existing hydroxyl group in the aromatic ring [1] and undergoes non-enzymatic polymerization with a variety of cellular constituents such as proteins and amino acids which lead to the formation of red, brown and black pigments [2]. PPO is located in the thylakoid membrane of chloroplasts and their phenolic substrates are located in the vacuoles in higher plants [1]. PPO has been studied in many of the vegetables and fruits such as: *Solanum lycocarpum* [3], persimmon [4], Jackfruit [5], Mamey [1], apple [6], red swiss chard [7], Cabbage [8] and banana [9].

The jackfruit belongs to the family Moraceae [Mulberry family]. About 70-80% of polyphenol oxidase are present in jackfruit waste. In common with other tropical fruits such as durian, banana, etc., it also possesses high nutritional value such as dietary fiber, minerals, and vitamins and free of saturated fats [10]. The aim of the study was to partially purify polyphenol oxidase present in the *Artocarpus heterophyllus* waste and to characterize the partially purified enzyme fraction in terms of optimum pH, thermal stability, optimum temperature, heat inactivation, optimum concentration, best substrate, electrophoresis profile and kinetic parameters [including km, Vmax and IC50 values].

MATERIALS AND METHODS

Materials and reagents

Jackfruit waste was collected from the Salem market, Tamil Nadu and it were stored at -8°C to avoid the oxidation reaction. DEAE – Cellulose, substrates, inhibitors from Hi Media Pvt, Ltd., and protein marker from Merck Genei were of analytical grade.

Enzyme extraction and purification

20 g of Jackfruit waste were homogenized with ice-cold 100 ml of sodium phosphate buffer [0.1 M, pH 7.0] containing 1 % PVP. The crude extract was then centrifuged at 10,000 rpm for 20 min at 4°C. After centrifugation, several precipitations with ammonium sulfate between 20-80%, 25-80%, 30-80%, 35-80%, 40-80% was examined to find the proper saturation point. The precipitation was diluted in sodium phosphate buffer [0.1 M, pH 7.0] twice the volume of the precipitate obtained.

The diluted sample was subjected to dialysis using 50 kDa MWCW dialysis bag at the 4°C for overnight in the sodium phosphate buffer [0.1 M, pH 7.0]. For further purification of PPO by column packed with DEAE- Cellulose [1.4 x 20 cm] equilibrated with sodium phosphate buffer [0.1 M, pH 6.8]. The dialyzed enzyme solution was loaded into the column for further purification. The desired sample was eluted with a gradient buffer solution pH ranging from 5.8-7.8 [sodium phosphate buffer]. The elution rate was adjusted to 10-15 ml/hr. Fraction of 3 ml per each was collected and active fraction was pooled out.

The protein concentration and enzyme activity of each fraction were detected using 0.1 M catechol as substrate and the fraction with higher activity and protein concentration were collected and pooled off for the further characterization studies. The protein concentration was determined by [11]. Lowry's method The values obtained were plotted against the fraction number. The final active fraction eluted was used for bioassay, sodium dodecyl sulfate –polyacrylamide gel electrophoresis [SDS- PAGE] and other kinetic studies.

Electrophoresis

SDS–PAGE [12] was performed using a discontinuous buffer system with a stacking gel of 6 % acrylamide and a separating gel of 15 % acrylamide. The samples were run at a constant voltage of 200 V. Gels were stained for protein by using Coomassie Brilliant Blue dye.

PPO activity assay and substrate specificity

PPO activity assayed in (3 ml) 0.1 M sodium phosphate buffer [pH 7.0] containing 0.1 M [Gallic acid [triphenol, 420 nm], catechol [diphenol, 420 nm] and L-tyrosine [monophenol, 400 nm]] with 50 µl of enzyme solution added to begin the reaction. The reaction was carried out at room temperature; the change in absorbance at 420 nm was recorded for every 60 s for 10 min using HitachiU2900 UV- Spectrophotometer. A total of 1 enzyme unit [U] was defined as a change in absorbance of 0.001 in 1 min [5]. The enzyme activity was expressed in U/mg.

Effect of pH and temperature

Optimum pH of jackfruit waste PPO was determined by measuring its activity in different pH, sodium phosphate buffer ranges from pH 6.2- 8.0. The assay mixture contains [3 ml] of 0.1 M catechol in 0.1 M sodium phosphate buffer [pH 7.0] and 50 µl. The relative activity detected under the above assay condition and the graph was plotted between pH and residual activity. The temperature optimum for jackfruit waste PPO was estimated by determining its activity across temperature ranging from 10°C- 70°C. The effect of temperature on enzyme activity was monitored by incubating the PPO in 0.1 M sodium phosphate buffer [pH 7.0] and the optimum temperature was detected by using standard assay condition and relative activity was found.

Thermal stability and heat inactivation

The thermal stability of the enzyme was monitored by incubating the enzyme at various temperatures [10–70 °C] for 30 min. After pre incubation, 50 µL of treated enzyme was immediately cooled on ice for 5 min and then assayed at pH 7.0 and 20 °C. The highest relative activity was defined as 100 %. For heat inactivation, the enzyme was incubated at 40-90 °C up to 25 min. The activity of the enzyme without pre incubation was defined as 100 %.

Effect of metal ions, surfactants and inhibitors

The effect of metal ions, surfactants and potential inhibitor of purified PPO activity was determined by using catechol as a substrate. The enzyme was pre incubated at room temperature for 60 min and experiment was carried out under standard assay condition as mentioned above. The inhibition type was identified by the Lineweaver–Burk plot and IC50 values were determined.

Determination of kinetic parameters

The Michaelis- Menten constant [Km] and maximum velocity [Vmax] was determined by varying the concentration of catechol [0.02 M-0.2 M] in 0.1M sodium phosphate buffer [pH 7.0]. The LB-Plot was drawn in order to determine the kinetic parameters.

RESULTS AND DISCUSION

PPO purification

PPO from jackfruit waste was purified by ammonium sulfate precipitation and DEAE- Cellulose column an anion exchanger. Ammonium sulfate precipitation [35-80 %] increase the jwPPO activity [1.19- fold], which when further purified by an anion exchanger [DEAE- Cellulose] showed 22.9 folds purity as shown in Table 1. The elution profile of the jwPPO on DEAE- Cellulose was shown in the Figure 1.

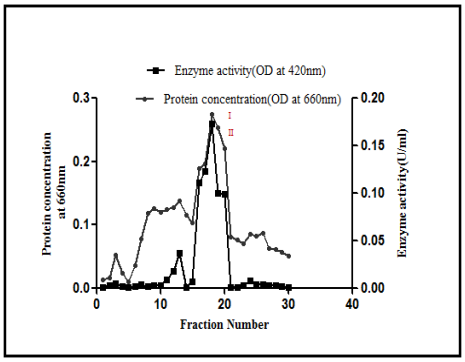

Fig. 1: Elution profile by Ion exchange chromatography – DEAE - Cellulose [an anion exchanger]

The protein with PPO activity is represented in the form of two peaks: First peak represents the protein concentration which was observed between the fractions of 13- 20, while the second peak represents the enzyme activity which was observed between the fractions of 14-21. The specific activity of purified PPO was found to be 1360 [µkat/mg] and a yield of 13.5 %. The obtained enzyme was used for further studies.

Electrophoresis and molecular weight determination

SDS-PAGE was used to monitor the progression of purification process, to determine the homogeneity and apparent molecular mass of jwPPO. The molecular weight and purity of purified jwPPO was analyzed by denaturing by SDS-PAGE. Coomassie brilliant blue staining of SDS-PAGE gel showed a single band. Based on the molecular weight marker range [35-100 kDa], the molecular weight of jwPPO was determined as 63 kDa as an average [Figure 2], which was similar to that of 65 kDa [5] but higher than that of PPOs from banana, cabbage 41 kDa [9], 53.1 kDa broccoli florets PPO [13], 41 kDa red swizz chard PPO [7] and beet root PPO 54 kDa [14]. These results confirmed that the purified homogeneous protein was PPO and suggested that jwPPO have a single polypeptide chain [monomer]. As similar to PPO separated from snake fruit [15].

Table 1: Purification of PPO from jackfruit waste

Purification step	Activity [µkat/ml]	Total activity [µkat/ml]	Total protein [mg/ml]	Specific activity [µkat/mg]	Purification [Fold]	Yield [%]
Crude extract	249	24900	4.2	59.28	1	100
Ammonium sulfate precipitation	275	20625	3.9	70.51	1.19	82.83
Dialysis	129	1290	0.4	322.5	5.44	5.18
Ion exchange [DEAE cellulose]	374	3366	0.275	1360	22.9	13.5

Effect of pH on jwPPO

The optimum pH at which the enzyme was more stable was determined. The activity of purified jwPPO was measured at various pHs using catechol as substrate. It was found that at pH 6.0 the enzyme activity was rapidly increasing, attained a steep at pH 6.8 and reached the maximum activity at pH 7.0. Then again drastically decreased at the pH of 7.2 [Figure 3]. The activity of PPO in pineapple and jackfruit was found to be at neutral pH 7 similar to that of the jwPPO [16, 5]. By considering this condition, the pH dependency varies due to several factors such as extraction method, phenolic compounds, buffer used for the assay and temperature [1]. The enzyme was active over a pH range of 6.2 - 7.4 with a half maximum activity at pH 6.6 [Figure 3].

Below and above pH 7.0 the activity decreased rapidly. By using catechol as a substrate most of the plant showed the maximum PPO activity between pH 6.0-8.0. Artichoke [17] showed the maximum

activity at pH 7.0 similar to jwPPO. But the PPO activity in Tobacco pH 6.0 [18] and Barbados cherry pH 7.2 [19] reported was not similar to jwPPO. jwPPO activity decreases below pH 6.2 and above pH 7.2 but the enzyme was still active at pH 7.8 with relative activity close to 80%.

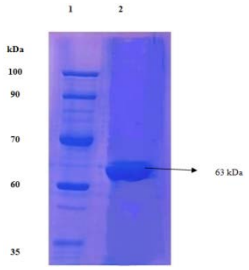

Fig. 2: SDS- PAGE of jackfruit waste PPO. The polyacrylamide gel of [15%] stained by Coomassie Brilliant Blue dye. Lane1: Marker [35-100 kDa], Lane2: Purified PPO

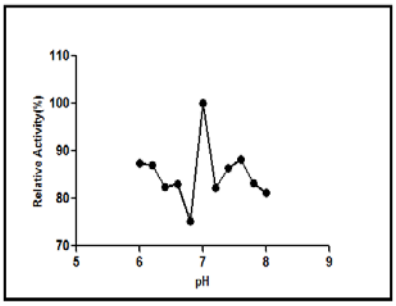

Fig. 3: pH stability of PPO from jackfruit waste. The substrate used to be 0.1M catechol in 0.1M sodium phosphate buffer [pH 7.0]. Activity was expressed as relative activity [%] compared with activity determined at pH 7.0.

Effect of temperature on jwPPO and heat stability

The effect of temperature between 10°C–70°C on jwPPO activity showed that optimum temperature for purified jwPPO at 20°C [Figure 4]. This optimum temperature was similar to grapes [25°C] [20], and Stanley plums [20°C] [21]. In contrast to our result, the optimum temperature of jackfruit PPO was reported as 8°C [5], pear was reported as 35°C [22], and persimmon was reported as 55°C [4].

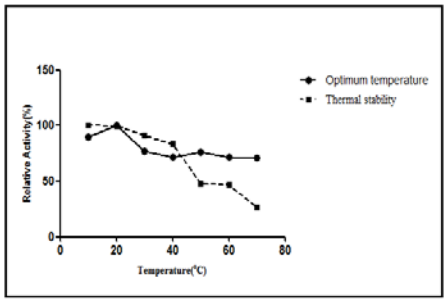

Fig. 4: Heat stability of PPO from jackfruit waste. The substrate used was 0.1 M catechol in 0.1 M sodium phosphate buffer [pH 7.0]. Activity was expressed as relative activity [%] compared with activity determined at 20°C and the stability of jwPPO was found between 10°C- 40°C.

Heat stability of PPO was analyzed by incubating the enzyme for 30 min at temperatures ranging from 10°C-70°C. In the 10°C -30°C ranges, relative activity was similar [Figure 4]. At 30°C the relative activity was maintained at 95 % at higher temperature the activity decreased [40°C-70°C] and the relativity activity was found to be between 30-40 %.

The jwPPO was found to be stable in the temperature ranging from 10°C-40°C. In contrast to the result, Chinese cabbage PPO was thermally stable between 50°C-70°C [8] and apple was reported to be stable between 30°C - 75°C, while above this temperature PPO become rapidly inactivated [6]. The PPO temperature optimum also depends on the conditions to which the fruit develop.

Heat stability of jwPPO was retained till 40°C as similar to a jackfruit PPO [5], at that temperature the jwPPO activity was retained. Then the enzyme activity decreased drastically with decrease in temperature from 50°C- 70°C [Figure 4].

Heat inactivation

Heat inactivation was performed between the temperatures ranging from 50°C- 80°C. PPO was stable below 40°C, while at the temperature > 50°C the activity of the enzyme was decreased [Figure 4]. The result is similar to pouteria sapota[1] reported that PPO was inactive between 65°C- 70°C.

The heat inactivation process was represented in [Figure 5a]. The semi-log plots of the remaining activity of PPO versus heating time were linear at 4°C–90°C, indicating a simple first-order process. The inactivation rate constants k was calculated from the slopes of these lines, and the k values were plotted in an Arrhenius plot [Figure 5b]. The result is similar to the jackfruit Arrhenius plot, reported that as the temperature increases the activity of the enzyme is inhibited [5].

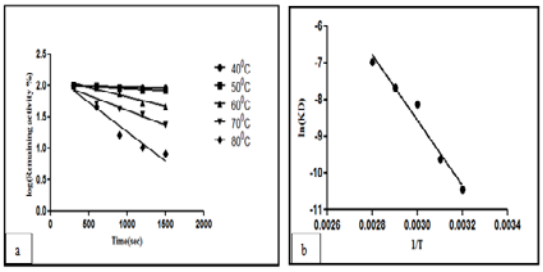

Fig. 5: [a] Heat inactivation of jwPPO. The enzyme was incubated at 40–90 °C up to 25 min. After pre incubation, 50 µL of treated enzyme was immediately cooled on ice for 5 min and then assayed at pH 7.0 and 20 °C. The activity of the enzyme without pre incubation was defined as 100%. [b] Arrhenius plot of heat inactivation rates of jwPPO.

Substrate specificity

The oxidation of substrate by purified jwPPO was determined spectrophotometrically at the specific wavelength for each substrate. OD for Gallic acid (Triphenol), catechol (Diphenol) was taken at the wavelength of 420 nm and OD for L-tyrosine (Monophenol) was taken at the wavelength of 400 nm. The Substrate specificity was detected at 0.1 M concentration for optimum pH and temperature. The jwPPO highly oxidize the catechol when compared to gallic acid and L-Tyrosine as represented in [Figure 6].

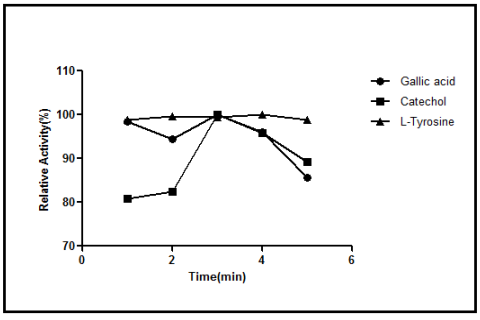

Fig. 6: Substrate specificity and Enzyme activity

Table 2: Substrate specificity, enzyme activity and Kinetic parameters for the oxidation of phenol substrate by jwPPO

Phenol type	Substrate	Wavelength	Relative activity [%]	Km [mM]	Vmax [U/ml]	Enzyme activity [U/mg]
Monophenol	L-Tyrosine	400	37.15	20.7	982	210.8
Diphenol	Catechol	420	100	15.82	2182	700.4
	Chlorogenic acid	400	-	-	-	-
	L-DOPA	475	-	-	-	-
Triphenol	Gallic acid	420	68.04	18.85	1514	408
	Methyl gallate	400	-	-	-	-
	Pyrogallic acid	430	-	-	-	-

From [Figure 6] the enzyme activity of various substrates was determined. Among the three different substrate catechol showed the highest enzyme activity. So, for further studies catechol was taken as the substrate. The enzyme activity of Gallic Acid [Triphenol], Catechol [Diphenol] and L-Tyrosine [Monophenol] was found to be 408 U/mg, 700.4 U/mg and 210.8 U/mg. Thus the substrate specificity was high in the order of Diphenol>Triphenol>Monophenol. The triphenol activity of jwPPPO was similar to that of the red swiss chard leaves reported that triphenol substrate activity was moderate when compare to the mono and diphenol substrate [23]. The result obtained was also similar to a *solanum lycocarpum* fruit [3], dormant saffron PPO [24], butter lettuce [25] and peppermint [26].

From Table 2, When comparing the activity of the three substrate catechol [diphenol] showed the highest activity towards the oxidation reaction [23]. These results indicate that the purified jwPPO had a diphenol activity much stronger than its monophenol activity and the diphenolase activity was higher towards o-diphenols than towards m- and p-diphenols. This characterization was also done in many other plant species like Barbados cherry [19] and Jackfruit [5].

Effect of different substrate concentration

The optimized substrate [i. e.] catechol was subjected to enzyme assay at different concentration ranging from 0.02 M - 0.2 M. The high oxidation of catechol by jwPPO was attained at the concentration of 0.1 M [Figure 7].

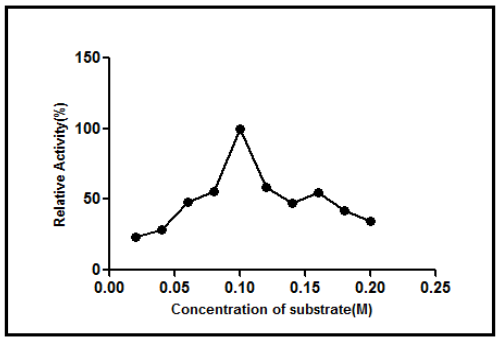

Fig. 7: Effect of different substrate [Catechol] concentration

The optimum substrate concentration of the plant PPO falls in between 0.08 M - 0.2 M. Thus the obtained result for jwPPO was similar to that of jackfruit PPO [5].

Enzyme kinetics

Michael's constant [Km] and maximum reaction velocities [Vmax] were determined using catechol as substrate at various concentrations and under optimum condition [pH and temperature]. Partially purified jwPPO has a Km value of 15.86 mM calculated from the Lineweaver-Burk plot for the substrate catechol. This value for catechol was similar to that of tea leaves [12.5 mM] [27], field bean seed [10.5 mM] [28] and cabbage [15.4 mM] [8]. The best substrate for each enzyme may be chosen based on a high substrate affinity [km] and high catalytic rate [Vmax]; an additional criteria for choosing the best substrate is by the highest catalytic efficiency [Vmax/km].

Vmax and Km values were determined by linear regression analysis of V versus [S] for each substrate [Table 2]. Catalytic efficiency was the lowest but substrate binding was the highest with catechol. The Vmax value indicated PPO had the highest affinity for catechol [Table 2], and it was found that catechol was the most efficient phenolic substrate for jwPPO. The result was similar to PPO from Lonicera japonicaThunb reported that, catechol has the high substrate binding capability when compared to the other phenol substrate [29, 30].

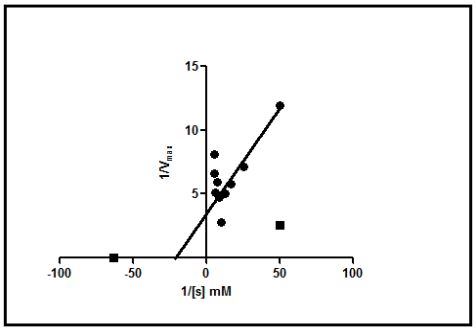

Fig. 8: LB-PLOT of optimized substrate catechol

Effect of metal ions on jwPPO activity

In order to determine the effect of metal ion as possible inhibitors of jwPPO activity, the jwPPO was incubated with 0.1 mM, 1 mM and 5 mM of different metal ion and assayed by using catechol as substrate, then the relative activity was measured using the standard protocol. The purified jwPPO activity in the absence of testing metal ions under optimum condition was set at 100 %.

As represented in Table 3, Mn^{2+} at 0.1 mM, 1 mM and 5 mM had a positive effect, whilst divalent cation includes Zn^{2+}, Ca^{2+} and Mg^{2+} generally had negative one. Among the divalent cation tested, Ca^{2+} at 5 mM showed the strongest inhibitory effect. and 76.92% of the enzyme activity was inhibited. K^{+} showed the maximum inhibition percentage of 65.25 at 5 mM. Mn^{2+} at 1 mM and 5 mM enhance the activity of jwPPO by 10-15 %, but ca^{2+} at higher concentration decreases the jwPPO activity. The jwPPO result was similar to PPO from Cleome gynandra L. [31]. The k+ and zn^{2+} metal ion strongly inhibit the activity of jwPPO when the concentration of metal ions increases [Table 3]. Thus the sulfur containing compounds is known to be a specific potent inhibitor of PPO by removing quinines and in turn preventing them from participating in melanizing reaction and reacting directly with the enzyme [32].

Effect of surfactants on jwPPO activity

For determining the effect of different surfactant on the jwPPO, it was incubated with the surfactants at optimum pH and temperature for 1 hr and the enzyme activity was determined under normal assay condition. The PPO activity without any surfactants was taken as 100 %.

Table 3: Effect of metal ions on jwPPO

S. No.	Metal Ion	Relative Activity [%]		
		0.1 mM	1 mM	5 mM
1.	K^{+}	101.09±0.08	73.44±0.13	65.25±0.03
2.	Zn^{2+}	115.79±0.03	96.34±0.04	78.34±0.06
3.	Ca^{2+}	105.42±0.03	89.26±0.09	76.68±0.50
4.	Mg^{2+}	99.70±0.41	91.22±0.29	87.76±0.20
5.	Mn^{2+}	89.72±0.23	97.21±0.06	111.03±0.06

* Data are represented in Mean ±SD, n=3

Table 4: Effect of Surfactant on jwPPO

S. No.	Surfactant	Relative Activity [%]		
		0.05 % (W/V)	0.15 % (W/V)	0.25 % (W/V)
1.	SDS	101.50±0.01	92.05±0.05	69.25±0.23
2.	CTAB	98.84±0.03	87.26±0.12	54.62±0.04
3.	Triton -X 100	42.78±0.08	71.39±0.16	98.69±0.39

* Data are represented in Mean ±SD, n=3

Table 5: Effect of different inhibitor

S. No.	Inhibitor	Relative Activity [%]		
		1 mM	3 mM	5 mM
1	EDTA	86.07±0.08	95.86±0.20	99.35±1.02
2	L-Cysteine	112.42±0.11	81.91±0.04	55.55±0.12
3	Sorbic Acid	110.04±0.07	95.60±0.05	98.97±0.08
4	Ascorbic Acid	80.54±0.50	64.06±0.26	57.86±0.35
5	Citric Acid	90.55±0.32	94.77±0.66	98.30±0.55

* Data are represented in Mean ±SD, n=3

Table 6: IC50 values and type of inhibition of jwPPO with different substrate

Compounds	Concentration [M]	Type of Inhibition	%Inhibition	IC50 [mM]
EDTA	1x10-3	Non-Competitive	13.85	0.019
	3 x10-3		3.95	
	5 x10-3		0.03	
L-Cysteine	1x10-3	Non-Competitive	0	0.100
	3 x10-3		18.05	
	5 x10-3		44.38	
Sorbic Acid	1x10-3	Non-Competitive	0	0.060
	3 x10-3		4.35	
	5 x10-3		0.97	
Ascorbic Acid	1x10-3	Competitive	19.17	0.100
	3 x10-3		35.80	
	5 x10-3		41.76	
Citric Acid	1x10-3	Non-Competitive	9.22	0.020
	3 x10-3		4.82	
	5 x10-3		1.11	

As given in the Table 4, CTAB [cation] showed the maximum inhibition at 0.25 % [W/V] with the residual percentage of 54.63 % and the minimum inhibition was shown by SDS with the residual activity of 69.38 % at 0.25 [W/V] %. Triton –X 100 activates the jwPPO activity by 98.84 %. The result attained was similar to PPO from Cleome gynandra L. [31], but it was against to jackfruit PPO reported [5].

Effect of inhibitor on jwPPO

jwPPO activity was measured in the presence of five different inhibitors namely; Ascorbic acid, L-cysteine, Citric acid, Sorbic acid and EDTA were taken at the concentration of 1 mM, 3 mM and 5 mM using catechol as substrate. The most effective inhibitor was as follows: Ascorbic acid and L-Cysteine inhibited [Table 5] 40 %- 50 % at 5 mM. In contrast, in the presence of citric acid, sorbic acid and EDTA jwPPO activity were activated rather than inhibition [Table 5].

From Table 6, the inhibition type of various inhibitors was determined. L-Cysteine, EDTA, Sorbic acid and citric acid shows Non- Competitive inhibition similar to jackfruit PPO [5] where as ascorbic acid shows competitive inhibition. The IC50 value was found to be high in L-Cysteine and ascorbic acid, and also the % inhibition was found to be high in ascorbic acid and L-cysteine. Thus the best inhibitor to arrest the oxidation reaction of jackfruit waste was determined as L-cystiene and ascorbic acid shown in Table 6. The inhibitor reaction mechanism differs, depending on the reducing agent employed. Ascorbate acts more as an antioxidant than as an enzyme inhibitor because it reduces the initial quinone formed by the enzyme to the original diphenol before it undergoes secondary reactions which leads to browning reaction [33].

CONCLUSION

Polyphenol oxidase [PPO] was purified and characterized from jack fruit waste using DEAE- Cellulose Column. The purity of jwPPO was

resulted in ~ 23 folds and its specific activity was found to be 1360 [µkat/mg]. Purified Polyphenol oxidase was found to be a monomer with a single polypeptide chain having a molecular weight of 63 kDa revealed by Coomasie Brilliant Blue staining. The optimum pH of jwPPO was found to be pH 7.0 and temperature was found to be 20°C. jwPPO shows the maximum stability between pH 6.4- 7.6 at 10°C- 40°C. jwPPO showed the enzyme activity towards Diphenol> Triphenol> Monophenol and it had high efficacy to oxidize the catechol. Thus the substrate specificity was found to be higher towards catechol [Diphenol] and has the optimum activity at 0.1 M concentration. The jwPPO activity was activated by Mn^{2+}, Triton X-100, EDTA, Sorbic acid and Citric acid, but inhibited by L-cysteine, Ascorbic acid, SDS, Cetyl trimethyl ammonium bromide [CTAB], K^{+}, Zn^{2+}, Ca^{2+} and Mg2+. Kinetic constant for PPO was found to be km= 15.82 mM and Vmax= 2182 U/ml min using catechol as substrate. The best substrate for PPO was identified as catechol [diphenol] and best inhibitor was L-cysteine and ascorbic acid. Further immobilization of PPO can be made which plays a major role in various fields like Bioremediation to treat the phenol contaminated soil, environmental biotechnology to treat the contaminated water and also in Biomedical engineering to create a biosensor to quantify the L-DOPA concentration.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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