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Comparison of polyphenol oxidase in fruits of *Solanum melongena* L. *(*purple*)* and *Solanum melongena* L. (green)

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Abstract

A comparative study of polyphenol oxidase (PPO) from two varieties of brinjal (local long green-bili badnea and purple-gundu badne) was conducted to determine differences and similarities of some of the characteristics of the PPO enzymes in terms of temperature and pH optima, substrate specificity, thermal inactivation and potency of some PPO inhibitors. PPO was partially purified by ammonium sulfate precipitation followed by dialysis. The optimum pH and temperature of the two PPOs was found to be similar. It is found that the PPO activity is found more in local long green-bili badnea than in purple-gundu badne. PPO activity is almost double the concentration in local long green-bili badnea compared to PPO found in purple-gundu badne. The data obtained in this study help to better understand fruit browning in two varieties of brinjal.

Keywords: *Solanum melongena*, polyphenol oxidase, brinjal, purification, characterization

Introduction

Polyphenol oxidase (*o*-diphenol: oxygen oxidoreductase, EC.1.10.3.1.) has been found in higher plants, and is responsible for enzymatic browning of raw fruits and vegetables. This reaction is important in food preservation and processing, and is generally considered to be an undesirable reaction because of the unpleasant appearance and concomitant development of an off flavour. Fruits and vegetables may also contain peroxidases (EC 1.11.1.7) which can contribute to or generate browning-like reactions (Aydin and Kadioglu, 2001). The activities of peroxidase have been reported to increase with senescence advancement. Polyphenol oxidases (PPO) (EC 1.10.3.1); are enzymes, belonging to a group of copper containing metallo proteins and are members of oxido-reductases, that catalyze the oxidation of a wide range of phenolic compounds by utilizing molecular oxygen. Multiple forms of PPO have been isolated from a wide variety of sources, including from tea leaf (Halder *et al*., 1998), the pulp of banana (*Musa sapientum* L.) (Yang *et al*.,2000), royal ann cherries (Benjamin and Montgomery, 1973), leaf and fruit endosperm of coffee (Mazzafera and Simon, 2000), rasberry fruits (González *et al*., 1999), tobacco (*Nicotiana tabacum*) (Shi *et al*., 2002), aerial roots of a tropical orchid, *Aranda* 'Christine 130 (Ho, 1999), grapes (Kimberly *et al*., 1981), tea (Vasyl *et al*., 2001), peppermint leaves (*Mentha piperita*) (Kavrayan and Aydemir, 2001), brinjal (Gosmi and Amarpurkar, 2003), Indian gooseberry (Latha *et al*., 2013). Brinjal or eggplant (*Solanum melongena* L.) is a very rich source of polyphenol oxidase (PPO). It is being used as vegetable for thousand years. The purpose of this study is to compare characteristic activities of polyphenol oxidase (PPO) from two very commonly found varieties of brinjal, *i.e*., Gundu badne-purple and local long green-bili badnea. The results of this study would provide an understanding of the browning of the two vegetables and means of prolonging the shelf-life. It was reported that fruits which are developed under long day treatment had higher phenol contents than in normal day treatment (Mehta and Bhavannarayana, 1980). Polyphenol oxidase obtained from (*Solanum melongena* L.) was characterized and studied by Todaro *et al*., 2011.

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Material and Methods

Plant material

Two varieties of fresh brinjal long local variety commonly called bili badanea, Sample-1 **(S-1),** Purple colored with white stripe, and oval shape commonly called Gundu badane, Sample-2 **(S-2)** were collected from College garden, Government Science College. Hassan.

Enzyme extraction and partial purification

Two varieties of fresh brinijal of ten grams each were cleaned, peeled and homogenized by grinding 50ml of 0.1M phosphate buffer (pH 7), 1% (W/V) Triton-X-100 and 0.1M NaCl. The crude extract samples were centrifuged at 30,000g for 20 min at 4ºC. Followed by solid ammonium sulphate precipitation and followed by dialysis (Dialysis membrane-70 LA393 from HIMEDIA). Extract was used as the PPO enzyme source.

Determination of PPO activity

PPO activity in two varieties of brinjal was determined by measuring the absorbance at 420 nm using a Spectrophotometer (Pharmatech, Model ELiCO SL 150 UV-VIS-Spectrophotometry).The activity was assayed in 3 ml of reaction mixture 0.2ml enzyme, 0.5ml substrate (Catechol RM6782 from HIMEDIA). "One unit of the polyphenol oxidase is defined as the enzyme which transfers 1umol catechol to quinones per minute under defined conditions".

Substrate concentration and specificity of PPO

The brinjal-PPO activity was determined using two different substrates, namely catechol and tyrosine.The highest enzyme activity was obtained with 10mM of catechol. Therefore, the concentration10mM catechol was used as the substrate in further experiments.

Protein estimation and determination of molecular weight

Protein content was estimated by the Lowry Method **(**Lowry *et al*., 1951).

Enzyme kinetics

Michaelis constant (Km) and maximum velocity (Vmax) values of the enzyme were calculated from Lineweaver- Burk graphs.

Effect of pH

The effect of pH on two varieties of brinjal. PPO activity was determined under standard laboratory containing 3ml reaction mixture (0.2ml enzyme, 0.5ml catechol. 2.3ml phosphate buffer of pH 4 to pH 8).

Effect of temperature

The optimum temperature of two varieties of brinjal for PPO activity was determined. The enzyme was measured at different temperatures (30-80°C), using 3ml reaction mixture (0.2ml enzyme, 0.5ml catechol,2.3ml phosphate buffer**).**

Effect of inhibitors and metallic ions on PPO activity

The effects of seven metal ions ($CaCl_2$, $CuSO_4$, $MgSO_4$, KCl, $ZnSO_4$, $BaCl_2$, NaCl), and effect of EDTA,SDS was evaluated on two varieties - PPO activity, using 3ml reaction mixture (0.2ml enzyme, 0.5ml catechol, 2.3ml phosphate buffer). The change in absorbance was measured Spectrophotometrically at 420 nm.

Results and Discussion

Extraction and purification of PPO

The total protein concentrations of the dialyzed extract, estimated using Lowry Method (Lowry *et al*., 1951) was 80µg for 0.2ml of enzyme for S-1 and 30µg for 0.2 ml of enzyme for S-2.

Polyphenol activity

It was observed that PPO activity was more in long local variety commonly called Bili badanea brinjal variety, Sample-1 **(S-1),** than Purple colored with white stripe brinjal variety, Sample-2 **(S-2)** Figure 1.

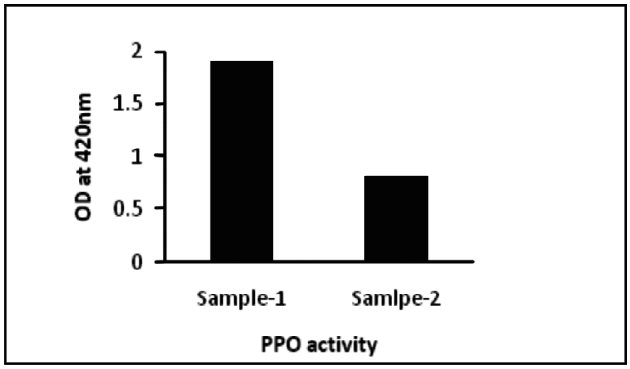

Figure 1: PPO activity

Molecular weight estimation

The analysis of SDS-PAGE gel revealed two bands in purified extracts as for (S-1) and one adjacent bands in (S-2) (Figure 2). We recommend accurate molecular weight of these two varieties has to be studied further.The molecular weight of PPO from other species has been reported as follows: *Dolichos lablab* seeds, PPO 120 ± 3 kDa (Ho, 1999),*Ocimum basilicum* L. (Vasyl *et al*., 2001) 54kDa,Chinese cabbage (Gosmi and Amarpurkar, 2003) 465kDa,tea leaf (Halder *et al*., 1998) 72kDa, Rape flower (Kimberly *et al*., 1981) 60.4kDa, Black pepper (Mehta and Bhavannarayana, 1980) 60kDa, Mango peel (*Mangifera indica*)136 kDa (Lowry *et al*., 1951) and Indian Gooseberry (Latha *et al*., 2013) 100 kDa. The molecular weight PPO from eggplant (*Solanum melongena* L.) was found to be 112kDa homodimer (Mishra *et al*., 2012).Our results indicate that the accurate molecular weight of these two varieties has to be studied further and then confirmed [*Green colored long local variety* Sample-1(S-1) Purple colored with white stripe, oval shape commonly called Gundu badane] Sample-2 (S-2).

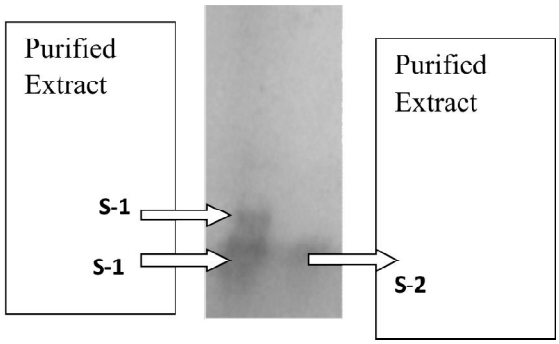

Figure 2 : Electrophoretogram of PPO from extracts of fruits of brinjal. Lane; lane 1 purified extract of (S-1), lane-2 purified extract of (S-2)

Enzyme kinetics and substrate specificity

Michaelis constants (Km) and maximum reaction velocities (Vmax) and specificity (Vmax/Km) of the brinjal PPO was determined at optimum pH 7.0 and 40°C, using catechol Km =70mM and Vmax = 0.008mM/sec (Line Weaver Burk Plot) as reported in Figure 3.

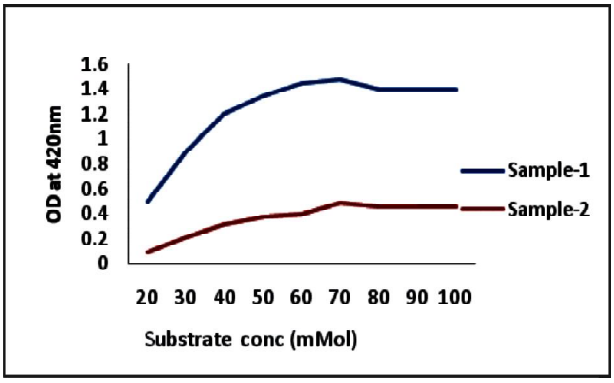

Figure 3: Km and Vmax

Optimum pH

The optimum pH exerts a strong effect on enzymatic activity. It is pH7 for both Sample-1 and Sample-2 PPO of brinjal varieties. We found that the enzyme activity is more in S-1 than S-2 (Figure 4).

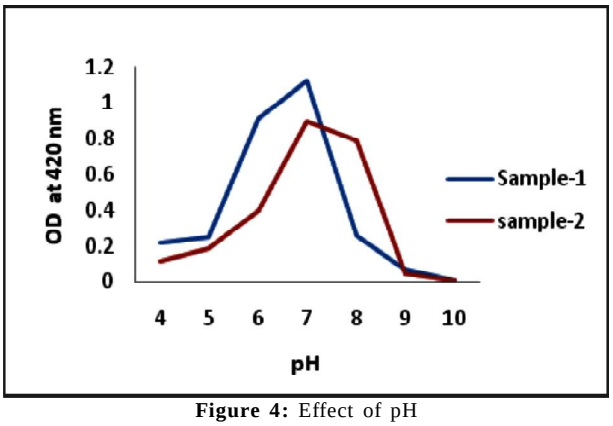

Figure 4: Effect of pH

Effect of temperature

Effect of temperature was assayed, using catechol as a substrate, over a temperature range of 30-80 °C at the optimum pH. The results are shown in Figure 5. Brinjal activity was found to increase with increasing temperature with the maximum activity being attained at 40 °C and dropped to a sub-minimum at 70°C. Polyphenoloxidase in eggplant (*Solanum melongena* L.) get completely inactivated after heat treatment at 75°C for 30 min or 80°C for 5 min (Fujita and Tono, 1988). It was reported earlier that the optimum temperature values were 45 °C for Hibiscus-PPO (Madani *et al*., 2011), 30 °C for aubergine (Dogan *et al*., 2002) and 56 °C for amasya apple (Oktay *et al*., 1995).

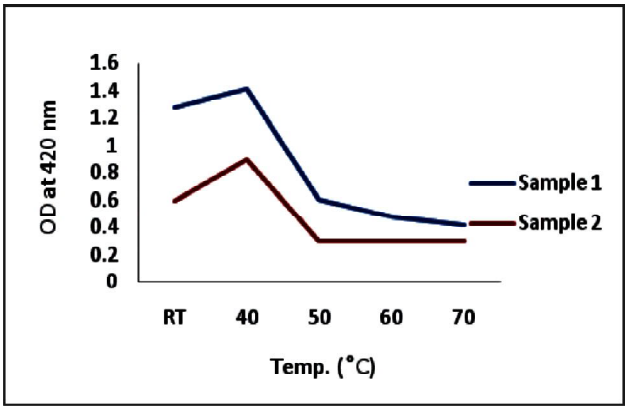

Figure 5: Effect of temperature

Effect of metallic compounds

We studied and evaluated effects of seven metal ions ($Mgso_4$, $CuSo_4$ KCl, , $BaCl_2$, NaCl). The results indicated that PPO is a copper containing enzyme, copper sulphate and zinc sulphate (10mM) serves as an activator for its activity. SDS and EDTA (10mM) showed inhibitory effects on the activity of PPO (Figure 6).

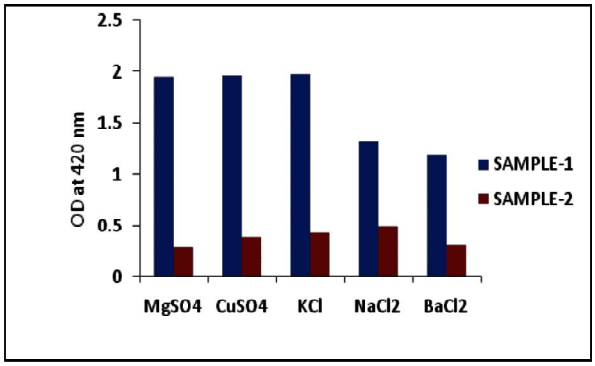

Figure 6: Effect of metal ions

In our study, we evaluated effects of seven metal ions ($Mgso_4$, $CuSo_4$ KCl, , $BaCl_2$, NaCl). The results indicated that PPO is a copper containing enzyme, copper sulphate and manganese

sulphate and chloride ions also (10mM) serves as an activator for PPO activity. The studies of the effect of different metal ions on polyphenol oxidase from eggplant fruit, Cu^{2+}, Mn^{2+} and Mg^{2+} were found to promote the activity of the enzyme. Four metal ions (Ca^{2+}, Na^{+}, Al^{3+} and Fe^{3+}) had an inhibitory effect on the enzyme (Xing-Hui, 2011). It is studied that browning in fruits was found to be dependent on phenolic content and PPO specific activity (Mishra *et al*., 2013). SDS and EDTA (10mM) showed inhibitory effects on the activity of PPO.

Conclusion

In this report, studies were carried out on the PPO from the extract of two varieties of brinjal (Gundu badne-purple and local long green-bili badnea). Ammonium sulphate precipitated fraction of the aqueous extract of brinjal fruits and dialyzed fractions demonstrated polyphenol oxidase activity and its characteristic physicochemical properties. It was found that concentration of PPO in local long green-bili badnea is more and about twice,as that of found in Gundu badne-purple.This study is helpful to understand the browning properties of two brinjal varieties.

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