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EXTRACTION, CHARACTERIZATION AND INHIBITION OF POLYPHENOL OXIDASE IN *SOLANUM MELONGENA*

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Keywords:

Polyphenol oxidase, *Solanum melongena*, Phenol, FTIR, Enzymatic Browning, Antioxidants

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ABSTRACT: Enzymatic browning is a process that occurs in fruits and vegetables by the enzyme polyphenol oxidase, which results in brown pigments reduce shelf life which indirectly causes huge domestic loss to the farmers during the post-harvesting process. In this context, the present study was carried out to preserve and increase the shelf life of Brinjal vegetables from enzymatic browning using ascorbic acid. The seeds of Brinjal hybrid and wild were treated with (0.5-2%) concentrations of Ascorbic acid; after 21 days of treatment, the plant material was extracted, Partially purified, and analyzed for enzyme activity. The polyphenol oxidase was extracted and determined for enzyme activity, phenol content, protein concentration as well as functional group determination using FTIR analysis. The polyphenol oxidase activity decreased with an increase in ascorbic acid concentration. The study revealed that the protein content in the Brinjal hybrid variety had a maximum of 221 µg/ml, whereas the wild brinjal variety possesses 180 µg /ml. The phenolic content of the hybrid Brinjal variety was found to be 67 µg/ml, whereas the wild brinjal variety showed 54 µg /ml. The result of this study Suggested that Ascorbic acid can be used as novel alternative inhibitors.

INTRODUCTION: Fruit and vegetables have a high content of fiber, vitamins, antioxidant compounds, which benefits the health of the consumers. However, changes in the antioxidant compounds may occur during harvesting, storage (fresh-cut fruits) of these fruits. These changes induce a pronounced loss of the antioxidant qualities and microbiological qualities [1] (Lindley, 1998). The major group of browning reactions is enzymatic browning which is a process that involves enzymatic action and is considered as an indicator of quality loss responsible for many fresh and processed fruits and vegetables such as dates, bananas, apricot, and potato.

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This process occurs naturally due to the action of the enzyme polyphenol oxidase (PPO) in the presence of oxygen on phenolic compounds and resulting in a brown compound called o-Quinones. During storage, o-Quinones polymerize nonenzymatically to produce heterogeneous deep dark polymers called melanin [2].

Thus, in the food industry, preservation against oxidation in food during storage and processing has become an increasing priority. Enzymatic browning is the main oxidative reaction. They involve two oxidoreductase enzymes: polyphenol oxidase (PPO) and peroxidase (POD) [3]. A broad range of plant species have significant PPO activity (Mayer, 2006), and many show differential expression in cells and tissues. Polyphenol oxidase (PPO) (monophenol, dihydroxy-L-pheny1alanine: oxygen oxido-reductase EC 1.14.18.1) is a copper-containing oxidoreductases enzyme that catalyzes the hydroxylation of monophenols to o-diphenols to o-quinones [4]. PPO enzyme is found in all living

organisms, including plants in the chloroplast, animals as well as microorganisms. In plants, it is involved in defense mechanisms. When a plant gets an injury or incision in the presence of oxygen, certain phenolic compounds are oxidized by the PPO enzyme to form a polymeric structure that prevents microbial contamination [5]. Because of the wide commercial applications of PPO in various fields and the development of more effective methods to prevent enzymatic browning, the properties of PPO, preservation conditions from its various sources need to be studied. The objective of our study was to extract and characterize polyphenol oxidase from eggplant and inhibition of PPO activity.

Brinjal (*Solanum melongena*), also known as eggplant, guinea squash, or aubergine is a warm-weather crop mostly cultivated in tropical and subtropical regions of the world. It is native to the "Indo-Chinese origin. It is an economic flowering plant and is one of the species of the Solanaceae or nightshade family, and it is included under the genus Solanum covering more than 1550 species *Solanum melongena* grown for its often purple edible fruit [6-7] and the contents of nutrients has lower carbohydrate and lipid content of 11.77 ± 1.55% and 1.65 ± 0.62% respectively were found in *Solanum melongena*.

Brinjal or eggplant (*Solanum melongena* L.) is a widely grown vegetable in South and South-East Asian countries. He also mentioned mineral contents like calcium, iron, sodium, and nitrogen were higher in the round variety of *Solanum melongena*, while magnesium, phosphorous, potassium was higher in the oval variety. The zinc contents were the same in both varieties. It is rich in total water-soluble sugars, free reducing sugars, and proteins, and it is a good source of minerals and vitamins [8].

During food processing, appropriate measures will prevent the process of browning in fruits. Many methods of polyphenol oxidase inhibition are known, such as sulfur dioxide, heating and sulfites, sodium chloride, exclusion of oxygen, boric acid, methylation of phenolase substrates, acids, and borates [9]. In general, their action is to decrease the rate of enzymic browning by lowering the tissue pH. The optimum pH of PPO lies within the range [6-7]; below pH 3, PPO activity is inhibited.

Ascorbic acid is the most significant inhibitor of the PPO enzyme because, in addition to its vitamin value, it has no corrosive action upon metals and no detectable flavour at the concentration used, which would interfere with the acceptability of the final processed product [10].

MATERIALS AND METHODS:

Sample Collection: The seeds of brinjal (wild and hybrid variety) were collected from the company National Seeds Corporations Ltd, Hebbal, Bangalore. Two varieties of fresh brinjal local variety commonly called brinjal PPL Hybrid (Pusa purple long seeds). Sample-1 shiny dark Purple colored, thin and long. Sample 2-brinjal Shyamal wild variety purple-colored oval-shaped.

Pretreatment with Ascorbic Acid: The seeds of brinjal (hybrid and wild variety) were surface sterilized by immersion in 0.5% sodium hypochlorite (NaOCl) solution for 5 min to prevent fungal infections and then washed three times with sterile, distilled water to remove any NaOCl residue. After washing, the seeds were pretreated with ascorbic acid by soaking for 24 h at room temperature in an ascorbic acid solution (0.5%, 1%, 1.5%, 2%). Seeds pretreated with distilled water was taken as control [10].

Collection and Processing of Soil Sample: The soil was collected from the farmyard of Rampura, Bangalore. The soil sample was sieved to remove root, pebbles, plant debris. The seeds of brinjal (wild and hybrid variety) were sown on various pots. After 30 days, the grown brinjal plant materials were collected.

Extraction of Polyphenol Oxidase Enzyme: Ten grams of plant material were homogenized in 80 mL of 0.1 M sodium phosphate buffer (pH = 7.0) containing 10 mM ascorbic acid and 0.5% polyvinyl-pyrrolidone and extracted with the aid of a magnetic stirrer for 1 h. The crude extract samples were centrifuged at 4000 rpm for 20 min. Pellet was discarded, and the supernatant was collected and used as crude enzyme extract [11].

Partial Purification: Solid $(NH_4)2SO_4$ was added to the supernatant to obtain 80% saturation (40 Celsius). After 1 h, the precipitated proteins were separated by centrifugation at 4000 rpm for 20 min. The precipitate was dissolved in a small amount of

50 mM phosphate buffer (pH 7.0) dialyzed in a cellulose bag at 40 Celsius in the same buffer for 24 hrs. The dialyzed extract was used as a partially purified PPO enzyme source in the subsequent experiments [11].

Determination of Enzyme Activity: PPO activity in two varieties of brinjal was followed by recording the absorbance increase of 3 mL reaction mixture at 420 nm using a UV/Vis spectro-photometer.

The 0.1 mL partially purified PPO enzyme extract was added to 2.9 mL of 10 mM catechol substrate prepared in 0.1 M KH_2PO_4 buffer (pH 6.8) at room temperature. 2.9 ml of 10 mM catechol substrate was taken as blank. Absorbance measurement was immediately started after adding enzyme solution. The alteration in absorbance for 5 min was monitored. One unit of PPO enzyme activity was described as the amount of enzyme that causes a change in absorbance of 0.001 per minute [12].

Estimation of Total Phenolic Content: The total phenolic content was determined using the Folin-Ciocalteu colorimetric method. Resorcinol (0.1 mg/ml) was used as a standard for the construction of a protein standard curve [13].

Determination of Protein Concentration: Protein concentrations were determined following the Lowry method. Bovine serum albumin (0.5 mg/ml) was used as a standard for the construction of a protein standard curve.

Characterization of PPO Enzyme:

Optimization of the Effect of pH on PPO Activity: The effect of pH on two varieties of brinjal for PPO activity was determined. The enzyme activity was measured at different pH (phosphate buffer of pH 4 to pH 8) using 3 ml mixture (0.2 ml enzyme, 0.5 ml catechol. 2.3 ml phosphate buffer).

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

Optimization of the Effect of Metallic Ions on PPO Activity: The effects of seven metal ions ($CuSO_4$, $MgSO_4$, EDTA, $BaCl_2$, NaCl) and the effect of EDTA, was evaluated on two varieties - PPO activity, using 3 ml reaction mixture (0.2 ml enzyme, 0.5 ml catechol, 2.3 ml phosphate buffer, 10 mM metal ions). The change in absorbance was measured Spectrophotometrically at 420 nm.

Fourier Transform- Infrared Spectroscopy (FT-IR) analysis: Partially purified PPO enzyme extract was dried in a Hot air oven. The dry sample (0.1 g) was used for the FT-IR analysis [14].

GCMS Analysis: The GC – MS analysis was carried out using a Clarus 500 Perkin – Elmer (Auto system XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perkin Elmer Turbo mass 5.2 spectrometers with an Elite – 5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30 m × 0.25 µm DF of capillary column.

The instrument was set to an initial temperature of 110 ºC and maintained at this temperature for 2 min. At the end of this period, the oven temperature was rose up to 280 °C, at the rate of an increase of 5 ºC/min, and maintained for 9 min.

Injection port temperature was ensured as 200 ºC and Helium flow rate as one ml/min. The ionization voltage was 70eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45-450 (m/z) [15].

RESULTS AND DISCUSSION: Brinjal PPL seeds (Hybrid variety) were treated with different concentrations of ascorbic acid (0.5%, 1%, 1.5%, 2%). The treated seeds were subjected to grown for 30 days time interval and extracted the PPO content.

The extract was screened for enzyme activity and further characterized for the optimization of parameters such as pH, temperature, and metal ions. Treated seeds were sown in soil and grown for 30 days **Fig. 1**.

At the end of the 30th day, the plants which were treated with 0.5%, 1%, 1.5%, 2% **Fig. 2** showed almost the same growth pattern but slightly less growth response when compared to control plants

CONTROL 0.5% 1% 1.5% 2%

FIG. 1: ASCORBIC ACID TREATMENT OF BRINJAL SEEDS FOR INHIBITION OF PPO ENZYME ACTIVITY

CONTROL 0.5% 1% 1.5% 2%

FIG. 2: ASCORBIC ACID TREATED BRINJAL PPL (HYBRID) PLANT GROWTH ON 30th DAY

Polyphenol Oxidase Activity: Polyphenol oxidase activity was decreased with the increase of ascorbic acid concentration **Table 1**. The higher the ascorbic acid concentration, the lower was the phenolase activity when compared with control.

TABLE 1: POLYPHENOL OXIDASE ACTIVITY OF BRINJAL WITH DIFFERENT ASCORBIC ACID CONCENTRATION

S. no	Ascorbic acid percentage	% PPO inhibition	
		Brinjal wild	Brinjal hybrid
1	0.5%	0.6%	Nil
2	1%	8.6%	36.42%
3	1.5%	37.93%	82.85%
4	2%	58.62%	90.6%

Ingraham, 1956; Schultz, 1959; Eskin *et al.,* (1971) reported Ascorbic acid itself is not an inhibitor for polyphenol oxidase; it must be oxidized indirectly by the enzyme before it can inhibit the enzyme activity. Therefore, if sufficient ascorbic acid is present, polyphenol oxidase will oxidize its natural substrate and the oxidation product will be immediately reduced by ascorbic acid. Byan *et al.*, 2012, reported 0.5% ascorbic acid lowered phenolase activity to one-fourth after 3 h, whereas 15% and 10% of phenolase activity was found to be inhibited after using 1% and 1.5% ascorbic acid, respectively. This means that 1 to 1.5% ascorbic acid is very effective in considerably reducing enzyme browning in apple slices. Whereas in the present study, 0.5% showed slightly 0.6% PPO inhibition in brinjal wild variety and no inhibition of PPO in brinjal hybrid variety. At 1% ascorbic acid concentration, 8% of PPO inhibition was found in the brinjal wild variety, whereas up to 36% of PPO inhibition was found in the brinjal hybrid variety. At 1.5% ascorbic acid concentration, up to 38% of PPO inhibition was found in brinjal wild, and up to 82% of PPO inhibition was found in brinjal hybrid. At 2% ascorbic acid concentration, up to 59% of PPO inhibition was found in brinjal wild, and up to 90% inhibition was found in brinjal hybrid.

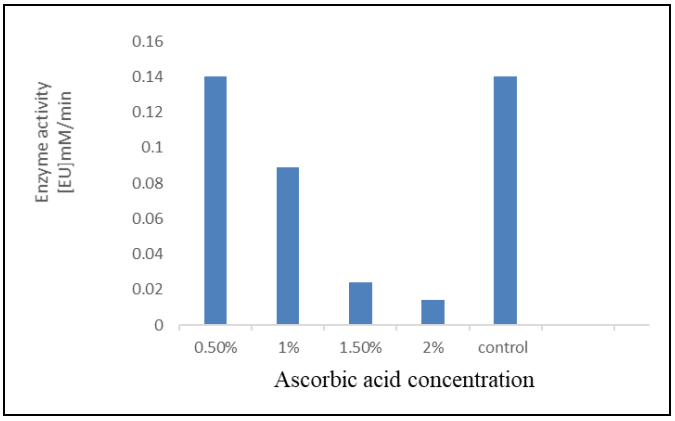


FIG. 4: INHIBITION OF POLYPHENOL OXIDASE ACTIVITY FROM BRINJAL PPL (HYBRID) VARIETY USING ASCORBIC ACID

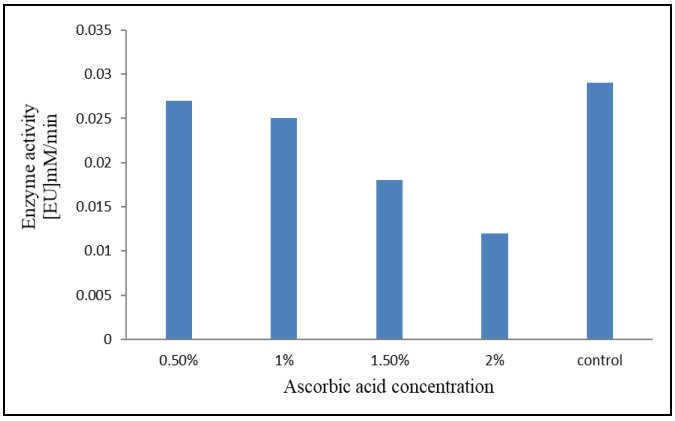


FIG. 5: INHIBITION OF POLYPHENOL OXIDASE ACTIVITY FROM BRINJAL SHYAMAL (WILD) VARIETY USING ASCORBIC ACID

The PPO inhibition pattern in brinjal hybrid and brinjal wild showed variation, but PPO activity in both the variety gradually decreased as ascorbic acid concentration increased. 2% ascorbic acid concentration reduced PPO activity up to 90% in brinjal hybrid and 60% in brinjal wild variety therefore, 2% ascorbic acid is considered effective when compared to control and 1%, 2% ascorbic acid concentration **Fig. 4** & **5**.

Protein Estimation: The results of total protein content in ascorbic acid-treated brinjal plant and control showed that ascorbic acid-treated brinjal plants had lower protein content as compared to the control sample **Table 2**.

The Brinjal hybrid variety had maximum protein content (221 µg/ml) followed by brinjal wild (180 µg/ml). The minimum amount of phenolics was recorded in the 2% ascorbic acid-treated brinjal sample hybrid (128 µg/ml) and wild (112 µg/ml), respectively.

TABLE 2: ESTIMATION OF PROTEIN CONCENTRATION OF BRINJAL

S. no	Brinjal variety	The concentration of ascorbic acid used	Protein estimated in µg/ml
1	Brinjal PPL (hybrid) [sample 1]	0.5%	218
		1%	203
		1.5%	185
		2%	128
		Control	221
2	Brinjal Shyamal (wild) [sample 2]	0.5%	168
		1%	143
		1.5%	122
		2%	112
		control	180

Phenolic Estimation: The total phenolics content in ascorbic acid-treated brinjal plant using control, showed that the ascorbic acid-treated brinjal plants had lower phenolic content as compared to the control sample **Table 3**. The Brinjal hybrid variety had maximum phenolic content (67 µg/ml) followed by brinjal wild (54 µg/ml). The minimum amount of phenolics was recorded in the 2% ascorbic acid-treated brinjal sample hybrid (19 µg /ml) and wild (23 µg/ml), respectively.

TABLE 3: ESTIMATION OF TOTAL PHENOLIC CONTENT FROM ASCORBIC ACID-TREATED BRINJAL PLANTS

Sl no	Brinjal variety	Concentration of ascorbic acid	Phenolic content in µg/ml
1	Brinjal PPL (Hybrid) [sample 1]	0.5%	65
		1%	49
		1.5%	30
		2%	19
		control	67
2	Brinjal shyamal (wild) [sample 2]	0.5%	49
		1%	42
		1.5%	37
		2%	23
		control	54

Effect of Temperature: Brinjal plants (hybrid) PPO activity, which was treated with ascorbic acid **Fig. 6**, peaked at 40 for catechol, and above 70 the PP0 activity was gradually decreased. Polyphenoloxidase in eggplant (*Solanum melongena* L.) is completely inactivated after heat treatment at 75 for 30 min or 80 for 5 min (Fujita and Tono, 1988). Effect of temperature on partially purified polyphenol oxidase enzyme from brinjal plants variety (hybrid) which was treated with ascorbic acid shows optimum activity at temperature 40 same as the standard (control) polyphenol oxidase enzyme characteristic hence the study reveals

ascorbic acid doesn't change the character of PPO but only inhibits its activity.

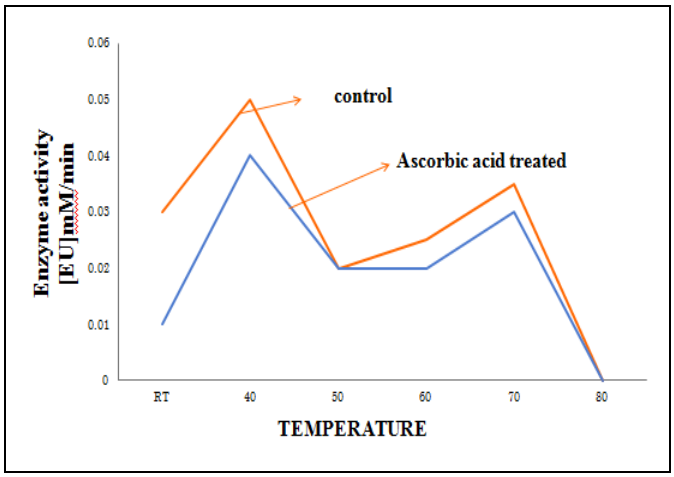


FIG. 6: EFFECT OF TEMPERATURE ON ASCORBIC ACID-TREATED POLYPHENOL OXIDASE ENZYME ACTIVITY FROM BRINJAL PLANT (HYBRID)

Brinjal plants (wild) PPO activity, which was treated with ascorbic acid **Fig. 7**, peaked at 40 for catechol, and above 60 the PPO activity was gradually decreased. Polyphenoloxidase in eggplant (*Solanum melongena* L.) is completely inactivated after heat treatment at 75 for 30 min or 80 for 5 min (Fujita and Tono, 1988).

Effect of temperature on partially purified polyphenol oxidase enzyme from brinjal plants variety (wild) which was treated with ascorbic acid shows optimum activity at temperature 40 same as the standard (control) polyphenol oxidase enzyme characteristic; hence the study reveals ascorbic acid doesn't change the character of PPO but only inhibits its activity.

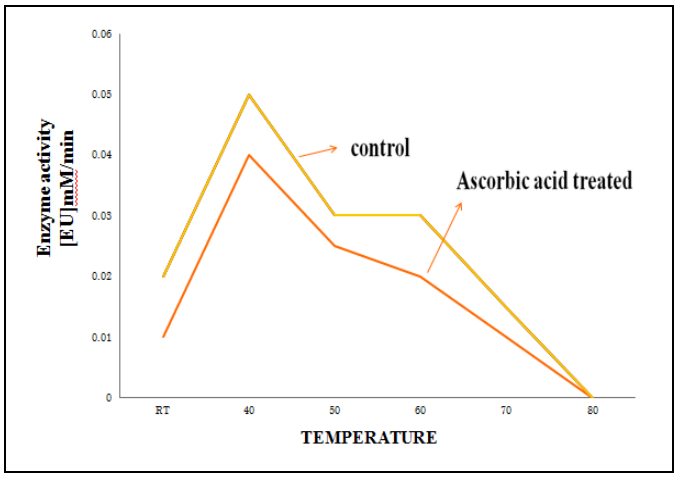


FIG. 7: EFFECT OF TEMPERATURE ON ASCORBIC ACID-TREATED POLYPHENOL OXIDASE ENZYME ACTIVITY FROM BRINJAL PLANT (WILD)

Effect of pH: The effect of pH on the polyphenol oxidase activity was found that below pH 3 the phenolase activity is very weak, while it has a considerable activity within the range of pH 5-7 **Fig. 8**. This is in agreement with Schultz (1959), who found that levels of pH below 2.5 to 2.7 were very suitable for the inactivation of enzyme browning in apple. Analysis on the pH stability test, the enzymatic activity of PPO extracts was determined. According to the analysis results, the PPO activity was nearly stable in the neutral condition. But the enzymatic activity of PPO extracts was decline near acidic and alkaline pH conditions.

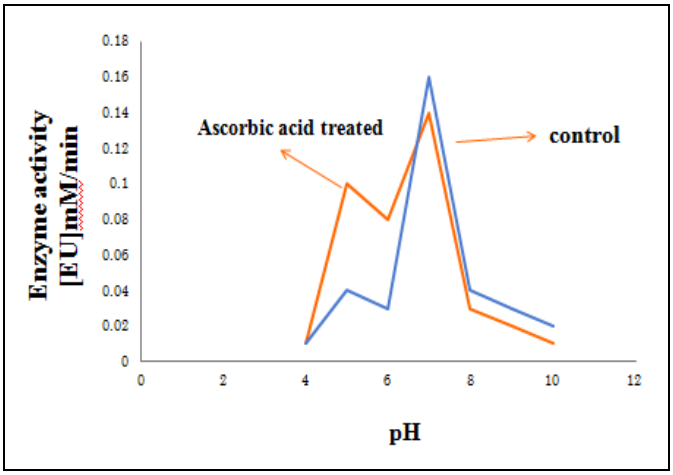


FIG. 8: EFFECT OF PH ON ASCORBIC ACID-TREATED POLYPHENOL OXIDASE ENZYME ACTIVITY FROM BRINJAL (WILD) PLANTS

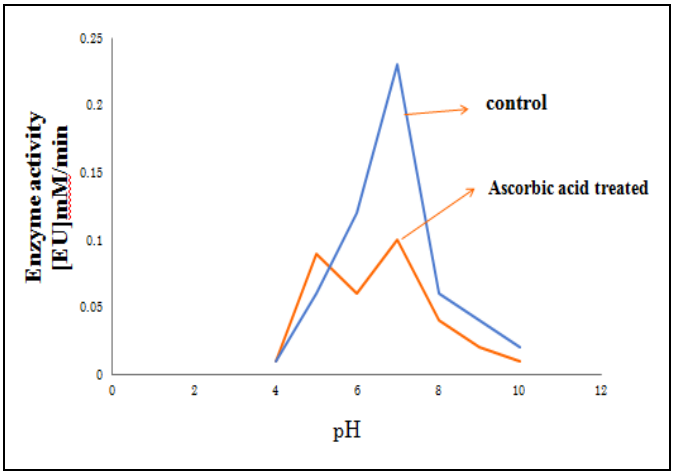


FIG. 9: EFFECT OF PH ON ASCORBIC ACID-TREATED POLYPHENOL OXIDASE ENZYME ACTIVITY FROM BRINJAL (HYBRID) PLANTS

The activity of PPO treated with ascorbic acid was nearly stable under pH 7.0. But above pH 7.0, the enzyme activity showed that the activity dropped over 50% loss and the enzyme activity lost about

70% between the pH ranges 8.0 – 10. The optimum pH for polyphenol oxidase activity was found to be 7. Effect of pH on partially purified polyphenol oxidase enzyme from brinjal plant (hybrid) **Fig. 9,** which was treated with ascorbic acid, shows optimum activity at pH 7 same as the standard polyphenol oxidase enzyme characteristic; hence the study reveals ascorbic acid doesn't change the characteristics of PPO but only inhibits its activity.

Effect of Metal Ions: In our study, we evaluated the effects of five metal ions ($Mgso_4$, $CuSo_4$, EDTA, $BaCl_2$, NaCl) on the PPO enzyme extracted from ascorbic acid-treated brinjal plants (hybrid) **Fig. 10** and control **Fig. 11**.

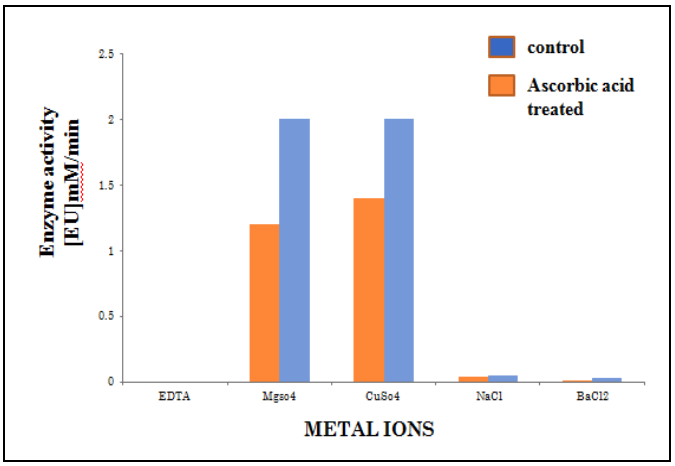


FIG. 10: EFFECT OF METAL IONS ON ASCORBIC ACID-TREATED POLYPHENOL OXIDASE ENZYME ACTIVITY IN BRINJAL (HYBRID) PLANTS

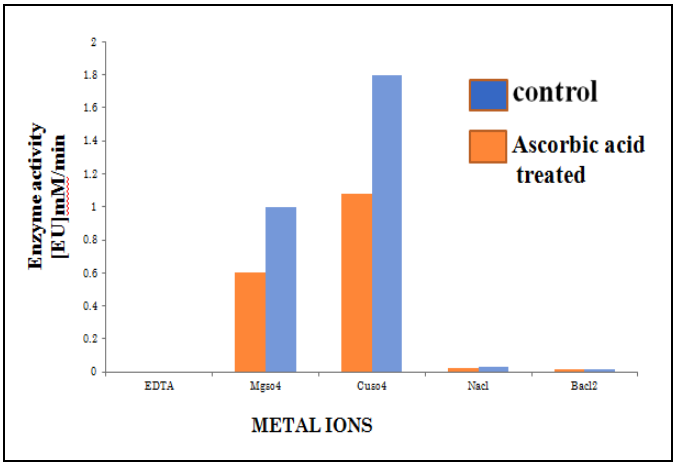


FIGURE 11: EFFECT OF METAL IONS ON ASCORBIC ACID-TREATED POLYPHENOL OXIDASE ENZYME ACTIVITY IN BRINJAL (WILD) PLANTS

The results indicated that PPO is a copper-containing enzyme, copper sulfate, and magnesium sulfate (10 Mm) showed increased PPO activity, whereas the EDTA, NaCl, $Bacl_2$ showed inhibitory effects on the activity of PPO. Latha *et al.*, 2013 evaluated the effects of seven metal ions ($Mgso_4$, $CuSo_4$, KCl, $BaCl_2$, NaCl) on PPO activity. The results indicated that PPO is a copper-containing enzyme; copper sulfate and zinc sulfate (10 mM) serves as an activator for its activity. EDTA (10 mM) showed inhibitory effects on the activity of PPO. Thus, the study reveals ascorbic acid does not change the characteristics of the effect of metal ions pattern on ascorbic acid-treated PPO but only inhibits its activity.

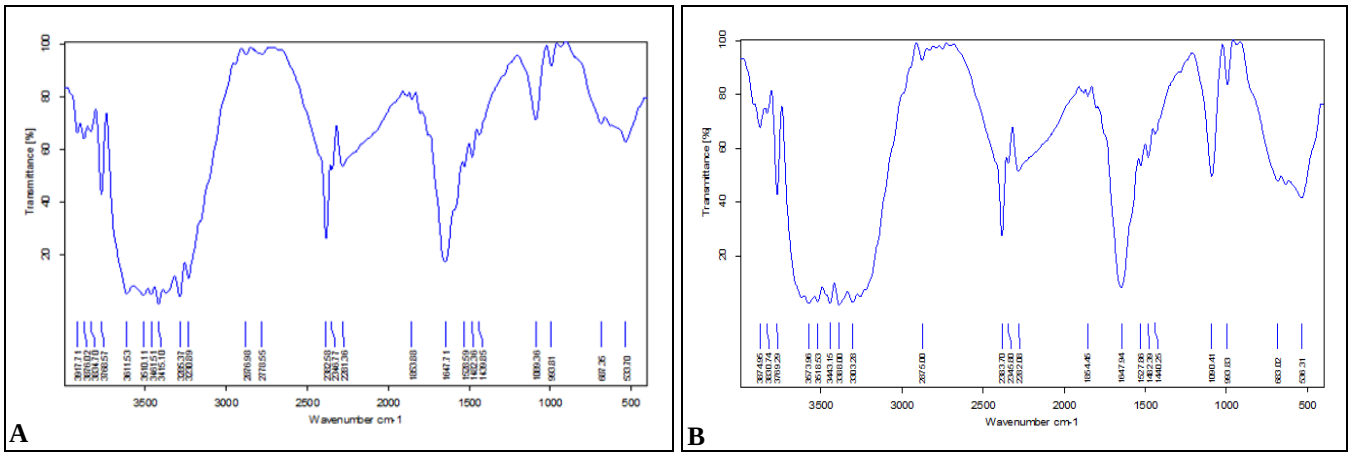


FIG. 12: FTIR ANALYSIS OF POLYPHENOL OXIDASE ENZYME (A) BRINJAL HYBRID CONTROL PLANT, (B) ASCORBIC ACID-TREATED BRINJAL HYBRID PLANT

FTIR: The FTIR spectrum of PPO enzyme extracted from brinjal (hybrid) **Fig. 12** plants control was determined with characteristic stretching vibration amide I, amide II, and amide III absorption at 1647.71 cm^{-1}, 1528.59 $cm^{-1,}$ and 1439.85 cm^{-1}. The specific N-H and C-H bending bands of PPO that was purified from the brinjal were recorded at 3230.89 cm-1 and 2876.98 cm^{-1}, respectively.

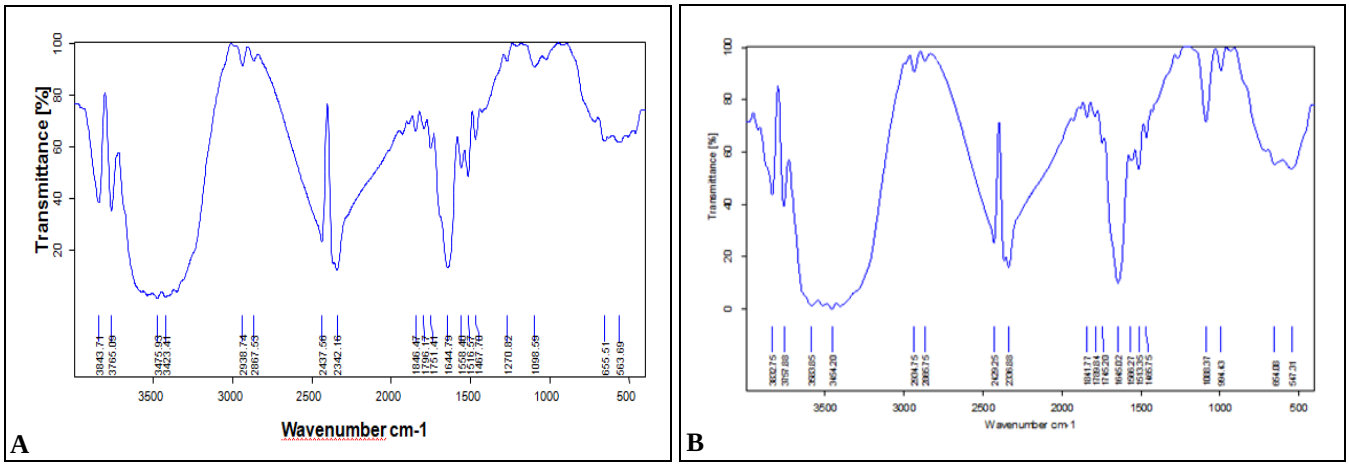


FIG. 13: FTIR ANALYSIS OF POLYPHENOL OXIDASE ENZYME (A) BRINJAL WILD PLANT SAMPLE, (B) ASCORBIC ACID-TREATED BRINJAL WILD PLANT

The FTIR spectrum of PPO enzyme extracted from ascorbic acid-treated brinjal (hybrid) plant sample was determined with characteristic stretching vibration amide I, amide II, and amide III absorption at 1647.94 cm^{-1}, 1527.96 cm^{-1}, 1440.25 cm^{-1}. The specific N-H and C-H bending bands of PPO that was purified from brinjal were recorded at 3330.28 cm^{-1} and 2875.00 cm^{-1}, respectively. Hatice aysun, *et al.*, 2015 worked on FTIR analysis of PPO enzyme and the studies revealed PPO has characteristic peaks, which include N–H vibration at 3198.95 cm^{-1} and C–H vibration at 2962.2 cm^{-1}. The spectrum shows stretching vibration of amide I, amid II, amid III adsorption bands at 1656.6 cm^{-1}, 1544.7 cm^{-1} and 1315.2 cm^{-1}, respectively. The FTIR spectrum of PPO enzyme extracted from brinjal (wild) **Fig. 13** plants control was determined with characteristic stretching vibration amide I, amide II & amide III absorption at 1644.79 cm^{-1}, 1558.40 cm^{-1} & 1270.82 cm^{-1}. The specific N-H and C-H bending bands of PPO that was purified from brinjal was recorded at 3423.41 cm^{-1} and 2867.3 cm^{-1}, respectively.

The FTIR spectrum of PPO enzyme extracted from ascorbic acid-treated brinjal (wild) plant was determined with characteristic stretching vibration amide I, amide II & amide III absorption at 1647.94 cm^{-1}, 1527.96 cm^{-1} & 1090.41 cm^{-1}.

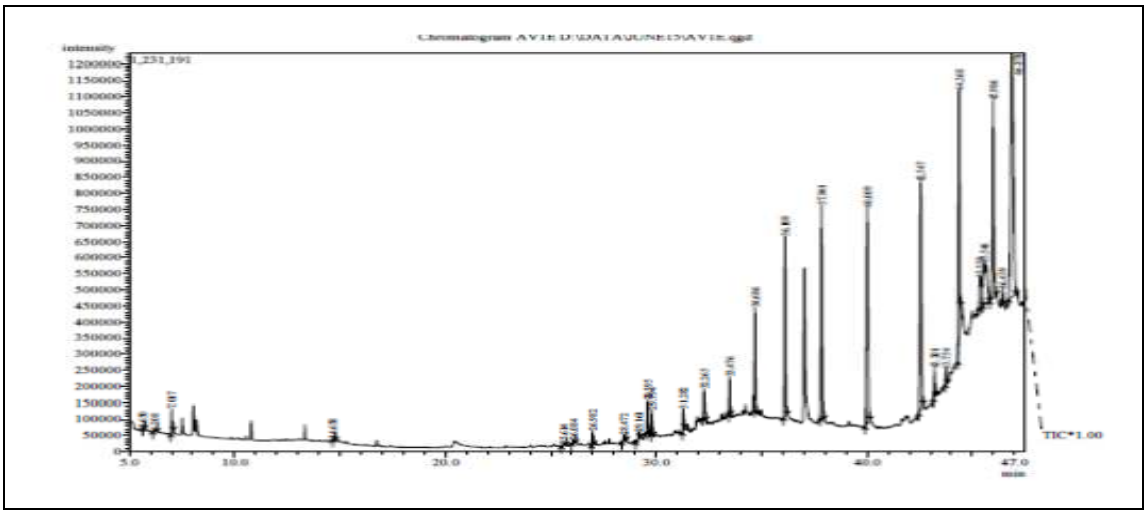
The specific N-H and C-H bending bands of PPO that was purified from brinjal were recorded at 3443.15 cm^{-1} and 2875.00 cm^{-1}, respectively. It was clear that wavenumbers of the characteristic peaks in the FTIR spectrum of PPO were shifted to higher/lower wavelengths. This is because of the presence of some impurities in the enzyme extract.

GCMS Analysis: GCMS analysis revealed the presence of 25 compounds in the extracts of *S. melongena* with their retention time and by interpretation of their mass spectra. The extract of *S. melongena* shows twenty-five peaks **Fig. 2**. Among these, there are seven major peaks that indicating the presence of seven major phytochemical constituents.

TABLE 3: PHYTOCOMPONENTS IDENTIFIED IN THE EXTRACTS OF *SOLANUM MELONGENA* BY GC-MS

S. no.	RT	Compound name	Molecular formula	Molecular weight	Peak area (%)
1	5.640	Benzeneethanamine	$C_{23}H_{24}FNO_3$	381	0.00
2	6.206	Methylbenzeneethanamine	$C_9H_{13}N$	135	0.05
3	7.014	Methoxy, Phenyl- ,Oxime	$C_8H_9NO_2$	151	1.21
4	14.638	N-Ethylformamide	C_3H_7NO	73	0.04
5	25.614	Propane, 2-methoxy-2-methyl	$C_5H_{12}O$	88	0.08
6	26.074	4-Undecene	$C_{12}H_{24}$	168	0.17
7	26.972	Neophytadiene	C_20H_{38}	278	0.74
8	28.462	1-Hexanol	$C_{10}H_{22}O$	158	0.34
9	29.151	2-Isopropyl-5-methyl-1-heptanol	$C_{11}H_{24}O$	172	0.33
10	29.585	Hexadecanoic acid	$C_{18}H_{36}O_2$	284	1.61
11	29.764	4- (3,5-Di-tert-butyl-4-hydroxyphenyl) butyl acrylate	$C_{21}H_{32}O_3$	332	1.25

12	31.282	3,7,11,15-Tetramethylhexadec-2-en-1-ol	$C_{20}H_{40}O$	296	1.09
13	32.255	Palmitic acid	$C_{18}H_{36}O_2$	284	1.45
14	33.466	Tetratetracontane	$C_{44}H_{90}$	618	1.86
15	34.676	Eicosane	$C_{20}H_{42}$	282	5.25
16	36.107	n-Hexatriacontane	$C_{36}H_{74}$	506	9.17
17	37.831	Dotiacontane	$C_{32}H_{66}$	450	10.80
18	40.008	Tetrapentacontane	$C_{54}H_{110}$	758	11.05
19	42.546	N-Tetratetracontane	C_44H_{90}	618	11.50
20	43.101	Squalene	$C_{30}H_{50}$	410	1.36
21	43.654	n-Tetracosane	$C_{24}H_{50}$	562	0.67
22	44.268	Tetracontane	$C_{40}H_{82}$	562	12.54
23	45.258	Hexacontane	$C_{60}H_{122}$	842	1.48
24	45.446	1,54-Dibromotetrapentacontane	$C_{54}H_{108}Br_2$	914	2.12
25	45.886	Tetrapentacontane	$C_{54}H_{110}$	758	10.11



PHYTOCOMPONENTS IDENTIFIED FROM ETHANOLIC EXTRACTS OF *SOLANUM MELONGENA*

CONCLUSION: The food industry still faces the major problem of how to prevent enzymatic browning while considering food safety, regulations, marketability of the treated products, and the cost associated with the prevention process. There are several inhibitors used by researchers to prevent enzymatic browning. The present work described the characteristics of the polyphenol-oxidases in brinjal plant variety (hybrid and wild) and the effect of ascorbic acid at different concentrations (0.5%, 1%, 1.5%, 2%) for inhibition of PPO enzyme.

Though several compounds are used as substrates for polyphenol oxidase, in this study, we selected the most commonly used substrates, such as catechol. Ammonium sulfate precipitated and partially purified dialyzed PPO enzyme extract of brinjal plant demonstrated polyphenol oxidase activity and its characteristic physicochemical properties**.** The findings revealed from both the brinjal variety (hybrid and wild), showed at 2% ascorbic acid concentration, up to 59% of PPO inhibition was found in brinjal wild, and up to 90% inhibition was found in brinjal hybrid. The PPO inhibition pattern in the brinjal hybrid and the brinjal wild showed variation, but PPO activity in both the variety gradually decreased as ascorbic acid concentration increased. 2% ascorbic acid concentration reduced PPO activity up to 90% in brinjal hybrid and 60% in brinjal wild variety; therefore, 2% ascorbic acid is considered effective when compared to control and 1%, 2% ascorbic acid concentration. To inhibit the activity of polyphenol oxidases in brinjal plants (hybrid and wild). The seeds of brinjal can be subjected to 2% ascorbic acid treatment.

Usually, brinjal plant processing in the food industry is a serious problem, it could be suitable for minimal processing if it possesses low PPO activity. The characterization of the PPO extracted from 2% ascorbic acid-treated plants showed optimal conditions like pH (7.0) and temperature

40 ºC for inhibition by ascorbic acid. This information could be useful in the production of minimally processed eggplant in the food industry. Ascorbic was found to be the most suitable organic acid to increase the quality and shelf life of eggplant because it was effective at 2% concentration, and it is unlikely to affect sensory parameters and, at the same time, may increase the product's nutritional value with low additional cost.

The PPO activity was inhibited by ascorbic acid treatment. Thus, ascorbic acid treatment can be implemented during the processing of eggplants fruits to prevent browning. However, a more proper procedure should be proposed to meet the growing consumer demand and acquire wider applications in the inhibition of PPOs. The result of this study showed that ascorbic acid was a novel alternative inhibitor to synthetic PPO inhibitors that could be utilized in various areas such as the food industry, health care & medicine to cure skin disorders.

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