CHAPTER 2

Comparative Evaluation of PPO Activity in Different Brinjal Cultivars
2.1. Introduction

The polyphenol oxidase (PPO) enzyme in plants oxidizes phenolics in the presence of oxygen on cut surface of fruits and vegetables, producing quinones which autopolymerise to form brown colour pigments (Madinez and Whitaker, 1995). During physical cutting, disruption of cellular structure leads to the release of PPO enzyme and its phenolic substrate. Many cultivars of brinjal (*Solanum melogena*) are available with varying morphological features such as colours (purple, green, purple with white and green stripes and patches), shapes (ovoid, obovate, oblong, cylindrical, club shaped), and calyx (spiny, non-spiny) (Raigón et al., 2008). These cultivars differ in the extent of post-cut browning which could be due to the variations in PPO activity or level of soluble phenolics. There are a few reports on characterization of PPO from brinjal. Roudsaria et al. (1981) reported purification of PPO using chromatography. Pérez-Gilabert and Carmona (2000) and Doğan et al. (2002) have characterized the ammonium sulfate precipitated PPO from brinjal. Concellón et al. (2004) have also reported the PPO activity of the crude extract during low temperature storage of brinjal. There are also reports about the phenolic compounds present in brinjal with chlorogenic acid as the major component (Luthria et al., 2010). In the current study, comparative evaluation of PPO enzyme activity, substrate status, and the overall browning index in different brinjal cultivars have been performed. The findings will help in understanding the contribution of these factors in post-cut browning process in fresh as well as stored brinjal.
2.2. Materials and methods

2.2.1. Chemicals

Agarose, ammonium sulfate, ascorbic acid, Bradford reagent, cetyl trimethyl ammonium bromide, chloroform, chlorogenic acid, disodium hydrogen phosphate, Folin-Ciocalteu reagent, gallic acid, isoamyl alcohol, 4-methyl catechol, sodium dihydrogen phosphate, polyvinyl pyrrolidone (PVP), polyvinyl polypyrrolidone (PVPP), sodium carbonate, and triton X-100 were procured from Sigma-Aldrich Inc., St. Louis, MO.

2.2.2. Brinjal cultivars

Eight major popular cultivars including ‘Pusa purple long’, ‘Ravaiya’, ‘Azad kranti’, ‘Arka navneet’, ‘Kalpatharu’, ‘Raveena’, ‘Anupam’, ‘Silki’ (listed in Table 2.1) of brinjal (Solanum melongena) were procured from a local supplier (4 kg of each variety). The vegetables were cleaned in potable water and stored at ambient (26 ±2 ºC) as well as 10 ºC temperature and 62% humidity. The samples were analysed periodically for browning index, PPO activity, and total soluble phenolics.

2.2.3. Determination of extent of browning

For browning measurement of cut brinjal, reflectance in visible spectrum region (360 to 780 nm) was recorded at 10 nm wave length interval using a Minolta CM-3600D spectrophotometer (Konica Minolta Sensing, Inc., Osaka, Japan). D65 lamp was used as reference light source and the detector was fixed at an angle of 10º with respect to the light source (Ramirez-Jiménez et al., 2000). The equipment was calibrated prior to analysis with a standard white tile and a black box for 100 and 0% reflectance,
respectively. The colour parameter used was CIE L (Lightness) which denotes the amount of light or luminance reflected from the sample. The browning/darkening was calculated as (100-L), which is opposite of lightness (Ramírez-Jiménez et al., 2000).

2.2.4. Extraction of PPO

Cut brinjal pieces (30 g) were frozen in liquid N\textsubscript{2}, ground to fine powder and homogenized in 100 ml of extraction solution for better extractability using a polytron homogenizer (Model PT3100, Kinematica AG, Switzerland). The extraction solution contained sodium phosphate buffer (pH 6.8, 0.05 M) with polyvinyl pyrrolidone (PVP) (1%), polyvinyl polypyrrolidone (PVPP) (2%), triton X-100 (1%) and ascorbic acid (30 mM). The extract was stirred for 30 min, filtered using muslin cloth, and centrifuged (5810R, Eppendorf, Hamburg, Germany) at 10000 g for 15 min at 4\textdegree C. The supernatant was collected and fractionated using ammonium sulfate precipitation (up to 80%). Initially, to remove most hydrophobic proteins, 20% ammonium sulfate precipitation was performed. Later, the supernatant of this fraction was saturated upto 80% ammonium sulfate and precipitated. This precipitate represented the total PPO. Based on earlier findings supernatant of 20% fraction was also parallely fractionated using 10% sequential increase of ammonium sulfate which resulted in precipitation of two isoforms of PPO called PPO 1 (precipitated at 30% ammonium sulfate fractionation) and PPO 2 (precipitated at 70% ammonium sulfate fractionation) (Englard and Seifter, 1990). The individual fractions were solubilized in 10 ml of phosphate buffer (pH 6.8, 20 mM) and dialysed using 10 kDa cut off membrane in 3 litre phosphate buffer (pH 6.8, 2 mM) at 4\textdegree C with three buffer changes at 4 h interval for removal of salt.
2.2.5. Assay of protein and PPO activity

The polyphenol oxidase (PPO) enzyme activity was determined spectrophotometrically using 4-methyl catechol as a substrate (ConSELLón et al., 2004). The enzyme assay was carried out taking 0.88 ml of phosphate buffer (pH 6.8, 50 mM), 0.1 ml substrate (0.1M) and 0.02 ml of enzyme extract (prepared as discussed later). The increase in absorbance at 420 nm was monitored at 30 sec interval for 3 min using a spectrophotometer (Model UV 4-100, Unicam, Cambridge, UK) and the average change in absorbance per min was calculated. One unit of enzymatic activity was defined as the amount of enzyme which caused a change in absorbance of 0.1/min. The PPO activity was expressed as U/g of brinjal weight. The specific activity was determined by expressing PPO activity/mg protein. Protein content of the brinjal extract was determined by the Bradford method (1976), using bovine serum albumin (BSA, Sigma Chemical, St. Louis, USA) as standard.

2.2.6. Estimation of total soluble phenolics

Brinjal pieces (7.5 g) were soaked in 50 ml of 80% methanol and homogenized using a polytron homogenizer (Model PT3100, Kinematica AG, Switzerland). The suspension was centrifuged at 10,000 g for 20 min and the supernatant was collected. A 25 µl of aliquot of the supernatant was mixed with equal volume of milli Q water and further mixed with 50 µl of 0.2 N Folín-Ciocalteu reagent. The suspension was incubated at ambient temperature (26±2°C) for 15 min and later mixed with 0.15 ml of sodium carbonate solution (0.2 g/ml). The reaction mixture was incubated in a water bath at 40°C for 20 min. Later placed on ice for 5 min and then the absorbance was measured at 755 nm using a spectrophotometer (Model UV 4-100, Unicam, Cambridge, UK). The total
phenolic content was calculated using gallic acid as a standard and expressed as mg GA equivalent/g of brinjal (Luthria et al., 2010).

2.2.7. Estimation of chlorogenic acid and HPLC profile of other phenolics

Chlorogenic acid was estimated using HPLC by the method of Luthria et al. (2010). Brinjal (7.5 g) was soaked in 50 ml of 80% methanol and homogenized using a polytron homogenizer. The suspension was centrifuged at 10,000 g for 20 min, the supernatant was collected and membrane (0.2 µm) filtered. A 60 µl aliquot was loaded on a reverse phase C18 column. The elution was carried out using a gradient of formic acid (0.1%) from 90 to 55% over methanol for 26 min at a flow rate of 1 ml/min. The detection was performed at 350 nm using a UV detector. The chlorogenic acid concentration in brinjal was calculated using pure commercially available chlorogenic acid (retention time 16 min in specified mobile phase) as standard. The phenolics profile of cut brinjal before and after browning was performed using HPLC of 80% methanol extract and using a gradient of acetic acid (0.2%) from 100% to 0% over methanol for 30 min at flow rate of 1 ml/min and the detection was carried out at 285 nm.

2.2.8. Statistical analysis

Experiments were repeated in three sets independently, each set having 3 replicates. The means and SD were calculated taking all the readings into consideration. Two way ANOVA was performed to ascertain the significance of difference of the means. Statistical analysis was performed using BioStat 2009 Version Professional 5.8.0.0 (AnalystSoft Inc., Canada)
Table 2.1. The different brinjal cultivars studied and their characteristics.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Fruit characteristics*</th>
<th>Picture</th>
<th>Cultivar</th>
<th>Fruit characteristics</th>
<th>Picture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pusa purple long (V1)(^{xx})</td>
<td>Club shaped Purple (60 ± 20 g)</td>
<td><img src="image1" alt="Picture" /></td>
<td>Kalpatharu MEBH 10 (V5)(^{xx})</td>
<td>Ovoid reddish purple with white stripes, spiny calyx (50 ± 15 g)</td>
<td><img src="image2" alt="Picture" /></td>
</tr>
<tr>
<td>Ravaiya MEBH39 (V2)(^{xx})</td>
<td>Ovoid Purple (40 ± 10 g)</td>
<td><img src="image3" alt="Picture" /></td>
<td>Raveena (V6)(^{xx})</td>
<td>Cylindrical Green (60±15 g )</td>
<td><img src="image4" alt="Picture" /></td>
</tr>
<tr>
<td>Azad kranti (V3)(^{xx})</td>
<td>Club shaped Purple (300±50 g)</td>
<td><img src="image5" alt="Picture" /></td>
<td>MHB Anupam (V7)(^{xx})</td>
<td>Club shaped green (300±50 g)</td>
<td><img src="image6" alt="Picture" /></td>
</tr>
<tr>
<td>Arka navneet (V4)(^{xx})</td>
<td>Obovate Purple (400±100 g)</td>
<td><img src="image7" alt="Picture" /></td>
<td>Silki (V8)(^{xx})</td>
<td>Ovoid, spiny calyx purple with green stripes (40 ± 10 g)</td>
<td><img src="image8" alt="Picture" /></td>
</tr>
</tbody>
</table>

*Fruit calyx is without spines unless mentioned. Values in parenthesis indicate average weight per fruit ± standard deviation.

\(^{xx}\) Coding of different cultivars used for ease of description
3. Results and Discussion

The varieties of brinjal display a wide range of shapes and colours, starting from pure white to purple, black, green, and variegated in different shades (Hazra and Banerjee, 2005). The fruit characteristics (shape, colour, and average weight) and photographs of different brinjal cultivars used in this study are detailed in Table 2.1. The local names of these cultivars differ in different parts of India. However, the shape is considered as a very stable genotype dependent character and cultivars can be identified from the morphology. Among the cultivars, the ‘Kalpatharu’ is maximally grown and available in most parts of India. In India during summer and winter season the average shelf life is reported to be 1-2 and 3-4 days, respectively (Dhaliwal, 2007). Under ambient temperature and storage (26 ± 2 °C) and 80% relative humidity the shelf life is about 8-10 days. The vegetable with bigger size have higher shelf life compared to the smaller varieties.

2.3.1. Extent of browning varied among cultivars

Browning discolouration started immediately after cutting and increased with time. The measurement of browning after 20 min of cutting was found to be suitable for comparison among the cultivars and shown in Figure 2.1a. The ‘Kalpatharu’ (V5) showed maximum browning followed by other cultivars (V3, V4, V6, and V8) which showed similar level browning and the overall extent was about 11 % lesser than V5. The browning in ‘Ravaiya’ (V2) and ‘Anupam’ (V7) was found to be further less and difference among them was insignificant (P≤0.05). These two cultivars showed minimum browning and were about 39 and 35% less compared to V5, showing maximum browning. As PPO and its phenolic substrates have been reported as major factors responsible for post-cut
browning in various other fruits and vegetables, it was interesting to know their status in these brinjal cultivars too.

2.3.2. Cultivars showing maximum browning exhibited higher PPO activity

The maximum PPO activity was observed in brinjal cultivars ‘Kalpatharu’ (V5) and ‘Raveena’ (V6) followed by ‘Silki’ (V8) (Fig. 2.1b). The cultivar V2 showed lesser PPO activity, whereas, others (V1, V3, V4, V7) showed the least PPO activity. A significantly high PPO activity was observed in two discrete ammonium sulfate precipitated fractions (20-30% and 50-70%) compared to other fractions (P≤0.05) and could be due to the presence of two isoforms of PPO (Fig. 4.1a). These two fractions after running on native PAGE were stained with the substrate (4-methyl catechol) in the presence and absence of inhibitor potassium metabisulfite (PMS) for ruling out the enzyme activity by any other proteins including peroxidase (Mayer, 2006). The results detailed in chapter 4 showed that these two are indeed PPO isoforms localized at different positions on gel (Fig. 4.1b). In all these cultivars PPO2 was found to be predominant, except ‘Ravaiya’ (V2). Comparatively, the overall activity of PPO2 was significantly less in 4 cultivars (V1, V4, V3, and V7). The total PPO activity has been observed more than the individual activity of PPO1 and PPO2 in all the cultivars. This also indicated that possibly total PPO activity could be representing both these activities together. The varietal difference in PPO activity has been reported in other fruits and vegetables including litchi which probably could be due to variations in its level of expression or bioactivity. Existence of isozymes of PPO has been reported in other fruits and vegetables including potato (Thygesen et al., 1995). In brinjal too different alleles of PPO have been reported in root, leaf and fruit (Shetty et al., 2011).
Figure 2.1. (a) Browning index in different brinjal cultivars. (b) The total PPO activity and activity of PPO1 and PPO2. (c) The total soluble phenolic content of different brinjal cultivars. The different letter superscript indicated significance of difference (P≤0.05). The initial 4 letter of cultivar name was used as their abbreviation in figures. U–unit of PPO activity (change in 0.01 absorbance/ min).
Figure 2.2. The phenolics profile of freshly cut brinjal (a) and brinjal after browning (b) using HPLC with detection at 280 nm. [1; benzoquinone and adducts (tR, 2-6 min), 2; dihydroxycinnamoyl amide (tR, 19 min), 3; dicaffeoylspermidine (tR, 22.5 min), 4; chlorogenic acid (5-caffeoylquinic acid, tR, 25.5 min)), 5; 5-caffeoylquinic acid (tR, 27.5 min), 6; 5-cis-caffeoylquinic acid(tR, 28 min)]. The reflectance spectrum (360-780 nm) of fresh (c) and brown (d) cut brinjal.
2.3.3. Phenolic concentration varied significantly among cultivars

The phenolic content in brinjal cultivars has been shown in Figure 2.1c. It was found to be the highest in brinjal ‘Arka navneet’ (V4). In the case of cultivars ‘Pusa purple long’ (V1), ‘Azad kranti’ (V3), and ‘Silki’ (V8) too, phenolic content was statistically similar to that of V4. The cultivar V2 and V5 showed about 28% less, whereas, V6 and V7 showed about 45% less phenolics than V4. Phenolic content has been reported to vary in different cultivars of fruits and vegetables (Camm and Towers, 1973).

2.3.4. Kinetics of changes in browning index, PPO activity and phenolic content in stored brinjal

The change in browning, phenolics and PPO activity was evaluated during ambient temperature (26 ±2º C) storage of different brinjal cultivars up to 12 days (Fig. 2.2). The dynamics of these parameters were found to significantly change during storage and profound cultivar based variations were observed.

2.3.4.1. Browning index, PPO activity and soluble phenolics increased during ambient temperature storage in four cultivars

Four cultivars ‘Pusa purple long’ (V1), ‘Ravaiya’ (V2), ‘Azad kranti’ (V3), and ‘Arka navneet’ (V4) showed increase in browning index and phenolics along with increase in PPO activity and phenolic content during storage (Fig. 2.2a, 2b, 2c, and 2d). Cultivar ‘Pusa purple long’ (V1) with respect to fresh brinjal samples did not show any significant increase in browning until day 8 and then increased significantly by about 50% on day 12 (Fig 2.2a). The PPO activity and soluble phenolics during the same period was increased by 160 and 44%,
Chapter 3

Storage days of 'Pusa purple long' (V1)

Browning

Phenolics

PPO activity

Storage days of 'Ravaiya' (V2)

Browning

Phenolics

PPO activity

Storage days of 'Azad kranti' (V3)

Browning

Phenolics

PPO activity

Storage days of 'Arka navneet' (V4)

Browning

Phenolics

PPO activity

Contd…….
Figure 2.3. Comparative profile of browning index, PPO activity, and total soluble phenolic content of brinjal cultivars (a–h). For each parameter, data points with different letter superscripts are significantly different (P≤0.05). GAE – Gallic acid equivalent. U – unit of PPO activity (change in 0.01 absorbance/ min). U – unit of PPO activity (change in 0.1 absorbance/ min).
respectively (Fig. 2.2a). The increase in PPO activity was found to be comparatively very high than the phenolic content and browning index. In case of ‘Ravaiya’ (V2), an increase in these parameters was observed during storage. The browning and PPO activity were steadily increased to about 72 and 100% during 12 days of storage (Fig. 2.2b). The increase in browning index and phenolic content was highest in this variety. The phenolic level was increased by 125% on day 12 (Fig. 2.2b). In brinjal ‘Azad kranti’ (V3), the browning and PPO activity were increased by 38 and 185%, respectively (Fig. 2.2c). Among these cultivars the increase in PPO activity was found to be highest in V3. The phenolic content was increased by about 50% during 12 days of storage (Fig. 2.2c). In cultivar V4, the browning, PPO activity and phenolics were increased by 41, 180, and 36%, respectively (Fig. 2.2d). The activity of oxidative enzymes like PPO is known to increase during storage in fruits (Jiang et al., 2004). Such increase in PPO activity, phenolics and browning during storage was reported in two cultivars of litchi fruit (Mishra et al., 2011). The change in total phenolics during storage could be due the physiological changes associated with senescence of brinjal. The increase in PAL activity, which is a regulatory enzyme in phenolics biosynthesis, could also be the reason for such an increase in total phenolics (Camm and Towers, 1973).

2.3.4.2. Browning index and soluble phenolics increased but PPO activity decreased in rest of the four cultivars during ambient temperature storage

In brinjal ‘Kalpatharu’ (V5), ‘Raveena’ (V6), ‘Anupam’ (V7), and ‘Silki’ (V8), both browning index and phenolic content were found to increase during storage, however, PPO activity decreased (Fig. 2.2e, 2f, 2g, and 2h). Such a decrease in PPO activity has been reported earlier at low temperature storage of brinjal (Concellón et al., 2004). With respect to fresh brinjal samples, the browning and phenolics were increased by 30 and
Chapter 3

(a) Browning index (100-L) over days of storage at 10 °C for different varieties:
- V1, V2, V3, V4, V5, V6, V7, V8

(b) PPO specific activity (U/mg protein) over days of storage at 10 °C for different varieties:
- V1, V2, V3, V4, V5, V6, V7, V8

(c) Days of storage at 10 °C

Contd......
Figure 2.4. Comparative profile of browning index (a), polyphenol oxidase (PPO) specific activity (b, c), and total soluble phenolics (d) in different eggplant cultivars during storage at 10 (±2)°C. For each parameter, data points with different letter superscripts are significantly different (P≤0.05). GAE – Gallic acid equivalent. U – unit of PPO activity (change in 0.1 absorbance/ min).
61%, respectively in V5, 36 and 56%, respectively in V6, 50 and 109%, respectively in V7, and 42 and 100%, respectively in V8 (Fig. 2.2e-h). The PPO activity decreased by 28% in V5, 46% in V6, 36% in V7, 27% in V8 (Fig. 2.2e-h). The increase in browning index even after the decrease in PPO activity signifies the role of free phenolics in post-cut browning in stored brinjal. Similar observation with respect to PPO activity has also been reported earlier in case of rambutan fruit (Yingsanga et al., 2008). In another report browning was reported to increase with increase in phenolic content where PPO activity remained unchanged during storage in longkong (Aglaia dookkoo) fruit (Lichanporn et al., 2009).

2.3.4.3. Cultivar based variation in PPO activity, phenolics and browning was storage temperature independent

During ambient temperature storage, all these cultivars underwent senescence which was determined in terms of loss of weight and firmness. The percentage weight loss and firmness in these cultivars after 12 days were found to be in the range of 12-23 and 18-31%, respectively. Similar percentage weight loss and firmness was observed after 21 days of storage in samples stored at 10ºC. This indicated that at low temperature storage eggplant senescence was delayed and an extension of shelf life up to 21 days was observed. The variation in PPO sp. activity, phenolic content, and browning was also analysed in eggplant fruit stored at 10ºC to assess if these changes were similar to those observed in case of ambient temperature stored samples. As observed in ambient temperature stored sample in group 1 cultivars (V1-V4), the browning, soluble phenolic content, and PPO sp. activity increased during storage (Fig. 2.4). However, in group 2 cultivars (V5-V8), the browning and soluble phenolics increased but PPO sp. activity
**Figure 2.5.** Correlation analysis of browning index with PPO activity and total soluble phenolics in different brinjal cultivars. The initial four letter of cultivar name was used as their abbreviation in figures.
decreased during storage. These findings indicated that the change in these parameters was senescence independent and could be the characteristic features of the specific cultivars. The decrease in storage temperature did not affect the enzyme activity and thus indicated the variety specific variation in the kinetics of PPO activity during storage.

2.3.4.4. Correlation analysis of browning with PPO activity and phenolics

In fresh samples of the majority of varieties, PPO activity was found to be comparatively well correlated with browning index. However, in stored samples phenolics were found to be the major contributors to the browning. In four cultivars (V1-V4) the PPO activity showed a positive correlation ($r$) in the range of 0.86 to 0.88, whereas, phenolics showed marginally higher correlation ($r$) in the range of 0.85 to 0.97 with browning (Fig. 2.4). The correlation of browning with phenolics was observed to be higher than the correlation between PPO activity and browning. Contrary to this in other four cultivars (V5-8) the browning increased with the increase in phenolics during storage but PPO activity decreased. In these four cultivars a strong negative correlation ($r$) was observed in the range of -0.80 to -0.91 among PPO activity and browning during storage (Fig. 2.4).

2.3.5. Chlorogenic acid content showed no correlation with browning and PPO activity

The concentration of chlorogenic acid in brinjal was reported to vary between 40-70% (Shetty et al., 2011). The HPLC chromatogram of 80% methanol extract which contained most of the soluble phenolics showed a single major peak which matched with chlorogenic acid standard compound and is shown in Figure 2.5. The chlorogenic acid content in brinjal cultivars has been compared with the phenolic content to observe the relative difference in their ratio among cultivars (Fig. 2.6). In most of the cultivars
Figure 2.6. (a) The HPLC profile of methanol extract of brinjal showing chlorogenic acid as the major phenolic (peak 2). The other major peaks found were N, N’-dicaffeoylspermidine (peak 1), and 3-acetyl-5-caffeoylquinic acid (peak 3) (Singh et al., 2009). (b) The HPLC chromatogram of standard chlorogenic acid.
Figure 2.7. Comparison of chlorogenic acid and total phenolics among cultivars of brinjal. For each parameter, data points with different letter superscripts are significantly different (P≤0.05). The initial four letter of cultivar name was used as their abbreviation in figures. GAE – Gallic acid equivalent.
Chapter 3

Storage days of 'Pusa purple long' (V1)

Storage days of 'Ravaiya' (V2)

Storage days of 'Azad kranti' (V3)

Storage days of 'Arka navneet' (V4)

Contd.....
Figure 2.8. The comparison of change in phenolics and chlorogenic acid during storage in brinjal cultivars (a-h). For each parameter, data points with different letter superscripts are significantly different (P<0.05). GAE – Gallic acid equivalent.
including V3 (‘Azad kranti’), V4 (‘Arka navneet’), (V5 ‘Kalpatharu’), and V8 (‘Silki’), the chlorogenic acid concentration was about 45-60% of the total soluble phenolics. In a few cultivars including V1 (‘Pusa purple long’), V6 (‘Raveena’), and V7 (‘Anupam’) the chlorogenic acid concentration was as high as about 70% of the total phenolics (Fig 2.6). Each fruit and vegetable was known to differ in concentration of phenolics. In apple the chlorogenic acid content was shown to differ among cultivars (Awad et al., 2000). The varietal difference is also common in many fruits and vegetables. The change in the concentration of chlorogenic acid compared to phenolic content in cultivars during storage is shown in Figure 2.7. Change in chlorogenic acid was also found to be different from change in phenolics during storage in majority of cultivars. In ‘Pusa purple long (V1), phenolics increased but chlorogenic acid content decreased during storage (Fig. 2.7a). In V2 (‘Ravaiya’), V3 (‘Azad kranti’), V7 (‘Anupam’), and V8 (‘Silki’) the increase in chlorogenic acid concentration was less compared to increase in phenolic content (Fig. 2.7b, c, g, h). In V4 (‘Arka navneet’) and V5 (‘Kalpatharu’), the chlorogenic acid concentration was almost stable, whereas, the phenolic content increased significantly during storage (Fig. 2.7d, e). Similarly, chlorogenic acid content did not show considerable correlation with PPO activity and browning in these cultivars.

2.4. Discussion

The extent of PPO activity and phenolic content differ in fruits and vegetables. These two factors seem to be the major determinants of browning in processed fruits and vegetables. The brinjal (Solanum melogena) is one of the vegetable displaying intense post-cut browning. Interestingly the post-cut browning in brinjal was found to be independent of its outer skin colour. The green and purple skin colour could be due to chlorophyll and anthocyanins, respectively. The ‘Kalpatharu’ (V5) and ‘Raveena’ (V6) are purple and
green in colour, respectively, and showed higher browning. The highly purple ‘Ravaiya’ (V2) brinjal on cutting showed comparatively less browning. Again the comparison among cultivars indicated the major role of PPO and phenolics in browning, particularly in case of fresh raw brinjal. For example in V5 and V6, high PPO activity reflected in the maximum browning with comparatively lesser concentration of phenolics. In cultivar ‘Raveena’ (V6) and ‘Anupam’ (V7) the phenolic concentration was almost same, but V6 showed higher browning which seems to be due to higher PPO activity. However, in V3 and V4, although less PPO activity was observed, browning was found to be higher, which could be due to the high concentration of phenolics (Fig. 2.1). Thus both these factors were found to play a role in browning of fresh and raw brinjal and the lesser concentration of any of these two components got compensated if other one was on higher side. If both these two factors are on comparatively lower side, as in case of cultivar V2 and V7, browning index was also low.

The profile of PPO activity change during storage resulted in assortment of these brinjal cultivars in two groups. The one group comprising of V1-V4 showed increase in PPO activity, whereas, the other group counting V5-V8 showed decrease in PPO activity during storage. The study of browning, phenolic content and PPO activity at lower temperature (10 °C) of storage in two representative cultivars (‘Arka navneet’ (V4) and ‘Pusa purple long’ (V5), one each from these two groups, also endorsed the findings that profile of change in PPO activity during postharvest storage and processing was variety specific and not related to the senescence of brinjal.

In stored brinjal, the correlation of browning with phenolics in V2, V3, and V4 was observed to be marginally higher than the correlation between PPO activity and browning. In other four cultivars (V5-V8) during storage, browning showed negative
correlation \((r)\) with PPO activity and high correlation with the phenolic content (Fig. 2.4). This indicated that the lower concentration of PPO is not a limiting factor for browning particularly in case of stored brinjal. The presence of a few molecules of enzyme could be sufficient to cause browning if level of phenolics is good enough.

The chlorogenic acid was found to be the major phenolic in brinjal and is also reported to be the major phenolic in potato (a close taxonomic relative). The varietal differences in the content of chlorogenic acid are common in many fruits and vegetables (Mishra et al., 2011). In apple the chlorogenic acid content was shown to differ among cultivars (Awad et al., 2000). The change in the content of chlorogenic acid during storage did not show any correlation with change in phenolics, PPO activity, or browning. This observation could be due to the fact that chlorogenic acid may not be the major natural substrate for PPO in brinjal. Chlorogenic acid is reported to be the major phenolic in potato (90% of total phenolics) and it did not show correlation with browning (Friedman, 1997). However, in their studies as in our finding the browning showed a correlation with PPO activity. Our observation that chlorogenic acid showed about only 31% substrate specificity with PPO (shown in chapter 3) compared to other phenolics also supported this hypothesis. The chlorogenic acid might be having other metabolic roles and less involved in the enzymatic browning of brinjal. The chlorogenic acid was shown to participate in the regulation of shoot, root and root hair development in *Hypericum perforatum* (Franklin and Dias, 2011). The inhibition of chlorogenic acid biosynthesis also showed inhibition of shoot, root and root hair development. Chlorogenic acid also reported to be antimicrobial and confers resistance to several major foliage-feeding insect pests (Friedman, 1997).