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REVIEW ARTICLE

PLANT POLYPHENOL OXIDASES: ISOLATION AND CHARACTERIZATION

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Abstract

Polyphenol oxidases are found both in prokaryotic and eukaryotic microorganisms, in mammals, invertebrates and plants. Most plants have multiple forms of PPO. The more recent articles regarding the polyphenol oxidases from different plants are reviewed. The main characteristics considered were the sources, structure and properties of polyphenol oxidase (PPO) as well as the inhibitors of this enzyme. The polyphenol oxidase was extracted and purified from different plants such as parsley, banana, grapes, durum wheat, etc and it was achieved a fold purification in the range 3.32 – 259 with a recovery between 5.11 – 44.3% The optimum pH and temperature for polyphenol oxidases from various sources of plants were in the range of 4.0 – 8.5 and 30ºC - 80ºC. The affinity of the enzyme varied depending on the substrate used, thus the polyphenol oxidases from plants had the highest affinity for 4-methylcatechol, catechol and pyrogallol. For the inhibition studies, there were tested many substances but the most effective inhibitors for almost every PPO were: ascorbic acid, citric acid, L-cysteine and sodium metabisulfite.

Keywords: polyphenol oxidase, plants, extraction, purification, substrate specificity, inhibitors, optimum pH, optimum temperature

Introduction

Polyphenol oxidases or tyrosinases (PPO) are enzymeswith a dinuclear copper centre, which are able to insertoxygen in a position *ortho*- to an existing hydroxyl groupin an aromatic ring, followed by the oxidation of the diphenolto the corresponding quinone. Molecular oxygen isused in the reaction. The structure of the active site ofthe enzyme, in which copper is bound by six or seven histidineresidues and a single cysteine residue is highly conserved.The enzyme seems to be of almost universaldistribution in animals, plants, fungi and bacteria. Muchis still unknown about its biological function, especially in plants, but also in fungi (Mayer, 2006). The importance ofPPO in browning reactions continues to occupy manyresearchers as indicated by an ACS Symposium (Lee and Whitaker, 1995), and very many subsequent publicationsdescribe browning reactions in a variety of species and theirtissues (Mayer, 2006). Enzymatic browning is one of the major problems contributing to the quality losses

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of fruits and vegetables. The occurrence of enzymatic browning is due to the action of polyphenoloxidase (PPO) on phenolic compounds that are naturally present in plants. The presence of copper atoms at the active site of the enzyme is essential for its catalytic activity (Zawistowski *et al.*, 1991). According to Mayer (1987) and Nagai and Suzuki (2001), PPO is a copper-containing enzyme that catalyses two different reactions: (1) hydroxylation of monophenol to o-diphenols (monophenol monooxygenase: E.C. 1.14.18.1) and (2) oxidation of odiphenols to o-quinones (diphenol oxidase: E.C. 1.10.3.2) in the presence of oxygen. o-Quinone will then undergo spontaneous polymerization, which produces melanins, the pigment with high molecular weight and dark in colour (Zaini *et al.*, 2013).

Characterization of polyphenol oxidases

Extraction and purification

Nowadays, the extraction and the purification of enzymes follow multiple steps. Ümit Ünal *et al.* in 2011 replied that they purified the PPO from tea leaves using a DEAE cellulose column, thusachieving 3.32 fold purification with a recovery of 5.11%. Halder *et al.*, 1998 studied the purification and characterization of PPO from tea leaves obtained three fractions on DEAE cellulose column, of which two of them were absorbed and one was unabsorbed. Madani *et al.* stated in 2011 that of a fresh weight basis the total protein content of *Hibiscus* petals crude extract and ammonium sulfate extract were 425 µg and 122 µg/100 g fresh petals. The purification steps resulted in 31-fold purification. The overall activity yield of the purified LjPPO was 33.8%, with specific enzyme activity of 4458.24 U/mg. Gao *et al.* studied in 2011 the purification and characterisation of polyphenol oxidase from leaves of *Cleome gynandra*, commonly known as spiderflower and determined a sequential purification with ammonium sulphate, dialysis, DEAE-Sepharose ion-exchange chromatography and Sephadex G-75 gel filtration chromatography that resulted in 37.8-fold enrichment in the specific activity and 44.3% recovery of the total activity. Queiroz *et al.*, 2011 achieved a 59-fold with 28.2% recovery of activity for cashew apple PPO. For the PPO from parsley, Doğru *et al.*, 2012 found that after the purification steps was found to have 1.65 fold purification after ammonium sulfate precipitation, and 26.92 fold purification after gel filtration chromatography. Altunkaya *et al.*, 2012 affirmed that the PPO from durum wheat (*Triticum durum* L.) was extracted and partially purified by ion-exchange chromatography on a column packed with diethyaminoethyl cellulose (DEAE). This procedure led to 26.33-fold purification with 24.7% recovery. Mishra *et al.*, 2012 found that the eggplant (*Solanum melongena*) is a rich source of PPO which affects it's sensorial quality due to enzymatic browning. They managed to obtain one isoform of PPO that had 259-fold purification using standard chromatographic procedures. Palma-Orozco *et al.*, researched in 2011the PPO from mamey (*Pouteria sapota*) and obtained two isoenzymes (PPO 1 and PPO 2) upon ammonium sulfate precipitation and hydrophobic and ion exchange chromatography. They found that PPO 1 was purified up to 6.6-fold with 0.28% yield, while PPO 2 could not be characterized because the enzyme activity was completely lost after 24 h of storage. Zaini *et al.*, 2013 investigated the membrane-bound polyphenol oxidase from Snake fruit [*Salacca zalacca* (Gaertn.) Voss] (mPPO) as an oxidative enzyme, thus causing the enzymatic browning. The mPPO from Snake fruit was purified to 14.1 folds with a recovery of 12.35%. Liu *et al.*, 2013 studied the PPO from the flower buds of *Lonicera japonica and* found that their purification steps resulted in 31-fold purification. The overall activity yield of the purified LjPPO was 33.8%, with specific enzyme activity of 4458.24 U/mg.

Molecular weight

Some authors have studied the partial purification and characterization of polyphenol oxidase from *Hibiscus rosa–sinensis* through the analysis of SDS-PAGE gel that revealed some bands in native conditions both on crude and purified extracts (Madani *et al.*, 2011). The same group mentioned that the crude extract showed several bands with a more distinct band corresponding to a molecular weight of 70.795 K Da while the partial purified extract showed less number of bands and with a

more distinct band corresponding to a molecular weight of 39.810 K Da. The molecular weight of PPO from other species has been reported as follows: cabbage 39 K Da (Fujita *et al.*, 1995), banana 62 K Da (Galeazzi *et al.*, 1981), field bean seed 120 K Da (Beena *et al.*, 2000), and Chinese cabbage 65 K Da (Nagai *et al.*, 2001). Their results indicated that the molecular weight of *Hibiscus*-PPO is similar to that of cabbage but lower than those of banana, field bean seed and Chinese cabbage. Dogru *et al.*, 2012 estimated by gel filtration chromatography on Sephadex G-100 column that the PPO from from parsley (*Petroselinum crispum*, Apiaceae) has a molecular weight of 237 kDa. Liu *et al.*, 2013 studied the polyphenol oxidase from the flower buds of *Lonicera japonica* and found that the molecular weight was ≈49 kDa which was calculated based on the standard molecular weight marker. Thus, the LC–ESI-MS analysis determined the molecular mass of LjPPO was 49.277 kDa. The result was higher than that of *Hevea brasiliensis* PPO (32 and 34 kDa) (Zhou *et al.*, 1991), but lower than that of *Cleome gynandra* L. leaves (52.6 kDa) (Gao *et al.*, 2011), potato PPO (57–60 kDa), tomato PPO (57–62 kDa) and broccoli florets PPO (53.1 kDa) (Gawlik-Dziki *et al.*, 2007), respectively. Jang *et al.*, 2011 studied the PPO from fresh-cut apple and replied that the main protein bands had a molecular weight of approximately 63 kDa. Mishra *et al.*, 2012 used a calibration curve that was prepared using proteins of known molecular weight in order to study the purified PPO from eggplant (*Solanum melongena).* They observed thatthe molecular weight of purified PPO was about 112 kDa.The purified enzyme from mamey (*Pouteria sapota*) displayed a molecular weight of ≈18 kDa in SDS–PAGE and 16.1 kDa by gel filtration (GF), suggesting that PPO is monomeric. After gel filtration GF elution, the enzyme was collected and measured for PPO activity, showing a specific activity similar to the PPO1 previously loaded (Palma-Orozco *et al.*, 2011). The calculated molecular weights were lower than those calculated using the same procedures, i.e. 39 kDa (Fujita *et al.*, 1995) and 41 kDa for PPOs from cabbage and banana (Yang *et al.*, 2000), respectively. While PPO from pineapple was determined to be ≈104 kDa by GF, using SDS–PAGE, this PPO displayed a molecular weight of ≈25 kDa, thus suggesting that in this fruit PPO is a tetramer (Das *et al.*, 1997). Zaini *et al.*, 2013 studied the mPPO from Snake fruit and the results showed that the purified enzyme migrated as a single protein band suggesting that mPPO from Snake fruit is a monomeric protein with estimated molecular weight of 38 kDa. The molecular size was lower than those of mPPO from other plants such as beet root (Gandia-Herrero *et al.*, 2004), apples (Tsurutani *et al.*, 2002), peach (Cabanes et al., 2007) and loquat fruit (Selles-Marchart *et al.*, 2006) with a molecular weight of 54, 65, 60, and 59.2 kDa, respectively. Gao *et al.*, 2011 studied the purification and characterisation of polyphenol oxidase from leaves of *Cleome gynandra* and found that the purified PPO is a monomeric protein of 52.6 kDa.

Optimum pH and stability

Different optimum pHs for PPO obtained from various sources have been reported in the literature. In general, vegetables and fruits show maximum activity at or near neutral pH values, but these values may vary with the source of enzyme and substrate within a relatively wide range of pH. Although, in most cases, pH optima have been reported between 4.0 and 8.5, it should be noted that the optimum pH can also be affected by the type of buffer and the purity of enzyme (Erat *et al.*, 2010, Wesche-Ebeling *et al.*, 1990, Zhou *et al.*, 1991).Queiroz *et al.*, 2011 studied the Cashew apple PPO that showed maximal activity at pH 6.5 and remained at approximately 63% at pH 7.0. PPO activity was not apparent at pH above 7.0 due to the oxidation of substrate. Similar pH values were found for PPO extracted from yacon root (Neves *et al.*, 2007) and medlar (Dincer *et al.*, 2002). According to the literature, optimal pH for PPO activity varies from 5.0 to 7.5 (Yoruk *et al.*, 2006). Ümit Ünal *et al.*, 2011 studied the polyphenol oxidase from tea leaf *(Camellia sinensis)* and the PPO activity as a function of pH was determined in a pH range of 4.03-7.00. As the pH increased from 4.03 to 6.02, the enzyme activity increased, with maximal activity occurring at pH 6.02, after which the activity started to decrease. The enzyme from tea leaf exhibited high activity in a broad pH range. In the pH range of

4.5-7.0 the enzyme activity was more than 63%. Madani *et al.*, 2011said that since pH exerts a strong effect on enzymatic activity, it was of interest to study the effect of pH on *Hibiscus*–PPO activity using 4-methylcatechol as a substrate. It was found that the optimum pH value for *Hibiscus*-PPO was 6.0 which was similar to that reported for aubergine (Dogan *et al.*, 2002) and for *Ferula* sp. (Erat *et al.*, 2006). In general, most plants show maximum PPO activity at or near neutral pH values (Betrosian *et al.*, 1960). However, different optimum pHs for PPO obtained from various sources are reported in the literature. For example, it is reported that optimum pH values are 5.5 for strawberry (Wesche-Ebeling *et al.*, 1990), 7.0 for Amasya apple (Siddiq *et al.*, 1992) 6.8 for cocoa beans (Lee *et al*., 1991) using 4–methylcatechol as a substrate. Altunkaya *et al.*, 2011 found that the optimum pH for durum wheat PPO was found to be 6.5. Their results revealed that the optimum pH of durum wheat PPO is similar to PPOs from other sources. Durum wheat PPO was found to be stable in the pH range from 6.0 to 6.8. The optimum pHs for parsley (*Petroselinum crispum*, Apiaceae) were found to be 4.0 for catechol, 4-methylcatechol, and catechin, 4.5 for L-dopa, 8.0 for dopamine and 8.5 for pyrogallol. Stability of PPO activity was studied ranging from 4.0 to 7.5 over a period of 4 days by using catechol as a substrate. It was found that the PPO activity of parsley was the highest at pH 6.5, but decreased at similar rates at each of the pH values studied (Doğru *et al.*, 2012). Liu *et al.*, 2013 found that the pH activity profile indicated that the optimum pH value for *Lonicera japonica* PPO activity was 7.5. This result was similar to that of that of red Swiss chard (Gao *et al.*, 2009), but higher than that of Ferula sp. (6.0) (Erat *et al.*, 2006) and medlar fruit (6.5) (Dincer *et al.*, 2002). Altunkaya *et al*., 2011 found that the optimum pH from freshly cut lettuce (*L. sativa*) was 7.0 which was the same value for the PPO from mamey (Palma-Orozco *et al.*, 2011). The effect of pH on PPO activity was investigated using catechol as a substrate in the pH 5.0–8.0 range. The optimum pH for pomegranate PPO activity was found to be about pH 6.0. The PPO activity decreased rapidly at pH below or above this optimum (Jaiswal *et al.*, 2010). The pH optimum of the enzyme from Snake fruit [*Salacca zalacca* (Gaertn.) Voss] was found to be at 6.5 as shown by Zaini *et al.*, 2013. They replied that there is no report on mPPO pH optimum using catechol as substrate, the results were compared with sPPO from other plant sources. This result is similar with previous work by Eidhin *et al.*, (2006) and Cho and Ahn (1999), who reported the pH optimum of 6.5 and 6.6 for apple and potato, respectively, using the same substrate for enzymatic activity evaluation. However, the pH optimum of artichoke and Barbados cherry were found to be at pH 7.0 (Dogan *et al.*, 2005; Kumar *et al.*, 2008), butter lettuce was at pH 5.5 (Gawlik- Dziki *et al.*, 2008) and Malatya apricot was at pH 8.5 (Arslan *et al*., 1998). The activity of purified PPO from leaves of *Cleome gynandra* was measured at different pH values using L-DOPA as substrate. The enzyme was active between the pH range 6.5–10.0, and exhibited maximal activity at pH 8.0 with a half maximal activity at pH 6.8. Below and above pH 8.0, its activity decreased rapidly. At pH 7.5 and 9.0, the enzyme lost about 30% of its activity, at pH 10.0 the activity dropped to only 32%, and below pH 6.0, the enzyme showed less than 22% activity (Gao *et al.*, 2011).

Optimum temperature and stability

An optimum temperature is necessary for the enzyme to achieve maximum activity. In practical production, avoiding proper temperature of reaction is an efficient way to inhibit enzyme activity (Liu *et al.*,2013). The PPO temperature optimum also depends on conditions to which the fruit develops, i.e. in tropical regions higher temperatures may be required to achieve the maximum activity **(**Palma-Orozco *et al.*, 2011). Altunkaya *et al.*, 2012 identified the temperature effects on PPO activity of durum wheat were studied between 10 and 80ºC with catechol used as substrate. The purified durum wheat PPO had an optimum activity at 40ºC. They observed a gradual decrease in the activity of PPO at temperatures exceeding 40ºC due to protein denaturation. Actually, 40% of the original activity remained at temperature of 60ºC. The effect of temperature on PPO from tea leaf *(Camellia sinensis)* activity was investigated in the range of 20-80°C and the results showed that the optimum temperature for PPO activity was found to be at 30°C. After 30°C, the

activity started to decrease gradually. However, the enzyme had a very high activity at a broad temperature range. The enzyme had still 72% of the maximal activity even at 80°C(ÜmitÜnal *et al.*, 2011). Madani *et al.*, 2011 assayed the effect of temperature using 4–methylcatechol as a substrate over a temperature range of 25-70°C at the optimum pH. *Hibiscus*-PPO activity was found to increase with increasing temperature with the maximum activity being attained at 45°C and dropped to a sub-minimum at 70°C. In the literature the reported optimum temperature values were 20°C for dog rose (Sakiroglu *et al.*, 1996), 30°C for aubergine (Dogan *et al.*, 2002) and 56°C for amasya apple (Oktay *et al.*, 1995) by using 4–methylcatechol as a substrate. Liu *et al*, 2013 investigated the PPO from *Lonicera japonica and* reported that the effect of temperature on the activity of LjPPO was determined over a temperature range from 5 to 65ºC at pH 7.5. The optimum temperature of *Lonicera japonica* PPO was 25ºC. Altunkaya *et al.*, 2011 studied the purification and characterization of polyphenol oxidase from freshly cut lettuce and discovered 4 isoenzymes. They concluded that the purified lettuce PPO1 and PPO4 isoenzymes had an optimum activity at 40 and 30 °C, respectively. There was a gradual decrease in the activity of PPO isoenzymes at temperatures exceeding 40°C due to protein denaturation. The optimum temperature of crude lettuce PPO has been reported previously as 40°C. For the PPO from parsley, Doğru *et al.*, 2012 depicted that theoptimum temperature was substrate-dependent and the enzyme activities were determined with each of the six substrates used in the experiment. They found that the optimum temperature is 40°C for catechol and 4-methyl catechol, 35°C for progallol and catechol, 45°C for L-dopa and dopamine. It has been reported that optimum temperature for PPO is 40°C for Chinese cabbage (Nagai *et al.*, 2001) and 12°C for Ferula sp. (Erat *et al.*, 2006), using catechol as a substrate. Palma-Orozco *et al.*, 2011 reported that the thermal stability of PPO from mamey *(Pouteria sapota)* was analyzed by incubating the enzyme for 30 min at temperatures ranging from 20 to 80ºC. In the 35–45ºC range, residual activity was similar. At 20ºC the residual activity (26%) was maintained, at higher temperatures the activity decreased (50–60ºC) and from 65 to 70ºC there was no activity. In contrast to Palma-Orozco *et al.*, 2011results, the PPO from apple was reported to be stable between 30 and 75ºC, while above this temperature it becomes rapidly inactivated (Ni Eidhin *et al.*, 2006). The same behavior was observed for PPO from Chinese cabbage; it was thermally stable between 50 and 70ºC and inactivated above this temperature (Nagai and Suzuki, 2001).

In contrast, PPO from banana was thermally stable at 30ºC and even at temperatures >60ºC, the PPO displayed a partial residual activity (Yang *et al.*, 2000). In this regard, PPO from medlar showed a thermal stability pattern similar to that of PPO in this study, as at temperatures >80ºC the enzyme was rapidly inactivated (Dincer *et al.*, 2002). Zaini *et al.*, 2013 showed that the optimum temperature of mPPO from Snake fruit [*Salacca zalacca* (Gaertn.) Voss] was at 30ºC and its activity decreased gradually as temperature increased. Casado-Vela *et al*, 2005 and Selles-Marchart *et al.*, 2006 reported slightly higher optimum temperature for mPPO from tomato (40ºC) and loquat (70ºC) fruit. In comparison to sPPO similar optimum temperature at 30ºC was obtained by Eidhin *et al.*, 2006 and Yang *et al.*, Fujita *et al.*, 2000 for apple and banana PPO, respectively. However, the optimum temperatures of longan fruit and butter lettuce were reported to be at 35ºC (Gawlik-Dziki *et al.*, 2008; Jiang, 1999), cabbage and Barbados cherry were at 40ºC (Kumar *et al.*, 2008; Nagai *et al.*, 2001) and artichoke was at 25ºC (Aydemir, 2004). Gao *et al.*, 2011 studied the polyphenol oxidase from *Cleome gynandra* and showed the effects of temperature on purified PPO activity and stability. It was estimated over a temperature range of 10–85ºC. The PPO activity increased progressively with the increment of temperature from 10 to 45ºC, but rapidly until 60ºC. Above 60ºC, the enzymatic activity gradually decreased, and between 60 and 80ºC only 60% of its maximum activity remained. When the temperature was below 15ºC, less than 10% of its maximum activity could be detected. Therefore, the optimal temperature for purified PPO activity was 60ºC, though the enzyme showed considerable activity over the temperature range of 55–75ºC.

Substrate specificity

The affinity of the enzyme varied depending on the substrate used. Tea PPO had a higher affinity for 4-methylcatechol. Of the substrates tested, the best substrate for tea PPO was 4-methylcatechol. The enzyme showed no activity against caffeic acid and gallic acid.Orenes-Pinero *et al.*, 2006 *Km* value for latent PPO from quince was found to be 1.2 mM, using 4-*tert*-butylcatechol as substrate.Duangmal *et al.*, 1999 reported the following *Km* values for taro PPO: 9.0 mM for 4-methylcatechol, 67.9 mM for catechol and 89.9 mM for pyrogallol. The same investigators reported the following *Km* values for potato PPO: 1.1 mM for 4-methylcatechol, 6.8 mM for catechol and 1.5 mM for pyrogallol (Madani *et al.*, 2011). Doğru *et al.*, 2012 found that the PPO of parsley had the greatest affinity toward catechol of the six substrates tested. When the V_{max} values for the six substrates were compared, it was found that the V_{max} for catechol was higher than those of other substrates. This observation was similar to that of the work on PPO from different sources such as Ferula sp. (Erat *et al.*, 2006). Gao *et al.*, 2011 showed that the purified *Cleome gynandra* PPO showed a decrease in relative substrate activity: diphenols > triphenols > monophenols. Among the diphenols tested, L-DOPA and catechol gave the highest relative enzymatic activity (>90%), whereas m-dimethylphenol, the lowest (36%), the rest, p-dimethylphenol and chlorogenic acid, in the middle. The PPO from Snake fruit specificity was studied in the presence of monophenol, diphenols and triphenols compounds as substrates. The relative activity was calculated by considering catechol as 100% activity. mPPO showed substrate preference for diphenols, with the highest activity detected in catechol (100±1.8%), followed by 4-methyl catechol (84.5 ±2.1%) and epicatechin (71.6±3.6%). However, the mPPO had a low activity towards L-DOPA (19.1±0.6%), chlorogenic acid (17.8±0.7%), caffeic acid (14.5±0.4%) and triphenols such as gallic acid (3.3±0.1%). No activity was detected with monophenol (p-cresol) as substrate. The results obtained in this study were in agreement with those reported by Onsa *et al.*, 2000, Selles-Marchart *et al.*, 2006 and Casado-Vela *et al.*, 2005 for mPPO from sago log, loquat fruit and tomato fruit, respectively (Zaini *et al.*, 2013).Queiroz *et al.*, 2011found that the optimal substrate for PPO from cashew apple (*Anacardium occidentale*, L.) was catechol.Later, Altunkaya *et al.*, 2012 showed that for the durum wheat PPO there were used several phenolic compounds as substrate. Among the substrates used, the greatest substrate specificity was observed with catechol.The order of affinity as substrate of durum wheat PPO was as follows: catechol> chlorogenic acid > caffeic acid> catechin > 4-methyl catechol. Mishra *et al.*, 2012 showed that the eggplant (*Solanum melongena*) enzyme had very low Km (0.34 mM) and high catalytic efficiency (3.3±106) with 4-methyl catechol. The substrate specificity was in the following order: 4-methyl catechol > tert-butylcatechol > dihydrocaffeic acid > pyrocatechol. Liu *et al.*, 2013 discovered that the crude enzyme solution from *Lonicera japonica* showeddiphenolase activity toward catechol, L-dopa and chlorogenic acid rather than monophenolase and triphenolase activity, and the best substrate was catechol because of the highest V_{max}/K_m value of 1000.By studying mamey fruit Palma-Orozco *et al.*, 2011 observedaffinity with various phenolic compounds but the pyrogallol showed to be the best substrate.

John *et al.*, 2011 investigated the PPO from *Mangifera indica* L. sap (latex) enzyme that showed activity with *o*-dianisidine, *p*-phenylenediamine, tetramethyl benzidine, diaminobenzidine and guaiacol in the presence of H_2O_2. However, 1mM *o*-dianisidine was found to be the best substrate for this enzyme followed by *p*-phenylenediamine.

Effect of inhibitors

The effect of inhibition was studied since the first studies on PPO. There are several inhibitors, such as ascorbic acid used by researchers to prevent enzymatic browning of plants.Theeffects of cysteine, ascorbic acid and sodium metabisulfite on tea PPO activity at various concentrations using catechol as the substrate wasstudied by ÜmitÜnal *et al.*, 2011 and the results were reported as percentage inhibition. From the results, it was concluded that the most potent inhibitors were sodium metabisulfite and ascorbic acid, because a

higher degree of inhibition was achieved. Cysteine was the least potent inhibitor. In the same year Madani *et al.*, 2011investigated the ascorbic acid and EDTA in order to study their inhibitory effects on the activity of PPO from *Hibiscus*. The ascorbic acid is a stronger inhibitor than EDTA while iron chloride did not show any effect on the *Hibiscus* PPO activity. Gao *et al.*, 2011 showed that the activity of PPO from *Cleome gynandra* was slightly increased by 0.1% SDS, heavily inhibited by Hg^{2+} and Pb^{2+}, and completely inhibited by 1.0 mM of ascorbic acid, L-cysteine, b-mercaptoethanol, sodium diethyldithiocarbamate and thiourea, and by 10 mM of dithioerythritol, sodium metabisulphite and sodium sulphite. Queiroz *et al.*, 2011studied the PPO from cashew apple (*Anacardium occidentale*, L.) and found that ascorbic acid, citric acid, sodium sulphite and sodium metabisulphite decreased PPO activity, while sodium chloride increased PPO activity. Later, Zaini *et al.*, 2013 investigated the effect of inhibition on the PPO from Snake fruit [*Salacca zalacca* (Gaertn.) Voss]. Among the chemical inhibitors tested, L-cysteine showed the best inhibitory effect, with an IC50 of 1.3±0.002 mM followed by ascorbic acid (1.5±0.06 mM), glutathione (1.5±0.07 mM), EDTA (100±0.02 mM) and citric acid (186±0.16 mM).For the inhibition studies of PPO from durum wheat (*Triticum durum* L.) Altunkaya *et al.*, 2012 found that among others inhibitors, ascorbic acid was found to be the most effective, followed by L-cysteine, oxalic acid and citric acid. Mishra *et al.*, 2012 investigated the eggplant (*Solanum melongena*) PPO inhibitors showing that cysteine hydrochloride, potassium metabilsulfite, ascorbic acid, erythorbic acid, resorcylic acid and kojic acid that showed competitive inhibition, whereas, citric acid and sodium azide showed mixed inhibition of PPO activity. Cysteine hydrochloride was found to be an excellent inhibitor with the low inhibitor constant of 1.8 mM. Several common PPO inhibitors had an inhibitory effect on the *Lonicera japonica* PPO. Among these inhibitors, SDS presented no effect at concentrations of 0.01–1 mM, but presented positive effect at the concentration of 10 mM. Tropolone and kojic acid inhibited the enzyme activity in some extent as the specific enzyme inhibitors. Ascorbic acid exhibited nearly complete inhibition of LjPPO at 10 mM and itmay act more as an antioxidant than as an enzyme inhibitor (Liu *et al.*, 2013). The most effective inhibitors for mamey PPO were as it follows: ascorbic acid, sodium metabisulfite, succinic acid, tropolone inhibited completely at 10 mM, as did kojic acid, EDTA and sodium chloride. In contrast, in the presence of citric acid, benzoic acid and potassium sorbate, PPO 1 was activated rather than inhibited (Palma-Orozco *et al.*,2011).

Conclusions

The increasing consumers trend for the selection of fruits and vegetables with high quality of flavor, texture and color is of great importance for achieving a high nutrional food. In order to respond to the market demands, fruits and vegetables processors make considerable efforts in order to improve the fruits and vegetables quality from harvesting through the end of the processing in food industry.

Thus, in food processing, enzymes can have an undesired effect on the quality of fresh products. One such enzyme is polyphenol oxidase (PPO), that catalyzes the oxidation of phenolic compounds. This enzyme is directly responsible for the production of dark pigments in fruits and vegetables, which cause a poor quality appearance. During harvest, the plants can be bruised thereby the PPO could be released into the cytoplasm of cells thus iniating the process of enzymatic browning. The data obtained from this review regarding the extraction, purification, optimum pH and temperature, substrate specificity, inhibitors may give an insight to understand the behaviour of polyphenol oxidases from plants in order to acknowledge the behaviour of this enzyme during various processes and to enable the proper procedure for inactivation.

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