Polyphenol Oxidase(s): Importance in Food Industry

6

Enzymes' role as a catalyst in plant physiology and metabolism is indispensable. Enzymes remain active throughout the life cycle of a crop including at postharvest stages. This is useful in species where the ripening occurs during postharvest storage, but sometimes it leads to deterioration of quality traits including color, taste, texture, and nutritional content. The combination of endogenous deteriorative enzymes and growth of microorganisms can significantly impact the quality of produce, shorten the shelf life, and affect the consumer acceptance of the produce. Despite their influence on quality traits, enzymes are used as biocatalysts in several food-processing applications to hydrolyze large molecules into simpler molecules. Additionally, enzymes are being used to produce and change molecules to improve the organoleptic quality of food products (Terefe et al. 2014). The enzymes responsible for quality losses vary among the different products like PPOs in cereals and fruits (Bhattacharya et al. 1999; Holderbaum 2010), lipoxygenase in certain vegetables, and polygalacturonase in apricots (Luh et al. 1978; Whitaker 1991). According to estimates, PPO is the single most damaging of enzymes in color deterioration of crop products with losses of up to 50% for tropical fruits and vegetables (Whitaker and Lee 1995).

The oxidative enzymes like polyphenol oxidases (PPOs) are responsible for changes in color and flavor in addition to loss of nutritional value (Whitaker 1991; Terefe et al. 2014). These undesirable changes in quality are a major limiting factor in handling and processing of crops since the products undergo rapid browning upon slicing, peeling, and damage due to mechanical reasons or herbivory. Some of the economically important edible products susceptible to undesirable browning include wheat-based products (Bhattacharya et al. 1999); fruits like apple (Holderbaum 2010), banana (Gooding et al. 2001), grape (Rathjen and Robinson 1992), pineapple (Das et al. 1997), and mango (Robinson et al. 1993); and vegetables such as eggplant (Perez-Gilabert and Carmona 2000; Shetty et al. 2011) and potato (Sanchez-Ferrer et al. 1993; Cho and Ahn 1999). Interestingly, the browning reaction enhances the quality of certain products like black tea (Eskin 1990; Ullah

A. Jukanti, *Polyphenol Oxidases (PPOs) in Plants*,
DOI 10.1007/978-981-10-5747-2_6

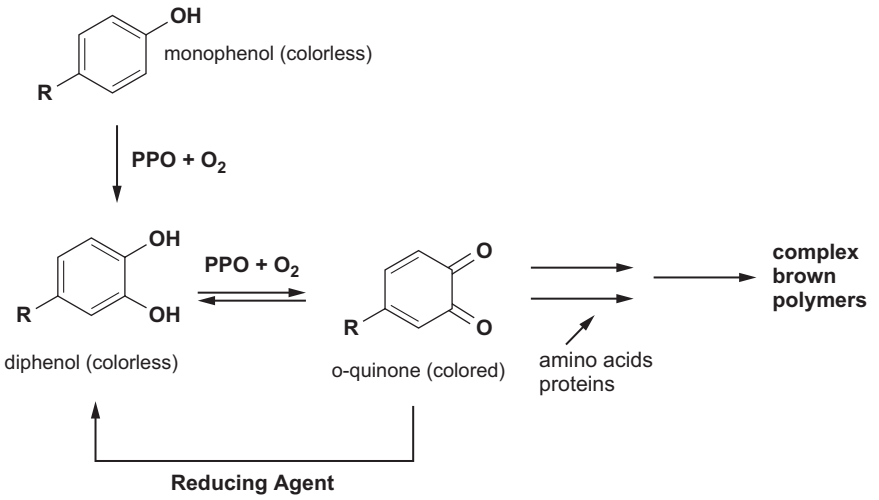

Fig. 6.1 The reaction catalyzed by polyphenol oxidase (Mayer and Harel 1979)

1991), coffee (Amorim and Silva 1968; Amorim and Melo 1991), and cocoa (Lee et al. 1991; Lopez and Dimick 1991).

As mentioned earlier, PPOs catalyze two important reactions: hydroxylation of monophenols to *o*-diphenols and oxidation of *o*-diphenols to *o*-quinones (Tomas Barberan and Espin 2001; Fig. 6.1). The *o*-quinones responsible for enzymatic discoloration of plant products are yellow compounds that are highly unstable and form complexes with amino acids or proteins (Ramaswamy and Riahi 2003). The polymerization of these intermediates and condensation of *o*-quinones produce the characteristic reddish-brown colored polymers called melanins that are responsible for the undesirable browning of food products (Tomas-Barberan and Espin 2001; Ramaswamy and Riahi 2003).

Phenols are the major substrates of PPOs. In food, phenolic compounds contribute to the bitterness, color, flavor, astringency, and oxidative stability of products (Tomas-Barberan and Espin 2001). Interaction between food proteins and oxidized products of phenols leads to covalent condensation resulting in changes in different characteristics of food proteins (Matheis and Whitaker 1984; Yoruk and Marshall 2003). The *o*-quinones interact with the side chains of amino acids, thereby causing a reduction in the nutritive value of food products (Matheis and Whitaker 1984; Felton et al. 1989, 1992). The amino acid side chain groups like –SH and $–NH_2$ are generally susceptible to binding by quinones. A reduction in lysine content was reported in casein and tomato protein in the presence of phenolic compounds and PPOs (Matheis and Whitaker 1984; Felton et al. 1989, 1992). Further, food proteins that have tyrosine or are linked to phenolic groups are modified by PPOs (Matheis and Whitaker 1984). Interestingly, redox cycling of quinones formed during PPO reactions generates free radicals that can damage DNA, amino acid, and proteins (Felton et al. 1992; Hill 1992).

More than 8000 polyphenolic compounds have been identified in various cereals, fruits, and vegetables (Pandey and Rizvi 2009). Cereals, fruits, and vegetables both in their raw and processed forms contain significant amounts of polyphenols. Polyphenols are secondary metabolites that are mostly implicated in plant defense against pathogens. The long-term consumption of polyphenol-rich foods has shown beneficial effects on human health owing to the high antioxidant activity of polyphenols (Heim et al. 2002). Further, several epidemiological studies demonstrated some protection by polyphenols against cancers, diabetes, and neurodegenerative and cardiovascular diseases (Arts and Hollman 2005; Graf et al. 2005). It is interesting that high polyphenol content is helpful for human health, whereas low enzymatic browning is relevant for food processing as to maintain original color, flavor, and nutritional value (Murata et al. 1995; Podsedek et al. 2000). Owing to their economic and nutritional importance, polyphenols and PPOs are a subject of scientific interest for plant breeders, pathologists, food technologists, and medical researchers.

Globally, wheat is the second most important staple food crop with an estimated annual production of 729 million tonnes in 2014 (http://www.fao.org/faostat). Wheat flour is used in preparation of several products including bread, crackers, cookies, biscuits, cakes, noodles, macaroni, and spaghetti. Wheat is mostly consumed in the form of flatbread (Middle East and Asia) and noodles (South and East Asia). Wheat noodles form an important part of the daily diet in several Asian countries, and about ~40 to 50% of wheat flour consumed is used to manufacture different kinds of noodles (Hou and Kruk 1998; Anderson and Morris 2001). The USA, Australia, and Canada are the key suppliers of wheat used in noodle manufacturing in Asia as their wheat is considered to possess desirable quality attributes. Brightness and color (cream/white or yellow) are two very important quality parameters of Asian noodles (Fig. 6.2). Therefore, tailor-made wheat varieties that meet the needs of discriminating buyers, particularly the Asian markets which imports significant quantities of wheat from the USA, Australia, and Canada, need to be developed.

The time-dependent darkening of wheat noodles due to PPOs is critical (Kruger et al. 1994; Baik et al. 1995; Crosbie et al. 1998; Hou and Kruk 1998), more importantly in raw noodles that are stored for several days before cooking (Mares and Campbell 2001). So far, the wheat improvement efforts have focused on developing cultivars with low or reduced PPO content. But even these varieties that contained low or reduced PPO levels possessed certain levels of PPO that caused browning reaction in the wheat products (Fig. 6.2). But collaborative efforts between breeders at Montana State University, Bozeman, USA, and University of Nebraska, Lincoln, USA, have been successful in identifying wheat lines (07OR1074) with negligible or zero PPO activity comparable to durum wheat (Hystad et al. 2015). Breeding efforts are now underway to incorporate these genotypes into wheat breeding programs. The major objective would be to develop varieties with zero/negligible PPO activity in addition to good milling and baking qualities that would be acceptable to the consumers.

The importance of PPOs in plant defense and their involvement in postharvest losses of horticultural crop produce due to enzymatic browning explains why PPOs

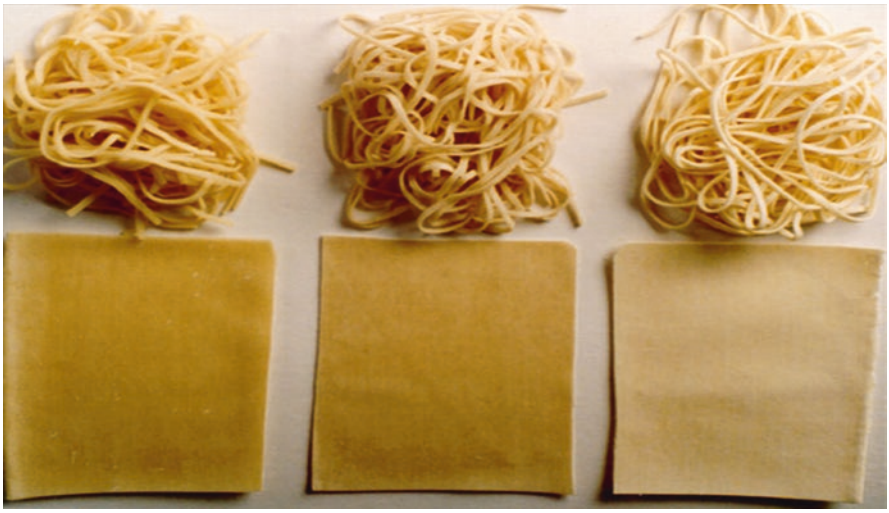

Fig. 6.2 Variation in end-product quality traits in different types of wheat varieties with different PPO levels (Source: Dr. J Martin, Montana State University, Bozeman, USA, with permission)

Fig. 6.3 Tissue prints showing enzymatic browning from eggplant fruits due to mechanical injury (sections **a**–**c**) and shoot/fruit borer infestation (**d**–**f**). Sections **a** and **d,** immunolocalization using immune serum (rabbit anti-GST–SmePPO1); sections **b** and **e,** pre-immune serum; sections **c** and **f,** secondary antibody conjugate (Adapted from Shetty et al. 2011)

have been studied and characterized in several diversified vegetable species (Mayer 2006). Among the crops of Solanaceae family, potato, eggplant (also called brinjal or aubergine), and peppers are the major vegetable crops of substantial economic value (Daunay 2008). Eggplant is an important vegetable crop of Asia; India and China are the leading producers with an annual production of 13.6 and 29.5 m tonnes, respectively, in 2014 (http://www.fao.org/faostat). It is an important ingredient of the Indian cuisine especially for the vegetarians. The PPOs of eggplant (rich in phenols) have been studied extensively due to their role in enzymatic browning of the fruit. Chlorogenic acid is the dominant phenol accounting for ~70 to 95% of total phenols in the flesh of the eggplant fruit (Whitaker and Stommel 2003; Singh et al. 2009). Shetty et al. (2011) have studied and characterized the multigene family of eggplant PPOs. They have demonstrated the browning reaction of eggplant fruit upon mechanical damage and herbivory (Figs. 6.3 and 6.4).

Apple is one of the major fruit crops of the world with an annual global production of ~84.6 million tonnes in 2014 (http://www.fao.org/faostat). The major

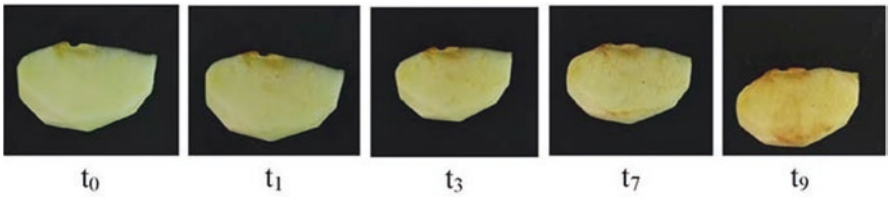

Fig. 6.4 The panel shows a fresh-cut apple slice at zero time (*t0*), after 1 (*t1*), 3 (*t3*), 7 (t7), and 9 (*t9*) days of storage ($T = 7.5$ °C; Adapted from Lunadei et al. 2011)

producers of apples during 2014 are China, USA, Poland, India, and Turkey. The industry consumes about ~40 to 60% of the apples produced, and the rest is sold as fresh produce in the market (Nicolas et al. 1994). Juice, sauce, and slices are the major processed products developed from apples. The spoilage of vegetables/fruits during postharvest manipulations or processing is up to ~25% of the harvested produce (Nicolas et al. 1994). The postharvest losses are estimated to be 30–40% of total production in developing countries (Singh et al. 2014). Postharvest deterioration in apples causes 5–25% losses of total production (Jijakli and Lepoivre 2004). The losses incurred are mostly due to physical injuries, disorders, or diseases and unsuitable conditions for processing. Brown flesh with flattened area on the fruit is a very common defect of apples (Nicolas et al. 1994). Further, browning of the skin or internal tissue is caused due to different conditions including bitter pit, scalds, water core, core flush, and internal breakdown. Improper processing of products (juice, sauce, or slices) could result in discoloration and ultimately reduces the consumer acceptance.

6.1 Control of Browning Reaction

6.1.1 Chemical Control

Browning or discoloration due to PPOs drastically reduces the nutritional and sensory qualities. The PPO-mediated undesirable brown discoloration in cereal products, fruits, and vegetables causes enormous economic impact necessitating its control to reduce the economic losses, maintain product quality, and extend the shelf life of the produce. Therefore, several mechanisms or approaches have been developed to control the undesirable browning. Broadly the available inhibitors could be divided into six classes (McEvily et al. 1992):

1. Reducing agents – ascorbic acid and analogs, sulfites;
2. Chelating agents – ethylenediaminetetraacetate, sodium diethyldithiocarbamate, and sodium azide;
3. Complexing agent – cyclodextrins, chitosan;
4. Acidulants – ascorbic acid, citric acid, malic acid, phosphoric acid;

5. Enzyme inhibitors – substrate analogs, halides; (vi) Enzyme treatments – proteases, and *o*-methyltransferase.

The chemical antibrowning agents eliminate or target different components necessary for the PPO reaction like phenolic substrates and intermediate products like quinones, oxygen, enzyme, or copper molecule (Nicolas et al. 1994; Ahvenainen 1996; Ferrar and Walker 1996; Queiroz et al. 2008). Though different PPOs may have an identical reaction against inhibitors, the inhibitors' effectiveness against different PPOs could vary significantly, thereby necessitating a specific control mechanism for individual PPO systems (Ferrar and Walker 1996).

Reducing agents like sulfites and ascorbic acid are most widespread compounds used in browning control. They control the browning either by preventing the accumulation of *o*-quinones or by forming stable colorless products (Eskin et al. 1971; Nicolas et al. 1994; Osuga et al. 1994; Ashie et al. 1996; Kim et al. 2000). Among the reducing agents, sulfur dioxide and sulfites (sodium sulfite, sodium bisulfite, or sodium metabisulfite) are the most potent inhibitors of browning reaction (Eskin et al. 1971; Sapers 1993; Kim et al. 2000). Sulfites have been used in the vegetable and fruit industry owing to their effectiveness and low price. It is assumed that sulfites directly inhibit PPOs and interact with quinones preventing them from further polymerization (Ashie et al. 1996). But due to adverse health effects of sulfites, the World Health Organization has recommended a limited use or nonuse of sulfites in treatment of fresh fruit and vegetables (Queiroz et al. 2008). Cysteine is another effective sulfur-containing compound used as antibrowning agent. Cysteine reacts with *o*-quinones to produce stable and colorless compounds (İyidoğan and Bayiindirli 2004). Though cysteine is effective in preventing browning at very low concentrations of 1–4 mM (Özoğlu and Bayiindirli 2002; İyidoğan and Bayiindirli 2004), its use in food processing is limited because it produces undesirable odor even at these low levels.

Ascorbic acid (vitamin C) is the best available alternative to sulfites and is often used as antibrowning agent in sliced fruits, canned fruits and vegetables, purees, and fruit juices (Yoruk and Marshall 2003). Ascorbic acid reduces the quinone prior to it undergoing secondary reactions that are responsible for browning and also decreases the pH (Guerrero-Beltrán et al. 2005). The pH optima for PPO reactions are usually in the range of 5–7.5; therefore, lowering of pH inhibits the enzyme activity. The inhibitory effect of ascorbic acid is temporary because it is oxidized irreversibly by reacting with the intermediates like *o*-quinones, endogenous enzymes, metals (e.g., copper), and pigments (Queiroz et al. 2008). But a combination of ascorbic and citric acid was shown to be more effective than ascorbic acid alone (Eskin et al. 1971; Sapers 1993). The acidic environment coupled with the stability of ascorbic acid in an acidic environment could be responsible for increased effectiveness of ascorbic acid. The PPO is inhibited by citric acid through its chelating action and by ascorbic acid via site-directed specificity toward histidine residues of the PPO (Golan-Goldhirsh et al. 1992; Osuga et al. 1994).

The most commonly found acids (like ascorbic, citric, malic, and phosphoric) have been used in food processing to control browning. These acids decrease the pH

to 3 or lower drastically reducing the enzyme activity (Eskin et al. 1971; Park and Luh 1985; Eskin 1990; Osuga et al. 1994). Kojic acid is found in fermented foods as a natural product and has shown antibrowning effect (Queiroz et al. 2008; İyidoğan and Bayiindirli 2004). Koji acid inhibits PPO and also bleaches melanin to colorless compounds. Limited usage of kojic acid in food industry is due to difficulty in large-scale production and higher cost. Aromatic carboxylic acids like benzoic and cinnamic acids inhibit PPO activity owing to their structural similarities with phenolic substrates and by forming a complex with copper (Marshall et al. 2000). β-cyclodextrins inhibit the enzyme activity by probably forming inclusion complexes with PPO substrates (Irwin et al. 1994), and they bind to the substrate's hydrophobic core. The inhibition of enzyme activity is dependent upon the substrate (Casado-Vela et al. 2006) and type of cyclodextrin used (López-Nicolás et al. 2007). Therefore, for effective PPO inhibition, suitable cyclodextrin should be used considering the dominant phenols present in the food product.

A limited number of inhibitors are available that can be used in the food-processing industry due to effective inhibition, undesirable odor/flavor, food safety concerns, and economic feasibility (Eskin et al. 1971; McEvily et al. 1992; Sapers 1993). Honey (Chen et al. 2000), procyanidins (Le Bourvellec et al. 2004), Maillard reaction products (Lee and Park 2005), pineapple juice (Lozano-De-Gonzalez et al. 1993), rhubarb juice (Son et al. 2000a), pectin preparations (Tong et al. 1995), and amino acids with glucose (Tan and Harris 1995) are some of the natural products that have been shown to exhibit a certain inhibitory effect on PPO activity. Honey obtained from different sources decreased the PPO activity by ~2 to 45% in fruit and vegetable products, and enhanced inhibition was observed in combination with ascorbic acid (Chen et al. 2000). Native procyanidins, natural flavanol polymers occurring in plants, inhibited PPO activity probably by forming enzyme–polyphenol–substrate complex (Le Bourvellec et al. 2004). The inhibitory effect of Maillard reaction products produced from amino acids and various sugars depended upon the amino acid [arginine > cysteine > histidine > lysine] and type of sugar [monosaccharides > disaccharides] (Lee and Park 2005).

Proteins, amino acids, and peptides can react with *o*-quinones and chelate the copper at the active site of PPO, thereby affecting PPO activity levels (Queiroz et al. 2008). Girelli et al. (2004) observed that certain glycyl dipeptides like glycylaspartic acid, glycylphenylalanine, glycylglycine, glycyllysine, glycyltyrosine, and glycylhistidine had impacted quinone formation at concentrations ranging from 1 to 50 mM. Different types of inhibition were exhibited by cinnamic acid and its derivatives: cinnamic acid [noncompetitive] > 4-hydroxycinnamic acid [competitive] > 4-methoxycinnamic acid [noncompetitive] (Shi et al. 2005). Cinnamic acid and its derivatives attach to a nonactive site region, thereby either changing the enzyme conformation or hindering the binding of substrate to the enzyme through steric hindrance. Concentration-dependent PPO inhibition was exhibited by several *p*-alkoxybenzoic acids, *p*-methoxybenzoic acid being the most potent inhibitor (Chen et al. 2005). Rhubarb juice and pectin contain oxalic acid, a natural PPO inhibitor that inhibits PPO activity through copper chelation (Son et al. 2000b; Yoruk and

Marshall 2003). Though several natural PPO inhibitors have been identified, they are not yet commercially utilized in the food-processing industry.

6.1.2 Physical Control

The above-described methods of PPO inhibition mostly involve the use of chemical compounds, but several other physical methods like heating, dehydration, irradiation, high pressure, and freezing/refrigeration (Ashie et al. 1996; Kim et al. 2000) have also been used for the control of adverse browning. But some of these methods may have certain disadvantages such as enzyme–substrate contact due to subcellular breakdown and texture deterioration (Macheix et al. 1990). Heat treatment is an effective method to stabilize foods due to its ability to destroy microorganisms and deactivate enzymes. Blanching is most commonly used in vegetables (Marshall et al. 2000), but it is rarely used for treating soft textured fruits or vegetables as it destroys thermosensitive nutrients like vitamins, proteins, and carbohydrates (Lado and Yousef 2002). Large quantities of water and energy requirement coupled with waste disposal problems make blanching technically unattractive. Generally, the catalytic activity of PPO is destroyed in the range of 70–90 °C, but the time needed for deactivation is very variable depending upon the product source (Chutintrasri and Noomhorm 2006). Pineapple PPO activity decreased by ~60% after exposure to 40–60 °C for 30 min, deactivation increased drastically >75 °C, and the residual was 7% and 1.2% at 85 °C and 90 °C for 5 min, respectively (Chutintrasri and Noomhorm 2006).

Good quality and increased shelf life of fruit and vegetable products can be achieved through high hydrostatic pressure (HHP) treatment (Kim et al. 2001). Compared to heat treatment, HHP causes less damage to nutrients like vitamins, pigments, and flavoring agents since HHP has limited or no impact on the covalent bondings in the compounds (Butz et al. 2003). Depending upon applied pressure and its duration, temperature, and protein system (e.g., type of protein, pH, ionic strength), HHP can affect the protein structure and cause protein denaturation or aggregation (Messens et al. 1997). New or analog products can be developed by HHP treatment of foods with no effect on flavor, color, or nutritional content and without any thermal degradation (Vardag and Körner 1995). Further, HHP can modify the functionality of an enzyme, affect the biological activity of the enzyme, and also change its substrate specificity (Hendrickx et al. 1998). The effectiveness of HHP treatment for several PPOs is greater at lower pH and is also influenced by the addition of antibrowning agents, salts, or sugars (Rapeanu et al. 2005). Since PPOs were found to be more resistant to HHP than heat treatment, a combination treatment of high pressure (>400 MPa) and mild heat treatment (~50 °C) was found to be best to inactivate the enzyme in banana puree (*Musa* sp.; Palou et al. 1999), lychee (*Litchi chinensis*; Phunchaisri and Apichartsrangkoon 2005), and strawberry (*Fragaria* × *ananassa*; Dalmadi et al. 2006). Interestingly, low pressure (≤400 MPa) also induced PPO activation in red raspberry (*Rubus idaeus*; Garcia-Palazon et al. 2004) and pear (Asaka and Hayashi 1991) and in apple juice (Anese et al. 1995).

Therefore, the level of pressure applied for inactivation of PPO activity is very critical.

Irradiation with gamma (γ) rays is a physical treatment involving direct exposure of fruit and vegetable products for food preservation, extending their shelf life, and improving their safety and quality (Lacroix and Ouattara 2000). Additionally, radiation treatment destroys the bacteria and fungi, guaranteeing total disinfection, and also delays the ripening process (Iemma et al. 1999). PPO activity in irradiated (1.0 kGy) fresh-cut lettuce was ~31% lower compared to untreated sample, but after 9 days PPO activity of the irradiated samples was ~54% higher than in the control sample (Zhang et al. 2006). Similarly, in fresh-cut celery at 1 kGy (0.5 kGy/h) treatment, the PPO activity was reduced by 73% in the treated sample, and after 9 days the PPO activity was only ~25% lower than in the control sample (Lu et al. 2005).

Pulsed electric field (PEF) is a nonthermal food preservation technology that is mostly focused to inactivate microorganisms and is used in processing of liquid foods (García et al. 2003, 2005; Evrendilek et al. 2004; Li and Zhang 2004). Electric field strength, time of PEF treatment (Zhong et al. 2007), electrical conductivity, and pH affect the inactivation of PPO. The average electric field strength of ~25 kV/min given for 744–6000 μs decreased the PPO activity by 70–97% in different fruit products (Zhong et al. 2007; Giner et al. 2001, 2002). The sensitivity of different enzymes to PEF treatment varied as pepsin > PPO > peroxidase > chymotrypsin and lysozyme (Yang et al. 2004). PEF treatment of PPO results in the loss of α-helix fractions and induces changes in its secondary structure (Zhong et al. 2007). Other technologies used for reducing PPO activity include supercritical carbon dioxide and ohmic and microwave heating (Queiroz et al. 2008). Supercritical carbon dioxide is a nonthermal treatment that physically destroys the microbial cells (Corwin and Shellhammer 2002) and induces enzyme inactivation by causing changes in the secondary and tertiary structure of the enzyme (Gui et al. 2007). Reduced PPO activity was observed when food products were treated with supercritical carbon dioxide (Gui et al. 2007) or in combination with HHP (Corwin and Shellhammer 2002).

PPO is an important enzyme in the food-processing industry, and its activity, in general, causes undesirable discoloration (browning reaction) of food products. The browning of food products decreases their nutritional quality, lowers consumer acceptance levels, and causes economic losses. Therefore, identification of safe antibrowning agents or developing improved technologies to control browning is very critical to enhance product value and minimize the economic losses. Though several technologies have been demonstrated, only a few like HHP have been adopted in food-processing industries. The knowledge about these technologies and the combination of technologies to be used to obtain a high-quality product is very essential.

References

Ahvenainen R (1996) New approaches in improving the shelf life of minimally processed fruits and vegetables. Trends Food Sci Technol 7:179–200

Amorim HWD, Melo M (1991) Significance of enzymes in non-alcoholic coffee beverage. In: Fox PF (ed) Food enzymology. Elsevier Science Publishing, New York, pp 189–209

Amorim HWD, Silva DM (1968) Relationship between the polyphenol oxidase activity of coffee beans and the quality of the beverage. Nature 219:381–382

Anderson JV, Morris CF (2001) An improved whole-seed assay for screening wheat germplasm for polyphenol oxidase activity. Crop Sci 41:1697–1705

Anese M, Nicoli MC, Dall'Aglio G, Lerici CR (1995) Effect of high pressure treatments on peroxidase and polyphenoloxidase activities. J Food Biochem 18:285–293

Arts ICW, Hollman PCH (2005) Polyphenols and disease risk in epidemiologic studies. Am J Clin Nutr 81:317–325

Asaka M, Hayashi R (1991) Activation of polyphenoloxidase in pear fruits by high pressure treatment. Agric Biol Chem 55(9):2439–2440

Ashie INA, Simpson BK, Smith JP (1996) Mechanisms for controlling enzymatic reactions in foods. Crit Rev Food Sci Nutr 36:1–30

Baik BK, Czuchajowska Z, Pomeranz Y (1995) Discoloration of dough for oriental noodles. Cereal Chem 72(2):198–205

Bhattacharya M, Luo Q, Corke H (1999) Time-dependent changes in dough color in hexaploid wheat landraces differing in polyphenol oxidase activity. J Agric Food Chem 47:579–3585

Butz P, García AF, Lindauer R, Dieterich S, Bognár A, Tausher B (2003) Influence of ultra high pressure processing on fruit and vegetable products. J Food Eng 56:233–236

Casado-Vela J, Sellés S, Bru R (2006) Influence of developmental stage, cultivar, and hexapeptide and cyclodextrin inhibitors on polyphenol oxidase activity from tomato fruits. J Food Biochem 30:623–640

Chen L, Mehta A, Berenbaum M, Zangeri AR, Engeseth NJ (2000) Honeys from different floral sources as inhibitors of enzymatic browning in fruit and vegetable homogenates. J Agric Food Chem 48(10):4997–5000

Chen Q-X, Song K-K, Qiu L, Liu X-D, Huang H, Guo H-Y (2005) Inhibitory effects on mushroom tyrosinase by *p*-alkoxybenzoic acids. Food Chem 91:269–274

Cho YK, Ahn HK (1999) Purification and characterization of polyphenol oxidase from potato: I. Purification and properties. J Food Biochem 23:577–592

Chutintrasri B, Noomhorm A (2006) Thermal inactivation of polyphenoloxidase in pineapple puree. LWT-Food Sci Technol 39(3):492–495

Corwin H, Shellhammer TH (2002) Combined carbon dioxide and high pressure inactivation of pectin methylesterase, polyphenol oxidase, *Lactobacillus plantarum* and *Escherichi coli*. J Food Sci 67(2):697–701

Crosbie GB, Huang S, Baclay IR (1998) Wheat quality requirements of Asian foods. Euphytica 100:155–156

Dalmadi I, Rapeanu G, Van Loey A, Smout C, Hendrickx M (2006) Characterization and inactivation by thermal and pressure processing of strawberry (*Fragaria ananassa*) polyphenol oxidase: a kinetic study. J Food Biochem 30:56–76

Das JR, Bhat SG, Gowda LR (1997) Purification and characterization of a polyphenol oxidase from the Kew cultivar of Indian pineapple fruit. J Agric Food Chem 45:2031–2035

Daunay MC (2008) Eggplant. In: Prohens J, Nuez F (eds) Handbook of plant breeding, vegetables II: Fabaceae, Liliaceae, Solanaceae, and Umbelliferae, vol 2. Springer, New York, pp 163–220

Eskin NAM (1990) Biochemistry of food spoilage: enzymatic browning. In: Eskin NAM (ed) Biochemistry of foods. Academic, New York, pp 401–432

Eskin NAM, Henderson HM, Townsend RJ (1971) Browning reactions in foods. In: Eskin NAM, Henderson HM, Townsend RJ (eds) Biochemistry of foods. Academic, New York, pp 69–83

Evrendilek GA, Li S, Dantzer WR, Zhang QH (2004) Pulsed electric field processing of beer: microbial, sensory, and quality analyses. J Food Sci 69(8):228–232

Felton GW, Donato K, Del Vecchio RJ, Duffey SS (1989) Activation of plant foliar oxidases by insect feeding reduces nutritive quality of foliage for noctuid herbivores. J Chem Ecol 15:2667–2694

Felton GW, Donato KK, Broadway RM, Duffey SS (1992) Impact of oxidized plant phenolics on the nutritional quality of dietary protein to a noctuid herbivore, *Spodoprera exigua*. J Insect Physiol 38:277–285

Ferrar PH, Walker JRL (1996) Inhibition of diphenol oxidases: a comparative study. J Food Biochem 20:15–30

García D, Gómez N, Condón S, Raso J, Pagán R (2003) Pulsed electric fields cause sublethal injury in *Escherichia coli*. Lett Appl Microbiol 36(3):140–144

García D, Hassani M, Manas P, Condon S, Pagan R (2005) Inactivation of *Escherichia coli* O157:H7 during the storage under refrigeration of apple juice treated by pulsed electric fields. J Food Saf 25:30–42

Garcia-Palazon A, Suthanthangjai W, Kadja P, Zabetakis I (2004) The effects of high phydrostatic pressure on β-glucosidase, peroxidase and polyphenoloxidase in red raspberry (*Rubus idaeus*) and strawberry (*Fragaria* × *ananassa*). Food Chem 88:7–10

Giner J, Gimeno V, Barbosa-Cánovas GV, Martin O (2001) Effects of pulsed electric field processing on apple and pear polyphenoloxidases. Food Sci Technol Int 7(4):339–345

Giner J, Ortega M, Mesegue M, Gimeno V, Barbosa-Cánovas GV, Martin O (2002) Inactivation of peach polyphenoloxidase by exposure to pulsed electric fields. J Food Sci 67(4):1467–1472

Girelli AM, Mattei E, Messina A, Tarola AM (2004) Inhibition of polyphenol oxidases activity by various dipeptides. J Agric Food Chem 52:2741–2745

Golan-Goldhirsh A, Osuga DT, Chen AO, Whitaker JR (1992) Effect of ascorbic acid and copper on proteins. In: D'Souza VT, Feder J (eds) The bioorganic chemistry of enzymatic catalysis: a homage to Myron L. Blender. CRC Press, Boca Raton, pp 61–76

Gooding PS, Bird C, Robinson SP (2001) Molecular cloning and characterization of banana fruit polyphenol oxidase. Planta 213:748–757

Graf BA, Milbury PE, Blumberg JB (2005) Flavonols, flavonones, flavanones and human health: epidemological evidence. J Med Food 8:281–290

Guerrero-Beltrán JA, Swanson BG, Barbosa-Cánovas GV (2005) Inhibition of polyphenoloxidase in mango puree with 4-hexylresorcinol, cysteine and ascorbic acid. LWT – Food Sci Tecnhol 38(6):625–630

Gui F, Wu J, Chen F, Liao X, Hu X, Zhang Z, Wang Z (2007) Inactivation of polyphenol oxidases in cloudy apple juice exposed to supercritical carbon dioxide. Food Chem 100(4):1678–1685

Heim KE, Tagliaferro AR, Bobilya DJ (2002) Flavonoid antioxidants: chemistry, metabolism and structure–activity relationships. J Nutr Biochem 13:572–584

Hendrickx M, Ludikhuyze L, Van Den Broeck I, Weemaes C (1998) Effects of high pressure on enzymes related to food quality. Trends Food Sci Technol 9:197–203

Hill HZ (1992) The function of melanin or six blind people examine an elephant. BioEssays 14:49–56

Holderbaum DF (2010) Enzymatic browning, polyphenol oxidase activity, and polyphenols in four apple cultivars dynamics during the fruit development. Hortic Sci 45(8):1150–1154

Hou G, Kruk M (1998) Asian noodle technology. In: Asian noodles. Tech Bull XX (12): 1–10

Hystad SM, Martin JM, Graybosch RA, Giroux MJ (2015) Genetic characterization and expression analysis of wheat (*Triticum aestivum*) line 07OR1074 exhibiting very low polyphenol oxidase (PPO) activity. Theor Appl Genet 128:1605–1615

Iemma J, Alcarde AR, Domarco RE, Spoto MHF, Blumer L, Matraia C (1999) Radiação gama na conservação do suco natural de laranja. Sci Agric 56(4):1193–1198

Irwin PL, Pfeffer PE, Doner LW, Sapers GM, Brewster JD, Nagahashi G, Hicks KB (1994) Binding geometry, stoichiometry, and thermodynamics of cyclomalto-oligosaccharide (cyclodextrin) inclusion complex formation with chlorogenic acid, the major substrate of apple polyphenol oxidase. Carbohydr Res 256:13–27

İyidoğan NF, Bayiindirli A (2004) Effect of L-cysteine, kojic acid and 4-hexylresorcinol combination on inhibition of enzymatic browning in Amasya apple juice. J Food Eng 62:299–304
Jijakli MH, Lepoivre P (2004) State of the art and challenges of post-harvest disease management in apples. In: Mukerji KG (ed) Fruit and vegetable diseases, vol 1. Kluwer Academic Publishers, Dordrecht, pp 59–94
Kim J, Marshall MR, Wei C (2000) Polyphenoloxidase. In: Haard NF, Simpson BK (eds) Seafood enzymes utilization and influence on postharvest seafood quality. Marcel Dekker, New York, pp 271–315
Kim Y-S, Park S-J, Cho Y-H, Park J (2001) Effects of combined treatment of high hydrostatic pressure and mild heat on the quality of carrot juice. J Food Sci 66(9):1355–1360
Kruger JE, Hatcher DW, DePauw R (1994) A whole seed assay for polyphenol oxidase in Canadian praire spring wheats and its usefulness as a measure of noodle darkening. Cereal Chem 71(4):324–326
Lacroix M, Ouattara B (2000) Combined industrial process with irradiation to assure innocuity and preservation of food products – a review. Food Res Int 33:719–724
Lado BH, Yousef AE (2002) Alternative food-preservation technologies: efficacy and mechanisms. Microbes Infect 4:433–430
Le Bourvellec C, Le Quéré J-M, Sanoner P, Drilleau J-F, Guyot S (2004) Inhibition of apple polyphenol oxidase activity by procyanidins and polyphenol oxidation products. J Agric Food Chem 52:122–130
Lee M-K, Park I (2005) Inhibition of potato polyphenol oxidase by Maillard reaction products. Food Chem 91:57–61
Lee PM, Lee K, Karim MIA (1991) Biochemical studies of cocoa bean polyphenol oxidase. J Sci Food Agric 55:251–260
Li S-Q, Zhang QH (2004) Inactivation of *E.coli* 8739 in enriched soymilk using pulsed electric fields. J Food Sci 69(7):169–174
Lopez AS, Dimick PS (1991) Enzymes involved in cocoa curing. In: Fox PF (ed) Food enzymology. Elsevier Science Publishing, New York, pp 211–236
López-Nicolás JM, Núñez-Delicado E, Sánchez-Ferrer A, García-Carmona F (2007) Kinetic model of apple juice enzymatic browning in the presence of cyclodextrins: the use of maltosyl b-cyclodextrin as secondary antioxidant. Food Chem 101:1164–1171
Lozano-De-Gonzalez PG, Barrett DM, Wrolstad RE, Durst RW (1993) Enzymatic browning inhibited in fresh and dried apple rings by pineapple juice. J Food Sci 58:399–404
Lu Z, Yu Z, Gao X, Lu F, Zhang L (2005) Preservation of gamma irradiation on fresh-cut celery. J Food Eng 67:347–351
Luh BS, Ozbilgin S, Liu YK (1978) Textural changes in canned apricots in the presence of mold polygalacturonase. J Food Sci 43:713–716
Lunadei L, Galleguillos P, Diezma B, Lleó L, Ruiz-Garcia L (2011) A multispectral vision system to evaluate enzymatic browning in fresh-cut apple slices. Postharvest Biol Technol 60:225–234
Macheix J, Fleuriet A, Billot J (1990) Phenolic compounds in fruit processing. In: Macheix J, Fleuriet A, Billot J (eds) Fruit phenolics. CRC Press, Boca Raton, pp 239–312
Mares DJ, Campbell AW (2001) Mapping components of flour and noodle colour in Australian wheat. Aust J Agric Res 52:1297–1309
Matheis G, Whitaker JR (1984) Modification of proteins by polyphenol oxidase and peroxidase and their products. J Food Biochem 8:137–162
Marshall MR, Kim JM, Wei CI (2000) Enzymatic browning in fruits, vegetables and seafoods. FAO, http://www.fao.org/ag/ags/agsi/ENZYMEFINAL/Enzymatic%20Browning.html
Mayer AM, Harel E (1979) Polyphenol oxidases in plants. Phytochem 18:193–215
Mayer AM (2006) Polyphenol oxidases in plants and fungi: going places? A review. Phytochemistry 67:2318–2331
McEvily AJ, Iyengar R, Otwell WS (1992) Inhibition of enzymatic browning in foods and beverages. Crit Rev Food Sci Nutr 32:253–273

Messens W, Camp JV, Huyghebaert A (1997) The use of high pressure to modify the functionality of food proteins. Trends Food Sci Technol 8:107–112
Murata M, Noda I, Homma S (1995) Enzymatic browning of apples on the market: relationship between browning, polyphenol content, and polyphenol oxidase. Nipon Shokuhin Kagaku Kogaku Kaishi 42:820–826. [in Japanese]
Nicolas JJ, Richard-Forget FC, Goupy PM, Amiot M-J, Aubert SY (1994) Enzymatic browning reactions in apple and apple products. Crit Rev Food Sci Nutr 34(2):109–157
Osuga D, Van Der Schaaf A, Whitaker JR (1994) Control of polyphenol oxidase activity using a catalytic mechanism. In: Yada RY, Jackman RL, Smith JL (eds) Protein structure-function relationships in foods. Chapman and Hall, New York, pp 62–88
Özoğlu H, Bayiindirli A (2002) Inhibition of enzymatic browning in cloudy apple juice with selected antibrowning agents. Food Control 13:213–221
Palou E, López-Malo A, Barbosa-Cánovas GV, Welti-Chanes J, Swanson BG (1999) Polyphenoloxidase activity and color of blanched and high hydrostatic pressure treated banana puree. J Food Sci 64(1):42–45
Pandey KB, Rizvi SI (2009) Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Med Cell Longev 2(5):270–278
Park EY, Luh BS (1985) Polyphenol oxidase of kiwifruit. J Food Sci 50:678–684
Perez-Gilabert M, Carmona FG (2000) Characterization of catecholase and cresolase activities of eggplant polyphenol oxidase. J Agric Food Chem 48:695–700
Phunchaisri C, Apichartsrangkoon A (2005) Effects of ultra-high pressure on biochemical and physical modification of lychee (*Litchi chinensis* Sonn.) Food Chem 93:57–64
Podsedek A, Wilska-Jeszka J, Anders B, Markowski J (2000) Compositional characterization of some apple varieties. Eur Food Res Technol 210:268–272
Queiroz C, Lúcia MLM, Fialho E, Valente-Mesquita VL (2008) Polyphenol oxidase: characteristics and mechanisms of browning control. Food Rev Int 24(4):361–375
Ramaswamy H, Riahi E (2003) High-pressure inactivation kinetics of polyphenol oxidase in apple juice. Appl Biotechnol Food Sci Pol 1:189–197
Rapeanu G, Loey AV, Smout C, Hendrickx M (2005) Effect of pH on thermal and/or pressure inactivation of Victoria grape (*Vitis vinifera sativa*) polyphenol oxidase: a kinetic study. J Food Sci 70(5):E301–E307
Rathjen AH, Robinson SP (1992) Aberrant processing of polyphenol oxidase in a variegated grapevine mutant. Plant Physiol 99:1619–1625
Robinson SP, Loveys BR, Chacko EK (1993) Polyphenol oxidase enzymes in the sap and skin of mango fruit. Aust J Plant Physiol 20:99–107
Sanchez-Ferrer A, Laveda F, Garcia-Carmona F (1993) Partial purification of soluble potato polyphenol oxidase by partitioning in aqueous two-phase system. J Agric Food Chem 41:1219–1224
Sapers GM (1993) Browning of foods: control by sulfites, antioxidants, and other means. Food Technol 47:75–84
Shetty SM, Chandrasekhar A, Venkatesh YP (2011) Eggplant polyphenol oxidase multigene family: cloning, phylogeny, expression analyses and immunolocalization in response to wounding. Phytochemistry 72(18):2275–2287
Shi Y, Chen Q-X, Wang Q, Song K-K, Qiu L (2005) Inhibitory effects of cinnamic acid and its derivates on the diphenolase activity of mushroom (*Agaricus bisporus*) tyrosinase. Food Chem 92:707–712
Singh AP, Luthria D, Wilson T, Vorsa N, Singh V, Banuelos GS, Pasakdee S (2009) Polyphenols content and antioxidant capacity of eggplant pulp. Food Chem 114:955–961
Singh V, Hedayetullah M, Zaman P, Meher J (2014) Postharvest technology of fruits and vegetables: an overview. J Postharvest Technol 2(2):124–135
Son SM, Moon KD, Lee CY (2000a) Rhubarb juice as a natural antibrowning agent. J Food Sci 65:1288–1289
Son SM, Moon KD, Lee CY (2000b) Kinetic study of oxalic acid inhibition on enzymatic browning. J Agric Food Chem 48:2071–2074

Tan BK, Harris ND (1995) Maillard reaction products inhibit apple polyphenoloxidase. Food Chem 53:267–273

Terefe NS, Buckow R, Versteeg C (2014) Quality-related enzymes in fruit and vegetable products: effects of novel food processing technologies, Part 1: High-pressure processing. Crit Rev Food Sci Nutr 54(1):24–63

Tomas-Barberan FA, Espin JC (2001) Phenolic compunds and related enzymes as determinants of quality in fruits and vegetables. J Sci Food Agric 81:853–876

Tong CBS, Hicks KB, Osman SF, Hotchkiss AT, Haines RM (1995) Oxalic acid in commercial pectins inhibits browning of raw apple juice. J Agric Food Chem 43:592–597

Ullah MR (1991) Tea. In: Fox PF (ed) Food enzymology. Elsevier Science Publishing, New York, pp 163–187

Vardag T, Körner P (1995) High pressure: a real alternative in food processing. Food Mark Technol 9:42–47

Whitaker JR (1991) Enzymes: monitors of food stability and quality. Trends Food Sci Technol 2:94–97

Whitaker JR, Lee CY (1995) Recent advances in chemistry of enzymatic browning. In: Lee CY, Whitaker JR (eds) Enzymatic browning and its prevention. Amer Chem Soc Symp Ser 600, Washington, DC, pp 2–7

Whitaker BD, Stommel JR (2003) Distribution of hydroxycinnamic acid conjugates in fruit of commercial eggplant (*Solanum melongena* L.) cultivars. J Agric Food Chem 51:3448–3454

Yang R-J, Li S-Q, Zhang QH (2004) Effects of pulsed electric fields on the activity of enzymes in aqueous solution. J Food Sci 69(4):FCT241–FCT248

Yoruk R, Marshall MR (2003) Physicochemical properties and function of plant polyphenol oxidase: a review. J Food Biochem 27:361–422

Zhang L, Lu Z, Lu F, Bie X (2006) Effect of γ irradiation on quality-maintaining of fresh-cut lettuce. Food Control 17:225–228

Zhong K, Wu J, Wang Z, Chen F, Liao X, Hu X, Zhang Z (2007) Inactivation kinetics and secondary structural change of PEF-treated POD and PPO. Food Chem 100:115–123