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Polyphenol Oxidase: Characteristics and Mechanisms of Browning Control

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Polyphenol oxidase, a copper-containing metalloprotein, catalyzes the oxidation of phenolic compounds to quinones, which produce brown pigments in wounded tissues. This enzymatic mechanism causes post harvest losses and mainly affects tropical fruits. In this article, some characteristics of polyphenol oxidase from different plants are reviewed and information about conventional and alternative methods to inactivate this enzyme is presented. Characterization of the polyphenol oxidase could help to develop or to choose more effective methods for controlling browning of vegetables and products.

Keywords enzymatic browning, fruits, vegetables, antibrowning agents, antibrowning treatments

Introduction

Polyphenol oxidases (PPOs) are a group of copper-proteins, widely distributed phylogenetically from bacteria to mammals, that catalyze the oxidation of phenolics to quinones which produce brown pigments in wounded tissues. PPO has been implicated in the formation of pigments, oxygen scavenging and defense mechanism against plant pathogens and herbivory insects. Phenolic compounds serve as precursors in the formation of physical polyphenolic barriers, limiting pathogen translocation. The quinones formed by PPOs can bind plant proteins, reducing protein digestibility and their nutritive value to herbivores. On the other hand, the oxidation of phenolic substrates by PPO is thought to be the major cause of the brown coloration of many fruits and vegetables during ripening, handling, storage and processing. This problem is of considerable importance to the food industry as it affects the nutritional quality and appearance, reduces the consumer’s acceptability and therefore causes significant economic impact, both to food producers and to food processing industry. It is estimated that over 50% of losses in fruits occur as a result of enzymatic browning and tropical and subtropical fruits and vegetables are the most susceptible to these reactions.

PPO has been regarded to be a critical enzyme in food technology and it has been intensively studied in several plants. It is known that plant PPOs are synthesized as preproteins and contain putative plastid transit peptides at the N-terminal region, which

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target the enzyme into chloroplast and thylakoid lumen. PPO from some plants has been described as a multiple gene family. Seven PPO genes were identified from tomato (PPOs A, A’, B, C, D, E and F), and five distinct PPO cDNAs were isolated from potato plants. In Fuji apple plants, the expression of two PPO genes is differentially regulated during vegetative and reproductive development and in response to wounding.

The mechanism of action proposed for PPO is based on its capacity to oxidize phenolic compounds. When the tissue is damaged, the rupture of plastids, the cellular compartment where PPO is located, leads to the enzyme coming into contact with the phenolic compounds released by rupture of the vacuole, the main storage organelle of these compounds. The active site of PPO consists of two copper atoms and the enzyme catalyzes two different reactions in the presence of molecular oxygen: the hydroxylation of monophenols (monophenolase activity) and the oxidation of o-diphenols to o-quinones (diphenolase activity) (Fig. 1). This reaction is followed by non-enzymatic polymerization of the quinones giving rise to melanins, pigments of high molecular mass, and dark color.

Plants PPOs have broad substrates specificities and are able to oxidize a variety of mono, di or polyphenols. Phenolic compounds are natural substances that contribute to the sensorial properties (color, taste, aroma and texture) associated with fruit quality. Structurally they contain an aromatic ring bearing one or more hydroxyl groups together with a number of other substituents (Fig. 2). Some of PPO substrates occur naturally in fruits and vegetables, e.g., apples, very suitable to enzymatic browning, are rich in chlorogenic acid, catechin and epicatechin.

Table 1 summarizes some characteristics of PPO extracted from different plant sources. Details about function and these characteristics kinetics can be seen in the reviews from Mayer and Yoruk and Marshall.

The inactivation of PPO is required to minimize product losses caused by browning. In this way, several methods and technologies have been studied. Heat treatment and addition of antibrowning agents are usually applied, but several researchers have proposed the application of other methods as alternatives to thermal processing for PPO inactivation. This review focuses on the studies in fruits and vegetables with non-conventional methods to prevent browning reactions.

### Browning Control

Since browning reduces nutritional and sensory qualities, several techniques and mechanisms have been developed to control PPO activity. These mechanisms act on one or more of the essential components necessary for the reaction to occur: oxygen, enzyme, copper or substrate.

![Figure 1](image.png)

**Figure 1.** Reactions of (a) hydroxylation and (b) oxidation catalyzed by PPO.
Chemical Antibrowning Effectors

According to the literature, different effectors can control enzymatic browning and these compounds are classified based on the inhibition mechanism as reducing agents, chelating agents, acidulants, enzyme inhibitors, enzyme treatments and complexing agents.\(^{(39)}\)

The most widespread agents used for browning control are sulphiting agents. Due to adverse health effects caused by these compounds, the World Health Organization (WHO) has recommended limiting, as much as possible, the use of SO\(_2\) in the treatment of foodstuffs, even to the point of contemplating the possibility of its complete suppression. According to the current indications of WHO, the acceptable daily intake of SO\(_2\) in foodstuffs has been established as 0.7 mg/kg of body weight. Several studies have been made to find an efficient inhibitor without toxic effects.\(^{(40)}\)

Among sulphur-containing agents, cysteine is an effective compound to prevent browning reaction. It reacts with o-quinones intermediates to produce stable and colourless products.\(^{(41)}\) Rapeanu et al.\(^{(29)}\) related that the most potent inhibitors for grape PPO were ascorbic acid, cysteine, and sodium metabisulfite because these compounds induced a high degree of inhibition, even at the lowest concentration used (0.05 mM). Cysteine inhibited PPO activity in mango puree at concentration about 0.2 mg/g\(^{(42)}\) and was effective in preventing the browning of apple juice at 1–1.8 mM\(^{(39)}\) and 4 mM.\(^{(41)}\) However, even at these concentrations, cysteine produces an undesirable odour, limiting its use in food processing.

Ascorbic acid (AA) is frequently used for browning control of food products and it has been shown to be more effective than its isomer isoascorbic acid.\(^{(39)}\) This vitamin acts as an antioxidant because it reduces the quinone produced before it undergoes secondary reactions that lead to browning and also contributes to decreasing pH.\(^{(42)}\) Since optimum pH to PPO reactions range from 5 to 7.5 (Table 1), lower values inhibit enzymatic activity.
Mango puree with the addition of AA inhibited more PPO activity than puree containing cysteine or 4-hexylresorcinol (4-HR). However, AA was added at higher concentrations than 4-HR and cysteine.\(^{42}\) The effect of AA can be considered temporary because it is oxidized irreversibly by reaction with intermediates, as pigments, endogenous enzymes and metals such as copper. In apple juice, inhibitory effect of AA at concentration of 1.8 mM was observed for 4 hours.\(^{39}\)

**Table 1**

Kinetic parameters of PPO extracted from different vegetable sources

<table>
<thead>
<tr>
<th>Source</th>
<th>Substrates with higher affinity</th>
<th>(K_m) (mM)</th>
<th>Optimum pH</th>
<th>Optimum temperature (°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple (cv. Amasya)</td>
<td>4-Methylcatechol, Catechol</td>
<td>3.1, 34.0</td>
<td>7.0, 6.0</td>
<td>15, 25</td>
<td>(25), (26)</td>
</tr>
<tr>
<td>Artichoke(^1)</td>
<td>Catechol, 4-Methylcatechol</td>
<td>10.2, 12.4</td>
<td>6.0, 25</td>
<td></td>
<td>(27)</td>
</tr>
<tr>
<td>Aubergine(^2)</td>
<td>Catechol</td>
<td>8.7</td>
<td>7.0</td>
<td>20</td>
<td>(28)</td>
</tr>
<tr>
<td>Banana (cv. Anamur)</td>
<td>Catechol</td>
<td>8.5</td>
<td>7.0</td>
<td>30</td>
<td>(29)</td>
</tr>
<tr>
<td>Cherry laurel(^3)</td>
<td>DHPPA(^4)</td>
<td>—</td>
<td>5.0</td>
<td>50</td>
<td>(12)</td>
</tr>
<tr>
<td>Grape (cv. Victoria)</td>
<td>Chlorogenic acid, Catechin, Catechol</td>
<td>3.2, 4.3</td>
<td>5.0, 7.0</td>
<td>25</td>
<td>(30)</td>
</tr>
<tr>
<td>Henry chestnut(^5)</td>
<td>Catechol</td>
<td>14.3</td>
<td>5.0</td>
<td>40</td>
<td>(31)</td>
</tr>
<tr>
<td>Loquat(^6)</td>
<td>Chlorogenic acid, 4-tert-catechol</td>
<td>1.0, 1.2</td>
<td>6.5, 30</td>
<td></td>
<td>(32)</td>
</tr>
<tr>
<td>Mango (cv. Tainong)</td>
<td>Catechol, Pyrogallol</td>
<td>6.3, 47.8</td>
<td>7.0, 30</td>
<td></td>
<td>(33)</td>
</tr>
<tr>
<td>Medlar(^7)</td>
<td>DHPPA, Epicatechin, L-DOPA</td>
<td>1.9, 4.0</td>
<td>6.5, 4.7</td>
<td>35, 20</td>
<td>(34)</td>
</tr>
<tr>
<td>Mulberry(^8)</td>
<td>Pyrogallol, 4-Methylcatechol</td>
<td>1.2, 9.2</td>
<td>7.5, 19.8</td>
<td>20, 35</td>
<td>(35)</td>
</tr>
<tr>
<td>Thymus(^9)</td>
<td>Pyrogallol, 4-Methylcatechol</td>
<td>5.5, 9.8</td>
<td>6.5, 18.0</td>
<td>35, 25</td>
<td>(36)</td>
</tr>
<tr>
<td>Peppermint</td>
<td>Catechol</td>
<td>6.3</td>
<td>7.0</td>
<td>30</td>
<td>(11)</td>
</tr>
<tr>
<td>Strawberry (cv. Elsanta)</td>
<td>Catechol</td>
<td>5.9</td>
<td>5.0</td>
<td>25</td>
<td>(37)</td>
</tr>
<tr>
<td>Persimmon</td>
<td>Catechol, 4-Methylcatechol</td>
<td>12.4, 14.6</td>
<td>7.5, 20–40</td>
<td></td>
<td>(38)</td>
</tr>
<tr>
<td>Yacon Root(^10)</td>
<td>Caffeic acid, Chlorogenic acid, 4-Methylcatechol</td>
<td>0.2, 1.1, 1.3</td>
<td>6.6, 1.1</td>
<td>30, 4.3</td>
<td>(39)</td>
</tr>
</tbody>
</table>

\(^1\)Artichoke (Cynara scolymus L.); \(^2\)Aubergine (Solanum melongena var. insanum); \(^3\)Cherry laurel (Laurocerasus officinalis Roem. ‘Globigemmis’); \(^4\)DHPPA – 3-(3,4-dihydroxyphenyl)propionic acid; \(^5\)Henry chestnut (Castanea henryi); \(^6\)Loquat (Eriobotrya japonica Lindl.); \(^7\)Medlar (Mespilus germanica L. Rosaceae); \(^8\)Mulberry (Morus alba L.); \(^9\)Thymus (Thymus longicaulis subsp. chaubardii var. chaubardii); and \(^10\)Yacon root (Smallanthus sonchifolius).
4-HR is generally recognized as safe (GRAS) for shrimp melanosis and inhibits browning by generating an inactive complex with the enzyme. Kinetic studies demonstrated that 4-HR inhibits PPO activity either by a competitive type,\(^{(43)}\) or a slow-binding inhibition mechanism,\(^{(44)}\) depending on the substrate. In mango purees, the main inhibition effect was observed at a concentration of 0.04 mg/g\(^{(42)}\) and in apple juice a concentration of 4 mM had long term inhibitory effect.\(^{(41)}\)

Kojic acid is found in many fermented Japanese foods. Although it occurs in certain foods as a natural fermentation product, the use of kojic acid in the food industry may be restricted due to the difficulty of large-scale production and its high cost. Iyidoğan and Bayindirli\(^{(41)}\) showed a significantly antibrowning effect of kojic acid in apple juice at concentrations ranging from 1 to 4 mM. This agent inhibited PPO and bleached melanin due to chemical reduction of the browning pigment to colourless compounds.

Aromatic carboxylic acids (benzoic and cinnamic acids) are PPO inhibitors due to their structural similarities with the phenolic substrates. Undissociated forms of these acids are able to inhibit PPO through complexation with copper at the active site of the enzyme.\(^{(21)}\) Rapeanu \textit{et al.}\(^{(29)}\) reported a weak inhibition at 0.5 mM (23%), when benzoic acid was tested in grapes.

\(\beta\)-cyclodextrins bind substrate in their hydrophobic core. The most important functional property of cyclodextrins is their ability to form inclusion complexes with a wide range of organic guest molecules, including PPO substrates.\(^{(45)}\) Özoğlu and Bayindirli,\(^{(39)}\) using concentration ranging from 0.3 to 1.8 mM, showed no effect of \(\beta\)-cyclodextrin, indicating that a higher amount of the compound was necessary to achieve inhibition. In tomato fruits, strength of inhibition was substrate-dependent following this order: chlorogenic acid, epicatechin and 4-tert-butylicatechol > caffeic acid and 4-methyl catechol > pyrocatechol, dopamine, protocatechuic acid and L-DOPA.\(^{(46)}\) In addition, PPO activity in apple juice was also affected by the type of cyclodextrin.\(^{(47)}\) Therefore, to achieve efficiency in PPO inhibition, food industries must consider the major phenolic compounds present in the product and the type of \(\beta\)-cyclodextrins used.

Sodium chloride is a strong oxidizing agent, which can generate chlorine dioxide under acidic conditions.\(^{(48)}\) Below pH values of 5, a strongly pH dependent PPO inhibitory effect is observed and the degree of inhibition increases with the acidity of the reaction medium.\(^{(48,49)}\) On the other hand, an activation effect is observed at higher pH values.\(^{(49,50)}\)

These differences in the mechanisms allow the use of combinations of antibrowning agents that may result in enhanced inhibition. Ascorbic acid, cysteine and cinnamic acid in combination showed a synergistic effect compared to the individual compounds in browning of cloudy apple juice.\(^{(39)}\) Iyidoğan and Bayindirli\(^{(41)}\) obtained 89% enzymatic inhibition in Amasya apple juice treated with 3.96 mM L-cysteine, 2.78 mM kojic acid and 2.34 mM 4-HR. Also, in apple juice, a synergic effect was observed between \(\beta\)-cyclodextrin and 4-HR which was not observed for the combination of \(\beta\)-cyclodextrin and methyl jasmonate. In the first combination, \(\beta\)-cyclodextrin acted to reduce the concentration of free substrate that could be oxidized while 4-HR interacted directly with the enzyme by a competitive mechanism, and consequently, the combination of the two inhibition mechanisms reduced PPO activity to a higher degree. In the second case, \(\beta\)-cyclodextrin would be complexed with substrate and methyl jasmonate, increasing the amount of free substrate and decreasing concentration of methyl jasmonate, which could inhibit PPO activity.\(^{(51)}\) The inhibition by methyl jasmonate observed in this study might be an exception since some authors have described PPO activation by this compound in several plants like tomato, tobacco, hybrid poplar (\textit{Populus trichocarpa} × \textit{deltoides}) and coffee leaves (\textit{Coffea}}
According to Guerrero-Beltrán et al., the synergistic effect of cysteine (0.3 mg/g) or AA (1 mg/g) with 4-HR (0.04–0.08 mg/g) may have the greatest potential for reducing PPO activity and improving color stability of mango puree during storage.

Other compounds have been studied in order to obtain alternatives to prevent/control enzymatic browning. Honey, procyanidins, and Maillard reaction products are natural agents proposed to have an inhibitory effect on PPO. Honeys contain a number of components known to act as preservatives; these include α-tocopherol, ascorbic acid, flavonoids and other phenolic compounds. Honeys from different floral sources reduced PPO activity over a range of 2–45% in fruit and vegetable homogenates and combination with ascorbic acid enhanced the inhibitory effect. Le Bourvellec et al. showed that native procyanidins, flavanol polymers that occur naturally in plants, inhibited PPO activity in cider apple juices and inhibition intensity increased with degree of polymerization of the procyanidins. The mechanism is probably due to the binding of the polyphenol to the protein affecting the catalytic activity of the enzyme or by forming an inactive enzyme-polyphenol-substrate complex. Maillard reaction products synthesized from various amino acids and sugar solutions were tested on potato PPO activity by Lee and Park and it was verified that inhibitory effect depends on the amino acid (arginine > cysteine > histidine > lysine) and the type of sugar used (monosaccharides > disaccharides).

Studies about inactivation of commercial PPO (mushroom tyrosinase) have been published. Girelli et al. measured PPO activity in the presence of various glycyldipeptides. Proteins, peptides and amino acids can affect PPO activities by reacting with the o-quinones and by chelating the copper at the active site of PPO. These authors found that glycylaspartic acid, glycyphenilalanine, glycyglycine, glycyllysine, glycytyrosine, and glycylhistidine affected the formation of quinone in all concentrations used varying from 1 to 50 mM. Shi et al. showed different inhibition types between cinnamic acid and its derivates. Strength and inhibition types were: cinnamic acid (non-competitive) > 4-hydroxycinnamic acid (competitive) > 4-methoxycinnamic acid (non-competitive). 2-Hydroxycinnamic acid had no inhibitory effect on the diphenolase activity. According to the authors these inhibitors were attached to a region different from the active site and hindered the binding of substrate to the enzyme through steric hindrance or by changing the protein conformation. Chen et al. demonstrated that PPO was inhibited by several p-alkoxybenzoic acids and this inhibition was concentration-dependent. Among the compounds investigated, p-methoxybenzoic acid was the most potent inhibitor. The inhibitory effects of cis- and trans-isomers of 3,5-dihydroxystilbene were studied by Song et al. Both compounds inhibited PPO activity and it was dose-dependent, but the cis-form had higher inhibitory ability than the trans-form because cis-form can bind more tightly to the active site of the enzyme than its isomer.

**Thermal Processing**

Heat treatment is the most widely used method for stabilizing foods because of its capacity to destroy microorganisms and to inactivate enzymes. Blanching is the most common method to inactivate vegetable enzymes. It causes denaturation and therefore inactivation of the enzymes but also causes destruction of thermosensitive nutrients and it is rarely used for soft fruits.

Water blanching can be disadvantageous because it results in losses in vitamins, flavour, colour, texture, carbohydrates and other water-soluble components. Requirements for large amounts of water and energy, coupled with waste disposal problems make blanching technically disadvantageous.
In general, exposure of PPO to temperatures of 70–90°C destroys their catalytic activity, but the time required to inactivation depends on the product.\(^{(63)}\) Chutintrasri and Noomhorm,\(^{(63)}\) studying thermal inactivation of pineapple PPO, described that the enzyme activity reduced approximately 60% after exposure to 40–60°C for 30 min. Denaturation increased rapidly above 75°C. Thus, residual activity was about 7% after 5 min at 85°C and 1.2% after 5 min at 90°C. The same profile was observed for Napoleon grape PPO,\(^{(64)}\) when, in this study, the enzyme was heated for 5 min at temperatures ranging from 30 to 100°C. From 70 to 80°C, 20% of activity remained and at 100°C, total inactivation was reached. In Victoria grape PPO,\(^{(29)}\) the complete inactivation was reported after 10 min at 70°C. The activity of PPO from *Castanea henryi* nuts after incubation at 70°C for 30 min was 8%.\(^{(30)}\) Valderrama et al.\(^{(65)}\) obtained fully inactivation of apple PPO after 10 min of treatment for 75°C. Maximum reduction of PPO activity in cloudy apple juice was 27.9% at 55°C for 60 min.\(^{(66)}\) PPO extracted from Elsanta strawberry was also thermosensitive. Its activity reduced 50% after 10 min of heating at 55°C and the enzyme was almost completely inactivated after 10 min or thermal treatment at 65°C.\(^{(36)}\)

**High Hydrostatic Pressure Treatment**

High hydrostatic pressure (HHP) treatment of fruit and vegetable products offers the chance of producing food of high quality, greater safety, and increased shelflife.\(^{(67)}\) HHP reduces microbial counts and inactivates enzymes.\(^{(68)}\) The treatment is expected to be less detrimental than thermal processes to low molecular mass food compounds such as flavouring agents, pigments and vitamins as covalent bondings are not affected by pressure.\(^{(69)}\) In apple-broccoli juice processed by HHP, it was observed that the content of ascorbic acid was more sensitive to treatment time than to pressure applied and there were no changes in carotene content during storage.\(^{(70)}\)

HHP can affect protein conformation and lead to protein denaturation, aggregation or gelation, depending on the protein system, the applied pressure, the temperature and the duration of the pressure treatment. HHP can be used to create new products (new texture or new taste) or to obtain analogue products with minimal effect on flavour, colour and nutritional value and without any thermal degradation.\(^{(71)}\) Pressure also can influence biochemical reactions by reducing molecular spacing and increasing interchain reactions.\(^{(21)}\)

HHP promotes protein denaturation which is associated with conformational changes. It can increase or reduce enzymatic biological activity or change its substrate specificity, modifying the functionality of an enzyme.\(^{(72)}\)

The effectiveness of treatment depends on the type of enzyme, pH, medium composition, temperature, time and pressure level applied.\(^{(72)}\) For several PPOs, it has been reported that pressure-induced inactivation proceeds faster at lower pH. In addition to pH, pressure inactivation is influenced by the addition of salts, sugars or chemical antihrowning effectors.\(^{(73)}\)

PPO is more resistant to pressure than thermal treatment, when compared to other enzymes like peroxidase,\(^{(74)}\) and the combination of these methods increases the inactivation effectiveness. According to Bayindirli et al.,\(^{(68)}\) the HHP processing time needed to inactivate enzymes is longer than that needed to kill bacteria in acid juices. The best results to decrease PPO activity were found at pressures higher than 400 MPa combined with mild heat (50°C). Palou et al.\(^{(75)}\) obtained reduction of PPO activity in banana puree after short steam blanching pretreatment, followed by HHP treatment (517 and 689 MPa/10 min). Phunchaisri and Apichartsrangkoon,\(^{(76)}\) studying the effect of HHP on lychee (*Litchi chinensis* Sonn.) PPO, observed 90% of inactivation when treated at 600 MPa and
60°C for 20 min. Damaldi et al.\(^{(36)}\) observed that 800 MPa for 15 min at 25°C was necessary to reduce 95% of enzyme activity, but when mild temperature (40–50°C) was used, the required pressure level for 80–100% inactivation of PPO fell to 550 MPa. Carrot juice PPO was inactivated around 96% after a combined treatment at 300 MPa and 70°C for 10 min.\(^{(67)}\) In this study, for producing high-quality carrot juice, the best pressure range and treatment time predicted was 395–445 MPa and 8–10 min, respectively.

The level of pressure applied is critical to change enzyme activity. Studies have shown that low pressure (up to 400 MPa) induced PPO activation in red raspberry (\textit{Rubus idaeus}),\(^{(77)}\) in pear (200–400 MPa/25°C/10 min),\(^{(78)}\) and in apple juice (100 MPa/1 min).\(^{(79)}\)

### Gamma Irradiation

Fruits and vegetables can be treated by \(\gamma\) irradiation to extend shelf life. Irradiation is a physical treatment involving direct exposure to electrons or electromagnetic rays, for food preservation and improvement of safety and quality.\(^{(80)}\) \(^{60}\)Co produces electromagnetic \(\gamma\) rays which are similar to light but with much higher energy. Radiation inactivates microorganisms (mainly bacteria, moulds and yeasts), guarantees complete desinfection and delays the ripening process and senescence.\(^{(81)}\) In addition, some authors noted that treatment with \(\gamma\) rays decreased ascorbic acid losses\(^{(82,83)}\) and stimulated synthesis of phenolic compounds during storage.\(^{(84)}\)

Low-dose \(\gamma\) irradiation is commonly applied in fruit and vegetable products. Treatment with a dose of 0.35 kGy decreased 1.5 and 1 log of total aerobic microorganisms and yeasts and molds in cut romaine lettuce, respectively. This dose did not adversely affect sensory attributes, such as visual quality and off flavor development.\(^{(85)}\) Zhang et al.\(^{(82)}\) related reduction on the spoilage from microorganisms at a dose of 1.0 kGy (0.5 kGy/h and 15°C) in fresh-cut lettuce and the shelf life was 9 days based on microbiological safety. At the same dose, PPO activity from lettuce was about 31% lower than in untreated samples stored at 4°C for three days, but after 9 days at the same conditions, PPO activity in irradiated samples was 54% higher than in control.

Lu et al.,\(^{(83)}\) studying the effects of irradiation on fresh-cut celery, found a decrease of 73% on PPO activity in the sample treated with 1.0 kGy (0.5 kGy/h) after 3 days kept under refrigeration (4°C). After 9 days, the enzyme showed an activity around 25% lower than the control sample.

Irradiation can also be used in addition with other methods or chemical antibrowning effectors. A combination of calcium ascorbate dipping and low dose ionizing radiation resulted in a microbiologically safe and high quality of fresh-cut apples rich in nutrients (calcium ascorbate and ascorbic acid).\(^{(86)}\)

### Pulsed Electric Field

Pulsed electric field (PEF) is an emerging non-thermal food-preservation technology that has been researched and developed close to the commercial stage. This process is conducted by introducing the food in a chamber containing two electrodes that apply high voltage pulses in the order of 20–80 kV for microseconds. The PEF applied to the food causes irreversible loss of the cell membrane functionality, a process known as electroporation, which leads to inactivation of microbial cells.\(^{(87,88)}\) Therefore, most studies on the PEF process have focused on the inactivation of microorganisms and have reported that this treatment can be used in the processing of liquid foods.\(^{(89,90,91,92)}\)
There are few studies about the effect of PEF on PPO. Zhong et al.,(93) showed that inactivation of commercial PPO depends on the electric field strength and time of treatment. The greatest reduction in PPO activity was 76.2% at 25 kV/min for 744 μs. Other studies reported a decrease of 97% on this enzyme activity in apple extract at 24.6 kV/cm for 6000 μs, 72% in pear at 22.3 kV/cm for the same treatment time,(94) and 70% in peach PPO at 24.3 kV/cm for 5000 μs.(95)

The sensitivity to PEF treatment varies from enzyme to enzyme. The sequence of sensitivity to PEF of five enzymes tested by Yang et al.(96) was: pepsin > PPO > peroxidase > chymotrypsin and lysozyme. The inactivation effect of PEF on enzymes was affected by electric field strength, electrical conductivity and pH.

PEF treatment also induces changes in secondary structure of PPO and peroxidase.(93) After PEF treatment, the loss of the relative α-helix fractions of PPO and peroxidase were calculated and these results showed that PPO was more susceptible to the treatment than peroxidase.

Other Technologies

Although most studies have focused on HHP, irradiation and PEF treatments, other techniques also hold promise to reduce PPO activity.

Supercritical carbon dioxide is a non-thermal technology with a pressurization step that ensures that the applied gas penetrates the microbial cells, and subsequent explosive decompression results in rapid gas expansion within the cells, physically destroying them.(97) Beyond the lethal effect on microorganisms, supercritical carbon dioxide also has an effect on enzyme inactivation due conformational changes caused by gas in the secondary and tertiary structure.(66) Cloudy apple juice exposed to supercritical carbon dioxide at 30 MPa and 55°C for 60 min presented a reduction of PPO activity over 60%, which was higher than that under atmospheric conditions at 55°C (27.9% of reduction), indicating that the combined effects of pressure, temperature and time occurred after carbon dioxide treatment. (66) An addition of carbon dioxide in the product before HHP treatment can also be used to inhibit PPO activity and the results found by Corwin and Shellhammer(97) showed a residual activity of 57.6% (500 MPa/3 min at 50°C). In this study addition of carbon dioxide decreased significantly the PPO activity at all pressures (0, 500, and 800 MPa) and temperatures (25 and 50°C) beyond the effects of pressure alone.

Ohmic heating is defined as a process in which electric currents are passed through foods with the main purpose of heating them. Ohmic heating is distinguished from other electrical heating methods by the presence of electrodes contacting the foods, the frequency, and the waveform of the electric field imposed between the electrodes. According to Castro et al.,(98) inactivation kinetics of PPO followed a first-order reaction for conventional and ohmic heating treatments. In this study, the presence of an electric field reduced the time needed for enzyme inactivation. Ohmic heating can also be used for blanching of food products. Icier et al.(99) treated pea puree by ohmic blanching and it inactivated the peroxidase enzyme at lower processing time than conventional water blanching.

Microwave heating is an alternative method for liquid food pasteurization. Compared to conventional heating methods, microwaves are able to heat products internally, have greater penetration depth and faster heating rates that would potentially improve retention of thermolabile constituents in the food.(100,101) Microwave energy induces thermal effects over microorganisms and enzymes similar to those of conventional heating mechanisms.(102) Matsui et al.(103) submitted solutions simulating the chemical constituents of
coconut water to a batch process in a microwave oven and observed that PPO activity in water and in sugar solution was reduced after treatment. In salt solution, PPO stability was significantly affected and the contact between salt and enzyme promoted a drastic reduction of the initial activity. At temperatures above 90°C, the combined effects of salts and microwave energy reduced enzymatic activity to undetectable levels. However, at 90°C, the inactivation effect can be due to temperature alone.

Conclusions

PPO is an important enzyme in the food industry and its activity, in general, causes decrease of nutritional value and consumer’s acceptance leading to economical losses. The improvement of methods to control browning is an important key to enhance product value and minimize post harvest losses.

Alternatives to heat treatment, such as high hydrostatic pressure, irradiation, pulsed electric fields, etc. have been studied to control enzymatic browning with minimal changes in sensory and nutritional characteristics. A disadvantage of these emerging technologies is the high initial cost necessary to acquire the equipment and to implement new production technologies. Furthermore, industry application of these technologies is dependent on their validation with experimental data to evaluate the effects of treatments on microorganisms, enzymes and biological tissues. Although benefits of some alternative technologies have been described by many authors, such as HHP, only a few industries have already adopted these food processing treatments. The knowledge of these alternatives is necessary to use one, or a combination of methods, to obtain a high quality product.

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